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SCHOOL OF ENVIRONMENTAL SCIENCES

DEPARTMENT OF HYDROLOGY AND WATER RESOURCES

Investigation of Coagulant Properties and Efficiency of *Diceriocaryum Eriocarpum* Plant for Turbidity Removal and Biosorbent for Heavy Metals Uptake in Aqueous Solution

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DECLARATION

I, Bassey Odo Jones, hereby declare that this dissertation for the Master in Environmental Sciences degree at the University of Venda, hereby submitted by me, has not been previously submitted for a degree at this or any other institution. This is my work in design and execution, and all reference materials contained herein have been duly acknowledged.

Signature _____ Date: 12/11/14

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Abstract

Environmental pollution over the past decades has reached crisis level due to the nature of pollutants that leach into the water bodies. Inadequate water services coupled with lack of potable drinking water infrastructure is one of the major challenges facing most of the rural communities in South Africa. This research was aimed at investigating the potential of a new and cost effective plant material as biosorbent in the removal of heavy metal ions from aqueous solution and also as coagulant for removal of turbidity from raw water. The material is intended for use in rural households where most of the domestic water supplies are untreated.

Therefore, this study proposed the use of mucilage from *Diceriocaryum eriocarpum* (DE) as biosorbent in the removal of heavy metals from aqueous solution and as coagulant in the removal of turbidity from raw water. To this end, modification of the DE mucilage was carried out by addition of chlorides during the extraction process. Extraction of the mucilage was carried out using KCl, NaCl solution and deionized water solely. Characterisation of the active agent in the mucilage was carried out using Fourier transform infared spectroscopy (FTIR). Improvement of the coagulation efficiency was carried out by optimizing various parameters. Assessing the biosorption capability of DE mucilage was also achieved by optimizing parameters. Optimization was achieved by varying one parameter while keeping the others constant.

Application and improvement of coagulation and biosorption potential of DE was greatly attributed to the functional group (active agent) present in the mucilage. FTIR results showed that the functional groups acting as the active agent in DE both in modified and unmodified mucilage were carboxyl, hydroxyl and carbonyl groups. The chloride used in the modification of the mucilage did not introduce new functional groups but rather expanded and increased the already existing functional groups in the mucilage.

The modified mucilage: potassium crude extract (PCE) and sodium crude extract (SCE) display high coagulation and biosorption efficiency more than unmodified mucilage; deionized water crude extract (DCE). It was observed that an increase in coagulant dosage, settling time and initial turbidity influence the coagulation efficiency of DE mucilage. Assessing the EC-levels, pH and high reduction in turbidity levels of the treated water samples showed that the treated water was of high quality. Coagulation mechanism for unmodified mucilage was suggested to be

strong repulsion force while modified mucilage mechanisms occur via double layer interaction and charge neutralization.

Results from biosorption experiment showed that, DCE, SCE and PCE display good binding affinity with heavy metal ions (Zn, Cd, Ni, Cr and Fe) in the aqueous solution. It was also observed that an increase in the aqueous solution pH, mucilage concentration and initial concentration of metal ions also increased the sorption efficiency of DCE mucilage. The DCE biosorbent was able to attain equilibrium rapidly within 8-10 minutes of contact time. Biosorption mechanism for both modified and unmodified mucilage seems to occur via electrostatic interaction and binding chelation.

Generally, it was observed that the modified mucilage was highly efficient in turbidity removal and also in the removal of metal ions more than the unmodified mucilage. This can be attributed to the presence of the salts enhancing the mechanism in the active agent (functional groups) of DE coagulant.

Hence, the best method for improving the coagulation and biosorption efficiency of DE plant is by using salt solution in extracting the mucilage. Coagulation efficiency was improved to 99% using modified mucilage coagulant. In light of this, the adoption of DE mucilage in treatment of raw water showed good results and can be adopted for household use in communities where there is need for potable drinking water.

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Chapter 1: Introduction

1.1. Background of the research

Environmental pollution has become of global concern and has attracted much attention over the past decades. Increased use of metals and chemicals in process industries such as mining has resulted in generation of large quantities of effluents that contain high levels of toxic heavy metals [Department of Environmental Affairs and Tourism (DEAT), 2006]. The presence of heavy metals pose environmental–disposal problems due to their non-degradable and persistence nature (Roa, 2005). Water is typically the prime environmental medium that has been affected by pollution as a result of human activities. This makes water treatment of high importance.

Drinking water treatment characteristically includes coagulation, sedimentation, filtration and disinfection (McCarthy and Zachara, 1989). Coagulation process independently is capable of removing either soluble organic, inorganic constituent or colloidal phase impurities. Thus coagulation process is significantly important in water treatment. Coagulation with subsequent flocculation has been shown to be suitable for the removal of particulate matter. It is also known to remove solution-phase organic and inorganic pollutants, thereby removing the microorganisms that are often attached to the particles (Miller *et al*., 2008).

Coagulation process does not only improve the quality of the treated water but it is also known to be cost effective, requiring less capital expenditure in the implementation. Coagulation process can easily be controlled and is particularly suited for application to water/aqueous solution treatment. The use of polymers in water/aqueous solution treatment is one such technological advancement. Polymers have been used effectively in water/aqueous solution treatment in several ways as primary coagulants, as coagulant aid, as filter aids, as filter conditioners and as sludge conditioners (Welday and Baumann, 1979).

Natural plant polymers (coagulants) show bright future in water/aqueous solution treatment because they have similar function like inorganic coagulant. Natural polymers seem to be the best means of obtaining low turbidity water, eliminating potentially hazardous particles without any mutagenic effect and producing water with no extreme pH conditions. Water treated with natural plants coagulants is also able to comply with the guidelines of drinking water standards

(Ndabigengesere *et al*., 1995; Okuda *et al*., 1999 and Ghebremichael *et al*., 2005). Biological materials have emerged as an economic and eco-friendly option in the removal of heavy metals from industrial effluent via biosorption. Most recent studies have reported that biomaterials of microbial and plant origin interact effectively with heavy metals and are less expensive, available and easy to use (Hanafiah and Ngah, 2008).

Diceriocaryum eriocarpum (DE) plant is a common species from grassland of the family Pedaliaceae with common name "Devil's thorns", locally called in Vhembe District 'Museto' (Fig. 1.1). It is widely spread throughout Limpopo Province of South Africa, and in Southern Africa in the Kalahari dune veld, sandy soils. It is also found in dune slopes and river banks, particularly trampled areas and abandoned fields, usually in sandy soils (Van Wyk and Malan, 1988).

Figure 1.1 *Diceriocaryum eriocarpum* plant (Botha, 2000)

DE species is a multi-purpose plant because of its various functions. All parts of the plant contain saponins and mucilage markedly soapy and slimy. If crushed plant is left overnight in water, the resultant mucilage from the leaves is a useful substitute for soap and shampoo (Van Wyk and Gericke, 2000). In terms of medicinal purposes, it can be used for: antibacterial, anti-

inflammatory (Luseba *et al*., 2006), ethno-veterinary medicines (Merwe *et al*., 2001) and parts of the plant have been used locally to treat diseases.

A recent study conducted by Malima (2010) revealed that DE leaves have the potential to purify raw water to potable water suitable for drinking. Result of the study showed that applying liquid mucilage squeezed from the leaves of DE into turbid raw water contributed to a decrease in turbidity of the raw water. Turbidity was reduced to less than or up to approximately 5 Nephelometric turbidity units (NTU), which according to World Health Organisation [WHO (2006)] guidelines is suitable for drinking. The pH values of the treated water fell within the target water quality range of 6-9, which shows no significant effects on health according to Department of Water Affairs and Forestry [DWAF (1996)]. Thus Malima (2010) study showed that DE mucilage can be used as coagulant in removal of turbidity and purification of untreated raw water.

According to Malima (2010), when DE plant mucilage was added to the raw water (river water), there was an increase in electrical conductivity which was attributed to the possible presence of chemicals in the plant or mobilization of ions in the water. The use of DE as a coagulant required at least 5 days to purify raw water and was ineffective in purification of low turbid waters (Malima, 2010). These scenarios indicate the need to investigate the coagulation properties of DE. The slow rate of turbidity removal by DE coagulant highlighted the need to improve its coagulation efficiency. This study is therefore focused on coagulation properties of DE, improving its efficiency for turbidity removal and assessing the biosorption capability of DE mucilage in the removal of heavy metals from aqueous solution.

1.2. Statement of the problem

Poor quality of water is an important vehicle for the transmission of pathogenic microorganisms that can cause infections, illnesses and deaths, which have devastating and debilitating effects on rural residents (WHO/ United Nation Children's Fund (UNICEF), 2000; Gray, 2008). Incidences of morbidity and mortality resulting from waterborne diseases especially among children are significantly high in settlements where water supply and sanitation fall below the levels recommended by DWAF (1996) as reported by Obi *et al*. (2005).

Inconsistent water services such as water treatment infrastructure together with poor hygiene and poor sanitation are among the serious challenges facing many rural communities of South Africa (Obi *et al*., 2002). The use of small water treatment plants (SWTPs) in some rural areas of South Africa in the provision of potable water is a major problem in terms of the quality of water particularly due to inadequate finances for purchasing chemicals for treatment, among other factors (Makungo *et al*., 2011). Conclusion drawn from Makungo *et al*. (2011) indicated that Mutshedzi WTP located in Vhembe district is not producing adequate quality of water suitable for drinking.

The Vhembe District of the Limpopo Province, South Africa (SA) is mostly rural and most of the villages lack proper water treatment systems. Village communities also depend on readily available water sources for their daily needs including drinking. Microbial quality of several untreated water sources in Vhembe exceed the acceptable guidelines for drinking water as stipulated by DWAF (1996) of South Africa (SA) (Obi *et al*., 2002, 2005). These make the untreated water sources unsafe for human consumption due to the presence of pathogenic microorganisms in the water (Obi *et al*., 2002, 2005).

The outbreak of acute diarrhea in Tshikuwi in 2006, a village in Vhembe District, was due to the use of untreated water as indicated by Bessong *et al*. (2009). According to WHO (2006), faeces can be a source of pathogenic bacteria, viruses, protozoa and helminth which are responsible for waterborne diseases associated with poor quality of water. It is noted that high turbidity in raw water is associated with pathogenic diseases such as cholera, typhoid fever, skin irritation and other related waterborne diseases (Gray, 2008). Malima (2010) review showed that Nzhelele River in Vhembe District had high turbidity, which exceeded the DWAF (1999) recommended guidelines of 1 NTU for domestic use. Turbidity removal is relatively important in achieving high quality of water for human use.

One of the most leading factors of polluted water in South Africa is wastewater from different sources such as mining, milling and surface finishing industries, which discharge a variety of toxic metals such as Cd, Cu, Zn and Pb into the environment (Roa, 2005). South Africa as a leading country in mining operations faces crucial environmental problems (DEAT, 2006; Akpor

and Muchie, 2010). It had been discussed that, heavy metal pollution of surface water bodies affects the quality of water, thereby contaminating drinking water sources and is dangerous to consumer's health. Due to the toxic effect of the heavy metals, there is need for them to be removed before using the water for domestic purposes. In view of the above, there is a considerable urgent need for alternative, feasible, relatively simple, easily accessible, locally available and cost effective methods for the removal of pollutants from polluted water sources particularly in rural communities.

1.3. Motivation

Complying with DWAF (1996) guidelines in terms of water quality is difficult due to inadequate or lack of water treatment systems/facilities in the rural villages. Therefore it is important to study the use of natural plants coagulants as alternatives for the treatment of water before use. This is because natural plants coagulants have been shown to be abundant and can be used sustainably to remove high and low turbidity in untreated water. They are able to reduce microorganisms in raw water and purify untreated water until it becomes suitable for drinking, following WHO (2006) guidelines (Ndabigengesere *et al.*, 1995; Okuda *et al.*, 1999; Ghebremichael *et al.*, 2005 and Yin, 2010).

Application of natural plants coagulants reduces high rate of death caused by consumption of untreated water. Most importantly, natural plants coagulants are considered safe and environmentally friendly, especially non-toxic, biodegradable, affordable if locally available, and easy to use and maintain (Yin, 2010). Ndabigengesere and Narasiah (1998) reported that natural coagulants of vegetable and mineral origin were in use in water treatment before the advent of chemical salts. They have not been able to compete effectively because of the fact that scientific understanding of their effectiveness and mechanism of action was lacking making their use to be discouraged without any scientific evaluation (Ndabigengesere *et al.*, 1995).

As reported in section 1.1, in previous years, DE was known only for medicinal purposes such as antibacterial, anti-inflammatory and ethno-veterinary medicines. The use of DE as a water coagulant has been recently initiated by the University of Venda hydrology research group led by Prof J. O. Odiyo. Therefore there is general lack of information regarding the use of DE for water purification. Understanding the properties and potentials of DE in turbidity removal will

enhance its application and usage in water treatment in the rural communities if locally available. Its usage will be beneficial for commercial use if it is found in large quantities and for rural communities where there is no water treatment plant.

There is increasing application of plant materials as biosorbents in removal of heavy metals contaminants from aqueous solution, as a low cost and easy method. The use of mucilage as biosorbent for heavy metals ions removal has been seen to be scarce. A few studies have reported the use of mucilage from *Oputia species, Cactus species* and *Zea Mays* L (Mane *et al.*, 2011; Fox *et al.*, 2012). No studies have been reported on the use of mucilage from *Diceriocaryum species* for the removal of heavy metals ions from aqueous solution. Therefore, this study proposes the additional use of mucilage from DE as biosorbent in the removal of heavy metals from aqueous solution.

1.4. Objectives of the study

1.4.1. Main objective

The main objective of this study is to investigate the coagulant properties of DE mucilage and improve its efficiency in turbidity removal, in addition to assessing its application as biosorption medium for the removal of heavy metal ions from aqueous solution.

1.4.2. Specific objectives

The specific objectives of this study include:

a) To identify the active agents present in DE mucilage.
b) To improve the coagulation efficiency of DE mucilage for turbidity removal.
c) To assess the biosorption capability of DE mucilage in removal of heavy metals ions from aqueous solution.

1.5. Hypotheses

The hypotheses of this study include:

- The mucilage of DE plant possesses active agents that enables it to act like a coagulant as well as a biosorbent.

- Coagulation efficiency of DE mucilage can be improved by using modified mucilage released from the plant.
- The mucilage of DE plant material can be used as biosorbent media in removal of heavy metals ions from polluted water.

1.6. Study Area

1.6.1. Location

The Vhembe District, which constitutes the study area for DE plant and water sampling, is situated at the north-western tip of South Africa in Limpopo Province. It lies approximately between the Longitude 28°54′20.66′′E and 31°04′31.19′′E and latitudes 22°7′26.59′′S and 23°26′6.49′′S in the Northern Region of the Limpopo Province of South Africa (Fig. 1.2).

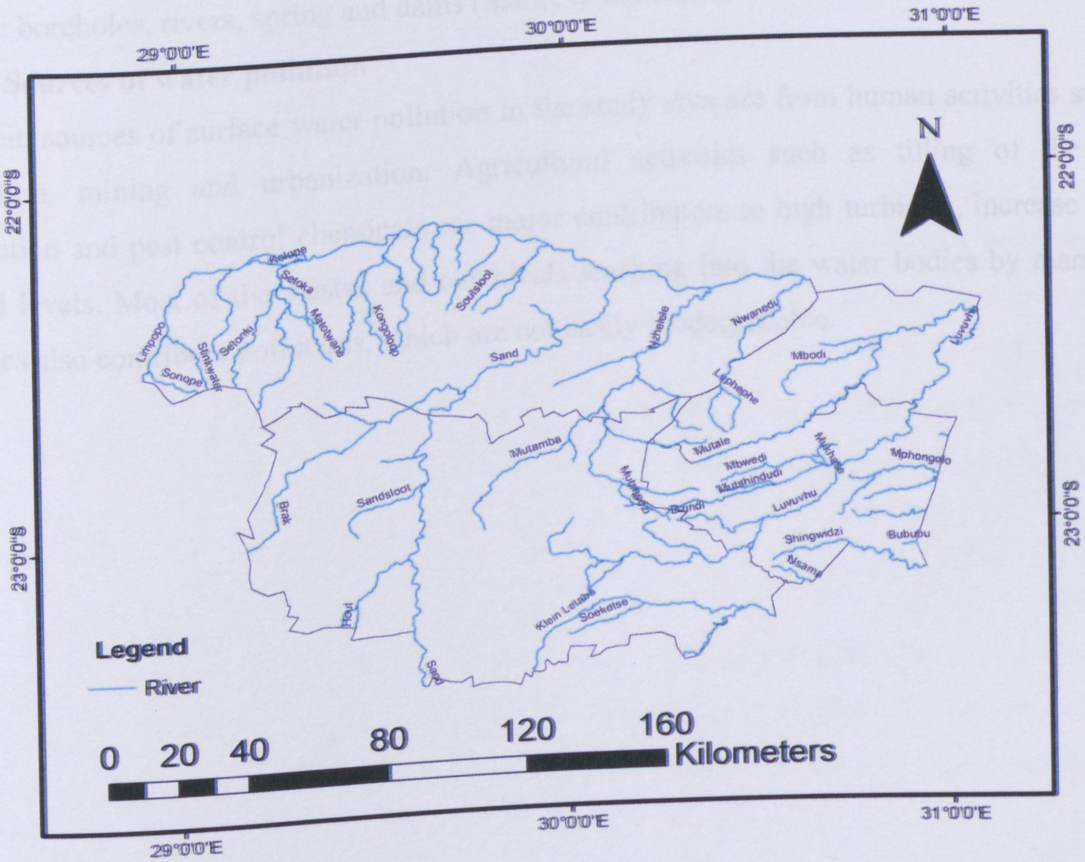

Figure 1.2: Map of Vhembe District

1.6.2. Land use

The study area is populated with existence of various urban and semi-urban settlements as well as numerous scattered rural villages. Land use here is predominantly commercial and subsistence agriculture, mining and rural settlements.

1.6.3. Water sources

In urban and rural regions of South Africa, 9.7 million (20%) of the people do not have access to adequate water supply such as potable water for drinking (Kahinda *et al.*, 2007). A few households in the rural villages in Vhembe District have indoor taps (Samie *et al.,* 2012). Most of the villages in Vhembe District only get reticulated water for about three days a week and sometimes rely on other unprotected sources for the rest of the week. Such water sources include: boreholes, rivers, spring and dams (Samie *et al.,* 2012).

1.6.4. Sources of water pollution

The main sources of surface water pollution in the study area are from human activities such as agriculture, mining and urbanization. Agricultural activities such as tilling of the land, fertilization and pest control chemicals are major contributors to high turbidity, increase in EC and pH levels. Most of the wastes and chemicals leaching into the water bodies by man-made activities also contribute pollutants, which are not easily biodegradable.

Chapter 2: Literature review

2.1.Preamble

This chapter presents a concise review of the literature relating to water pollution with more emphasis on heavy metals pollution and their effects on human. General overview has been carried out on biosorption and coagulation using various natural plants, their active agents, mechanisms, factors influencing their efficiency in turbidity removal and biosorption. The literature review also covered the methods used in extraction and purification of plant materials, methods used in determining active agents in plants and the methods used to improve the coagulation efficiency of natural plants.

2.2. Status of water resources in South Africa

It has been reported that almost 30% of the population in South Africa do not have access to adequate supply of potable water, and the water supply infrastructure is not meeting up the demands of the people (Tutu *et al.*, 2008; Kahinda *et al.*, 2010). Water supplies continue to decrease while water demand and population growth are increasing.

Population growth is accompanied by many human activities such as agriculture, mechanisation, urbanisation, industries and mining which have great negative impacts on the ecosystem and water quality in particular (Saad, 2011). This situation impacts negatively especially in areas with water scarcity and associated increases in water pollution and limited social and economic development and are linked closely to the prevalence of poverty, hunger and diseases associated with using polluted water (Falkenmark, 1994).

2.2.1. Water borne diseases

In Africa there are over 50 communicable diseases associated with water and inadequate sanitation (Connolly *et al.*, 2004). Waterborne diseases such as dysentry, cholera, diarrhea, typhoid, gastro-enteritis and hepatitis are the primary causes of diseases and poor health in SADC region (Said *et al.*, 2009).

During June- July 2006, there was an outbreak of diarrhoea in Tshikuwi, a village in the Venda area of Vhembe District in Limpopo Province of South Africa due to consumption of untreated water as reported in Bessong *et al.* (2009). Information gathered from the local clinic indicated

that 37 cases of diarrhoea in children aged less than 5 years and 23 cases of diarrhoea in individuals aged more than 5 years were recorded in June 2006 (Bessong *et al*., 2009). Children younger than the age of 5 years, especially those in areas devoid of sanitation are extremely prone to devastating effect of diarrhoea since diarrhoea may be transmitted by poor water quality (Esrey *et al*., 1991; Parashar *et al*., 2003).

A study conducted by Samie *et al*. (2009) in Vhembe District revealed that *E. Histolyticaldispar, Cryptosporidium, Giardia* and *Cyclospora* are common parasitic causes of diarrhoea in Vhembe District. *Campylobacter species* and *Aeromonos* are the most common bacterial causes of diarrhoea. In the study, the factors associated with the transmission of these pathogens to human include low socio-economic status, low level of education, use of unchlorinated well or river water and low level of personal hygiene. There is a strong link between diarrhoeal diseases and unsafe drinking water and poor personal hygiene, with ripple effect on the health of affected communities (Moomba and Kaleni, 2002).

Another study conducted by Gundry *et al*. (2009) in Vhembe District revealed that Vhembe District has higher incidences of childhood dysentry disease than the national average resulting from unimproved ground and surface water sources. Cholera is an infection caused by the bacteria *Vibrio* cholera. People become infected by drinking water or eating food contaminated by bacteria, poor sanitation as well as personal and domestic hygiene practices. Cholera epidemic spread to seven of the nine provinces in South Africa, in 2001, the total number of cases were 106 224 and the total number of fatalities were 228 cases (Nevondo and Cloete, 2002).

Studies have reported the improvements of water services including potability, access to water and water treatment infrastructure in South Africa as well as Vhembe District but the quality of the water is still unacceptable after upgrades (Majuru *et al*., 2012). Samie *et al*. (2012) also reported that water samples consumed by households in rural community of Vhembe District were contaminated with potential bacterial pathogens. The study indicated that household treatment of water before use is essential to reduce the risk of water borne diseases (Samie *et al*., 2012). It is well known that the health of a community is significantly affected by poor drinking water quality, which poses a great threat to human life.

Currently, people in Vhembe district rarely have access to potable drinking water and they depend on untreated water sources. Recent studies carried out by Samie *et al.* (2012) still show that microbial quality of water used for purposes such as drinking, bathing, washing, etc, are generally poor. 58% of the water samples used by the households were contaminated with faecal coliforms with many diarrhoea-causing agents (Samie *et al.*, 2012).

Treatment of water before use still remains the best approach in overcoming related water-borne diseases often associated with untreated water sources. However, natural plant based material having both coagulant and anti-microbial properties used in the treatment of water are numerous. Extensive research on DE has shown that it has anti-microbial and coagulant properties. The major advantage of DE over other natural plants coagulants is that it is locally available in Vhembe District and can also be easily accessed by rural households without any financial implications.

2.2.2. Heavy metals

Recently, the term heavy metals has been used as a classification or definition for those metals with toxic properties that accumulate in human bodies (Diaz, 1999). Generally there are more than 20 heavy metals, all of which have tendencies to accumulate in the environment. The most harmful metals to human health are mercury, cadmium, lead, arsenic, chromium, copper, and zinc (Cavus and Gulten, 2008; Soghoian and Sinert, 2013). Some metal ions such as sodium, potassium, magnesium, and calcium are essential to sustain biological life.

Some transition metals including manganese, iron, cobalt, copper, zinc and molybdenum are important for optimal growth, development, and reproduction in human bodies. Some trace metals are naturally found in the body and function mostly as catalysts for enzymatic activity in human bodies (DEAT, 2006 and Roa, 2005). Furthermore, all essential trace metals become toxic when their concentrations become excessive and some can even be toxic in minute concentrations (Roa, 2005). These metals can cause chronic or acute poisoning and should be eliminated as much as possible from the living environment (Roa, 2005 and Saad, 2011).

The content of heavy metals in waste is primarily a consequence of the intended use of heavy metals in industrial products and at the end of their useful life, all products end up being

pollutants to the extent that they are not attractive for recycling (Bellmann and Khare, 2000). Cadmium, zinc, copper, nickel, lead, mercury and chromium are often detected in industrial aqueous solution (Kadirvelu *et al*., 2001). They originate from metal plating, mining activities, smelting, battery manufacture, tanneries, petroleum refining, paint manufacture, pesticides, pigment manufacture, printing and photographic industries, etc., (Kadirvelu *et al*., 2001). Heavy metals can be accumulated in living tissues, causing various diseases and disorders (Hanafiah and Ngah, 2008).

2.3. Different techniques for the removal of heavy metals from aqueous solutions

Several researchers have reviewed the methods used in the removal of heavy metals from aqueous solution. The methods are generally applicable for heavy metals removal in aqueous solution including wastewater treatment. Some of these techniques include: chemical precipitation, ion-exchange, adsorption, membrane filtration, coagulation-flocculation, flotation and electrochemical, biosorption methods (Fu and Wang, 2011). The most common usable remediation techniques with fewer disadvantages are chemical remediation including precipitation, reverse osmosis, and ion exchange, biosorption and coagulation.

2.3.1. Chemical precipitation

Chemical precipitation is a widely used remediation treatment process for removal of dissolved metals from wastewater solution containing toxic metals by transforming dissolved contaminants into insoluble solids (Akpor and Muchie, 2010). Chemical precipitation in water treatment involves the addition of chemicals to alter the physical state of dissolved and suspended solids and to facilitate their removal by sedimentation or filtration (Akpor and Muchie, 2010; Oncel *et al.*, 2013).

Precipitation of heavy metals in water has been practiced as a prime method of treatment of industrial wastewater for many years by adding sodium hydroxide or lime as coagulants or flocculants to increase the particle size (Akpor and Muchie, 2010). Effectiveness of chemical precipitation process is dependent on several factors. These factors include; concentration of ionic metals present in the solution, the precipitant used and the presence of other constituents that may inhibit the precipitation reaction, pH and alkalinity of the water (Dhananjay and Mulimani, 2009).

Chemical precipitation has many disadvantages, making it an unfeasible method. These include: the high cost of waste disposal due to the large volumes of sludge generated to be disposed. That is why a combination of precipitation with other chemical treatment techniques such as ion exchange has also been used in order to achieve more effective removal (Munoz and Guieysse 2006). This has been applied to treat acid mine drainage from gold mines in South Africa by precipitation of heavy metals with lime and sulphides, followed by ion exchange (Feng *et al.*, 2000).

2.3.2. Ion exchange

Ion exchange is a very similar process to the biosorption method in removal of heavy metals. It is widely applied due to its many advantages such as high treatment capacity, high removal efficiency and fast kinetics (Kang *et al.*, 2004). It is a reversible chemical reaction wherein an ion from wastewater solution is exchanged for a similarly charged ion attached to an immobile solid particle (Akpor and Muchie, 2010; Fu and Wang, 2011).

These solid ion exchange particles are either naturally occurring inorganic materials or synthetically produced organic resins (Harland, 1994). The uptake of heavy metals ions via ion-exchange resins can also be affected by different factors such as temperature, pH, initial concentration, contact time (Pehlivan, 2009). The major disadvantages of this technique are that it is highly expensive and also requires expert in operating it.

2.3.3. Reverse osmosis

Reverse Osmosis is a membrane process that acts as a molecular filter, in which the fluid passes through a membrane, allowing the fluid that is being purified to pass through it while the dissolved and particulate matter is left behind (Akpor and Muchie, 2010). A significant advantage of reverse osmosis over other traditional water treatment technologies is its ability to reduce the concentration of other ionic contaminants, as well as dissolved organic compounds (Ucun *et al.*, 2003). It is a very effective method but it is very costly because the membranes are expensive both to procure and operate. Another setback of reverse osmosis method is the high power consumption due to the pumping pressures and the restoration of the membranes (Fu and Wang, 2011).

2.4. Biosorption

Biosorption of heavy metals from aqueous solutions is a relatively new process that has been confirmed as a very promising process in the removal of heavy metal contaminants (Fu and Wang, 2011). It is progressively becoming an ideal alternative for decontamination of heavy metals containing effluent (Fu and Wang, 2011). Biological materials have emerged as economic and eco-friendly option in the removal of heavy metals from industrial effluent via biosorption.

The use of natural plant material as biosorbent in the removal of heavy metals from wastewater as well as aqueous solution has gained more attention lately due to low cost and high biosorption efficiency. The plant material often used are bio-solids (Hanafiah and Ngah, 2008). New methods have also shown that bio-liquids are also able to act as biosorbents in the uptake of heavy metals. As indicated in section 1.3, only a few studies have shown the use of bio-liquid such as mucilage in the uptake for heavy metals. This also highlights the need of investigating the efficiency of the new method by using DE mucilage (bio-liquid) as biosorbent in the removal of heavy metals from aqueous solution.

Most recent studies have reported that biomaterials of microbial and plant origin interact effectively with heavy metals and are less expensive, affordable and easy to use (Hanafiah and Ngah, 2008). The major advantages of biosorption are its high effectiveness in reducing the heavy metal ions and the metal ions loading into the biosorbent is often high leading to a very efficient metal uptake and inexpensive (Ahluwalia and Goyal, 2007). Biosorption processes are particularly suitable to treat dilute heavy metal aqueous solution and due to their similar characteristics as an ion-exchanger, acting as a chemical substance, the process is very rapid and takes place between a few minutes to a few hours (Fu and Wang, 2011).

A major setback of biosorption is early saturation which can be a problem when metal interactive sites are occupied (Ahluwalia and Goyal, 2007). The sorption capacity of different biosorbents from plant origin whose efficiencies have been documented in literature, include, rice husk, sawdust, peanut husk, sugar beet pulp, wheat bran, groundnut shells, carrot residues, cock powder, coirpith, nipah palm, banana pith, carica papaya, etc, (Ahluwalia and Goyal, 2007; Hanafiah and Ngah, 2008). Biosorbent can easily be produced using inexpensive growth media or obtained as a by-product from industry (Ahluwalia and Goyal, 2007).

2.4.1. Functional groups

Functional groups present in the cell wall of biological plant materials and their associated ionic state are responsible for biosorption of metal ions by plant biomass (Bhatti *et al*., 2007). Plants biosorbent primarily contain acidic and basic functional groups and the acidic group are more dominating than the basic group (Bhatti *et al*., 2007; Gilbert *et al*., 2011). Gilbert *et al*. (2011) observed that the FTIR study of defatted *C. Papaya* seed showed that the acidic groups are more than the basic group and may be basically responsible for the sorption of Pb and Cd ions by *C. Papaya* sorbent.

Carboxylic groups (-COOH) are one of the important groups in biological biosorbent responsible for the uptake of metal ions (Bhatti *et al*., 2007). Fox *et al*. (2012) observed that carboxyl, carbonyl and hydroxyl functional groups of the mucilage in *Opuntia* species were involved in reacting with arsenate resulting in increase in sorption efficiency. Application of brown seaweed (*Sargassum fluitans*) confirmed that carboxyl groups were involved in the uptake of Fe^{2+} and Fe^{3+} and sulfonate groups were responsible for the uptake of Fe^{3+} only (Ahluwalia and Goyal, 2007). Hanafiah and Ngah (2008) review also documented that the presence of carboxyl groups in cassava biosorbent increase the binding of cadmium to the sorbent surface.

Several studies have indicated that MO seed biosorbent has a heterogenous complex chemical structure containing proteinacious amino acid possessing both negative and positive charged adsorption sites (Kumari *et al*., 2006; Sharma *et al*., 2006). Kumari *et al*. (2006) results showed that protein/amino acid-arsenic interactions was responsible for the sorption process of arsenic metal ions. Sharma *et al*. (2006) also indicated that the availability of carboxyl ligands of amino acids in shell *Moringa oleifera* seed promote interaction with cadmium ions. It can be explained that, the amino acids group of MO seed depending on the pH, possess both negatively and positively charged sites, thus are capable of attracting anionic or cationic species of metal ions (Kumari *et al*., 2006).

Vinod *et al*. (2010) illustrated that the acidic groups present in gum kondagogu biosorbent was responsible for the uptake of multiple metal ions species; Cd, Ni, Cr, Zn, Cu, Fe, Pb, Co, Se and As It can be concluded that the availability of acidic groups, which are mostly negatively charged, promotes the interaction and sorption of positively charged metal ions.

2.4.2. Biosorption mechanisms

Generally, mechanism of biosorption involves adsorption process such as ionic state, chemical structure, hydrogen bonding, acid-base interactions, hydrophobic interactions, precipitation ion-exchange, physical biosorption, complexation, coordination/chelation and electrostatic interactions (Farooq *et al.,* 2010). This mechanism process is possible with the aid of the functional groups present in the plant biomass. It is due to the presence of certain functional groups, such as amine, carboxyl, hydroxyl, phosphate, sulfhydryl, carbonyl, etc., on the cell wall of the plant biomass that contribute to the high sorption rate of metals ions by plant (Wang, 2002).

Biosorption of cadmium by sawdust biomass suggested that ion-exchange was considered as the predominating mechanism of biosorption (Hanafiah and Ngah, 2008). Vinod *et al*. (2010) also suggested that electrostatic attraction to negatively charged functional groups present in gum kondagogu was the biosorption mechanism responsible for the uptake of metal ions.

Fox *et al*. (2012) proposed that mechanism of biosorption between mucilage and metal ion (As) was via hydrogen bond bridging which was between the protons associated with the As species and the ionized carbonyl and carboxyl groups in the mucilage. While Sharma *et al*. (2006) mentioned that shell *Moringa oleifera* seed metal ion binding of Cd(II) appears to be via ion exchange involving electrostatic attraction between negatively charged groups of amino acids and metallic cations.

2.5. Factors affecting the efficiency of plant biosorbent

2.5.1. Initial concentration of metal ions

Several studies have documented that increase and decrease in the initial concentration of heavy metals ions also have influence on the uptake of metal ions by plant biomass. According to Sharma *et al*. (2006), it was observed that sorption of Cd(II) by shell Moringa oleifera (MO) seed increase with increase in the concentration of Cd(II) from 10-100 lg/ml. The result of this study showed that, sorption activity reached an optimal at 25 lg/mg and further increase in the concentration of the metal ion beyond 25 lg/mg showed no further increase in the metal uptake (Sharma *et al*., 2006). Similar result was also recorded by Gilbert *et al*. (2011) that increasing

metal ions concentration from 50-500 mg/L also increase the amount of metal ions removed by C. Papaya biosorbent.

Bhatti *et al*. (2007) explained that, at very low concentrations of metal ions, the sorption surface area of the sorbent (plant biomass) to the total metal ions available is high and thus increases the removal efficiency of metal ions. The reverse is the case when metal ions concentrations are increased beyond optimum (Bhatti *et al*. 2007). This results in the saturation of the binding sites of the sorbent as the amount of the sorbent remains constant (Bhatti *et al.,* 2007). Qi and Aldrich (2008) reported that the sorption of heavy metals by plant biomass by increasing the concentration of the metal ions had little effect on the surface charge properties of the plant biosorbent.

2.5.2. Biomass concentration

Concentrations of plant biomass strongly have been noted to influence the amount of metal ions removed from aqueous solutions. Kumari *et al*. (2006) showed that an increase in the biomass dosage of MO from 0.5-2.0 g display high sorption potential in the uptake of both arsenic(III) and arsenic(V) and no further increase in the sorption efficiency of MO was observed with further increase in the biomass dosage from 2.0 g onwards. Increasing the biosorbent dosage of *C. Papaya* decreased the amount of both metal ions (Pb and Cd) in the aqueous solution (Gilbert *et al*., 2011). Shell Moringa oleifera seed (SMOS) used as biosorbent in the removal of heavy metal ions from aqueous solution showed that sorption efficiency increased with the increased in the MO dosage from 2.0-4.0 g (Sharma *et al*., 2006). Further increase in the sorbent dosage yield no increase in the sorption uptake (Sharma *et al*., 2006).

The above results explains that biomass concentration of natural plant biosorbent strongly influence the uptake of metal ions from aqueous solution. The increase in the uptake of the metal ions with increase in the biomass dosage is greatly attributed to the availability of more sorption sites in the plant biomass to adsorp metal ions (Mohanty *et al*., 2005). The attainment of equilibrium and no further increase in sorption activity with further increase in the biomass dosage beyond optimum can be attributed to poorer biomass ultilization (Mohanty *et al*., 2005; Bhatti *et al*., 2007). Bhatti *et al*. (2007) explained that, as biomass concentration rises, the maximum biosorption capacity drops resulting in lower efficiency.

2.5.3. Effect of particle size of biosorbent

The variations in particle size of natural plant biosorbent also influenced the sorption efficiency of plant biomass. A fundamental review by Hanafiah and Ngah (2008) highlighted that the removal efficiency of heavy metal ions by plant biomass increase with a decrease in sorbent size. Bhatti *et al.* (2007) reveal that the effect of altering the sorbents particle size of MO on the sorption efficiency showed that, there was a more dominant removal of Zn(II) by the smaller particles more than larger particles.

Different sizes of MO plant particles ranging from 105-420 mm were use to study the effect of particles size on the uptake of metal ions [As (III) and As (V)] (Kumari *et al.*, 2006). It was observed that decrease in particles size (105 mm) had favourable effect on the removal of metals ions more than larger particles sizes (420 mm) (Kumari *et al.*, 2006). Gilbert *et al.* (2011) also showed that with increasing particle size (75 < 300 < 500 < 750 mm) of defatted C. papaya seed biosorbent, sorption efficiency decreased.

High biosorption capacity observed by smaller particles can be due to the increase in the total surface area which provided more sorption sites for the metal ions (Bhatti *et al.*, 2007; Hanafiah and Ngah, 2008). These indicate that smaller particles have larger surface area more than large particle size. Gilbert *et al.* (2011) mentioned that smaller particle sizes of biosorbent have high potential of diffusion and adsorption thus intra-particle diffusion is reduced as particle size reduces due to the shorter mass transfer zone causing faster rate of adsorption and saturation (Gilbert *et al.*, 2011).

2.5.4. pH

Change in metal solution pH is an important parameter influencing biosorption of heavy metals by plant sorbent. Biosorbents contains various functional groups (hydroxyl, carboxyl, carbonyl, amino groups and etc) acting like active site in the sorption of heavy metals. It has been observed that change in aqueous solution pH also affect the behaviour of these functional groups. For instance, in extreme acidic conditions, the carboxylic groups' ionization remains constant in a neutral form. Indicating that the negatively charged carboxylic group are protonated acting like positively charged species

With increasing the pH of the solution to basic/alkaline conditions, the carboxylic group becomes ionized. This result in deprotonation of the functional group to behave as negatively charged moieties (Farooq *et al*., 2010). As the pH increase from highly acidic to slightly alkaline conditions, the positive charged species of carboxylic groups of plant biomass is converted to negative one. It is able to start attracting positively charged metal ions thereby competing between hydrogen ions and positively charged metal ions leading to high adsorption activity. It should also be highlighted that, when the pH of metal solutions is under basic/alkaline conditions, the carboxylic groups becomes ionized while the hydroxyl group becomes neutral.

Result obtained from Kumari *et al*. (2006) reported that, optimum sorption of Arsenic (V) was at pH range of 2.6-6.5 while optimum sorption of arsenic (III) was from pH 2.0-7.5 by MO biomass. Optimum biosorption at less alkaline pH can be attributed to the availability of negatively charged arsenic ions to interact with positively charged amino acids present in the MO biomass (Kumari *et al*., 2006).

MO's structural composition contains positively charged amino groups (proteins) as well as carboxylic groups as the active site in binding with metal ions. Kumari *et al*. (2006) explained that with increase in the pH conditions, the carboxylic group of the amino acids would gradually become deprotonated as carboxylate ligands simultaneously protonating the amino group.

Metal ions precipitate as metal hydroxide in basic pH conditions due to the increase in the hydroxyl ions leading to low adsorption activity. For example, cadmium is present in aqueous solution as free Cd^{2+} species in an acidic pH condition and at pH 7.5 cadmium starts to precipitate as $Cd(OH)_2$ and is no longer available for biosorption (Farooq *et al*., 2010). Bhatti *et al*. (2007) results showed that uptake of zinc ions by MO increases with increase in the solution pH but with further increase in pH to 7, the sorption capacity reduced due to precipitation of Zn in the solution. It has also been noted that when pH of a solution exceeds 8, metal ions are precipitated out and are no longer available for uptake by plant biomass (Farooq *et al*., 2010).

Hanafiah and Ngah (2008) documented that maximum removal of cadmium by sawdust biomass occurred at pH above 4, indicating that at pH less than 3, carboxylic groups are still able to attract Cd^{2+} ions. Another study conducted by Qi and Aldrich (2008) showed that sorption of

heavy metal ions by tobacco dust started at pH 4-5 and decreased at pH 9-10. The study explained that the ionic state of functional groups such as carboxyl, phosphate and amino-groups present in the tobacco dust promotes reaction with metal ions via electrostatic attraction (Qi and Aldrich, 2008).

The reaction between the positively charged metal ions and negatively charged binding sites in tobacco dust at pH 4-5 leads to rapid uptake of metal ions and increase the removal efficiency (Qi and Aldrich, 2008). Majority of the metal ions are positively charged except the oxyanions of some metals like chromate and arsenate which are charged negatively (Hanafiah and Ngah, 2008; Farooq *et al*., 2010).). Therefore change in the pH condition of metal ions solution can affect the complex formation causing change in biosorption efficiency of the plant biomass (Farooq *et al.,* 2010).

2.6.Coagulation

Coagulation is the process whereby destabilization of a given suspension solution is effected (Bratby, 2006). Coagulation and flocculation are essential processes in a number of diverse disciplines including biochemistry, manufacturing of cheese and rubber, and water and aqueous solution treatment (Lee *et al*., 2012). Coagulation-flocculation followed by clarification is the most widely used process for treatment of water and aqueous solution using coagulants as displayed in Fig 2.1.

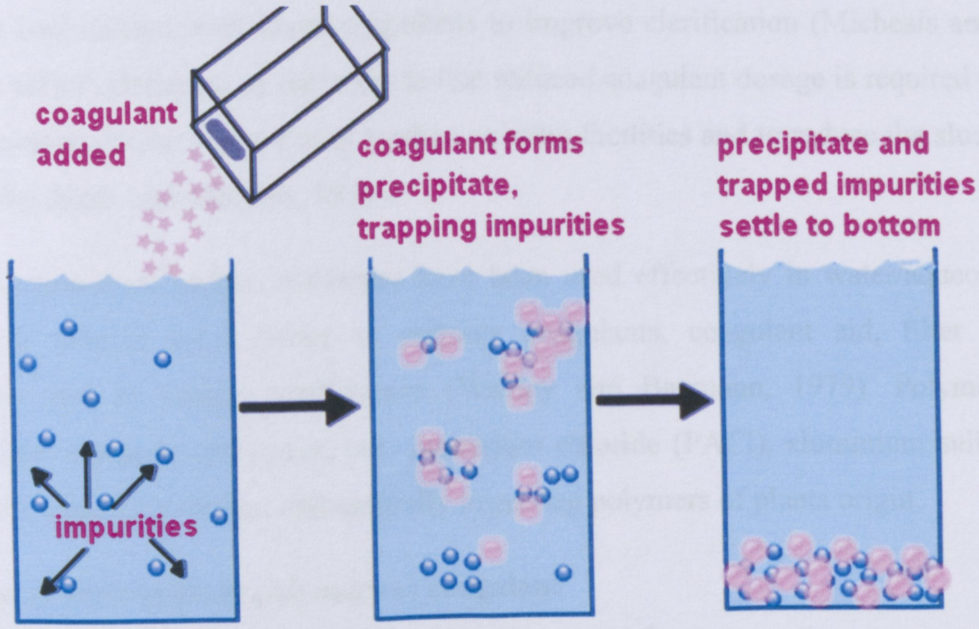

Figure 2.1: Coagulation process (Source: http://chemistry.tutorvista.com/physical-chemistry/flocculation.htmL)

Coagulants are agents that are used to assist with the removal of colour and turbidity present in untreated raw water. They do this by forming settleable particles in the form of flocs, which are then removed in downstream clarification or filtration treatment processes (Gebbie, 2006). Coagulation involves reaction between coagulant species, natural organic materials (NOM) molecules and surface particles (Pernitsky, 2003).

Coagulation process is used to destabilize suspended particles and to react with dissolved organic material in raw water (Bratby, 2006). Pernitsky (2003) reported that particles can be effectively destabilized by neutralization of surface charge by positively charged coagulant species to produce large amounts of flocs that will settle rapidly. Coagulation has also been shown to be most effective in removing NOM in high and intermediate molecule ranges (Kim, 2005).

2.7. Use of Polymers as coagulants

Polymers are polyelectrolytes with high molecular weight and they also possess such characteristics of simple electrolytes as electrical charges or ionizable groups or neutral polyelectrolyte (Yin, 2010). The use of polymers in water/aqueous solution treatment is one of such technological advancement. Polymers are used in water/aqueous solution treatment either

alone or in conjunction with metal coagulants to improve clarification (Micheals and Morelos, 1955). The major advantage of polymers is that reduced coagulant dosage is required to allow an increased treatment rate without over loading existing facilities and to reduce the sludge volume produced (Micheals and Morelos, 1955).

As already mentioned earlier, polymers have been used effectively in water/aqueous solution treatment in several ways either as primary coagulants, coagulant aid, filter aids, fitter conditioners and as sludge conditioners (Welday and Baumann, 1979). Polymers can be classified into inorganic polymers; polyaluminium chloride (PACl), aluminium sulfate (alum), etc, synthetic organic polymer; and naturally occurring polymers of plants origin.

2.7.1. Use of natural plant polymers as coagulant

The use of natural plant materials such as MO, *Zea mays, Opuntia* species, etc, as coagulants for water/aqueous solution purification has been practiced since in the early centuries. Studies such as Jahn (1988) indicated that dry seed suspension of MO were applied as natural coagulant to purify highly turbid Nile water in the early centuries. Nilanjana (2005) also highlighted that powdered roasted grains of *Zea mays* were used by soldiers in Peru as a means of settling impurities in the 16th and 17th century.

Previous researchers also revealed that the use of natural plant coagulants have not only been known to purify water but also have been able to show bright future in water/aqueous solution treatment. It is because they have similar functions like inorganic coagulants and are also able to comply with the recommended WHO (1996) guidelines. Many studies have proposed the use of natural plant coagulants as alternative coagulants over inorganic coagulants due to their cost effectiveness especially if locally available (Yin, 2010). They are also environmentally friendly due to the small volume of biodegradable sludge they produce which can also be applied as fertilizers in farms (Ndabigengesere *et al*., 1995; Okuda *et al*., 1999; Ghebremichael *et al*., 2005 and Yin, 2010).

Turbidity removal by a known number of identified natural plant coagulants have been so impressive in purification of both raw and prepared water. Different studies have used Guar gum (Pritchard *et al*., 2010), *Moringa oleifera* (Muyibi and Evison, 1995; Ndabigengesere *et al*.,

1995; Ndabigengesere and Narasiah, 1998; Okuda *et al*, 1999, 2001a&b; Abaliwano *et al*., 2008; Pritchard *et al*, 2009 & 2010), Cactus species (Diaz, 1999 and Zhang *et al*., 2006), and Common beans (Antov *et al*., 2009). In each of the above studies, they showed that natural plants coagulants performance in turbidity removal was up to 80-99%. Pritchard *et al*. (2010) and Abaliwailo *et al*. (2008) also showed the potential of natural plant coagulants to be able to reduce the microorganisms (coliforms) in water via their anti-microbial properties. The use of natural plants coagulants as coagulants aids in water/waste treatment has shown high performance efficiency in turbidity removal.

2.7.2. Coagulation mechanism

Polymeric (including plants) can be collectively termed 'polyelectrolytes' because of their cationic, anionic or non-ionic properties (Bache and Gregory, 2007). Yin (2010) review showed that coagulation mechanisms of aggregation particulates in a solution can occur via four ways. These include; double layer compression, sweep flocculation, adsorption and charge neutralization, and adsorption and interparticle bridging (Yin, 2010).

The presence of salts or suitable coagulants can cause compression of the double layer as demonstrated in Fig. 2.2 resulting in destabilization of the particles. Sweep flocculation occurs when coagulants encapsulate suspended particulates in a soft colloidal floc (Fig. 2.2). Adsorption and charge neutralization refer to the sorption of two particulates with oppositely charged ions. Interparticle bridging occurs when a coagulant provides a polymeric chain, which sorbs particulates during coagulation.

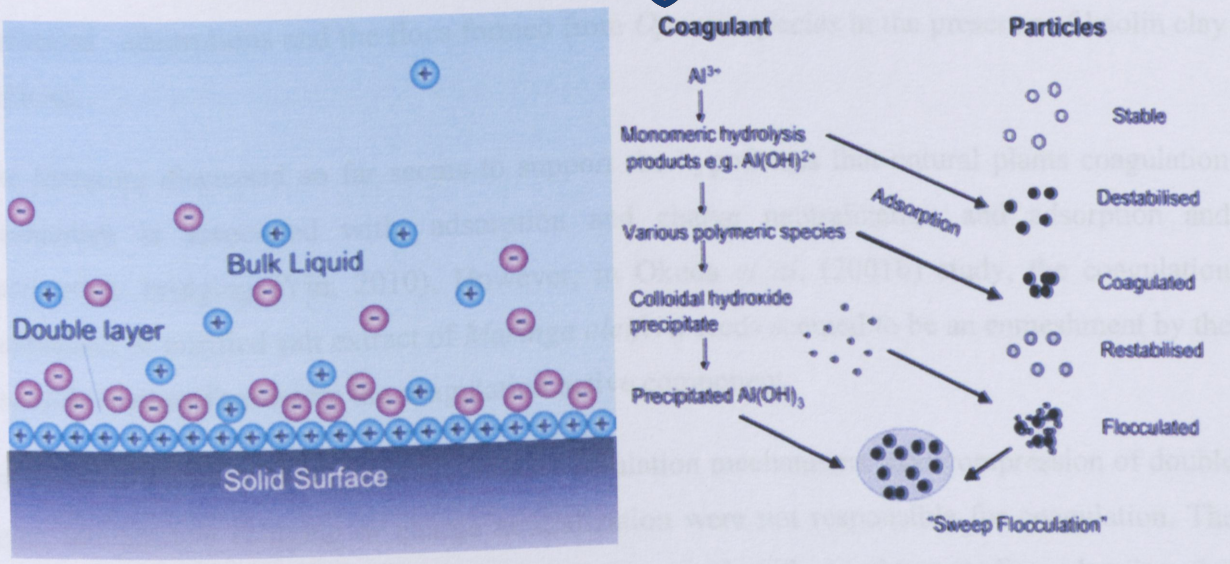

Figure 2.2: Double layer and sweep flocculation mechanisms occurring during coagulation (Duan and Gregory, 2003)

The review study carried out by Yin (2010) further highlighted that natural plant coagulation mechanisms are generally associated with two of these mechanisms: (i) adsorption and charge neutralization, and (ii) adsorption and interparticle bridging. These two mechanisms provide underlying principles to the inner workings of plant based coagulants and the existence of background electrolytes in aqueous medium can facilitate the coagulating effect of polymeric coagulants (Yin, 2010).

A study carried out by Ndabigengesere *et al.* (1995) on the coagulation mechanism of MO seeds in turbid water showed that the predominant mechanism of coagulation appears to operate via adsorption and charge neutralization. The use of MO seeds for softening hard water indicated that the mechanism of coagulation was found to be adsorption and conversion of soluble hardness-causing ions to be insoluble products by precipitation reactions (Muyibi and Evison, 1995).

Miller *et al.* (2008) suggested that coagulation mechanism of *Opuntia species* is adsorption and bridging whereby particles are bound to a polymer like material from the plant. The study further explained that natural electrolytes within the *Opuntia species* pads, particularly the divalent cations, are known to be important for coagulation with anionic polymers which may have

facilitated adsorptions and the flocs formed from *Opuntia species* in the presence of kaolin clay particles.

The literature discussed so far seems to support the hypotheses that natural plants coagulation mechanism is associated with: adsorption and charge neutralization and adsorption and interparticle bridging (Yin, 2010). However, in Okuda *et al.* (2001b) study, the coagulation mechanism of purified salt extract of *Moringa oleifera* seeds seemed to be an enmeshment by the insoluble matters formed by the coagulation active component.

Okuda *et al.* (2001b) highlighted that other coagulation mechanisms like: compression of double layer, interparticle bridging or charge neutralization were not responsible for coagulation. The result obtained in the latter study did not correspond with previous studies, showing that coagulation mechanism of MO extracted with salt solution is not the same with distilled water extract used by previous studies. The salting-in process may have influenced the coagulation mechanism of MO (Okuda *et al.*, 2001b).

2.7.3. Zeta potential (ζ)

Zeta potential measurement is used to determine the mechanisms of coagulation of both natural and chemical coagulants. It is also a very significant systematic parameter in studies of the effects of various coagulants on the removal of colloidal particlulate in water/aqueous solution (McLellon *et al.*, 1972). Zeta potential provides background information necessary for preparing stable colloidal suspensions in many applications (Hackley *et al.*, 1995). Some of these applications include; food preparations, agriculture, ceramics, pharmaceuticals, paints, as well as water/aqueous solution treatment (Hackley *et al.*, 1995).

Miller *et al.* (2008) described zeta potential measurement as a measure of the surface charge particles, which indicates the degree of repulsion between adjacent and similarly charged particles in dispersion. Ndabigengesere *et al.* (1995) reported that zeta potential measurement is used to determine the mechanism of coagulation, which depends upon the electrostatic forces between charges carried by colloidal particles. Miller *et al.* (2008) suggested the use of zeta potential measurements and transmission electron microscopy images of flocs to determine the

coagulation mechanism of *Opuntia species*. Zeta potential is a useful factor for better understanding of the electrokinetic character of a colloid and its stability.

2.7.4. Active agent of coagulation in natural plants coagulants

According to Yin (2010), the active agents in natural coagulants are mostly either polysaccharides or proteins and can either be cationic, anionic or non-ionic. The potential of a natural plant to act as a coagulant seems to be associated with their polyelectrolytes and biochemical properties (Yin, 2010).

Studies carried out on the active agent of MO by different researchers have produced different answers on the exact nature of MO seed active agent. Ndabigengesere *et al.* (1995) reported that the active agent in aqueous MO seed extract are dimeric cationic proteins, having a molecular weight of 13 kDa and isoelectric point (p/) between 10 and 11. Gassenschmidt *et al.* (1995) reported that the coagulating agent of MO is protein with molecular mass of 6-5 kDa and p/ greater than 10.

The above studies have described the active agent of water-extract of MO seed to be proteinaceous. Okuda *et al.* (2001a) reported that the active agent of salt solution extract of MO seed is not protein, polysaccharides nor lipid but an organic polyelectrolyte with a molecular weight of 3.0 kDa. Ghebremichael *et al.* (2005) in their findings suggested that the coagulating agent of both water and salt solution extract of MO seed is cationic protein with p/ greater than 9.6 kDa. Most studies seem to agree that the active agent of MO seed is proteinaceous but the conclusion from Okuda *et al.* (2001a) studies seems to be in divergence from results of other studies.

It has been established that mucilage is a complex carbohydrate with a great capacity to absorb water (Saenz *et al.*, 2004). Miller *et al.* (2008) reported that mucilage found only in some plants such as *Opuntia* species, aloe and okra is a highly branched carbohydrate polymer thought to hold water tightly. The high coagulation capability of *Opuntia species* is most likely attributed to the presence of mucilage. It is attributed to the viscous and complex carbohydrate stored in cactus inner and outer pads that has great water retention capacity (Nobel, 2002; Sepulveda *et al.*, 2007).

Mucilage in *Opuntia* species contains varying proportions of L-arabinose, D-galactose, L-rhamnose, D-xylose and galactoronic acid (Saenz *et al*., 2004). Miller *et al*. (2008) reported that these individual components of mucilage independently displayed no coagulation activity but galacturonic acid added independently was able to reduce turbidity by more than 50%. Miller *et al*. (2008) further mentioned that combination of all these components could only account for 50% of the turbidity removal.

Conclusion drawn from Miller *et al*. (2008) was that, *Opuntia species* is able to significantly reduce turbidity with removal efficiency ranging from 92 to 99% while individual components isolation and combination account for 50% of the turbidity removal. It is likely that there are additional components of the *Opuntia species* that are not found in the mucilage but contribute to the coagulation activity in this plant. The exact nature of the active agent of *Opuntia species* is not known but was suggested to be galacturonic acid from the mucilage.

2.8. Factors affecting the efficiency of natural plants coagulants

2.8.1. Turbidity

Turbidity is the amount of cloudiness in a fluid. The turbidity of natural water is made up of a complex mixture of particles consisting principally of various colloidal clays (Pressman and Birkner, 1967). These clay particles are often contaminated of colloids from domestic and industrial wastes, live decaying algae and their decomposing products, bacterial cells, decaying organic matter, and colour colloids (Pressman and Birkner, 1967).

Clay particles constitute the majority of the particles encountered often available in turbid water (Black and Sidney, 1961). The primary goal of coagulation-flocculation is turbidity removal (Ndabigengesere and Narasiah, 1998). Therefore, turbidity removal is one of the important steps in water treatment process, which is generally achieved using coagulants (Katayon *et al*., 2006). Clay particles suspended in water are known to be negatively charged and the size of particles also influence the destabilization of colloidal suspensions (Black and Sidney, 1961; Black and Manuel, 1969). Understanding the surface properties such as; surface areas, surface charge, surface shape of colloids is the basis for the full understanding of stability in colloidal particles (Black and Sidney, 1961). Understanding the interactions and properties of coagulant species and colloidal particles also play a key role in coagulation efficiency of natural plant coagulants.

Water quality has effect on the coagulation efficiency of natural plant because of the effect of particle size on the kinetics of the destabilization process, making it faster the smaller the size (Black and Sidney, 1961).

2.8.2. pH

pH is defined as the negative logarithm of the hydrogen ion concentration. pH is considered as one of the most important single variables influencing the effectiveness of polymer (Welday and Baumann, 1979). McLellon *et al.* (1972) explained that destabilization of biocolloids and chemical precipitate reactions can be accomplished only if the pH is maintained thoroughly during any one coagulation-flocculation experiment. This proves that pH play a crucial part in control of coagulation.

The variation of pH of known concentration of coagulant would definitely influence the efficiency of turbidity removal from the surface water. Flocculation efficiency of natural plants coagulants is very sensitive to pH, and reaches a maximum at pH 7 for some natural plants (Divakaran and Pillai, 2001, 2002). Prichard *et al.* (2010) concluded that maximum reduction in turbidity (80%) was observed at pH 6.5 and was considered as the optimum pH at which the active coagulation component in *Moringa oleifera* seeds acts. Abaliwano *et al.* (2008) reported that MO coagulant performance improved with decrease in pH as the net positive charge increase with the presence of hydrogen ion. Both the last two quoted studies showed that increase and decrease in pH can alter the effectiveness of the active agent of MO in turbidity removal.

Earlier work by Ndabigengesere and Narasiah (1998) showed that the use of MO seeds to treat high turbid water did not alter the pH of the treated water and the pH value (7.6) remained almost constant. Zhang *et al.* (2006) indicated that the variation of pH altered the final turbidity in cactus species and the optimum pH was observed at pH 10 and the worst effect appeared at about pH 6.

The differences in the coagulation performance of MO and cactus at different pH could be due to different cationic, anionic or non-ionic polyelectrolytes properties of these plants. Diaz, (1999) suggested that there is no reason to consider using *Prosopis juliflora* as active coagulant at a pH

other than neutral thereby proposing that this plant is most effective in turbidity removal when the pH of the sample water is neutral.

Miller *et al*. (2008) showed that in a model turbid water containing negatively charged ions (i.e both the colloidal particles and the natural coagulant has similar charge), 98% turbidity removal was achieved at pH 8-10. The study further explained that, it is attributed to greater electrostatic repulsion between particles and the coagulant in the absence of positively charged ions (Miller *et al.*, 2008). According to Ndabigengesere and Narasiah (1998), pH does not affect the treated water when MO coagulant is used to treat the water and Muyibi and Evison (1995) study also supports this observation. The results in Muyibi and Evison (1995) report that, the efficiency of softening hardwater with MO seeds is not dependent on the pH but dependent on the permanent hardness of the raw water. Diaz (1999) argued that pH changes do alter the final turbidity in terms of the overall removal and stressed that the changes are not considerable.

2.8.3. Temperature

Pritchard *et al*. (2009) showed that an increase in temperature of the untreated water results in an increase in the coagulation efficiency of MO, which is adversely affected at low temperatures. The lower the temperature the more viscous the water would be and this in turn affects the rate of flocculation (Bache and Gregory, 2007). The relationship between cactus species dosage and the turbidity removal efficiency with different temperatures was described in Zhang *et al*. (2010), which showed that the coagulation effect of temperature at 10°C was a little worse than that at 20°C and 35°C. This study verified that turbidity removal efficiency increases with the increase in temperature.

2.8.4. Plant material extract

Effectiveness of turbidity removal using natural plant also depends on the part of the plant extracted for coagulation. Studies have shown that various forms/parts of natural plants coagulants posses different percentages of coagulation activity than other parts of the plants. For example, Ndabigengesere *et al*. (1995) showed that the coagulation activity was absent when green pods, dried pods, bark of pods and bark of seeds of MO were used in the coagulation test while non-shelled seeds and shelled seeds of MO showed coagulation activities.

Further studies reported that the shelled seed of MO is more effective and efficient than non-shelled seeds in turbidity removal and the active agent (cationic protein) are present in seeds (Ndabigengesere *et al*., 1995 and Okuda *et al*., 1999). A study by Miller *et al*. (2008) reported that coagulation activity was present in fresh pad, outer pad without skin, outer pad with skin, inner pad and dry pad of *Opuntia* species, while the skin, whole pad macerated and whole pad dried at 120°C displayed no coagulation activity.

The above studies proved that not all the parts or forms of natural plants coagulants possess coagulation ability. Some parts or forms of plants possess coagulation activity but are present in trace amounts while some parts are very well represented. This may be attributed to the presence of active agent of coagulation in that part and absence of the same in the other part of the plant.

2.8.5. Optimum dosage

Dosage of MO has been found to influence the coagulation efficiency (Pritchard *et al*., 2010). This is because the nature of the water sample and the colloidal particles could be flocculated with optimum dosages of polymer (Pressman and Birkner, 1967). Pritchard *et al*. (2010) reported that little or no coagulation occurred at low coagulant doses of MO but as the dose level increased, an optimum point was reached that corresponded to maximum reduction in turbidity. Pritchard *et al*. (2010) indicated that this reduction in turbidity could be attributed to MO coagulant relying on inter-particle collisions to destabilize the suspension by charged neutralization or patch charge micro-bridging.

Zhang *et al*. (2010) reported that using optimum dosage of cactus coagulant (50 mg/L) achieved removal efficiency of 94% turbidity. Ndabigengesere and Narasiah (1998) showed that, at optimum dosage of MO seed, turbidity decreased from 105 NTU to 10 NTU corresponding to a turbidity removal of 90%. Abaliwano *et al*. (2008) have also shown that reduction in bacteria counts was highly significant with increased dosage of MO leading to decrease in the E.coli bacteria counts.

The above results from different studies can further be explained by Pressman and Birkner (1967). Their study states that natural waters contain stabilizing elements which can increase the flocculation dosage requirements. Similar studies like Muyibi and Evison (1995) and Katayon *et*

al. (2006) conducted using MO have documented different optimum dosages with similar turbidity values. According to Katayon *et al*. (2006), the difference in the optimum dosages of MO may be due to the difference in experimental procedures used such as; settling, mixing velocity gradient and mixing durations and also may be due to using different species of MO.

Optimizing of MO dosage showed that increasing dosage of coagulant did not improve the removal of turbidity but increased the residual turbidity of the coagulated sample (Katayon *et al*, 2006). Pressman (1967) explained that with increasing dosage beyond the optimum, a charge reversal takes place and dispersion of turbidity particles becomes operative. Muyibi and Evison (1995) also explained that, large doses which eventually lead to overdosing result in the saturation of the polymer bridge site leading to re-stabilization and destabilization particles due to insufficient number of particles to form more inter-particle bridge. The study further stated that, cationic polyelectrolyte being the active and dominating charge in MO can ultimately cause charge reversal and subsequent re-stabilization of destabilized particles (Muyibi and Evison, 1995). Finally, it can be mentioned that the efficiency of coagulation process also depends on the increase in the coagulant concentration in the untreated water.

2.9. Methods used for extraction of active ingredients from plant

Extraction is the first important step for the recovery and purification of active ingredients of plant materials (Jianyong *et al*., 2001). The process of extraction of active substances from plant material by means of solvent generally occurs in two main stages: dissolution of material near the surface and diffusion of solute from the porous plant residue into the solution (Ponomarev, 1976).

The traditional techniques of solvent extraction of plants materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of materials and the rate of mass transfer (Jianyong *et al*., 2001). Ndabigengesere *et al*. (1995) reported that coagulation activity of extracts from MO using different solvents such as: petroleum ether, hexane, chloroform, acetone and methanol displayed no coagulation activity but only water extract displayed coagulation activity.

Okuda *et al*. (1999) reported that extraction of active agents from MO seeds using salts solution showed better coagulation activity than using distilled water. The study further stated that solvent also had effect on the nature of the active agent in the plant (Okuda *et al*., 1999). This verifies the fact that traditional techniques of solvent extraction of plant materials are related to the correct choice of solvents. It also depends on the plant species and the mode of extraction may have effect on the coagulation efficiency and chemical composition of the plant.

Traditional techniques are known to require long extraction hours and have low efficiency and many natural products are thermally unstable and may degrade during thermal extraction (Jianyong *et al*., 2001). Smelcerovic *et al*. (2006) review discussed that three test variables (extraction temperature, extraction time, and solvent concentration) have effect on the extraction efficiency of active compounds from *Hypericum* (H)*perforatum*) and most significantly, higher temperatures may also provoke decomposition of some active compounds in the plants.

Recently, it has been shown that the extraction process of organic compounds, phytochemicals and bioactive components of plants can be improved with the use of ultrasound (Jianyong *et al*., 2001; Smelcerovic *et al*., 2004; 2006). Ultrasound has proven to be a much simpler and more effective means than the traditional extraction method of refluxing boiling solvents (Jianyong *et al*., 2001). High concentration of saponins extracted from ginseng roots was achieved via ultrasound extraction (Jianyong *et al*., 2001).

Mason (1996) explained that the improvement of solvent extraction from plant material by ultrasound is due to the mechanical effects of acoustic cavitation, which enhances both solvent penetration into the plant material and the intracellular product release by disrupting the cell walls. Documented data established that ultrasound in extraction processes are mass transfer intensification, cell disruption, improved penetration and capillary effects (Vinatoru *et al*., 1999). Ultrasound extraction can be carried out at low temperatures, avoiding thermal damage to extracts and loss of volatile components in boiling (Jianyong *et al*., 2001).

Comparison of direct sonication extraction method of *H. perforatum* with conventional maceration, indirect sonication, soxhlet extraction, and accelerated solvent extraction (ASE) was investigated by Smelcerovic *et al*. (2006). The study reported that highly selective liquid

chromatography/tandem mass spectrometry analysis showed that the content of six investigated active compounds (hypericin, pseudohypericin, hyperoside, rutin, quercitrin and hyperforin) in extracts obtained by direct sonication was significantly higher than extracts obtained by other methods. Smelcerovic *et al*. (2006) further indicated that conventional maceration gave the lowest amount of analyzed active compounds and soxhlet extraction gave better results than ASE or indirect sonication.

The yield of 2 hours ultrasound assisted extraction of saponin from the ginseng roots were comparable to those achieved with the conventional method by Jianyong *et al*. (2001). The result showed that the extraction rate of ultrasound assisted process was about three times faster than the conventional method and soxhlet extraction gave better results than ASE or indirect sonication. Data from the above mentioned studies proved that direct ultrasound is more effective in extraction than other conventional methods. It also has more advantages in terms of amount of solvent, temperature and time consumed. Also, soxhlet extraction is effective in the extraction of active ingredients from plants but always requires a longer time compared to ultrasound that uses few hours. Soxhlet extraction can also be used as a substitute for direct ultrasound.

2.10. Methods used for purification of plant extract

In the past years, similar methods and processes have been used to purify crude extract of natural plant. Studies such as those reported by Ndabigengesere *et al*. (1995) and Okuda *et al*: (1999) used methods described by Jirgensons (1962); Franks (1988) and Scopes (1987) to purify crude extract of MO. The processes involved in those methods include: dialysis, delipidation, ultrafiltration, lypholisation, ion-exchange, chemical precipitation and electrophoresis. These multiple steps method involves 80-100% of ammonium sulphate added to the plant extract to get precipitates of the active agent (proteins).

This purification method involving multiple steps complicates the use of plants extracts in large-scale treatment (Ghebremichael *et al*., 2005). Studies have also identified that one of the critical parameters in this purification method is the concentration of ammonium sulphate used for precipitating the protein in the interfacial phase and the concentration should be less than the one, which causes 'salting out' of any protein (Aparna and Gupta, 2001).

There is an increasing use of chromatographic methods in purification of the active agents in natural plant extract. Ghebremichael *et al.* (2005) suggested that using simple, scalable purification method involving a single fractionation step is sufficient to remove the majority of proteins from crude extract of MO to produce a sample enriched in coagulating proteins. The study further indicated that the use of standard cation exchange chromatography, which is well established in batch and continuous flow application can be used for large volume purification treatment methods.

Preliminary chromatographic separation allows the purification of carbohydrates from complex mucilage matrix (Nunzio *et al.*, 2009). Liquid chromatography coupled with electrospray-ionization tandem mass spectrometry (LC–ESIMS/MS) is gaining an increasing importance as a powerful technique for carbohydrate purification and characterization in complex samples (Nunzio *et al.*, 2009).

Many researchers have applied different advanced methods in their studies to purify plants extract. Studies such as Warrand *et al.* (2003) showed that a combination of ion-exchange and size exclusion chromatography enables purification of three distinct polysaccharides from viscous flaxseed mucilage. This method enables complete fractionation of large amounts of mucilage and is convenient for purification of large amounts of samples (Warrand *et al.*, 2003). Root mucilage of maize (*Zea mays* L.) was purified using Sephadex size-exclusion chromatography and purification process allowed subsequent analysis of the polysaccharides derived from this mucilage (Osborn *et al.*, 1999).

Conclusion from the above review shows that there is no standard method for the purification of plant extract. Different methods and different equipment produce best results but the use of chromatographic method seems to be more appropriate and an easy way of purification. The major advantage is that it enables convenient purification of large amounts of samples.

2.11. Methods used for plant extract analysis

Instruments such as Gas-liquid chromatography, Thin layer chromatography, Liquid chromatography-mass spectrometry, Gas chromatography-mass spectrometry, High performance liquid chromatography, FTIR, etc, have been used for plant extract analysis (Fernandez and

Winkelmann, 2005). Accuracy is often a problem due to background, contamination and interfering compounds (Fernandez and Winkelmann, 2005). Pothitirat and Gritasanapan (2009) reported that analytical methods of α-mangostin by using UV-spectrophotometry and TLC-densitometry showed low cost in terms of solvents, number of samples and time consumed but less accuracy and precision.

Kacurakova and Wilson (2001) reviewed that FTIR is more beneficial in structural evaluation (configurational and conformational analysis), systematic fingerprinting of carbohydrates in various physical states, and allows special applications such as analysis of crude samples of natural products.

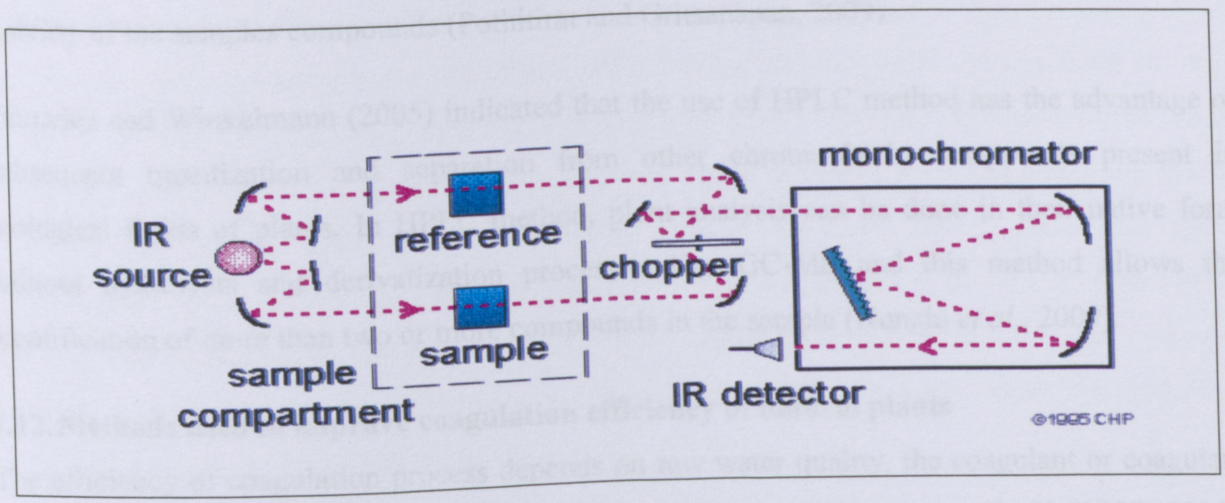

Figure 2.3 Basic components of FTIR (Saad, 2011)

FT-IR analysis is carried out by inserting the sample into the sample compartment (Fig. 2.3). This is followed by infrared ray source where the infrared energy is emitted from a glowing black-body source and then passes through an aperture which controls the amount of energy presented to the sample, which ultimately goes to the detector. Then from there, the beam enters the interferometer where the spectral encoding takes place.

The resulting interferogram signal then exits the interferometer to pass through the sample (analyte) whereby it is transmitted through the surface of the sample. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed. The beam then passes to the detector for final measurement. Finally the measured signal is digitized and

sent to the computer where the Fourier transformation takes place and the infrared spectrum is then presented to the user for interpretation (Thermo Nicolet Corporation, 2001; Saad, 2011).

Studies such as: Fernadez and Winkelmann (2005); Nunzio *et al.* (2009) and Pothitirat and Gritsanapan (2009) have proved the HPLC method to be accurate and simple, specific, precise and sensitive for quantitative analysis of bioactive compounds, phytochemical, etc, in plant. These major advantages make use of HPLC one of the methods of preference for plant extract analysis. Pothitirat and Gritsanapan (2009) reported that HPLC method promoted high precision, sensitivity and accuracy for quantitative analysis of raw materials of *G. mangostana* fruit rind and its extract. The study further indicated that HPLC method is not altered by volatility or stability of the samples compounds (Pothitirat and Gritsanapan, 2009).

Fernadez and Winkelmann (2005) indicated that the use of HPLC method has the advantage of subsequent quantization and separation from other chromophoric compounds present in biological fluids of plants. In HPLC method, plant analysis can be done in their native form without hydrolysis and derivatization process unlike GC-MS and this method allows the identification of more than two or more compounds in the sample (Nunzio *et al.*, 2009).

2.12. Methods used to improve coagulation efficiency of natural plants

The efficiency of coagulation process depends on raw water quality, the coagulant or coagulant aids used, operational factors including mixing conditions, coagulant dose and pH (WHO, 2004). Different studies have used different operational methods to improve the coagulation efficiency of natural plant coagulant in turbidity removal.

2.12.1. The increase of ionic strength of the coagulant

Study conducted by Okuda *et al.* (1999) on improvement of the performance of MO seeds revealed that, MO extracted from salt solution showed better coagulation activity than distilled water extract. Okuda *et al.* (1999) reported that MO salt solution dosage was 7.4 times lower than using distilled water extract for the removal of kaolin in water. MO salt solution extract efficiency was more than 95% and only required 4 mL/L to treat turbid water sample (50 NTU) while distilled water extract required 32 mL/L of dosage to be able to remove only 78% of the turbid water sample (Okuda *et al.*, 1999).

Another study conducted by Ghebemichael *et al*. (2005) also confirmed that using salt solution extract of MO seed showed coagulation activity higher than the water extract. The study explained that the higher coagulation performance of salt water extract of MO seed can be attributed to the precipitate observed when salt solution extract was mixed with water. Ghebemicheal *et al*. (2005) indicated that the precipitate could act as nuclei for floc formation and also the amount of protein in the salt solution extract was higher (two-fold) than the water extract of MO seed. Okuda *et al*. (1999) attributed the higher coagulation activity in salt solution extract to be due to the salting-in mechanism in proteins wherein, a salt increases protein-protein dissociations leading to increasing protein solubility as the salt ionic strength increases.

2.12.2. The use of purified plant extract

Studies have documented that the use of purified extract of natural plants coagulants do not only improve the coagulation performance but also have a positive impact on the quality of the water. The use of crude extract has been reported to be ineffective in low turbid water and crude extract also generate organic matter in treated water, which can cause odour, taste and colour (Ndabigengesere and Narasiah, 1998; Okuda *et al*., 1999; Abaliwano *et al*., 2008). Jahn (1988) reported that water treated with crude extract of MO should not be stored for more than 24 hours. Therefore, crude extract is not suitable for large water supply systems where the hydraulic residence time is very high (Ghebremichael *et al*., 2005).

Studies such as Ndabigengesere and Narasiah (1998), Abaliwano *et al*. (2008) and Antov *et al*. (2009) have shown that the use of purified extract of natural plant coagulant can improve the coagulation efficiency. The major advantage of purification is that it reduces the organic load in treatment systems without requiring more complex protein production methods (Ghebremichael *et al*., 2005). Results of coagulation experiments with purified proteins showed that the optimal dosage was 0.5 to 1 mg/L, which was 50 to 100 times lower than the optimal dosage for alum or the crude water extract of the shelled dry MO seeds (Ndabigengesere and Narasiah, 1998).

The highest turbidity removal was obtained by purified crude extract of common bean (*Phaseolus vulgaris*), which was almost 22 times higher than the performance of the crude extract (Antov *et al*., 2009). Ndabigengesere and Narasiah (1998) suggested that purified extract of MO seed can be used as coagulant in water and aqueous solution treatment only after adequate

purification of the active proteins. It is because with purified extract, no increase of organic matter, phosphates or nitrates was noticed in treated water (Ndabigengesere and Narasiah, 1998).

2.12.3. The combination of natural plant coagulant and inorganic coagulant

The use of natural plant as coagulant aid in conjunction with aluminium chloride ($AlCl_3 \cdot 6H_2O$) has also improved the efficiency of natural plant in water and aqueous solution treatment. The use of $AlCl_3 \cdot 6H_2O$ (Aluminium chloride + water) and cactus solely to treat sewage water showed a maximal removal efficiency of turbidity and COD removal than when $AlCl_3 \cdot 6H_2O$ and cactus were used synchronously (Zhang *et al.*, 2006). A study conducted by Bhuptawat *et al.* (2006) showed that both 50 and 100 mg/L of MO obtained overall COD removal of 50% but on combination of 10 mg/L of alum with 50 and 100 mg/L of MO seed obtained a maximum overall removal of 64% of COD.

2.12.4. Application of filtration

In water treatment, the pilot process involves coagulation-flocculation, sedimentation and filtration. Filtration is an essential step in the production of high-quality water. It is because filtration improves the removal of suspended solids, both flocs and turbidity and slightly improves the process as a whole within/via the filter media (Raghuvanshi *et al.*, 2002; Sanchez-Martin *et al.*, 2010).

The main advantage of filtration is that it can successfully treat water of high and low turbidity, remove microorganisms in water, absorb chemicals, oxidize iron, manganese and ammonia (WHO, 2004). It also effectively removes organics including certain pesticides in raw water depending on the filter medium (WHO, 2004). The efficiency of water treatment process (coagulation-flocculation, sedimentation and filtration) using plants based coagulants was evaluated by Raghuvanshi *et al.* (2002). The study revealed that in the filter water, turbidity obtained when Nirmali seed and maize were used as coagulant aids was approaching zero NTU (Raghuvanshi *et al.*, 2002).

The use of tannin-based coagulant to treat surface water and aqueous solution showed that filtration improved the removal of suspended solids and also removed flocs formed from turbidity and slightly improved the process as a whole (Sanchez-Martin *et al.*, 2010). Studies

have also indicated that the majority of COD removal occurred during the filtration process and was likely to be attributed to the relatively strong MO flocs that formed, which are non-settleable but filterable.

Chapter 3: Methodology

3.1. Preamble

This chapter addresses the research approach used to characterize the active agents in DE mucilage so as to determine its coagulation potential and biosorption capability. The process involved include extraction, precipitation and determination of functional groups. Different parameters were optimized to improve the coagulation efficiency of DE mucilage. This chapter ends with methods for biosorption studies; different parameters were also optimized to assess application of DE mucilage as biosorbent medium for removal of heavy metals from aqueous solution.

3.2. Sample collection and preservation

The plant materials (Fig. 3.1) were collected from Siloam village located in Nzhelele catchment area; from farm lands and nearby bushes close to human settlements in Vhembe District. Plant samples were put in a sampling bag and transported to the laboratory. Botanists from Botanical Laboratory in the Department of Botany, University of Venda identified the plant to be *Dicerocaryum eriocarpum* species of the Pedecialeace family. Fresh leaves of the plant were kept in the fridge at 4°C prior to analysis. It should be noted that fresh plant leaves were collected after every two days throughout the duration of this experiment (May-November, 2012) in order to extract pure viscous mucilage.

Figure 3.1: Plant samples collected from farm land

The raw water used in this study for coagulation experiments was collected from downstream of Mvudi River, Vhembe District using a 5 litre (L) plastic container. The water was sampled after a heavy rainfall to obtain high turbid water. The water samples were collected according to the commonly accepted sampling protocols of Hermond and Fechner-Levy (2000). The collected water samples were kept in a cooler box and transported to the laboratory. The samples were filtered in the laboratory and kept in the fridge at 4°C to prevent bacterial activity prior to application in the coagulation experiments. The map of the sampling sites for both the plant sampling and water sampling is given in Fig. 3.2.

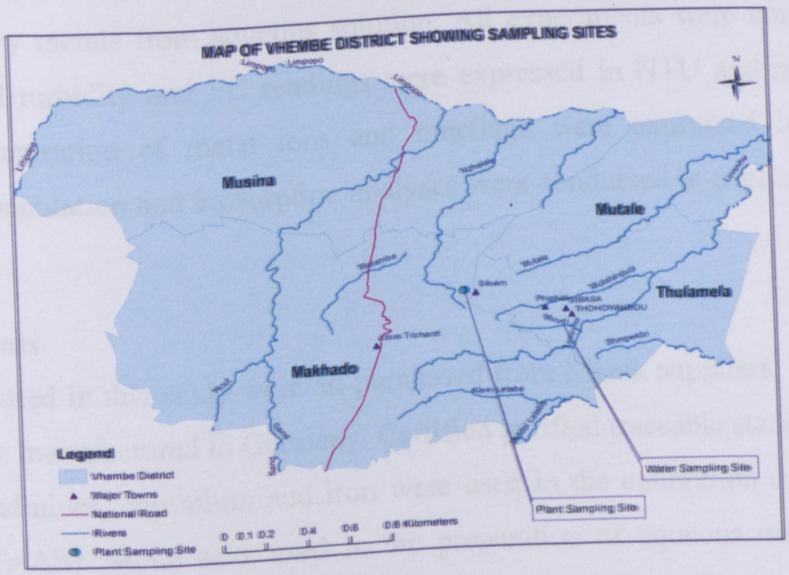

Figure 3.2: Sampling site

pH, EC and turbidity were recorded in the field using field meters. The field measurement of raw water was carried out according to the standard operating procedures of turbidity, pH and EC given in Armand and LaNeave (2012). The field measurement of EC and pH were carried out using a portable Thermo Scientific Orion (5 star) equipped with a pH electrode, integrated with a standard conductivity cell. Turbidity measurement was carried out using TB200 Portable turbidimeter (Orbeco-Hellige).

The pH electrode was calibrated according to International Union of Pure and Applied Chemistry (IUPAC) recommendations against three buffer solutions of pH 4, 7 and 9. The EC electrodes were checked using a standard buffer solution and all reported potentials were

corrected relative to the standard hydrogen electrode (SHE). The turbidity meter was calibrated using the turbidity standard solutions ranging from 0.01 to 1000 NTU.

3.3. Experimental protocol

The experimental protocol of the research was subdivided into three parts; firstly, it was the characterisation part, which involved the identification of the functional group present in the plant's mucilage. Secondly, it was the coagulation studies, which involved the application of DE mucilage in the purification and removal of turbidity in raw water.

Lastly, the biosorption experiment involved the use of DE mucilage as biosorbent medium in the removal of heavy metals from aqueous solution. All experiments were conducted under room temperature. All turbidity and EC readings were expressed in NTU and mS/cm, respectively, while the concentration of metal ions and mucilage were expressed in mg/L and %v/v, respectively. Coagulation and biosorption analyses were conducted in triplicate to ensure quality assurance.

3.3.1. Chemicals

The chemicals used in this study were all purchased from Merck suppliers, Johannesburg, South Africa and were manufactured in Germany. Certified purified traceable standard of 1000 mg/L of zinc, nickel, cadmium, chromium and iron were used in the calibration of Atomic Absorption Spectrometry (AAS). Metal salts used in the preparation of aqueous metal solution include; $Cr(NO_3)_3$, $Fe(NO_3)_2$, $Ni(NO_3)_2$, $Zn(NO_3)_2$ and $Cd(NO_3)_2$. HNO_3 and NaOH used in pH adjustment were also purchased from the same source as well as NaCl and KCl salts used in the extraction process. No further purification was carried out on the chemicals afterward.

3.4. Plant Materials

3.4.1. Plant preparation and extraction of mucilage from DE leaves

Plant leaves on the stem (Fig. 3.3a) were detached (Fig 3.3b) and washed thoroughly with tap water and rinsed with de-ionized water to remove dirt, soil particles and debris that may have been deposited on the leaves from the soil or air.

Figure 3.3: (a) Plant leaves on the stem (b) Plant leaves detached from its stem

50 g of washed leaves (Fig. 3.4 a-b) were suspended in 100 mL of boiled de-ionized water and stirred for 30 min; the mixture was kept for 1 hr under room temperature. This process was able to extract thick viscous-slimy foamy mucilage from the DE leaves. The final concentrate of pure transparent mucilage-solution was recovered by filtration using 0.45 µm filter paper with the aid of a suction pump as indicated in Fig. 3.5a-b. After filtration, 90 mL of mucilage fluid was recovered for further experiments.

Figure 3.4: (a) Extraction of thick viscous foamy slimy mucilage (b) mucilage released from the leaves

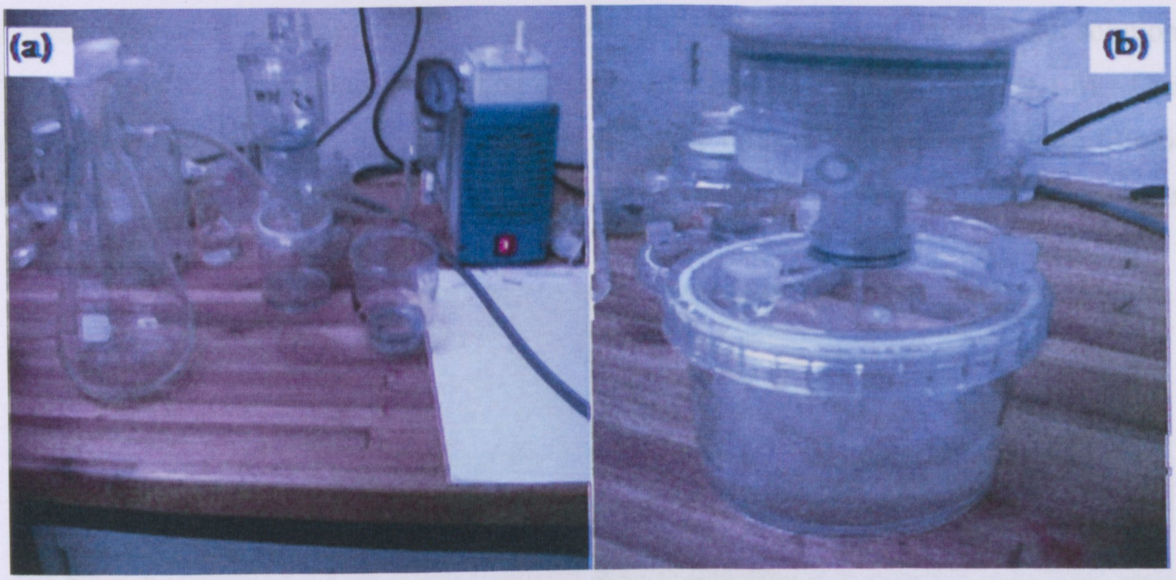

Figure 3.5 a-b: Fitration of thick viscous foamy slimy mucilage

3.4.2. Preparation of modified agent

In this study, DE mucilage was modified by introducing chloride salts into DE mucilage. This was carried out by using potassium chloride (KCl) and sodium chloride (NaCl) only. A mol L^{-1} solution of KCl and NaCl was prepared from their respective salts by weighing 7.8 g of KCl and 5.8 g of NaCl salts and dissolving each salt in 100 mL of de-ionized water as described by Ndabigenesere *et al*. (1995) and Okuda *et al*. (2001a&b) and displayed in Fig. 3.6.

Figure 3.6 Preparation of salt solution

This was followed by soaking 50 g of washed fresh DE leaves in 100 mL of each salt solution as shown in Fig. 3.7a-b, following the method described in the paragraph above using 1 M KCl and 1 M NaCl solution replacing de-ionized water. This was followed by filtration. After filtration, 90 mL of the mucilage extracted from each chloride salt was recovered for further experiments.

Figure 3.7 a-b: Soaked leaves in different salt solutions to release mucilage

3.4.3. Characterization

The characterisation of the functional group present in the mucilage is of critical importance because functional groups plays a significant role in the performance of the natural polymers. The filtrate mucilage was in liquid form as presented in Fig. 3.8 and was converted to solid form for characterization analysis using FTIR instrument. Ethanol was added to the filtrate mucilage in a quantity that was three times the volume of the total filtrate as described by Mudadi *et al.* (1993). The mixture was stirred for 30 minutes and kept overnight at a room temperature to allow it form a precipitate.

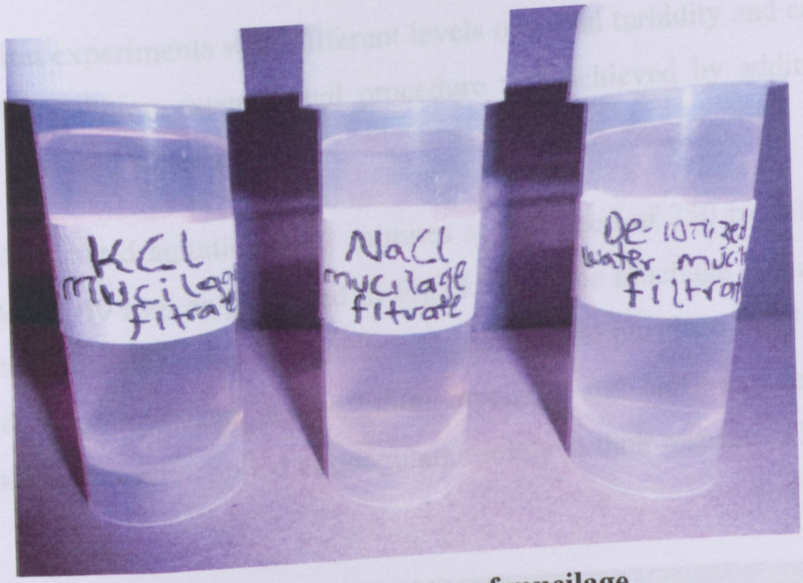

Figure 3.8: Filtrates of mucilage

The mixture was transferred into the centrifuge tube and after centrifugation, a greyish thread-like substance (solid) was produced as precipitate mucilage and recovered by filtration using pore size of 0.45 µm cellulose acetate filter paper. The recovered precipitate was washed again with de-ionized water and oven dried at 70°C. The dried precipitate was ground to a fine powder and stored in a dry place until required for characterization.

This same process was applied solely and then to all the filtrates, which included; deionized - water mucilage, KCl-solution mucilage and NaCl- solution mucilage. The FTIR measurement was carried out using the same procedure described by Saad (2011).

The characterization of the functional groups present in dried mucilage precipitates prepared from KCl – solution mucilage, NaCl – solution mucilage as well as deionzed water mucilage filtrate was conducted using FTIR spectroscopy on an FTIR spectrometer (Tensor 27, Burker, Germany). This was conducted in the analytical chemistry laboratory of the University of the Witwatersrand.

3.5. Coagulation studies

3.5.1. Procedure for coagulation experiment

Coagulation activity of DE mucilage was verified by jar test experiment using a 550 mL volume beakers equipped with mechanical stirrers. The beakers were filled with 500 mL of turbid water

samples. Coagulation experiments with different levels of initial turbidity and coagulant dosages were performed. Coagulation experimental procedure was achieved by addition of coagulant dosage to 500 mL of turbid water sample.

This was followed by rapid agitation for 2 minutes at the speed of 250 rpm, followed by slow agitation for 30 min at 40 rpm as presented in Fig. 3.9a-b. The mucilage filtrate extracted from various solutions/solvents were also applied as coagulant in the purification of raw water. These include; deionized-water mucilage, KCl-solution mucilage and NaCl-solution mucilage. The mucilage filtrate solutions were applied as coagulants solely in their respective liquid form.

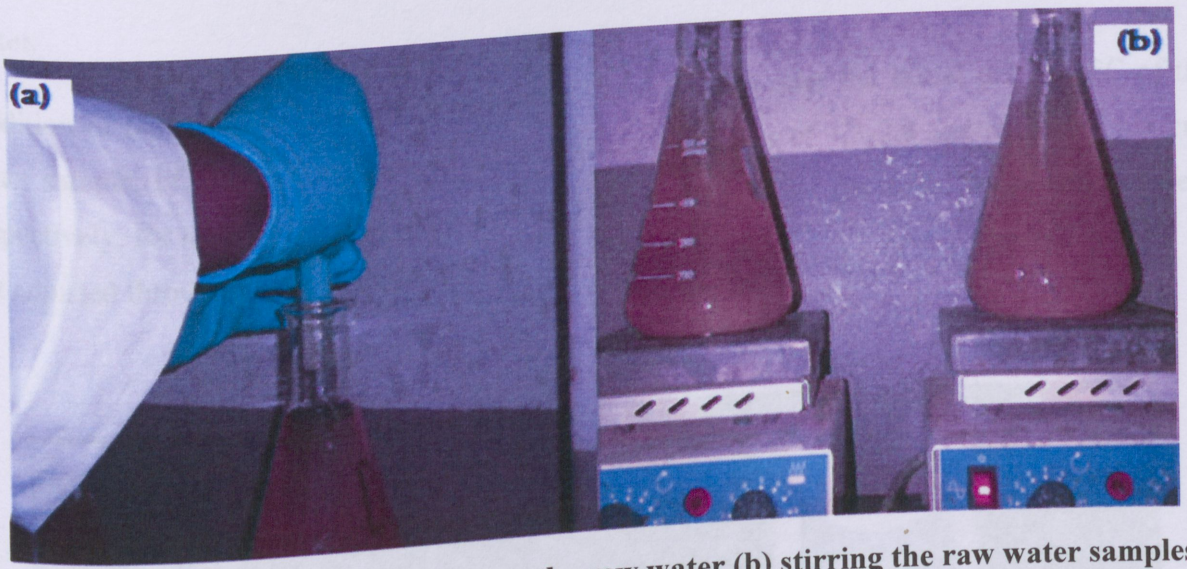

Figure 3.9: (a) Addition of coagulant to the raw water (b) stirring the raw water samples

After sedimentation, the treated water samples were assessed in accordance with the standard of drinking water. Turbidity, pH and EC were compared with South African drinking water standards (DWAF, 1996/1999) as well as international standards (WHO, 1996 and USEPA, 2009) before and after coagulation.

Note: In this study; the crude mucilage extracted using different solvents are identified as follows;

- De-ionized water mucilage filtrate, represented as—DCE
- NaCl solution mucilage filtrate is represented as —SCE
- KCl solution mucilage filtrate is represented as — PCE

Where S: represents sodium chloride, D: de-ionized water, P: potassium chloride, C: crude and E: extract.

3.5.2. Turbid water

The turbid water used in this study to assess the coagulation efficiency of DE plant so as to improve its efficiency in turbidity removal was collected from Mvudi River as earlier mentioned in section 3.2. The turbidity of the raw water was very high as shown in Fig. 3.10 (a) and (b) with initial turbidity of 630 NTU. Tap water was used to dilute the turbid raw water in order to obtain the desired turbidity levels. The desired turbidity levels prepared were within the range of 65 to 380 NTU in order to assess the performance of DE mucilage in both low and high turbid water.

Addition of tap water to dilute the raw turbid water did not alter the initial pH and EC of the water sample. EC and pH maintained a level within the range of 7.3-7.8 and 00.2-00.8 mS/cm, respectively, for all the water samples. Both the pH and the EC of the turbid water samples were not adjusted throughout the experiment.

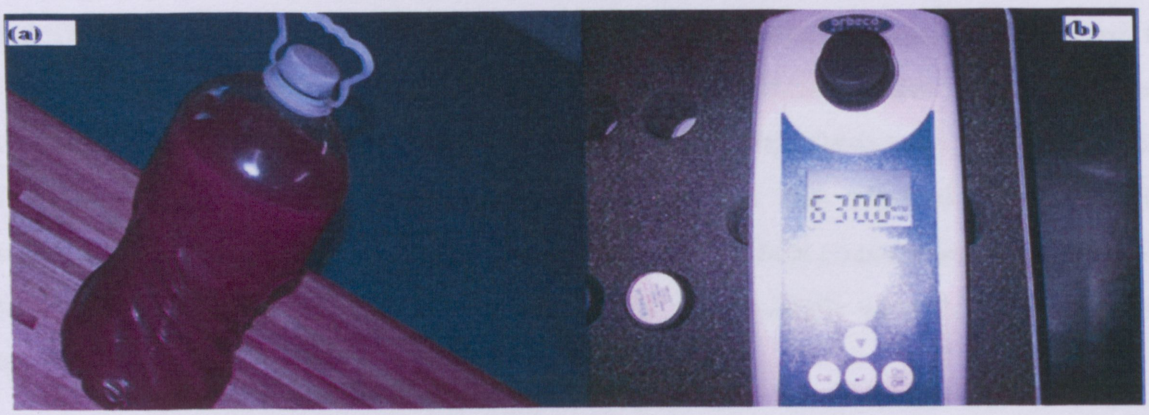

Figure 3.10: (a) represents undiluted raw water sample collected from Mvudi River and (b) represent turbidimeter displaying the initial turbidity of the raw water measured

3.5.3. Optimization of parameters for removal of turbidity from water

Settling time, coagulation efficiency of modified and unmodified mucilage, changes in coagulant dosages and changes in initial turbidity were optimized. Optimization was achieved by varying one parameter while keeping the others constant.

The influence of the optimized parameters were evaluated by calculating the turbidity removal efficiency (R) using the formula below:

$$R = \frac{NTU_{initial} - NTU_{final}}{NTU_{initial}} X\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

a) The effect of settling time (sedimentation)

The effect of settling time was determined to understand the impact of sedimentation on the coagulation efficiency of DE mucilage. The same dosage (10 mL) of coagulant (SCE, PCE and DCE) was applied to each turbid water sample, with initial turbidity maintained at 209.9 NTU. The influence of sedimentation on coagulation efficiency was assessed at different settling time intervals ranging from minimum to maximum settling time (2-18 hrs).

b) Coagulation efficiency of modified and unmodified mucilage

Comparative studies were carried out to assess the efficiency of mucilage extracted from potassium chloride solution, sodium chloride solution and deionized water. It was achieved by applying the same dosage (10 mL) of DCE, SCE and PCE coagulants each into 500 mL turbid water sample. The initial turbidity of the sampled water was adjusted to 209.9 NTU as demonstrated in Fig. 3.11. The dosage of each coagulant was kept constant at 10 mL per 500 mL of turbid water. The pH and EC levels of the turbid water sample were 7.3 and 00.2 mS/cm, respectively.

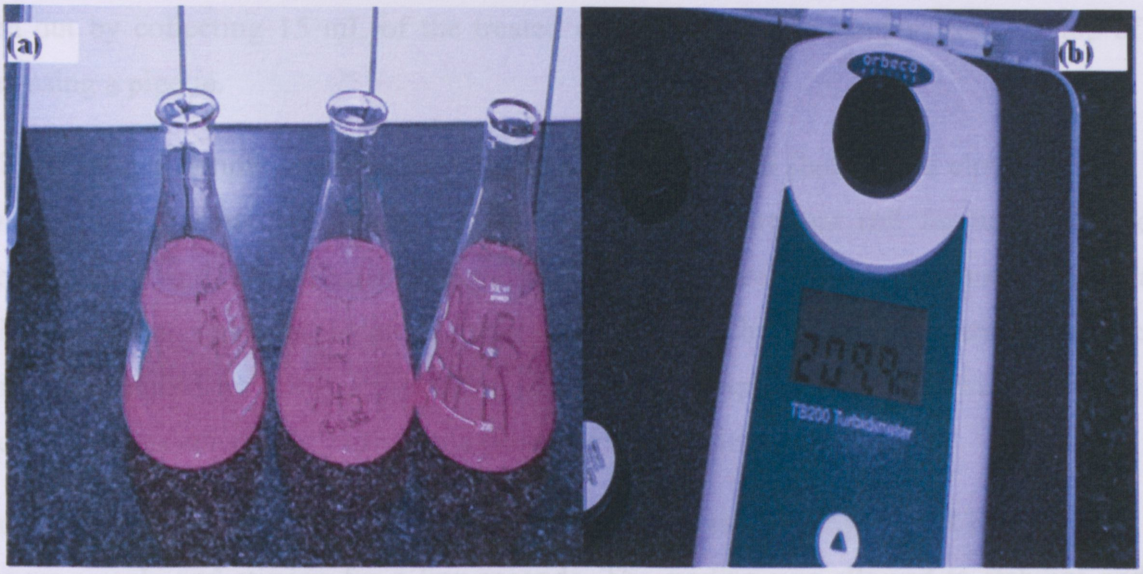

Figure 3.11: (a) represents prepared turbid water with initial turbidity of 209.9 NTU and (b) represent turbidimeter showing the initial turbidity of the sampled water

c) The effect of changes in coagulant dosage

The effect of dosage of the coagulant was assessed. The dosages of the coagulant were adjusted from 5, 10, 20 and 40 mL per 500 ml turbid water. Each coagulant dosage was added to 500 mL of turbid water samples. The turbidity of the water samples were maintained at 160 NTU as well as the pH (7.2) and EC level (00.2).

d) The effect of changes in initial turbidity

Coagulation experiments with different levels of initial turbidity of raw water varying from 50 NTU to 500 NTU were assessed. Initial turbidity of the raw water were adjusted using tap water to different turbidity levels of 65, 220 and 380 NTU from the 630 NTU stock raw water sample. The dosage of the coagulant was kept constant at 20 mL while the pH and EC levels were maintained at values of 7 and 00.3, respectively.

3.5.4. Analytical techniques

The turbidity of untreated and treated raw water samples were measured using a TB200 Portable Turbidimeter (Orbeco-Hellige). The pH and the EC of the water samples were measured using a portable Thermo Scientific Orion (5 star) equipped with a pH electrode, integrated with a standard conductivity cell. The analytical measurements of turbidity in the water samples was

carried out by collecting 15 mL of the treated or untreated water sample from the top of the beaker using a pipette.

The 15 mL water sample was filled into a turbidity sample cell (bottle) and closed tightly. The sample cell was inserted carefully into the turbidity compartment cell for turbidity reading immediately to avoid condensation of particles in the sample cell. The compartment cell infrared ray lights detector was used to measure the light scattered by suspended particles in order to determine the turbidity. The average initial turbidity was displayed on the turbidity screen. The turbidity tube was constantly washed and rinsed with de-ionized water.

EC and pH analytical measurements were carried out by submerging the pH/EC electrode into the water sample and stirring for a few seconds. The reading was allowed to stabilize and the result displayed on the screen of the meter was then recorded. All the electrode probes were always rinsed with deionized water after each measurement.

3.6. Biosorption experiment

Biosorption involves the use of biological materials that form complexes with metal ions in their functional groups (Krishnani *et al*., 2008). The developed crude mucilage extracts applied in coagulation experiment (sub-section 3.5.1) was also applied as biosorbent for heavy metals ions removal from aqueous metal ion solutions. This was aimed at studying their capability and understanding their chelating properties prior to applying them in real environment for optimum removal conditions.

3.6.1. Preparation of solutions

All metal solutions were prepared from their respective nitrate salts by weighing out the appropriate amount of salts and dissolving in de-ionized water. The metals used in this study include Zn, Cr, Ni, Fe and Cd. These metal ions were chosen for this experiment because they are often detected in industrial aqueous solution and constitute environmental hazards.

A 1000 mg/L stock solution containing each metal salt was prepared by weighing out the required mass of appropriate metal chloride salts, from which the 100 mg/L multi-component working standard solution was obtained by serial dilution for each of the metal salts. A 1 mol L^{-1} solution of NaOH and HNO_3 were prepared by weighing out the required amount and dissolving

with deionized water. pH adjustment for the biosorption experiments was conducted using HNO_3 and NaOH solution. De-ionized water was used for the preparation of all solutions.

3.6.2. Preparation of metal ions aqueous solution

Stimulated spiked metal ions solution was prepared by making a solution of multi-component standard involving all the prepared metal ions. A 10 mg/L concentration was prepared by extracting 10 ml of 100 mg/L of each metal ion and transferring it to the 100 ml volumetric flask. The 100 ml volumetric flask was topped to the mark using ultra-pure water.

The volumes were prepared carefully, using the following basic equation 3.2.

$C_1V_1=C_2V_2$ .....(3.2)

where C_1, is initial concentration, V_1, is initial volume, C_2, is final concentration, V_2, is the final volume. With the above equation, 7.5, 12.5 and 25 mg/L initial concentration of metal ions was achieved. This was used as multi-component working standard for initial concentration of metal ions in this study. Heavy metal ions standards used in calibration of the Atomic Absorption Spectrometry (AAS) were also prepared using a similar method.

3.6.3. Biosorption experimental procedure

A known concentration of mucilage was added to each flask of aqueous solution containing multiple metal ions followed by stirring with magnetic stirrer. After the agitation using magnetic stirrer at 70 rpm; the mixtures were filtered through 0.45 µm pore nitrate cellulose filter membrane. The samples were refrigerated at 4°C prior to analysis by AAS.

The extracted mucilage was studied in order to determine its efficiency as a biosorbent medium. To this end, the following parameters were optimized; sample contact time, sample pH, biosorbent concentration, initial concentration of the heavy metals and biosorption efficiency of modified and unmodified mucilage. Optimization was achieved by varying one parameter while keeping the others constant. The influence of these parameters was evaluated by calculating the extraction/recovery efficiency using the following formula:

$$\%\ Removal = \left(\frac{Co-Ce}{Co}\right) \times 100 \quad \text{.....(3.3)}$$

where: Co is the initial concentration (mg/L) and Ce is the equilibrium metal ion concentration (mg/L).

a) The effect of contact time

Contact time biosorption experiments were conducted to determine the time needed for biosorption to reach equilibrium. Biosorption was studied at various time intervals (2, 4, 6, 8, 10 and 30 min) at the initial concentration of 7.5 mg/L of metal ion following the same procedure described in section 3.6.2. The dosage of the biosorbent was maintained at 20% v/v and pH of the metal solution kept at 6. The mixture was agitated at different time intervals and recovery was determined at the end of each time.

b) The effect of pH

The effect of change in pH condition of the metal ion solution on the biosorption efficiency was determined at four different pH values (2, 4, 6 and 8). The initial concentration of the metal was kept constant at 7.5 mg/L. The concentration of biosorbent (20% v/v) and contact time (10 min) were also maintained throughout the experiment.

c) The effect of biosorbent dosage

The influence of increase in the mucilage dosage on the removal efficiency of metal ions was investigated. The proportion of the mucilage varied from 10 to 50% v/v and equilibrated for 10 min at initial metal ion concentration of 7.5 mg/L in solution while maintaining a constant pH of 6.0.

d) Effect of initial concentration of heavy metals

Initial concentrations of the metals were adjusted in 500 mL flasks: 7.5, 12.5 and 25 mg/L. Each experiment was repeated three times. The pH (6.0), contact time (10 min) and the mucilage concentration (20% v/v) were kept constant.

e) Comparative study to assess the biosorption efficiency of modified and unmodified mucilage

Further studies were also carried out to investigate the effectiveness of using modified and unmodified mucilage in removal of metal ions from aqueous solution. The pH (6.0), contact time (10 min), the mucilage concentration (20% v/v) and initial concentration of metal ion (12.5

mg/L) were kept constant. This was to evaluate the efficiency of using mucilage extracted from NaCl, KCl solution and de-ionized water as biosorbents in removal of metals ions.

3.6.4. Analytical techniques

Heavy metals analyses were conducted using AAS (Perkin Elmer Analyst 400, Germany). This was carried out in the Earth Sciences laboratory, University of Venda. The AAS measurement was carried out using the same procedure described by Maboladisoro (2004).

The AAS with air-acetylene flame as the atomizer was used to measure the concentration of total trace metals (Zn, Cd, Ni, Cr and Fe) recovered after biosorption. The instrument was calibrated using the prepared Zn, Cd, Ni, Cr and Fe standard solutions. The instrument was then blanked using the prepared blank solutions before each standard solution was analyzed. The measurement of Zn, Cd, Ni, Cr and Fe concentrations were done in triplicates and the mean concentrations were calculated.

Chapter 4: Results and Discussion

4.1. Preamble

This chapter presents the results for all the experimental work conducted. These results relate to: characterization of the active agents present in DE mucilage; functional groups determination, coagulation studies; improving coagulation efficiency in turbidity removal by optimization of parameters, and biosorption studies; optimization of various parameters to assess the capability of DE mucilage in removal of metal ions. The results presented in this chapter are the summaries of large quantity of data presented in appendices of this document.

4.2. Characterization of mucilage

Figure 4.1 (a-c) shows FTIR spectra of DE mucilage; (a) DCE, (b) PCE and (c) SCE. FTIR spectra provide a chemical fingerprint of materials by correlating their absorption frequencies with known absorption frequencies of bonds. The FTIR spectra of SCE, PCE and DCE were all measured within the range of 400-4000 cm^{-1}. The characteristics of the peaks for DCE, PCE and SCE were significantly similar as observed in Fig 4.1a-c. The same functional groups present in the DCE were also present in PCE and SCE; carboxylic (-COOH), hydroxyl (O-H) and carbonyl groups (C=O).

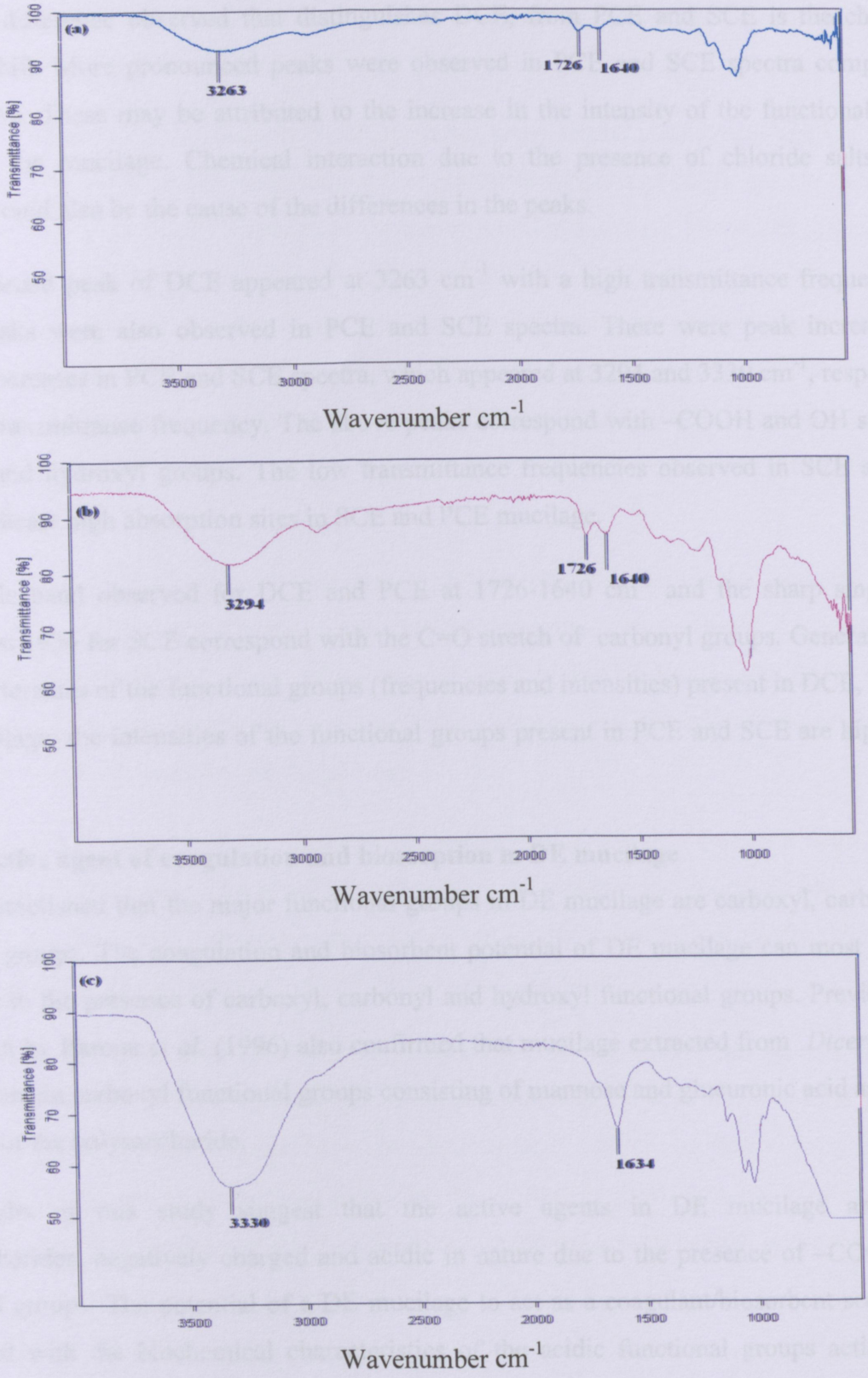

Figure 4.1: FTIR spectra of (a) DCE, (b) PCE and (c) SCE

The main difference observed that distinguishes DCE, from PCE and SCE is the change in intensity shift. More pronounced peaks were observed in PCE and SCE spectra compared to DCE spectra. These may be attributed to the increase in the intensity of the functional groups present in the mucilage. Chemical interaction due to the presence of chloride salts in the mucilage could also be the cause of the differences in the peaks.

The first broad peak of DCE appeared at 3263 cm^{-1} with a high transmittance frequency and similar peaks were also observed in PCE and SCE spectra. There were peak increases and intensity increases in PCE and SCE spectra, which appeared at 3294 and 3330 cm^{-1}, respectively, with low transmittance frequency. The above peaks correspond with –COOH and OH stretch of carboxyl and hydroxyl groups. The low transmittance frequencies observed in SCE and PCE spectra indicate high absorption sites in SCE and PCE mucilage.

The double band observed for DCE and PCE at 1726-1640 cm^{-1} and the sharp single peak observed at 1634 for SCE correspond with the C=O stretch of carbonyl groups. Generally, from the characteristics of the functional groups (frequencies and intensities) present in DCE, PCE and SCE mucilage; the intensities of the functional groups present in PCE and SCE are higher than DCE.

4.2.1. Active agent of coagulation and biosorption in DE mucilage

Having established that the major functional groups in DE mucilage are carboxyl, carbonyl and hydroxyl groups. The coagulation and biosorbent potential of DE mucilage can most likely be attributed to the presence of carboxyl, carbonyl and hydroxyl functional groups. Previous work carried out by Barone *et al*. (1996) also confirmed that mucilage extracted from *Diceriocaryum* species contain carboxyl functional groups consisting of mannose and glucuronic acid as the core structure of the polysaccharide.

The results of this study suggest that the active agents in DE mucilage are mostly polysaccharides, negatively charged and acidic in nature due to the presence of –COOH, C=O and –OH groups. The potential of a DE mucilage to act as a coagulant/biosorbent seems to be associated with the biochemical characteristics of the acidic functional groups acting as the dominant group in the mucilage. Natural plant biosorbent contain acidic and basic functional

groups and the acidic groups are more present and dominating in the uptake of metal ions than the basic groups (Bhatti *et al.*, 2007; Gilbert *et al.*, 2011).

Similar results were also obtained in different studies using natural plant biosorbent in the uptake of metal ions. Vinod *et al.* (2010) identified that gum kondagogu is an acidic gum with hydroxyl, acetyl, carbonyl and carboxylic groups as the major functional groups. The acidic groups present in gum kondagogu were responsible for uptake of multiple metal ions species (Pb, Cd, Ni, Cr, Fe, Cu, Zn, Co, Se and As) due to the presence of the negatively charged functional groups (Vinod *et al.*, 2010). The presence of carboxyl groups in cassava sorbent increased the removal efficiency of cadmium in aqueous solution (Hanafiah and Ngah, 2008). Ahluwalia and Goyal (2007) documented that carboxyl groups in brown seed weed was involved in the uptake of Fe^{2+} and Fe^{3+}.

Evaluating the role of *Zea may L.* and cactus plant mucilage in the uptake of metal ions also confirmed that the binding of metal to the mucilage was due to the carboxyl functional groups acting as active sites (Morel *et al.*, 1986; Fox *et al.*, 2012). In addition, availability of the acidic groups in DE mucilage, which are mostly negatively charged can be responsible for the binding and sorption of positively charged metal ions.

The ability of DE plant to act as coagulant in the removal of turbidity from raw water is highly attributed to the presence of the mucilage. It has been documented that mucilage found in natural plants are complex carbohydrate polymers with great ability to hold water tightly (Nobel, 2002; Saenz *et al.*, 2004; Sepulveda *et al.*, 2007; Miller *et al.*, 2008).

Miller *et al.* (2008) reported that the high coagulation activity of *Opuntia species* was highly related to the presence of the mucilage in the plant. Mucilage extracted from several plant such as Zea mays, okra and cactus plant have also demonstrated high coagulation activity in removal of turbidity from water. A conclusion can be drawn that turbidity removal efficiency of DE mucilage is attributed to the COOH, -OH and C=O functional groups present in the mucilage.

4.3. Coagulation studies results

4.3.1. Effect of settling time

Removal of turbidity in raw water involves coagulation, sedimentation and filtration. As displayed in Fig. 4.2 and Appendix A1, increase in settling time influenced the coagulation efficiency of DE mucilage. It was observed that, after 0-6 hrs of sedimentation, the turbidity removal efficiency of DE mucilage was generally low. SCE, PCE and DCE removal efficiencies were 79, 69 and 34%, respectively.

The low coagulation efficiency observed in the first 0-6 hrs of settling time for DCE can be attributed to the negatively charged colloidal particles naturally present in raw water. In addition, DCE mucilage is negatively charged and reacting with negatively charged colloidal particles in the water sets up repulsion forces. Thus it reduces the tendency for the particles to agglomerate and settle easily.

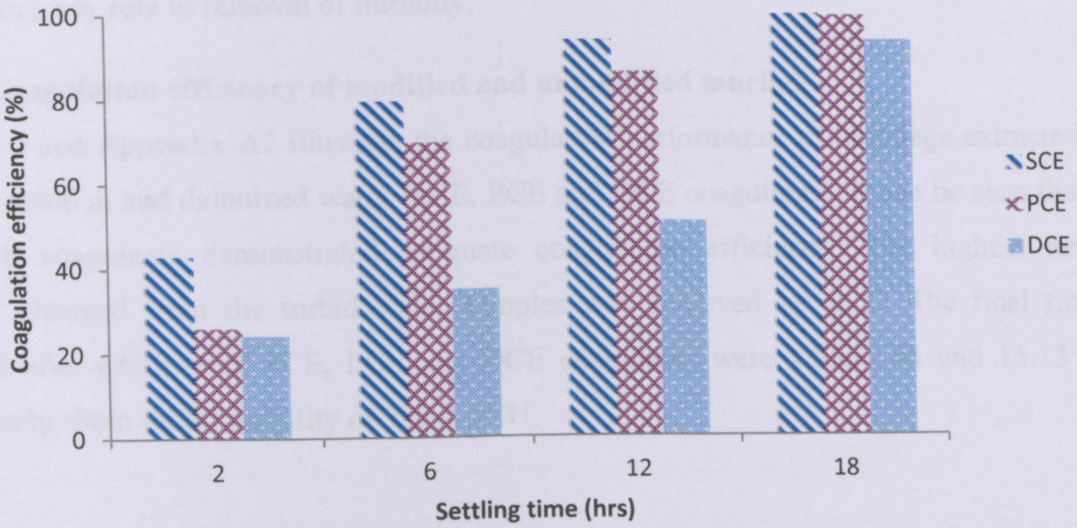

Figure 4.2: The effect of settling time on coagulation efficiency

Parameters held constant: pH (7.3), EC (00.2 mS/cm), initial turbidity (209.9 NTU), dosage (20 mL)

To overcome the repulsion forces, increase in settling time is required. It can further be explained as follows: with an increase in settling time, flocs are able to aggregate together into large particles in order to settle easily. With a decrease in settling time; small flocs would be

formed and aggregate together but do not easily settle. The mucilage was able to increase the particles sizes of the colloidal particles present in the turbid water. This was attributed to the aggregation of the small particles inside the viscous mucilage sites to form large particle sizes, which can easily settle.

Generally, the results from this study showed that coagulation efficiency was greatly enhanced by increase in settling time from 6 to 18 hrs. SCE and PCE coagulants showed the best performance in terms of turbidity removal as presented in Fig. 4.2. SCE and PCE recorded coagulation efficiencies of 99% and 98%, respectively, while DCE coagulation efficiency increased greatly with an increase in settling time to 92%.

This study also confirms that sedimentation alone without coagulation can only remove large coarse suspended solids as previously reported by Ndabigengesere *et al.* (1995). With addition of coagulants, the tiny colloidal particles in the raw water are entrapped. This results in the formation of insoluble precipitate (large flocs) that can easily settle and therefore sedimentation time plays a key role in removal of turbidity.

4.3.2. Coagulation efficiency of modified and unmodified mucilage

Figure 4.3 and Appendix A2 illustrate the coagulation performance of mucilage extracted from chloride solution and deionized water (SCE, PCE and DCE coagulants). It can be seen that PCE and SCE coagulants demonstrated adequate coagulation efficiency. The highest turbidity removal obtained from the turbid water samples was achieved by SCE. The final turbidity recorded after addition of SCE, PCE and DCE coagulants were 1.95, 2.84 and 15.13 NTU, respectively, from initial turbidity of 209.9 NTU.

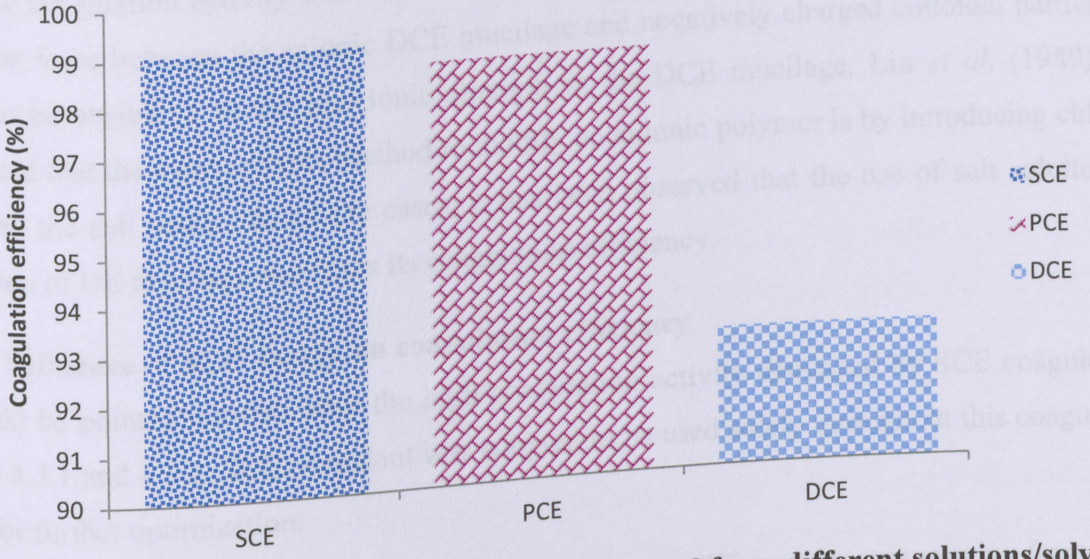

Figure 4.3: Coagulation efficiency of mucilage extracted from different solutions/solvents Parameters held constant: pH (7.3), EC (00.2 mS/cm), initial turbidity (209.9 NTU), dosage (20 mL) and settling time (18 hrs)

There was no huge significant difference in the coagulation efficiency of PCE and SCE coagulants. SCE was 13 times better than DCE while PCE was 12 times better than DCE in turbidity removal. This indicates that, the coagulation efficiency of DE mucilage was greatly improved in modified crude extract than unmodified crude extract. Similar results have been previously reported by Okuda *et al.* (1999) and Ghebremichael *et al.* (2005) where extraction of MO using salt solution improved its coagulation efficiency more than distilled water extract.

The increase in coagulation activity in modified mucilage is largely attributed to the chloride salts present in the mucilage thus increasing the ionic strength of the mucilage. PCE and SCE coagulants were able to trap the negatively charged colloidal particles easily due to their K^+ and Na^+ ions and retaining them inside the mucilage cell walls. The charge neutralization between the positively charged ions present in the mucilage and the negatively charged colloidal particles enhance large flocs formation, rapid sedimentation and improved coagulation activity. This is to be expected since it was also observed in the FTIR spectra results of PCE and SCE that there were more pronounced peaks compared to DCE spectra.

The low coagulation activity observed in unmodified mucilage (DCE) can be attributed to the repulsion force between the anionic DCE mucilage and negatively charged colloidal particles. It can also be attributed to the low ionic strength in the DCE mucilage. Lin *et al.* (1989) also confirmed that the most suitable method to modify an anionic polymer is by introducing chloride salts into the cell walls. In all the cases, it has been observed that the use of salt solutions in extraction of DE mucilage increases its coagulation efficiency.

4.3.3. Influence of SCE dosage on coagulation efficiency

It should be pointed out that after the high coagulation activity displayed by SCE coagulant in section 4.3.1 and 4.3.2, SCE coagulant was adopted to be used solely throughout this coagulation study for further optimization.

Results of coagulation experiments using SCE coagulant at different dosages ranging from 5 mL to 40 mL is displayed in Fig. 4.4. At the dosage of 20 mL, optimum removal efficiency of 78.8% was recorded within 12 hrs of settling time. Further increase in the dosage of SCE coagulant to 40 mL decreased the removal efficiency to 74.8%. The results obtained from this study show that SCE dosage influences coagulation efficiency.

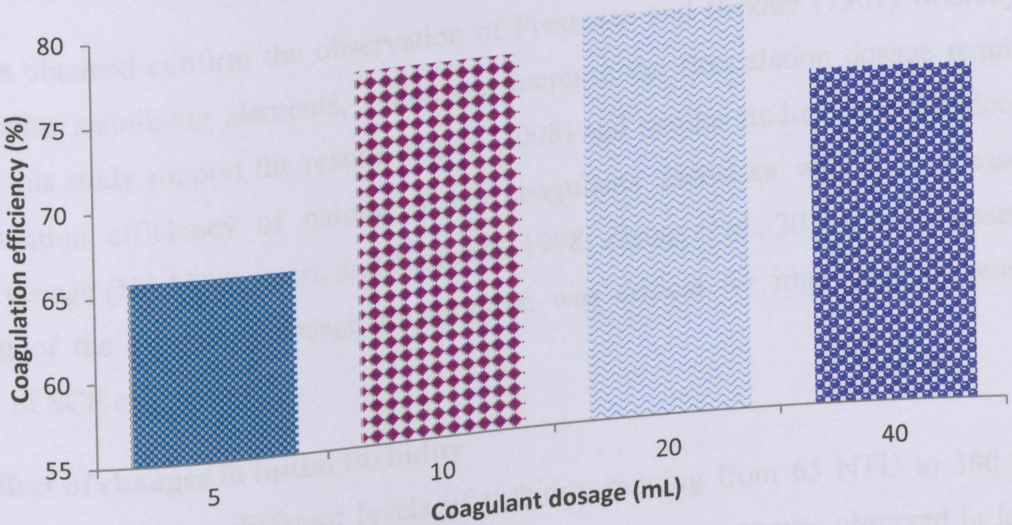

Figure 4.4: Influence of SCE dosage on coagulation efficiency
Parameters held constant: pH (7.3), EC (00.2 mS/cm), initial turbidity (160 NTU) and settling time (12 hrs)

The decrease in the coagulation efficiency of SCE when 40 mL dosage was added to the turbid water can be attributed to the early saturation of the mucilage sites by colloidal particles (cell walls). The addition of 40 mL dosage made the turbid water sample to be intense due to the high volume of viscous mucilage. It lead to dispersion of colloidal particles due to insufficient number of particles to form inter-particle bridging. It also reduced the mechanical strength of SCE coagulant, reduced the formation of flocs and delayed sedimentation. The results in this study are consistent with the results reported by Ndabigengesere *et al.* (1995) and Okuda *et al.* (2001a).

At low dosage of 5 mL, the coagulation efficiency was 65.8% showing that little coagulation occurred at low coagulant dosages. With an increase in SCE dosage from 5 mL to 20 mL, maximum reduction in turbidity was observed corresponding to 78.8%. The low coagulation efficiency observed when 5 mL was added can be attributed to the diffusion of mucilage in the particles easily. In addition, at low dosage coagulant can easily diffuse into the colloidal particles, resulting in low aggregation and less formation of flocs. While at optimum dosage, the mucilage is able to cover the surface of the colloidal particles without diffusing into it. It causes the formation of large flocs and precipitation leading to high reduction in turbidity and rapid sedimentation.

The results obtained confirm the observation of Pressman and Birkner (1967) whereby natural water contains stabilizing elements, which can increase the flocculation dosage requirements. Results in this study support the results of Lin (2008) and similar studies have also documented that coagulation efficiency of natural plants coagulants increases with an increase in the coagulant dosage (Ndabigengesere and Narasiah, 1998; Zhang *et al.*, 2010). It was observed that controlling of the coagulant dosage to optimum was critical for improving the coagulation efficiency of SCE coagulant.

4.3.4. Effect of changes in initial turbidity

The results of optimizing different levels of turbidity ranging from 65 NTU to 380 NTU are presented in Fig. 4.5. Figure 4.5 shows that the worst performance was observed in low turbid water (65 NTU) giving a coagulation efficiency of 34.3%. Coagulation efficiency improved greatly with an increase in the initial turbidity from 220 NTU to 380 NTU (high turbid water).

Corresponding to coagulation efficiency of 67% to 71% within 12 hrs of settling time. It proves that SCE coagulant is more effective as coagulant in high turbid water than in low turbid water.

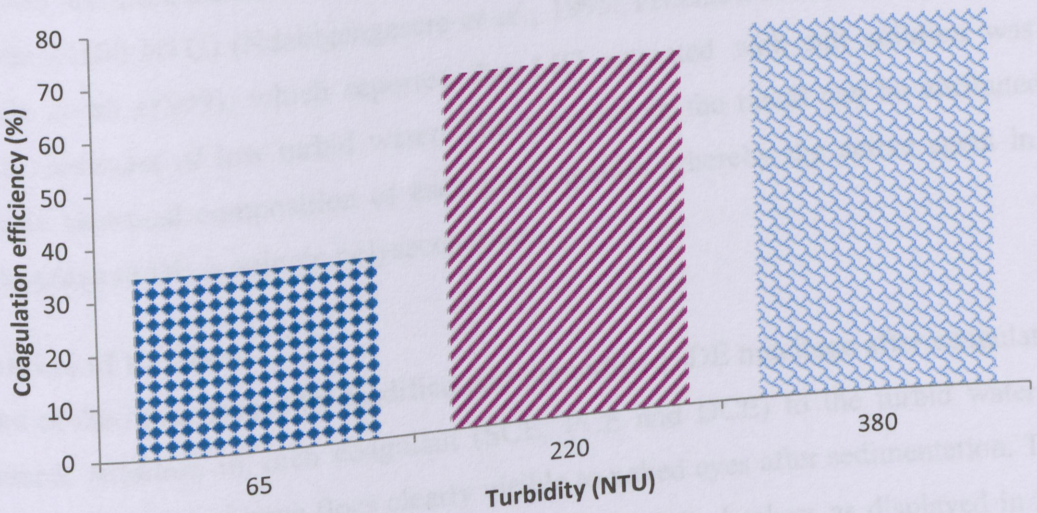

Figure 4.5: Effect of initial turbidity on the coagulation efficiency Parameters held constant: pH (7), EC (00.3 mS/cm), dosage (20 mL) and settling time (12 hrs)

The increase in coagulation efficiency of SCE in high turbid water sample (220-380 NTU) can be attributed to the increase in the number of particles in the water that increase aggregation and flocs formation. In addition, the efficiency of SCE coagulant in removal of turbidity is also dependent on the nature and amount of colloidal particles present in the turbid water. For example, water samples with high turbidity enhance large flocs formation, rapid sedimentation and increase in coagulation efficiency.

The low coagulation efficiency observed in low turbid water (65 NTU) can be attributed to insufficient numbers of colloidal particles present in the turbid water to form large flocs. In addition, it can also delay sedimentation due to the slow rate of small particles in forming large flocs rapidly. Therefore, understanding of the interactions and properties of coagulant species

and colloidal particles in the water sample play a major role in improving the coagulation efficiency of natural plant coagulants.

Most studies conducted on plant based coagulants have reported similar results showing that natural plants are more efficient in turbidity removal in high turbid water (>100 NTU) than low turbid water (<100 NTU) (Ndabigengesere *et al.*, 1995; Pritchard *et al.*, 2010). The exception was Okuda *et al.* (1999), which reported that MO extracted with salt solution was highly efficient in treatment of low turbid water. The variation in the result can be attributed to the difference in chemical composition of each plant species whereby the active agent in MO is cationic protein and DE is anionic polysaccharide.

4.3.5. Nature of the flocs

The nature of the flocs formed by modified and unmodified DE mucilage after coagulation was also assessed. Addition of each coagulant (SCE, PCE and DCE) to the turbid water sample enhances the formation of large flocs clearly visible to naked eyes after sedimentation. The flocs formed in the treated water sample settle in the bottom of the beakers as displayed in Fig. 4.6. The flocs formed by PCE and SCE were tiny orange colour particles and spherical in shape. They compare with the pattern of flocs formed by MO coagulant (Ndabigengesere and Narasiah, 1998). The flocs by DCE coagulant was thick brownish cobweb-like in structure.

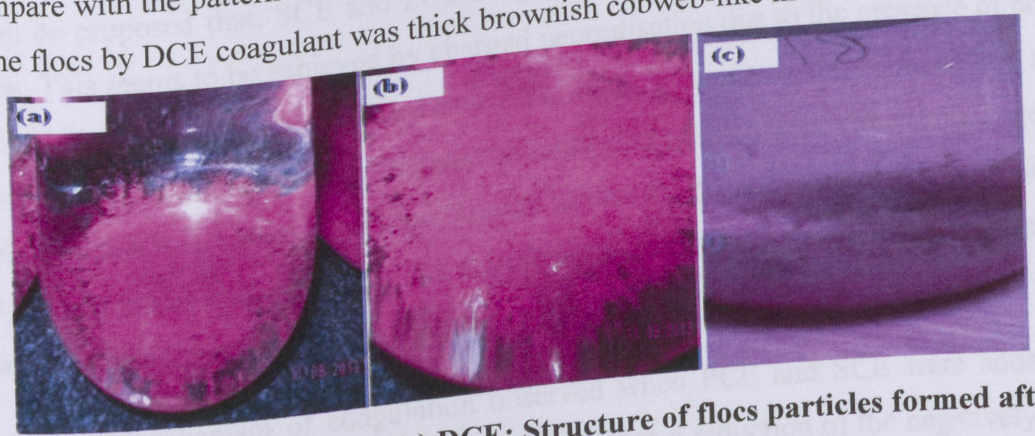

Fig. 4.6: (a) PCE (b) SCE and (c) DCE: Structure of flocs particles formed after coagulation

4.3.6. Mechanism of coagulation

The difference in the nature of the flocs particles formed by each coagulant suggests that the coagulation mechanism of mucilage extracted from various solvents differs. It appears that, the

DCE coagulant and the colloidal particles have similar charge, which is negative charge. Thus, it can be suggested that, the mechanism of coagulation of DCE is via strong repulsion between particles and the mucilage.

Even with the strong repulsion force between the coagulant and colloidal particles, it was still possible for coagulation activity to occur. It can be attributed to the shear stress induced during slow mixing during coagulation, which was able to overcome the repulsion between particles (coagulant and colloidal particles). Thus, it enables the particles to aggregate together after encounter of each other over time (Lin *et al.*, 1989). This strongly implies that particles collide many times before sticking occurs linking the colloidal particles to the polymer chain of the mucilage; resulting in formation of flocs inside the mucilage walls.

The above also agrees with the observation of Miller *et al.* (2008), where anionic mucilage from *Opuntia* specie was used in removal of turbidity in water. Although Miller *et al.* (2008) suggested that, the coagulation mechanism of opuntia species, which is also negatively charged is via adsorption and bridging, whereby clay particles do not contact one another but are bound to polymer-like material from opuntia spp.

It can also be proposed that, SCE and PCE coagulation mechanisms occurred via double layer interaction. This seems to be achieved by charged neutralisation due to the presence of positively charged ions Na^+ and K^+ in the modified mucilage. Thus, it increases the opposite and negative charge interaction between particles. Increase in ionic strength of the modified mucilage lead to less repulsion force between the particles. Results in this study are in agreement with Singley *et al.* (1971) where it was mentioned that the effectiveness of double layer is influenced greatly by the overall ionic concentration of the solution.

Another distinct mechanism of coagulation observed when PCE and SCE were added to the turbid water sample is via charge neutralization. It leads to a reduction of the negatively charged potentials of both mucilage and colloidal particles and enhances rapid particles aggregation. It decreases the size of the polymer chain (mucilage) and permits closer cluster of the polymer and particles to settle easily. The above factors explain that flocs formed in PCE and SCE treated water sample were via electrostatic patch while DCE flocs were formed via enmeshment. This

study agrees with Lin *et al*. (1989) observation where electrostatic patch or charge neutralisation can cause coagulation faster than enmeshment. It explains the high coagulation activity in PCE and SCE more than DCE.

4.3.7. Quality of the water

It is noteworthy to mention that the quality of the treated water samples was assessed according to the drinking water guidelines of DWAF (1996/1999), WHO (2006) and USEPA (2009). The above drinking water guidelines are presented in appendix B2.

(a) Turbidity

The turbidity values as presented in appendix B1 show that after treatment with modified mucilage coagulant, the turbidity values exceeded the DWAF (1996) guideline but were within the acceptable drinking water standard of WHO (1996) and USEPA (2009). With the aid of filtration using membrane filter paper with a pore size of 0.05 µm, the final turbidity of the water samples treated with unmodified mucilage coagulant improved greatly. Thus, it was suitable for drinking according to the required guidelines of WHO (1996) and USEPA (2009).

(b) pH

As it was observed, all the water samples treated with SCE coagulant had pH values ranging from 6.2 to 7.5 (Appendix B1), while the water sample treated with PCE coagulant also recorded a pH-value within the range of 6.9-7.3. The pH value of water samples treated with SCE and PCE coagulants complied with the stipulated guidelines by DWAF (1996), WHO (2006) and USEPA (2009) standard. Significantly, the water sample treated with DCE coagulant had a pH value within the range of 7.4-7.6. Thus, it did alter the initial pH of the water samples and was within the accepted limits stipulated by the above the guidelines.

The decrease in the pH value of SCE and PCE treated water can be attributed to precipitation of insoluble salts used during the modification process. In addition, it can also be attributed to the series of hydrolytic reactions between the PCE, SCE and the water sample. This can also result in decrease in pH due to the production of hydrogen ions in the treated water samples.

(c) Electrical conductivity

The EC-level of the treated water samples using modified and unmodified mucilage coagulant were also assessed and the results are presented in Appendix B1. Water sample treated with DCE coagulant did comply with the USEPA standard having an EC value within the range of 0.02-00.3 mS/cm. While the water samples treated with SCE and PCE coagulants exceeded the USEPA standard but complied with DWAF (1999) standard. EC levels of the water treated with SCE and PCE increase from initial EC of 0.02 mS/cm to 5.8 and 4.7 mS/cm, respectively (Appendix B3).

Increase in the EC levels of SCE and PCE treated water samples can be attributed to the presence of chloride ions in mucilage resulting in increase in the EC levels. The concentration of the chloride salts used during the extraction process did not cause any discomfort in taste of the water. Significantly, even with the slight increase in the EC of treated water samples, the quality of the treated water maintained a characteristic of high quality water.

4.3.8. Comparing the efficiency of DE with other plants

DE coagulation efficiency has been able to compete with the performance of other plant coagulants especially when it has been modified. The major setback of using DE is the long settling time unlike other natural plants coagulants that require a maximum of 1 hr for sedimentation time. This may be due to the nature of the functional groups present in the mucilage. Another major advantage of DE coagulant over other plant coagulants like MO is the quality of the treated water.

The quality of the treated water is potable after days of treatment without the development of organic load and odour unlike MO as reported by Ndabigengesere and Narasiah, (1998) and Okuda *et al*. (2001a). Similar result was also recorded in Saenz *et al*., (2004) when *Opuntia* spp mucilage was used in treatment of water. This result achieved in this study and Saenz *et al*., (2004) may be the major characteristics of using natural plant coagulants that contain mucilage as their active polymer in clarification of water.

4.4. Biosorption studies

The removal of metal ions from aqueous solution by DE mucilage is expressed as removal/recovery efficiency using equation 3.3. The results are presented graphically (Fig. 4.8-4.12) and in Appendix B3. It should be pointed out that, unmodified mucilage (DCE) was used mostly in this biosorbent study to optimize parameters. Modified mucilage (PCE and SCE) was only used for comparative study.

4.4.1. Effect of contact time

The effect of contact time on the removal of multiple metal ions (Zn, Ni, Fe, Cr and Cd) was investigated at different time intervals ranging from 2 to 30 min using DCE biosorbent. The results in Fig. 4.7 show that, sorption increased rapidly at the beginning and became very slow at the end. Maximum uptake rate was observed within 10 minutes of the experiment and further increase in time had little or no impact on the sorption of the metal ions.

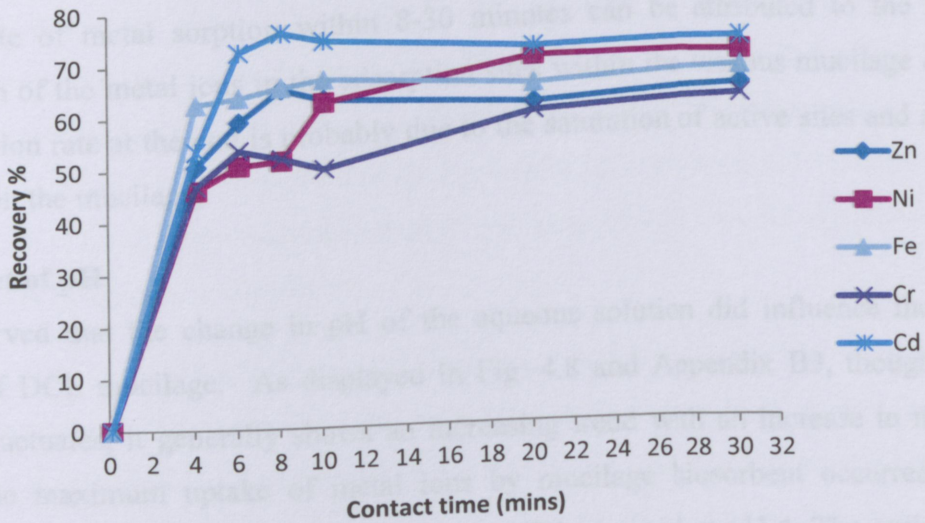

Figure 4.7: Effect of contact time on uptake of metal ions by DCE
Parameters held constant: pH (6), initial metal ion concentration (7.5 mg/L) and biosorbent concentration (20%v/v)

Biosorption by DCE was rapid and attained equilibrium faster. These results are in line with those of Chen and Chen (2001) and Miretzky *et al.* (2008). Miretzky *et al.* (2008) reported that

within 7 min of contact time, the uptake efficiency of positively charged metal ions by *Opuntia strptacantha* increased to 86.5%. Ogaji *et al.* (2012) also reported that increase in the agitation (stress) from 75 to 1500s resulted in a decrease in the viscous nature of the grewia gum.

The above explains the fact that mucilage extracted from natural plants have the tendency to reduce their viscous properties when exposed to agitation/stress within a short period of time. It can be suggested that, the viscous nature of the DCE mucilage decreases with an increase in the contact time. In addition, it reduces the mucilage polymer chain sites as well as the binding interaction between the mucilage and the metal ions. Each metal ion reached equilibrium at different contact time. For example Zn, Cd and Fe achieved equilibrium at 8-10 minutes, recording removal efficiencies of 64.7% for Zn and Fe, 78.7% for Cd, while Ni and Cr reached equilibrium at 20 and 30 minutes, recording removal efficiencies of 69.2% and 60.9% (Fig. 4.8), respectively.

The sorption efficiency slightly decreases with an increase in contact time beyond 10 min for Cr. The high rate of metal sorption within 8-30 minutes can be attributed to the high rate of accumulation of the metal ions in the adsorption sites within the viscous mucilage of DCE. The slow adsorption rate at the end is probably due to the saturation of active sites and attainment of equilibrium in the mucilage.

4.4.2. Effect of pH

It was observed that the change in pH of the aqueous solution did influence the biosorption efficiency of DCE mucilage. As displayed in Fig. 4.8 and Appendix B3, though biosorption efficiency fluctuates, it generally shows an increasing trend with an increase in the pH of the solution. The maximum uptake of metal ions by mucilage biosorbent occurred at pH 4-8. Optimum binding of mucilage with Zn, Ni, Fe and Cd occurred at pH 4. The optimum binding with Cr occurred at pH 6. The minimum uptake of all the metal ions were observed at pH 2.

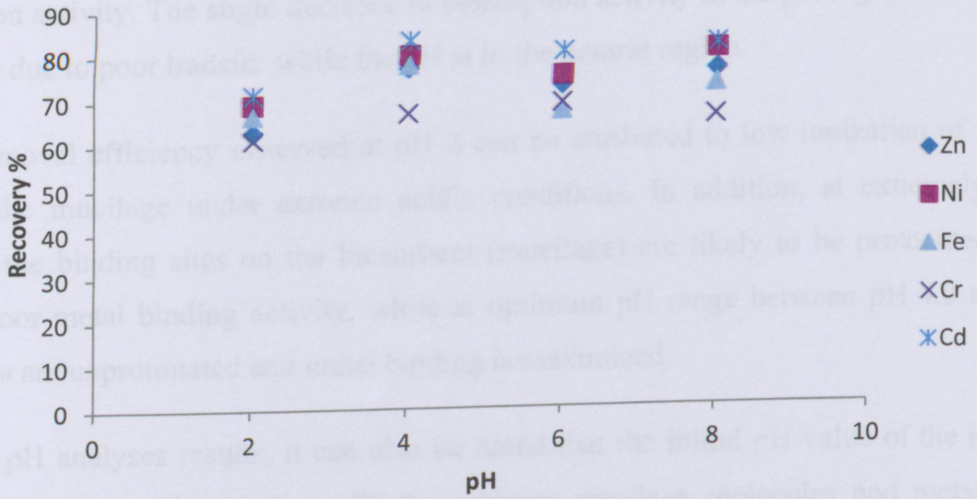

Figure 4.8: Effect of pH on the uptake of metal ions

Parameters held constant: Initial metal ion concentration (7.5 mg/L), biosorbent concentration (20%v/v) and contact time (10 min)

The results in this study agree with those of previous study by Qi and Aldrich (2008), where sorption of heavy metal ions by tobacco dust started at pH 4-5 and decreased at pH 9-10. The results also agree with previous studies of Bhatti *et al.* (2007) and Sharma *et al.* (2006). The uptake of Zinc ions by MO increased with increase in the solution pH (Bhatti *et al.*, 2007). Biosorption of Cd (II) by MO seeds occurred at pH range of 4.5-7.5 with optimum binding with Cd ions at pH 6.5 (Sharma *et al.*, 2006).

The increase in the removal efficiency with increase in pH from 2-8 could be explained by the fact that, in extreme acidic conditions (pH 2), the carboxylic groups binding sites in the mucilage are less ionized and remain constant. Thus, the negatively charged carboxylic group acts like positively charged species due to protonation and cannot attract positively charged metal ions species.

With an increase in pH of the solution to less acidic/basic conditions; the carboxylic groups become ionized and are converted to negatively charged species due to deprotonation. It is able to start attracting positively charged metal ions. Thus the multiple metal ions (Zn^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+} and Fe^{2+}/Fe^{3+} and the H^{+} in the mucilage compete for the same adsorption site resulting to

high sorption activity. The slight decrease in biosorption activity in the pH regions between pH 4 to 6 may be due to poor transfer while the pH is in the neutral region.

The low removal efficiency observed at pH 2 can be attributed to low ionization of functional groups in the mucilage under extreme acidic conditions. In addition, at extremely low pH conditions, the binding sites on the biosorbent (mucilage) are likely to be protonated. Thus it results in poor metal binding activity, while at optimum pH range between pH 4.0 to 8.0, the binding sites are unprotonated and metal binding is maximized.

From these pH analyses results, it can also be noted that the initial pH value of the metal ions solution can influence the binding affinity between mucilage molecules and metal ions. In addition, change in pH solution also influences the behavior of the functional groups present in the mucilage.

4.4.3. Effect of biosorbent concentration

The results showed that the removal efficiency of heavy metal ions increased with an increase in the concentration of mucilage. For example, Fig. 4.9 and Appendix B3 show that increase in mucilage concentration from 10 to 50%v/v generally resulted in increase in the removal of all the metal ions. Removal efficiency of Cd increased from 77.4% to 89.7%, 71.3% to 87.6% for Ni, 66.1% to 87.8% for Zn, 57.6 % to 83.4% for Fe and 46.6% to 77.4% for Cr. The increase in the biosorption efficiency with biosorbent concentration is due to the greater availability of the biosorption sites within the mucilage concentration to bind with metal ions.

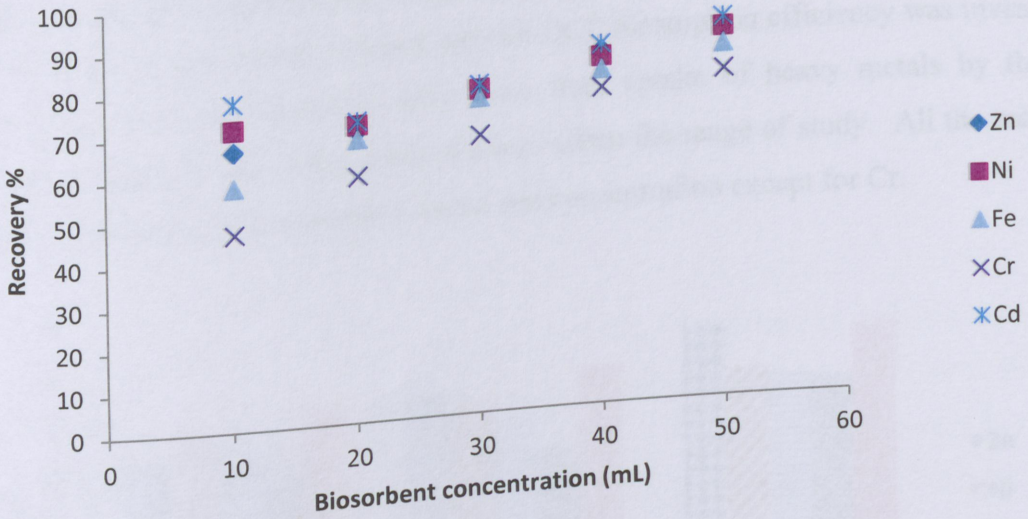

Figure 4.9: Effect of biosorbent concentration on the uptake of metal ions Parameters held constant: pH (6), initial metal ion concentration (7.5 mg/L) and contact time (10 min)

The result achieved in this study are in contrast to the result obtained by Mane *et al.* (2011) using *Opuntia* species-mucilage for the removal of heavy metals ions. Mane *et al.* (2011) reported that 10% v/v of mucilage concentration provided the optimal effectiveness for metals removal more than 20% v/v. The variation in the results can be attributed to different experimental conditions such as: difference in the concentration of the mucilage, plant species (*Opuntia* species), concentration of the metal ions, variation in polysaccharide composition, contact time and pH.

This study can confirm that DCE biosorbent is pseudoplastic in nature. At higher concentration, the mucilage extract are more pronounced and available compared to lower concentration and this agrees with results of Kuhn *et al.* (2014). This is because at low concentration, the surface area of the mucilage is small, thereby not enhancing metal ion binding activity due to insufficient binding sites in the mucilage. Medina-Torres *et al.* (2000) also reported that, there is a clear tendency for mucilage to form macromolecular networks at high concentration than at low concentration. Therefore, increase in the concentration of mucilage results in high biosorption efficiency more than low concentration.

4.4.4. Effect of initial concentration of heavy metals

The effect of initial concentration of metal ions on DCE biosorption efficiency was investigated. The results (Fig. 4.10 and Appendix B3) show that, uptake of heavy metals by the DCE increased with increase in the initial concentration within the range of study. All the metal ions were adsorbed uniformly with increase in metal ion concentration except for Cr.

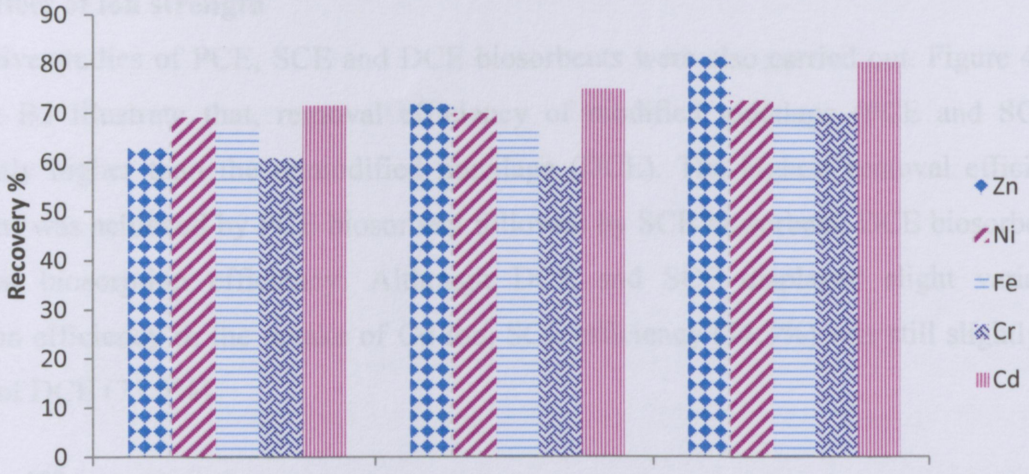

Figure 4.10: Effect of initial concentration of metal ions

Parameters held constant: pH (6), biosorbent concentration (20%v/v) and contact time (10 min)

The uptake of Cr showed a slight decrease with increase in the initial concentration from 7.5 to 12.5 mg/L. It later increased significantly with increase in metal ion initial concentration from 12.5 to 25 mg/L. The variation in the removal of Cr with increase in the initial concentration from 7.5 to 12.5 can be attributed to the influence of the presence of other metal ions in the solution. Tsezo and Volesky (1981) reported that the uptake of Thorium and Uranium was found to be influenced by the presence of Fe^{2+} and Zn^{2+} in solution. Veglio and Beolchini (1997) also confirm that the removal of one metal ion may be influenced by the presence of other metal ions. These can be attributed to several metal ions competing against each other for open sites available on the biosorbent thus influencing the uptake one metal ions in the presence of other metal ions in solution.

The results achieved in this study concur with literature whereby biosorption capacity increase with increase in the initial concentrations of the heavy metals (Mishra *et al*., 1997; Van Wyk and Gericke, 2000; Chuah *et al*., 2005). Van Wyk and Gericke (2000) discussed that a natural biosorbent such as rice husk also increased the adsorption capacity of Zn (II) with increment in the initial concentration of the heavy metals.

4.4.5. Effect of ion strength

Comparative studies of PCE, SCE and DCE biosorbents were also carried out. Figure 4.11 and Appendix B3 illustrate that, removal efficiency of modified mucilage (PCE and SCE) was significantly higher than the unmodified mucilage (DCE). The highest removal efficiency of metals ions was achieved by PCE biosorbent followed by SCE biosorbent. DCE biosorbent gave the lowest biosorption efficiency. Although DCE and SCE displayed slight variation in biosorption efficiency in the uptake of Cd ion, SCE efficiency (74.3%) was still slightly higher than that of DCE (73.6%).

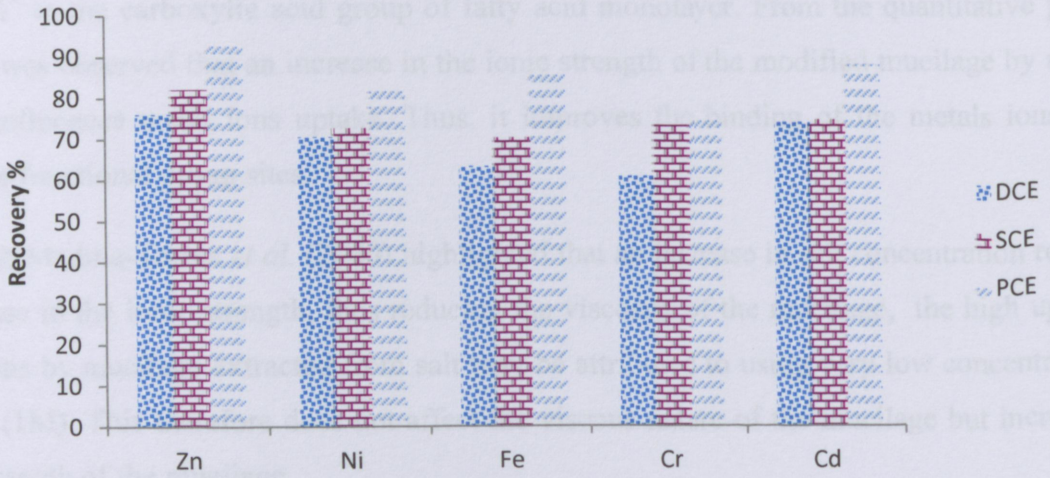

Figure 4.11: Effect of ionic strength on the removal efficiency of mucilage
Parameters held constant: pH (6), initial metal ion concentration (12.5 mg/L), biosorbent concentration (20%v/v) and contact time (10 min)

It is noteworthy to mention that, from the illustration in the FTIR spectra (Fig. 4.1) there was an increase in broad peak of the hydroxyl, carboxyl and carbonyl group for PCE and SCE compared to DCE. With SCE having the broad large peak followed by PCE displaying satisfactory

structural stability. Similar results were also obtained by Ghanem *et al.* (2009) indicating that levels of rhamnose and uronic acid in *Kosteletzkya virginica* plant increased when exposed to salt.

These results also compare with Chubar *et al.* (2004) where cork oak powder was modified with salts such as NaCl and $CaCl_2$ solely for the removal of Cu, Zn and Ni from aqueous solution. The modified salt extract showed greater sorption capacity than the unmodified cork with NaCl displaying high biosorption efficiency than $CaCl_2$. The high biosorption activity of PCE and SCE can be attributed to the conversion of the binding sites in the functional group from H^+ form to Na^+ and K^+ forms, thus increasing the density of biosorption sites in the mucilage (Chubar *et al.*, 2004).

Increase in biosorption activity in PCE more than SCE can be attributed to K^+ exhibiting a stronger ionic binding affinity to the carboxylic anions groups in the mucilage more than Na^+. It also compares with the observation of Tang and Allen (2009) in the binding affinity of Na^+ versus K^+ to the carboxylic acid group of fatty acid monolayer. From the quantitative point of view, it was observed that an increase in the ionic strength of the modified mucilage by addition of salt influences metal ions uptake. Thus, it improves the binding of the metals ions in the mucilage functional group sites.

Although Medina-Torres *et al.* (2000) highlighted that an increase in salt concentration results in a decrease in the ionic strength, thus reducing the viscosity of the mucilage, the high uptake of metal ions by mucilage extracted from salt may be attributed to using very low concentration of the salt (1M). This therefore does not affect the viscous nature of the mucilage but increase the ionic strength of the mucilage.

4.5. Mechanism of biosorption

Metal ions are attracted and bound to the plant biosorbent by a complex process that comprises of a number of mechanisms via adsorption. Mucilage in *Diceriocaryum eriocarpum* species has been characterized to be a polymer polysaccharide containing carboxylic acid, carbonyl, and hydroxyl functional group. The presence of these functional groups contribute to the binding chelation of metal ions as the main biosorption mechanism.

Another mechanism of biosorption in mucilage can be via electrostatic interaction. It is because, positively charged metal ions can bind by way of electrostatic attraction with the negatively charged surface of DE mucilage. Lastly, this study can assume that a combination of these two mechanisms; electrostatic interaction and binding chelation provide underlying principles to the inner working of mucilage biosorbent.

Chapter 5: Conclusion and Recommendations

5.1. Preamble

This chapter summarizes the outputs of the study on the biosorption and coagulation efficiency of modified and unmodified DE mucilage and its functional groups. It presents the potential and efficiency for DE mucilage in turbidity and heavy metals removal

The general aim of this study was to investigate the coagulant properties of DE mucilage and assess its application as biosorption medium for the removal of heavy metals ions from aqueous solution, in addition to improving its efficiency in turbidity removal.

5.2. Conclusion

In order to assess the ability of mucilage extracted from DE plant to act as coagulant and as biosorbent, characterisation tests were conducted on modified and unmodified mucilage. The DE mucilage was modified before characterisation by introducing different chloride (KCl and NaCl) in similar proportions into the mucilage before extracting it. The dominating functional groups that were present after characterisation and acting like active agent in mucilage were carboxyl, hydroxyl and carbonyl groups. The modified mucilage showed superior removal efficiency in both biosorption and coagulation compared to the unmodified mucilage.

The ability of mucilage to remove turbidity in raw water as well as removal of metal ions from aqueous solution was highly dependent on the functional groups present in the mucilage. Significantly, this study has established that DE has the potential to purify water up to 99% using modified mucilage coagulant. The turbidity of the treated water did comply with the international standards for drinking water. This coagulation study has demonstrated that all the water quality parameters assessed in the treated water using modified mucilage coagulant did not pose any health effect to consumers.

This study has proven that both modified and unmodified mucilage from DE plant can be successfully applied as biosorbent medium in the removal of metal ions from aqueous solution. This is attributed to the high active biosorption sites in the viscous mucilage biosorbent. This study has shown that DE mucilage performance is comparable to that of other bio materials used as biosorbent. The introduction of anions and cations (Na^+Cl^- and K^+Cl^-) into negatively charged mucilage did not decrease the uptake of metal but rather increased the removal efficiency of the metals ions. The major setback of using mucilage

medium as biosorbent is associated with its liquid form. Thus it cannot be regenerated and re-used after it has been used in the first batch since it is in liquid form.

5.3. Recommendations

Thus, we recommend that modified DE mucilage be used as a coagulant in turbidity removal. It guarantees satisfactory water clarification corresponding with the potable drinking water standards. Thus, the water can be used for drinking without any negative ill-health impacts on humans. Therefore DE mucilage can be adopted as coagulant for removal of turbidity from raw water especially in rural areas where there is no access to potable water.

However, application of DE mucilage as biosorption medium in removal of heavy metals from aqueous solution is still a preliminary finding. It is recommended that further investigations still need to be carried out to assess its metals ions binding ability in acid mine drainage raw water to be able to propose its application for commercial use.

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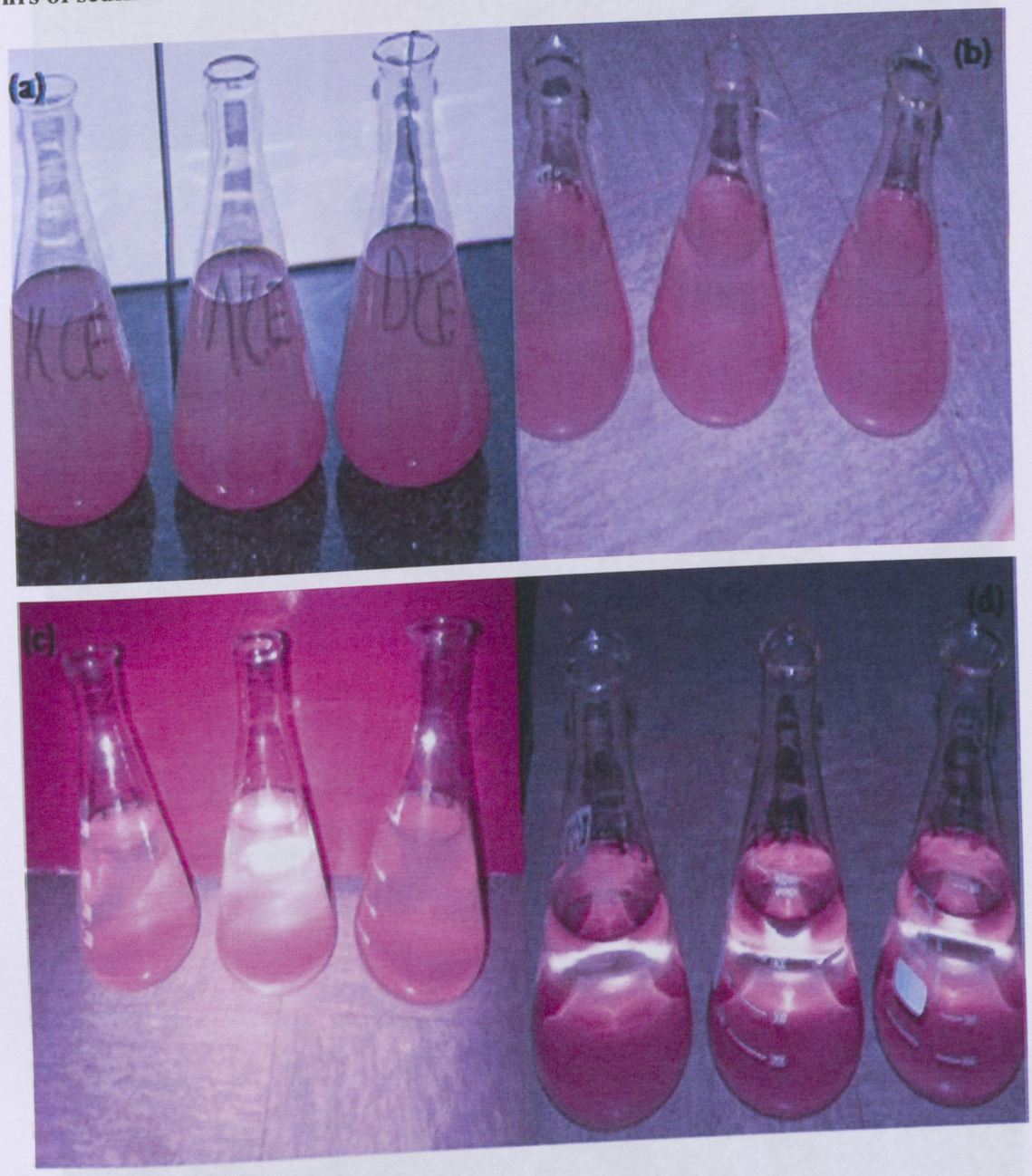
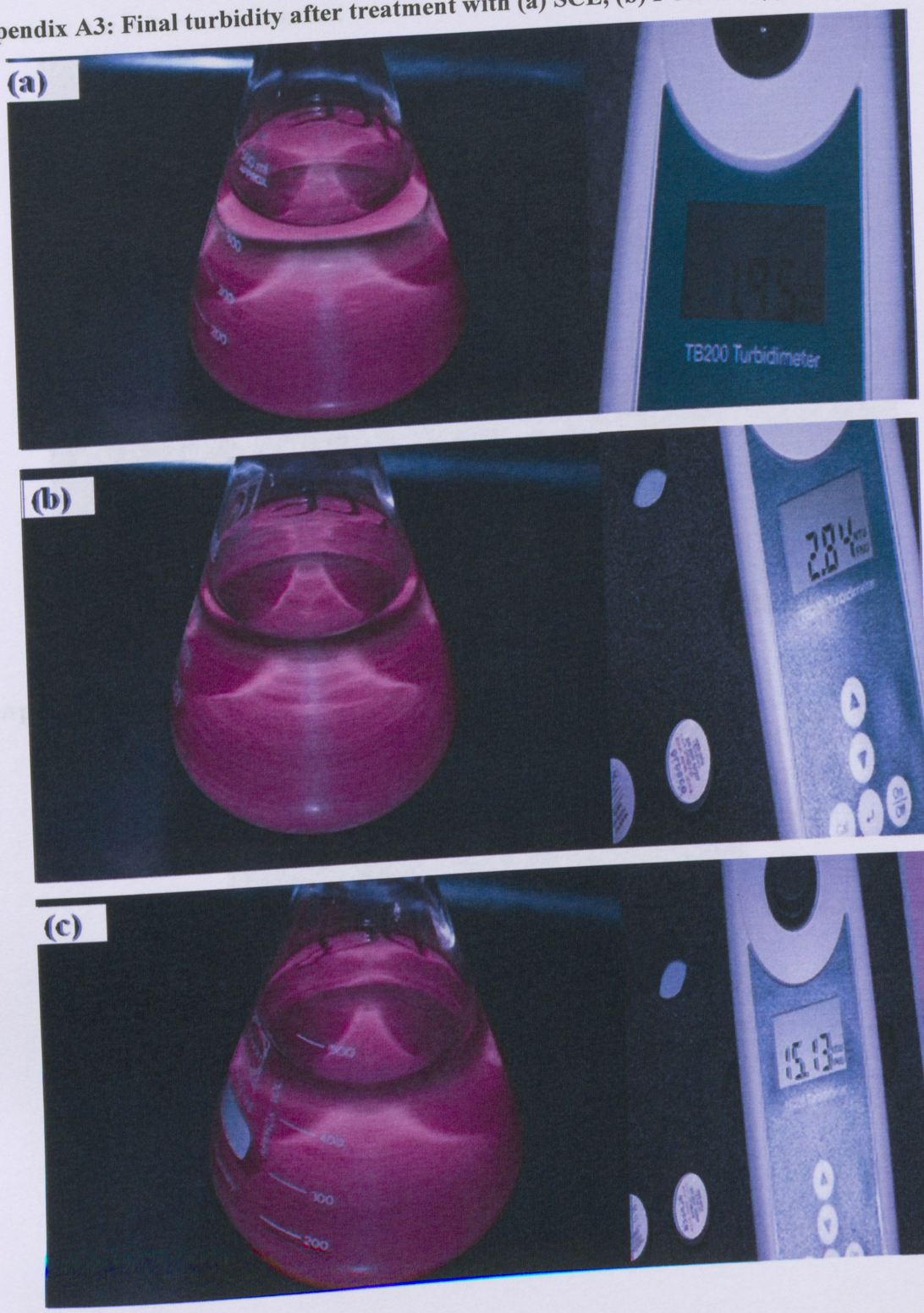
Appendices

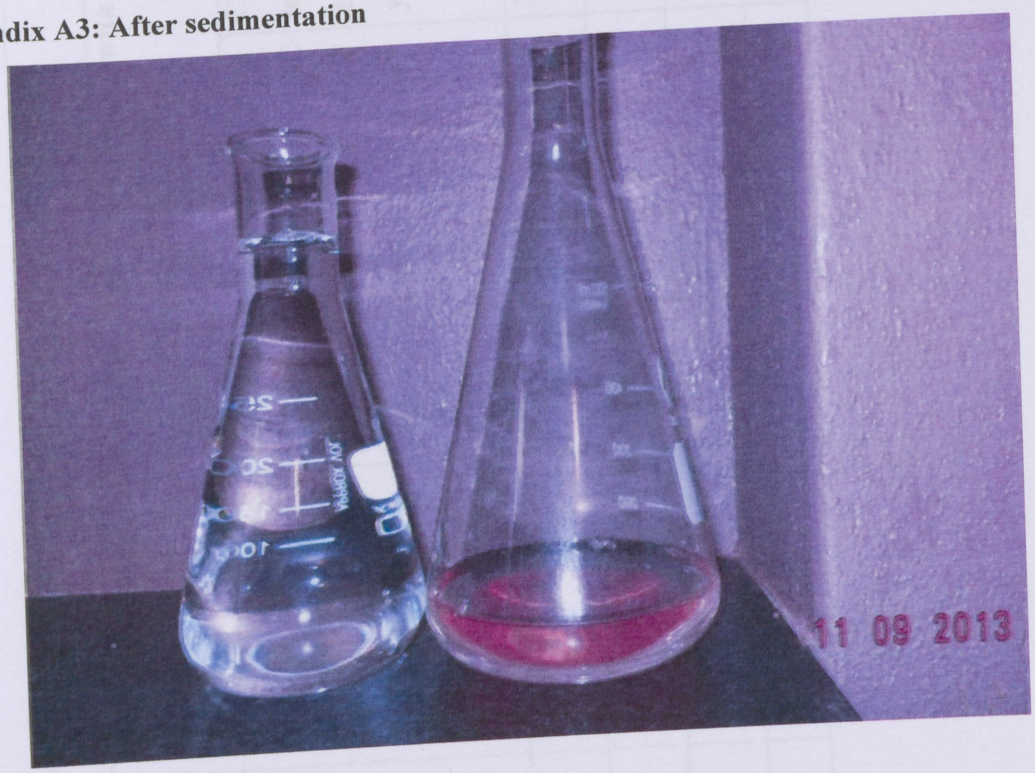
Apendix A1: After 2 hrs of sedimentation (a), after 6 hrs of sedimentation (b), after 12 hrs of sedimentation (c) and (d) after 18 hrs of sedimentation



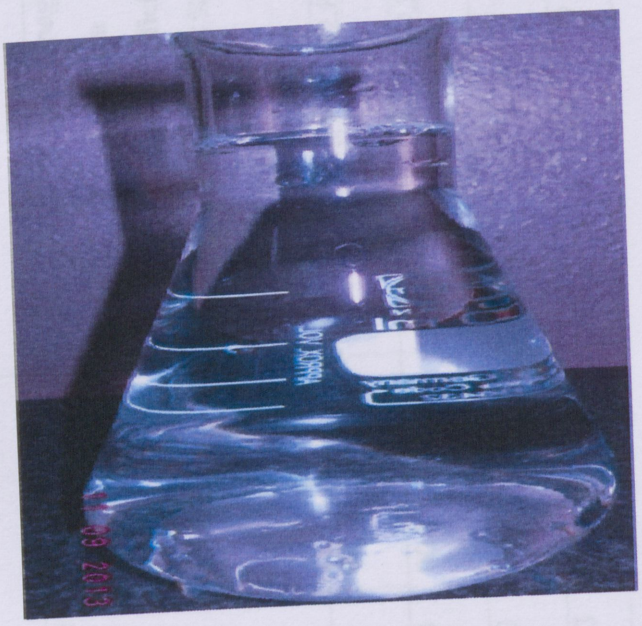
Apendix A3: Final turbidity after treatment with (a) SCE, (b) PCE and (c) DCE



Apendix A3: After sedimentation



Apendix A4: Water sample after treatment with DE mucilage



Appendix B1: Results of coagulation experiment and parameters optimized

Settling time (hrs)/Coagulation efficiency of modified and unmodified mucilage																
	Before treatment				After treatment with SCE coagulant				After treatment with PCE coagulant				After treatment with DCE coagulant			
Settling time (hrs)	Initial turbidity (NTU)	Initial pH	Initial EC (mS/cm)	Coagulant dosage (mL)	Final turbidity (NTU)	Final pH	Final EC (mS/cm)	Coagulation efficiency (%)	Final turbidity (NTU)	Final pH	Final EC (mS/cm)	Coagulation efficiency (%)	Final turbidity (NTU)	Final pH	Final EC (mS/cm)	Coagulation efficiency (%)
2	209.9	7.3	00.2	20	120	7.1	0.1	42.8	155.5	6.9	1.02	25.9	159.2	7.4	00.3	24.
6	209.9	7.3	00.2	20	43.6	7.3	2.8	79.2	64.1	7.1	1.06	69.4	137	7.4	00.2	34.
12	209.9	7.3	00.2	20	14.3	7.5	4.5	93.1	30.3	7.3	3.6	85.5	104.2	7.6	00.2	50.3
18	209.9	7.3	00.2	20	1.95	7.1	5.8	99	2.84	7.1	4.7	98.6	15.1	7.4	00.2	92.7

Effect of coagulant dosage (SCE)							
Coagulant dosage (mL)	**Initial turbidity (NTU)**	**Initial pH**	**Initial EC (mS/cm)**	**Final turbidity (NTU)**	**Final pH**	**Final EC (mS/cm)**	**Coagulation efficiency (%)**
5	160	7.2	00.2	54.6	7.4	0.10	65.8
10	160	7.2	00.2	36.5	7.1	2.1	77.1
20	160	7.2	00.2	33.8	6.2	4.0	78.8
40	160	7.2	00.2	40.3	6.6	4.7	74.8

Effect of initial turbidity after treatment with SCE coagulant							
Initial turbidity (NTU)	**Coagulant dosage (mL)**	**Initial pH**	**Initial EC (mS/cm)**	**Final turbidity (NTU)**	**Final pH**	**Final EC (mS/cm)**	**Coagulation efficiency (%)**
65	20		00.3	42.6	6.2	2.1	34.3

	Effect of initial turbidity after treatment with SCE coagulant						
	Coagulant dosage (mL)	**Initial pH**	**Initial EC (mS/cm)**	**Final turbidity (NTU)**	**Final pH**	**Final EC (mS/cm)**	**Coagulation efficiency (%)**
220	20	7	00.3	72	7.4	1.08	67.2
380	20	7	00.3	110.1	7.3	1.01	71.02

Appendix B2

Guidelines for drinking water according to DWAF (1996/1999), WHO (2006) and USEPA (2009) standards			
Parameters (unit)	DWAF	World Health Organisations	USEPA
Turbidity (NTU)	0-1	0-5	0-5
pH	6-9	6.0-8.5	5.5-9.0
EC (mS/cm)	0-70	-	1 mS/cm

Appendix B3: Result of biosorption experiment and parameters optimized

Parameters optimized	Initial concentration (mg/L)	Concentration of biosorbent (%v/v)	Recovery concentration (mg/L)					Biosorption efficiency (%)				
Contact time			Zn	Ni	Fe	Cr	Cd	Zn	Ni	Fe	Cr	Cd
4	7.5	20	3.71	4.05	2.81	4	3.49	50.5	46	62.5	46.7	53.5
6	7.5	20	3.08	3.71	2.73	3.49	2.08	58.9	50.5	63.6	53.5	72.3
8	7.5	20	2.63	3.65	2.6	3.62	1.82	64.9	51.3	65.3	51.7	75.7
10	7.5	20	2.8	2.84	2.51	3.79	1.96	62.7	62.1	66.5	49.5	73.3
20	7.5	20	2.96	2.3	2.69	3.08	2.17	60.5	69.3	64.1	58.9	71.1
30	7.5	20	2.78	2.31	2.53	2.93	2.12	62.9	69.2	66.2	60.9	71.7
Parameters optimized	**Initial concentration (mg/L)**	**Concentration of biosorbent (%v/v)**	**Recovery concentration (mg/L)**					**Biosorption efficiency (%)**				
pH			Zn	Ni	Fe	Cr	Cd	Zn	Ni	Fe	Cr	Cd

2	7.5	20	2.8	2.32	2.51	2.92	2.16	62.7	69	66.5	61	71.2
4	7.5	20	1.77	1.55	1.72	2.52	1.31	76.4	79.3	77	66.4	82.5
6	7.5	20	2.1	1.93	2.52	2.39	1.54	72	74.2	66.4	68.1	79.4
8	7.5	20	1.88	1.53	2.12	2.65	1.44	74.9	79.6	71.7	64.6	80.8
Concentration of biosorbent (%v/v)			Zn	Ni	Fe	Cr	Cd	Zn	Ni	Fe	Cr	Cd
10	7.5		2.54	2.15	3.18	4	1.69	66.1	71.3	57.6	46.6	77.4
20	7.5		2.33	2.21	2.47	3.12	2.15	68.9	70.5	67	58.4	71.[illegible]
30	7.5		1.82	1.75	1.91	2.57	1.67	75.7	76.6	74.5	65.7	77.7
40	7.5		1.22	1.33	1.57	1.89	1.12	83.7	82.2	79	74.8	85

Parameters optimized	**Initial concentration (mg/L)**	**Concentration of biosorbent (%v/v)**	**Recovery concentration (mg/L)**					**Biosorption efficiency (%)**				
Concentration of biosorbent (%v/v)			Zn	Ni	Fe	Cr	Cd	Zn	Ni	Fe	Cr	Cd

50	7.5		0.91	0.93	1.24	1.69	0.77	87.8	87.6	83.4	77.4	89.7
Initial concentration of metal ions (mg/L)			Zn	Ni	Fe	Cr	Cd	Zn	Ni	Fe	Cr	Cd
7.5		20	2.78	2.31	2.53	2.93	2.12	62.9	69.2	66.2	60.9	71.7
12.5		20	3.51	3.74	4.15	5.11	3.12	71.9	70	66.8	59.1	75
25		20	4.6	6.8	7.3	7.49	3.86	81.6	72.8	70.8	70	80.7
Modified and unmodified extracts			Zn	Ni	Fe	Cr	Cd	Zn	Ni	Fe	Cr	Cd
DCE	10	20	2.98	3.69	4.6	4.9	3.2	76.1	70.4	63.2	60.6	73.6
SCE	10	20	2.24	3.42	3.73	3.37	3.21	82	72.6	70.1	73	74.3

Parameters optimized	**Initial concentration (mg/L)**	**Concentration of biosorbent (%v/v)**	**Recovery concentration (mg/L)**					**Biosorption efficiency (%)**				
Modified and			Zn	Ni	Fe	Cr	Cd	Zn	Ni	Fe	Cr	Cd

unmodified extracts												
PCE	10	20	0.77	2.3	1.69	3.09	1.55	93.8	81.6	86.4	75.2	87.6