EFFICIENCY OF SUGARCANE BAGASSE INOCULATED WITH *MICROSPORUM* AND *ALTERNARIA* SPECIES IN THE REMOVALOF HEAVY METALS FROM REFINERY EFFLUENT

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MAY, 2017

DECLARATION

I hereby declare that this work in this dissertation entitled "Efficiency of sugarcane bagasse inoculated with *Microsporum* and *Alternaria* species in the removal of heavy metals from refinery effluent" was carried out by me in the Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, under the supervision of Prof. D.A. Machido and Dr. M.B. Tijjani. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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Signature Date

CERTIFICATION

This dissertation entitled "EFFICIENCY OF SUGARCANE BAGASSE INOCULATED WITH *MICROSPORUM* AND *ALTERNARIA* SPECIES IN THE REMOVAL OF HEAVY METALS FROM REFINERY EFFLUENT" by Linda Elsee Ofeh AMEH, meets the regulations governing the award of Masters of Science (Microbiology) of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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This research work is dedicated to God Almighty.

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ABSTRACT

In this study, sugarcane bagasse was inoculated with single and co-culture of *Microsporum* and *Alternaria* species to assess its efficiency in the removal of heavy metals from refinery effluent. The proximate composition of sugarcane bagasse was determined according to the method of Association of Analytical Chemist (AOAC, 2010) while the heavy metal content in the refinery effluent was determined following the method of American Public Health Association (APHA, 2005). Evaluation of the potential of sugarcane bagasse to support the growth of the test fungi was carried out using modified method of Machido *et al*. (2015). Sorption capacity of inoculated/uninoculated sugarcane bagasse was assessed and one-way Analysis of Variance was used to compare means. The removal rate of the heavy metals from the various treatments, were as follows: 89-92% of Pb, 86-94% of Cr and 99% of Cd. This corresponds to adsorption of: 0.9-1.0mg/g of Pb, 0.7-0.9mg/g of Cr and 1.8mg/g of Cd respectively. Uninoculated sugarcane bagasse was able to remove 90% Pb, 88% Cr and 99% Cd; inoculated bagasse with *Alternaria* species removed 89% Pb, 94% Cr and 99% Cd; inoculated bagasse with *Microsporum* species removed 92% Pb, 91% Cr and 99% Cd while inoculated bagasse with co-culture of the fungal species removed 91% Pb, 88% Cr and 99% Cd. Inoculated bagasse with *Microsporum* species had the highest removal of Pb and the least was recorded for inoculated bagasse with *Alternaria* species; inoculated bagasse with *Alternaria* species had the highest removal of Cr while uninoculated and inoculated bagasse with co-culture had the least. However, there was no statistical difference ($P>0.05$) between inoculated and uninoculated sugarcane bagasse implying that the incorporation of fungal biomass as component of the sorbents did not result in any measurable differences in the percentage or quantity of the targeted metal ions removed or adsorbed. The relative affinities of the sorbents for the targeted metal ions were also determined using the Langmuir equation.

It was noted that, all the experimental sorbents exhibited high affinity for Cr ions (b = 0.03), followed by Cd ions (b = 0.13) and Pb ions (b = 0.27).

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

It has been established, that effluents from petroleum refineries are contaminated with toxic organic compounds (Zhu *et al.*, 2001; Bako *et al.*, 2002) and heavy metal ions (Beddri and Ismail, 2007; Marcus and Ekpete, 2014). The harmful impacts of these toxic pollutants on the recipient environment (Alluri *et al.*, 2007; Varsha *et al.*, 2010; Mudhoo *et al.*, 2011; Marcus *et al.*, 2013; Ho *et al.*, 2014) and on human health has been widely reported in several studies (Ramzan *et al.*, 2011; Tokar *et al.*, 2011; *Ho et al.*, 2014). There are also reports on the inefficiency and high cost of conventional methods of waste treatments such as precipitation, reverse osmosis, electrolytic extraction, cementation, electrodialysis, filtration amongst others used in the removal of these heavy metal ions from such effluents (Iqbal *et al.*, 2005; Otokunefor and Obiukwu, 2005; Ezzouhri *et al.*, 2009; Wang and Chen, 2009; Hemambika *et al.*, 2011).

Over the years, there has been constant search for efficient, cost effective and eco- friendly means of treating effluents from industries prior to their discharge into the environment. Studies have shown that adsorption is the most effective and sustainable technique for removal of heavy metal ions even at very low concentrations (Allen and Brown, 1995). Hence, recent studies on the adsorption of heavy metals have been centered on the use of biological materials which are available in large quantities as well as certain waste products from industrial and agricultural operations (Vieira and Volesky, 2000; Agarwal *et al.*, 2006). Based on the metal binding capacities of various biological materials, biosorption is employed to separate heavy metals from wastewater (Vilar *et al.*, 2007) and by definition;

biosorption is the ability of biomaterials to accumulate heavy metals from waste water through physico-chemical pathways (Fourest and Roux, 1992). Assessment of the potential of large amounts of readily available and inexpensive biomaterials serving as effective heavy metal ions scavengers gained considerable attention and as such, many biomass types have been tested for their metal binding capabilities (Martin and Ayele, 2002; Bishnoi *et al*., 2004; Sharma *et al*., 2006; Ahluwalia and Goyal, 2007). The major challenge for the biosorption field however, remains the selection of the most promising types of biomass from the various types available (Kratochvil and Volesky, 1998).

As compared to conventional methods for the treatment of liquid wastes, the biosorption process offers: low operating cost, minimization of the volume of chemical and/or biological sludge to be disposed of, high efficiency in detoxification of very diluted effluents, no nutrient requirements amongst others. These advantages provide very strong incentives for developing full-scale biosorption processes to clean up heavy metal pollution (Kratochvil and Volesky, 1998).

Biomass of fungal origin have been of paramount interest, and therefore, given considerable attention out of the numerous microbial biomasses assessed (Volesky and May, 1995; Niyogi *et al*., 1998; Tang and Cheng, 2003; Nirmal *et al*., 2010; Hemambika *et al*., 2011; Joshi *et al*., 2011; Kumar *et al*., 2012; Rao and Bhargavi, 2013). In practice, the operation commences by loading the biomaterials (biosorbents) into sorption columns, whereby a metal bearing effluent is passed through the packed-bed and the heavy metal ions are taken up from the liquid by the biosorbents which provides an effective tool for the continuous sorption of heavy metal ions (Kratochvil and Volesky, 1998). Plant derived biomasses are made up of proteins, carbohydrates, phenolic compounds and other constituents, which contain functional groups, such as carboxyl, hydroxyl and amine, capable of binding metal ions in solution(Choi and Yun, 2006; Abdulsalam *et al.,* 2014). It is conceivable therefore, thatthe decomposition

of plant derived biomasses by fungi could enhance its capacity to bind and remove heavy metal ions from solution. Such enhancement in the sorption capacity of the biomass could be brought about by generating additional reactive functional groups on the surfaces of the biomaterials and providing substrate for the growth and accumulation of fungal biomass which serves as additional sorbent. This approach completely eliminates the need for laboratory media to generate fungal biomass refuted to be very efficient in the sorption of heavy metal ions from solution (Nirmal *et al*., 2010; Hemambika *et al*., 2011; Joshi *et al*., 2011; Kumar *et al*., 2012; Rao and Bhargavi, 2013).

1.2 Statement of Research Problem

The establishment of industries such as tanneries, textile factories, electroplating, petroleum refineries and other petrochemical industries in Nigeria heralded the generation of immense quantities of toxic liquid wastes (Machido *et al*., 2015) and have been reported to produce large volume of effluents containing wide range of toxic heavy metals (Emoyan *etal*., 2005; Atubi, 2011; Lekwot *et al*., 2012; Ho *et al*., 2014) often at much higher levels that are considered dangerous to the receiving environment and human health (Balaji *et al*., 2005; Srivastava *et al*., 2006; Alluri *et al.*, 2007; Machido *et al*., 2015). This is a serious cause for concern to both environmental and public health authorities.

Over the years, conventional techniques used in the removal of heavy metals from industrial effluents have provento be inefficient, especially at low heavy metal concentration (Iqbal *et al*., 2005; Otukunefor and Obiukwu, 2005; Ezzouhri *et al*.,2009). These methods are also costly and less eco-friendly (Nasir *et al*., 2005).

Burning of agricultural wastes as a method of waste disposal causes environmental pollution that could contribute to global warming as well as change in weather and climate thereby posing a greatthreat to the environment and humanity in general (Ballesteros *et al*., 2006).

1.3 Justification

Studies have reported the potential of biomaterials in the removal of heavy metal ions from dilute solutions as are found in effluents from industries (Joshi *et al*., 2011; Kumar *et al*., 2012). Thus, the need to assess, the potential of oneof the readily available types such as sugarcane bagasse as a possible sorbent for the removal of heavy metal ions from refinery effluents.

Due to the inefficiency of conventional treatment practices (Wang and Chen, 2009; Hemambika *et al*., 2011), biosorption, has been considered a suitable and a more effective technique for heavy metal removal from industrial effluents using microorganisms most especially fungi; and the use of sugarcane bagasse for the growth of these fungi serves as an additional sorbent for heavy metal removal.

Utilisation of this source of biomass (sugarcane bagasse) as biosorbents for heavy metal removal from industrial effluents and also, as a medium for fungi growth provides an alternative means for its disposal in an eco-friendly way (Lovett *et al*., 2006).

1.4 Aim

The aim of this study is to assess the efficiency ofsugarcane bagasseinoculated with *Microsporum*and *Alternaria* species in the removal of lead, chromium and cadmium from refinery effluent.

1.5 Objectives

The objectives of this study were to:

1. Determine the proximate composition of sugarcane bagasse.
2. Determine the physico-chemical properties of the refinery effluent.

3. Evaluate the potentialof sugarcane bagasse in supporting growth of *Microsporum* and *Alternaria* species
4. Assess the effect of growth of *Microsporum* and *Alternaria* speciesseparately and as co-culture on the capacity of sugarcane bagasse in the removal of lead, chromium and cadmium ions from the refinery effluent.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Industrial Wastes

Industrial waste is defined as an unwanted or residual material generated by manufacturing or industrial processes. Industrial wastes can either be solid, semi-solid, liquid or gaseous. Industrial wastes often contain organic and inorganic compounds, various toxic metals and harmful gases. These wastes are generated at every stage in the production process depending on the type of industry, the raw materials used and the type of technology utilized (Agoyi, 2002). If improperly treated before discharge, due to lack of highly efficient and economic technology, these wastes can pose dangerous health and environmental consequences (Ezeonuegbu *et al*., 2014). High levels of toxic contaminants have been found in animals and humans, particularly people that work in the farms, oil and gas sector, because they are continually exposed to such wastes. Waste water from manufacturing or chemical processes in industries contributes to water pollution (Agoyi, 2002). The effects of water pollution are not only devastating to people but also to animals, fish, and birds. Polluted water is unsuitable for domestic, recreational, agricultural, and industrial purposes. It diminishes the aesthetic quality of lakes and rivers. More seriously, contaminated water destroys aquatic life and reduces its reproductive ability (Wahaab, 2000; Balaji *et al.*, 2005).

2.1.1 Types of industrial waste

Industrial waste can be categorized into biodegradable and non-biodegradable:

Biodegradable: these are waste materials that can be broken down to non-poisonous substances in nature in due course of time by the action of microorganisms. These types of

waste are generated from food processing industries, dairy, textile mills etc. some examples are paper, leather, wool, animal bones, wheat, etc(Agoyi, 2002).

Non-biodegradable: these are waste materials that cannot be broken down into non-poisonous or harmless substances in nature. Examples are plastics, polythene bags, synthetic fibres, silver foils, radioactive wastes etc (Agoyi, 2002).

2.1.2 Sources of industrial wastes

Common sources of industrial wastes include industries such as electroplating, plastic, petroleum refining, textile, dyeing and printing, paper mill, pharmaceutical, food, leather tanning, agro-based, mining and metallurgical and a host of others. Several materials and contaminants generated by these industries have been reported by several studies (Singh *et al*., 2005; Jonathan *et al*., 2008; Radic *et al*., 2010; Ahmed *et al*., 2011).

2.1.3 Impact of industrial wastes on the receiving environment

Industrial wastes, whether solid, semi-solid or liquid are sometimes discharged into the environment either partially treated or without any prior treatment. The receiving environment could be soils (landfilled) and rivers (streams, lakes) and could find their way to ground water (Ezeonuegbu *et al*., 2014). The discharge of industrial wastes mainly as effluents into rivers diminishes the quality of such rivers and poses danger to aquatic life forms and even humans when such waters are used for consumption, domestic and recreational purposes. The usage of such waters for irrigation purposes on farmlands reduces plant growth and crop yield (Ezeonuegbu *et al*., 2014).

2.2 Petroleum Refinery

Petroleum refinery is an industrial process plant where crude oil is processed and refined into more useful petroleum products, such as gasoline, diesel, fuel, asphalt base, heating oil, kerosene, and liquefied petroleum gas (Ali, 2012).

2.2.1 Major products of petroleum refinery

Al Zarooni and Elshorbagy, (2006) classified refineries into either a hydro-skimming unit comprised of three sub-units (a crude distillation unit, which fractionates crude oil into various components, a desulphurising unit, which reduces the sulphur content of some fractions such as kerosene and naphtha and a reforming unit for producing reformate) or a complex unit, which incorporates an additional catalytic cracking unit into the hydro-skimming refinery such as heavy fuel oil, lubricating oils, wax, asphalt. This classification is based on the way crude oil is distilled and separated into fractions called distillates and residue (Page *et al*., 2002)

• Liquefied petroleum gas (LPG)

• Gasoline (also known as petrol)

• Naphtha

• Kerosene and related jet aircraft fuels

• Diesel fuel

• Lubricating oils

• Paraffin wax

• Asphalt and tar

• Petroleum coke

Petroleum refineries also produce various intermediate products such as hydrogen, light hydrocarbons, reformat and pyrolysis gasoline (Page *et al*., 2002). These are not usually

transported but instead are blended or processed further on-site. Chemical plants are thus often adjacent to oil refineries. For example, light hydrocarbons are steam-cracked in an ethylene plant, and the produced ethylene is polymerized to produce polyethylene (Page *et al*., 2002).

Petroleum refineries are complex systems of multiple operations that depend on the type of crude oil refined and the desired products. For these reasons, no two refineries are alike. Depending on the size, crude oil, products and complexity of operations, a petroleum refinery can be a large consumer of water, relative to other industries and users in a given region. Within a refinery, the water network is as unique to the refinery as its processes. This section describes the typical sources of water supplied to a refinery and the typical discharges of water from a refinery. It also provides an overview of the types of contaminants contained in the raw water and the methods used to remove these contaminants (Kuehn *et al*., 1995).

2.3 Petroleum Refinery Effluent

Petroleum refinery effluents (PRE) are wastes originating from industries primarily engaged in refining crude oil and manufacturing fuels, lubricants and petrochemical intermediates (Harry, 1995). Due to the ineffectiveness of purification systems, wastewaters may become seriously dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem (Ali, 2012).

Although concerted efforts have been made to replace fossil fuels, crude oil remains an important raw material. The need to satisfy the ever-increasing global energy demand, which is expected to soar by 44% over the next two decades (Doggett and Rascoe, 2009), makes the processing of crude oil and the generation of PRE globally important issues. The process of refining crude oil consumes large amounts of water. Consequently, significant volumes of wastewater are generated. Coelho *et al*. (2006) reported that the volume of PRE generated

during processing is 0.4-1.6 times the amount of the crude oil process. World oil demand is expected to rise to 107mbpd over the next two decades, and oil will account for 32% of the world's energy supply by 2030. Biofuels, including ethanol and biodiesel, are expected to account for 5.9mbpd by 2030, and the contributions from renewable energy sources like wind and solar power are estimated to be 4-15% (Doggett and Rascoe, 2009). These data clearly indicate that effluents from the oil industry will continually be produced and discharged into the world's main water bodies. These pollutants pose serious toxic hazards to the environment.

Petroleum refinery effluents can vary greatly depending on the type of oil being processed, the plant configuration, and operation procedures (Saien and Nejati, 2007). They enter into waterways and adversely affect water quality. Some of the more common of these are nickel, vanadium, copper, cadmium, lead, chromium, zinc and selenium. Of late, mercury has also started to appear as an impurity in our natural gas and crude streams (Mubin and Ismail, 2007).

2.3.1 Composition of refinery effluents

Physically, refinery effluent is usually turbid and is characterized by a black color and a solid content and the suspended solids can lead to the development of sludge deposits and anaerobic conditions when discharged into the receiving environment(Wake, 2005). Chemically, refinery effluents are characterized by the presence of large quantities of crude oil products, polycyclic aromatic hydrocarbons, phenols, metal derivatives, surface-active substances, sulfides, naphthylenic acids, oxalic acid, and other chemicals (Mizell and Romig, 1997). These effluents are a major source of aquatic environmental pollution industries (Wake, 2005).

Biological component of refinery effluents include micro-organisms such as fungi, bacteria, algae, protozoa and plants. Toxic compounds produced by the first four pass through wastewater treatment facilities that have not been designed to remove them and interfere with their operation making the biological treatment process ineffective (Liu and Lipta, 2000).

2.3.2 Heavy metals associated with refinery effluents

Petroleum refinery effluents have been known to contain various heavy metals in varying concentrations which are non-biodegradable and can persist in the environment for a long period of time. Heavy metals associated with refinery effluents include; lead, chromium, cadmium, nickel, zinc, copper, arsenic, vanadium, selenium and mercury (Mubin and Ismail, 2007). Heavy metals in refinery effluents mainly originate from the feedstock (Ali, 2012). Others are from corrosion products of the equipment and pipes, from process chemical additives and materials like catalysts and others used in processes downstream of the primary distillation (Ali, 2012).

2.3.3 Adverse effects of effluents

Petroleum refinery effluents (PRE) are priority pollutants due to their high content of polycyclic aromatics contents, which are toxic and tend to be more persistent in the environment (Wake, 2005). They encompass a wide range of contaminants at varied concentrations that are generally harmful. Decreased productivity of algae (a very important link in the food chain) observed for PRE-receiving water bodies have been attributed to such effects (Pardeshi and Patil, 2008). The minimum amount of dissolved oxygen necessary for normal life in an aquatic environment is about 2mg/L (Attiogbe *et al.*, 2007), and the discharge of waste waters with high organic matter content into water bodies results in the excess consumption of oxygen by the bacteria. This is in an attempt to oxidise the effluent,

thus depleting oxygen from the water faster than it dissolves back into the water from the air (Attiogbe *et al.*, 2007). This problem leads to the inadequate maintenance of higher life forms. In addition, oxygen availability is important because the end products of chemical and biochemical reactions in anaerobic systems often produce aesthetically displeasing colours, tastes and odours in water (Attiogbe *et al*., 2007).

Oil and grease are sticky in nature; they tend to aggregate, clogging drain pipes and sewer lines, causing unpleasant odours and corroding sewer lines under anaerobic conditions (Chen *et al.*, 1999; Xu and Zhu, 2004). They also interfere with unit operations in municipal wastewater treatment plants because they float as a layer on top of the water. They also stick onto pipes and walls consequently blocking strainers and filters (Xu and Zhu, 2004). Phenolic compounds pose a significant threat to the environment due to their extreme toxicity (Kavitha and Palanivelu, 2004), stability, bioaccumulation and ability to remain in the environment for long periods. They generally are carcinogenic, causing considerable damage and threaten the eco-system in water bodies along with human health (Abdelwahab *et al*., 2009).

The nitrogen and sulphur components of the effluent are highly toxic and are represented in the form of ammonia and hydrogen sulphide (H_2S), respectively (Altas and Büyükgüngör, 2008). In aqueous form, H_2S exists in equilibrium with bisulphide (HS^-) and sulphide (S^{2-}), and the latter sulphide is the most reduced form (Altas and Büyükgüngör, 2008). Sulphide has a high oxygen demand,thus contributing significantly to oxygen depletion (Poulton *et al*., 2002). This results in mass fish mortality when the threshold limit exceeds 0.5 mg/L for freshwater or saltwater fish (Altas and Büyükgüngör, 2008). The degradation of water quality through PRE discharges and the quest for a cleaner environment caused by the increased awareness of the hazardous composition of PRE has resulted in various environmental protection agencies setting maximum limits of discharge for each component of the waste.

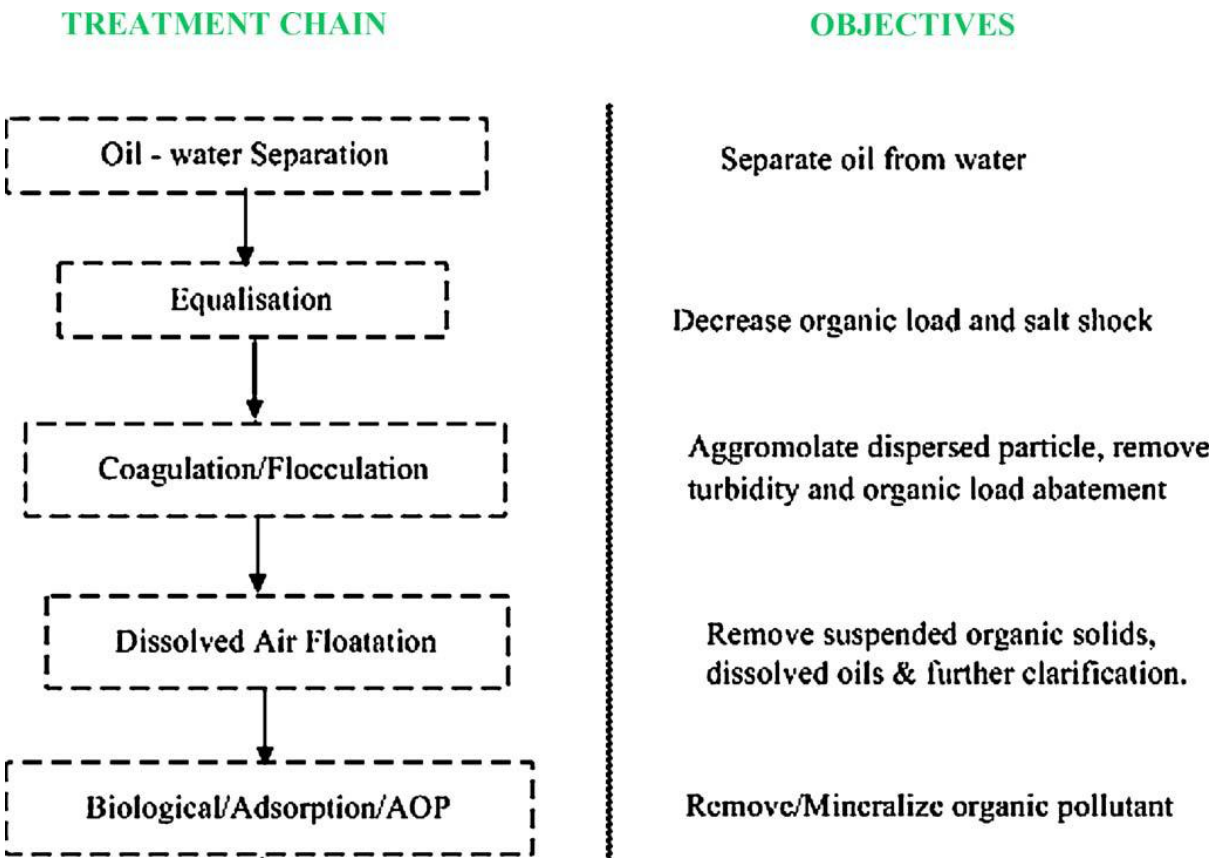


Figure2.1: Schematic diagram of generic sequence for treating petroleum refinery effluent (Diya'uddeen *et al*., 2011).

2.4 Impact of Refinery Effluents on the Physicochemical Properties of a Water body

Water pollution is commonly defined as the physical, chemical or biological change in water quality which has an adverse impact on living organisms in the environment or which makes a water resource unsuitable for one or more of its beneficial uses (Ezeonuegbu *et al*., 2014). The nature of a pollutant and the quantity of it are important considerations in determining its environmental significance (Walakira, 2011). Identifiable parameters peculiar to effluent characteristics and its effect on the quality of a water body are biodegradable organic substances such as carbohydrates and fats. These compounds are readily biodegraded when discharged into the receiving water body and are quickly decomposed through the action of natural microbial populations (Walakira, 2011). The dissolve oxygen in the water body is utilized through oxidation of these organic substrates by microbial respiration process. The rate of biodegradation of organic matter is proportional to the rate of dissolved oxygen consumption by microorganisms. As a result of this relationship, the organic content of wastewater is usually measured in terms of oxygen consumed during its oxidation, termed Biochemical Oxygen Demand (BOD) (Ezeonuegbu *et al*., 2014).

Chemical oxygen demand (COD) is the measure of the total quantity of oxygen required to oxidize all organic material into carbon dioxide and water. COD values are always greater than BOD values but COD measurement takes less time (few hours) compared to BOD measurement which usually takes five days (BOD_5) (Walakira, 2011).

Other important parameters include; plant nutrients, (particularly phosphorus and nitrrogen) (Mosley *et al*., 2004), pathogenic organisms (Ezeonuegbu *et al*., 20014), turbidity (Galadima, 2012), pH (Salequzzaman *et al*., 2008), electrical conductivity (Nadia, 2006), and heavy metals (Krishna *et al*., 2012).

2.5 Heavy Metals

The term “heavy metals” refers to any metallic element that has a relatively high density (greater than 5mg/ml) and is toxic or poisonous even at low concentrations (Lenntech, 2004; Chen and Wang, 2008; Elen *et al.*, 2011). According to the World Health Organization (WHO, 2006) amongst the most toxic heavy metals are cadmium, chromium, copper, lead, mercury and nickel. Most of the point sources of heavy metal pollutants are industrial wastewater from mining, metal processing, tanneries, petroleum refining, pharmaceuticals, pesticides, organic chemicals, rubber and plastics, lumber and wood products. Even if they are present in dilute, undetectable quantities, their recalcitrance and consequent persistence in water bodies imply that through natural processes such as biomagnification, concentrations may become elevated to such an extent that they begin exhibiting toxic characteristics (Lenntech, 2004). Apart from environmental issues, technological aspects of metal recovery from industrial waste waters must also be considered whereby environmentally hazardous, or which are of technological importance, strategic significance or economic value, be removed/recovered at their source using appropriate treatment systems (Chen and Wang, 2008).

Heavy metals in industrial wastewater include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold, and nickel (Ahalya *et al.*, 2003). These pollutants are widespread, non-biodegradable and persistent, can accumulate in food chain, and thus may pose a significant danger to human health ((Srivastava *et al.*, 2006, Dimple, 2014).

Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). Working in or living near an industrial site which utilizes these metals and their compounds increases one’s risk of exposure, as does living near a site where these metals have been improperly disposed. Subsistence lifestyles can also impose higher risks of

exposure and health impacts because of hunting and gathering activities (Occupational Health Safety Administration, 2004).

2.5.1 Lead

Lead is one of the limited classes of elements that can be described as purely toxic. Lead is a heavy metal poison which forms complexes with oxo-groups in enzymes to affect virtually all steps in the process of hemoglobin synthesis and porphyria metabolism (Grazyna and Pawel, 2010). Toxic levels of lead in human have been associated with encephalopathy, seizures, hallucinations, delusions, poor memory, irritability, nervous breakdown, heart and kidney failure and mental retardation (Ogwuegbu and Muhanga, 2005; Grazyna and Pawel, 2010). Lead affects children by leading to the poor development of the grey matter of the brain, thereby resulting in poor intelligence quotient (Udedi, 2003). Lead is present in petroleum-based effluents and many other industrial facilities.

2.5.2 Chromium

There are two stable oxidation states of chromium found in the environment, which include, hexavalent and trivalent forms. These forms have contrasting toxicities, mobility and bioavailability. The hexavalent form of chromium is more toxic than trivalent chromium and is often present in wastewater as chromate (CrO_4^{2-} and dichromate ($Cr_2O_7^{2-}$). This is of serious environmental concern as Cr(VI) persists indefinitely in the environment complicating its removal (Ho *et al.*, 2014). The persistent nature makes it accumulate in the food chain, which with time reaches harmful levels in living beings resulting in serious health hazards such as irritation in lungs and stomach, cancer in digestive tract, low growth rates in plants and death of animals (Lenntech, 2004; Occupational Health Safety Administration, 2004; Sen and Gosh, 2010; Ho *et al.*, 2014). Hexavalent chromium compounds are powerful oxidants and are used as pigments for photography. They are also used in the manufacture

ofdyes, paints, inks,and plastics. They can also be used for stainless steel production, textile dyes, wood preservation, leather tanning, and as anti-corrosion coatings (Nwanko, 2010). While trivalent chromium compounds are relatively innocuous and immobile, hexavalent forms move readily through soils and aquatic environments. Low level exposure can irritate the skin and cause ulceration while long-term exposure can cause damage to the kidney, liver, circulatory and nerve tissues (Nwanko, 2010; Ali, 2012).

2.5.3 Cadmium

Cadmium is toxic at extremely low levels. In humans, long term exposure results in renal dysfunction, characterized by tubular proteinuria (World Health Organisation, 2007). High exposure can lead to obstructive lung disease, cadmium pneumonitis, resulting from inhaled dusts and fumes. It is characterized by chest pain, cough with foamy and bloody sputum, and death of the lining of the lung tissues because of excessive accumulation of watery fluids. Cadmium is also associated with bone defects, viz; osteomalacia, osteoporosis and spontaneous fractures, increased blood pressure andmyocardic dysfunctions (World Health Organisation, 2007). Depending on the severity of exposure, the symptoms of its effects include nausea, vomiting, abdominal cramps, dyspnea and muscular weakness. Severe exposure may result in pulmonary odema and death. Pulmonary effects (emphysema, bronchiolitis and alveolitis) and renal effects may occur following subchronic inhalation exposure to cadmium and its compounds (Young, 2005). It has also been classified as a human carcinogen. It is usually present in the environment at low levels; however, human activity has greatly increased those levels (International Programme on Chemist Safety, 1992).Cadmium can travel long distances from the source of emission by atmospheric transport. It is readily accumulated in many organisms, notably molluscs and crustaceans (World Health Organisation, 2007).

Human exposure occurs mainly from the consumption of contaminated food, active and passive inhalation of tobacco smoke and inhalation by workers in the non-ferrous metal industry (International Program on Chemist Safety, 1992).

2.6 Methods of Heavy Metal Removal from Industrial Effluents

Due to the discharge of large amounts of metal contaminated wastewater, industries bearing heavy metals, such as Cadmium, Chromium, Copper, Nickel, Arsenic, lead, and Zinc, are the most hazardous among the chemical-intensive industries. Because of their high solubility in the aquatic environments, heavy metals can be absorbed by living organisms. Therefore, it is necessary to treat metal contaminated wastewater prior to its discharge to the environment. Several techniques have been used over the years to remedy the problems of environmental pollution caused by effluent discharged from various industrial processes. Amongst these techniques are: conventional and biological methods (Ezeonuegbu *et al*., 2014).

2.6.1 Conventional methods

The removal of heavy metals from waste water has since been a problem for most industries that are involved in the discharge of these pollutants into the environment. Various techniques which include conventional methods have long been devised but these treatment techniques are inherently problematic in their application to metal-bearing waste water. They are costly to the user as the active agent cannot be recovered for reuse in successive treatment cycles (Hemambika *et al*., 2011). Also, the end product is usually a low volume, highly concentrated metal bearing sludge that is difficult to dewater and dispose of (Atkinson *et al*., 1998). As a result of the high cost of these techniques, most industries are unable to meet up with effluent standards and as such, dispose their effluent into the receiving environment

without any prior treatment(Iqbal *et al*., 2005; Otokunefor and Obiukwu, 2005; Ezzouhri *et al.,* 2009; Wang and Chen, 2009; Hemambika *et al*., 2011). Amongst these techniques are:

2.6.1.1 Chemical precipitation

Chemical precipitation is effective and by far the most widely used process in industries (Ku and Jung, 2001; Islamoglu *et al.,* 2006) because it is relatively simple and inexpensive to operate. In precipitation processes, chemicals react with heavy metal ions to form insoluble precipitates (Islamoglu *et al.,* 2006). The formed precipitates can be separated from the water by sedimentation or filtration. And the treated water is then decanted and appropriately discharged or reused. Simple precipitation of metals as insoluble hydroxides, carbonates, or sulfides is used in about 75% of electroplating facilities to treat wastewater (Ku and Jung, 2001). This process is quite simple as the pH of the effluent is increased by using lime (CaO) or caustic soda (NaOH) to precipitate and hence immobilize the heavy metals as their respective hydroxides. Precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers(Ku and Jung, 2001).

2.6.1.2 Membrane filtration

Membrane filtration technologies with different types of membranes show great promise for heavy metal removal for their high efficiency, easy operation and space saving (Fenglian and Wang, 2011). The membrane processes used to remove metals from wastewater are ultrafiltration, reverse osmosis, nanofiltration and electrodialysis (Fenglian and Wang, 2011).

*Ultrafiltration:*Ultrafiltration (UF) is a membrane technique with a pressure-driven purification process in which water and low molecular weight substances permeate a membrane while particles, colloids, and macromolecules are retained. Since the pore sizes of UF membranes are larger than dissolved metal ions in the form of hydrated ions or as low

molecular weight complexes, these ions would pass easily through UF membranes. Typically, ultrafiltration will remove high molecular-weight substances, colloidal materials, and organic and inorganic polymeric molecules (Mortaheb *et al.,* 2009). The primary removal mechanism is size exclusion, although the electrical charge and surface chemistry of the particles or membrane may affect the purification efficiency (Mortaheb *et al.,* 2009; Fenglian and Wang, 2011).

Reverse osmosis:The reverse osmosis (RO) process uses a semi-permeable membrane, allowing the fluid that is being purified to pass through it, while rejecting the contaminants. Reverse osmosis is one of the techniques able to remove a wide range of dissolved species from water. It accounts for more than 20% of the world's desalination capacity (Shahalam *et al*., 2002). Reverse osmosis is an increasingly popular wastewater treatment option in chemical and environmental engineering. The efficiency of appropriate RO systems to remove heavy metals has been investigated, but these have yet to be widely applied (Shahalam *et al*., 2002). In the reverse osmosis process cellophane-like membranes separate purified water from contaminated water. RO is when a pressure is applied to the concentrated side of the membrane forcing purified water into the dilute side. The rejected impurities from the concentrated side will be washed away in the reject water. Applications that have been reported for RO processes include the treatment of wastewater containing organic substances from electroplating and metal finishing, pulp and paper, mining and petrochemical, textile, and food processing industries, radioactive wastewater, municipal wastewater, and contaminated groundwater (Williams *et al*., 1990).

*Nanofiltration:*Nanofiltration (NF) is the intermediate process between UF and RO. NF is a promising technology for the rejection of heavy metal ions such as nickel chromium, copper and arsenic from wastewater. NF process benefits from ease of operation, reliability and

comparatively low energy consumption as well as high efficiency of pollutant removal (Fenglian and Wang, 2011).

Electrodialysis: Electrodialysis (ED) is another membrane process for the separation of ions across charged membranes from one solution to another using an electric field as the driving force. In most ED processes, ion-exchange membranes are used. The membranes are actually of two basic types: cation-exchange and anion-exchange membranes. The membranes are cation- or anion-selective, which basically means that either positive ions or negative ions will flow through. Cation-selective membranes are polyelectrolytes with negatively charged matter, which rejects negatively charged ions and allows positively charged ions to flow through (Sadrzadeha *et al*., 2009).

This process has been widely used for the production of drinking and process water from brackish water and seawater, treatment of industrial effluents, recovery of useful materials from effluents and salt production (Sadrzadeha *et al*., 2009). ED has also proven to be a promising method in heavy metal wastewater treatment. The energy requirement is directly proportional to the concentration of salts in the water being treated. Being a flexible unit operation, electrodialysis can operate in batch or continuous mode. However, it is not economical for treatment of very dilute solutions or to produce effluent of less than 1 ppm of pollutant level and the membrane may not have a perm-selectivity of unity and therefore allow passage of the co-ions (different ions of the same charge). Additionally, the life of membrane is limited and they may be fouling. pH adjustment at low values is also required to prevent the decrease in membrane performance due to scale formation(Ali, 2012).

2.6.1.3 Coagulation/ flocculation

Coagulation is the destabilization of colloids by neutralizing the forces that keep them apart. Many coagulants are widely used in the conventional wastewater treatment processes such as

alum, ferrous sulfate and ferric chloride, these are mixed rapidly then stirred to encourage formation of floc prior to settling resulting in the effective removal of wastewater particulates and impurities by charge neutralization of particles and by enmeshment of the impurities on the formed amorphous metal hydroxide precipitates (Fenglian and Wang, 2011). The coagulant's role here is to destabilize the colloidal suspension by reducing the attractive forces, thereby lowering the energy barrier and enabling particles to aggregate (Thomas *et al*., 1999). Coagulation is one of the most important methods for wastewater treatment, but the main objects of coagulation are only the hydrophobic colloids and suspended particles. In order to remove both soluble heavy metal and insoluble substances efficiently by coagulation, sodium xanthogenate group was grafted to polyethyleneimine (Chang and Wang, 2007). A number of coagulation mechanisms including charge neutralization, double layer compression, bridging and sweep, have been postulated dependent on the physical and chemical properties of the solution, pollutant and coagulant (Thomas *et al.,* 1999). Flocculation is the agglomeration of the destabilized particles by chemical joining and bridging. Once suspended particles are flocculated into larger particles, they can usually be removed or separated by filtration, straining or floatation. Today many kinds of flocculants, such as polyaluminium chloride (PAC), polyferric sulfate (PFS) and polyacrylamide (PAM), are widely used in the treatment of wastewater, however, it is nearly impracticable to remove heavy metal very well from wastewater directly by these current flocculants Generally, coagulation/flocculation can't treat the heavy metal wastewater completely (Chang and Wang, 2007). Therefore, coagulation flocculation must be followed by other treatment techniques.

2.6.1.4 Floatation

Flotation has nowadays found extensive use in wastewater treatment. Floatation has been employed to separate heavy metals from a liquid phase using bubble attachment, originated in mineral processing. Dissolved air flotation (DAF), ion flotation and precipitation flotation are the main flotation processes for the removal of metal ions from solution. DAF is to allow micro-bubbles of air to attach to the suspended particles in the water, developing agglomerates with lower density than water, causing the flocs to rise through the water and accumulating at the surface where they can be removed as sludge (Lundh *et al.,* 2000).

Ion flotation has been shown a promising method for the removal of heavy metal ions from wastewaters. The process of ion flotation is based on imparting the ionic metal species in wastewaters hydrophobic by use of surfactants and subsequent removal of these hydrophobic species by air bubbles (Yuan *et al*., 2008).

Precipitate flotation process is another alternative of flotation method, based on the formation of precipitate and subsequent removal by attachment to air bubbles. Depending on the concentration of the metal solution, the precipitation may proceed via metal hydroxide formation or as a salt with a specific anion (sulfide, carbonate, etc.)(Capponi *et al.*,2006).

2.6.1.5 Ion exchange

Ion-exchange processes have been widely used to remove heavy metals from wastewater due to their many advantages, such as high treatment capacity, high removal efficiency and fast kinetics (Kang *et al*., 2004). Ion-exchange resin, either synthetic or natural solid resin, has the specific ability to exchange its cations with the metals in the wastewater. When the resin capacity is used up, it is regenerated by using high concentrations of the original ion that is exchanged from the resin. The resins of ion exchange consist of an organic or inorganic

network structure with attached functional groups. Among the materials used in ion-exchange processes, synthetic resins are commonly preferred as they are effective to nearly remove the heavy metals from the solution (Alyüz and Veli, 2009). A key advantage of the ion exchange process is that the metal contaminants can be recovered and reused. Another advantage is that ion exchange may be designed to remove certain metals only, providing effective removal of these metals from highly contaminated wastewater. A disadvantage is that the resins may be fouled by some organic substances. Ion exchange is suitable for the following (Ali, 2012):

. Removal and recovery of heavy metals from very dilute solutions

. Recovering plating solution drag from rinse water

. Return of the purified water for reuse

. Concentrating effluents prior to conventional treatment

. Removing trace pollutant after a conventional treatment process

The economics of ion exchange are related to the capacity of the resin to exchange ions and to the quantity of regenerate required. The regenerating solution containing the concentrated metal salts requires further treatment prior to its reuse. Moreover a filtration, carbon absorption step must precede the ion exchange process to prevent resins fouling and pH adjustment is required for final disposal or reuse (Ali, 2012).

2.6.1.6 Cementation

Cementation is a metal –replacement process in which a solution containing the dissolved metallic ions comes in contact with a more active sacrificial metal such as iron (iron or aluminum scraps) for an oxidation-reduction spontaneous reaction to occur. The use of metal scraps for heavy metals treatment can lower the chemical costs and simplify the treatment

operation by eliminating the need for careful dosage of chemical. It needs at least four hours for complete treatment (Younesi *et al.,* 2006).

2.6.1.7 Evaporation / Distillation

Distillation is the oldest demineralization process. It consists of evaporating a part or all of the water from a saline solution and subsequent condensation of mineral-free vapor. The energy requirements are very high for the system. One modification of the simple distillation process is multiple-effect evaporation, where water is evaporated in different stages at different pressures. Another modification is distillation with vapor compression. This system utilizes the latent heat of the compressed steam to preheat and evaporate the incoming water. Both modifications conserve energy, but the energy requirements are quite large. Evaporation concentrates the total salts and impurities in the recovered solution however; it has been proved effective for recovering valuable metal salts from electroplating waste solutions without sludge formation .This technique needs relatively high capital costs and evaporating costs. Additionally these systems are complex and their application in wastewater treatment is not feasible (Ali, 2012).

2.6.1.8 Solvent extraction technique

Solvent extraction is a powerful technique used mainly for recovering /separating metal ions from aqueous solutions having higher concentrations to obtain high pure solutions. The principle of solvent extraction is that when a metal ion solution is contacted with a solvent, the metal ion is distributed between the two phases (Jia *et al.,* 2004). The disadvantage of this method is that the large amount of solvent of the extracted phase should be refreshed in a costly stripping step. It may not be advisable to adopt this technique to remove heavy metals whose concentration is very less as the recovery cost will be very high (Mellah and Benachour, 2007)

2.6.1.9 Adsorption

Adsorption is now recognized as an effective and economic method for heavy metal wastewater treatment (Gottipati and Mishra, 2012). The adsorption process offers flexibility in design and operation and in many cases will produce high-quality treated effluent. In addition, because adsorption is sometimes reversible, adsorbents can be regenerated by suitable desorption process. .Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a molecular or atomic film (the adsorbate). Adsorption is operative in most natural physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, synthetic resins and water purification. Among these methods, adsorption is currently considered to be very suitable for wastewater treatment because of its simplicity and cost effectiveness (Kwon *et al.*, 2010).

2.6.2 Biological Methods

Biological methods of heavy metal removal from waste water, have gained research attention in recent years, and have since served as an effective alternative method in place of conventional methods. Biosorption is the ability of biomaterials to accumulate heavy metals from waste water through metabolically mediated or physico-chemical pathways based on the metal binding capacities of various biological materials (Choi and Yun, 2006). Living as well as dead (metabolically inactive) biological materials have been sought to remove metal ions from aqueous solutions. It was found that various functional groups present on their cell wall offer certain forces of attractions for the metal ions and provide a high efficiency for their removal (Choi and Yun, 2006). The mechanisms of uptake by living systems (bioaccumulation) and removal by dead ones (biosorption) are entirely different.

2.6.2.1 The use of biosorbents

The assessment of the metal-binding capacity of some types of biomass has gained momentum since 1985 (Volesky and Holan, 1995). Availability is a major factor to be taken into account to select biomass for clean-up purposes. The economy of environmental remediation dictates that the biomass must come from nature or even has to be a waste material. A large number of microorganisms, including actinomycetes, cyanobacteria, and other bacteria, algae, fungi and yeasts, have the ability to accumulate heavy metals in different ways depending on the nature of the microorganisms and on the concentration of heavy metals in the environment (Payne, 2000). Additionally, various agricultural wastes, among other kinds of biomass, have been tested for metal sorption capacity.Agricultural products and by-products is an abundant waste material, and needs proper disposal. When disposed by burning in situ, it generates CO_2 and other forms of pollutions. This creates a need for the conversion of agricultural products and by-products to useful, and hopeful, value-added products (Ballesteros *et al*., 2006).

Biosorption can be classified into two principle categories namely active (metabolically dependent) and passive (non-metabolic dependent) processes (Malik, 2004).

Metabolism-dependent (bioprecipitation, biomineralization, bioaccumulation) intracellular uptake of metal ions involves the use of living and active biological materials.

The passive or non-metabolic dependent (biosorption) process involves the use of dead or inactive biomaterials (Ezeonuegbu *et al*., 2014). Generally, biosorbents could be classified into the following categories:

1. Bacteria

The cell walls of bacteria are efficient metal chelators though a wide spectrum of uptake capacities may be exhibited. Metal binding may be at least a two-stage process first involving interaction between metal ions and reactive groups followed by inorganic deposition of increased amounts of metals although extracellular polysaccharides are involved in metal binding (Malik, 2004), the main components responsible for metal binding capacity of the cell wall in Gram-positive bacteria are anionic functional groups present in the peptidoglycan, teichoic acids and teichuronic acid while in Gram-negative bacteria, peptidoglycan, phospholipids and lipopolysaccharides play the major role (Vijayaraghavan and Yun, 2008). The carboxyl groups of glutamic acid of peptidoglycan are the major site of metal deposition. In some bacteria, metabolism-independent biosorption may be the most significant proportion of total uptake.

2. Algae

Metabolism-independent accumulation of metals is often rapid and usually completes in 5–10 minutes. Their cell wall, is similar to that of fungi and contain, xylans, pectin, mannans, alginic acids or fucinic acids and are responsible for metal uptake from solutions (Fosso-Kankelu and Mulabe-Bafubiandi, 2014).

3. Fungi and yeasts.

Metabolism-independent binding of metal ions to fungal and yeastcell walls is usually rapid and large amounts may be bound. The cell wall of fungi is dominated by polysaccharide completed with proteins, lipids, polyphosphates and inorganic ions (figure 2.2). The biosorption capacity of dead biomass may be greater, equivalent to, or less than that of living cells and this may depend on the killing process used. As in other microbes, a variety of

ligands may be involved including carboxyl, amino, phosphate, hydroxyl and sulphydryl groups. In certain fungi, and especially yeasts, greater amounts of metals may be taken up by transport than by biosorption. However, for many filamentous fungi, it appears that general biosorption accounts for a major portion of metal uptake (Fosso-Kankelu and Mulabe-Bafubiandi, 2014).

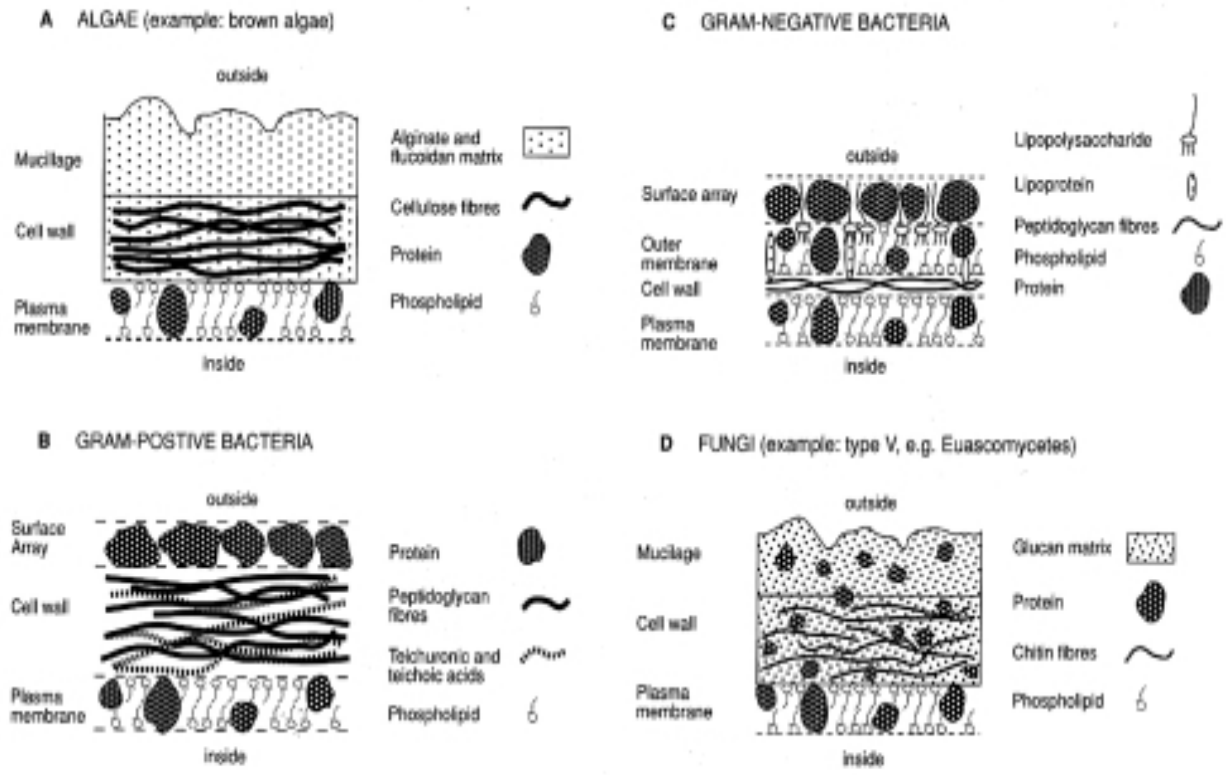


Figure 2.2: Cell wall structure in various microorganisms A: algae (brown algae), B: Gram-positive bacteria, C: Gram-negative bacteria and D: fungi (*Euascomycetes*). (Vieira and Volesky, 2000).

4. Agricultural wastes

In this case, a variety of by-products are incorporated, e.g. wool, olive cake, sawdust, pine needles, almond, rice husk, coconut shell, amongst others. Either protein-based animal fibres, or cellulose-based plants fibres bear many amino, carboxylic or hydroxyl functional groups, which play a major role in metal binding (Choi and Yun, 2006).

2.7 Sugarcane Bagasse as a Heavy Metal Scavenger

After sugarcane is milled for juice extraction, bagasse is obtained as a residue which corresponds to about 25% of the total weight and contains 60-80% of carbohydrates (Betancur and Pereira, 2010). About 54 million tons of sugarcane bagasse is produced annually throughout the world. In general, sugar factories generate approximately 270kg of sugarcane bagasse (50% moisture) per metric ton of sugarcane (Xu *et al*., 2006). Sugarcane bagasse can be considered either as a waste affecting the environment when discarded as agricultural waste or burned for energy supply in sugar and ethanol mills (Himmel *et al*., 2007; Pauly and Keegstra, 2008). Both alternatives are, however, pollutant and inefficient in making use of the chemical energy available in the biomass (Deschamps *et al*., 1996). Bagasse consists of cellulose 43.8%, hemicellulose 28.6%, lignin 23.5%, ash 1.3%, and other components 2.8% (Luz *et al*., 2007). The two former components are hydrophilic, and latter is hydrophobic (Sun *et al*., 2004).

This waste has been classified as a low cost adsorbent as it is readily available with little or no cost. Sugarcane bagasse just like other agricultural waste materials from either industrial or agricultural operations is considered to be good metal scavenger from solution and waste water as it contains certain functional groups. These functional groups are associated with cellulose, hemicellulose and lignin as major constituents. Cellulose-based plants fibres bear many amino, carboxylic orhydroxyl functional groups, which play a major role in metal

binding. Lignin is a natural amorphous crosslinked resin that has an aromatic three-dimensional polymer structure containing a number of functional groups such as phenolic, hydroxyl, carboxyl, benzyl alcohol, methoxyl, and aldehyde groups, making it potentially useful as an adsorbent material for removal of heavy metals from waste water (Oliveira *et al*., 2008).

Metal uptake is believed to occur through sorption process involving carboxylic and phenolic functional groups which result in formation of surface complexation mechanism between metal ions and lignocellulosic adsorbents. Cellulosic surfacebecomes partially negatively charged when immersed inwater and, therefore, possess columbic interaction withcationic species in water (Laszlo and Dintzis, 1994). The high binding capacities of cationic species onthe adsorbents are mainly the results of columbicinteractions (Weixing *et al*., 1998).

Gupta *et al*. (2003) used sugarcane bagasse fly ash in the removal of cadmium and nickel from wastewater. They reported that as much as 90% removal of cadmium and nickel was achieved in about 60 to 80 minutes respectively, under batch test condition. Several literatures have reported the use of sugarcane bagasse as an adsorbent for heavy metal removal from solutions (Nasim *et al*., 2001; Khan *et al*., 2004; Ibrahim *et al*., 2006; Homagai *et al*., 2010; Aril *etal*., 2013; Kong *et al*., 2014).

2.8 Mechanisms of Biosorption of Heavy Metals

The mechanisms involved in biosorption are often difficult to characterize, except perhaps in the simplest laboratory systems. The elucidation of the mechanism of biosorption is necessary to enable the technology to be developed (Pagnanneli *et al*., 2002). Such mechanisms are complicated and not fully understood. An extensive literature exists concerned with the mechanism and modeling of biosorption referring to specific elements

and microbial strains (Pagnanneli *et al*., 2002). The key factors controlling and characterizing these mechanisms are:

1. The type of biological ligands available for metal sequestering
2. The status of the biomass, i.e. living /non-living
3. The chemical, stereo-chemical and coordination characteristics of the targeted metals and metal species
4. The characteristics of the metal solution such as pH, and the presence of competing co-ions

The variety of structural components present in biomass means that many functional groups are able to interact with metal species, e.g. carboxyl, phosphate, hydroxyl, amino, thiol, etc., to varying degrees and influenced by physico-chemical factors. For biosorption, defined as a physicochemical process independent of metabolism, such mechanisms as complexation; chelation; co-ordination; ion exchange; precipitation; reduction may be important and, in these cases, biosorption can be rapid and reversible with biomass properties analogous to conventional ion exchange resins (Gadd, 2008).

2.8.1 Complexation

The complex formation of metal ions with organic molecules involves ligand centres in the organic species i.e. the presence of an atom or atoms having lone pair electrons to donate. Complexation may be electrostatic or covalent and the simplest case is complexation by a mono-dentate ligand such as RNH_2^- . To approach and elucidate biosorption mechanisms, a significant part of the recent advances in biosorption are based on the classification of elements according to the hard-soft acid-base classification (Pearson's classification). "Hard acids", metals such as Na, K, Ca, Mg, often essential nutrients for microbial growth, bind

preferentially to oxygen containing "hard bases", ligands such as OH^-, HPO_4^{2-}, CO_3^{2-}, R-COO^-, and =C=O.

Soft acids, metals such as the precious metals Ag, Au, Pt, Pd are bound covalently to the cell wall by "soft bases", ligands containing nitrogen or sulfur. Several mechanisms might be involved in the immobilisation of metals and it is now evident and confirmed by several researchers, that the biosorption of precious metals is a two-step mechanism comprising first covalent bonding and then *in-situ* reduction. Borderline ions on the "hard/soft" classification such as the transition elements: Mn^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+} exhibit a significant degree of covalent bonding with nitrogen or sulfur containing "soft" ligands (Gadd, 2008).

2.8.2 Chelation

Organic molecules containing more than one functional group with donor electron pairs can simultaneously donate these to a metal atom. This can result in the formation of a ring structure involving the metal atom a process termed 'chelation' (Gadd, 2008). The structure of a metal ion chelated with ethylenediamine tetracarboxylic acid (EDTA), a hexa-donating compound with two nitrogen and four oxygen donor atoms is shown on figure 2.3.

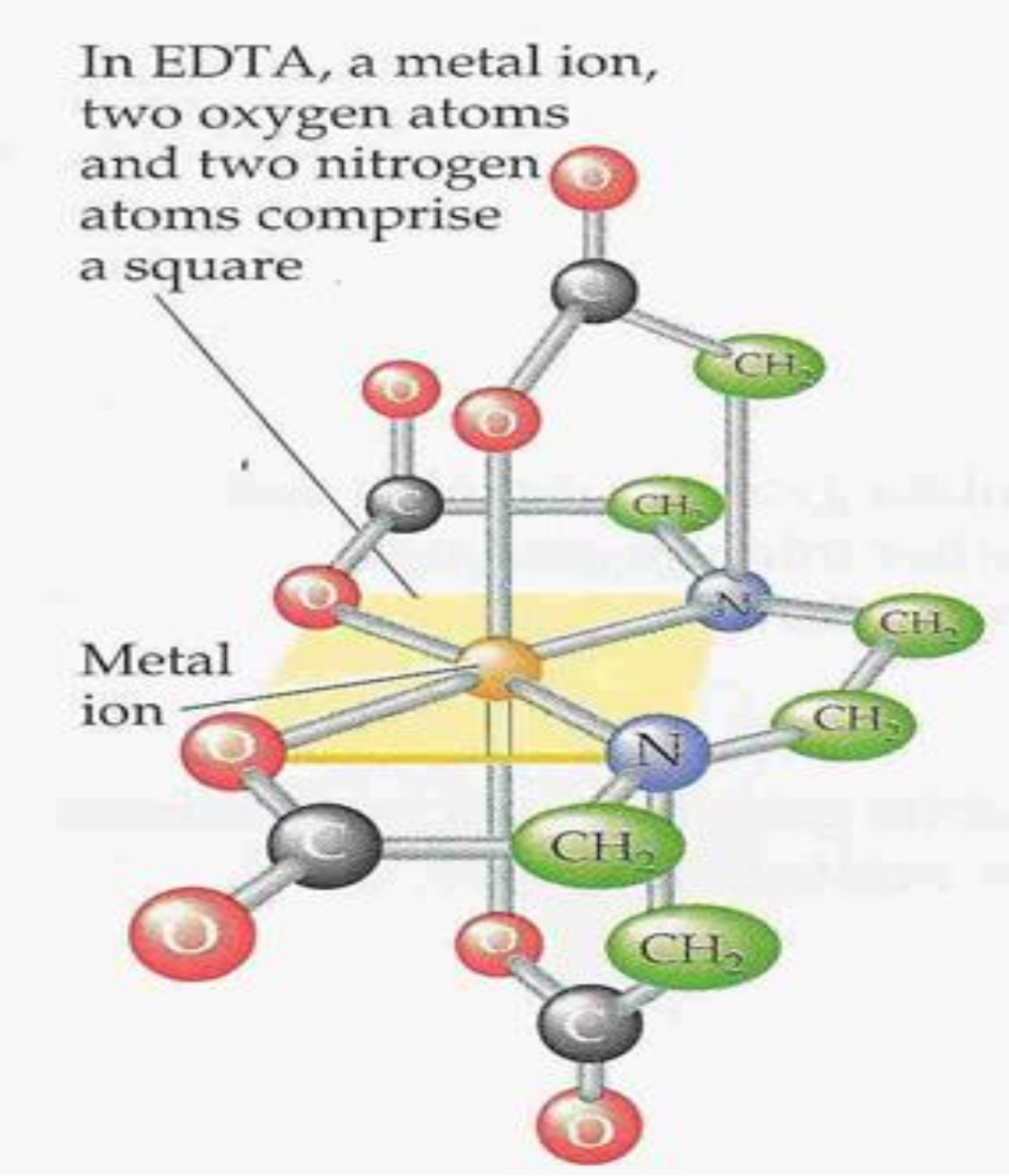


Figure 2.3: Schematic diagram of bonding in a metal-EDTA complex ion (Gadd, 2008)

In general, since a chelating agent may bond to a metal ion in more than one place simultaneously, chelated compounds are more stable than complexes involving monodentate ligands. Stability tends to increase with the number of chelating sites available on the ligand.

2.8.3 Ion exchange

Ion exchange is the replacement of an ion in a solid phase in contact with a solution by another ion. More specifically, it is the replacement of an absorbed, readily-exchangeable ion by another (Gadd, 2008). The gram-positive bacteria, principally members of the genus *Bacillus,* have enhanced capacity for metal binding because of a significant negative charge density. This is due to the structure of the cell wall with teichoic and teichuronic acids attached to the peptidoglycan network. The phosphodiesters of teichoic acid and the carboxylate groups of the teichuronic acid thus contribute ion-exchange capacity to the cell wall (Gadd, 2008).

Many examples have established the existence of an ion exchange mechanism in metal ion removal by biosorption. However, it has been suggested by many researchers that ion exchange is neither the sole nor the main mechanism for metal biosorption.

2.8.4 Precipitation

Metal precipitation is also involved in biosorption, where precipitates formed may remain in contact with or inside the microbial cells or may be independent of the solid phase of the microbial cell. In the later case, the presence of the solid phase-microbial cell or biofilm also plays a favourable role in the phenomenon of precipitation. The term precipitation in most cases refers to the formation of insoluble inorganic metal precipitates (Ramoundaki *et al.*, 2003). However, in the case of metal biosorption by microbial cells, organic metal precipitates may also be formed. This may be more easily understood when metals are bound to extracellular polymeric substances (EPS) excreted by some prokaryotic (bacteria, archaea)

and eukaryotic (algae, fungi) microorganisms. Purified products from isolated cells such as glucan, mannan, and chitin accumulate greater quantities of cations than the intact cells, proving that biomolecules can form metal precipitates (Gadd, 2008).

2.8.5 Reduction

The removal of toxic hexavalent chromium from aqueous solution by biosorption by different biomass types has been extensively reported. This removal is often associated with the simultaneous reduction of Cr(VI) to Cr(III), thus inactivated fungal biomass e.g. *Aspergillus niger*, *Rhizopus oryzae*, *Saccharomyces cerevisiae* and *Penicilliumchrysogenum* remove Cr(VI) from aqueous solutions by reduction to Cr(III) when contacted with the biomass (Donghee *et al*., 2005). The biosorption mechanism is a two-step process: initiation of the uptake at discrete points by chemical bonding, then reduction of the metal ions (Lin *et al*., 2005).

It is likely that the various mechanisms involved in biosorption can operate simultaneously to varying degrees. Metabolic activities such as respiration, nutrient uptake, and metabolite release will alter the microenvironment around the cells which, in turn, may affect adsorption, ion exchange, complexation and precipitation (Malik, 2004; Sen and Ghosh, 2010). Extracellular polymeric materials are capable of binding and entrapment of metal and radionuclide species: such phenomena may be significant in biofilms (White and Gadd, 1998). A variety of oxido-reductive transformations that alter metal or radionuclide speciation can lead to enhanced deposition in and around cell walls, or even volatilization (Hockins and Gadd, 2007). Living cells also possess a variety of specific and non-specific transport systems for intracellular accumulation (Gadd, 2008). Thus, depending on the system, multiple mechanisms may be involved in metal removal from solution and their dissection, modelling and analysis may be difficult. The diversity of chemical structure

encountered in organic pollutants mean that molecular size, charge, solubility, hydrophobicity, and reactivity, all affect biosorption as well as the type of biosorbent and wastewater composition.

2.9 Factors Affecting Biosorption of Metals

Biosorption depends on many factors but some of these factors are related to the biomass and metal and the others are related to environmental conditions. The major factors that affect the biosorption process are:

1. Temperature

In contrast to bioaccumulation process, biosorption efficiency remains unaffected within the range 20-35°C,(Aksu and Kutsal, 1992) although high temperatures, e.g. 50°C, may increase biosorption in some cases, but these high temperatures may cause permanent damage to microbial living cells and then decreasing metal uptake (Ahalya *et al*., 2003; Goyal *et al*., 2003). Adsorption reactions are generally exothermic and the extent of adsorption increases with decreasing temperature. The maximum biosorption capacity for Ni and Pb by *Saccharomyces cerevisiae* was obtained at 25°C and found to decrease as the temperature was increased to 40°C (White *et al*., 1997).

2. Characteristics of biomass

The nature of the biomass or derived product may be considered one of the important factors, including the nature of its application such as: freely-suspended cells, immobilized preparations, living biofilms etc. Physical treatments such as boiling, drying, autoclaving and mechanical disruption will all affect binding properties while chemical treatments such as alkali treatment often improve biosorption capacity, especially evident in some fungal systems because of de- acetylation of chitin to form chitosan-glycan complexes with higher metal affinities (Wang and Chen, 2006). Growth and nutrition on the biomass, and age of the

cells can also influence biosorption due to changes in cell size, wall composition, extracellular product formation, etc.

3. The surface area to volume ratio

It may be important for individual cells or particles, as well as the available surface area of immobilized biofilms. In addition, the biomass concentration may also affect biosorption efficiency with a reduction in sorption per unit weight occurring with increasing biomass concentration.

4. Acidity

pH seems to be the most important parameter in the biosorption processes. Biosorption is similar to an ion-exchange process, i.e. biomass can be considered as natural ion-exchange materials which mainly contain weakly acidic and basic groups (Deng and Wang, 2012). Therefore, pH of solution influences the nature of biomass binding sites and metal solubility; it affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions. Metal biosorption has frequently been shown to be strongly pH dependent in almost all systems examined, including bacteria, cyanobacteria, algae, and fungi. Competition between cations and protons for binding sites means that biosorption of metals like Cu, Cd, Ni, Co and Zn is often reduced at low pH values (Deng and Wang, 2012). Generally, the heavy metal uptake for most of the biomass types decline significantly when pH of the metal solutions is decreased from pH 6.0 to 2.5. At pH less than 2, there are minimum or negligible removal metal ions from solutions. The metal uptake increases when pH increases from 3.0 to 5.0. Optimum value of pH is very important to achieve highest metal sorption, and this capacity will decrease with further increase in pH value.

5. Biomass Concentration

Concentration of biomass in solution affects the specific uptake (Modak and Natarajan, 1995). At a given equilibrium concentration, the biomass adsorbs more metal ions at low cell densities than at high densities (Ali, 2012). Thus, electrostatics interaction between the cells plays an important role in metal uptake. At lower biomass concentration, the specific uptake of metals is increased because an increase in biosorbent concentration leads to interference between the bindings sites. High biomass concentration restricts the access of metal ions to the binding sites. Fourest and Roux (1992) invalidated this hypothesis attributing the responsibility of the specific uptake decrease to metal concentration shortage in solution. Hence this factor needs to be taken into consideration in any application of microbial biomass as biosorbent.

6. Initial Metal Ion Concentration

The initial concentration provides an important driving force to overcome all mass transfer resistance of metal between the aqueous and solid phases (Zouboulis *et al*., 1997). Increasing amount of metal adsorbed by the biomass will be increased with initial concentration of metals. Optimum percentage of metal removal can be taken at low initial metal concentration. Thus, at a given concentration of biomass, the metal uptake increases with increase in initial concentration.

7. Metal Affinity to the Biosorbent

Physical/chemical pretreatment affects permeability and surface charges of the biomass and makes metal binding groups accessible for binding. It can be manipulated by pretreating the biomass with alkalis, acids detergents and heat, which may increase the amount of metal uptake (Ali, 2012).

2.10 Advantages of Biosorption Process

Overall, compared with the conventional heavy metal removal methods, the potential advantages of biosorption process includes (Aksu and Kutsal, 1992):

1. Use of naturally abundant renewable biomaterials that can be cheaply produced;
2. Ability to treat large volumes of wastewater due to rapid kinetics;
3. High selectivity in terms of removal and recovery of specific heavy metals;
4. Ability to handle multiple heavy metals and mixed wastes;
5. High affinity, reducing residual metals to below 1 ppb in many cases;
6. Less need for additional expensive reagents or laboratory media which typically cause disposal and space problems;
7. Operation over a wide range of physiochemical conditions including temperature, pH, and presence of other ions
8. Relatively low capital investment and low operational cost
9. Greatly improved recovery of bound heavy metals from the biomass;
10. Greatly reduced volume of hazardous waste produced.
11. The process is independent of physiological constraints of living cells.
12. Process is very rapid, as non-living material behaves as an ion exchange resin, metal loading is very high.
13. The conditions of the process are not limited by the living biomass, no aseptic conditions required.
14. Process is reversible and metal can be desorbed easily thus recycling of the materials is quite possible.

However, there are certain disadvantages as well;

1. Irrespective of the value of the metal, it needs to be desorbed from the material to be further re-employed.

2. The characteristics of the biosorbents (living) cannot be biologically controlled.

Several important parameters that affect the performance of living biosorbent must be taken into account, these are:

1. The physiological state of the organism,
2. The age of the cells,
3. The availability of micronutrients during their growth, and
4. The environmental conditions during the biosorption process (such as pH, temperature and the presence of certain co-ions).

2.11 Comparison of Sorption Performance ("b")

A steep initial slope of a sorption isotherm indicates a sorbent which has a high capacity for the sorbate in the low residual (final, ***Cf***) concentration range (high affinity). This affinity is indicated by the coefficient ***b*** in the Langmuir equation which is often conveniently fitted to experimental sorption results although it does not correspond to the biosorption (ion exchange) phenomena. The lower the value of langmuirian **"*b*",** the higher the affinity (Kratochvil and Volesky, 1998).

It is relatively simple and easy to obtain laboratory equilibrium sorption data for a single sorbate. A small amount of the sorbent tested is brought into contact with solution containing the given sorbate. However, the 'environmental' parameters in the sorption system (particularly pH) may have to be carefully controlled unless an appropriate (ion exchange) sorption model is available to compensate for the changing proton concentration in the system (Kratochvil and Volesky, 1998).

2.12 *Microsporum* species in Heavy Metal Uptake

The genus, *Microsporum*, has been placed under the phylum, "Fungi imperfecti or Deuteromycota". The genus produces hyphae with large and rough-walled with multicellular, spindle shaped conidia which are formed at the ends of hyphae. The macroconidia are abundant and may have 1-15 septations. Microconida are lesser in number. Macroconidia are hyaline, multiseptate,variable in forms, fusiform, with a thin or thick echinulate to verrucose cell walls, spindle shaped to obovate and may be numerous or scarce (Graser *et al*., 2006).

Their shape, size and cell wall features are important characteristics for species identification. However, the essential distinguishing feature of this genus is the echinulations on the macroconidial cell wall. The thickness of the cell wall and shape varies depending on the species. Microconidia are hyaline, single- celled, pyriform to clavate, smooth walled, about 2.5-3.5 by 4-7μm in size (Caddell, 2002). Specie colonies vary from moderately slow growing, matted, and furrowed to fast growing, powdering or velveting, and light tan, furrugineous, yellowish to cinnamon in colour. The reverse side of the colony may vary from reddish brown, ferrugineous, yellowish to reddish black (Kane *et al*., 1997).

On the basis of anamorph morphology, approximately (18) species of *Microsporum* are considered valid members of these genera (Kane *et al*., 1997).

Microsporum species have been isolated from industrial dumpy soil (Mohammadian *et al*., 2015) and waste waters such as refinery effluents (Machido *et al*., 2015) and just like most fungi species, they have the ability to remove heavy metals from aqueous solutions and as such, have been employed in biosorption processes. Their bioaccumulation/biosorption capacity has been attributed to their metabolic activity and functional groups in their cell wall composition with binding properties to heavy metal ions when in aqueous solutions (Choi and Yun, 2006). Their metal uptake capacity has been reported by several literatures

(Ezeonuegbu *et al*., 2014; Machido *et al*., 2015; Mohammadian *et al*., 2015; Abdumajeed *etal*., 2016)

2.13 *Alternaria* species in Heavy Metal Uptake

This genus, *Alternaria*, belongs to the class of fungi known as Ascomycetes. Species of this genus are commonly considered as saprophytic contaminants. Species are ubiquitous in the environment, living in the soil, water and on organic debris. Hyphae are septatae and dark. Conidiophores are septate and of variable length and sometimes have a zigzag appearance. Conidia are large and have both transverse and longitudinal septations, sometimes produce germ tubes, and are found singly or in chains; they are usually rather round at the end nearest the conidiophore, while narrowing at the apex, producing a club-like shape. Surface is at first greyish white and woolly and later becomes greenish black or brown, suede-like or velvet with a light border. May eventually, become covered by short, greyish aerial hyphae. Reverse is black on PDA plate (Kirk *et al*., 2008).

Alternaria species have been isolated from petroleum polluted soils, and showed the highest resistance to 10% petroleum containing medium (Mohsenzadeh *et al*., 2012), and also, from refinery effluents (Machido *et al*., 2015).Their bioaccumulation capacity has been reported by several literatures (Mohsenzadeh *et al*., 2012; Machido *et al*., 2015; Tatah *et al*., 2016; Okwute *et al*., 2017). Metal uptake capacity by this fungal species has been attributed to a non-specific binding of the metals to all surfaces, slime layers and extracellular matrices, metabolism dependent intracellular uptake and enzymatic modification of the metal ions to less toxic form (Ezeonuegbu *et al*, 2014). In addition, these fungal species are reported to possess specific genes for resistance to heavy metal ions. Mostly, these genes are chromosomal but others are acquired through plasmids and the plasmid encoded genes are reported to be induced by the presence of specific metal ions (Kumar *et al*., 2012). The genes

encoding the synthesis of metal binding proteins such as metallothionein are a good example (Rao and Bhargavi, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

Kaduna Refining and Petrochemical Company (KRPC) is located in Chikun local government area of Kaduna State, Nigeria. The refinery occupies an area of 2.9 square kilometers and is located on an undulating land about 700 meters above sea level (Ezeonuegbu *et al*., 2014).

3.2 Collection and Handling of Samples

3.2.1 Sugarcane bagasse

Sugarcane bagasse wascollected in clean polythene bagsfrom markets in Zaria metropolis, Kaduna State, Nigeria.This was transported to the Department of Microbiology, Ahmadu Bello University, Zaria, where it was sundriedand taken to the market for milling and then preserved in clean polythene bags until needed.

3.2.2 Refinery effluent

The refinery effluent sample was collected in clean ten liter plastic gallon from waste retention pond of the Kaduna Refining and Petrochemical Company (KRPC). This was done by carefully dipping a wide mouth plastic container into the retention pondand withdrawing the effluent. The retained effluent was carefully transferred into the ten litre plastic gallon until filled. The sample collected was transported immediately to the Department of Microbiology, Ahmadu Bello University, Zaria for further analyses.

3.3 Fungal Isolates

Fungal isolates of heavy metal resistance (*Microsporum* and *Alternaria* species),earlier identified by Machido *et al*. (2015) were used in this study and were collected from the Department of Microbiology, Ahmadu Bello University, Zaria. The isolates were reactivated and re-authenticated following standard procedures (by culture and microscopy) and identification was achieved using an appropriate taxonomic guide of Mycology (Hakeem and Bhatnagar, 2010; Thippaswamy *et al.*, 2012). Reactivation was carried out by aseptically preparing fresh plates of Potato Dextrose Agar (PDA)in accordance to manufacturer's instructions. This was followed by inoculation of the isolates on the PDA plates aseptically, and incubating at room temperature in a dark cupboard for a period of seven days. Re-authentication was carried out by slide culture method using lactophenol cotton blue inoculum preparation. A scalpelwas sterilized by passing it over a Bunsen burner flame three times. It wasthen allowed to cool after which it was used to cut the PDA into blocks. Each block was placed on previously sterilized glass slides using the scapeland was aseptically inoculated with the test isolatesand sterile cover slip was placed on each agar at the point of inoculation. The glass slides containing inoculated isolates were placed in sterilized glass petri dishes containing sterile cotton wool wetted with distilled water. The petri dishes were covered and incubated in a dark cupboard for growth to occur. After the occurrence of growth, the cover slips were carefully removed; a drop of lactophenol cotton blue solution was added on the growth surface of each and placed on sterile glass slides inverted and mounted on a microscope and viewed at ×10 and ×40 objective lens (Guimaraes *et al.,* 2006). Microscopic features were compared with those on mycology atlas for identification.Authenticated isolates were sub-cultured on fresh PDA slants until required.

3.4 Preparation of Fungal Spore Inoculum

Sporulated cultures of the fungal isolate grown on potato dextrose agar slants for seven days were used.Spores of the fungal isolates were picked using a sterilized inoculating needle. The spores were transferred into sterilized bottles containing sterile potato dextrose broth. The bottles were placed in a shaker and incubated at room temperature for a period of two days.(Modified method of Byaruagaba-Bazirake *et al*., 2013)

3.5 Determination of the Proximate Composition of Sugarcane bagasse

Moisture, ash, cellulose, hemicellulose, lignin, protein and lipid content of the sugarcane bagasse sample were carried out atDepartment of Food Science, Institute of Agricultural Research, A.B.U, Zaria, following standard procedures of the Association of Analytical Chemist (AOAC, 2010)

3.5.1 Determination of moisture content

The crucible was washed and dried in an oven at 100°C for one hour; the weight was noted as (W_1). Two grams of the sugarcane bagasse sample was weighed into the crucible and the weight was taken as (W_2), this was dried at 100°C to constant weight (W_3).

$$\text{Moisture content} = W_2 - W_3 \times 100 = \text{weight of moisture} \times 100$$

Where; W_1 = weight of empty crucible

W_2 = weight of crucible and weight of sample before drying

W_3 = weight of sample after drying to constant weight

3.5.2 Determination of ash content

A pre-heated and cooled crucible was weighed. The sample was charred on a Bunsen flame inside a fume cupboard. The charred sample was placed in a muffle furnace set at 550°C

fortwo hours until a light grey ash was obtained. The sample was then removed and cooled in a desiccator before taken the weight.

$$\text{Ash content} = W_3 - W_1/W_2 - W_1 \times 100$$

Where W_1 = weight of empty crucible

W_2 = weight of crucible + weight of sample

W_3 = weight of crucible + weight of sample after ashing

3.5.3 Determination of cellulose content

The cellulose content of the sample was determined according to the method of Badu *et al.* (2011). Two grams of the sugarcane bagasse sample was placed in a 250ml beaker; 100ml of 17.5% NaOH solution was added and stirred at 25°C for 30 minutes. The content of the beaker was then filtered, washed with 25ml of 9.5% NaOH solution and 20ml portions of 100ml distilled water. The residue was washed with distilled water again and 40ml of 10% acetic acid and further with one litre distilled water. The residue was then dried at 105°C for 24 hours and cooled before weighing.

3.5.4 Determination of holocellulose content

This was determined following the method of Badu *et al.*(2011). To 2g of the sugarcane bagasse sample, 180ml distilled water; 8.6g sodium chloride and 6.0ml ethanoic acid were added. The mixture was then digested in a 250ml conical flask under reflux at 70°C for 3 hours. It was allowed to cool and then filtered and the residue washed with 20ml portions of 100ml distilled water. The residue was then dried at 105°C for 24 hours and allowed to cool before weighing to obtain the weight of holocellulose present in the sample.

3.5.5 Determination of hemicellulose content

This was determined using the method of Badu *et al.*(2011). On the bases of solubility in 17.5% NaOH solution, holocellulose is subdivided into insoluble cellulose and soluble hemicellulose. Therefore, the hemicellulose was determined as the difference between the weight of holocellulose and weight of cellulose present.

3.5.6 Determination of lignin content

The lignin content of the sample was determined according to the method of Badu *et al.* (2011). To 1g of the sugarcane bagasse sample, 14ml of cold 72% sulphuric acid was added and stirred. The mixture was left to stand for 2 hours. After the 2 hours, the mixture was washed in a one litre conical flask and diluted to 3% sulphuric acid. The mixture was then boiled for 4 hours under reflux. The insoluble materials were allowed to settle and filtered. The residue was then washed and dried in an oven at 105°C for 2 hours after which it was allowed to cool before weighing to obtain the lignin content.

3.5.7 Determination of protein content

This was determined according to the standard method of AOAC (2010) using Kjeldahl's method. Two gram of sugarcane bagasse sample was weighed into Kjeldah's flask and 5g of anhydrous sodium sulphate was added. Two hundred and fifty millilitres H_2SO_4 was then added with few boiling chips. The content of the flask was heated in the fume chamber until clear solution was obtained. The solution was allowed to cool and transferred into 250ml volumetric flask and was made up to the level of distilled water.

The distillation process was carried out using a well cleaned Kjeldahl apparatus. One hundred milliliters conical flask containing 5ml of 2% boric acid and two drops of methyl red indicator was placed under the condenser. 5ml of the digest was transferred using a pipette

into the distillation unit. The digest was then washed down with distilled water followed by addition of 5ml of 60% NaOH solution, this was distilled until volume of the distillate (ammonium sulphate) reached 100ml. The solution in the flask was then titrated with 0.04 HCl acid until the first permanent pink colour appeared. The blank was also titrated the same way.

$$\%\ \text{nitrogen} = V_s \times V_b \times N_{acid} \times 0.04 \times 100$$

Where; VS = volume (ml) of acid required to titrate sample

Vb = volume (ml) of acid required to titrate the blank

N acid = normality of acid (0.1N)

W = weight of sample (gram)

Note: most proteins contain 16% nitrogen, so that 16mg nitrogen = 100mg protein

1mg nitrogen = 6.25

Therefore protein (%) = N × 6.25 (conversion factor for protein)

3.5.8 Determination of lipid content

This was determined using the standard method of AOAC (2010). Five hundred milliliters round bottom flask together with a soxhlet extractor and a reflux condenser was set up and 300ml of petroleum ether was poured into the flask. Two grams of sugarcane bagasse sample was weighed into a labeled thimble and sealed with cotton wool, before fitting it into the extraction tube of the soxhlet extractor. The soxhlet extractor was then allowed to reflux for 6 hours after which the thimble was removed with care and the petroleum ether collected on top was drained into a clean conical flask for reuse. After the flask was free of ether, it was removed and dried in a desiccator before weighing.

$$\%\ \text{Fat content} = \frac{W2 - W1}{W}$$

Where; W = weight of sample used

W_1 = weight of empty extracting flask

W_2= weight of flask and extracted oil

3.6 Determination of the Physico-Chemical Properties of the Refinery Effluent

The physicochemical parameters analysed include: pH, temperature, turbidity, Electrical Conductivity (EC), Total Dissolved Solids(TDS), Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Nitrate, Phosphate, Sulphate and oil and grease by following the Standard methods suggested by American Public Health Association (APHA, 2005), while the heavy metals; lead (Pb), Chromium (Cr), and Cadmium (Cd) were analysed using atomic absorption spectrophotometer (model AA240S Varian Technologies, USA).

3.6.1 pH, temperature, electrical conductivity and total dissolved solids

The pH, temperature, electrical conductivity and total dissolve solids of the effluent were determined at the point of collection using the HANNA combo tester (H198130, Denver, USA), a water proof tester that offers high accuracy of pH, EC, TDS and temperature in a single test. Briefly, following the manufacturer's instructions, the electrodes connected to each meter were submerged in a clean bucket containing the sample. The values read for each parameter by the tester was observed and recorded.

3.6.2 Dissolved oxygen and biological oxygen demand

Dissolved oxygen and biological oxygen demand weredetermined using HANNA instrument (HANNA 3200, Denver USA). Dissolved oxygen was done by inserting the instrument into the sample at the point of collection while the BOD was carried out after the sample was transferred into a BOD bottle and left to stand for five days at temperature of (20^oC). The instrument was inserted into the incubated sample and read. Initial DO value was

thensubtracted from the final value gotten after incubation to obtain the BOD_5(Radojavic and Bashkin, 1999).

3.6.4 Nitrates

This was carried out by the method described by APHA (2005). The sample was neutralized to pH 7.0 and 100ml of the sample was then placed in a beaker and allowed to evaporate to dryness in a water bath. The residue was dissolved using a glass rod with 2ml disulphonic acid reagent and then transferred to Nessler's tube. Six millilitres of ammonium hydroxide (NH_4OH) and EDTA was then added drop wise till the residue dissolved. A blank was also prepared the same way using distilled water instead of the sample. The colour development was then read at 410nm with a light path of 1cm. The nitrate concentration was calculated using the formula:

$$\text{Mg/L of nitrate} = \frac{\text{mg nitrate N} \times 100}{\text{ml sample taken}}$$

3.6.5 Sulphates

One hundred millilitres of the sample was dispensed into Erlenmeyer flask, 5ml conditioning reagent was added and mixed using a magnetic stirrer. A spoonful of barium chloride crystals was then added while the solution was still being stirred. The turbidity was measured at 30seconds after stirring at interval of 4 minutes using turbidity meter (Hach 2100N, USA). A blank was ran without barium chloride added. The sulphate concentration was calculated using the formula below and sulphate concentration was estimated by comparing the reading with a standard curve (APHA, 2005).

$$\text{Mg/L of Sulphate} = \frac{\text{mg } S0_4 \text{ x } 1000}{\text{ml sample taken}}$$

3.6.6 Phosphates

3.6.6.1 Preliminary sample treatment

One drop (0.05ml) phenolphthalein indicator was added to 100ml of the sample. Strong acid solution (hydrochloric acid) was then added drop wise to discharge the pink colour.

3.6.6.2 Colour development

One hundred millilitres of treated sample was poured into a flask and 4.0ml of molybdate reagent was added and mixed thoroughly. Ten drops (1ml) stannous chloride reagent was then added. After 10 minutes, the colour was measured photometrically at 690nm and compared with the standard curve. Distilled water was used as blank. Calculation was carried out using the formula by (APHA, 2005):

$$\text{Mg/L of phosphate} = \frac{\text{mg } PO_4 \times 1000}{\text{ml sample taken}}$$

3.6.7 Oil and grease

The partition-Gravimetric was used for the analysis of oil and grease. Fifty millilitres of the sample was acidified to pH 2 using 5ml of hydrochloric acid, shaken vigorously for 3 minutes. The sample was transferred into a separating funnel held to a retort stand, and then 15ml of hexane was used to carefully rinse the beaker before pouring the sample into the funnel which separated the content into layers of oil, grease and water. The water being of higher density was collected below leaving oil and solvent at the surface. Twenty grams of anhydrous was added to the filter cone before the emulsified solvent was collected. Extraction was done twice using 15ml of the organic solvent in each process. An additional 20ml of solvent was used to wash the filter paper and evaporation was carried out using water

bath until all the solvent evaporated. The flask was transferred to a desiccator before weighing. The oil and grease content was determined as follows:

$$A - B \times 1000\text{ml of } \frac{\text{oil and grease}}{\text{ml sample oil + grease (Mg/L)}}$$

Where, A and B are the initial and final weight of the flask and its contents.

3.6.8 Determination of heavy metal contents in the effluent

The concentrations of heavy metals (lead, cadmium and chromium) in the effluent sample were determined using the fast sequential atomic absorption spectrophotometer (Model AA240S Varian Technologies, USA)following themethod of APHA (2005). Briefly, the instrument's settings and operational conditions were done in accordance with manufacturer's specifications by calibrating with analytical grade metal standard stock solution.

Five millilitres of each sample was digested in a beaker by adding 37.5ml nitric acid and 12.5ml hydrochloric acid, heated to almost dryness and topped up to 50ml with distilled water. The digested samples were filtered to remove any insoluble materials that could clog the atomizer. The lead, chromium and cadmiumcontent in the filtrate was then determined using the AAS (Model AA240S Varian Technologies, USA).

3.7 Evaluation of the Potential of Sugarcane Bagasse inSupporting Growth of theTest Fungi

This was carried out by weighing 20g of sugarcane bagasse into four separate glass Petri dishes followed by supplemental addition of 200ppmof ammonium nitrate, magnesium sulphate and potassium hydrogen phosphate. The Petri dishes and their contents were

autoclaved at 121^0Cfor 15 minutes. Ten milliliter sporessuspension of isolates of *Microsporum* and *Alternaria* specieswere aseptically inoculated into duplicate plates of the freshly sterilized sugarcane bagasse. All inoculated plates were incubated aerobically in dark cupboard under ambient laboratory conditionsfor fourteen days with continuous monitoring for appearance of fungal growth. The cultures were then harvested dried and weighed using a top loading balance(Mettler PM16-K) to obtain the dry weight of the partially digested bagasse (Modified method of Machido *et al*., 2015)

3.8 Preparation of Experimental Sorbents

The sorbents used for the sorption experiments in this study were prepared by weighing five batches of 200g each of ground sugarcane bagasse into separate Aluminum columns measuring 5cm in diameter x 25cm in depth to serve as fixed bed columns and as substrate for growth and accumulation of mycellial biomass of *Microsporum* and *Alternaria* species. Four of the five fixed bed columns thus prepared were sterilized by autoclaving and the fifth was left unsterilized.

Of the four sterile columns, two were inoculated with 50ml of spore suspensions prepared from reactivated pure cultures of *Microsporum* and *Alternaria* species. A third column was inoculated with a mixture of spore suspensions of both fungal isolates and the fourth was left uninoculated. The unsterilized column was also left uninoculated. The uninoculated columns served as the controls. Inoculated columns were incubated aerobically under ambient laboratory conditions for 14 days to obtain the experimental sorbents (Machido *et al*., 2015) as shown in Plate I.

3.9 Sorption Process

One litre of effluent sterilized by autoclaving was carefully dispensed into each of the sorption columns prepared as described in section 3.8 after two weeks of incubation.Twenty millilitre of filtrate was collected from each sorption column in sterile and well labeled plastic sample bottles one hour after introducing the effluent into the sorption columns.The residual concentrations of Pb, Cr and Cd in the filtrates were then determined using Atomic Absorption Spectrophotometer(Model AA240S Varian Technologies, USA). This exercisewas repeated at hourly intervals for 6 hours (Machido *et al*., 2015). The experimental set up for the sorption studies is shown in Appendix VII.

3.10 Determination of Sorption Performance

The difference in residual heavy metal concentration before and after biosorption process was used to assess the capacity of heavy metal removal from each column. The heavy metal removal (R) from the effluent was calculated and expressed in percentages using the equation by Ezeonuegbu *et al*. (2014):

$$\%\ \text{Heavy metal removal (R)} = \frac{\text{Ci} - \text{Cf}}{\text{Ci}} \times 100$$

Where Ci = initial concentrations and Cf = final concentrationsof heavy metal ionsin the effluent, before and after biosorption processrespectively.

The quantityof heavy metal ion sorbed per unit mass (mg/g) of biomass (qt) was calculated using the equation by Ezeonuegbu *et al*. (2014):

$$\text{qt} = \frac{\text{Ci} - \text{Cf}}{\text{M}} \times \text{V}$$

Where Ci = initial concentrations and Cf = final concentrations of heavy metal ions in the effluent, before and after biosorption processrespectively, V =volume of effluent used and M = mass of adsorbent used respectively during the sorption process.

The relative affinity (b) of individual sorbents for sorbates was calculated using the Langmuirian equation byKratochvil and Volesky (1998):

$$Q = qmax(bcf)/(1 + bcf)$$

Thus,

$$b = \left(\frac{Q}{qmax} \times Cf\right) - (Q \times Cf)$$

Where Q = quantity metal ion adsorbed at time t, q_{max}= maximum adsorption at time t and Cf is the final concentration of metal ion at time t.

3.11 DataAnalysis

Data obtained from the study were analyzed using statistical package for social science, version twenty two ($SPSS_{22}$) and one way Analysis of Variance (ANOVA) was used to comparemeans at 95% confidence interval.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Sugarcane Bagasse

Result of proximate analysis of samples of sugarcane bagasse revealed that it is composed of moisture(3.52%), ash(0.75%), protein(4.81%), fat (0.2%), carbohydrate (64.7%)and crude fiber(26.02%) as indicated in Table 4.1. Further analysis of the crude fiber components have shown that, it is made up of cellulose, hemicellulose, lignin, ash and other constituents to be 46.5%, 23.61%, 21.4%, 0.75% and 3.43% respectively (Figure 4.1).

4.2 Physicochemical Properties of the Refinery Effluent

Physicochemical analysis of therefinery effluent revealed that both the temperature and pH of the raw refinery effluent were within acceptable level specified by Federal Ministry of Environment (FEMENV), Nigeria. However, values recorded in respect of parameters such as electrical conductivity (717μS/cm), oil and grease, (37mg/l), chemical oxygen demand (COD) (874mg/l), biological oxygen demand (BOD) (31mg/l), Sulphate (73.2mg/l) and phosphate (33.7mg/l) were all found to exceed the permissible limit set by FEMENV. Similarly, the levels of lead (Pb) (1.294mg/l), Chromium (Cr)(11.31mg/l) and Cadmium (Cd) (1.9mg/l) in the refinery effluent werealso found to far exceed the allowable limits set by FEMENV by factors of 129, 377 and 63 respectively On the other hand, the levels of dissolved oxygen (DO) (2.93mg/l) and nitrate (1.79mg/l) were far below the level considered safe by FEMENV (Table 4.2).

Table 4.1 Proximate Composition of Sugarcane Bagasse

Parameters	Percentage Composition (%)
Moisture	3.52
Ash	0.75
Protein	4.81
Fat	0.20
Crude Fibre	26.02
Carbohydrate	64.70

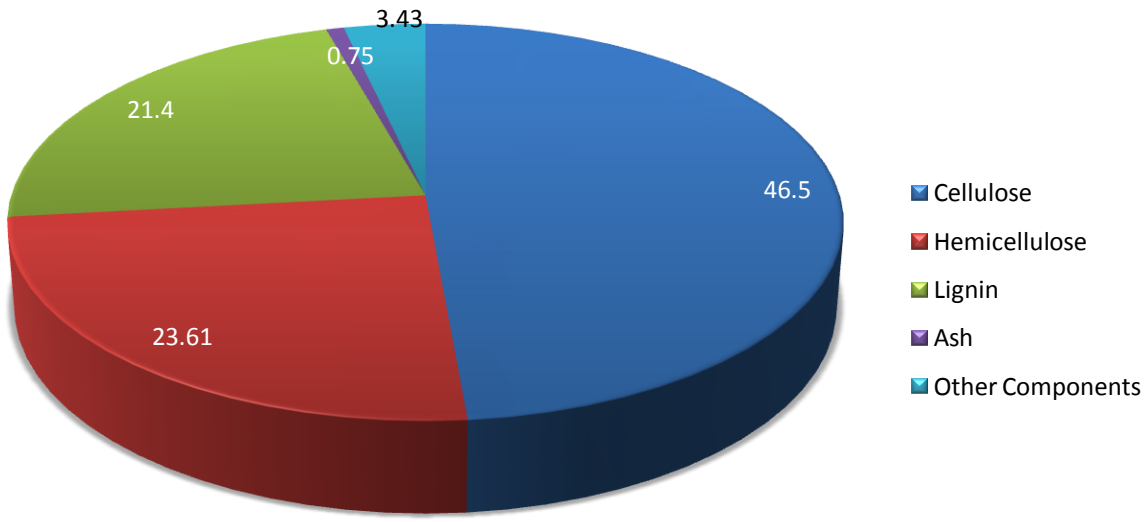


Figure 4.1: Crude Fibre Contents of Sugarcane Bagasse

Table 4.2 Physicochemical Properties of theRefinery Effluent

Physicochemical Parameters		Values	Permissible Limit (FMENV)
pH		8.8	6-9
Temperature (^{0}C)		29.6	40
Electrical Conductivity (μS/cm)		717	400
Total Dissolved Solids (mg/L)		258	500
Biological Oxygen demand (mg/L)	31		10
Dissolved Oxygen (mg/L)		2.93	10
Chemical Oxygen Demand (mg/L)		874.	50
Oil and Grease (mg/L)	37		10
Nitrate (mg/L)	1.7		10
Phosphate (mg/L)	33.7		5
Sulphate (mg/L)		73.2	50
Lead (mg/L)		1.294	0.01
Chromium (mg/L)		11.31	0.03
Cadmium (mg/L)		1.9	0.03

FMENV = Federal Ministry of Environment, Nigeria, (1999)
μS/cm = Micro siemens per centimeter
mg/L = Milligram per liter

4.3 Evaluation of the Potential of Sugarcane Bagasse in Supporting Growth of the Test Fungi

The capacity of sugarcane bagasse to support the growth of the two fungalspecies used in this study was demonstrated as visible growth of each with the bagasse serving as substrate. Growth of *Alternaria* species was rapid with initially olivaceous green to black surface coloration but turned grey with age of the culture (Plate Ia) as against the coloration exhibited by the uninoculated bagasse (Plate Ib). On the other hand, growth of *Microsporum* species was slow at first, exhibiting white/whitish yellow, flat, cottony or wooly surface appearance (Plate Ic). In general, the bagasse as substrate appeared to support a more luxuriant growth of *Alternaria species* as compared to *Microsporum species* under similar incubation conditions. This is further demonstrated by the proportion of the bagasse utilized by the two fungi during the14 days period of growth (Table 4.3).

4.4 Percentage of Heavy Metal ions Removed bytheSorbents

The mean percentage of Pb, Cr and Cd ions removed by the different sorbents ranged from 89% - 92%, 86% - 94% and 99% respectively. It was observedthat the sorbents varied in their capacity to remove the targeted metal ions from the solution. For instance, sorbents A and D had the highest mean percentage removal of Pb (92%) and the lowest recorded for sorbent C (89%). On the contrary, highest efficiency of Cr removal was recorded for sorbent C (94%) while the lowest was noted for sorbent A (86%). However, the five sorbents exhibited similar efficiency in the removal of Cd which was at 99%. In general, the five experimental sorbents exhibited highest efficiency in the removal of Cd ions but appeared least efficient in the removal of Cr ions (Figure 4.2).



Plate Ia: *Alternaria* species growth on sugarcane bagasse medium (incubation period: 14days)

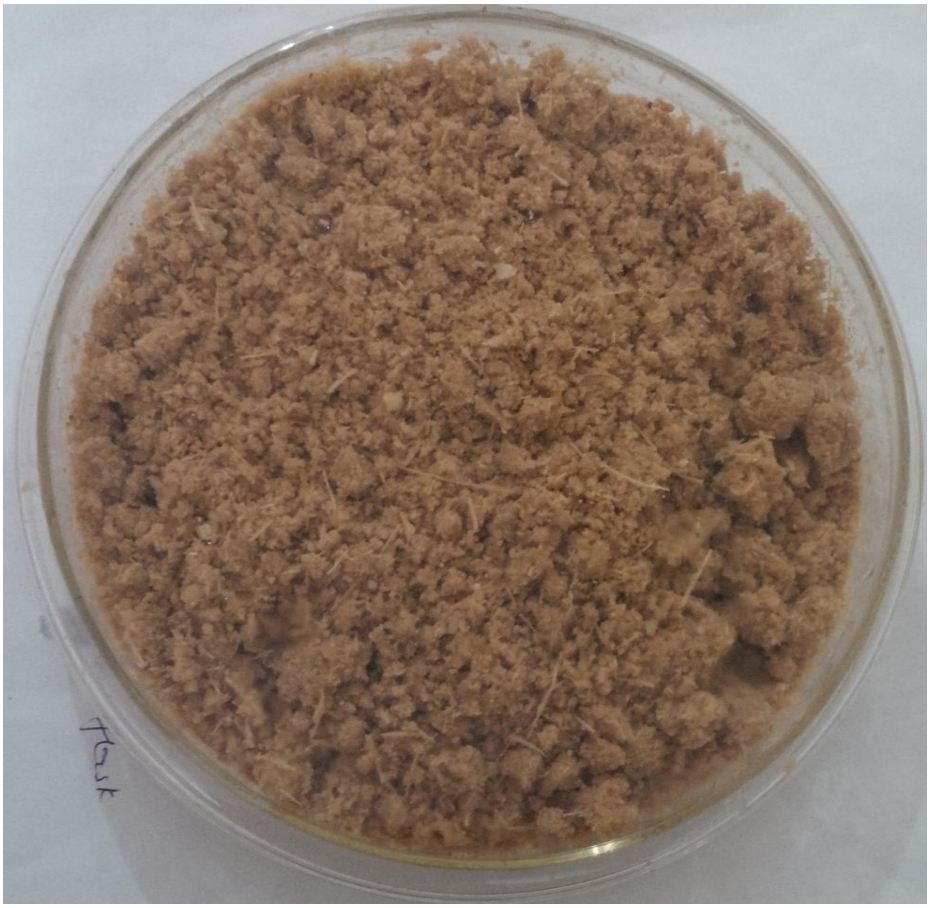


Plate Ib: un-inoculated sugarcane bagasse medium

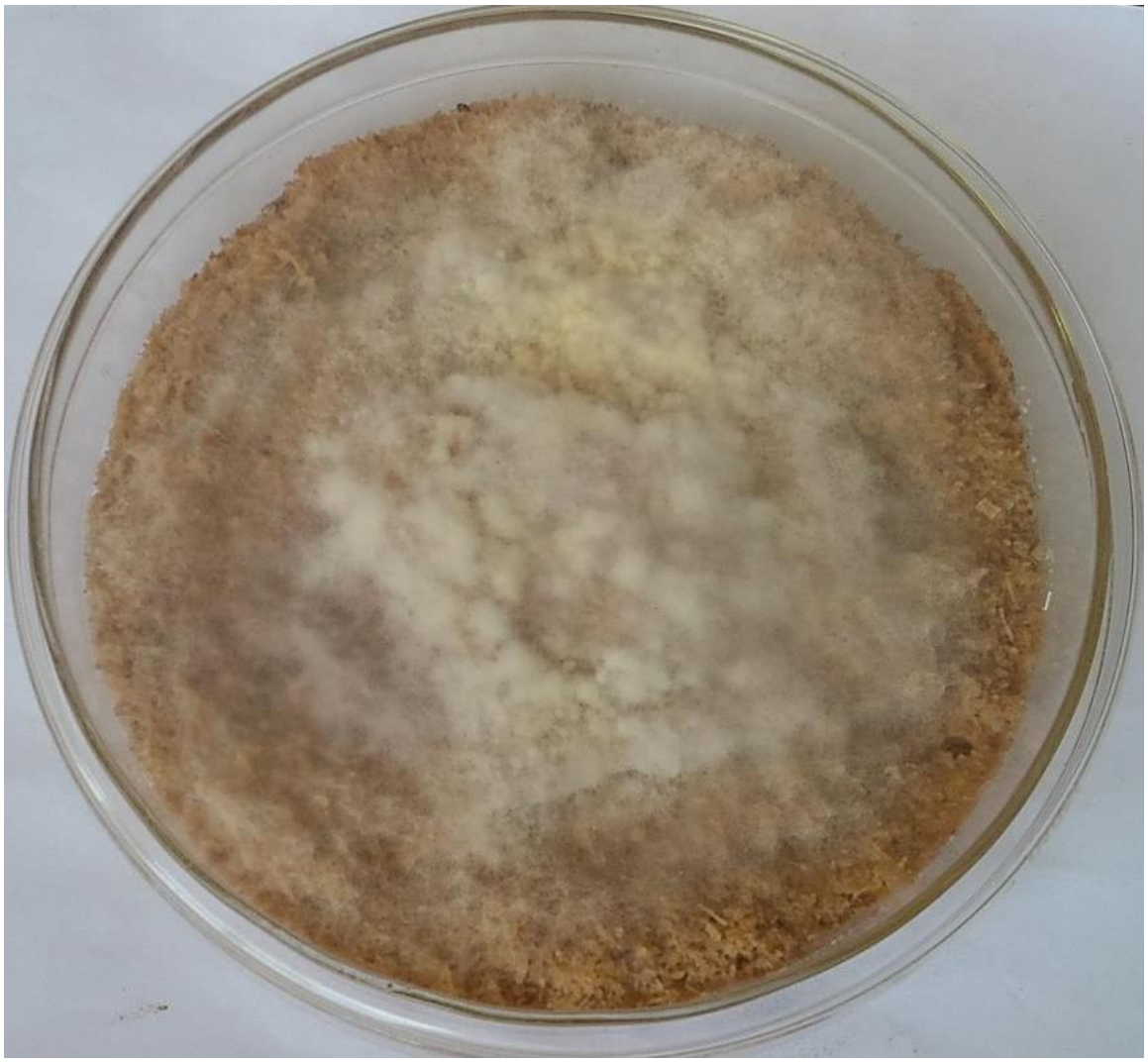


Plate Ic: *Microsporum* species growth on sugarcane bagasse medium (incubation period: 14days)

Table 4.3 Percentage ofsugarcane bagasse to growth of *Alternaria* **and** *Microsporum* species **over 14 days period**

Fungi Tested	Weight of sterile bagasse (g)	Weight of bagasse after fungal growth (g)	% of bagasse utilized for growth
***Alternaria* species**	297. 3	270.6	9.0
***Microsporum* species**	303.5	286.1	5.7

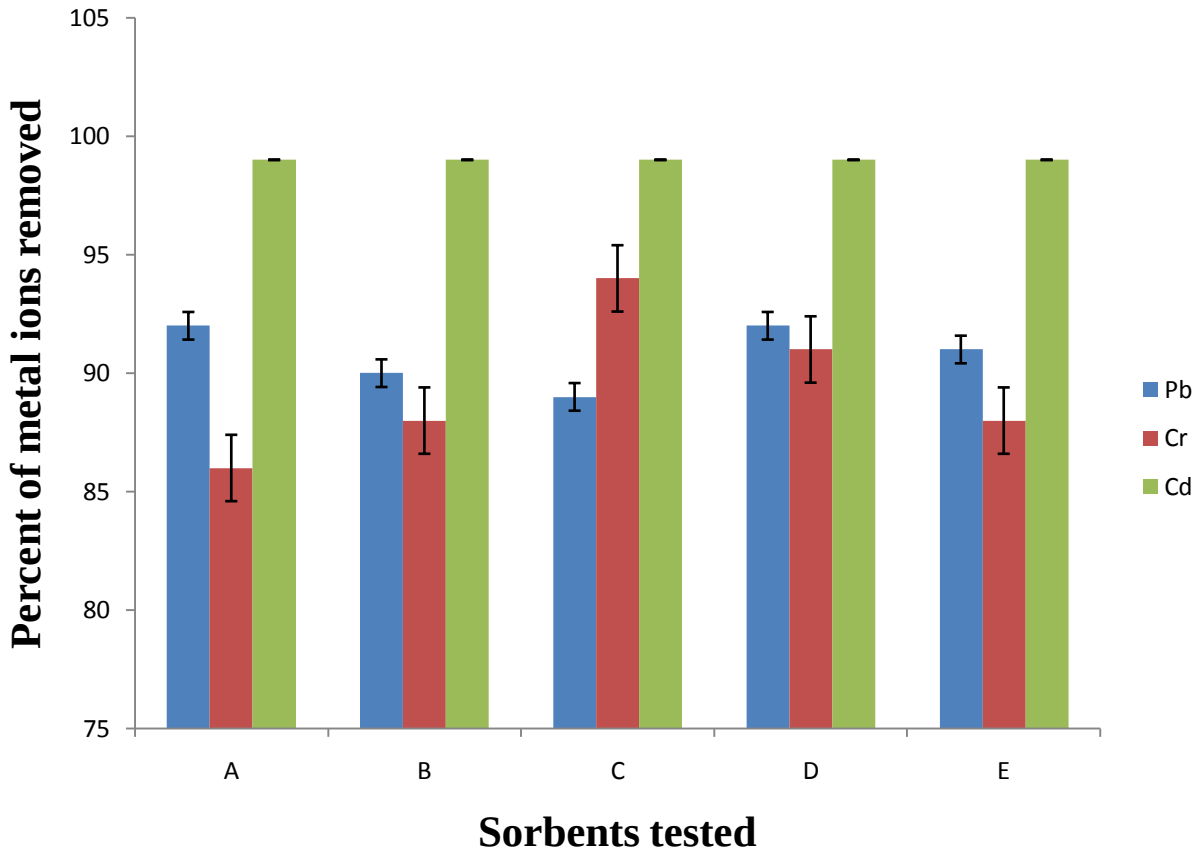


Figure 4.2: Percentage of Pb, Cr and Cd removed by sorbents (P>0.05). Each data represents the means±SE

A= Unsterilized and uninoculated sugarcane bagasse, **B**= Sterilized but uninoculated sugarcane bagasse, **C**= Sterilized sugarcane bagasse inoculated with *Alternaria* species, **D**= Sterilized sugarcane bagasse inoculated with *Microsporum* species, **E** = Sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

4.5 Quantity of Heavy Metal ions Adsorbedby Sorbents

The mean quantity of Pb, Cr and Cd ions adsorbed per gram of the different experimental sorbents ranged from 0.9-1.0mg/g, 0.7-0.9mg/g and 1.8mg/g respectively as shown in figure 4.3. It was observed that the sorbents varied in their capacity to adsorb the targeted metal ions from the solution. Sorbents A and D had the highest mean amounts of Pb adsorbed (1.0mg/g) while sorbent C adsorbed the least quantity of the same metal ion (0.85mg/g). Similarly, highest sorption of Cr was recorded for sorbent C (0.9mg/g) while the lowest amounts of the same metal ion adsorbed was observed for sorbent E (0.7mg/g). In general, it was observed that the five experimental sorbents exhibited highest and same level of efficiency with regards to sorption of Cd ions (1.8mg/g).

4.6 Relative Affinity of Sorbents for Heavy Metal ions Expressed in LangmuirianCoefficient (b)

The Langmuirian coefficient (b) was used as a measure of the affinity of different sorbents for sorbates. Usually, low value of the coefficient (b) signifies higher affinity of sorbents for the sorbates.

It was observed that the five experimental sorbents exhibited the highest affinity for Cr ions followed by Cd and Pb ions in decreasing order (Figure 4.4). However, it was observed that the experimental sorbents varied in their affinity for Pb ions with sorbent C exhibiting the lowest affinity (b = 0.27) in comparison to sorbents A (b = 0.23), B (b = 0.23), D (b = 0.23) and E (b =0.25). No variations were observed in the affinity of the experimental sorbents for Cr and Cd ions.

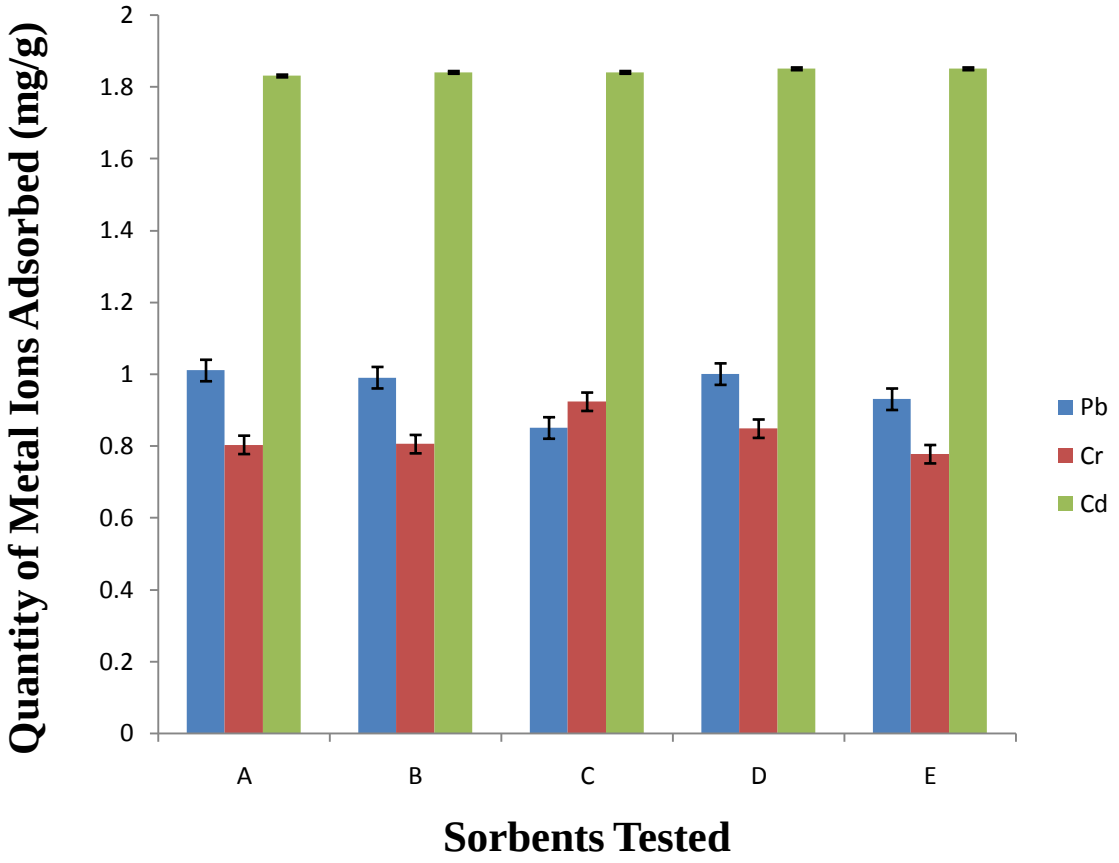


Figure 4.3: Quantity of Pb, Cr and Cd adsorbed by sorbents (P > 0.05). Each data represents the means±SE

A= Unsterilized and uninoculated sugarcane bagasse, **B**= Sterilized but uninoculated sugarcane bagasse, **C**= Sterilized sugarcane bagasse inoculated with *Alternaria* species, **D**= Sterilized sugarcane bagasse inoculated with *Microsporum* species, **E** = Sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

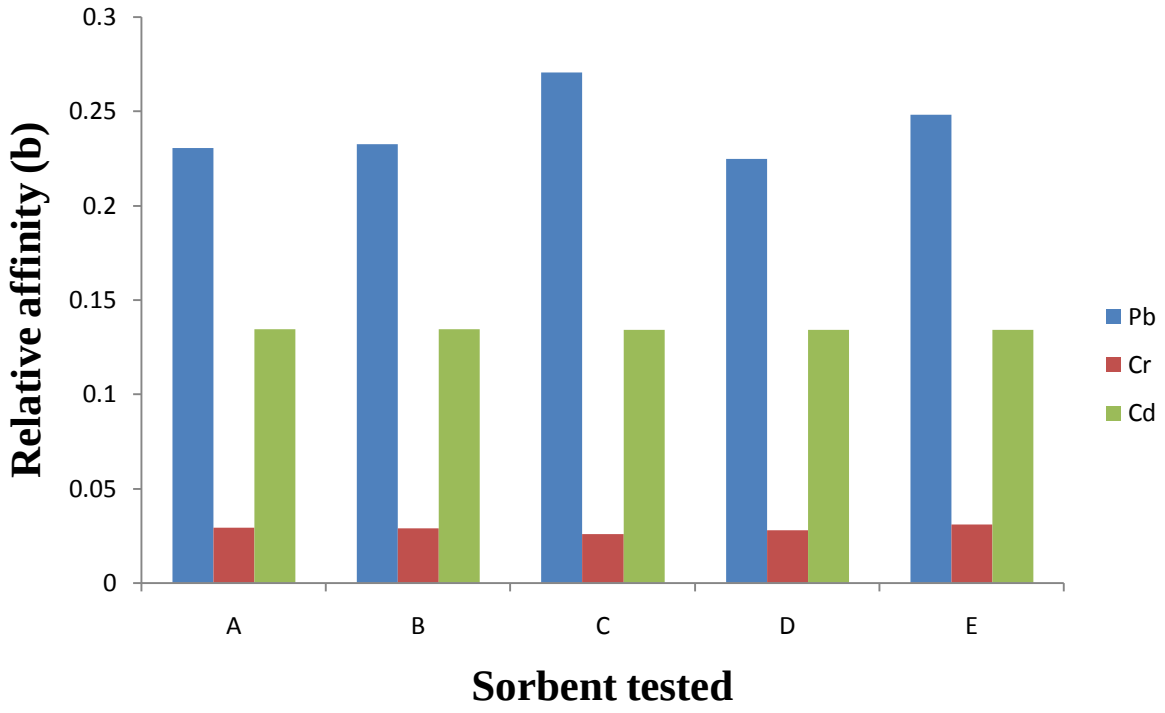


Figure 4.4: Relative affinity (b) of sorbents for Pb, Cr and Cd ions

A= Unsterilized and uninoculated sugarcane bagasse, **B**= Sterilized but uninoculated sugarcane bagasse, **C**= Sterilized sugarcane bagasse inoculated with *Alternaria* species, **D**= Sterilized sugarcane bagasse inoculated with *Microsporum* species, **E** = Sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

4.7 Effects of Contact Time on Adsorption of Heavy Metal ions

Data obtained revealed a general trend of decrease adsorption of Pb with increased contact time for all the five experimental sorbents with maximum adsorption recorded after the first hour of contact. This was followed by a slight decline at the second hour for all except sorbent D. At the third hour, the sorption capacity of sorbent C and to a lesser extent, sorbent E declined at faster rate compared to sorbents A, B, and D. However, it was noted that experimental sorbents varied in the rate at which sorption of Pb ions decline with contact time. Sharp and consistent decline in adsorption of Pb was observed in sorbent C and E throughout the 6 hour period of the study compared to sorbents A, B and D. On the other hand, only slight decline in rate of sorption of Pb ions was noted for sorbent D during the first 3 hours of contact. Similar observation was made for sorbent A during the first 5 hours of contact (Figure 4.5).

The capacity of the sorbents to bind and retain Cr ions appeared to be less affected by the length of period of contact between the sorbents and the effluent; especially during the first 4 hours of the sorption process. During the period, the amount of Cr ions sorbed by all the five sorbents dropped only slightly from10-11mg/g to 7.8-9.5mg/g giving a decline ranging between 1.5 - 2.2mg/g. However, extending the period of contact between the sorbents and effluent by an hour resulted in a sharp decline in the sorption capacity of the sorbents to varying degrees. Sorption rates of 4, 5 and 6mg/g were recorded for sorbents E, A and B, and D respectively at 5 hours after the start of the sorption process. During the corresponding period, the sorption capacity of sorbent C remained relatively high at 7.4mg/g within the 6 hours after the start of the sorption process (Figure 4.6).

With regards to sorption of Cd ions, all the five sorbents tested exhibited maximum sorption capacity (1.9mg/g) within the first I hour of contact between the sorbents and the effluent.

This was followed by a gradual but consistent decline in the amount of Cd ions sorbed with every hour to a minimum sorption capacity of 1.78mg/g for all the sorbents tested. There was also no noticeable differences recorded in the capacity of the sorbents throughout the 6 hour period of the sorption process as shown in Figure 4.7.

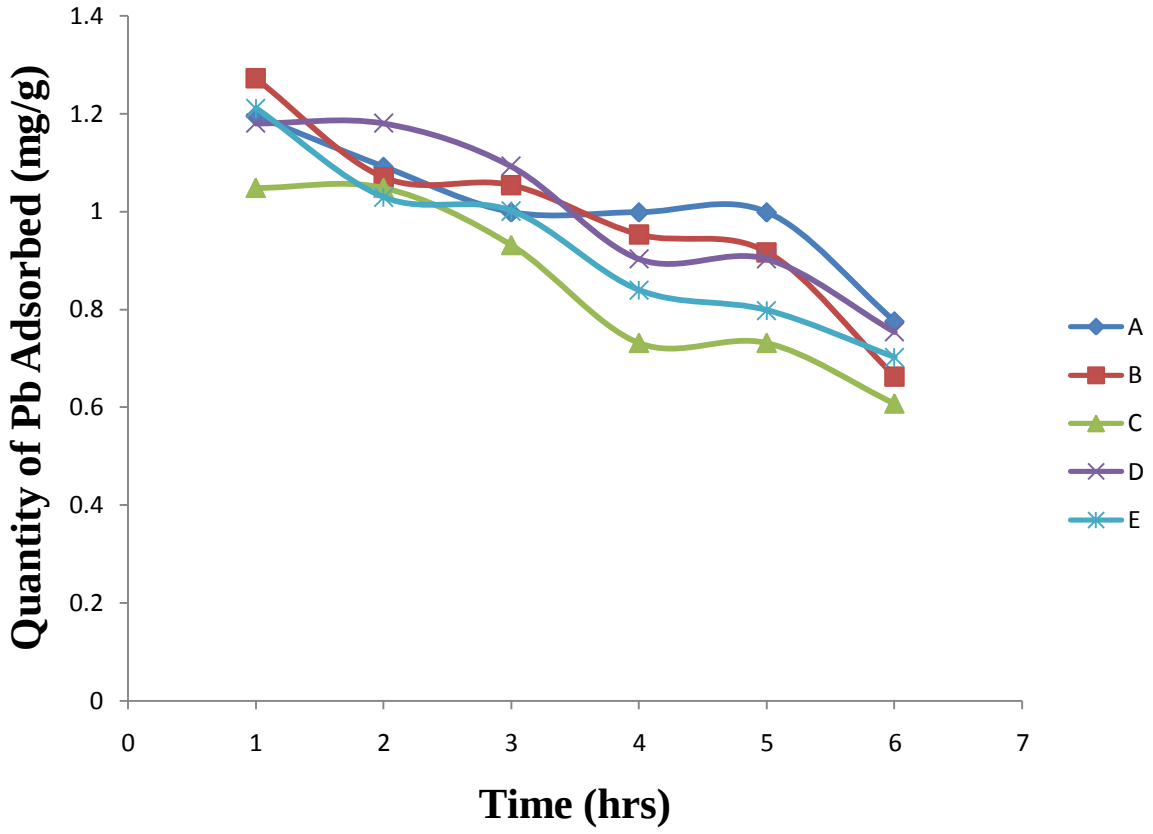


Figure 4.5: Effect of contact time on adsorption of Pb ions on sorbents

A= Unsterilized and uninoculated sugarcane bagasse, **B**= Sterilized but uninoculated sugarcane bagasse, **C**= Sterilized sugarcane bagasse inoculated with *Alternaria* species, **D**= Sterilized sugarcane bagasse inoculated with *Microsporum* species, **E** = Sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

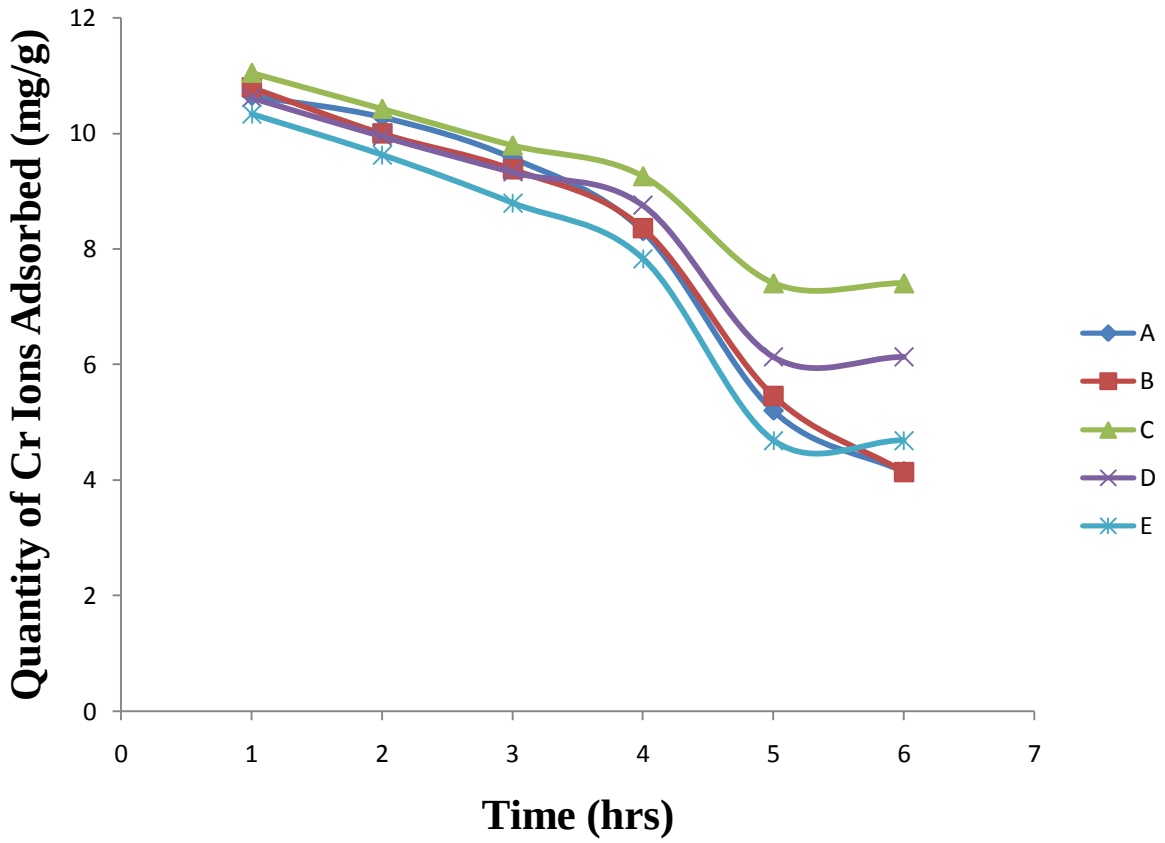


Figure 4.6: Effect of contact time on adsorption of Cr ions on sorbents

A= Unsterilized and uninoculated sugarcane bagasse, **B**= Sterilized but uninoculated sugarcane bagasse, **C**= Sterilized sugarcane bagasse inoculated with *Alternaria* species, **D**= Sterilized sugarcane bagasse inoculated with *Microsporum* species, **E** = Sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

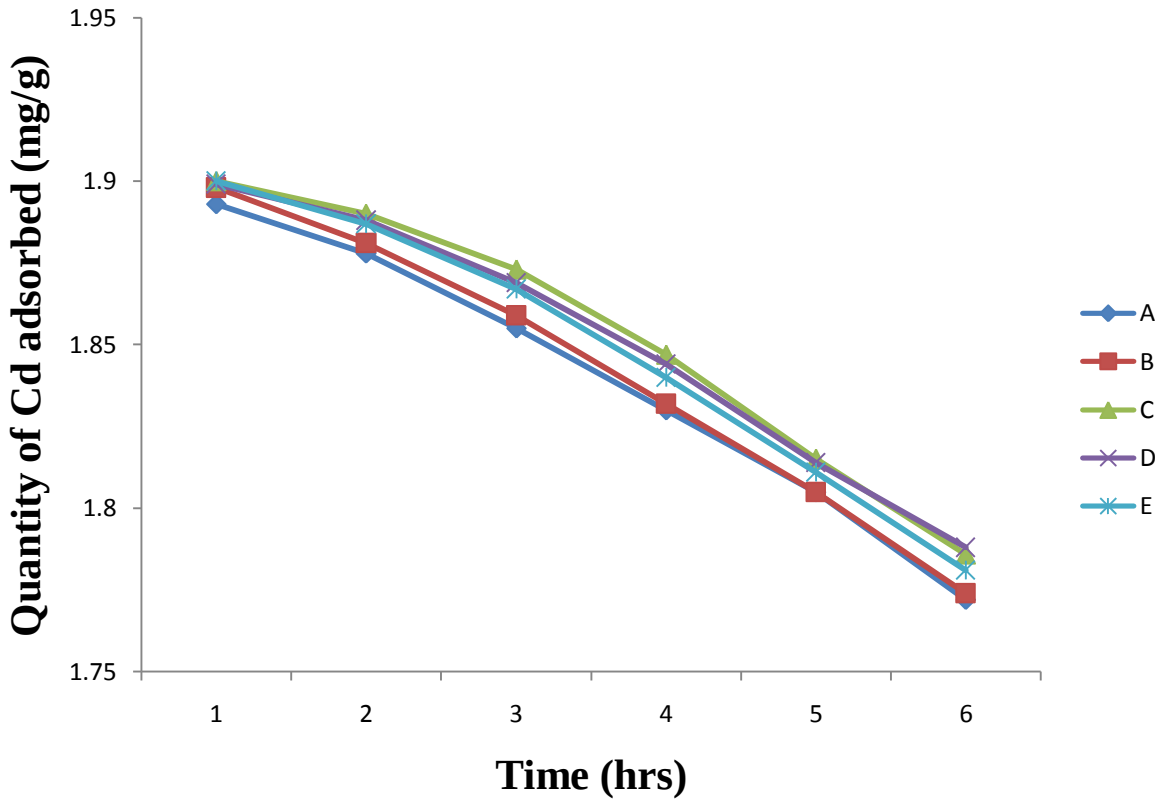


Figure 4.7: Effect of contact time on adsorption of Cd on sorbents

A= Unsterilized and uninoculated sugarcane bagasse, **B**= Sterilized but uninoculated sugarcane bagasse, **C**= Sterilized sugarcane bagasse inoculated with *Alternaria* species, **D**= Sterilized sugarcane bagasse inoculated with *Microsporum* species, **E** = Sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Composition of Sugarcane Bagasse

The proximate analysis of sugarcane bagasse used in this study tend to suggest that sugarcane bagasse has the potential of acting both as asuitable low cost biosorbent for the removal of heavy metal ions in solution and providing a suitable and cheap medium for the growth and generation of biomass of filamentous fungi such as *Microsporum* and *Alternaria*species for use as biosorbents. This view is supported bythe high proportions of cellulose (46.5%), hemicellulose (23.61%), lignin (21.4%), carbohydrate (64.7%), protein (4.81%), fats (0.2%), ash (0.75%) and other components (3.43%) making up the proximate composition of the bagasse.These components, have been shown to possess various functional groups that could serve sites capable of binding and removing heavy metal ions in solution with high affinity (Choi and Yun, 2006;Sud *et al*., 2008;Betancur and Pereira, 2010; Soliman *et al*., 2011;Abdulsalam *et al.,* 2014). These same components have also been used as major constituents of several laboratory media used for the cultivation of filamentous fungi in the laboratory (Prescott *et al*., 2013). The high content of carbohydrate (64.7%),protein (4.81%), lipid (0.20%) and ash (0.75%) in the bagasse used in this study, strongly suggest that it could provide the energy, carbon, nitrogen, phosphorus and sulphur which constitute important requirements for growth of most fungi(Prescott *et al*., 2013). This implies that, sugarcane bagasse could provide alternative growth medium on which large quantities of fungal biomass could cheaply be generated for use as biosorbents for heavy metal removal from wastewater and other purposes. The low moisture content is an indication that the substrate is of good yield and of high quality as moisture content is used to assess the shelf life and

quality of substrates. The ash content shows that the substrate indeed, contains a reasonable amount of mineral contents as it always represents a rough measure of the inorganic mineral elements in a sample such as calcium, sodium, potassium and chloride. The lower the ash content, the higher the quality of the material (Organic Materials Review Institute, 2002).

Similar results were obtained by Luz *et al*. (2007)who reported values of 43.8%, 28.6%, 23.5%, 1.3% and 2.8% for cellulose, hemicellulose, lignin, ash and other components respectively. However,Betancur and Pereira, (2010), reported values of 35%, 25%, 22% and 20% for cellulose, hemicellulose, lignin and ash respectively while Soliman *et al*. (2011), reported45%, 28% and 18% for cellulose, hemicellulose and lignin. The difference in variation could be due to the ratio/proportion of various constituents of biomass materials which differ greatly among biomass categories and also from species to species (Harman *et al*., 2007).

5.2 Physicochemical Properties of the Refinery Effluent

Of the physical and some of the chemical properties of the effluentdetermined,only electrical conductivity was found to be above the permissible limit (400µS/cm) set by Federal Ministry of Environment (FMENV), Nigeria. The high level of electrical conductivity could be due to the high concentration of organic and inorganic compounds (dissolved ions) from the various chemicals used in primary distillation process by the refinery plant. On the other hand, the temperature and pH recorded for the effluent could be considered within limits that could permit the growth of wide range of water dwelling life forms present in the effluent recipient water bodies. Similar observations have been reported by Ezeonuegbu *et al*. (2014) while working on refinery effluent from the same source.

The levels of other chemical properties of the effluentthat are considered important indices of effluent quality such asbiological oxygen demand (BOD), dissolved oxygen (DO),

chemical oxygen demand (COD), oil and grease, nitrate (NO_3), phosphate (PO_4) and sulphate (SO_4) clearly signal a threat of pollution (Otukunefor and Obiukwu 2005, Atubi 2011, Marcus and Okoye 2013, Marcus and Ekpete, 2014). With a BOD and COD at 3 and 18 times higher and DO at 3 times less than the permissible level suggest that the effluents require adequate treatment with the view to averting the possibility of over loading the effluent recipient river with organic and inorganic pollutants. The undesirable consequences of releasing untreated refinery effluents charged with high levels of organic materials into surface water bodies have been documented (Marcus and Okoye 2013, Marcus and Ekpete 2014).

Of even greater significance is the high level of Pb, Crand Cd contents of the refinery effluentobserved in this study as against the permissible standard set by FMENV.The presence of Pb, Cr and Cd at 1.294mg/l, Cr at 11.31mg/l and Cd at (1.9mg/l) represents 129, 377 and 63 times higher concentrations of Pb, Cr and Cd as compared with FMENV standard of effluent quality. The high levelscould have originated from feedstock, corrosion products of equipment and pipes, chemical additives and catalyst used in petroleum refining processes (Beddri and Ismail, 2007).This strongly suggest that, continuous release of the effluent containing the observed levels of these heavy metals into the environment without adequate treatment could have far reaching consequences on the environment (Alluri *et al.,* 2007; Varsha *et al*., 2010; Mudhoo *et al.,* 2011; Marcus *et al.,* 2013; Ho *et al.,* 2014) as well as posing serious dangers to public health (Ramzan *et al.,* 2011; Tokar *et al.,* 2011; *Ho et al.,* 2014). The health hazards associated with exposure to these Pb, Cr and Cd have been widely reported (Ogwuegbu and Muhanga, 2005; Young, 2005; Gosh; Grazyna and Pawel, 2010; Ho *et al.,* 2014).

5.3 Evaluation of the Potential of Sugarcane Bagasse to Support Growth of the TestFungi

The capacity of sugarcane bagasse to support the growth of *Alternaria* and *Microsporum*species as demonstrated on plates IIa and IIc attest to the fact that the constituents of the bagasse can supply adequate amounts ofnutrients essential for their growth. The ability of these fungalspecies to grow and produce measurable amounts of biomass at the expense of sugarcane bagasse on the other hand, indicates that these fungi species posses the requisite enzymes enablingthem to utilize the available carbon, nitrogen, sulphur and phosphorous for cellular growth (Walker and White, 2005). Although, both fungal species actively grew and produced measurablebiomass on sugarcane bagasse,*Alternaria* species was found to exhibit faster and more luxuriant growth compared to *Microsporum* species. Also, higher proportion of the bagasse was found to have been lost following the growth of *Alternariaspecies* (9%) as compared to 5.7% recorded following the growth of *Microsporumspecies* over the same period of incubation. This observation could be attributed to several factors that may include the the slow ability of *Microsporumspecies* to hydrolyze carbohydrate polymers (hemicellulose, cellulose and lignin) found in sugarcane bagasse (Lee *et al*., 2002; US Dept of Energy, 2006; Dashtban *et al.*, 2009).

The fact that sugarcane bagasse supported the growth of these fungi suggests that it could provide a cheaper alternative to costly laboratory media for large scale production of fungal biomass for various uses (Dashtban *et al*., 2009). Given the quantity of sugarcane bagasse generated annually in this country, a tremendous quantity of fungal biomass could be generated for use as sorbents for removal of heavy metals from toxic industrial effluents. The potential of fungal biomasses in the sorption of heavy metal ions from industrial effluents have been reported (Joshi *et al*., 2011; Kumar *et al*., 2012; Rao and Bhargavi 2013, Ezeonuegbu *et al*., 2014).

5.4 Assessment of the Effect of Growth of the Test Fungi Separately and as Co-Culture on the Sorbent Capacity of Sugarcane Bagasse

The assessment of capacity of the five experimentalsorbents in the removal of Pb, Cr and Cd ions from raw refinery effluent expressed both in percentile and quantitative terms revealed the sorbent, as having performed equally well.All the sorbents investigated were able to remove 89 to 92% of Pb, 86 to 94% of Cr and 99% of Cdfrom therefinery effluent over a period of 6 hours. This corresponds with adsorption of 0.9 to 1.0mg/g of Pb, 0.7 to 0.9mg/g of Cr and 1.8mg/g of the sorbents respectively. These observationscould be attributed tohigh carbohydrates, hemicellulose, cellulose and lignin contents ofthe sugarcane bagasse earlier reported. These biomolecules have been reportedto possess various functional groups such as carboxyl (COO^-), hydroxyl (OH^-) and phosphate (PO_4^{3-}) that could serve as attachmentsites to which the heavy metal ions are boundand removed from solution with varying degree of affinity (Choi and Yun, 2006;Sud *et al*., 2008; Betancur and Pereira, 2010; Soliman *et al*., 2011;Abdulsalam *et al.*, 2014). Since sorbent A and B were exclusively composed of these constituents, their performance in the removal of Pb, Cr and Cd could be explained by the chemical and physical processes by which adsorption of metal ions is mediated(Ahluwalia and Goyal, 2007;Beddri and Ismail, 2007).Sorbents C, D and E on the other hand, consisted the bagasse and live fungal, whose performance is driven by physical and chemical processes along with metabolic energy dependent processes (bioaccumulation) mediated by the live cells of *Alternaria*and *Microsporum*species present as constituents of these sorbents (Iqbal *et al*., 2005; Joshi *et al*., 2011; Ezeonuegbu *et al*., 2014).

From the observations made in this study, inoculation of the bagasse with *Alternaria* species and *Microsporum* species did not result in measurable differences in the sorption capacity of sugarcane bagasse, i.e. no significant difference existed ($P>0.05$). This could be attributed to various factors such as, competition by other metals present in the effluent (Gupta *et*

*al.,*2003) which could have succeeded in binding to the available sorption sites on the cell walls of the fungi species, thereby leaving little or no room for the targeted heavy metals (Kratochvil and Volesky, 1998). It could also be that the fungal species had higher affinity for other heavy metals than the targeted metals in the effluent. Metabolic activities of the fungi such as respiration, nutrient uptake, and metabolites released could have led to changes in the pH of the micro-environment around the cells which in turn affected removal of the targeted heavy metals from the effluent by the fungal cells (Malik, 2004; Sen and Ghosh, 2010). Also, fungal cell growth could have been inhibited by high levels of metals which have been reported to affect biosorption in living systems (Sen and Ghosh, 2010).

The Langmuirian coefficient "b" has been used to access the binding energy of different sorbents for sorbates (heavy metals) in solution. Usually, lower value of the coefficient b, indicates higher affinity of the sorbent for the sorbate (Kratochvil and Volesky, 1998). The results of the relative affinity of the different sorbents for the targeted heavy metals indicated that all the sorbents had highest affinity for Cr ions. This indicating that the sorbents (i.e sugarcane bagasse and the fungi species) were able to bind and strongly retain the Cr ions onsorbentssurfaces more than Cd and Pb ions.

5.5 Effect of Contact Time on Adsorption of Heavy Metal ions by Sorbents

Contact time is one of the important parameters of biosorption and results indicated decrease in the amounts of metal ions adsorbed per unit mass of sorbents with increase incontact time. This trend suggestsa decline in the number of available sorption sites with time progressively towards a point at which all available sorption sites were saturated.The rapid decline in adsorption by some of the treatments is an indication that adsorption sites on the sorbents were being saturated during the sorption process, as a result, less binding sites were available for metal ions in solution (Nwankwo, 2010) subsequently leading, to slower rate of

adsorption. Also, the rapid initial metal uptake by sorbents could be due to solution concentration being high at the beginning of the sorption process (Manish *et al*., 2013). Literatures have reported that uptake of heavy metals in aqueous solutions lies in theproperties of the adsorbent, nature of molecules of the sorbatestransferred from the solution to the solid phase, initial concentration of the adsorbate, presence of other metals and natureof the adsorbents (Mahvi*et al*., 2005a;Manish *et al*., 2013).

According to Tor-resdey *et al*. (2004) adsorption (uptake) of metal ion onto sorbent materials is characterized initially by physical adsorption, surface precipitation, ionic exchange and chemical reaction with surface sites and the rate of uptake is dependent on the availability of the overall electronegative charges on the sorbent which in turn can display several binding sites (Naja and Volesky, 2011).

Nasim *et al*. (2001) reported similar trend in the adsorption of Cr using sugarcane bagasse, while Kong *et al*. (2014) reported similar trend in the adsorption of Pb and Cd using acrylic-modified sugarcane bagasse-based adsorbents.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the observations made in this study, the following conclusions were drawn:

1. The proximate composition of sugarcane bagasse determined in this study is an indication of its potentials both as a source of low cost sorbent and as a cheap medium for growth of fungal biomass for heavy metal removal from solutions.
2. The refinery effluent is intensely contaminated with organic and inorganic pollutants (high biological oxygen demand, low dissolve oxygen, high oil and grease, high phosphate and sulphate) as well as toxic heavy metals (lead, chromium and cadmium) considered dangerous to the environment and public health.
3. The 9% and 5.7% utilization of sugarcane bagasse by *Alternaria* and *Microsporum* species respectively proved it to be a suitable medium for the growth of fungi.
4. Inoculation of sugarcane bagasse with *Alternaria* and *Microsporum* species had no significant difference in the sorbent capacity to remove or adsorb lead, Chromium and cadmium from refinery effluent.

6.2 Recommendations

From the observations made in this study, the following recommendations were drawn:

1. Studies on proximate composition should be carried out on other readily available agricultural waste materials to determine their capacity for metal binding.
2. Regulatory authorities should ensure strict adherence to recommended standard of the levels of heavy metals in effluent released into the environment.
3. The use of sugarcane bagasse should be promoted as a cheaper alternative to costly laboratory media for large scale production of fungal biomass for various uses.
4. Similar studies using biosorption technique should be carried out using other microorganisms in combination with other agricultural waste materials so as to assess their capacity of heavy metal removal from industrial effluents.

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APPENDICES

Appendix I: Removal of Pb by experimental sorbents as influenced by time

Contact time(hrs)	% of Pb removed by sorbents				
	A	B	C	D	E
1	92.3±4.98[a]	98.3±4.98[a]	81.0±4.98[a]	91.2±4.98[a]	93.6±4.98[a]
2	91.3±4.98[a]	84.1±4.98[a]	100.0±4.98[a]	100.0±4.98[a]	85.0±4.98[a]
3	91.5±4.98[a]	98.5±4.98[a]	88.8±4.98[a]	92.5±4.98[a]	97.3±4.98[a]
4	100.0±4.98[a]	90.4±4.98[a]	78.5±4.98[a]	82.7±4.98[a]	83.8±4.98[a]
5	100.00±4.98[a]	96.2±4.98[a]	100.0±4.98[a]	100.0±4.98[a]	100.0±4.98[a]
6	77.7±4.98[a]	72.3±4.98[a]	83.0±4.98[a]	83.5±4.98[a]	83.7±4.98[a]

Means with same superscripted alphabet across rows are not statistically different (P>0.05).
Values are expressed as means±SE (standard error of means).

A: unsterilized sugarcane bagasse

B: sterilized sugarcane bagasse

C: sterilized sugarcane bagasse inoculated with *Alternaria* species

D: sterilized sugarcane bagasse inoculated with *Microsporum* species

E: sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

Appendix II:Removal of Cr by experimental sorbents as influenced by time

Contact time(hrs)	% of Cr removed by sorbents				
	A	B	C	D	E
1	94.2±6.58[a]	95.4±6.58[a]	97.7±6.58[a]	93.8±6.58[a]	91.4±6.58[a]
2	96.5±6.58[a]	92.6±6.58[a]	94.4±6.58[a]	93.7±6.58[a]	93.2±6.58[a]
3	93.1±6.58[a]	93.8±6.58[a]	94.0±6.58[a]	93.8±6.58[a]	91.4±6.58[a]
4	86.8±6.58[a]	89.1±6.58[a]	94.6±6.58[a]	93.8±6.58[a]	89.1±6.58[a]
5	62.6±6.58[a]	65.3±6.58[a]	80.0±6.58[a]	70.1±6.58[a]	51.9±6.58[a]
6	79.8±6.58[a]	94.2±6.58[a]	100±6.58[a]	100±6.58[a]	100±6.58[a]

Means with same superscripted alphabet across rows are not statistically different (P>0.05).
Values are expressed as means±SE (standard error of means).

A: unsterilized sugarcane bagasse

B: sterilized sugarcane bagasse

C: sterilized sugarcane bagasse inoculated with *Alternaria* species

D: sterilized sugarcane bagasse inoculated with *Microsporum* species

E: sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

Appendix III:Removal of Cd by experimental sorbents as influenced by time

Contact time(hrs)	% of Cd removed by sorbents				
	A	B	C	D	E
1	99.6±0.34[a]	99.9±0.34[a]	100±0.34[a]	100±0.34[a]	100±0.34[a]
2	99.2±0.34[a]	99.1±0.34[a]	99.5±0.34[a]	99.4±0.34[a]	99.3±0.34[a]
3	98.8±0.34[a]	98.8±0.34[a]	99.1±0.34[a]	99.0±0.34[a]	99.0±0.34[a]
4	98.7±0.34[a]	98.5±0.34[a]	98.6±0.34[a]	98.7±0.34[a]	98.6±0.34[a]
5	98.6±0.34[a]	98.5±0.34[a]	98.3±0.34[a]	98.4±0.34[a]	98.4±0.34[a]
6	98.2±0.34[a]	98.3±0.34[a]	98.4±0.34[a]	98.6±0.34[a]	98.3±0.34[a]

Means with same superscripted alphabet across rows are not statistically different (P>0.05).
Values are expressed as means±SE (standard error of means).

A: unsterilized sugarcane bagasse

B: sterilized sugarcane bagasse

C: sterilized sugarcane bagasse inoculated with *Alternaria* species

D: sterilized sugarcane bagasse inoculated with *Microsporum* species

E: sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species.

Appendix IV: Quantity of Pb adsorbed by experimental sorbents as influenced by time

Contact time(hrs)	Quantity of Pb adsorbed by sorbents				
	A	B	C	D	E
1	1.20±0.10[a]	1.27±0.10[a]	1.05±0.10[a]	1.18±0.10[a]	1.21±0.10[a]
2	1.10±0.10[a]	1.07±0.10[a]	1.05±0.10[a]	1.18±0.10[a]	1.03±0.10[a]
3	0.10±0.10[a]	1.05±0.10[a]	0.93±0.10[a]	1.09±0.10[a]	1.00±0.10[a]
4	0.010±0.10[a]	0.95±0.10[a]	0.73±0.10[a]	0.90±0.10[a]	0.84±0.10[a]
5	0.10±0.10[a]	0.92±0.10[a]	0.73±0.10[a]	0.90±0.10[a]	0.84±0.10[a]
6	0.78±0.10[a]	0.66±0.10[a]	0.61±0.10[a]	0.75±0.10[a]	0.70±0.10[a]

Means with same superscripted alphabet across rows are not statistically different (P>0.05).
Values are expressed as means±SE (standard error of means).

A: unsterilized sugarcane bagasse

B: sterilized sugarcane bagasse

C: sterilized sugarcane bagasse inoculated with *Alternaria* species

D: sterilized sugarcane bagasse inoculated with *Microsporum* species

E: sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

Appendix V:Quantity of Cr adsorbed by experimental sorbents as influenced by time

	Quantity of Cr adsorbed by sorbents				
Contact time(hrs)	A	B	C	D	E
1	10.65±1.29[a]	10.79±1.29[a]	11.05±1.29[a]	10.61±1.29[a]	10.33±1.29[a]
2	10.28±1.29[a]	9.99±1.29[a]	10.43±1.29[a]	9.95±1.29[a]	9.63±1.29[a]
3	9.57±1.29[a]	9.38±1.29[a]	9.79±1.29[a]	9.33±1.29[a]	8.79±1.29[a]
4	8.31±1.29[a]	8.34±1.29[a]	9.26±1.29[a]	8.75±1.29[a]	7.83±1.29[a]
5	5.20±1.29[a]	5.46±1.29[a]	7.41±1.29[a]	6.13±1.29[a]	4.69±1.29[a]
6	4.148±1.29[a]	5.15±1.29[a]	7.41±1.29[a]	6.13±1.29[a]	4.69±1.29[a]

Means with same superscripted alphabet across rows are not statistically different (P>0.05).
Values are expressed as means±SE (standard error of means).

A: unsterilized sugarcane bagasse

B: sterilized sugarcane bagasse

C: sterilized sugarcane bagasse inoculated with *Alternaria* species

D: sterilized sugarcane bagasse inoculated with *Microsporum* species

E: sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

Appendix VI:Quantity of Cd adsorbed by experimental sorbents as influenced by time

	Quantity of Cd adsorbed by sorbents				
Contact time(hrs)	A	B	C	D	E
1	1.89±0.03[a]	1.90±0.04[a]	1.9±0.18[a]	1.90±0.18[a]	1.9±0.01[a]
2	1.88±0.01[a]	1.88±0.03[a]	1.89±0.23[a]	1.89±0.94[a]	1.89±0.29[a]
3	1.86±0.05[a]	1.86±0.12[a]	1.87±0.17[a]	1.87±0.28[a]	1.87±0.21[a]
4	1.83±0.01[a]	1.83±0.19[a]	1.85±0.09[a]	1.84±0.15[a]	1.84±0.38[a]
5	1.81±0.03[a]	1.81±0.03[a]	1.82±0.25[a]	1.81±0.19[a]	1.81±0.41[a]
6	1.77±0.18[a]	1.77±0.15[a]	1.79±0.09[a]	1.79±0.31[a]	1.78±0.39[a]

Means with same superscripted alphabet across rows are not statistically different (P>0.05).
Values are expressed as means±SE (standard error of means).

A: unsterilized sugarcane bagasse

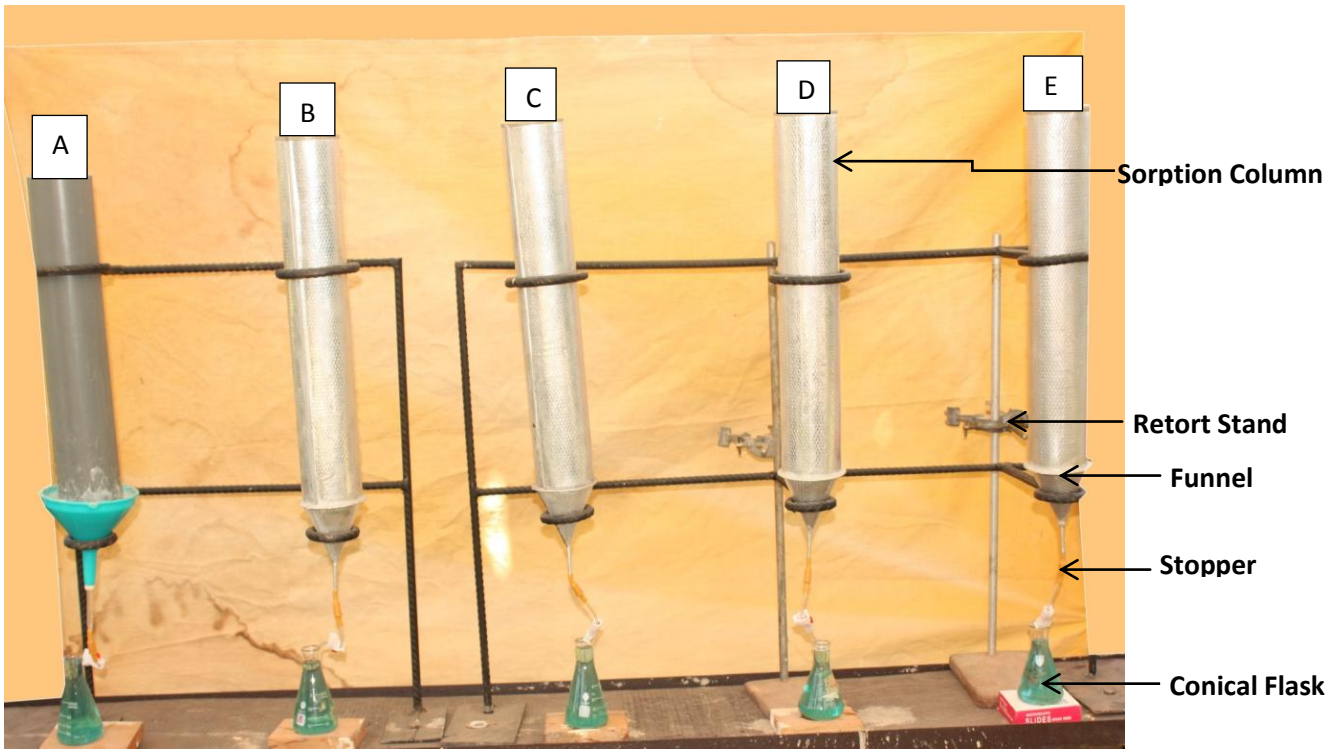
B: sterilized sugarcane bagasse

C: sterilized sugarcane bagasse inoculated with *Alternaria* species

D: sterilized sugarcane bagasse inoculated with *Microsporum* species

E: sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

Appendix VII: Experimental setup used for sorption of heavy metals



Source: Modified method of Machido *et al.*, 2015

Key:

A: unsterilized sugarcane bagasse

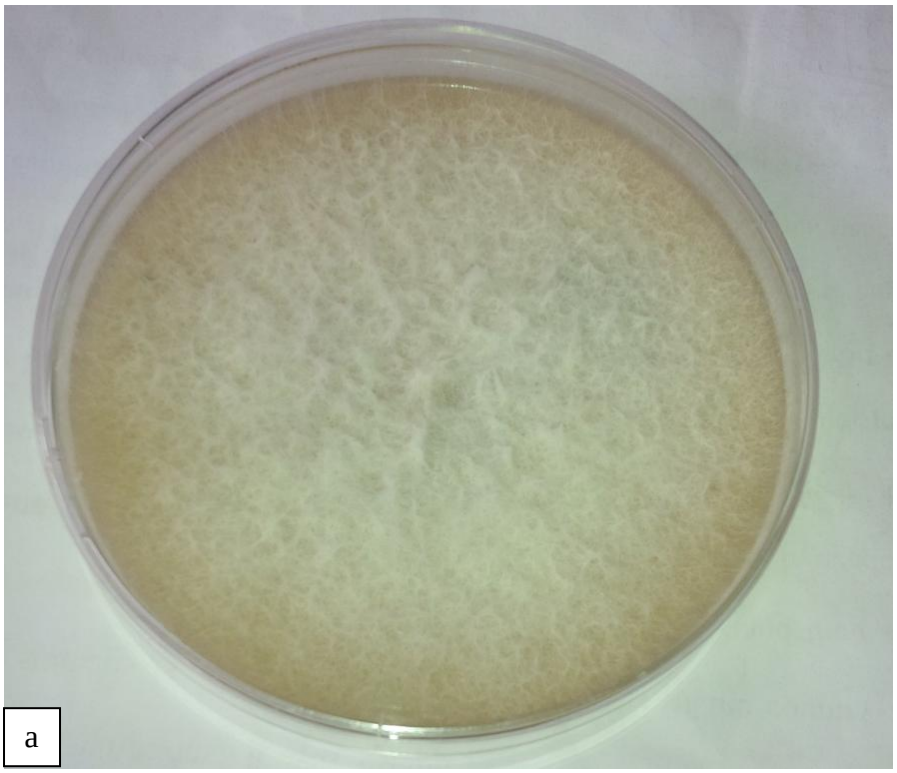
B: sterilized sugarcane bagasse

C: sterilized sugarcane bagasse inoculated with *Alternaria* species

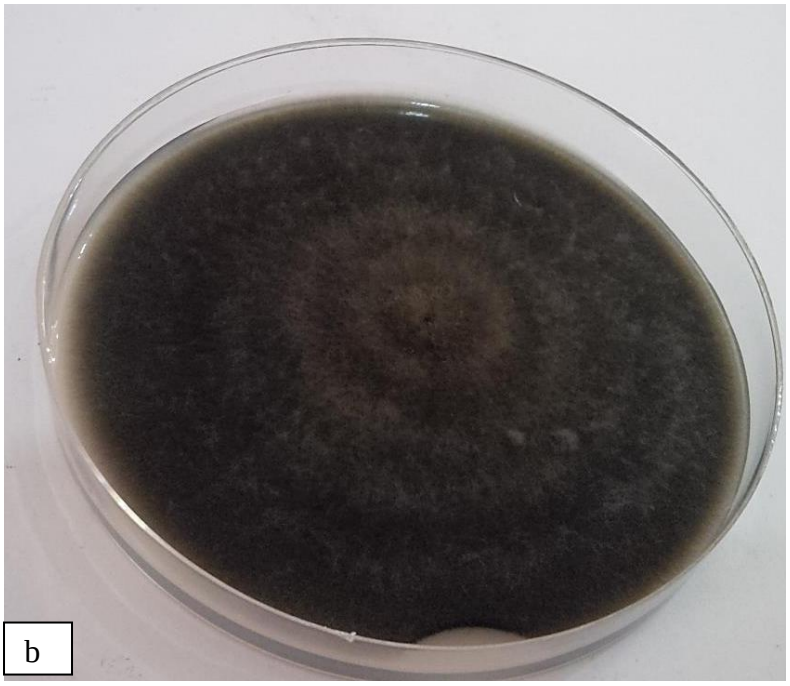
D: sterilized sugarcane bagasse inoculated with *Microsporum* species

E: sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species

Appendix VIII: Plates showing pure cultures of (a) *Microsporium* and (b) *Alternaria* species on PDA

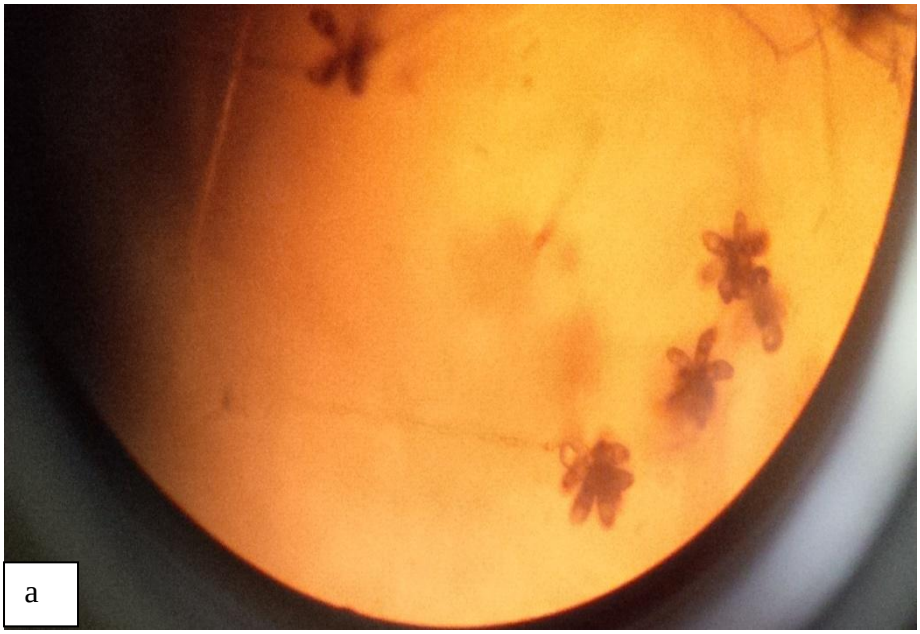


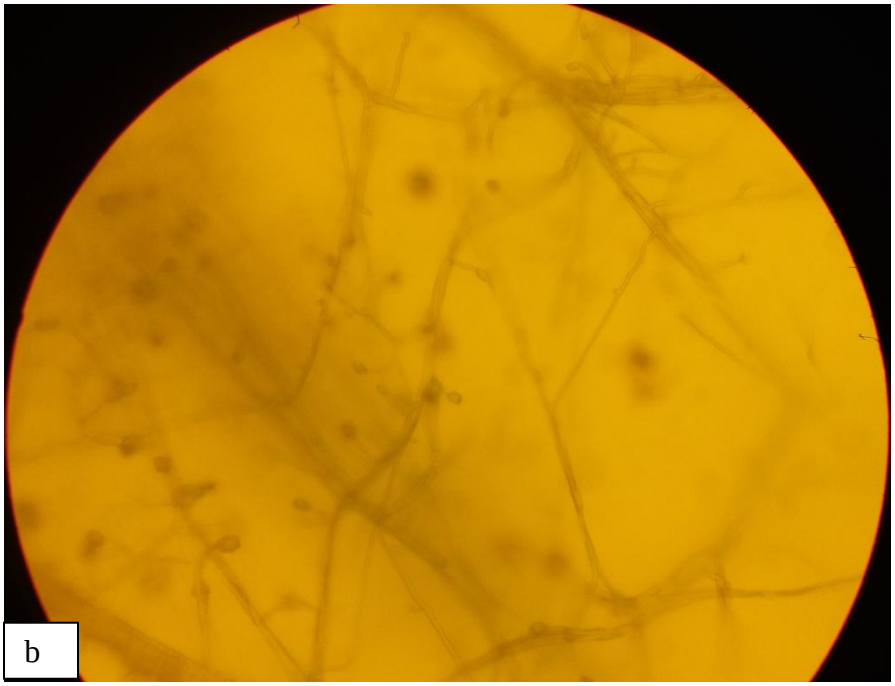
a



b

Appendix IX: Microscopic view of a three day old slide culture of (a) *Alternaria*and (b) *Microsporum*species at ×40 objective





Appendix X: Sugarcane bagasse (a) Un-milled (b) milled



a



b