REMOVAL OF HEAVY METALS FROM REFINERY EFFLUENT USING SUGARCANE BAGASSE INOCULATED WITH ASPERGILLUS FLAVUS AND TRICHOPHYTON SPECIES

BY

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AUGUST, 2017

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BY

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P13SCMC8039

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,

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AUGUST, 2017

DECLARATION

I hereby declare that the work in this dissertation entitled "Removal of heavy metals from refinery effluent using sugarcane bagasse inoculated with *Aspergillus flavus* and *Trichophyton* species" 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 Prof. H. I. Inabo. 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.

Murjanatu Abdullahi MUHAMMAD ________________ ____________

Signature Date

CERTIFICATION

This dissertation entitled "REMOVAL OF HEAVY METALS FROM REFINERY EFFLUENT USING SUGARCANE BAGASSE INOCULATED WITH *ASPERGILLUS FLAVUS* AND *TRICHOPHYTON* SPECIES" by Murjanatu Abdullahi MUHAMMAD, 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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DEDICATION

This research work is dedicated to Almighty Allah the One and Only God, and His Prophet Muhammad S. A. W., my late father, Malam Muhammad Abdullahi (May you continue to rest in Allah's eternal peace and mercy) and to the service of humanity.

ACKNOWLEDGEMENTS

To Almighty Allah, my Creator and Provider, Who saw me through all the hurdles and gave me the strength to see this work to the end.

I wish to express my special appreciation and gratitude to my honorable supervisors, Prof. D.A. Machido and Prof. H.I. Inabo, for their tremendous and relentless efforts in making this research work a reality, you have been great mentors I would forever keep in my memory. I am also grateful to the postgraduate coordinator, Prof. M. Aminu-Mukhtar, the Head of Department of Microbiology, Prof. I.O. Abdullahi, and to all my lecturers in the Department of Microbiology, Ahmadu Bello University, Zaria. To the people in the various labs, Mallam Shittu, M. Danlami, Mr. Onuh, I say a big thank you. This work would not have been easier without your help and support.

My sincere love and appreciation also goes to my friends and senior colleagues, Mrs. Munirah Shaibu Ahmed of Microbiology Department, and Mrs. Mercy Otori, for your selfless support and encouragement.

My deepest heartfelt gratitude to my mum and my brothers Ibrahim M. Abdullahi, Zaynul-Abideen M. Abdullahi, Ma-amoon M. Abdullahi, and my beloved niece Hajara (Ummi) Muhammad, and the rest of my family members for all their sacrifices and patience which has brought me this far.

My special thanks to my beautiful friends and roommates: Donna Bala Tige (My Darling daughter), Maimuna Muhammad Ahmad, Mariam Ocheja, Munirah Ibrahim Gebi, Ruqaiya Imran, Hasnah Baba, Madam Emilia Okafor, Mary Okobia (my special editor), Maggie and Stella. I am grateful for your assistance towards my peaceful and enjoyable stay in school and towards the success of my work. To my wonderful colleagues and friends, Linda (Zulai) Ameh (My special tutor), Saleem Abubakar Charanchi (My lil brother), Hussaini Ibrahim, Fatima Muhammad Jami'u, Zainab Abubakar, Zainab Azeez, and Titilayo Mansurat, the Departmental work and laboratory couldn't have been easy and fun without you. I am so grateful for your kind advice, meaningful contributions and help.

To all those who contributed to the success of my academic pursuit and to this research work in whichever way, thank you so much and may Almighty Allah continue to bless you and your families.

ABSTRACT

Physicochemical properties of most refinery effluents have been reported to consist of a variety of hazardous substances including heavy metals, which pose a threat to the ecosystem. Studies were carried out with the aim of testing the ability of two fungal isolates, namely *Trichophyton* species and *Aspergillus flavus* in removal of Nickel (Ni), Mercury (Hg) and Arsenic (As) from refinery effluent, with sugarcane bagasse serving both as growth medium and as a component of the experimental biosorbents. The physicochemical properties of the effluents were determined following standard procedures. The Proximate composition of the sugarcane bagasse as well as its ability to support growth of the *Aspergillus flavus* and *Trichophyton* species were determined. The performances of the experimental sorbents in the removal of Ni, Hg and As were also determined using fixed bed sorption columns. It was observed that the raw refinery effluent was highly turbid (111NTU) with high electrical conductivity (717µS/cm) and chemical oxygen demand (874.10mg/l). The levels of Nickel (1.149mg/l) and Mercury (484.780mg/l) were also found to be higher than permissible limits, while Arsenic was not detected. It was also noted that the sugarcane bagasse consists chiefly of cellulose (46.5%), hemicellulose (23.61%) and lignin (21.4%) with capacity to support luxuriant growth of *Trichophyton* sp. and *Aspergillus flavus*. The optimum time of Nickel and Mercury uptake by all the treatments was observed to be 1hr after the start of the experiment, and equilibrium was reached between 5-6hrs and at 4hrs for Nickel and Mercury adsorption respectively. The study also showed that both fungal isolates as well as the sugarcane bagasse had the ability to remove Nickel and Mercury ions from refinery effluent with more than 90% efficiency. The unsterilised and un-inoculated sugarcane bagasse removed more Mercury ions (97.6%) than the fungal cultures, while in Nickel removal; the sterilised sugarcane bagasse inoculated with *Aspergillus flavus* had the highest performance (97.1%). It was therefore concluded that the refinery effluent was highly polluted and hazardous to the environment and aquatic organisms, thus, improved treatment is required before releasing into receiving water. Also, sugarcane bagasse is a suitable medium for growth of fungi as well as removing heavy metal ions from refinery effluent. Data analysis carried out using one-way Analysis of Variance showed that there was a significant difference between the performance of the sorbents in the removal of Ni ions only ($p=0.00$); while no marked difference was observed statistically ($p>0.05$) between the performance of un-inoculated sugarcane bagasse in removing and adsorbing the heavy metal ions from solution with that of the sugarcane bagasse inoculated

with the test fungal isolates used in this study both separately and as co-culture, as each of the sorbents proved to be very efficient.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The presence of heavy metals and many other toxic organic compounds in waste effluents from refineries and other petrochemical industries have been widely reported (Sulemanov, 1995; Zhu *et al.,* 2001; Bako *et al.,* 2002; Jardao *et al.,* 2002; Van-Hamme *et al.,* 2003; Beddri and Ismail, 2007; Essiet *et al.,* 2010; Atubi, 2011; Adewuyi and Olowu, 2012; Galadima and Garba, 2012; Machido *et al*., 2014; Marcus and Ekpete, 2014). Investigations have also revealed that physicochemical parameters of these effluents measured were far and above the World Health Organisation (WHO) maximum permissible limits, such as in River Romi in Kaduna, and River Delimi in Jos (Galadima and Garba, 2012; Lekwot *et al.,* 2012; Sabo *et al*., 2013), and hence, constitute a threat to the environment (Alluri *et al*., 2007; Varsha *et al*., 2010; Mudhoo *et al*., 2011; Marcus *et al*., 2013; Ho *et al*. 2014) and human health (Howard, 2002; Tokar *et al.,* 2011; Silins and Högberg, 2011; Ramzan *et al.,* 2011; Galadima and Garba, 2012; Lekwot *et al.,* 2012; Chikogu *et al.,* 2012; Ho *et al.,* 2014). It is a known fact that polluted effluents are continuously released in large quantities in water bodies (Adewuyi and Olowu, 2012; Galadima and Garba, 2012; Lekwot *et al.,* 2012), the situation made worse by the inefficiency and high cost of conventional methods of treatment used in removing heavy metal ions from such effluents (Bishnoi and Garima, 2005; Iqbal *et al.,* 2005; Otokunefor and Obiukwu, 2005; Ezzouhri *et al.,* 2009; Lekwot *et al.,* 2012).

As a result, several attempts have been made in the past twenty years to devise efficient, cost effective and environmentally friendly methods that could be used in treating waste effluents emanating from petroleum related industries before they are released into the environment.

Adsorption has been found to be the most effective and versatile technique for heavy metals removal even at very low concentrations (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011; Dhokpande and Kaware, 2013; Lakherwal, 2014; Gunatilake, 2015), thus, resulting in the extensive use of natural materials mostly in form of agricultural and industrial wastes that are available in the environment in large quantities (Soliman *et al*., 2011; Dhokpande and Kaware, 2013). Various biological materials have been found to have metal binding capacities, hence, use of biosorption in separating heavy metals from wastewater (Bishnoi and Garima, 2005; Dhokpande and Kaware, 2013; Lakherwal, 2014). Thus, many efforts were directed at assessing the potential of extremely large pool of readily available and cheap biomaterials for use as sorbents in removal of heavy metals from industrial effluents (Ahluwalia and Goyal, 2007; Dhokpande and Kaware, 2013) and many have proven to be very effective with above 90% removal (Dhokpande and Kaware, 2013). Several researches have been carried out to test a good number of biomass types for their capabilities to bind metal ions under various conditions, and such works have been reported by Martin and Ayele (2002), Bishnoi and Garima (2005), Sharma *et al.* (2006), Ahluwalia and Goyal (2007) and Dhokpande and Kaware (2013). A wide range of effective adsorbents that include agricultural wastes, various algae, bacteria, fungi and other biomass have also been reported (Dhokpande and Kaware, 2013; Ezeonuegbu *et al.,* 2014; Kumar *et al.,* 2015; Machido *et al*., 2016). However, the major challenge to biosorption technology remains the selection of the most promising type of biomass from the wide range of types available.

The biosorption process has some advantages over conventional methods for removing toxic metals from industrial effluents which include: 1) low operating cost; 2) minimization of the volume of chemical and/or biological sludge to be disposed of; 3) high efficiency in detoxification of much diluted effluents, and 4) no nutrient requirements. These advantages have served as the primary incentives for developing full-scale biosorption processes to clean up heavy metal pollution (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011).

Biomass of fungal origin have so far proved to be the most promising and interesting out of the several biomass that have been tested, which is why most researches have been carried on them (Volesky and May, 1995; Niyogi and Ramakrishna, 1998; Sudha and Abraham, 1998; Fernandes and Nazareth, 1999; Tang and Cheng, 2003; Bishnoi and Garima, 2005; Dhokpande and Kaware, 2013; Rao and Bhargavi, 2013). Practically, biosorbents obtained from fungal biomass are packed in sorption columns which are effective devices that will continuously remove heavy metals (Kratochvil and Volesky, 1998). This is done by passing the effluent containing heavy metals over the loaded sorbent material, and the metals are taken up from the liquid by the biosorbent (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011; Dhokpande and Kaware, 2013). This has been shown to be achievable by means of ion exchange process in the sorption column (Dhokpande and Kaware, 2013; Machido *et al*., 2016).

1.2 Statement of Research Problem

Reports by Galadima and Garba (2012), Lekwot *et al*. (2012) and Lakherwal (2014) showed that industries such as tanneries, textile factories, petroleum refineries and other petrochemical industries produce large volumes of effluents containing wide range of toxic heavy metals.

These heavy metals are often at much higher levels that are considered dangerous to the environment and human health (Jarup, 2003; Galadima and Garba, 2012; Machido *et al*., 2014; Ezeonuegbu *et al*., 2015; Machido *et al*., 2016). This is a serious cause for concern to both environmental and public health authorities (Gunatilake, 2015).

Most of the conventional methods available for the treatment of such wastes have been shown to be inefficient in the removal of these metal ions especially at low concentrations (Iqbal *et al*., 2005; Otokunefor and Obiukwu, 2005; Ezzouhri *et al*., 2009). In addition, these methods are costly and above all, less environmentally friendly (Machido *et al*., 2016). There is therefore, the need to search for more efficient, cheaper and more environmentally friendly method by which toxic heavy metal ions could be removed from waste effluents prior to their discharge into the environment.

1.3 Justification

Reports of earlier investigations have indicated the potential of biomass in the removal of heavy metal ions from dilute solutions as are found in waste effluents from industries, and the most complete summary of metal biosorption results has been published. There is, therefore, the need to investigate the potential of some of the many available biomass such as sugarcane bagasse as sorbent for the removal of heavy metal ions from refinery waste effluents. Use of sugarcane bagasse as adsorbent and source of nutrient for growth of the fungal biomass would help to reduce the nuisance caused by this material to the environment, as it constitutes a major source of waste.

Although not all potentially applicable biosorbents have been systematically examined as yet, a substantial body of evidence has been collected which identifies ion exchange as the principal

mechanism of metal biosorption. Since sorption of heavy metals onto these biomaterials is attributed to their constituents, mainly proteins, carbohydrates and phenolic compounds which contain functional groups, such as carboxyl, hydroxyl and amine, capable of binding the metal ions, it is conceivable that partial decomposition of sugarcane bagasse by fungi could enhance its capacity to bind and remove heavy metal ions from solution. This enhancement could be brought about by generating additional reactive functional groups on the surfaces of the sugarcane bagasse particles and providing substrate for the growth and accumulation of fungal biomass which would serve as additional sorbent.

1.4 Aim and Objectives

The aim of this study was to remove Nickel, Mercury and Arsenic from raw refinery effluent using sugarcane bagasse inoculated with *Aspergillus flavus* and *Trichophyton* species.

The specific objectives of this study were to:

1. Determine the physicochemical properties of the refinery effluents.
2. Determine the proximate composition of the sugarcane bagasse.
3. Determine the capacity of the sugarcane bagasse to support the growth of *Aspergillus flavus* and *Trichophyton* sp.
4. Determine the effect of inoculation of fungal species *Aspergillus flavus* and *Trichophyton* sp. separately and as co-culture on the performance of the sugarcane bagasse in the removal of Ni^{2+}, Hg^{2+} and As^{2+} ions from refinery effluent.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wastewater

Wastewater is defined as the used water released from residence, commercial and industrial establishments, such as petroleum refineries and other industries (International Petroleum Industry Environmental Conservation Association –IPIECA, 2010) together with such groundwater, surface water and storm water as may be present (Boyd, 2000; Roefer *et al*., 2000). Refineries and petrochemical industries can generate a significant amount of wastewater that has been in contact with hydrocarbons. This wastewater is also known as effluent, and it is made up of both process and non-process wastewater (IPIECA, 2010). Refinery or industrial effluents released often contain a considerable amount of large quantity of crude oil products, polycyclic and aromatic hydrocarbons, phenols, heavy metal derivatives, surface active substances, sulphides, naphthalene acids and other chemicals (Atubi, 2011; Diya'uddeen *et al*., 2011). The uncontrolled disposal of untreated refinery effluent into water bodies renders the water unsafe for use, as well as poses a lot of health hazards for humans, animals and the ecosystem in general (Järup, 2003; Martin and Griswold, 2009; Essiet *et al*., 2010; Atubi, 2011; Diya'uddeen *et al*., 2011; WHO, 2011; Galadima *et al*., 2012; Machido *et al*., 2014; Kumar *et al*., 2015). This makes it necessary for such effluent to undergo treatment before it is released into water bodies (Machido *et al*., 2014).

2.1.1 Methods of Wastewater Treatment

So many methods are used for treating industrial wastewater. The most common methods are the conventional methods used to remove toxic wastes from industrial effluents as mentioned by

Alluri *et al*. (2007), IPIECA (2010), Diya'uddeen *et al*. (2011), and Gunatilake (2015) include thermal and catalytic cracking, oily wastewater treatment, precipitation and coagulation, reverse osmosis, elecrodialysis, ultrafilteration, photocatalysis, etc. Other physicochemical means of refining wastewater include complexation, solvent extraction, ion exchange, foam floatation, cementation, activated carbon adsorption, electrodeposition and membrane operations (Gunatilake, 2015). These treatment processes according to Diya'uddeen *et al*. (2011) are composed of three main stages namely: primary, secondary and tertiary treatment processes. Heavy metal removal from contaminated industrial wastewater is specifically achieved by electrodialysis, reverse osmosis, ultrafilteration and photocatalysis (Gunatilake, 2015). Biological methods are also employed in the removal of toxic substances from effluents which include the use of microorganisms for settling solids in the solution (Gunatilake, 2015). Other conventional methods such as activated sludge, trickling filters and also the use of stabilisation ponds are widely used (Diya'uddeen *et al*., 2011; Gunatilake, 2015). Diya'uddeen *et al*. (2011) explained elaborately the method of photocatalytic degradation- its principles and parameters as a major and successful method of treating petroleum refinery effluent.

The above conventional methods proved effective to a certain extent only (Ezzouhri *et al*., 2009) and their efficiency is drastically reduced (Gunatilake, 2015) which leads to incomplete processing, as well as produce toxic sludge which would further be more hazardous to the environment (Dhankar and Hooda, 2011; Gunatilake, 2015). This may be due to poor economic and national development (Ezzouhri *et al*., 2009), as most of these industries are centered towards making profits rather than caring for the environment (Lokhande *et al*., 2011). In addition, some of the industries lack adequate facilities as well as trained staff that would carry out these processes efficiently.

2.2 Heavy Metals

Heavy metals are defined as those metals found in all groups in the periodic table, except the metals of group 1 and 2 (Volesky and May, 1995; Dhankar and Hooda, 2011). However, most authors define heavy metals in terms of density and specific gravity (Duffus, 2002), their atomic mass (Rand *et al*., 1995) or atomic number (Lyman, 1995), or even in terms of toxicity (Järup, 2003; Dhankar and Hooda, 2011; Lakherwal, 2014; Gunatilake, 2015). Heavy metals have also been defined by Järup (2003), Martin and Griswold (2009), Lakherwal (2014) and Gunatilake (2015) as elements that have atomic weight within the range of 63.5-200.6; and a specific gravity greater than 5.0g/cm^3. Heavy metals are also metals which occur as natural constituents of the earth crust and are persistent environmental contaminants (Galadima and Garba, 2012), as they cannot be degraded by biological or chemical processes (Alluri *et al*., 2007; Galadima and Garba, 2012; Lakherwal, 2014). They also tend to bioaccumulate and permanently become part of the environment when they are introduced from industrial sources (Dhokpande and Kaware, 2013).

There are many examples of heavy metals in nature and particularly in industrial wastewater. These include: Lead (Pb), Chromium (Cr), Mercury (Hg), Uranium (U), Selenium (Se), Zinc (Zn), Arsenic (As), Cadmium (Cd), Silver (Ag), Gold (Au) Nickel (Ni), Iron (Fe), (Alluri *et al*., 2007; Dhankar and Hooda, 2011; Galadima and Garba, 2012; Lakherwal, 2014), Copper (Cu), Tin (Sn), Molybdenum (Mo), Cobalt (Co), Manganese (Mn), Titanium (Ti) and Aluminium (Al) (Gunatilake, 2015). Other heavy metals such as Scandium (Sc) and Platinum (Pt) have also been reported (Lakherwal, 2014) but are not of major significance as the others mentioned above. These metals have been and are still studied and their effects constantly accounted for by various

national and international bodies, which are concerned with public health issues (Järup, 2003; Lakherwal, 2014).

2.2.1 Occurrence and Sources of Heavy Metals in the Environment

Heavy metals occur in low concentrations when found in natural aquatic ecosystems, mainly obtained from weathering of soils and their associated bedrocks (Sabo *et al*., 2013). Generally, heavy metals are introduced into the environment and bodies of living things through contaminated air, water, food or soil from various sources (Ayodele and Abubakar, 2001; Ibeto and Okoye, 2010). In recent times, an unprecedented increase in level of these heavy metals is brought about by human activities such as: mining operations resulting in the formation of acid mine drainages (Sabo *et al*., 2013); production of waste solutions from electroplating industries which release heavy metals such as Cadmium (Cd), Zinc (Zn), Lead (Pb), Chromium (Cr), Nickel (Ni), Copper (Cu), Vanadium (V), Platinum (Pt), Silver (Ag) and Titanium (Ti) (Alluri *et al*., 2007; Sabo *et al*., 2013; Gunatilake, 2015); enormous generation of coal from coal-based power generations and also nuclear power generation resulting from special waste generation and processing; as well as Uranium (U) mining, serving as source of Uranium wastes (Dhankar and Hooda, 2011; Sabo *et al*., 2013; Gunatilake, 2015). Other industrial activities that result in releasing wastes containing heavy metals include wood-processing industries which produce Arsenic-containing waste from a chromated Copper-Arsenate wood treatment (Gunatilake, 2015), inorganic pigment manufacturing industries which deposit Cadmium and Chromium compounds (Alluri *et al*., 2007), petroleum refining industries which generate conversion catalysts contaminated with Nickel (Ni), Vanadium (V) and Chromium (Cr), as well as photographic operations that produce films with high concentrations of Silver (Ag) and Ferrocyanide (Adesina and Adelasoye, 2014; Gunatilake, 2015).

2.2.2 Uses of Heavy Metals

Heavy metals are essentially very useful in many aspects, especially in many industrial activities. Some of them are used as trace elements which are essential in maintaining metabolic activities in the body of living things (e.g. Nickel, Iron, Copper, Zinc and Selenium), as mentioned by Järup (2003). They are also known to be useful in making of antiseptics, self-cleaning ovens, golf clubs, cars, plastics, solar panels, mobile phones, particle accelerators, nuclear reactors, electronic devices, weapons, etc (Galadima and Garba, 2012). Adeyemi (2009) also stated some of the uses of these heavy metals such as treatment of wood (timber), production of pesticides, glass manufacturing as well as in the production of pharmaceutical and non-ferrous alloys. Other uses of heavy metals include production of batteries, roofing sheets, pipes, cables and electric wires, ammunition (e.g. bullets), solder and aluminium foils used in wrapping food items (Galadima and Garba, 2012).

2.2.3 Toxicity of Heavy Metals

Heavy metals have been shown by many researches to be toxic and harmful when introduced into human, animal or plant bodies (Gunatilake, 2015), due to their non-degradable nature, hence, are persistent environmental contaminants (Alluri *et al*., 2007; Essiet *et al*., 2010; Galadima and Garba, 2012; Dhokpande and Kaware, 2013; Kumar *et al*., 2015). Some of them when introduced into the living environment in small quantity can cause serious damage to tissues and systems of the living things (Galadima and Garba, 2012). The most hazardous threats of metals to public health are mostly encountered, according to Järup (2003) and Lakherwal (2014), from such heavy metals as Arsenic (As), Mercury (Hg), Lead (Pb) and Cadmium (Cd).

The threat posed by heavy metals is that they usually prevail in the ecosystem by a phenomenon known as bio-magnification, which occurs when they increase in concentration at every level of food chain and are passed onto the next higher level (Alluri *et al*., 2007). Some of the serious health effects associated with heavy metals as reported by Galadima and Garba (2012) and Gunatilake (2015), include cancer, organ damage, nervous system damage and in severe cases death may occur in humans if they are absorbed and accumulated in the body. In plants, stunted or poor growth and development may be resulted due to heavy metal accumulation in the tissues as observed by Essiet *et al*. (2010) Adesina and Adelasoye (2014), and Kumar *et al*. (2015).

The harmful effects of heavy metals as explained by Järup (2003) and Lakherwal (2014) may be in form of acute or chronic poisoning. The acute poisoning by heavy metals is the most dangerous and occurs due to exposure to large amounts of heavy metal at once. It is mostly fatal in children such as swallowing a toy made with a high quantity of a certain heavy metal, e.g. lead; or by consuming food obtained from a source highly contaminated by a heavy metal, e.g. mercury-contaminated fish (Järup, 2003). These could lead to a serious health effects such as confusion, numbness, nausea, vomiting, damages to cardiovascular and gastrointestinal systems, lungs, kidneys, liver inflammation, and failure to the central nervous system, which could lead to coma and in more severe cases, death may occur (Järup, 2003; Lakherwal, 2014). The chronic heavy metal poisoning occurs due to long-term exposure to lower levels of heavy metals Lakherwal, 2014). The symptoms may be severe and often less obvious, developing more slowly over time. These include headache, weakness, muscle and joint pains, constipation, fatigue and neurological damage (Järup, 2003), as well as pre-dispose the body to risk of cancer (Lakherwal, 2014).

The harmful effects of exposure to heavy metals both chronic and acute are wide and occur in different parts of the ecosystem and cannot be completely covered. This exposure to heavy metals is usually associated with their presence in soil, air and water (Galadima and Garba, 2012), which may result from their presence in large quantities in industrial wastes such as tanneries, refinery effluents, etc (Lakherwal, 2014; Machido *et al*., 2014).

For this particular research work, the heavy metals in focus are Nickel (Ni), Mercury (Hg) and Arsenic (As).

2.2.4 Nickel

This is a silvery-white, hard, malleable, ductile and lustrous metal with a slight golden tinge that takes a high polish, and is also a fairly good conductor of heat and electricity (Commission on Isotopic Abundances and Atomic Weights (CIAAW), 2013). It is a chemical element that belongs to the group 10 transition elements (Kittel, 1996).

Nickel was first discovered and classified as a chemical element in 1751 by Axel Fredrik Cronstedt (Kerfoot, 2000), and it occurs in tiny amounts in the earth's crust, usually in ultramafic rocks mainly obtained by mining processes. It has atomic number, molecular weight and atomic density of 28, 58.71g/mol and 8.9g/cm^3 at 20°C respectively; with a melting and boiling point of about 1,455^{o}C and 2913°C respectively (Kittel, 1996; Sigel *et al*., 2008). Rasmussen *et al*. (1998) mentioned that Nickel has great industrial uses, due to its slight magnetic properties, and also forms a lot of complex compounds, most of which are blue or green (e.g Nickel hydrochloric acid) in colour (Greenwood and Earnshaw, 1997; Housecroft and Sharpe, 2012). The content in soil can be as low as 0.2mg/ml or as high as 450mg/ml in some clay and loamy soils, and the average is around 20mg/ml (Sigel *et al*., 2008; Galadima and Garba, 2012). Tea leaves have also

been reported by Kuck (2006) to be another rich source of Nickel, whereby dried tea leaves may contain about 7.6mg of the element per kg. Nickel is mined mostly in countries like Russia, Australia, New Caledonia, Cuba, Canada and South Africa (Sigel *et al*., 2008).

Uses of Nickel date for as far back as 3500BCE (Rosenberg, 1968), and in modern times, it is used chiefly in alloys as it is known to be an excellent alloying agent used for making different types of metal alloys such as Meteorites, Pentlandites, Kamacite, Taenite, Millerite, Niccolite, Nickel-glance, etc. (Rasmussen *et al*., 1998). These alloys are mainly characterized by strength, ductility resistance to corrosion and heat (CIAAW, 2013). It is also used largely in producing stainless steel (about 65%) (Kerfoot, 2000; Kamerud, 2013; Buttice, 2015), electroplating, making detergents, as hydrogenating agent in some chemical reactions, as well as in making rechargeable batteries and jewelries (Davis, 2000; Kuck, 2016). In biological systems, Nickel serves as an essential nutrient for some microorganisms and plants (Rajendran *et al*., 2003) where it activates certain enzymes (Sigel *et al*., 2008; Sydor and Zamble, 2013). For example, the enzyme urease found in plants, hydrogenases, methyl coenzyme M reductase in methanogenic Archaea (Ragsdale, 2014), as well as a rare class of bacterial dismutase (Szilagyi *et al*., 2004).

Nickel also has some harmful effects, which constitute a threat to the plant, animal, human and environmental health. Adesina and Adelasoye (2014) explained that Nickel constitutes one of the major soil pollutants, which results in a reduction in plant quality and yield, as well as destruction of important soil microbes. Humans may be exposed to Nickel by breathing air, eating food or smoking cigarettes (United States Environmental Protection Agency (USEPA) report, 2011). Thysse *et al*. (2007) reported that skin contact with Nickel-contaminated soil or water may also result in exposure. Imminent accumulation of Nickel in body cells may result due

to consumption of food items like vegetables cultivated in soils polluted from Nickel-plated faucets, mining and smelting which may introduce Nickel into wastewaters, and also from Nickel-steel alloy cookware and Nickel- pigmented dishes which may release Nickel into food (Trumbo *et al*., 2001; Kamerud *et al*., 2013). Smokers also tend to have a higher Nickel uptake through their lungs due to considerable amount of the metal present in cigarettes. Skin contact with Nickel and Nickel compounds may result also from jewelry, shampoos, detergents and coins (Nestle *et al*., 2002; Kasprzak and Salnikow, 2003; Buttice, 2015). A less common form of chronic exposure to Nickel is through haemodialysis as traces of Nickel ions may be absorbed into the plasma from the chelating action of albumin (Buttice, 2015). The atmosphere may also be polluted by Nickel refining and fossil fuel combustion. Nickel is essential in small quantities, but when the uptake is too high it becomes dangerous to health (Dunnick *et al*., 1995).

Uptake of large quantity of Nickel can result in a number of health hazards which include development of cancer of the lungs, nose, larynx and prostate, sickness and dizziness after exposure, respiratory failure, birth defects, asthma and chronic bronchitis, allergic reactions such as skin rashes mainly from jewelry (Stellman, 1998; Kasprzak and Salnikow, 2003; Thysse *et al*., 2007), and heart disorders (Barceloux and Barceloux, 1999; Centre for Disease Control and National Institute for Occupational Safety and Health (CDC-NIOSH), 2015). Nickel fumes are reported to be respiratory irritants and may cause pneumonitis. Exposure to Nickel and its compounds may result in the development of a dermatitis known as “Nickel itch” in sensitized individuals (Thysse *et al*., 2007; American Academy of Dermatology, 2015; Nickel Producers Environmental Research Association (NiPERA), 2016), which, once acquired, persists indefinitely. Nickel and its compounds have also been listed by the United States National Toxicology Programme (NTP), the International Agency for Research on Cancer (IARC), and

other relevant organisations as being reasonably carcinogenic agents at different levels (Stellman, 1998).

Release of Nickel into the environment by power plants and trash incinerators results in the presence of the metal and its compounds in air (Buttice, 2015), where it settles after a long period of time to the ground or fall down after reactions with raindrops, which may also end up in surface water and becomes a part of wastewater streams (Kuck, 2016; Housecroft and Sharpe, 2012). High concentration of Nickel on sandy soils can clearly damage plants (Adesina and Adelasoye, 2014); while it diminishes the growth rates of algae in surface waters. It can cause various types of cancer in animals, especially those that live near the refineries (Galadima and Garba, 2012; Adesina and Adelasoye, 2014).

2.2.5 Mercury

Mercury is a heavy, silvery-white liquid metal (CIAAW, 2013; Senese, 2007). It is a chemical element denoted by the symbol Hg, commonly known as ‘Quicksilver’. It has atomic number of 80, atomic weight of 200.592g/mol, atomic density of 13.534g/cm^3, freezing, melting and boiling point of -38.83^{o}C, -234.3210K and -629.88K (356.73^{o}C) respectively (Norrby, 1991; Lide, 2006; Senese, 2007; CIAAW, 2013). It is a heavy metallic, block-d element of the transition metals series, found in period 6 and group 12 of the periodic table (Wang *et al*., 2007). Mercury is also surrounded in the periodic table by Cadmium from above, Cyanide from below, Gold from the left and Thallium from the right (Senese, 2007; CIAAW, 2013); and is the only known metal that commonly exists in liquid form under standard conditions of temperature and pressure, hence, it was formerly known as ‘Hydrargyrum’, which means ‘water-silver’ in Greek due to its similarity to water (liquid) and shiny form like Silver (Simons, 1968; Cox, 1997; Lide, 2006; Senese,

2007). It is a poor conductor of heat, but fairly conducts electricity (Lide, 2006; Wang *et al*., 2005). A decrease in volume is observed when in the frozen state, while the density increases from 13.534g/cm^3 to 14.184g/cm^3 as it changes from liquid to the solid state, in which it exhibits malleability and ductility (Simons, 1968). Mercury is an extremely volatile element, and exists in the +2, 1 and -2 oxidation states as is a property of the transition metals (Norrby, 1991; Lide, 2006; Senese, 2007; Wang *et al*., 2007).

The discovery of Mercury has not been specifically attributed to any individual in particular, but is said to have been discovered since around 1500BC in China, Tibet and also the Egyptian tombs (Stillman, 2003; Centre for Environmental Health Sciences, 2012). The Chinese were also said to believe then that the use of Mercury prolonged life (Liu *et al*., 2008; Centre for Environmental Health Sciences, 2012); and the metal was said to be named after the Roman god, Mercury, who was known for his speed and mobility (Stillman, 2003), and is also associated with the planet Mercury, because the astrological symbol of the planet is the same as that of the Alchemical symbol for the metal (Cox, 1997; Stillman, 2003). Stillman (2003) and Hesse (2007) stated that Mercury is the only element which has its planetary name same as its common name.

Mercury is an extremely rare element which occurs in the Earth's crust with an average crustal abundance of only 0.08mg/ml (Ehrlich and Newman, 2008), due to its failure to blend geochemically with those elements that constitute the majority of the crustal mass (Rytuba, 2003; Ehrlich and Newman, 2008). The richest ores of Mercury may contain up to 2.5% Mercury by mass, or the lowest of 0.1% Mercury, which is 12,000 times the average crustal abundance (Ehrlich and Newman, 2008). It rarely occurs freely in nature (Ehrlich and Newman, 2008); and is mostly found in form of ores like Cinnabar or Mercury Sulphides (HgS), which is th

e commonest ore of Mercury in nature, Corderoite, Livingstonite, as well as other ores which are found in hot springs or volcanic regions (Rytuba, 2003; Ehrlich and Newman, 2008). The metal is mined since 2500yrs ago in Almadin in Spain, Monte Amiata in Italy and Idrija in the present day Slovenia (Stillman, 2003; Hesse, 2007; Healey and Blainey, 2011; Centre for Environmental Health Sciences, 2012).

Mercury is a very useful metal that has been in use since ancient times, and is still used in our modern times for many purposes. It was used by the Romans and the ancient Egyptians in making cosmetics, while ancient Greeks used it in making ointments, and was greatly used in making amalgams or alloys since 500BC (Hesse, 2007; Healey and Blainey, 2011). Mercury was also reported by Lew (2008) to be used in the pyramids of ancient times Egypt, in decorating the tombs of their Emperors. The historical Islamic Spain was also reported to have abundant Mercury which was used to decorate the mosques (Lew, 2008).

In our modern times, Mercury is widely used in the industry. For example, it is used in manufacture of some chemicals, like chlorine and caustic soda (sodium hydroxide) (Commodity Research Bureau, 2000; Leopold, 2002), hydrogen, as well as metallic sodium (Wang *et al*., 2007), thermometers, Neon-sign lamps, Mercury-vapour lamps and fluorescent lamps in optical spectroscopy (Surmann and Zeyat, 2005); manufacture of pesticides and batteries, as well as in the paper industry (Alluri *et al*., 2007). Mercury is also used in liquid mirrors and transit telescopes in some laboratories (Gibson, 1991). Mercury (II) fulminate is used in producing primary explosives that are mainly used as a primer or cartridge in firearms (Galadima and Garba, 2012). Some cosmetic industries are also known to use Mercury in making skin-care products such as whitening agents and mascara (McKelvey *et al*., 2011; Galadima and Garba, 2012). It is also widely used in the field of medicine where mercuric compounds have been used

in dentistry for making dental amalgams (Leopold, 2002; Galadima and Garba, 2012), which were used to refill porous spaces in teeth. The use of these materials are however limited these days due to the awareness of the toxicity of the element. Parker *et al*. (2004), also stated some of the uses of Mercury in medicine such as the production of Thiomersal, an organic compound used in trace amount these days, to preserve vaccines e.g. the influenza vaccine, mostly administered to children. Mercury is also used in producing diuretic materials and also stimulant laxatives, eye-drops, nasal sprays, etc. Mebromine or Mercurochrome is also used to make topical antiseptics (Parker *et al*., 2004). The Chinese are also known to use cinnabar in their various traditional medicines in present times, even though this has not been fully established (Liu *et al*., 2008).

Other uses of Mercury include its use formerly in making Sphigmomanometers used for measuring blood pressure (Parker *et al*., 2004), in Frensel lenses (Pearson, 2008), in switches (Shelton, 2004), for cooling nuclear reactors and making space propulsion systems (Collier, 1987); and also as a component of the early used digital computers. Mercury has been identified as a very useful element that had been widely used for a long time in different aspects of life, especially in the middle of the 20th century.

The use of Mercury and its compounds, is however reduced to a greater extent in present times, and regulated in most countries due to the harmful effects associated with the element, such that a minute quantity can damage life (Galadima and Garba, 2012). This problem is essentially brought about by Mercury poisoning, which mostly occurs as a result of occupational exposures, contamination of rivers and agricultural farmlands with Mercury based chemicals as well as eating of exposed aquatic plants and animals like fishes (major source of methyl Mercury exposure), intoxicated meat and inhalation in air are also consistent contributing factors (Eisler,

2006; Galadima and Garba, 2012). Cinnabar is said to cause significant Mercury intoxication when heated or consumed in overdose, and can have adverse effects at the therapeutic doses (Liu *et al*., 2008). Some harmful effects of Mercury and its compounds mentioned by Alluri *et al*. (2007) include damage to the nervous system and protoplast poisoning when ingested above 0.01mg/ml. Gunatilake (2015) also reported that Mercury level above 0.00003mg/ml, can lead to a disorder known as Rheumatoid arthritis, and also diseases of kidneys and circulatory system. Mercury has also been included among a list of ten most hazardous chemicals which are of major public health concern (WHO, 2011).

2.2.6 Arsenic

Arsenic is a metallic, grey coloured, p-block element denoted by the symbol 'As' (CIAAW, 2013). It usually occurs as either yellow, grey or black solid, with the grey colour being the most commonly found (Norman, 1998). It is found in period 4, group 15 (pnictogens) of the periodic table, with atomic number 33, atomic weight of about 75g/mol (CIAAW, 2013), and a specific gravity of 5.7g/cm^3 (Adeyemi, 2009; Galadima and Garba, 2012). Galadima and Garba (2012) reported that pure form of Arsenic exists in two solid forms: yellow (with specific gravity of 1.97g/cm^3) and limetallic (specific gravity, 5.73g/cm^3). Arsenic can vaporise or turn to gaseous state (sublime) at a temperature of 614°C (Gokcen, 1989); and occupies the same group as Phosphorus (P) in the periodic table, as they are both found in the same column (Rieuwerts, 2015). Arsenic is found in between the element Germanium from the left, and Selenium from the right in the periodic table (CIAAW, 2013). It exists as a solid metalloid or semi-metal (Galadima and Garba, 2012), and occurs as a pure elemental crystal. It is found in many minerals, usually in combination with Sulphur and other metals (Rieuwerts, 2015).

The word Arsenic originated from the Syriac word ‘Al-zarniqa’ (Harper, 2010), from the Persian word ‘Zarnikh’, which means ‘Yellow’, or literally ‘gold-coloured’, and hence, was called ‘yellow orpiment’. It was later adopted into Greek as ‘Arsenikon’, or ‘Arsenikos’, (which means ‘male’ or ‘virile’). The Greek word ‘Arsenikos’ was adopted in Latin as Arsenicum, which is known as Arsenic in French, from which the English word Arsenic was taken (Harper, 2010). It was first isolated in the year 1250 by Albertus Magnus or Albert the Great, from soap heated with Arsenic trisulphide (Emsley, 2001). In 1649, Johann Schröder published two ways of preparing the metal.

Arsenic occurs naturally and abundantly in the Earth’s crust, and is the 53rd most abundant element found (Rieuwerts, 2015). It is present in more than 200 different minerals, the most common of which is called Arsenopyrite (FeAsS) which is an Arsenic sulphide, Realgar (As_2S_2), Orpiment (As_2S_3) and Arsenolite (As_4O_6), native Arsenic in ores of Copper, Lead, Cobalt, Nickel, Zinc, Silver, and Tin and also as nickel glance (NiAsS) or mispickel (Galadima and Garba, 2012); and usually exists as Arsenate, Arsenic pentoxide, Arsenic trichloride, Arsenico, Arsenicum album, Arsenite, etc (Adeyemi, 2009). Arsenic is chemically very similar to Phosphorus, such that it sometimes substitutes for it in biochemical reactions and is thus poisonous (Galadima and Garba, 2012). According to Adeyemi (2009), Arsenic is widely distributed and found at an average concentration of 2mg/kg in the Earth’s crust. About one-third of Arsenic found in the atmosphere is of natural origin, which is generated mostly from volcanic actions and Arsenic-containing vapour that is generated from solid or liquid forms of Arsenic salts at low temperature. (Green Facts, 2001). Elemental Arsenic is found in nature in form of crystals, although it is rare (Emsley, 2001). Inorganic Arsenic of geological origin is found in groundwater used for drinking in several parts of the world like Bangladesh, India Chile, China

and Taiwan (Järup, 2003; Adeyemi, 2009). Organic Arsenic compounds are mainly found in sea-living organisms, and a few of these found in species that live on land (Environmental Health Criteria, 2001).

Arsenic is usually introduced into the atmosphere by common anthropogenic sources such as mining, metal smelting use of Arsenic-containing agricultural chemicals, as well as weathering of Arsenic-containing rocks, such as Arsenopyrite, which lead to the contamination of air, water and soil (Adeyemi, 2009). It is released into the atmosphere primarily as Arsenic trioxide (AsO_3), and exists mainly adsorbed on dust particles, which are usually dispersed by wind and returned to the earth by rainfall, or dry deposition on the soil by other means (Environmental Health Criteria, 2001).

Arsenic is a very useful metal, and its uses date as far back as many centuries ago. It was known to be used frequently by rulers in ancient times to get rid of individuals they considered a threat to them, hence, it was prominently known then as 'King of Poisons' or 'Poison of Kings'(Vahidnia *et al*., 2007). It was used during the Bronze Age to form arsenical bronze, a very strong alloy of Arsenic with Bronze (Charles, 1967; Lechtman, 1996). Arsenic trioxide known as 'White Arsenic' was also mixed with vinegar and chalk to form a whitening agent that was used greatly by women in the Victorian era to improve their facial complexion, making their skin to look paler (Lechtman, 1996).

In modern times, it is still used in different areas such as agriculture, industry, medicine, and many other areas of life. It has been estimated that about 70% of the total world's Arsenic production is used in timber treatment as copper chrome arsenate for preservative purposes (Järup, 2003; Rahman *et al*., 2004), 22% in agricultural chemicals (pesticides) and the remainder

in glass, pharmaceuticals and non-ferrous alloys (Green Facts, 2001; Adeyemi, 2009; Galadima and Garba, 2012). It is a very important alloying agent, used in making very hard alloys, commonly with lead in producing car batteries and ammunition, and also as semi-conductor in electronic devices (Grund *et al*., 2005). Arsenic is also used as a dyeing agent, and some pigments of Arsenic origin such as Paris green and Scheele's Green have been converted to be used as insecticides (Peryea, 1998).

Biologically, Arsenic has a lot of uses from ancient times, and is still used in some areas despite its serious safety concerns. Some species of bacteria use Arsenic compounds as their respiratory metabolites, and is also used in trace quantities as an essential requirement in the diets of rats, hamsters, goats, chickens, and presumably many other species of animals including humans (Nechman *et al*., 2005). It is also found in foods like sea food, mushrooms and cereals or grains, especially rice (Nechman *et al*., 2005). In the field of Medicine, Arsenic has been in use since ancient times, and also a number of its compounds were used during the 18th, 19th and 20th centuries. A very good example of these is Arsphenamine, commonly used for treatment of syphilis and Trypanosomiasis (Gibaud and Jaouen, 2010), and also arsenic trioxide, which is known to be used in destroying cancerous cells, especially in the blood (acute promyeloctic leukemia (Huet *et al*., 1975). Other uses include its use in Chinese medicines to help in digestion, treating diseases like asthma, rheumatism, haemorrhoids, cough, insomnia, food poisoning and as a general tonic and pain killer (Holleman *et al*., 1985).

In spite of its many uses, Arsenic is generally regarded as a toxic element, and its toxicity to living organisms has long been acknowledged, right from ancient times when it was used for murder as reported by Vahidnia *et al*. (2007). More recently, awareness of its risk to plants, animals and human health as well as being a key contaminant in the environment has greatly

increased (Järup, 2003; Rajendran *et al*., 2003; Adeyemi, 2009). Arsenic has also been listed by WHO (2011) among the 10 chemicals of major health concern, and has been identified worldwide for its toxic effects. According to a report by Green Facts (2001), inorganic Arsenic compounds are more hazardous to health than the organic compounds; and the most toxic of these compounds are those in which the element lost all its three 4p electrons, that is, when it exists in the +3 oxidation state as As^{3+} (Galadima and Garba, 2012).

Exposure to Arsenic occurs from food, soil, water and air brought through natural sources as well as human activities ranging from occupational to indiscriminate use of materials laden with particles from the different sources mentioned above. Chronic exposure to high level of Arsenic and its compounds especially in drinking water can lead to several carcinogenic, mutagenic and teratogenic implications (Adeyemi, 2009). Arsenobetaine, an organic form usually found in fish, is one means of exposure through food (Järup, 2003). Other means of exposure as reported by Järup (2003) include smelting of non-ferrous metals, energy production from fossil fuels, contaminated soils (e.g. mine-tailings), as well as manufacture and use of pesticides and wood preservatives. Arsenic concentration in air in cities has been found to be higher than in rural areas, with much higher concentrations near industrial sources (Antman, 2001; Järup, 2003). Inorganic Arsenic is acutely toxic, and the intake of large quantities leads to gastrointestinal symptoms, severe disturbance of cardiovascular and central nervous system (Järup, 2003). It was known to have caused the death of over 20 people in Bradford Sweet poisoning in 1858, when it was accidentally used to adulterate some foodstuffs (Turner, 1999). Another toxic effect of Arsenic include a disorder which is often confused with Guillain-Barre syndrome, which is an anti-immune disorder that occurs when the body's immune system mistakenly attacks part of the Peripheral Nervous System, resulting in nerve inflammation that causes muscle weakness

(Vahidnia *et al*., 2007). Disorders such as bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy have also been observed (Järup, 2003). Ingestion of inorganic arsenic may induce peripheral vascular disease, which in its extreme form leads to gangrenous changes (Järup, 2003; Gunatilake, 2015).

Exposures to concentrations of Arsenic above 0.05mg/l in drinking water lead to excessive risk of mortality from lung, bladder and kidney cancer, and a higher risk to skin cancer (Gunatilake, 2015). There is also an increased risk of skin cancer and other skin lesions, such as hyperkeratosis and pigmentation changes (Järup, 2003). Occupational hazards of exposure to Arsenic by inhalation, such as smelter workers, pesticide manufacturers and miners in many different countries consistently demonstrate an excess lung cancer as observed by Järup (2003), Martin and Griswold (2009) and Gunatilake (2015). Lower level exposure to arsenic can cause nausea and vomiting, decreased production of red and white blood cells, abnormal heart rhythm, damage to blood vessels, and a sensation of "pins and needles" in hands and feet (Martin and Griswold, 2009). Death can result from exposure to high level of Arsenic (United States Department of Labour Publications, 2004; Martin and Griswold, 2009) and from damages of various body organs.

The major mechanism by which Arsenic exerts its toxic effect is through an impairment of cellular respiration by inhibition of various mitochondrial enzymes and the uncoupling of oxidative phosphorylation (Galadima and Garba, 2012), coagulating proteins, formation of complexes with coenzymes, as well as inhibiting the production of adenosine triphosphate (ATP) during respiration (Institute of Environmental Conservation and Research, 2000). It is also readily transferred through food chains and is not known to serve any essential biological function (Järup, 2003).

2.2.7 Presence of Nickel, Mercury and Arsenic in Industrial and Refinery Effluent:

The three heavy metals discussed above namely, Nickel, Mercury and Arsenic have been confirmed to be present in industrial and refinery effluent in several research studies (Uzoekwe, and Oghosanine, 2011; Galadima and Garba, 2012; Dhokpande and Kaware, 2013; Littlepage, 2013; Adesina and Adelasoye, 2014; Ezeonuegbu *et al*., 2014; Kong *et al*., 2014; Lakherwal, 2014; Gunatilake, 2015; Machido *et al*., 2016). Also, their presence has been observed to be in concentrations higher than the maximum limits permitted by many environmental regulation authorities, both nationally and internationally as mentioned by Järup (2003), Chikogu *et al*. (2012), Galadima and Garba, (2012), Machido *et al*. (2016) and many others. Above all, their toxicity on exposure in the environment such as in food, water, air soil and working places; and consequent health hazards have been documented in several literatures (Järup, 2003; Rajendran *et al*., 2003; Martin and Griswold, 2009; Galadima and Garba, 2012; Ezeonuegbu *et al*., 2014; Machido *et al*., 2014; Machido *et al*., 2016). This in turn, makes it necessary to devise measures by which exposure to these heavy metals, as well as their presence in the environment would be minimised easily and efficiently.

2.3 Methods of Heavy Metals Removal from Refinery Effluent

The well-known methods for removing heavy metals from industrial effluents are those that have been outlined earlier for industrial wastewater treatment. Such methods as mentioned by Dhokpande and Kaware (2013), Lakherwal (2014) and Gunatilake (2015) include physicochemical methods like ion exchange, ultrafiltration, electrocoagulation, electrodialysis, membrane separation (or filtration), nanofilteration, reverse osmosis, photocatalysis, flocculation, floatation, precipitation and adsorption; and biological methods which include

activated sludge, trickling filters, stabilisation ponds, anaerobic methods, and bioadsorption or biosorption. It is, however, a known fact that most of these methods mentioned are not very efficient in the removal of heavy metals ions (Gunatilake, 2015) and have several disadvantages such as high reagent requirement, unpredictable metal ion removal, generation of toxic sludge, high cost of operation, etc. (Lakherwal, 2014). Furthermore, removal of heavy metals by these methods is in most cases, is in such a way as only high concentrations of the heavy metals are removed, while very low concentrations persist, which are hazardous to the ecosystem even at such low concentration since they are non-degradable (Lakherwal, 2014; Gunatilake, 2015; Machido *et al*., 2016). Consequently, more simpler and efficient methods were sourced for, and the biological methods, specifically Adsorption has gained a wider application due to its inherent low cost, simplicity, versatility and robustness (Lakherwal, 2014), and therefore, several studies have been carried out to explore various aspects of this method as a means of bioremediation.

2.3.1 Bioremediation

Bioremediation is the term used to describe a process whereby biological systems (i.e. living things of either plant, animal or microbial origin) are employed to remedy or cleanse the environment from damages resulting from various pollutants. In most cases, Bioremediation is often associated with microorganisms. Adams *et al*. (2015) defined it as a process of using microbial metabolism in the presence of optimum environmental conditions (e.g. temperature and pH) and sufficient nutrients to breakdown contaminants or pollutants, notably petroleum hydrocarbons. Pollutants could be in gaseous, soluble or insoluble forms (Dhankar and Hooda, 2011). However, bioremediation is a general term for all living things that might be used to reduce environmental pollution.

There are two major aspects of bioremediation measures, commonly known as Biostimulation and Bioaugmentation (Adams *et al*., 2015).

2.3.2 Biostimulation

Biostimulation is defined as the addition of limiting nutrients to support microbial growth so as to enhance their growth and ability to carry out the remediation process efficiently (Adams *et al*., 2015).

2.3.3 Bioaugmentation

This is a process by which living cells of microbes, e.g. fungi or bacteria, which are capable of degrading contaminants are added or introduced in a system or an environment where Bioremediation is aimed to take place (Adams *et al*., 2015).

2.3.4 Adsorption

Adsorption is a process that takes place when a gas or liquid solute accumulates or adheres on the surface of a solid or a liquid, forming a molecular or atomic film on the surface (Lakherwal, 2014). Dhankar and Hooda (2011) also defined Adsorption as a mechanism by which ions and molecules physically adhere or bond onto the surface of another molecule, i.e. on to two-dimensional surfaces. It is basically a mass transfer process which involves the binding of substances by physical and/or chemical interactions to solid surface (Gunatilake, 2015). The heavy metal ions to be removed, which accumulate on the interface of another material are called the **adsorbates** (sorbates), while the solid surface on which the metal ions accumulate is called **adsorbent** (sorbent), which may be in form of agricultural waste (e.g. sugarcane bagasse), or microbial biomass-e.g. bacteria or fungi (Dhankar and Hooda, 2011). Adsorption processes are

often carried out using glass, or metallic materials known as sorption or filtration columns, in which a known amount of the adsorbents are added, followed by a known volume of the fluid containing the adsorbate, which is collected through a controlled outlet after sorption. The adsorbent along with bound metal ions remain in the column, which are collected later on and treated appropriately (Machido *et al*., 2016). Adsorption is operative in most natural physical, biological, and chemical systems, and is widely used in many industrial applications (Lakherwal, 2014). Recently, it has been recognised as an effective and economic method for the removal of heavy metals from wastewaters from industrial effluents. This is because it has many advantages over the conventional methods, as discussed by Sharma *et al*. (2013), Lakherwal (2014) and Adams *et al*. (2015), which include the following:

1. It offers simplicity and flexibility in design and operation so as to produce high quality treated effluents of desired standards for disposal, thus, it is suitable for water treatment.
2. It is a very effective method, which is capable of removing heavy metals even at low concentration.
3. The adsorbents used are mostly by-products of industrial and agricultural activities, hence, are easy and cheap to obtain.
4. Conversion of these materials into adsorbents for wastewater treatment would help to reduce the cost of waste disposal
5. Materials used for adsorptions can be used more than once.
6. Production or generation of toxic sludge is minimised or controlled entirely when adsorption is used.
7. Heavy metal ions or sorbates can be recovered by desorption process easily after the whole process.

8. It is a versatile process; can be achieved through various means by a wide range of materials regardless of the area.

2.3.5 Types of Adsorbents:

Several low-cost adsorbents that have been explored and tested by many researchers include various industrial wastes, microbial biomass as well as agro and horticultural waste by-products like sugarcane bagasse, rice husk, orange peels, almond shell, sawdust, soybean hulls, cottonseed hulls, rice bran, coconut tree sawdust, sago waste, banana pitch carbon, coconut husk, palm pressed fibre, clay and some bio adsorbents (Sharma *et al*., 2013), waste tea leaves, sand, egg shell, activated carbon, zeolites, olive stones, bone gelatin beads, leaf mould, moss, iron oxide-coated sand, modified wool, modified cotton (Lakherwal, 2014), red mud, coal, photocatalyst beads, nano particles, activated sludge, biomass of algae, bacteria, fungi, etc. (Gunatilake, 2015). Other adsorbents reported by Bishnoi and Garima (2005) and Dhankar and Hooda (2011) include tannin-rich tree barks, peanut skin, plant weeds, peat, fly ash, china clay, silica, active alumina, metal oxides, betonites, etc. most of these materials are abundantly and easily available in our environment for the elimination of heavy metals from wastewater (Sharma *et al*., 2013).

Activated carbon, which is the most widely used adsorbent, is a highly porous, amorphous solid, which consists of micro crystallites with a graphite lattice, usually prepared in small pellets or a powder. It can remove a wide variety of toxic metals (Lakherwal, 2014). However, its use for this purpose is limited in most cases due to the high cost, since it cannot be used more than once, and cannot also efficiently remove some heavy metals from solution, especially at very low concentrations (Dhankar and Hooda, 2011), thus, more attention is given to the low-cost agricultural and industrial wastes. Of the different biological methods, bioaccumulation and

biosorption have been demonstrated to possess good potential to replace conventional methods for the removal of heavy metals from solutions (Dhankar and Hooda, 2014).

2.3.6 Biological Modes of Adsorption

Biological methods which involve use of natural materials as adsorbents is essentially in two major forms as described by Bishnoi and Garima (2005), namely: Biosorption, and Bioaccumulation. The major difference between these two processes is that Biosorption mechanisms involve the use of dead biomass, whereas Bioaccumulation is used to describe the process involving living cells (Dhankar and Hooda, 2011).

2.3.7 Biosorption

Dhankar and Hooda (2011) defined Biosorption or Bioadsorption as a mechanism of binding and concentration of selected heavy metal ions or other molecules on to certain natural or biological material. It involves the surface binding of metal ions to the cell wall and extracellular materials, thus, mostly occurs outside the cell (Bishnoi and Garima, 2005). Biosorption removes heavy metals from wastewater based on the ion exchange principle, where attraction between the metals and the surface of biological materials gives rise to binding of the metal ions (Soliman *et al.*, 2011). Biosorption mechanisms involve the use of both living and dead biomass (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011), and is not dependent on cell metabolism. The sorption process involves transfer of ions from solution phase to the solid phase, which actually describes a group of processes such as adsorption and precipitation reaction (Gunatilake, 2015).

Biosorption seems to be preferred more than Bioaccumulation for the majority of metal removal studies reported. This is due to reasons such as;

- Toxicity limitations, which are encountered when using living cells are absent

- There are no requirement of growth media and nutrients in the feed solution
- It is cost effective; as it does not involve maintenance of cells
- Biosorbed metals can be easily adsorbed and recovered by desorption
- Regenerated biomass can be easily reused for other studies
- It is flexible to environmental conditions and toxicant concentrations
- The process occurs more rapidly, and offers very high uptake capacity of metal ions as biomass are reported to accommodate an amount of toxicant nearly as high as their dry weight.
- Mathematical expressions can be easily used to model the various metal uptake reactors for biosorption studies (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011).

Biosorption processes are, however, poor in terms of selectivity and the biomass need to undergo modifications and pre-treatments for better results.

2.3.8 Mechanisms of Biosorption

The biosorption process involves a solid phase (biosorbent) and a liquid phase (solvent: normally water) containing dissolved species to be sorbed (sorbate: metal ions). Due to the high affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms (Bishnoi and Garima, 2005). The process continues until equilibrium is established between the amount of solid-bound metal ions and those still remaining in the solution. The absence of available binding sites on the biosorbent molecules for remaining metal ions to attach to in the medium gives rise to an imbalance between the two phases which creates a driving force for the solute species (Dhankar and Hooda, 2011). The heavy metals adsorb on the surface of the biomass; thus the biosorbent becomes enriched with metal ions. The distribution of the

sorbate between the solid and liquid phases is determined by sorbent affinity for the sorbate (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011).

Biosorption is basically carried out by various mechanisms that essentially take place in the cell wall (Bishnoi and Garima, 2005), where the mechanisms responsible for the pollutant uptake will differ according to the biomass type. Dhankar and Hooda (2011) also explained that the biosorbent behaviour of biomaterials towards metallic ions is a function of the chemical make-up of the cell wall. The mechanisms of biosorption are generally based on physicochemical interactions between the metal ions and the functional groups (basically carboxylate, hydroxyl, amines and phosphryl groups found within cellular components such as polysaccharides, lipids and proteins) present on the cell surface (Rajendran *et al*., 2003; Bishnoi and Garima, 2005; Alluri *et al*., 2007). These interactions enumerated by Rajendran *et al*. (2003) include electrostatic interactions, covalent bonding, extracellular precipitation, ion exchange, metal ion chelation or complexation, or a combination of some of these processes. Biosorption is a process which requires minimal energy for activation, which in most cases does not exceed 21 kJ/mol of energy, and allows for metal desorption followed by regeneration and reuse of the biosorbent in successive sorption/desorption cycles. Biosorption is currently considered as one of the most promising technologies that can be used for sequestration of toxic and pollutant metals even in very dilute conditions, due to its many advantages as mentioned above (Dhankar and Hooda, 2011).

2.3.9 Biosorption Models

Adsorption isotherms are described in many mathematical forms called ‘models’. Some of these models are based on a simplified physical picture of adsorption and desorption, while others are

purely empirical and are intended to correlate the experimental data in simple equations with two or, at most, three empirical parameters: the greater the number of empirical parameters, the better the fit between experimental data (Dhankar and Hooda, 2011).

Biosorption isotherms of metal uptake are generally described in simple terms by the following equation reported by Bishnoi and Garima (2005):

$$Q_e = V[C_i - C]/W$$

Where: V is the volume in litre of solution contacted with the sorbent;

C_i and C are initial and equilibrium (final) concentrations of the sorbate in mg L^{-1};

W is the amount of biosorbent usually expressed as dry weight (g);

Q_e is the uptake of metal per unit weight of adsorbent, expressed as weight per unit dry weight (mg/g).

Some of the Biosorption models are used to describe simple, single components, while others are used to describe complex, multi-component experimental set-ups, which may be derived from the simpler models (Dhankar and Hooda, 2011).

Some of the known adsorption isotherm models that have been used to describe the Biosorption equilibria are as follows:

- Langmuir Isotherm Model
- Freundlich Model
- Brunauer-Emmett-Teller Model
- Scatchard Model (Bishnoi and Garima, 2005).

The Langmuir Isotherm Model- This was developed in 1918, and it describes adsorption at constant energy of adsorption, when maximum adsorption occurs and a saturated monolayer of

solute molecules is present on the adsorption surface, and there is no migration of adsorbate molecules in the surface plane. It is described by the following mathematical equation:

$$Q_e = (Q_{max} bC_f)/(1 + bC_f)$$

Where: Q_{max} is the maximum uptake of ions

b is the Langmuir's constant related to energy of adsorption;

C_f is the equilibrium or residual concentration of metal ions (Bishnoi and Garima, 2005).

Freundlich Model- is basically empirical, which was developed in 1907 for heterogeneous surfaces (Dhankar and Hooda, 2011), and it is a useful means of data description. It is represented by the following equation:

$$Q = K_f C_f^{1/n}$$

Where, Q is the uptake of metal ions per unit weight of Biosorption;

C_f is the equilibrium concentration of metal ions in solution;

K_f and n are the Freundlich constants, denoting adsorption capacity and intensity of adsorption respectively.

It should be noted that the higher the value of K_f and n, the lower the value of b; and this confers higher affinity of the biomass to the adsorbates (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011).

Brunauer-Emmett-Teller Model- This model represents the multi-layer adsorption at the adsorption surface, and is based on the assumption that a Langmuir Isotherm applies to each layer. It has the form,

$$C_e/(C_s - C_e) = 1/BQ^o + (B - {}^{1}/_{BQ^o})(C_e/C_s)$$

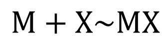
Where, C_s is the saturation concentration of the metal ions;

C_e is the equilibrium of metal ions concentration;

Q^o is the amount of ions adsorbed per unit weight of biomass for monolayer biosorption;

B is a constant which relates to the energy of interaction with the surfaces (Bishnoi and Garima, 2005).

Scatchard Model – Was developed to describe the attraction of proteins for small molecules and ions, and is also used to the equilibria of biosorption. In this model, interaction between the metal ions and cell surface binding sites of the sorbents is described by this equation:



$$k = [MX]/[M][X]$$

Where, k is the association constant;

M is the metal ion under consideration;

X is the binding site on the biomass surface

MX is the metal ion that has been biosorbed on biomass (Bishnoi and Garima, 2005).

2.3.10 Bioaccumulation

Bioaccumulation can be defined as the uptake of toxicants by living cells (Dhankar and Hooda, 2011). It involves the uptake of metal ions into the cell across the cell membrane, and is also referred to as intracellular uptake or active uptake which is facilitated by production of cellular metal-binding proteins (Bishnoi and Garima, 2005), since it occurs within the cell and is metabolism dependent. In general, the use of living organisms may not be an option for the continuous treatment of highly toxic organic/inorganic contaminants. Reasons for these may be;

- The toxicant can transport into the cell and accumulate intracellularly, across the cell membrane and through the cell metabolic cycle

- Organisms used may be sensitive to the toxic metals, which may bring about interruption in metabolic cycles, resulting in death of the organisms
- It constitutes a higher cost of operation, due to maintenance of the living cells used.
- Occurs slowly due to intracellular accumulation which is time-consuming.
- Other parameters such as temperature, pH, metal ion concentration as well as time of exposure to the metals may also interfere with the overall operation (Dhankar and Hooda, 2011).
- Metabolic energy is often needed to maintain the culture for the living organisms, thus is not easily maintained.
- Regenerability and re-usability of biomass is almost impossible, as the toxic metal ions accumulate within the cells.
- It is also not possible to recover the metal ions by elution (Dhankar and Hooda, 2011).

Bioaccumulation is, however, better than Biosorption in terms of high selectivity of biomass without any pre-treatment.

2.3.11 Mechanism of Metal Uptake by Living Cells:

Metal ion uptake by living cells is a function of some factors such as,

- ✓ Age of cell
- ✓ Culture conditions and Composition of growth media
- ✓ Contact time
- ✓ pH of metal solution
- ✓ Temperature (Dhankar and Hooda, 2011)

Biosorption of metals by non-growing cells of some fungi including *Penicillium*, *Aspergillus*, and *Mucor* species have been observed to reach equilibrium in 1-4hrs, and the biosorption kinetics of the metals is usually biphasic in nature, consisting of an initial rapid phase, which lasts for about 10mins and contributes up to about 90% of the biosorption; and a second, slower phase, that lasts for about 4hrs (Bishnoi and Garima, 2005). Metal ion uptake by bioaccumulation is essentially an active mode, which depends on the cell metabolic cycle (Rajendran *et al*., 2003). Metal ions are transported into the cell across the cell wall, by means of transporting agents of the cell (Dhankar and Hooda, 2011). Once inside the cell, the microbes can exhibit tolerance to the heavy metal toxicity by mechanisms such as compartmentalisation, mineralisation, formation of complexes, synthesis of binding proteins, anion efflux by enzymes such as ATPase, reduction of the oxidised metal ion, e.g. Mercury reduction and decreased uptake (Rajendran *et al*., 2003). Filamentous fungi according to Gadd (2009) might not be able to achieve effective metal uptake by active mode at a high concentration of the metal ions.

2.4 Sugarcane Bagasse

Sugarcane *(Saccharum officinarum)* is a perennial grass, which belongs to family *Gramineae* and has a stem structure resembling other monocotyledonous plants, and is also different from many grasses as it does not possess a hollow stem (Lacey, 1980). It thrives well in tropical and frost-free, warm temperate areas, and provides the cheapest form of energy food with the lowest unit of land area per unit of energy produced. It is one of the few plants which store its carbohydrate reserves as sucrose (Zafar, 2015). It was originally produced mainly for chewing, but has now become useful in many other things especially in sugar and ethanol production, as well as for many other industrial purposes, supplying more than half of the world's sugar consumption (Verheye, 2006). Brazil and India are the world's two largest sugarcane growers

with production of 300 and 285 Mt/yr. respectively, accounting for nearly 75% of the future increase in sugarcane production (Kadam, 2000).

Sugarcane Bagasse is a by-product of sugarcane. It is the fibrous residue left or generated after the sugar has been extracted from the cane (Kadam, 2000; Parani *et al*., 2016). It may be formed either as a result of chewing directly, or due to industrial processing to extract sugar. As an agricultural by-product, it is generated in large quantity in countries that produce it, and requires no special treatment or processing before it is used as substrate material (Parani *et al*., 2016).

2.4.1 Uses of Sugarcane Bagasse

Sugarcane bagasse is very useful, and has been employed in many agricultural and industrial processes. Some these include the production of fuel grade bioethanol for various industrial activities (De souza *et al*., 2012; Zafar, 2015), as local manure and component of animal feed (Kadam, 2000; Irfan *et al*., 2011; Zafar, 2015; Parani *et al*., 2016); also as raw material for generating electricity (Kadam 2000; Irfan *et al.* 2011), pulp and paper production (Daud *et al*., 2007) generating fermentation products (Ojumu *et al*., 2003; Bhattacharya *et al.,* 2011) as well as precursor in activation of carbon for industrial purposes (Lori *et al*., 2007). In recent years, several studies have been carried out to explore the use of sugarcane bagasse as a low-cost adsorbent in bioremediation processes such as in removing heavy metals and hydrocarbons from industrial effluents (Ahluwalia and Goyal, 2007; Sharma *et al*., 2013; Kong *et al*., 2014; Parani *et al*., 2016). Another important use of sugarcane bagasse is its use as a source of nutrient for microbial growth (Ojumu *et al*., 2003; Cunha *et al*., 2012; Oliveira *et al*., 2012; Parani *et al*., 2016). Sidana and Farooq (2014) carried out a study where capacity of sugarcane bagasse was tested as a potential medium for designing fungal cultures, and suggested that sugarcane bagasse

can be used as a growth medium for fungal cultures (sometimes even better than laboratory media), which reduces cost of laboratory media, minimises bacterial contamination, as well as free the environment from nuisance of waste by the bagasse generated. Hasan *et al*. (2015) also carried out a research in which sugarcane bagasse was used along with other low-cost wastes to serve as a growth supplement for pink oyster mushrooms (*Pleuretus djamor*), and concluded that it was a suitable material for growth of the mushrooms.

2.4.2 Sugarcane Bagasse as a Suitable and Effective Biosorbent

As a low-cost biosorbent, sugarcane bagasse has been proved to be a very good adsorbent that can efficiently remove heavy metals and other contaminants from industrial effluents. Soliman *et al*. (2011) and Kong *et al*. (2014) used sugarcane bagasse for heavy metals removal, and in their studies observed that sugarcane bagasse uses the Langmuir-Freundlich Isotherm model, of first order kinetics, which depend on various environmental conditions such as time, temperature, pH, etc. It has also shown higher efficiency in many studies than other biosorbents including microbial agents.

2.4.3 Components of Sugarcane Bagasse

Sugarcane bagasse as a waste product obtained from sugarcane mainly composed of cellulose, hemicellulose, pentosan, and lignin (Sharma *et al*., 2013), as well as other components like ash, moisture, reducing sugars, etc. In most of the studies and researches carried out using sugarcane bagasse, the proximate analysis reveals that lignocellulosic components namely; cellulose, hemicellulose and lignin constitute the major part. Cellulose has the highest percentage (about 35-60%), followed by hemicellulose (20-40%); and 10-30% lignin (Rodríguez-Chong *et al*., 2004; Dawson and Boopathy, 2008; Carvalho *et al*., 2009; Irfan *et al*., 2011; De Souza *et al*.,

2012) . Studies carried out by De Souza *et al*. (2012) show that sugarcane bagasse consists of 48.6% cellulose, 31.1% hemicellulose, 19.1% lignin, with other components such as ash having 1.2%. In a report by Dawson and Boopathy (2008), proximate composition of sugarcane bagasse was shown to consist of cellulose 30%; hemicellulose 23%; and 22% lignin. Irfan *et al*. (2011) also reported proximate composition of sugarcane bagasse as having about 40% cellulose; 23% lignin; ash and moisture content 0.9% and 7.1% respectively. Rodriguez-Chong *et al*. (2004) examined the composition of sugarcane bagasse which comprised of 38.9% cellulose, 20.6% hemicellulose and 23.9% lignin. In another study, the cellulose content was found as 42.5%, hemicellulose 24.88%, lignin 20.90% and ash content was 1.64% (Carvalho *et al*., 2009). The ash content generally has a low value, while the moisture content generally varies. These variations in composition of the sugarcane bagasse might be due to reasons such as differences in the varieties of sugarcane cultivated in various parts of the world, portion of fibre on the cane of the sugarcane, cleanliness of the cane supplied, harvesting practices, as well as maturity of the sugarcane before it was harvested (Carvalho *et al*., 2009; Irfan *et al*., 2011; Zafar, 2015). The value of the sugarcane bagasse depends largely on its calorific value, which is also affected by its composition of sugars such as carbohydrates, glucose, and galactose (Zafar, 2015).

2.5 Fungi

Fungi are a large group of organisms within a wide range of species that include molds, yeasts, yeast like fungi and dimorphic fungi (Abdullah and Saadullah, 2015). They constitute a kingdom of eukaryotic organisms, also known as *Eumycota* or true fungi. They are primarily terrestrial organisms, although a few are found in freshwater or marine. In Microbiology, fungi are described as eukaryotic organisms that are spore-bearing, have absorptive nutrition, lack chlorophyll, and reproduce both sexually and asexually (Willey *et al*., 2008). They are also

obligate aerobes, and grow optimally in acidic pH. Fungi exist primarily as filamentous mass of hyphae called a mycelium. The body or vegetative structure of a fungus is called a thallus, while the fungal cell is usually encased in a cell wall of chitin. They are of great importance to humans in both beneficial and harmful ways (Willey *et al*., 2008). Fungi grow best in dark, moist habitats where there is little danger of desiccation, but they are mostly found wherever organic material is available (Strohl *et al*., 2001; Willey *et al*., 2008). Most fungi are heterotrophic saprophytes**,** and obtain their nutrients from dead organic materials (Betsy and Keogh, 2005). Examples of some fungal genera include *Aspergillus*, *Penicillium*, *Trichophyton*, *Mucor*, *Microsporum*, *Saccharomyces*, *Alternaria*, *Amanita*, *Trichoderma*, *Rhizopus*, *Fusarium*, *Curvularia, Nigrospora Geotrichum, Rhizoctonia, Candida, Chaetophoma,* etc. (Strohl *et al*., 2001; Betsy and Keogh, 2005; Willey *et al*., 2008).

2.5.1 Types of Fungi

Fungi have been classified into the following groups:

1. Chytridiomycetes
2. Zygomycota,
3. Ascomycota,
4. Basidiomycota,
5. Urediniomycetes,
6. Ustilaginomycetes,
7. Glomeromycota, and
8. Microsporidia

2.5.2 Importance of Fungi

Fungi have a lot of importance in different aspects of human lives. These may be both beneficial and harmful. Some importance of fungi include the following:

- In Agriculture, importance of fungi is seen in the mutual relationship formed between the fungi called *Mycorrhizae* and the roots of plants. These help the plants roots to absorb water and minerals from the soil (Betsy and Keogh, 2005). Another mutual relationship is also formed between fungi and soil organisms like ants, as well as between the nitrogen fixers and root nodules of legume plants (Betsy and Keogh, 2005).
- Some fungi (e.g. mushrooms) are beneficial to humans and are used as food in some parts of the world (Betsy and Keogh, 2005).
- They are used in the preparation of food (e.g. yeast) such as in bread and beer production where fermentation occurs in making the food materials (Betsy and Keogh, 2005).
- Fungi are also used in producing antimicrobial agents, e.g. antibiotics to fight off bacterial diseases (Betsy and Keogh, 2005). An example of these is *Penicillium* species.
- Some fungi e.g. *Saccharomyces cerevisiae* are used in the industry to produce important materials, e.g. enzymes like cellulase, ethanol, and many others which are useful industrially.
- In the field of bioremediation, fungi are widely used (a process called Mycoremediation) especially in removing hydrocarbons and heavy metals from the environment. This is due to their ability to extend their hyphae through hydrocarbons and accumulate them through their tissues. Similarly, they have the ability accumulate heavy metals and modify them from toxic to non-toxic forms (Willey *et al.,* 2008). Other toxic substances like dead organic materials and harmful wastes are also degraded or decomposed by fungi

(Saprophytism), converting them into harmless materials, thereby reducing pollution caused to the environment as a result of their presence (Bishnoi and Garima, 2005; Willey *et al.,* 2008).

- In the field of Medicine, fungi have been associated with many diseases of humans and animals known as Mycoses (Besty and Keogh, 2005; Willey *et al.,* 2008). Examples include skin diseases, e.g. athlete's foot, respiratory diseases, food poisoning, etc. which has been considered hazardous to health.
- Some fungi can have a lot of harmful effects because they feed on plants and animals products, which lead to spoilage of food materials due to decay at different stages.
- Production of Mycotoxins by some fungi, e.g. aflatoxin production by *Aspergillus flavus* in rice, corn, sorghum, peanuts, soybeans and other cereals results in diseases called Aflatoxicoses in animals like Poultry, swine, cattle, sheep, dogs and even humans (Willey *et al*., 2008).
- Other medically important fungal infections include Aspergillosis (caused by *Aspergillus* species), superficial mycoses (caused by *Trichosporon beigelii*), cutaneous mycoses (caused by *Trichophyton rubrum*), and subcutaneous mycoses (caused by *Madurella mycetomatis*). Other fungal infections of skin, hair, and nails caused by fungi include Candidiasis (caused by *Candida albicans*), and Cryptococcosis (caused by *Cryptococcus neoformans* which affects the lungs, skin, bones, viscera, and central nervous system (Willey *et al*., 2008).
- Fungi have also been implicated in many plants diseases, e.g. tomato blight, cassava mosaic disease, etc. (Besty and Keogh, 2005; Willey *et al*., 2008).

2.5.3 Fungi as Agents of Heavy Metal Bioremediation

An area in which fungi are used extensively is their exploration in bioremediation of polluted environment. In nature, the responses of microorganisms to toxic metal ions can dramatically alter metal ion abundances and elemental speciation, leading to a range of transformations such as mobilization, immobilization and mineral neogenesis (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011). Many studies have been carried out since the discovery of the methods of Biosorption using different groups of microorganisms, among which, many fungal genera are inclusive. This is because, as stated by Al-Bahry *et al*. (2013), microorganisms can grow in extremely harsh environments such as oil reservoirs with extreme conditions, where they adapt, propagate and grow. This is the basis for use of fungi in Bioremediation especially in industrial effluents that contain hazardous substances such as heavy metals. Accumulation of heavy metals by fungal biomass may be particularly relevant because of its potential low cost application in bioremediation and recovery of metals (Bishnoi and Garima, 2005). Other microbes like bacteria (Al-Bahry *et al*., 2013) and algae (Otitoju and Otitoju, 2013) have also been tested and used in many studies.

Fungi were found to be more suitable and easy to use for Biosorption purposes, due to the fact that they were found to be more tolerant, as shown by *Aspergillus* and *Penicillium* species which have been observed by Ezzouhri *et al*. (2009) in their study. This may be due to the components present in their cell walls which have a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix and also because they mostly show a filamentous or hyphal growth (Alluri *et al*., 2007). They therefore, offer the advantage of having cell wall material which shows excellent metal-binding properties (Ezzouhri *et al*., 2009). A good example is the fungi *Saccharomyces cerevisiae*, which can remove toxic metals, recover

precious metals and clean radio-nuclides from aqueous solutions to various extents. It also has the ability to differentiate between different metals such as Selenium, Antimony and Mercury based on their toxicity, and is thus useful in analytical measurements (Wang and Chen, 2006; Alluri *et al*., 2007). Furthermore, as fungi play fundamental roles in the natural environment especially regarding decomposition, transformation and nutrient cycling (Dhankar and Hooda, 2011), knowledge of their responses in high metal concentration states may be particularly relevant, in this case, to the detoxification of Nickel, Mercury and Arsenic polluted habitats. Prompted by the combined abilities of fungi to render metals soluble from insoluble compounds and accumulate them from the dissolved state, this study sought to evaluate the accumulation and possible solubilisation of the heavy metals under study at certain concentrations and remove them from petroleum refinery effluent by free-living filamentous fungi.

Many research works including some mentioned above, have been published in which different genera of fungi have been shown to accumulate and biosorb different types of heavy metals. For example, *Phanerochaete chrysosporium* was used by Haluk and Ulki (2001) to remove Ni(II) and Pb(II) ions from solution; *Aspergillus niger* was reported to remove Cadmium ions in a study by Barros *et al*. (2003); Bhainsa and D'Souza (1999) reported accumulation of Uranium (IV) ions by *Aspergillus fumigatus*; *Aspergillus terreus* was also reported to bioaccumulate Copper ions from solution (Ruchi *et al*., 2003), while accumulation of Gold (Au) by *Penicillium chrysogenum* was reported by Niu and Volesky (1999). More recently, Ezeonuegbu *et al*. (2014) investigated the resistance of fungal genera isolated from refinery effluent such as *Aspergillus*, *Penicillium*, *Fusarium*, *Curvularia* and *Nigrospora* to Cadmium, Lead and Nickel ions. Machido *et al*. (2016) also explained the accumulation of the same heavy metal ions by *Trichoderma,*

Microsporum, Geotrichum, Rhizoctonia, Nigrospora and *Chaetophoma* species from refinery effluent.

2.5.4 Fungal Isolates Used in This Study:

***Trichophyton* species:**

Fungi of the genus *Trichophyton* are a diverse group genus in the phylum *Ascomycota* (Duncan and Horan, 2003), which consist of mostly filamentous and parasitic organisms, commonly known as dermatophytes. Shathele (2014) defined dermatophytes as a group of related fungi that utilise keratin and are adapted to infecting superficial layers of the skin of animals and man causing ringworm or Tinea. There are about 24 species within the genus. They can infect hair, skin, and nails, and most of them fungi reside in the soil in tropical areas and are involved in decomposition (Abdullah and Saadullah, 2015); however, the dermatophytes can infect living hosts (Centre for Food Security and Public Health (CFSPH, 2013)). Some species of this genus include *Trichophyton mentagrophytes, Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton verrucosum, Trichophyton soudanense, Trichophyton schoenleinii, Trichophyton concentricum* and *Trichophyton equinum* (Willey *et al*., 2008; CFSPH, 2013; Shathele, 2014; Abdullah and Saadullah, 2015). T. *rubrum* is the most common among the genus, as it has become the most widely distributed dermatophyte of man (Willey *et al*., 2008; Schaechter *et al*., 2009). It frequently causes chronic infections of skin, nails and rarely scalps (Shathele, 2014). The microscopic mode of identification is by the presence of Microconidia with smooth walls, while macroconidia are less distinctive, and in most cases may be absent, depending on the species (CFSPH, 2013). Some of the cultural characteristics include having colonies which are flat to slightly raised, white to cream, suede-like to downy, with a yellow-brown to wine-red

reverse (Schaechter *et al*., 2009). Most cultures show scanty to moderate numbers of slender clavate to pyriform microconidia (Shathele, 2014).

Importance of *Trichopyton* species is mostly known from the clinical perspective (CFSPH, 2013); where by majority of the species are known to cause diseases in humans and animals (Besty and Keogh, 2005; Shathele, 2014). Some diseases caused by these fungi as mentioned by Willey *et al*. (2008), Schaechter *et al*. (2009), Shathele (2014) and Abdullah and Saadullah (2015) include *Tinea pedis* (athlete's foot) of the toes, *Tinea Manuum* (Ringworm of the hands), *Tinea Cruris* (Jock Itch), *Tinea Corporis* (Ringworm of the Body), *Tinea Barbae* (Ringworm of the Beard), *Tinea Unguium* (Ringworm of the Nails) and *Tinea Capitis* (Ringworm of the Scalp).

There is very scarce literature published on the industrial uses of *Trichophyton* species. Therefore, there is the need to explore fungi of this genus to establish other importance they can offer other than being pathogens. Some species of this genus have been determined by Schaechter *et al*. (2009) to resist extreme conditions like high temperature, and also some species were isolated from refinery effluent (Ezeonuegbu *et al*., 2014), as well as proved to be resistant to and even be able to biosorb some heavy metals (Machido *et al*., 2016) within the refinery effluent.

Aspergillus flavus

Aspergillus is also a large genus of phylum *Ascomycota* (Duncan and Horan, 2003), composed of more than 180 accepted anamorphic species, with teleomorphs described in nine different genera. The genus is subdivided in 7 subgenera, which in turn are further divided into Sections (Rodriguez *et al*., 2007). The genus is easily identified by its characteristic conidiophore, but species identification and differentiation is complex, for it is traditionally based on a range of

morphological features. Macromorphological features which are considered include conidial and mycelial colour, colony diameter, colony reverse colour, production of exudates and soluble pigments, presence of sclerotia and cleistothecia. Micromorphology characterization is mainly dependent on seriation, shape and size of vesicle, conidia and stipe morphology, presence of Hülle cells, and morphology of cleistothecia and ascospores. *A. flavus* is morphologically similar to *A. nomius* and *A. parasiticus*, which makes identifying the organism a bit complex, but can be easily differentiated based on certain features (Rodriguez *et al*., 2007). *Aspergillus flavus* is very much known for its carcinogenic aflatoxin production, as well as its ability to cause infection called aflatoxicosis through food materials, mostly grain crops like maize and rice (Abdullah and Saadullah, 2015), hence, its importance in foodborne infections. It produces detectable aflatoxin B1 and B2 as well as cyclopiazonic acid (Rodriguez *et al*., 2007). It also causes Aspergillosis, which is a respiratory disease associated with fungi of the genus *Aspergillus* (Strohl *et al*., 2001).

Industrially, A. *flavus* is a very useful organism used for different purposes. These include biofuel production such as bioethanol, as well as enzyme production. In a study by Bhattacharya *et al*. (2011), *Aspergillus flavus* was found to be the best α-amylase enzyme producing fungus when used in fermentation. It was also employed for production of cellulase enzyme by Ojumu *et al*. (2003) and Cunha *et al*. (2012). In the area of Biosorption, A. *flavus* has been reported in many research works, where it was used for adsorption and removal of heavy metals and other hazardous substances from solution obtained from different industrial locations (Ezzouhri *et al*., 2009; Pandey *et al*., 2012; Dhokpande and Kaware, 2013; Ezeonuegbu *et al*., 2014; Machido *et al*., 2014).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Collection and Handling of Samples

3.1.1 Sampling of Refinery Effluent

Samples of Refinery effluent were collected from the Kaduna Refinery and Petrochemical Company that is located in Chikun Local Government Area, Kaduna State. The effluent is usually discharged from pipes into large stabilisation ponds for degradation of hydrocarbons and oily substances. However, heavy metals still persist after this treatment. After this retention period, it is then discharged into the nearby river.

The effluent was collected at the point where it was released from the processing unit before it undergoing treatment (i.e. stabilisation pond) in a new, 5 litre plastic gallon. Temperature and pH of the effluent was determined at the point of collection, and the sample thus collected was transported immediately to the Department of Microbiology laboratory in Ahmadu Bello University, Zaria for analysis.

3.1.2 Sugarcane Bagasse

Sugarcane bagasse was collected from Samaru, Zaria in new polythene bags and was taken to the laboratory of the Department of Microbiology, Ahmadu Bello University Zaria for analysis. In the laboratory, it was air-dried and chopped into smaller pieces using a sharp pair of scissors, after which it was ground using the milling machine to finer particles.

3.1.3 Fungal Isolates

Fungal isolates used in the study were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, from previous studies on refinery effluent carried out by Machido *et al.* (2014). The isolates were then resuscitated and re-authenticated following standard procedures. These include re-culturing on freshly prepared Potato Dextrose Agar (PDA) plates, followed by microscopic examinations. The authenticated isolates were sub-cultured onto fresh Sabaroud Dextrose Agar (SDA) slants and kept at room temperature for future use.

3.2 Determination of the Physicochemical Properties of the Refinery Effluents

Determination of the physicochemical properties of the effluents was carried out so as to have an idea of both the chemical and physical properties of the effluent which determines its level of pollution. It also explains the conditions in which the fungal isolates were initially found. These parameters were determined by using the Standard methods outlined by the American Public Health Authority, APHA (1999). In determining these parameters, two basic techniques were used, which were: Gravimetric and Volumetric technique. The Gravimetric technique was used to determine parameters like BOD, COD and also Oil and Grease; while the volumetric technique was used to determine other parameters like sulphates, phosphates, nitrates, turbidity and heavy metals. The physicochemical parameters determined were as follows:

3.2.1 Determination of pH, Temperature, Electrical Conductivity and Total Dissolved Solids:

The pH, Temperature, Electrical Conductivity and Total Dissolved Solids of the effluent were determined at the point of collection using the HANNA combo tester (H198130, Denver, USA), which is a water proof tester that offers high accuracy in determining these parameters in a single test. Following the manufacturer’s instructions, the electrodes were connected to each metre and

submerged in a clean bucket containing the sample. The values given by the tester for each parameter was observed and recorded.

3.2.2 Determination of Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD):

To determine these parameters, the Azide Modification of the Winkler Method was used. This is because it helps to most effectively remove any interference that may be caused by nitrites. The test procedure was as follows: 2ml of manganese sulphate solution was added to the sample in a 250-300ml BOD bottle and then 2ml alkali-iodide-azide reagent was added well below the surface of the liquid. The BOD bottle lid was carefully used to close the bottle, so as to exclude air bubbles and then mixed by inverting the bottle several times. When the precipitation settled, a clear supernatant above floc was left, and the BOD bottle was shaken again. When the settled solution has produced 100ml of clear supernatant, 2ml of concentrated H_2SO_4 was added and the lid was used to close the bottle again. The solution was mixed by gentle inversion and then 200ml was pipetted for titration. Titration was done with 0.025 $Na_2S_2O_3.H_2O$ to a pale straw colour; afterwards 1-2ml starch solution was added and titration was done again to the first disappearance of the blue colour.

The test depends on the principle that oxygen oxidizes Mn^{+4} to a higher state of valence under alkaline conditions and that manganese in higher states of valence is capable of oxidizing I^- to free I_2 under acidic conditions. Thus, the amount of free iodine released was equivalent to the dissolved oxygen originally present. The iodine was measured with standard sodium thiosulphate solution and was interpreted in terms of dissolved oxygen. The BOD test involves taking an initial dissolved oxygen (DO) reading and a second reading after five days of incubation at ambient temperature. After this time, the second bottle was tested for dissolved oxygen using the

same method that was used for the first bottle. The BOD is expressed in milligrams per litre of DO using the following equation:

DO (mg/L) of first bottle - DO (mg/L) of second bottle = BOD (mg/L)

The BOD was determined by subtracting the initial value of DO from the final value to obtain the BOD_5 (Radojevic and Bashkin, 1999).

3.2.3 Determination of Nitrate Content of the Effluent:

The nitrate content was determined by neutralizing the sample to pH 7.0, and a volume of 100ml was measured in a beaker and allowed to evaporate to dryness on a water bath. The residue was dissolved using a glass rod and 2ml disulphuric acid reagent was added. It was then transferred to Nessler's tube, to which 6ml of ammonium hydroxide (NH_4OH) and Ethyldimethyl tetraacetate (EDTA) solution was added in drops until the residue dissolved. A blank was also prepared in the same way using distilled water instead of the sample. The colour development was read at 410nm with a light path of 1cm. The nitrate concentration was calculated using a standard curve that has been previously prepared.

3.2.4 Determination of Sulphate Content of the Effluent:

This was done by dispensing 100ml of the sample into Erlenmeyer flask, and a magnetic stirrer was used to stir the mixture of the solution with 5ml conditioning reagent. A spoonful of barium chloride ($BaCl_2$) crystals was added while the solution was still being stirred. The turbidity was measured at 30 seconds after stirring at interval of 4 minutes using turbidity meter (Hach 2100N, USA). A blank was run with no barium chloride added. The sulphate concentration in the sample was estimated by comparing the turbidity reading with a standard curve previously prepared.

3.2.5 Determination of Phosphate Content of the Effluent:

In order to determine the phosphate content of the effluent sample, some preliminary treatments were first carried out. These include the following:

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

Colour Development: 100ml of treated sample was poured into a flask and 4.0ml of molybdate reagent was added and mixed thoroughly. Ten drops (0.5ml) of stannous chloride reagent was added. The colour was measured photometrically at 690nm after 10 minutes, and compared with the standard curve. Distilled water was used as blank. The phosphate concentration was determined from the previously prepared standard curve.

3.2.6 Determination of Oil and Grease:

The Partition or Gravimetric method was used for the analysis of oil and grease. It was done by taking 50ml of the sample, which was then acidified to pH 2.0 using 5ml of hydrochloric acid, and it was then shaken vigorously for 3mins. The sample was passed through a separating funnel held by 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 sodium sulphate was added to the filter cone and 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:

$$\text{Oil and Grease} = \frac{(A-B)\times 1000\text{ml of oil and grease}}{\text{amount of sample (ml)}+\text{grease (mg/l)}}$$

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

3.2.7 Determination of Chemical Oxygen Demand (COD):

Here, the permanganate method was used, where by a blank was prepared to each water sample, and 10ml of tetraoxosulphate(vi) acid (H_2SO_4) was added. After that, an extra 10ml of potassium per manganate ($KMnO_4$) was added until a pink colouration appeared. The samples and blanks were incubated at 27^oC for 4hrs. A 3mg potassium iodide (K_2I) solution was later added to the samples and their blanks, followed by adding 5drops of starch indicator to each. Each sample and blank was then titrated with sodium trioxosulphate (Na_2SO_3), and the end point was indicated by the disappearance of the blue colour, making the solution colourless. The COD was determined by the following equation:

COD = (A-B) x 1000 (mg/l) / a x volume of sample used

Where; A = volume of 0.0125N $KMnO_4$ for blank

B = volume of 0.0125N $KMnO_4$ for sample

a = volume (ml) of sodium trioxosulphate required for 10ml of $KMnO_4$

3.2.8 Determination of Total Dissolved Solids (TDS):

For TDS determination, the laboratory apparatus to be used, which include measuring cylinder, funnels, dishes and beakers were washed thoroughly with detergent solution, rinsed with distilled

water and placed in the hot air oven to dry at 105°C temperature, followed by cooling them in the desiccators. The dishes were weighed with the analytical balance (A (g)). The samples were then individually mixed and stirred, measuring 30ml of each with a measuring cylinder and poured into each dish, placed in the water bath and evaporated to dryness. They were then dried in the oven at 105°C, after which they were again placed in the desiccators to cool. The weight of the dry dishes and contents was obtained by using the analytical balance (B (g)). The TDS was determined as:

$$\text{Total Dissolved Solids} = \frac{(B-A)\times 10^6}{\text{amount of sample (ml)}}$$

Where, A = weight of dish alone (g); B = weight of dish + contents (g)

3.2.9 Determination of Total Suspended Solids (TSS):

For determination of TSS, filter paper was washed thoroughly with distilled water and transferred into the hot air oven to dry at 105°C, then cooled in the desssicators. The initial weight was taken on the analytical balance and recorded as W_1, in grams. The filter paper was then folded into a conical shape with clean, dry hands, and 50ml of the effluent sample was poured to strain off and retain the suspended solids therein. The filter paper was dried again in the oven at 105°C. The filter paper and suspended solids was finally taken to the desssicators to cool before recording the final weight as W_2 in grams. The amount of the total suspended solids in the effluent was determined as:

$$\text{Suspended Solids (mg)} = \frac{(W_2 - W_1)\times 10^6}{\text{amount (ml)of sample}}$$

Where, W_1 = initial weight of filter paper (mg)

W_2 = final weight of filter paper + suspended solids (mg).

3.2.10 Determination of Turbidity:

The turbidity of the effluent was determined by using a Partech Model DRT100B Turbimeter, following the manufacturer's instructions.

3.2.11 Determination of Heavy Metals Content of Refinery Effluent:

The concentration of heavy metal ions (Nickel, Mercury and Arsenic) in the sample was analysed using the fast sequential atomic absorption spectrophotometer (Model AA240S, Varian technologies, USA) following the method modified by APHA (1999). The instrument's setting and conditions of operation was carried out in accordance with manufacturer's specifications by calibrating with analytical grade of metal standard stock solution. After that, 5ml of each sample was digested in a beaker by adding 37.5ml of nitric acid followed by 12.5ml hydrochloric acid. This was 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 filtrate was then analysed for heavy metals content using the AAS.

3.3 Determination of Proximate Composition of Sugarcane Bagasse Sample

The proximate composition of substrate was determined using standard methods as outlined by the Association of Official Analytical Chemists, AOAC, (2010); where by its major components were assessed. Such major components that were determined include the following:

3.3.1 Determination of Moisture Content:

This was determined by washing and drying crucibles in an oven at 100^oC for 1 hour, and the weight was recorded as W_1. After this, 2gs of the sugarcane bagasse was separately weighed into

crucibles and their weight was recorded as W_2 before and during drying at 100°C to a constant weight W_3.

$$\text{Moisture content (\%)} = \frac{(W_3 - W_1) \times 100}{(W_2 - W_1)}$$

Where: W_1 = weight of empty crucible, W_2 = weight of sample and crucible before drying

W_3 = weight of sample after drying to a constant weight.

3.3.2 Determination of Protein Content:

The protein content was determined according to the standard methods of AOAC (2010), using Kjeldahl's method. It was carried out by weighing 2gs of the sugarcane bagasse into Kjeldahl's flask and adding 5gs of anhydrous sodium sulphate, after which 25mls of H_2SO_4 was added with a few boiling chips. The mixture was heated in the fume chamber until a clear solution was obtained. This solution was allowed to cool, transferred into a 250ml volumetric flask and was made up to the level with distilled water.

The distillation unit was carried out using a well cleaned Kjeidahl apparatus. It was done by putting 5ml of 2% boric acid into a 100ml conical flask; and to this, 2 drops of methyl red indicator was added under the condenser. Using a pipette, 5ml of the digest transferred into the apparatus through the small funnel on the distillation unit. The digest was washed down with distilled water followed by addition of 5ml of 60% sodium hydroxide solution; and it was then distilled until the volume of the distillate (ammonium sulphate) reached 100ml. The solution in the conical flask was then titrated with 0.04M hydrochloric acid (HCl), until a permanent pink

colour was seen. The blank was also titrated in the same way and the time value for the samples was obtained.

Percentage Nitrogen = $(V_s \times V_b \times N_{acid} \times 0.04 \times 100)/W$

Where; Vs = Volume (ml) of acid required to titrate the sample; Vb = volume (ml) of acid required to titrate the blank; N acid = normality of the acid (0.1N); W = weight of sample in grams.

Since most proteins contain about 16% Nitrogen, then, 16mg Nitrogen = 100mg of protein.

1mg Nitrogen = 6.25mg of protein. Therefore, percentage protein = N x 6.25 (conversion factor for proteins).

3.3.3 Determination of Lipid (fat) Content:

A round bottom flask of 500ml capacity was set up along with a Soxhlet extractor and a reflux condenser, and 300ml of petroleum ether was poured into the flask. Using the top-loading balance, 2gs of the sugarcane bagasse was weighed into a labeled thimble and sealed with cotton wool, after which it was fit into the extraction tube of the soxhlet extractor. The soxhlet extractor was allowed to reflux for a period of about 6 hours after the assembly was made, and then it was removed with care and the petroleum ether collected on top and drained into a container for re-use. After the ether has dried from the flask, the sample was then removed and dried in dessicator before it was weighed.

The fat content was calculated as follows:

$$\text{Fat content (\%)} = \frac{(W_2 - W_1) \times 100}{W}$$

Where: W= weight of sample used; W_1= weight of empty extracting flask; W_2= weight of flask + extracted oil.

3.3.4 Determination of Ash Content:

The ash content of the sugarcane bagasse was determined by pre-heating, cooling and weighing a crucible. The sample was then charred on a Bunsen flame inside a fume cupboard, and then placed in a muffle furnace set at 550°C for 2hrs until it turned to a white or light grey ash. The ash was then removed and weighed after it was cooled in desiccators. Ash content was calculated using the formula:

$$\text{Ash content (\%)} = \frac{(W_3 - W_1) \times 100}{(W_2 - W_1)}$$

Where; W_1 = weight of empty crucible; W_2 = weight of crucible + weight of sample before ashing; W_3 = weight of crucible + weight of sample after ashing.

3.3.5 Determination of Carbohydrate Content:

Carbohydrate content was obtained by method of difference having gotten other component of the material. That is;

100 - (% of moisture + % of protein + % of fat + % of ash)

3.3.6 Determination of Cellulose Content:

Approximately 2g of sugarcane bagasse was weighed and transferred into a 250 ml beaker, and 100 ml of 17.5% NaOH solution was added to it and stirred at 25°C for 30 minutes. The content of the beaker was then filtered, washed with 25 ml 9.5% NaOH solution and 20 ml portions of

100 ml distilled water. The residue was again washed with distilled water and 40 ml of 10% acetic acid and then with 1 Litre distilled water again. The residue was then then dried at 105°C for 24 hours to constant weight.

3.3.7 Determination of Hemicellulose Content:

Hemicellulose content was determined as the difference between the weight of holocellulose (which is made up of insoluble cellulose and soluble hemicellulose) and cellulose, based on its solubility in 17.5% NaOH solution. The holocellulose was first determined by weighing 2g of the sugarcane bagasse and adding 180ml distilled water, 8.6g sodium chloride, 6.0ml ethanoic acid and 6.6g sodium chloride respectively. The mixture was then digested in a 250 ml conical flask under reflux at 70°C for 3 hours. It was then allowed to cool, filtered and the residue washed with five 20 ml portions of 100 ml distilled water, the residue was then dried at 105°C for 24 hours to attain constant weight.

The Hemicellulose Content in grams = $\{(\text{Weight of holocellulose})\text{g} - (\text{Weight of cellulose})\text{g}\}$

3.3.8 Determination of Lignin Content:

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

3.4 Assessing Growth of *Trichophyton* sp. and *Aspergillus flavus* on Sugarcane Bagasse:

This was done by placing 20g of sugarcane bagasse in large petridishes followed by supplemental addition of 20ml, 200mg/ml of ammonium nitrate (NH_4NO_3), magnesium sulphate ($MgSO_4$) and potassium hydrogen phosphate (K_2HPO_4). The Petri-dishes and their contents were autoclaved at 121°C for 15mins, under 151b/sq. inch, to kill any contaminating microbes. Spores suspensions of the isolates of *Aspergillus flavus* and *Trichophyton* species were inoculated onto duplicate plates of the freshly sterilized sugarcane bagasse. All inoculated plates were incubated for 7days under aerobic conditions in a dark cupboard under ambient laboratory conditions.

The cultures were monitored continuously for the 7days period, after which they were harvested, dried and weighed using the top loading balance to obtain the dry weight of the partially digested bagasse.

3.5 Assessment of Heavy Metals Removal by the Sorbents

3.5.1 Preparation of the Experimental Sorbents:

This was carried out using a set of five (5) fabricated Aluminum sorption columns measuring 5cm in diameter x 50cm depth packed with 200g of the sugarcane bagasse each. This was followed by addition of 200ml of 200mg/ml solution of Ammonium nitrate (NH_4NO_3), Magnesium sulphate ($MgSO_4$) and Potassium Hydrogen Phosphate (K_2HPO_4) to provide a supplemental source of nitrogen, phosphorus, sulfur, potassium, and magnesium required for the growth of the fungi with the sugarcane bagasse as source of carbon and energy.

One of the sorption column thus prepared (A) was left unsterilized and un-inoculated and then moistened with water to serve as the negative control. The second column (B) was sterilized but

un-inoculated to serve as positive control. The third (C), fourth (D) and fifth (E) columns were sterilized and inoculated with *Trichophyton* species culture; *Aspergillus flavus* culture and co-culture of the two fungal isolates respectively (Plate I). The whole set up was incubated aerobically at 25°C for 2 weeks to allow growth and accumulation of fungal biomass intimately mixed with the bagasse to obtain the experimental sorbents.

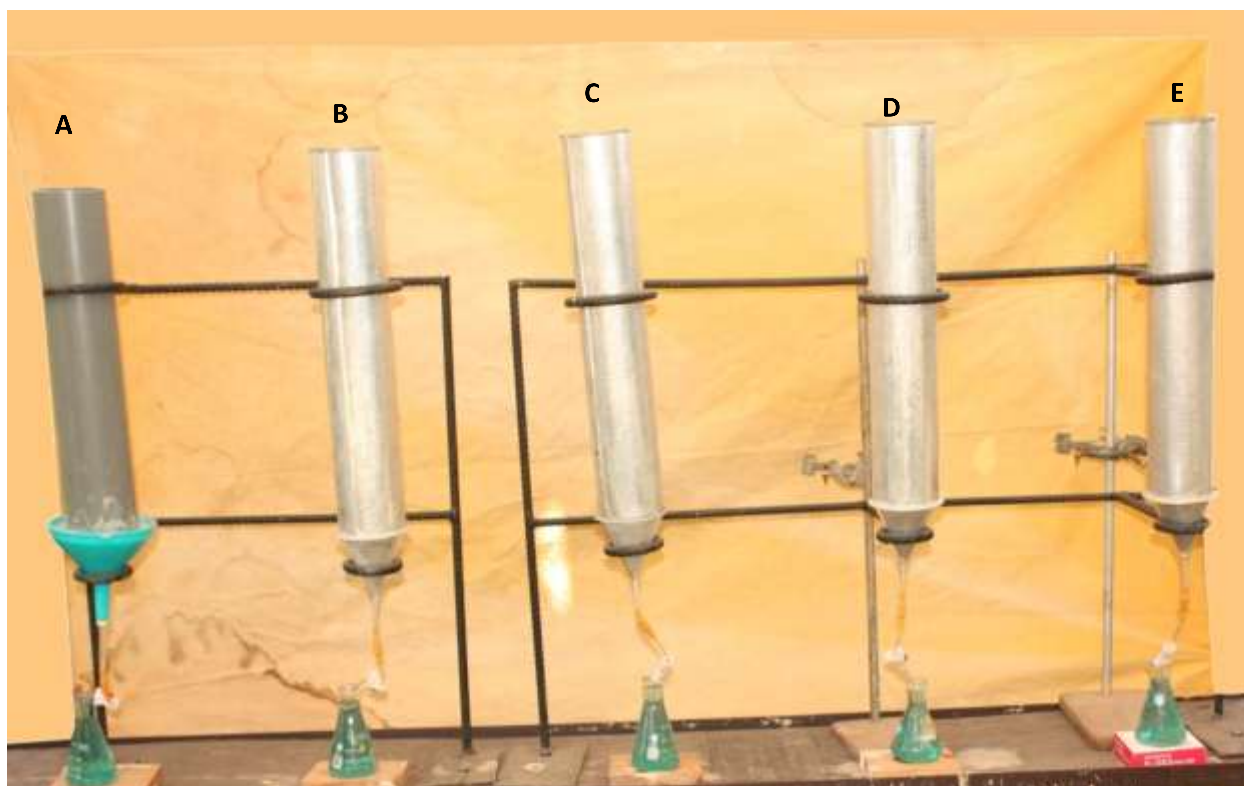


Plate I: Experimental Setup of the Sorption Process

3.5.2 The Sorption Process:

The sorption process commenced by pouring 1 liter of raw refinery effluent previously sterilized by autoclaving to completely submerge the fixed bed columns (sorbents) and allowed to stand for 1 hour. After the first hour, 20ml of filtrates were drawn from each column. This was repeated at hourly intervals for 6 hours. Samples of filtrates thus obtained were the analysed to

determine the residual concentrations of the target heavy metal ions using Atomic absorption spectrophotometer (AA240FS).

3.5.3 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 from the effluent (R) was calculated and expressed in percentages using the equation:

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

Where C_i = initial concentrations and C_f = final concentrations of heavy metal ions in the effluent before and after biosorption process respectively.

The amount of heavy metal ion sorbed per unit mass (mg/g) of sorbent (qt) was calculated using the equation:

$$Q_t = \frac{(C_i - C_f)}{M} \times V$$

Where C_i = initial concentrations and C_f = final concentrations of heavy metal ions in the effluent before and after biosorption process respectively, 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:

$$Q = \{Q_{max}(bC_f)/(1 + bC_f)\}$$

Thus,

$$b = \left\{\left(\frac{Q}{Q_{max}} \times C_f\right) - (Q \times C_f)\right\}$$

Where Q = quantity metal ion adsorbed at time t; Q_{max} = maximum adsorption at time t and C_f is the final concentration of metal ion at time t.

3.6 Data Analysis

Data obtained from results was analysed by one-way Analysis of Variance (ANOVA) at 5% confidence interval, using the SPSS Version 21 Package.

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Properties of Refinery Effluent

Results obtained from the effluent of Kaduna Refinery and Petrochemical Company show that physical parameters such as temperature (29.6^{o}C) and total dissolved solids (258mg/l) was found to be within the normal range, while the electrical conductivity (717 µS/cm), suspended solids (120mg/l) and turbidity (111NTU) were found to be higher than values set by FMENV (Table 4.1).

Results of chemical parameters such as pH (8.83) showed the refinery effluent to be slightly alkaline but still below the upper limit set by FMENV. Other parameters such as biochemical oxygen demand (1.2mg/l), dissolved oxygen (2.9mg/l) were found to be below the permissible limits. However, the chemical oxygen demand (874.1mg/l), phosphate (33.7mg/l) and sulphate (73.2mg/l) were much higher than values set for refinery effluents. But, values recorded for oil and grease (3.7mg/l) and nitrate (1.67mg/l) were within the levels recommended for refinery effluents (Table 4.1). The Mercury (484.780mg/l) and Nickel (1.149mg/l) contents of the refinery effluent were found to be far in excess of the level considered safe for discharge into the environment (Table 4.1).

Table 4.1: Physicochemical Properties of Refinery Effluent

Parameters Determined	Refinery Effluent Values	FMENV Standard
Temperature (^{o}C)	29.6	40.0
Electrical conductivity (µS/cm)	**717**	400
Total Dissolved Solids (mg/l)	258	500
Suspended Solids (mg/l)	**120**	30
Turbidity (NTU)	**111**	5
pH	8.83	6.0-9.0
Biological Oxygen Demand (mg/l)	1.20	10
Dissolved Oxygen (mg/l)	2.90	10
Chemical Oxygen Demand (mg/l)	**874.10**	50
Oil and Grease (mg/l)	3.70	10
Phosphates (mg/l)	33.70	5
Sulphate (mg/l)	**73.20**	50
Nitrates (mg/l)	1.67	10
Nickel (mg/l)	**1.149**	0.07
Mercury (mg/l)	**484.78**	0.002
Arsenic (mg/l)	0.00	NA

KEY:

FMENV: Federal Ministry of Environment, Nigeria, NA: Not Available, µS/cm: micro siemens per centimetres,

4.2 Proximate Composition of Sugarcane Bagasse:

The proximate analysis show that sugarcane bagasse consists of the following components: carbohydrates (64.7%), crude fibre (26.02%), fat (0.2%), crude protein (4.81%), moisture content (3.52%) and ash content (0.75%) which are the major components of the bagasse (Table 4.2).

Further analyses revealed contents of the crude fibre fraction as cellulose (46.5%), hemicellulose (23.61%) and lignin (21.4%) as the major components (Fig 4.2). The ash content (0.75%) and other components (7.74%) account for the remaining portion of the crude fibre fraction (Fig 4.1).

4.3 Capacity of Sugarcane Bagasse to Support the Growth of the Experimental Fungi

The sugarcane bagasse plate inoculated with *Trichophyton* species shows a luxuriant growth of the organism, which indicates that sugarcane bagasse was able to support growth of the organism (Plate II) when compared to the un-inoculated bagasse (Plate III). Similarly, the substrate has capacity to support the growth of *Aspergillus flavus* by the luxuriant nature of growth shown by the organism (Plate IV) compared to the un-inoculated bagasse in plate III.

The growth of *Aspergillus flavus* on the sugarcane bagasse was observed to be faster and more luxuriant after the period of inoculation and incubation than that of *Trichophyton* species (Table 4.3), as it was able to utilise 52.5% of the substrate, higher than what was utilised by *Trichophyton* sp. (32.5%) as shown in table 4.3 below:

Table 4.2: Proximate Composition of Sugarcane Bagasse

Components	Amount (%)
Carbohydrates	64.7
Crude Fibre	26.02
Fat	0.2
Protein	4.81
Ash	0.75
Moisture	3.52

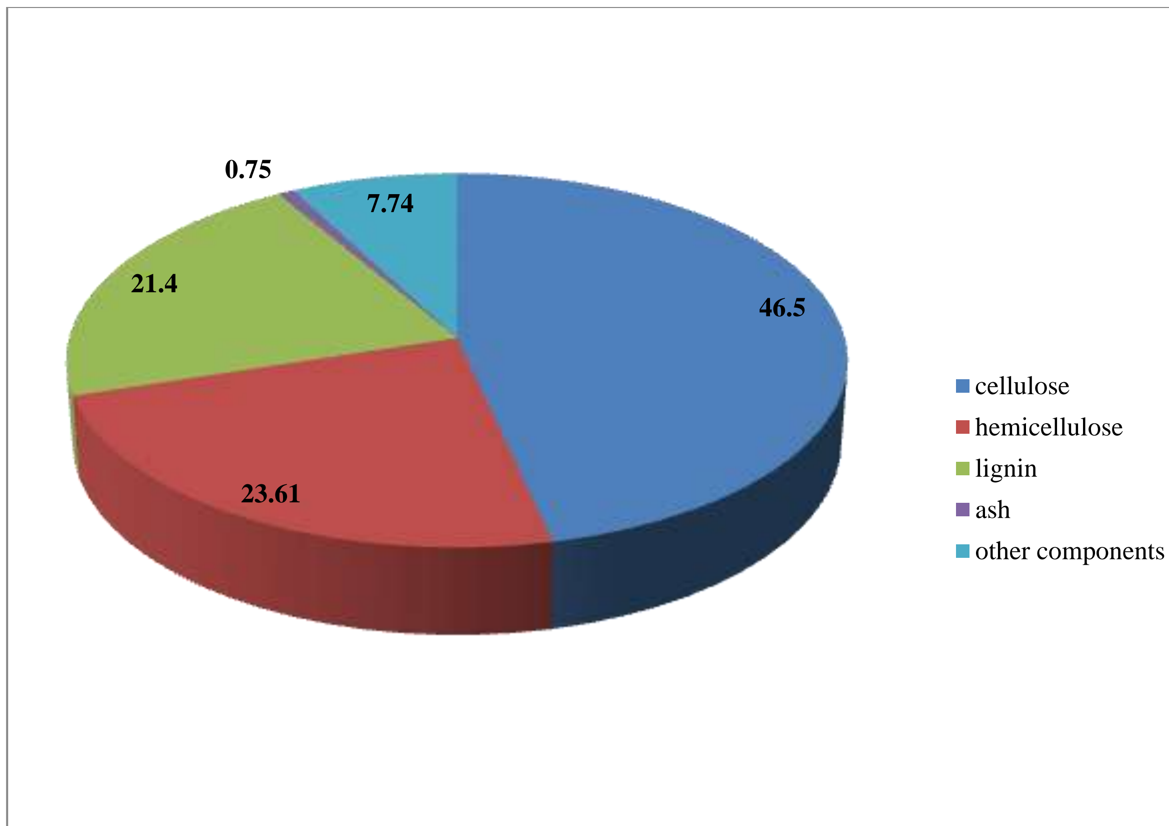


Fig 4.1 Crude Fibre Components of Sugarcane Bagasse Used for the Sorption Process

Plate II: *Trichophyton* species Grown on Sugarcane Bagasse

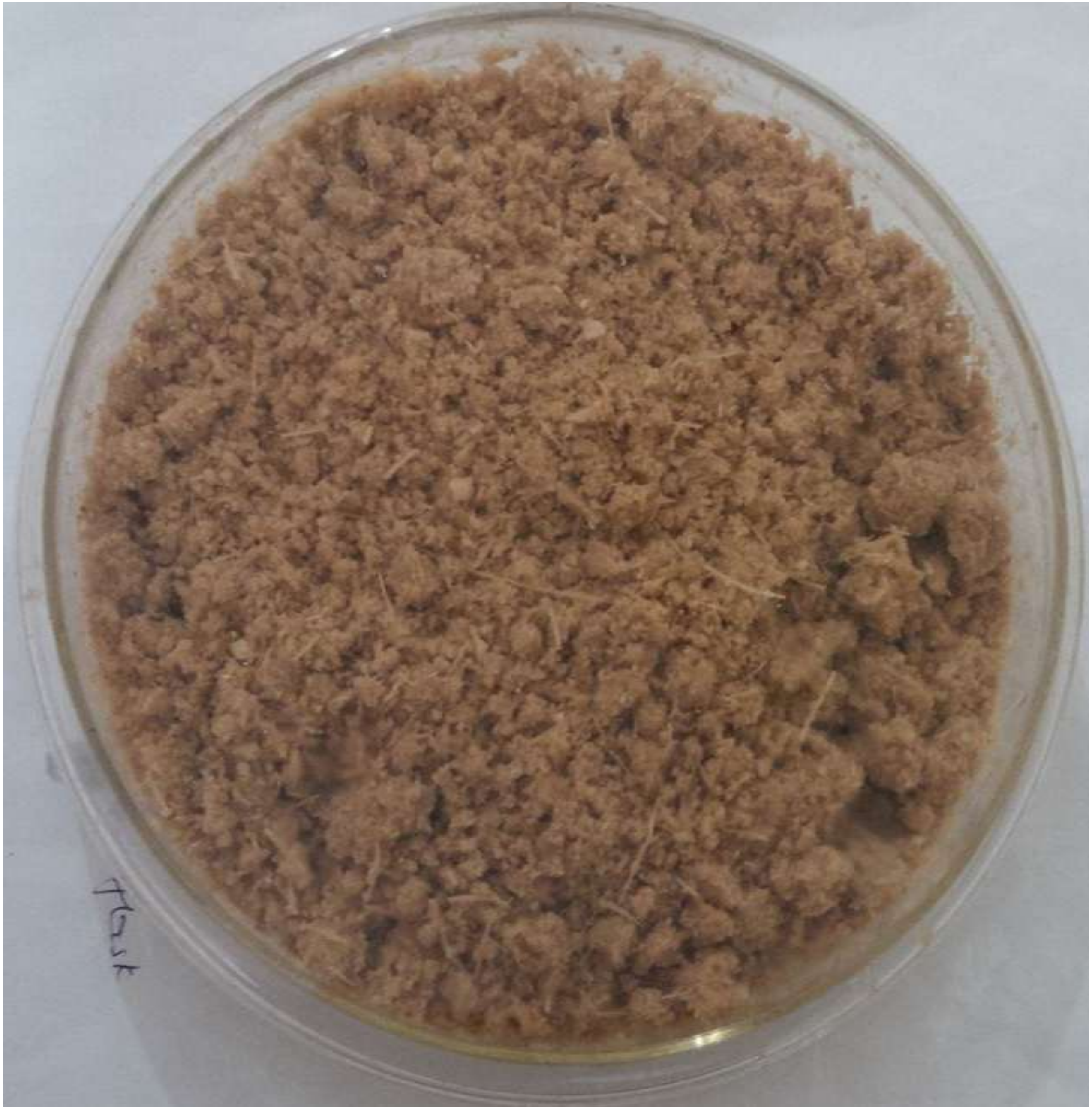


Plate III: Un-inoculated Sugarcane Bagasse

Plate IV: *Aspergillus flavus* Grown on Sugarcane Bagasse

Table 4.3: Amount of Sugarcane Bagasse Decomposed by Test Organisms after 14days Incubation at 25°C

Organisms	W_1 (g)	W_2 (g)	W_3(g)	Proportion of Bagasse metabolized (%)
***Trichophyton* sp.**	20.0	13.5	6.5	32.5
Aspergillus flavus		9.5	10.5	52.5

KEY:

W1: Initial amount of bagasse (g) before incubation; W2: Final amount of bagasse (g) after incubation; W3: Amount of sugarcane bagasse (g) utilised after incubation.

4.4 Removal of Nickel and Mercury by the Experimental Sorbents

This was expressed in percentage and it shows that more than 90% of the metals were removed efficiently.

Removal of Nickel was highest by the sorbent containing sterilised sugarcane bagasse inoculated with the co-culture of *Trichophyton* sp. and A. *flavus* (E) and the sorbent containing sterilised sugarcane bagasse inoculated with A. *flavus* (D), with more than 97.2% and 97.1% respectively, while A removed the least (91.7%), which is shown in the order E>D>B>C>A (Fig. 4.2). There was a significant difference between the sorbents used in removal of Ni ions by the type of sorbents used ($p<0.05$) as shown in Appendix IV.

The performance of all the experimental sorbents was also very efficient in Mercury removal as there was no significant difference ($p>0.05$) in the values obtained which were above 97% in each case (Appendix V). The unsterilised and un-inoculated sugarcane bagasse (A) had the highest removal (97.6%), followed by sterilised un-inoculated bagasse (B), with 97.4%. Sterilised sugarcane bagasse inoculated with co-culture of A. *flavus* and *Trichophyton* sp. (E) removed the least (97.1%). The order of Mercury ions removal by the sorbents was in the order A>B>C>D>E (Fig. 4.2).

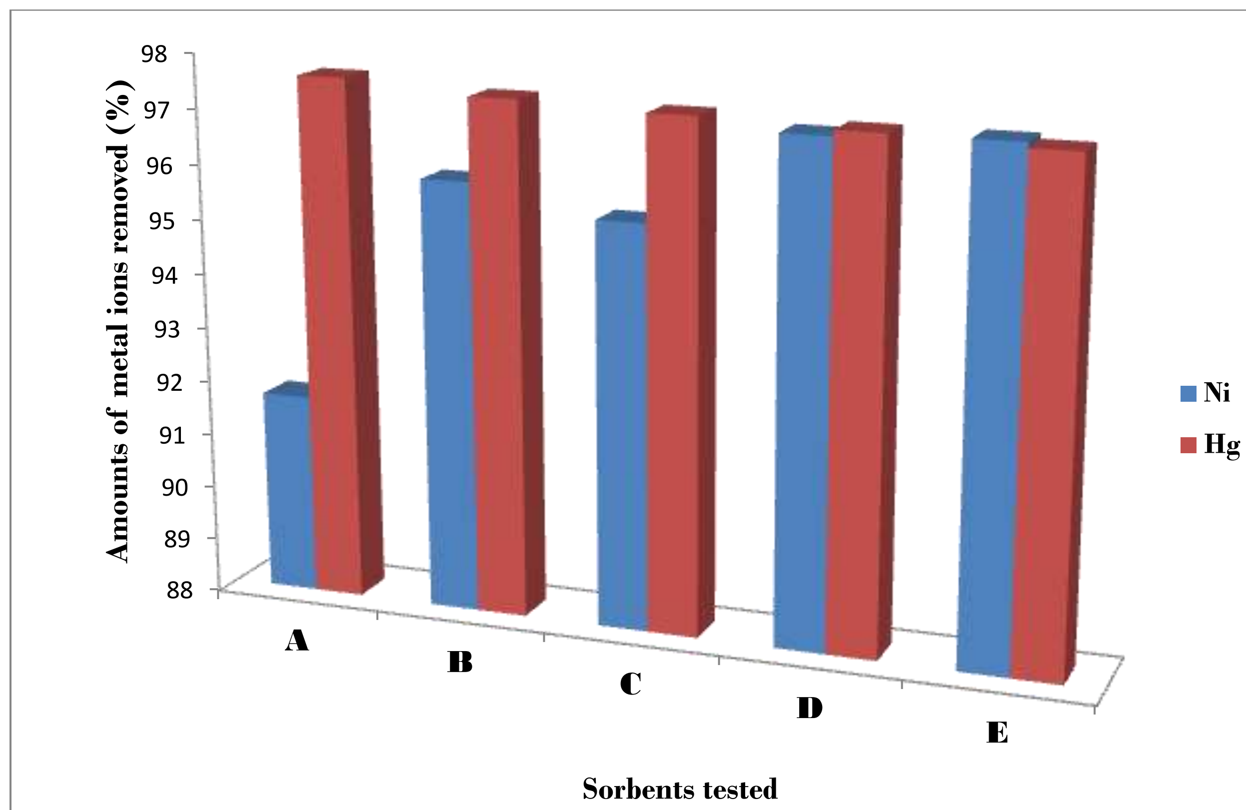


Fig. 4.2: Percentage Removal of Ni and Hg by Sorbents Using Sorption Columns Containing the Different Sorbents.

KEY:

A: Column containing unsterilised sugarcane bagasse only; B: Column containing sterilised sugarcane bagasse only; C: Column containing sterilised sugarcane bagasse + *Trichophyton* sp.; D: Column containing sterilised sugarcane bagasse + *Aspergillus flavus*; E: Column containing sugarcane bagasse + *Trichophyton* sp. and *Aspergillus flavus*; Ni: Nickel; Hg: Mercury.

4.5 Amount of Nickel and Mercury Adsorbed by Sorbents

The heavy metals Ni and Hg were efficiently adsorbed by all the sorbents as shown in Table 4.5. The amount of Nickel adsorbed has shown that sugarcane bagasse inoculated with A. *flavus* (D) (1.03mg/g) adsorbed the highest amount of Nickel, followed by the co-culture of A. *flavus* and *Trichophyton* sp. (E), which adsorbed 1.02mg/g. Sorbent A, which contained the unsterilised and un-inoculated sugarcane bagasse adsorbed the lowest amount of Nickel (0.89mg/g). The order of Nickel ions adsorption was D>E>B>C>A (Table 4.4).

Similarly, Mercury ions were adsorbed efficiently by all the sorbents, with A (containing the unsterilised and un-inoculated sugarcane bagasse) having the highest value (437.40mg/g), followed by B (sorbent containing sterilised un-inoculated sugarcane bagasse) with a value of 434.84mg/g. A value of 428.45mg/g was recorded from sorbent E, which contained the co-culture of A. *flavus* and *Trichophyton* sp. inoculated on sterilised sugarcane bagasse. In this experiment, Mercury ions were adsorbed by the different sorbents in the order A>B>C>D>E (Table 4.4).

There was also no significant difference ($p>0.05$) between the sorbents used in terms of metal adsorption (Appendix VI and VII).

Table 4.4: Amount of Heavy Metal Ions Sorbed by the Experimental Sorbents

Experimental Sorbents	Amount (Q) of Metal Ions Sorbed (mg/g)	
	Nickel	**Mercury**
A	0.89	**437.40**
B	1.01	434.84
C	0.97	432.73
D	**1.03**	430.86
E	1.02	428.45

KEY:

A: Column containing unsterilised sugarcane bagasse only; B: Column containing sterilised sugarcane bagasse only; C: Column containing sterilised sugarcane bagasse + *Trichophyton* sp.; D: Column containing sterilised sugarcane bagasse + *Aspergillus flavus;* E: Column containing sugarcane bagasse + *Trichophyton* sp. and *Aspergillus flavus;* Q: Equilibrium or residual concentration of metal ions (mg/g).

4.6 Effect of Time on Sorption of Nickel Ions

Adsorption of Nickel in the various sorption columns shows a clear decrease with increase in time in column A only, the maximum adsorption of Nickel ions was observed after 1hr of passing the effluent through the column (1.086mg/g), and the minimum after 6hrs (0.681mg/g) as shown in figure 4.3 below. The other columns B, C, D and E did not show a steady decrease from the first to the last hour. This implies that a higher amount of Ni was removed (1.086mg/g) after 1hr of passing the effluent through the column (Fig. 4.3).

4.7 Effect of Time on Sorption of Mercury Ions

The adsorption capacity of Mercury ions by the sorbents in the columns also shows a sharp decrease from time 1-4hrs of passing the effluent through the columns (Fig. 4.4). The highest adsorption in all the columns was after 1hr of passing the effluent in the columns and it continued to decrease up to the 4th hr. The value of Q became constant after 4hrs in all the sorbents. Therefore, maximum adsorption of Mercury ions in this experiment was observed from 1-4hrs (Fig. 4.4).

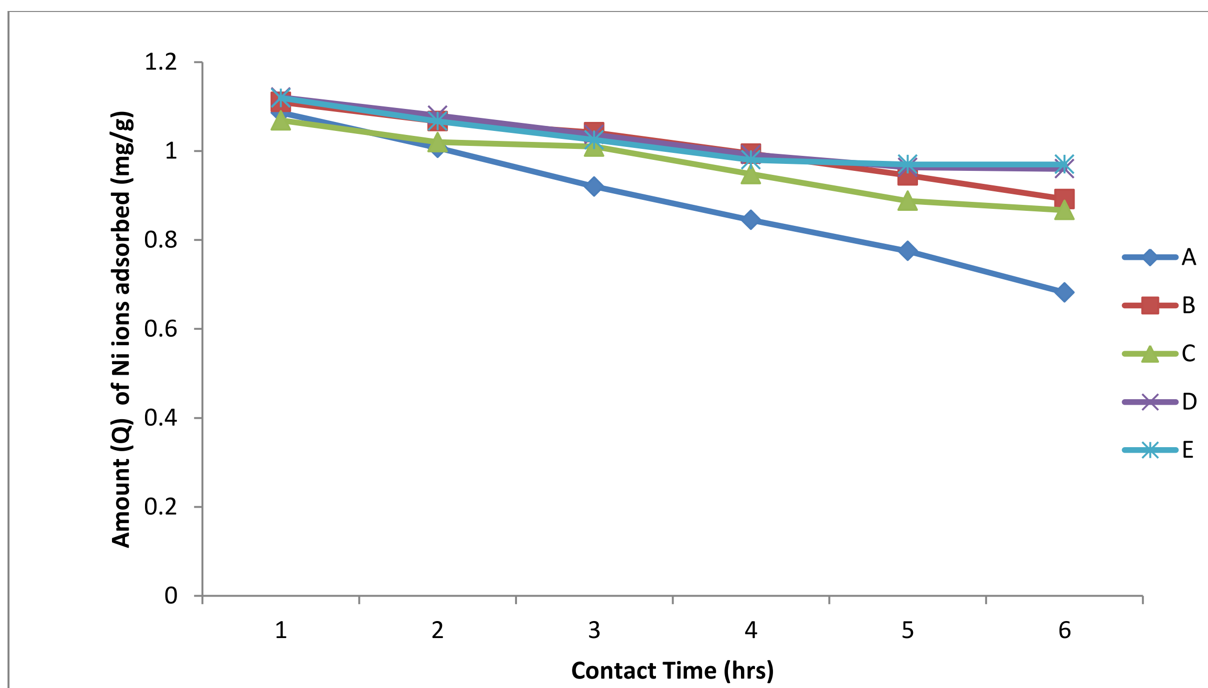


Fig 4.3 Effect of Contact Time on Nickel Ions Adsorption in the Sorption Columns.

KEY:

A: Column containing unsterilised sugarcane bagasse only; B: Column containing sterilised sugarcane bagasse only; C: Column containing sterilised sugarcane bagasse + *Trichophyton* sp.; D: Column containing sterilised sugarcane bagasse + *Aspergillus flavus*; E: Column containing sugarcane bagasse + *Trichophyton* sp. and *Aspergillus flavus*; Q: equilibrium residual concentration of metal ions (mg/g); Ni: Nickel

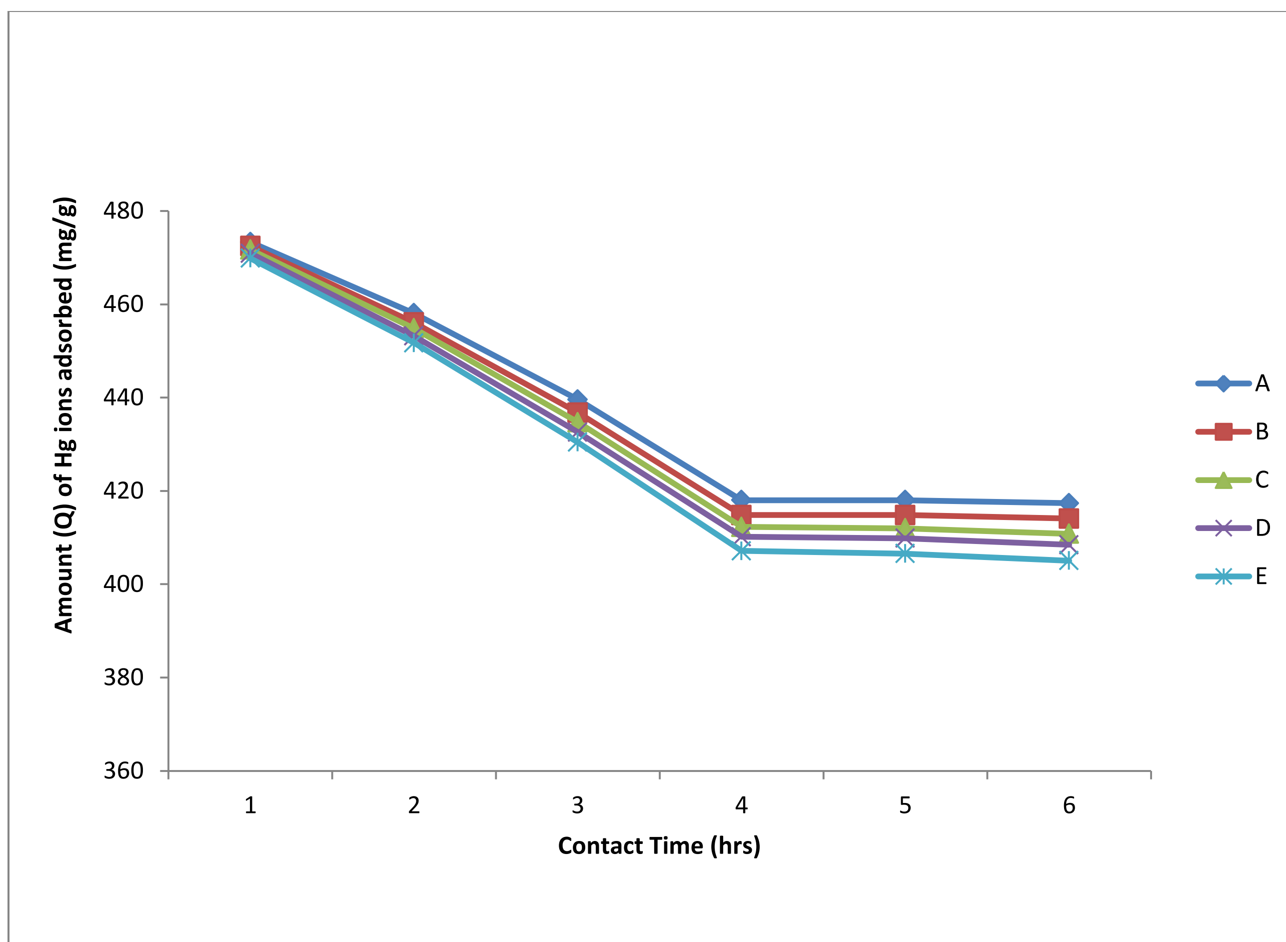


Fig 4.4 Effect of Contact Time on Mercury Ions Adsorption in the Sorption Columns.

KEY:

A: Column containing unsterilised sugarcane bagasse only; B: Column containing sterilised sugarcane bagasse only; C: Column containing sterilised sugarcane bagasse + *Trichophyton* sp.; D: Column containing sterilised sugarcane bagasse + *Aspergillus flavus*; E: Column containing sugarcane bagasse + *Trichophyton* sp. and *Aspergillus flavus*; Q: equilibrium or residual concentration of metal ions (mg/g); Hg: Mercury.

4.8 Relative Affinity of Sorbents for the Target Metals Ions

The relative affinity, **b** is used to measure the degree of interaction or attraction between the sorbates and sorbents, which enables adsorption to take place. Higher value of b indicates lower attraction between a metal and sorbent and *vice-varsa*. In this study, the values of b are lower for Mercury ions, which show that the sorbents had higher affinity for Mercury than Nickel ions (Fig. 4.5).

The affinity for Ni ions by the sorbents was highest in sorbent A, the unsterilised and un-inoculated sugarcane bagasse (b = 3.26) and lowest in D, which contained the sterilised sugarcane bagasse inoculated with A. *flavus*, with a value of b = 19.72 (Fig. 4.5). Similarly, the highest affinity for Hg ions was highest in the column containing the unsterilised and un-inoculated sugarcane bagasse (A) and the column consisting of sterilised and un-inoculated sugarcane bagasse (B), both showing a value of b = 0.07; while the column which contained sterilised sugarcane bagasse (C), recorded the lowest affinity between the Mercury ions and the sorbent (b = 1.11) as shown in Fig. 4.5 below:

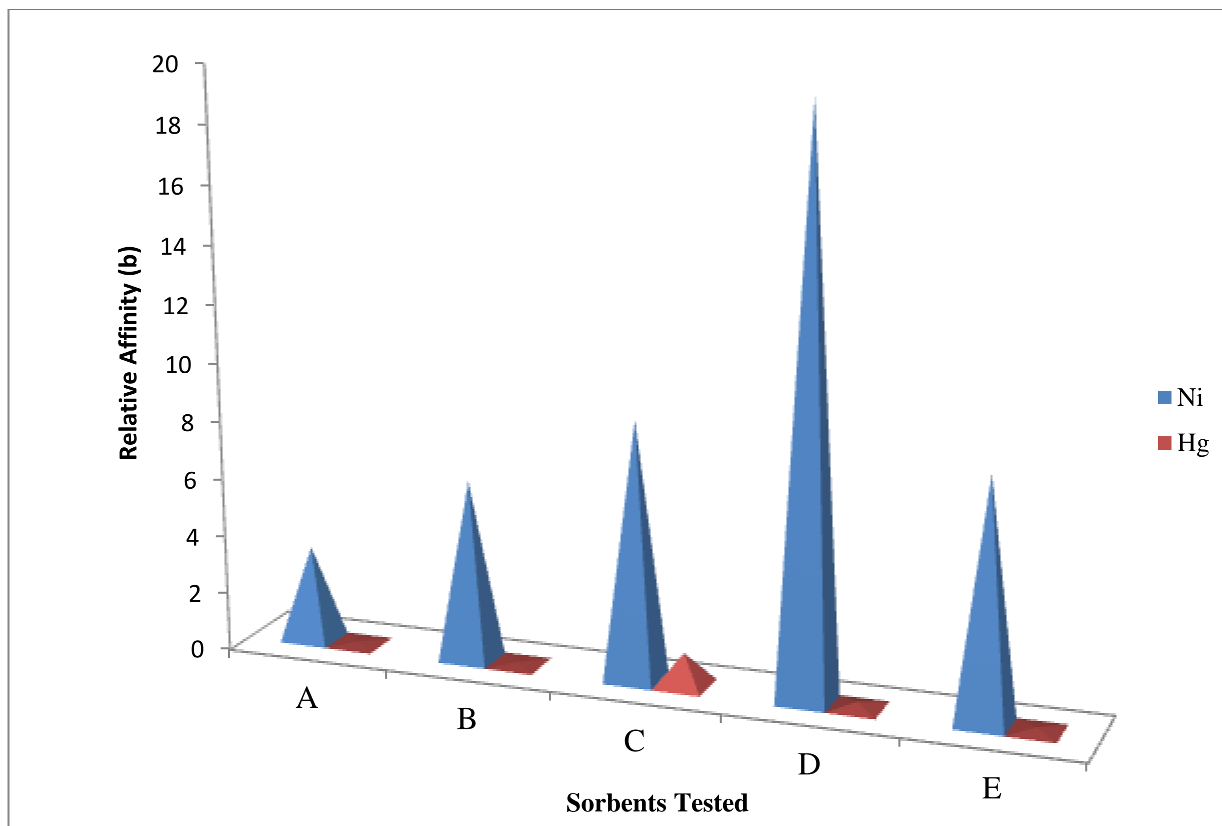


Fig 4.5 Relative Affinity (b) between Heavy Metals and Sorbents in Sorption Columns

KEY:

A: Column containing unsterilised sugarcane bagasse only; B: Column containing sterilised sugarcane bagasse only; C: Column containing sterilised sugarcane bagasse + *Trichophyton* sp.; D: Column containing sterilised sugarcane bagasse + *Aspergillus flavus*; E: Column containing sugarcane bagasse + *Trichophyton* sp. and *Aspergillus flavus*; b: Langmuire's Constant, defines the affinity between the sorbates (heavy metals) and Sorbents; Ni: Nickel; Hg: Mercury

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical Properties of the Refinery Effluent:

The temperature of the refinery effluent was found to be moderate and seems favourable for survival of living things in the aquatic environment. This could be because the processes that occurred in the processing plants did not lead to the production of high temperature products. As a result, the effluent poses no threat to the environment and the aquatic organisms when released into the receiving water body with regards to temperature. The range obtained was lower than what was obtained by Lokhande *et al*. (2011) in Mumbai, India, who obtained a temperature of 31.4^{o}C, and Chikogu *et al*. (2012) in Kaduna, Nigeria who recorded a temperature range of 30-38°C. The Total Dissolved Solids, made up of soluble materials of both organic and inorganic materials were also not in any way hazardous to the environment, which might have been as a result of low quantity of organic materials in the processing unit of the refinery. Chikogu *et al*. (2012) had a contrary result to this, where by the total dissolved solids was found to be higher than permissible limits.

The effluent also had a normal pH which was slightly alkaline but within the permissible limit. The reason for this could be that the materials processed from the refinery contained substances which were alkaline in nature, thus, giving the effluent its alkaline pH despite the high dissolution and mixing of various chemicals in the effluent during the rainy season, which was the time of collection of this sample (Thipeswamy *et al*., 2012). This result is in contrast with results obtained by Atubi (2011) in Warri, Chikogu *et al*. (2012) in Kaduna refinery and Sabo *et al*. (2013) in River Delimi, Jos where acidic pH was reported in all cases. Oil and grease and

nitrates were also normal in the effluent and may not cause any hazardous changes in the environment. This is because the refinery processes from which this effluent was released contained little amount of organic hydrocarbons. This implies that the refinery effluent consists of minimum organic pollutants, which is tolerable for the receiving environment. Chikogu *et al*. (2012) obtained similar results on oil and grease from Kaduna refinery, and contrary results on nitrates, which were found to be high.

The electrical conductivity was very high, almost twice the permissible range given by the FMENV. This could be due to the presence of charged particles of dissolved salts and various chemicals including heavy metals in high quantity within the water body, which is evident in the high chemical oxygen demand obtained. This result was found to be similar to the results obtained by Chikogu *et al.* (2012) from River Romi in Kaduna State, where by a value of 300µS/cm was obtained, but higher than that of Atubi (2011) from Iffie River in Delta state, and Sabo *et al*. (2013) from River Delimi in Jos, Nigeria, where a value of 196µS/cm and 104µS/cm was recorded respectively.

The total suspended solids are formed as a result of the presence of insoluble materials like sand, silt, chemical and organic compounds, etc. The high amount of these obtained in the effluent could be the due to the presence of different types of insoluble materials in the effluent, which may arise from insoluble chemicals available in the refinery operations, which made the effluent to be also highly turbid (111NTU). Similar results were obtained by Atubi (2011) and Mbaneme *et al*. (2013) in Okirika Mainland, Rivers State, Nigeria, where high turbidity was also recorded. The biological oxygen demand (BOD) was within permissible limits, while the dissolved oxygen (DO) value was considered to be too low, which may be due to the high turbidity and high amount of suspended solids. This indicates that a considerable amount of oxygen is needed for

proper aeration of the effluent before releasing to the environment. Results of the BOD and DO are in line with those obtained by Chikogu *et al*. (2012) from River Romi in Kaduna state.

Chemical oxygen demand (COD) and value of heavy metals recorded in high amount in this study could be as a result of the high amount of inorganic chemical pollutants released from the processing unit of the refinery, and is also evident in the high electrical conductivity and high suspended solids. This shows clearly that the pollution status of the refinery effluent was due to presence of inorganic chemicals in high quantity, rather than organic materials.

There was also high amount of sulphates and phosphates in the effluent which further confirms the chemical nature of the pollutants found in the effluent. The presence of sulphates and phosphates in high quantity helped greatly in reducing the amount of DO and BOD, making it too low and dangerous for aquatic animals to survive in the water, if allowed to flow into a nearby river, as it could also lead to eutrophication and subsequent death of the receiving water body due to lack of oxygen, if such condition were allowed to prevail.

Presence of Nickel in refinery effluent may be associated to the fact that the element is a known catalyst widely used in most modern oil refineries, in order to improve the selectivity and efficiency of refining processes (European Nickel Institute, 2007). The amount of Nickel obtained (1.149mg/l) might have been as a result of excessive use of the metal in the various processes mentioned above. This result compares to that of Olatunji and Osibanjo (2012), who obtained a value ranging from 0.75mg/l-1.12mg/l in North Central Nigeria. The results were however, higher than the results obtained by Olaniyi *et al*. (2012), who obtained a value of 0.80mg/l in effluent of a vegetable processing company. The high level of Mercury recorded in this study may be due to the fact that elemental Mercury as well as various Mercury compounds

which include Mercuric chloride ($HgCl_2$), Mercuric sulphides (HgS_2), Mercuric Selenide, dimethylMercury ($(CH3)_2Hg$), diethylMercury, as well as asphaltenes or tars, that exist either as soluble, insoluble, gaseous, solid and liquid states are used highly in the processing of crude oil (Sanchéz and Bain, 2013). Another source of Mercury in refinery effluent may also be from the different treatment plants like the coal-fired plants, which are considered to be the largest Mercury generators, whereby only a small portion of the Mercury generated would be discharged in a soluble form (Littlepage, 2013). Mercury has been reported in refinery effluent by several researchers (Chikogu *et al*., 2012; Olaniyi *et al*., 2012; Nwaichi and Warigbani, 2013; Lakherwal, 2014), and in most cases, found to be higher than the permissible limits. This high quantity is a source of worry, since Mercury is known to be listed by various regulatory bodies as one of the most hazardous heavy metals (Galadima and Garba, 2012; Chikogu *et al*., 2012; Lakherwal, 2014). The result obtained was also much higher than most results obtained from various researches carried out (Chikogu *et al*., 2012; Nwaichi and Warigbani, 2013). The high concentration of these metals in water bodies could have adverse effect on the aquatic life and man in general (Olaniyi *et al*., 2012).

5.2 Proximate Composition of Sugarcane Bagasse

The results obtained from compositional analyses of sugarcane bagasse had shown that cellulose, hemicellulose and lignin were the most abundant. This is because generally, plant biomass, which is known as the most common source of Carbon, consists primarily of these three components (Badu *et al*., 2011). These variations in composition of the sugarcane bagasse might be due to reasons such as differences in the varieties of sugarcane cultivated in various parts of the world, portion of fibre on the cane of the sugarcane, cleanliness of the cane supplied, harvesting practices, as well as maturity of the sugarcane before it was harvested (Carvalho *et*

al., 2009; Irfan *et al*., 2011; Zafar, 2015). The value of the sugarcane bagasse depends largely on its calorific value, which is also affected by its composition of sugars such as carbohydrates, glucose, galactose (Zafar, 2015), etc. which constitute most of the major nutrients needed for organisms to utilise for growth in this research. The materials found in sugarcane bagasse contain different functional groups which provide binding sites for positive metal ions making it possible for them to be adsorbed from solution (Soliman *et al*., 2011).

In most studies carried out which involved sugarcane bagasse, the proximate analysis reveal that cellulose has the highest percentage (about 35-60%), followed by hemicellulose (20-40%); and 10-30% lignin (Rodríguez-Chong *et al*., 2004; Dawson and Boopathy, 2008; Carvalho *et al*., 2009; Irfan *et al*., 2011; De Souza *et al*., 2012) . Studies carried out by De Souza *et al*. (2012) show that sugarcane bagasse consists of 48.6% cellulose, 31.1% hemicellulose, 19.1% lignin, with other components such as ash having 1.2%. In a report by Dawson and Boopathy (2008), proximate composition of sugarcane bagasse was said to consist of cellulose 30%; hemicellulose 23%; and 22% lignin. Irfan *et al*. (2011) also reported proximate composition of sugarcane bagasse as having about 40% cellulose; 23% lignin; ash and moisture content 0.9% and 7.1% respectively. Rodriguez-Chong *et al*. (2004) examined the composition of sugarcane bagasse which comprised of 38.9% cellulose, 20.6% hemicellulose and 23.9% lignin. In another study, the cellulose content was found as 42.5%, hemicellulose 24.88%, lignin 20.90% and ash content was 1.64% (Carvalho *et al*., 2009). The ash content generally has a low value, while the moisture content generally varies.

5.3 Sugarcane Bagasse Utilisation by the Test Organisms

The growth of the test fungal isolates A. *flavus* and *Trichophyton* species on sugarcane bagasse plates shows in each case, a pure culture of the organisms with no sign of any contaminant. This could be due to the fact that sugarcane bagasse as a growth medium does not essentially meet the needs for growth of every microbe, most especially bacteria (Sidana and Farooq, 2014), and therefore, it can be used effectively to minimise culture media contamination by other microorganisms.

Fungi are also known to grow generally well on most organic materials ranging from plant, animal and human materials; and every fungal species requires major elements namely Carbon, Nitrogen and energy source to grow and survive. Sugarcane bagasse as a residual material from sugarcane contains these materials in complex form such as carbohydrates, cellulose and lignin, which can be degraded by the fungi to grow and reproduce (Hasan *et al*., 2015). Sidana and Farooq (2014) explained that sugarcane bagasse allows for growth of fungi with production of more spores, as well as less bacterial contamination as is seen in other media, and also observed that *Aspergillus* species had the maximum ability of spore formation than other fungi grown with the same media. Ojumu *et al*. (2003) reported sugarcane bagasse as a supporting medium for growth of A. *flavus*, while Bhattacharya *et al*., (2011) also recorded A. *flavus* grown on sugarcane bagasse to be the best α-amylase producer in an investigation on the α-amylase production. Hasan *et al*. (2015) also reported a remarkable growth yield of Pink Oyster Mushrooms (*Pleurotus djamor*) using a supplement of sugarcane bagasse and wheat bran. A study carried out by Parani *et al*. (2016) in India had also shown 26.0%-60.3% degradation of sugarcane bagasse cellulose by Oyster Mushrooms. This is evident as other studies carried out

with different species of the genus *Aspergillus* (e.g. A. *niger*), have shown its great efficiency to grow with sugarcane bagasse as substrate (Oliveira *et al*., 2012).

However, there seems to be very scarce literature on the use of sugarcane bagasse to grow fungi of the *Trichophyton* genus. This study also provides evidence that *Trichophyton* sp. can also grow efficiently on sugarcane bagasse, as shown from the plates and table 4.3 above. Similarly, investigation of fungal growth on media designed with sugarcane bagasse proved that several fungal species from different genera, including *Trichophyton* species, could be efficiently grown on sugarcane bagasse substrate, based on their various degradative abilities of cellulose and other complex substances available in the substrate (Sidana and Farooq, 2014).

5.4 Removal of Ni and Hg Ions by Sorbents

The removal of Ni and Hg carried out in this study, which was expressed in percentage shows an overall efficiency range of 91-97% which indicates that it was effective for all the sorbents. However, removal of Hg ions was higher than Nickel. This may be because the sorbents have more binding sites on their various functional groups to pick up the Hg ions from the solution more than the Ni ions.

The nature or type of sorbent seem to have effect in the removal of Ni ions only ($p<0.05$), which means that in this case, the sorbent containing co-culture of A. *flavus* and *Trichophyton* sp. might have done better than the other sorbents due to the use of the two fungal isolates at the same time. The nature of sorbent used however, did not show any significance in the removal of Hg ions from the effluent ($p>0.05$), as the unsterilised bagasse (which contains impurities as well as other microbial contaminants) in this case has shown more efficiency in removing the Hg ions; while reverse is the case for Ni removal. *Aspergillus flavus* was found to remove a higher

percentage of the metal than *Trichophyton* sp. because even in the removal of Hg, there was just a little notable difference between the organisms. This result is in accordance with one obtained by Pandey *et.al* (2007), where 85% removal of Ni ions was observed by fresh biomass and chemically treated leached biomass of *Calotropis procera*.

Overall, the unsterilised bagasse which contains other microbes from the environment removed the highest percentage of heavy metal (97.6% Hg) in this experiment. This indicates that combining microbial biomass with organic wastes in bioremediation process is feasible, but not explicitly more beneficial (Adams *et al*., 2015). Sugarcane bagasse alone, supplemented with necessary nutrients under optimum conditions, is capable of removing a large amount of the heavy metals without any influence from microorganisms, as observed by Hamzah *et al*. (2014), where by sugarcane bagasse was reported to remove 100% of petroleum hydrocarbons and crude oil from polluted soil. Dadrasina and Agamuthu (2013) also observed that biowastes had potential to remediate diesel contaminated soil, and that the rapid removal of hydrocarbons was attributed to the presence of nutrients from the organic wastes, which contain microorganisms and some nutrients.

5.5 Amount of Ni and Hg Ions Adsorbed by Sorbents

This experiment shows that the different sorbents used (sugarcane bagasse, *Aspergillus flavus* and *Trichophyton* species) singly and in combination with one another all had the capacity to adsorb and accumulate Mercury and Nickel ions from the effluent. Each of these sorbents adsorbed a considerable amount of both metals compared to their initial concentration in the effluent. However, the amount of Mercury (437.39mg/l) adsorbed within the 6hrs period of the experiment was more than that of Nickel. This may be owing to the fact that Mercury was found

in much higher quantity in the effluent than Nickel. It may also be as a result of the ability of the various functional groups in the sugarcane bagasse such as amines, carboxyl ions, etc. to adsorb the Hg ions from the solution faster than the Ni ions before equilibrium was attained. The two fungal isolates in this experiment also adsorbed the heavy metals considerably, which may be as a result of the ability of the organisms to tolerate and uptake a high amount of the heavy metals from the environment they were previously isolated.

The best adsorbent in Mercury biosorption was the unsterilised bagasse, while the least was by the co-culture of the fungal isolates. In Nickel adsorption, a reverse case occurred where by *Aspergillus flavus* growing on sugarcane bagasse performed better than other sorbents, followed by the fungal co-culture of A. *flavus* and *Trichophyton* sp. inoculated on sugarcane bagasse. This may be because the metal ion binding capacity of microbial cells is dependent on certain factors such as age, composition of growth media, contact time, pH, temperature, etc. (Bishnoi and Garima, 2005). This result is similar to that of Pandey *et al*. in (2012) who carried out a research on Biosorption characteristics of *Aspergillus flavus* in removal of Nickel from an aqueous solution, and found out that the organism can adsorb up to 10 mg/L, and has tolerance to accumulate high level of the metal. Therefore, bio-removal carried out by this fungus could serve as an economical means of treating leachate, effluent and the polluted water areas charged with toxic metallic ions. The unsterilised bagasse adsorbed the least amount of Ni ions in the experiment. The mean residual concentration of metal ions in the effluent (Q) as seen in the experiment was reduced greatly after the 6hrs period of bioremediation, and this shows that the removal capacity of fungal biomass used is as good as or even better than other conventional methods. This is owing to the fact that some fungal genera including *Aspergillus* have long been reported to have biosorptive capabilities for accumulating Ni, Hg, As and a host of different

heavy metals from polluted areas (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011). Statistical analysis by ANOVA had also shown that there was a strong relation between the amount of Ni ions adsorbed and the type of sorbent used ($p = 0.00$), as shown in Appendix VI; while there was no significant difference between the amount of and Hg ions adsorbed and the type of sorbents used ($p>0.05$) in the sorption columns (Appendix VII).

5.6 Effect of Contact Time on Adsorption of Heavy Metal Ions by Sorbents

From the chart in fig.4.3 and 4.4, time of contact of adsorbents with the sorbates in the columns has an effect on the amount of heavy metals adsorbed by the various sorbents. The adsorption of Nickel by the sorbents (Fig. 4.3) shows a variation of adsorption with time in all the columns, and reached its lowest at maximum time (6hrs). The adsorption factor, Q, given as the equilibrium solid phase concentration, has the highest value after 1hr of adding the effluent in the sorption columns, thus, following a linear trend in the graph which moves downwards. This indicates that the optimum time for adsorption of Nickel ions was averagely 1-2hrs after the addition of effluent in the column, and the order of adsorption of Nickel ions could be taken as D>E>B>A>C. At maximum time of 6hrs, the order of adsorption became E>D>B>C>A, where the co-culture of the two fungal isolates adsorbed more Nickel ions, while bagasse with no inoculation of organisms (A) removed the least Nickel ions than the other sorbents. Equilibrium stage of adsorption was attained in column D and E 5hrs after the start of the experiment, as shown on the chart.

Results from fig 4.4 reveal the amount of Hg adsorbed by the sorbents with respect to time with almost a similar trend as in removal of Ni ions in fig 4.4. In this case, however, it should be noted that the adsorption of the Hg ions reached a clearly constant rate at exactly 4hrs of adding

effluent. This means that the sorbents accumulation of the metal reached equilibrium, such that very little amounts of Hg ions were accumulated by the sorbents. The optimum time for accumulation of Hg ions by all the sorbents was, as in Ni, also 1hr, which decreased significantly as time increased from 1hr to 4hrs. Therefore, average optimum time of adsorption was from 1-4hrs. The order in which Hg ions were adsorbed by the different groups was A>B>C>D>E.

The reasons for this could be because at the beginning of experiment, high amount of nutrients were available for growth and multiplication of microbial cells in column A, C, D and E and therefore, high activity of adsorption and accumulation of the metals occurred at the generation time, which decreased considerably as microbial cells increased, due to inadequate nutrients, as well as saturation of cells and surface of the other sorbent (sugarcane bagasse), which have been occupied by other metals. Another reason could be that as most metal ions were adsorbed at the beginning of the experiment, very little were left to be absorbed as time increased. These results agree with that of Abioye *et al*. (2012), where high degradation of motor oil using organic wastes was observed during the first 15days of experiment. The biosorption of heavy metals is a biphasic process, which involves an initial rapid phase that contributes up to 90% of the whole process within the first 10mins, and a slower phase which can last for up to 4hrs (Bishnoi and Garima, 2005). Soliman *et al*. (2011), who also used sugarcane bagasse and carried out biosorption to remove Fe(III) from polluted water samples, found out that the biosorption process was fast and occurs maximally within the first 60mins.

5.7 Relative Affinity of the Sorbates for the Sorbents

It can be observed from Fig. 4.5 that the values of b (which is known as the Langmuire's constant), that Ni had higher values than Hg, which is evident that the sorbents have higher

affinity to Hg ions more than Ni ions. This is because it has been established that the higher the adsorption capacity and intensity of a sorbent to a sorbate, the lower the value of b and, in turn, the higher the affinity and *vice-versa* (Bishnoi and Garima, 2005; Dhankar and Hooda, 2011). This may be attributed to the nature of the elements and their electron acceptance capability, as well as amount that can be accumulated by the fungal cells and sugarcane bagasse particles, which will facilitate their binding and accumulation to the sorbents. Biosorbents usually have a high affinity for the sorbate particles and so, they are attracted and bound by different mechanisms on the sorbent, and the process will continue until an equilibrium is established (as seen above) between the amount of solid-bound sorbates, and the sorbates that still remain in the solution (Dhankar and Hooda, 2011). The degree of affinity of the sorbent for the sorbate usually determines the distribution of the sorbate between the solid and liquid phase in the experiment (Dhankar and Hooda, 2011).

CHAPTER SIX

6.0 CONCLUSIONS, RECOMMENDATIONS AND CONTRIBUTIONS TO KNOWLEDGE

6.1 Conclusions:

From the study carried out, the following conclusions were made:

1. Physicochemical properties of the Kaduna Refinery and Petrochemical Company were generally found to be tolerable. However, some of the parameters like turbidity, electrical conductivity, total dissolved solids, Chemical Oxygen Demand, and heavy metals (Nickel and Mercury) were found to be much higher than the permissible limits outlined by the Nigerian Federal Ministry of Environment, which suggests that the effluent is hazardous to the receiving environment and needs proper treatment before it is released.
2. The proximate composition of sugarcane bagasse carried out has shown that sugarcane bagasse consists mainly of carbohydrates, crude fibre, fat, protein, moisture and ash contents. Cellulose, hemicellulose and lignin have also been found as components of the crude fibre.
3. Plates of sugarcane bagasse inoculated with *Trichophyton* species and *Aspergillus flavus* showed that both organisms have the ability to utilise the sugarcane bagasse as source of nutrient, with A. *flavus* exhibiting more growth than *Trichophyton* sp.
4. The different types of sorbents used for this experiment (both inoculated and un-inoculated) proved to be very efficient in the removal of Ni and Hg ions from the refinery effluent.

6.2 Recommendations:

- Strict adherence to regulations for release of hazardous effluents from refineries should be ensured by government in order to limit the indiscriminate discharge of such effluents into the environment.
- The use of sugarcane bagasse and other agricultural wastes should be employed in Nigeria for studies of bioremediation in order to reduce cost of using conventional and hazardous techniques, as well as reduce the nuisance posed by these wastes to the environment.
- The capacity of fungi of all genera grown on sugarcane bagasse should be explored and studied in the removal of heavy metal ions from refinery and other industrial effluents.
- Further studies should be carried out to test the capacity of culture containing more than two fungal isolates in the removal of more heavy metal ions from different industrial effluents would be tested.

6.3 Contributions to Knowledge:

This study has contributed to knowledge in the following ways:

The physicochemical properties of the effluent KRPC discharged had a high turbidity of 111NTU, Total Dissolved Solids of 258mg/l and Chemical Oxygen Demand of 874.10mg/l, which were all higher than permissible limits. Heavy metals Ni and Hg were also found in high concentration of 1.149mg/l and 484.780mg/l respectively.

The fungal isolates, *Aspergillus flavus* and *Trichophyton* sp. respectively utilised 52.5% and 32.5% of sugarcane bagasse after incubation for a period of 14days.

Removal of Ni and Hg by both the inoculated sugarcane bagasse with A. *flavus* and *Trichophyton* sp. (singly and in co-culture), as well as by the un-inoculated sugarcane bagasse in this study was very efficient; where by above 90% removal of the heavy metals was achieved in all cases, with no significant difference between the type of sorbent used.

REFERENCES

Abdullah, B.A. and Saadullah, A.A.M. (2015). Isolation and Identification of Filamentous Fungi from Indoor Swimming Pools in Duhok City. Biology Department, College of Science, University of Duhok. pp 1-6. A Paper Presented at the 5th International Conference on Agricultural, Ecological and Medical Sciences (AEMS-2015), Dubai, UAE. https://www.researchgate.net/publication/289378243_Isolation_and_Identification_of_Filamentous_Fungi_from_Indoor_Swimming_Pools_in_Duhok_City

Abioye, O.P., Agamuthu, P. and Abdul-Aziz, A.R. (2012). Biodegradation of Used Motor Oil in Soil Using Organic Waste Amendments. *Biotechnology Research International,* **2012**(2012): 1-8. DOI: 587041. http://dx.doi.org/10.1155/2012/587041

Adams, G.O., Fufeyin, P.T., Okoro, S.E. and Ehinomen, I. (2015). Bioremediation, Biostimulation and Bioaugmentation: A Review. *International Journal of Environmental Bioremediation and Biodegradation,* **3**(1): 28-39.

Adesina, G.O. and Adelasoye, K.A. (2014). Effect of Crude Oil Pollution on Heavy Metal Contents, Microbial Population in Soil, and Maize and Cowpea Growth. *Agricultural Sciences,* **5**(1): 43-50.

Adewuyi, G.O. and Olowu, R.A. (2012). Assessment of Oil and Grease, Total Petroleum Hydrocarbons and some Heavy Metals in Surface and Ground Water within the Vicinity of NNPC Oil Depot in Apata, Ibadan Metropolis, Nigeria. *International Journal of Review and Revised Applied Sciences*, **13**(1): 166-174.

Adeyemi, A.O. (2009). Bioaccumulation of Arsenic by Fungi. *American Journal of Environmental Sciences*, **5**(3):364-370.

Ahluwalia, S.S and Goyal, D. (2007). Microbial and Plant Derived Biomass for Removal of Heavy Metals from Wastewater. *Bioresource Technology,* **98**(12): 2243-2257.

Al-Bahry, S.N., Elsahfie, A.E., Al-Wahaibi, Y.M., Al-Bimani, A.S., Joshi, S.J., Al-Maaini, R.A., Al-Alawai, W.J., Sugai, Y. and Al-Mandhari, M. (2013). Microbial Consortia in Oman Oil Fields: A Possible Use in Enhanced Oil Recovery. *Journal of Microbiology and Biotechnology*, **23**(1): 106–117.

Alluri, H.K., Srinivasa, R., Ronda, H.K.,Vijaya, S.S., Jayakumar, S.B., Suryanarayana, V. and Venkateshwar, P. (2007). Biosorption: An Eco-friendly Alternative for Heavy Metal Removal. *African Journal* of *Biotechnology*. **6**(25): 2924-2931.

American Academy of Dermatology, USA. (2015). Position Statement on Nickel Sensitivity. Pages 1-2. https://www.aad.org/Forms/Policies/Uploads/PS/PS-Nickelsensitivity.pdf. Retrieved 8th Nov, 2017.

American Public Health Association, APHA. (1999). Standard Methods for the Examination of Water and Wastewater. (20th edition). Washington D.C.Antman, K.H. (2001). The History of Arsenic Trioxide in Cancer Therapy. *The Oncologist*, **6**(2): 1-2.

Association of Official Analytical Chemists, AOAC. (2010). *Official Methods of Analysis of Chemistry*. (18th edition). Washington D.C.

Atubi, A. O. (2011). Effect of Warri Refinery Effluent on Water Quality from Iffie River, Delta State, Nigeria. *American Review of Political Economy*. pp. 45-56.

Ayodele, J.T. and Abubakar, M.B. (2001). Trace Metal Levels in Tiga Lake, Kano, Nigeria. *Tropical Environmental Research*, **3**:230-237.

Badu, M., Twumasi, S.K. and Boadi, N.O. (2011)**.** Effects of Lignocellulosic in Wood Used as Substrate on the Quality and Yield of Mushrooms. *Food and Nutrition Sciences*, **2**:780-784.

Bako, S.P., Chukwunonso, D. and Adamu, A.K. (2002). Bio-remediation of Refinery Effluents by Strains of *Pseudomonas aeruginosa* and *Penicillium janthinellum*. *Applied Ecology and Environmental Research*, **6**(3): 49-60.

Barceloux, D.G. and Barceloux, D. (1999). Nickel. *Clinical Toxicology*, **37**(2): 239-258.

Barros, L.M., Macedo, G.R., Duarte, M.L., Silva, E.P. and Lobato, A.K.C.L. (2003). Biosorption of Cd using the fungus A. *niger*. *Brazilian Journal of Chemical Engineering*, **20**: 229-239.

Beddri, A.M. and Ismail, Z.B. (2007). Removal of Heavy Metals in Refinery Effluents Using Water Hyacinth. JURUTERA, March 2007. pp. 14-21.

Bhainsa K.C. and D'Souza, S.F. (1999). Biosorption of Uranium (VI) by *Aspergillus fumigatus*. *Biotechnological Techniques*, **13**: 695-699.

Bhattacharya, S., Bhardwaj, S., Das, A. and Anand, S. (2011). Utilization of Sugarcane Bagasse for Solid- State Fermentation and Characterization of α**-**Amylase from *Aspergillus flavus* Isolated from Muthupettai Mangrove, Tamil Nadu, India. *Australian Journal of Basic and Applied Sciences*, **5**(12): 1012-1022.

Betsy, T. and Keogh, J. (2005). *Microbiology Demystified*. The McGraw-Hill Publishing Companies, New York. pp. 168-169.

Bishnoi, N. R. and Garima. (2005). Fungus- An Alternative for Bioremediation of Heavy Metal Containing Wastewater: A Review. *Journal of Scientific and Industrial Research,* **64**: 93-100.

Boyd, J. (2000). Unleashing the Clean Water Act, the Promise and Challenge of the TMDL Approach to Water Quality. *Resources*, **139.**

Buttice, C. (2015). Nickel Compounds. In: Colditz, G.A. *The SAGE Encyclopaedia of Cancer and Society*. (2nd Edition). Thousand Oaks: SAGE Publications Inc. pp.828-831.

Carvalho, W., Canilha, L., Castro, P.F. and Barbosa, L.D.F.O. (2009). Chemical Composition of Sugarcane Bagasse. 31st Symposium on Biotechnology for Fuels and Chemicals. *Biomass*, pp. 3-32.

Centre for Disease Control and National Institute for Occupational Safety and Health, CDC-NIOSH. (2015). Pocket Guide to Chemical Hazards: Nickel Metal and Other Compounds by the Centre for Disease Control and National Institute for Occupational Safety and Health, CDC-NIOSH, USA. https://www.cdc.gov/niosh/npg/npgd0445.html.

Centre for Food Security and Public Health, CFSPH. (2013). Dermatophytosis: Ringworm, *Tinea*. A Report for the Centre for Food Security and Public Health, Iowa State University, USA. www.cfsph.iastate.edu/Factsheets/pdfs/dermatophytosis.pdf.

Charles, J.A. (1967). Early Arsenical Bronzes- A Metallurgical View. *American Journal of Archaeology,* **71**(1): 21-26.

Chikogu, V., Caleb, A. I. and Lekwot, V. E. (2012). Public Health Effects of Effluent Discharge of Kaduna Refinery into River Romi. *Greener Journal of Medical Sciences*, **2**(3): 064-069.

Commission on Isotopic Abundances and Atomic Weights, CIAWW. (2013). Standard Atomic Weights. Collier (1987). *Introduction to Nuclear Power*. Taylor and Francis, p.64. ISBN 1-56032-682-4. www.ciaaw.org/atomic-weights.htm

The Commodity Research Bureau (CRB). (2000). Commodity Yearbook (Annual). pp.14-173. ISSN 1076-2906.

Cox, R. (1997). The Pillar of Celestial Fire. First World Publishing, p.260.

Cunha, F.M., Esperança, M.N., Zangirolami, T.C., Badino, A.C. and Farinas, C.S. (2012). Sequential Solid-state and Submerged Cultivation of *Aspergillus niger* on Sugarcane Bagasse for the Production of Cellulase. *Bioresource Technology,* **112**: 270-274.

Dadrasnia, A. and Agamuthu, P. (2013). Potential Biowastes to Remediate Diesel Contaminated Soils. *Global NEST Journal,* **15**(4): 474-484.

Daud, S., Salleh, S.S.M., Salleh, M.N., Kasim, F.H. and Saad, S.A. (2007). Analysis of Chemical Composition in Sugarcane Bagasse and Rice Straw for Their Suitability Using in Paper Production. *School of Materials, Bioprocess and Environmental Engineering, Universiti Malaysia Perlis*: 291-292.

Davis, J.R. (2000). Uses of Nickel. ASM Specialty Handbook: Nickel, Cobalt, and Their Alloys. *ASM International*, pp.7-13.

Dawson, L. and Boopathy, R. (2008). Cellulosic Ethanol Production from Sugarcane Bagasse without Enzymatic Saccharification. *BioResources*, **3**(2): 452-460.

De Souza, A.P., Leite , D.C C., Pattathil, S., Hahn, M.G. and Buckeridge, M.S. (2012). Composition and Structure of Sugarcane Cell Wall Polysaccharides: Implications for Second-Generation Bioethanol Production. *Bioenergy Resource*, DOI: 10.1007/s12155-012-9268-1.

Dhankhar, R. and Hooda, A. (2011). Fungal Biosorption – An Alternative to Meet the Challenges of Heavy Metal Pollution in Aqueous Solutions. *Environmental Technology*, **32**(5): 467-491, DOI:10,1080/09593330.2011.572922.

Dhokpande, S. R. and Kaware, J. P. (2013). Biological Methods for Heavy Metal Removal- A Review. *International journal of Engineering Science and Innovative Technology,* **2**(5): 304-309.

Diya'uddeen, B.H., Daud, M.A. and Abdul Aziz, A.R. (2011). Treatment Technologies for Petroleum Refinery Effluents. *Elsevier*, **89**: 95-105.

Duffus, J.H. (2002). 'Heavy Metals'- A Meaningless Term? *Journal of Pure and Applied Chemistry*, **74**(5): 793-807. Doi:10.1351/pac200274050793.

Duncan, M. and Horan, N. (2003). *Hand Book of Water and Wastewater Microbiology.* Academic Press, An Imprint of Elsevier 84 Theobald's Road, London WC1X 8RR, UK pp. 84-87. http://www.academicpress.com.

Dunnick, J.K., Elwell, M.R., Radovsky, A.E., Benson, J.M. (1995). Comparative Carcinogenic Effects of Nickel Sulfides, Nickel Oxide or Nickel Sulfate Hexahydrate Chronic Exposures in the Lungs. *Cancer Research*, **55**(22): 5251-5256.

Ehrlich, H.L. and Newman, D.K. (2008). *Geomicrobiology*. CRC Press, p.265. ISBN 978-0-8493-7906-2.

Eisler, R. (2006). *Mercury Hazards to Living Organisms.* CRC Press. ISBN 978-0-8493-9212-2.

Emsley, J. (2001). Nature's Building Blocks: *An A-Z Guide to the Elements.* Oxford: Oxford University Press. pp. 43, 513, 529.

Environmental Health Criteria for Arsenic and Arsenic Compounds. (2001). Environmental Health Criteria, 224, Chapter 1: Summary Section 2.

Essiett, U. A., Effiong, G., Ogbemudia, F. O. and Bruno, E. J. (2010). Heavy Metal Concentrations in Plants Growing in Crude Oil Contaminated Soil in Akwa Ibom State, South-Eastern Nigeria. *African Journal of Pharmacy and Pharmacology*, **4**(7): 465-470.

European Nickel Institute. (2007). *Importance of Nickel Compounds*: Catalysts in Oil Refining. A report Prepared for the European Nickel Institute. The Weinberg Group LLC, Brussels, Belgium. Pg 1.

Ezeonuegbu, B. A., Machido, D. A. and Yakubu, S. E. (2014). Resistance to Some Heavy Metals among Fungal Flora of Raw Refinery Effluent. *Journal of Applied Sciences and Environmental Management,* **18**(4): 623-627.

Ezzouhri, L., Castro, E., Moya, M., Espinola, F. and Lairini, K. (2009). Heavy Metal Tolerance of Filamentous Fungi Isolated from Polluted Sites in Tangier, Morocco. *African Journal of Microbiology Research*, **3**(2): pp. 035-048.

Fernandes, O.S. and Nazareth, S.W. (1999). Studies on Lead Sorption by *Fusarium solani. Pollution Research*, **18**:211-216.

Gadd, G.M. (2009). Biosorption: Critical Review of Scientific Rationale, Environmental Importance and Significance for Pollution Treatment. *Journal Chemical Technology Biotechnology*, **84**:13-28.

Galadima, A. and Garba, Z. N. (2012). Heavy Metals Pollution in Nigeria: Causes and Consequences. *Elixir Pollution,* **45**: 7917-7922.

Gibaud, S. and Jaouen, G. (2010). Arsenic-based Drugs: From Fowler's Solution to Modern Anticancer Chemotherapy. *Topics in Organometallic Chemistry*, **32**: 1-20.

Gibson, B.K. (1991). Liquid Mirror Telescopes: History. *Journal of the Royal Astronomical Society of Canada*, **85**: 158.

Gokcen, N.A. (1989). The Arsenic System. *Bulletin of Alloy Phase Diagrams,* **10**: 11-22.

Green Facts. (2001). Facts of Health and the Environment – Chemical Substances. www.greenfacts.org/en/index.htm. Retrieved 12-020-2016.

Greenwood, N.M. and Earnshaw, A. (1997). Chemistry of the Elements. (2nd edition). Butterworth-Heinemann. ISBN. 0-08-037941-9.

Grund, S.C., Hanusch, K. and Wolf, H.U. (2005). Arsenic and Arsenic Compounds. *Ullman's Encyclopedia of Industrial Chemistry*, Weinheim: Wiley-VCH, doi:10.1002/14356007.a03113.pub2.

Gunatilake, S.K. (2015). Methods of Removing Heavy Metals from Industrial Wastewater. *Journal of Multidisciplinary Engineering Science Studies (JMESS)*, **1**(1): 12-18. ISSN: 2912-1309.

Haluk, C. and Ulki, Y. (2001). Biosorption of Ni(II) and Pb(II) by *Phanerochaete chrysosporium* from a Binary Metal System Kinetics. *Water SA*. **27**: 15-20.

Hamzah, A., Chia-Wei, P., Pek-Hoon, Y. and Nurul, H. (2014). Oil Palm Empty Fruit Bunch and Sugarcane Bagasse Enhance the Bioremediation of Soil Artificially Polluted by Crude Oil: Soil and Sediment Contamination. *An International Journal,* **23**(7): 751-762.

Harper, D. (2010). Arsenic. *Online Entymology Dictionary*. Retrieved 2010-05-15.

Hasan, M.T., Khatun, M.H.A., Sajib, M.A.M., Rahman, M.M., Rahman, M.S., Roy, M., Miah, M.N. and Ahmed, K.U. (2015). Effect of Wheat Bran Supplement with Sugarcane Bagasse on Growth, Yield and Proximate Composition of Pink Oyster Mushroom (*Pleurotus djamor*). *American Journal of Food Science and Technology*, **3**(6): 150-157.

Hesse, R.W. (2007). *Jewelry Making Through History.* Greenwood Publishing Group. p. 120. ISBN 0-313-33507-9.

Ho, Y.C., Show, K.Y., Guo, X.X., Norli, I., Alkarkhi, F.M. and Morad, N. (2014). Review on Industrial Discharge and their Effects on the Environment. *Universiti Tunku Abdulrahaman, Universiti Sains Malaysia*. Pp 1-33.

Holleman, A.F., Wiberg, E. and Wiberg, N. (1985). *Arsenic*. Lehrbuch der Anorganischen Chemie (in German), 91-100th edition, Walter de Gruyter. Pages 675-681.

Housecroft, C.E. and Sharpe, A.G. (2012). *Inorganic Chemistry*. (4th edition). Prentice Hall, p.764.

Howard, H. (2002). *Human Health and Heavy Metal Exposure*. In: *Life Support*: The Environment and Human health. Michael McCally MIT Press. pp 1-13.

Huet, P.M., Guillaume, E., Cote, J., Légaré, A., Lavoie, P. and Viallet, A. (1975). Noncirrhotic Presunisoidal Portal Hypertension Associated with Chronic Arsenical Intoxication. *Gastoenterology*, **68**(5.1):1270-1277.

Ibeto, C.N. and Okoye, C.O.B. (2010). High Levels of Heavy Metals in Blood of Urban Population in Nigeria. *Research Journal of Environmental Sciences*, **4**(4):371-382.

Institute of Environmental Conservation and Research, (2000). Position Paper against Mining in Rapu-Rapu. A Report of the Institute of Environmental Conservation and Research, Ateneo De Naga University, Phillippines. www.adnu.edu.ph/institutes/inecar/rapu-rapu/research01.asp.

International Petroleum Industry Environmental Conservation Association, IPIECA. (2010). Petroleum Refining Water/Wastewater Use and Management (Operation Best Practice Series). A Report of the International Petroleum Industry Environmental Conservation Association, London. Pp. 2-20.

Iqbal, M., Moh'd I.A, and Farrukh, A. (2005). Biosorption of Ni, Cr and Cd by Metal Tolerant *Aspergillus niger* and *Penicillium* species Using Sing and Multi-metal Solution. *Indian Journal of Experimental Biology.* **44**:73-7.

Irfan, M., Syed, Q., Abbas, S., Sher, M.G., Baig, S. and Nadeem, M. (2011). FTIR and SEM Analysis of Thermo-Chemical Fractionated Sugarcane Bagasse. *Turkish Journal of Biochemistry*, **36** (4):322–328.

Jardao, C., Pereira, M. and Pereira, J. (2002). Metal Contamination of River Waters and Sediments from Effluents of Kaolin Processing in Brazil. *Water, Air and Soil Pollution,* **140**(1): 119-138, ISSN 0049-6979.

Jarup, L. (2003). Hazards of Heavy Metal Contamination. *British Medical Bulletin,* **68**: 167-182.

Kadam, K.L. (2000). Environmental Life Cycle Implications of Using Bagasse-Derived Ethanol as a Gasoline Oxygenate in Mumbai (Bombay). A Report Prepared for the National Energy Technology Laboratory Pittsburgh, Pennsylvania, USA and USAID, New Delhi, India, pp 1-78.

Kamerud, K.L., Hobbie, K.A. and Anderson, K.A. (2013). Stainless Steel Leaches Nickel and Chromium into Foods during Cooking. *Journal of Agricultural and Food Chemistry*, **61**(39): 9495-9501.

Kasprzak, S.J. and Salnikow, F.W.K. (2003). Nickel Carcinogenesis. *Mutation Research*, **533**(1-2): 67-97.

Kerfoot, D.G.E. (2000). Nickel. *Ullmann's Encyclopedia of Industrial Chemistry.* doi:10.1002/14356007.a17_157.

Kittel, C. (1996). *Introduction to Solid State Physics*. Wiley. p.449. ISBN 0-471-14286-7.

Kong, W., Ren, J., Wang, S. and Chen, Q. (2014). Removal of Heavy Metals from Aqueous Solutions Using Sugarcane Bagasse-based Adsorbents: Equilibrium and Kinetic Studies. *Bioresources,* **9**(2): 3184-3196.

Kratochvil, D. and Volesky, B. (1998). Advances in the Biosorption of Heavy Metals. *Trends in Biotechnology,* **16:291**–300.

Kuck, P.H. (2016). The Role of Non-Fuel Minerals in the US Economy. Mineral Commodity Summaries: Nickel. *United States Geological Survey*. https://minerals.usgs.gov/minerals/pubs/mcs/2012/mcs2012.pdf.

Kumar, V., Singh, S., Kashyap, N., Singla, S., Bhadrecha, P., Kaur, P., Datta, S., Kalia, A.and Singh, J. (2015). Bioremediation of Heavy Metals by Employing Resistant Microbial Isolates from Agricultural Soil Irrigated with Industrial Waste Water. *Oriental Journal of Chemistry,* **31**(1): 357-361.

Lacey, J. (1980). *The Microbiology of the Bagasse of Sugarcane*. Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, England, pp. 2442-2461.

Lakherwal, D. (2014). Adsorption of Heavy Metals: A Review. *International Journal of Environmental Research and Development.* **4**(1): 41-48.

Lechtman, H. (1996). Arsenic-Bronze: Dirty Copper or Chosen Alloy? A View from the Americas. *Journal of Field Archaeology,* **23**(4): 477-514.

Lekwot, V. E., Adamu, I. B. and Ayuba, K. N. (2012). Effects of Effluent Discharge of Kaduna Refinery on the Water Quality of River Romi. *Journal of Research in Environmental Science and Toxicology,* **1**(3): 41-46.

Leopold, B.R. (2002). Chapter3: Manufacturing Processes Involving Mercury, Use and Release of Mercury in the US. National Risk Management Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, Cincinnati, Ohio.

Lew, K. (2008). *Mercury*. The Rosen Publishing Group. p.10.

Lide, D.R. (2005). CRC Handbook of Chemistry and Physics. (86th edition). Boca Raton (FL): CRC Press. pp.125-126.

Lide, D.R. (2006). The Elements-CRC Handbook of Chemistry and Physics. (86th edition). Boca Raton, (F.L): CRC Press. Page 5585. ISBN 0-8493-0486-5.

Littlepage, T. (2013). *Mercury in Crude Oils*. 2013 MCA Spring Seminar Series. Pages 1-5.

Liu, J., Shi, J.Z., Yu, L.M., Goyer, R.A. and Waalkes, M.P. (2008). Mercury in Traditional Medicines: Is Cinnabar Toxicologically Similar to Common Mercurials? *Experimental Biology and Medicine (Maywood)*, **233**(7): 807-810.

Lokhande, R.S., Singare, P.U. and Deepali S. Pimple, D.S. (2011). Study on Physico-Chemical Parameters of Waste Water Effluents from Taloja Industrial Area of Mumbai, India. *International Journal of Ecosystem*, **1**(1): 1-9.

Lori, J.A., Lawal, A.O. and Ekanem, E.J. (2007). Proximate and Ultimate Analysis of Bagasse, Sorghum and Millet Straws as Precursors for Active Carbons. *Journal of Applied Sciences*, **7**(21): 3249-3255.

Lyman, W.J. (1995). Transport and Transformation Processes. In: *Fundamentals of Aquatic Toxicology*, Taylor and Francis, London, pp. 449-492. ISBN 978-1-56032-090-6.

Machido, D.A., Yakubu, S.E. and Ezeonuegbu, B.A. (2014). Composition of Fungal Flora in Raw Refinery Effluent, Effluent Retention Pond and a Treated Effluent Recipient River. *Journal of Applied Sciences and Environmental Management,* **18**(4): 592-596.

Machido, D.A., Ezeonuegbu, B.A. and Yakubu, S.E. (2016). Capacity of Isolates of Six Genera of Filamentous Fungi to Remove Lead, Nickel and Cadmium from Refinery Effluent. *Journal of Environment and Earth Science,* **6**(8): 72-76.

Marcus, A.C., and Ekpete, O.A. (2014). Impact of Discharged Process Wastewater from an Oil Refinery on the Physicochemical Quality of a Receiving Water body in Rivers State, Nigeria. *International Organisation of Scientific Research- Journal of Applied Chemistry* **7**(12): 1-8.

Marcus, A.C., Okoye, C.O.B. and Ibeto, C.N. (2013). Organic Matter and Trace Metals Levels in Sediment of Bonny River and Creeks around Okrika in Rivers State, Nigeria. *International Journal of Physical Sciences,* **8**(15):652-656.

Martin, J. and Ayele, J. (2002). Removal of Some Heavy Metal Cations from Aqueous Solution Using Spruce Sawdust. *Environment Technology,* **23**(10):1157-1173.

Martin, S. and Griswold, W. (2009). Human Health Effects of Heavy Metals. *Centre for Hazardous Substance Research,* **1**(15): 5-6.

Mbaneme, F.C.N., Okoli, C.G. and Ekweghi, C. (2013). The Impact of Refinery Effluent in the Physiochemical Regime of Ekerekana Creek in Okirika Mainland, Rivers State, Nigeria. *American Journal of Environment, Energy and Power Research*, **1**(10): 255-271.

McKelvey, W., Jefferey, N., Clark, N., Kass, D. and Parsons, P.J. (2011). Population-Based Inorganic Mercury Biomonitoring and the Identification of Skin Care Products As a Source of Exposure in New York City. *Environmental Health Perspective*, **119**(2): 203-209.

Mudhoo, A., Sharma, S.K., Garg, V.K. & Tseng, C-H. (2011). Arsenic: An Overview of Applications, Health, Environmental Concerns and Removal Processes. *Critical Reviews in Environmental Science & Technology,* **41**:435–519.

Nechman, K.E., Graham, J.P., Price, L.B. and Silbergeld, E.K. (2005). Arsenic: A Roadblock to Potential Animal Waste Management Solutions. *Environmental Health Perspectives,* **113**(9): 1123-1124.

Nestle, O., Speidel, H. and Speidel, M.O. (2002). High Nickel release from 1- and 2- euro coins. *Nature*, **419**(6903).

Nickel Producers Environmental Research Association, NiPERA (2016). Dermal Exposure: Nickel Alloys. A report by Nickel Producers Environmental Research Association, USA. www.nipera.org/AboutNiPERA.aspx.

Niu, H. and Volesky, B. (1999). Characteristics of Au Biosorption from Cyanide Solution. *Journal of Chemical Techniques and Biotechnology*, **74**: 778-784.

Niyogi, S., Abraham, T.E. and Ramakrishna, S.V. (1998). Removal of Cr(vi) Ions from Industrial Effluents by Immobilized Biomass of *Rhizopus arrhizus*. *Journal of Scientific and Industrial Research,* **57**:809-816.

Norman, N.C. (1998). Chemistry of Arsenic, Antimony and Bismuth. *Springer,* p.50. ISBN 978-0-7514-0389-3.

Norrby, L.J. (1991). Why is Mercury Liquid? Or, Why Do Relativistic Effects Not Get into Chemistry Textbooks? *Journal of Chemical Education*, **68**(2): 110.

Nwaichi, E.O. and Warigbani, T.Z. (2013). Phenol Removal from Refinery Effluent by *Hevea Brasilliensis*. *Research Journal in Engineering and Applied Sciences*, **2**(1): 50-53.

Ojumu, T.V., Solomon, B.O., Betiku, E., Layokun, S.K. and Amigun, B. (2003). Cellulase Production by *Aspergillus flavus* Linn Isolate NSPR 101 Fermented in Sawdust, Bagasse and Corncob. *African Journal of Biotechnology*, **2**(6): 150-152.

Olaniyi, I., Raphael, O. and Nwadiogbu, J.O. (2012). Effect of Industrial Effluent on the Surrounding Environment. *Archives of Applied Science Research*, **4**(1): 406-413.

Olatunji, O.S. and Osibanjo, O. (2012). Determination of Selected Heavy Metals in Inland Fresh Water of Lower River Niger Drainage in North Central Nigeria. *African Journal of Environmental Science and Technology*, **6**(10): 403-408.

Oliveira, A.F., Matos, V.C. and Bastos, R.G. (2012). Cultivation of *Aspergillus niger* on Sugarcane Bagasse with Vinasse. *Biological Sciences Journal,* **28**(6): 889-894.

Otitoju, O. and Otitoju, G.T.O. (2013). Heavy Metal Concentrations in Water, Sediment and Periwinkle (*Tympanotonus fuscastus*) Samples Harvested from the Niger Delta Region of Nigeria. *African Journal of Environmental Science and Technology*, **7**(5): 245-248.

Otokunefor, T.V. and Obiukwu, C. (2005). Impacts of Refinery Effluents on the Physicochemical Properties of a Water Body in the Niger Delta. *Applied Ecology and Environmental Research,* **3**(1):61-72.

Pandey, P.K., Choubey, S., Verma, Y., Pandey, M., Kamal, S.S.K. and Chandrashekar, K. (2007). Biosorptive Removal of Ni(ii) from Wastewater and Industrial Effluent. *International Journal of Environmental Research and Public Health,* pp 332-339.

Pandey, A.K., Jamaluddin, A.K.A. and Pandey, A. (2012). Biosorption Characteristics of *Aspergillus flavus* in Removal of Nickel from an Aqueous Solution. *International Journal of Green and Herbal Chemistry,* **2**: 47-51.

Parani, K., Rajalakshmi, S., Selvi, T.I and Parvathi, K. (2016). Biodegradation of Sugarcane Bagasse Treated Using White Rot Fungi. *Advances in Biological Research* **10** (1): 22-26.

Parker, S.K., Schwartz, B., Todd, J. and Pickering, L.K. (2004). Thimerosal-Containing Vaccines and Autistic Spectrum Disorder: A Critical Review of Published Original Data. *Pediatrics*, **114**(3): 793-804.

Pearson, L.F. (2008). *Lighthouses*. Osprey Publishing. p.29.

Peryea, F.J. (1998). *Historical Use of Lead Arsenate Insecticides Resulting in Soil Contamination and Implications for Soil Remediation.* 16th World Congress of Soil Science, Montpellier, France.

Ragsdale, S.W. (2014). Chapter 6. *Biochemistry of Methyl Co-M Reductase. The Nickel Metalloenzyme That Catalyses the Final Step in Synthesis and the First Step in Anaerobic Oxidation of the Greenhouse Gas, Methane*. In: Kroneck, P.M.H. and Torres, M.E.S. The Metal-Driven Biogeochemistry of Gaseous Compounds in the Environment. *Metal Ions in Life Sciences.* (14th Edition). Springer, pp.125-145.

Radojevic, M. and Bashkin, V.N. (1999). Practical Environmental Analysis. Illustrated Edition, Page 138. Royal Society of Chemistry, Cambridge City.

Rahman, F.A., Allan, D.L., Rosen, C.J. and Sadowsky, M.J. (2004). Arsenic Availability from Chromated Copper Arsenate (CCA)-Treated Wood. *Journal of Environmental Quality*, **33**(1): 173-180.

Rajendran, P., Muthukrishnan, J. and Gunasekaran, P. (2003). Microbes in Heavy Metal Remediation. *Indian Journal of Experimental Biology,* **41**: 935-944.

Ramzan, M., Malik, M.A., Iqbal, Z., Arshad, N., Khan, S.Y. and Arshad, M. (2011). Study of Hematological Indices in Tannery Workers Exposed to Chromium in Sheikhupura (Pakistan). *Toxicology & Industrial Health,* pp.19.

Rand, G.M., Wells, P.G. and McCarty, L.S. (1995). *Introduction to Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment*, (2nd Edition). Taylor and Francis, London, pp. 3-70. ISBN 978-1-56032-090-6.

Rao, P.R. and Bhargavi,C. (2013). Studies on Biosorption of Heavy Metals Using Pretreated Biomass of Fungal Species. *International Journal of Chemistry and Chemical Engineering*, **3**(3):171-180.

Rasmussen, K.L., Malvin, D.J., Wasson, J.T. (1998). Trace Elements Partitioning between Taenite and Kamacite – Relationship to the Coding Rates of Iron Meteorites. *Meteoritics*, **23**(2):107-112.

Rieuwerts, J. (2015). *The Elements of Environmental Pollution*. Routledge Abingdon and New York.

Rodríguez-Chong A., Ramírez, J.A., Garrote, G. and Vázquez, M. (2004). Hydrolysis of Sugarcane Bagasse Using Nitric Acid: A Kinetic Assessment. *Journal of Food Engineering* **61**:143-152.

Roefer, P., S. Synder, R.E. Zegers, D.J. Rexing and J.L. Frank (2000). Endocrine-Disrupting Chemicals in Source Water. *Journal of American Water Works Association*, **92**(8).

Rosenberg, S.J. (1968). *Nickel and its Alloys*. National Bureau of Standards.

Ruchi, G., Saxena, R.K. and Rani, G. (2003). Fermentation Waste of *Aspergillus terreus*: A Promising Copper Bio-Indicator. *Process of Biochemistry* **39**: 1231-1235.

Rytuba, J.J. (2003). Mercury from Mineral Deposits and Potential Environmental Impact. *Environmental Geology*, **43**(3): 326-338. Doi: 10.1007/s00254-002-06.

Sabo, A., Gani, A.M. and Ibrahim, A.Q. (2013). Pollution Status of Heavy Metals in Water and Bottom Sediment of River Delimi in Jos, Nigeria. *American Journal of Environmental Protection*, **1**(3): 47-53.

Sanchéz, G.S. and Bain, E. (2013). *Mercury in Extraction and Refining Process of Crude Oil and Natural Gas*. A Research Thesis (M.Eng) Submitted to the School of Engineering, University of Aberdeen, page 22.

Schaechter, M., Baldauf, S.L., Baross, J.A., Baulcombe, D.C., Haselkorn, R., Hopwood, D.A., Ingraham, J.L., Laskin, A.I., Levin, B.R., Schmidt, T.M., Summers, W.C. and White, J.F. (2009). *Encyclopedia of Microbiology*, (3rd Edition). Elsevier Incorporation, San Diego, USA. pp. 383-384, 743.

Senese, F. (2007). Why is Mercury a Liquid at STP? *General Chemistry Online*. Frostburg State University.

Sharma, P., Kumari, P., Srivastava, M.M, and Srivastava, S. (2006). Removal of Cd from Aqueous System by Shelled *Moringa oleifera* Seed Powder. *Bioresource Technology* **97**: 299-305.

Sharma, P.K., Ayub, S. and Tripathi, C.N. (2013). Agro and Horticultural Wastes as Low Cost Adsorbents for Removal of Heavy Metals from Wastewater: A Review. *International Refereed Journal of Engineering and Science (IRJES),* **2**(8): 18-27.

Shathele, M.S. (2014). Mycological Studies of *Trichophyton mentagrophyes* var. *mentagrophyes* causing Ringworm in Horses in Al-Ahsa Province, Kingdom of Saudi Arabia. *Journal of Medicine and Medical Sciences,* **5**(8): 157-161.

Shelton, C. (2004). *Electrical Installations*. Nelson Thornes. p.260.

Sidana, A. and Farooq, U. (2014). Sugarcane Bagasse: A Potential Medium for Fungal Cultures. *Chinese Journal of Biology*, **2014**: 1-5. http://dx.doi.org/10.1155/2014/840505

Sigel, A., Sigel, H., Ronald, K.O. (2008). Nickel and its Surprising Impact in Nature. *Metal Ions in Life Sciences,* **2.** Wiley. ISBN 978-0-470-01671.

Silins, I. and Högberg, J. (2011). Combined Toxic Exposures and Human Health: Biomarkers of Exposure and Effect. *International Journal of Environmental Research & Public Health,* **8**:629-647.

Simons, E.N. (1968). *Guide to Uncommon Metals*. Frederick Muller, p.111.

Sloane, J. (2016). Mercury- Element of the Ancients. Institute of Environmental Health Sciences (IEHS) Bulletin, Dartmouth College, USA. https://www.dartmouth.edu/~toxmetal/mercury/history.html

Soliman, E.M., Ahmed, S.A. and Fadl, A.A. (2011). Reactivity of Sugarcane Bagasse As a Natural Solid Phase Extractor for Selective Removal of Fe(III) and Heavy-Metal Ions from Natural Water Samples. *Arabian Journal of Chemistry*, **4**: 63-70.

Stellman, J.M. (1998). Encyclopaedia of Occupational Health and Safety Chemical Industries and Occupations. International Labour Organisation. Page 133.

Stillman, J.M. (2003). *Story of Alchemy and Early Chemistry*. Kessinger Publishing. pp.7-9.

Strohl, W.A., Rouse, H. and Fisher, B.D. (2001). *Lippincott's Illustrated Reviews: Microbiology.* p.265.

Sudha, B. R. and Abraham, T.F. (1998). Studies on Biosorption of Cr(vi) by Dead Fungal Biomass. *Journal of Scientific and Industrial Research,* **57**:821-824.

Suleimanov, R.A. (1995): Conditions of Waste Fluid Accumulation at Petrochemical and Processing Enterprises and Prevention of their Harm to Water Bodies. *Madistina Trudai. Promyshlennaia Ekologiia,* **12**:31 – 36.

Surmann, P. and Zeyat, H. (2005). Voltammetric Analysis Using a Self-Renewable Non-Mercury Electrode. *Analytical and Bioanalytical Chemistry*, **383**(6): 1009-1013.

Sydor, A.M. and Zamble, D.B. (2013). Nickel Metallomics: General Themes Guiding Nickel Homeostasis, in: Metallomics and the Cell. *Metal Ions in Life Sciences*. (12th edition). Springer, pp.12.

Szilagyi, R.K., Bryngelson, P.A., Maroney, M.J., Hedman, B., *et al*. (2004). SK-Edge X-ray Absorption Spectroscopic Investigation of the Nickel-Containing Superoxide Dismutase Active Site: New Structural Insight into the Mechanism. *Journal of the American Chemical Society,* **126**(10): 3018-3019.

Tang, T.W. and Cheng, P. (2003). Biosorption of Metal Ions with *Penicillium chrysogenum. Applied Biochemistry and Biotechnology,* **104:119**-128.

Thipeswamy, B., Shivakumar, C.K. and Krishnapa, M. (2012). Bioaccumulation Potentials of *Aspergillus niger* and *Aspergillus flavus* for Heavy Metal Removal from Paper Mill Effluent. *Journal of Environmental Biol*ogy, **33**:1063-1068.

Thysse, J.P., Linneberg, A., Menne, T. and Johansen, J.D. (2007). The Epidemiology of Contact Allergy in the General Population- Prevalence and Main Findings. *Contact Dermatitis*, **57**(5): 287-299.

Tokar, E.J., Benbrahim-Tallaa, L. and Waalkes, M.P. (2011). Metal Ions in Human Cancer Development. *Metal Ions on Life Science,* **8**:375-401.

Trumbo, P., Yates, A.A., Schlicker, S. and Poos, M. (2001). Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. *Journal of the American Dietetic Association*, **101**(3): 294-301.

Turner, A. (1999). View-Point: The Story So Far: An Overview of Developments in UK Food Regulation and Associated Advisory Committees. *British Food Journal,* **101**(4): 274-283.

United States Department of Labour Publications. (2004). Occupational Safety and Health Topics: Heavy Metals. A report of the United States Department of Labour Publications, Washington, D.C.

United States Environmental Protection Agency, USEPA. (2011). Methods to Develop Cancer Risk Estimates for Chromium and Nickel Compounds. Research Triangle Park, NC: United States Environmental Protection Agency, Office of Air Quality Planning and Standards, Health and Environmental Impacts Division.

Uzoekwe, S.A. and Oghosanine, F.A. (2011). The Effect of Refinery and Petrochemical Effluent on Water Quality of Ubeji Creek, Warri, Southern Nigeria. *Ethiopian Journal of Environmental Studies and Management,* **4**(2): 107-116.

Vahidnia, A., Van der Voet, G.B. and De Wolff, F.A. (2007). Arsenic Neurotoxicity: A Review. *Human and Experimental Toxicology,* **26**(10): 823-832.

Van-Hamme, J. D., Singh, A. and Ward, O. P. (2003). Recent Advances in Petroleum Microbiology - Microbiology. *Molecular Biology Review,* **67**(4): 503-549.

Varsha, M., Nidhi, M., Anurag, M., Singh, R.B. and Sanjay, M. (2010). Effect of Toxic Metals on Human Health. *The Open Nutraceuticals Journal*, **3**:94-99.

Verheye, W. (2006). Growth and Production of Sugarcane. Soils. In: Plant Growth and Crop Production. *Encyclopedia of Life Support Systems*, **2**: 1-10.

Volesky, B. and May, H. (1995). Biosorption of Heavy Metals by *Saccharomyces cerevisiae. Applied Microbial Technology,* **42**:797-807.

Wang, X., Andrews, L., Riedel, S. and Kaupp, M. (2007). Mercury Is a Transition Metal: The First Experimental Evidence for HgF4. *Angewandte Chemie International Edition*, **46**(44): 8371-8375.

Wang, J. and Chen, C. (2006). Biosorption of Heavy Metals by *Saccharomyces cerevisiae*: A Review. *Biotechnological Advances*, **24**: 427-451.

World Health Organisation. (2011). Adverse Health Effects of Heavy Metals in Children: Ten Chemicals of Major Public Health Concern. *World Health Organisation Bulletin*, pp.1-10

Willey, M.J., Sherwood, L.M. and Woolverton, C.J. (2008). *Prescott, Harley and Klein's Microbiology,* 7th Edition. McGraw-Hill Companies Inc. New York, pp. 629-631.

Zafar, S. (2015). Composition of Bagasse: Energy Potential of Bagasse. *BioEnergy Consult*. http://www.bioenergyconsult.com/author/salman/. Retrieved on November, 10, 2016.

Zhu, X., Venosa, A.D., Suidan, M.T. and Lee, K. (2001). *Guidelines for the Bioremediation of Marine Shorelines and Fresh Water Wetlands.* – In: Annual Report, pp. 1 – 126. U.S. Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory Land Remediation and Pollution Control Division. 26 W. Martin Luther King Drive, Cincinnati, OH 45268.

APPENDICES

APPENDIX I: Effect of Contact Time on Nickel Removal and Quantity Adsorbed by Experimental Sorbents

Contact Time (hrs)	A				B				C				D				E			
	Ci	Q	Qmax	Ni%	Ci	Q	Qmax	Ni%	Ci	Q	Qmax	Ni%	Ci	Q	Qmax	Ni%	Ci	Q	Qmax	Ni%
1	1.149	1.086	5.43	94.5	1.149	1.11	5.55	96.6	1.149	1.069	5.345	93.0	1.149	1.121	5.605	97.6	1.149	1.119	5.595	97.4
2	1.086	1.007	5.035	92.7	1.11	1.067	5.335	96.1	1.069	1.02	5.1	95.4	1.121	1.08	5.4	96.3	1.119	1.067	5.335	95.4
3	1.007	0.92	4.6	91.4	1.067	1.042	5.21	97.7	1.02	1.01	5.05	99.0	1.08	1.037	5.185	96.0	1.067	1.025	5.125	96.1
4	0.92	0.845	4.225	91.8	1.042	0.994	4.97	95.4	1.01	0.948	4.74	93.9	1.037	0.992	4.96	95.7	1.025	0.98	4.9	95.6
5	0.845	0.775	3.875	91.7	0.994	0.945	4.725	95.1	0.948	0.888	4.44	93.7	0.992	0.963	4.815	97.1	0.98	0.97	4.85	99.0
6	0.775	0.681	3.405	87.9	0.945	0.892	4.46	94.4	0.888	0.867	4.335	97.6	0.963	0.96	4.8	99.7	0.97	0.97	4.85	100

KEY:

A: unsterilized sugarcane bagasse; B: sterilized sugarcane bagasse; C: sterilized sugarcane bagasse inoculated with *Trichophyton* species; D: sterilized sugarcane bagasse inoculated with *Aspergillus flavus*; E: sterilized sugarcane bagasse inoculated with *Trichophyton* species and *Aspergillus flavus*; Ci: equilibrium liquid phase concentration (mg/l); Q: equilibrium solid phase concentration (mg/l); Qmax: amount of metal adsorbed at time t (mg/g); Ni%: percentage of Nickel removed at time t.

APPENDIX II: Effect of Contact Time on Mercury Removal and Quantity Adsorbed by Experimental Sorbents

Contact Time (hrs)	A				B				C				D				E			
	Ci	Q	Qmax	Hg%	Ci	Q	Qmax	Hg%	Ci	Q	Qmax	Hg%	Ci	Q	Qmax	Hg%	Ci	Q	Qmax	Hg%
1	484.78	473.31	2366.6	97.63	484.78	472.46	2362.3	97.46	484.78	471.72	2358.6	97.31	484.78	470.92	2354.6	97.14	484.78	469.86	2349.3	96.9
2	473.31	458.05	2290.3	96.78	472.46	456.09	2280.4	96.53	471.72	454.86	2274.3	96.42	470.92	453.20	2266.0	96.24	469.86	451.72	2258.6	96.1
3	458.05	439.60	2198.0	95.97	456.09	436.79	2183.9	95.77	454.86	434.7	2173.5	95.57	453.20	432.60	2163.0	95.45	451.72	430.43	2152.1	95.2
4	439.60	418.03	2090.1	95.09	436.79	414.81	2074.0	94.97	434.7	412.29	2061.4	94.84	432.60	410.15	2050.7	94.81	430.43	407.13	2035.6	94.5
5	418.03	418.03	2090.1	100	414.81	414.81	2074.0	100	412.29	411.99	2059.9	99.93	410.15	409.87	2049.3	99.93	407.13	406.54	2032.7	99.8
6	418.03	417.37	2086.8	99.84	414.81	414.07	2070.4	99.82	411.99	410.80	2053.9	99.71	409.87	408.44	2042.2	99.65	406.54	405.05	2025.3	99.6

KEY:

A: unsterilized sugarcane bagasse; B: sterilized sugarcane bagasse; C: sterilized sugarcane bagasse inoculated with *Trichophyton* species; D: sterilized sugarcane bagasse inoculated with *Aspergillus flavus*; E: sterilized sugarcane bagasse inoculated with *Trichophyton* species and *Aspergillus flavus*; Ci: equilibrium liquid phase concentration (mg/l); Q: equilibrium solid phase concentration (mg/l); Qmax: amount of Mercury adsorbed at time t (mg/g); Hg%: percentage Mercury removed at time t.

APPENDIX III: Percentage of Heavy Metals Removed from the Refinery Effluent by the Experimental Sorbents as Influenced by Time of Contact

Contact Time (hrs)	Percentage of Heavy Metal Removed from Effluent by:									
	A		B		C		D		E	
	Ni	**Hg**	**Ni**	**Hg**	**Ni**	**Hg**	**Ni**	**Hg**	**Ni**	**Hg**
1	94.52	97.63	96.61	97.46	93.04	97.31	97.56	97.14	97.39	96.92
2	92.73	96.78	96.13	96.53	95.42	96.42	96.34	96.24	95.35	96.14
3	91.36	95.97	97.66	95.77	99.02	95.57	96.02	95.45	96.06	95.29
4	91.85	95.09	95.39	94.97	93.86	94.84	95.66	94.81	95.61	94.59
5	91.72	100	95.07	100	93.67	99.93	97.08	99.93	98.98	99.86
6	87.87	99.84	94.39	99.82	97.64	99.71	99.69	99.65	100	99.63
mean%/6hrs	**91.68**	**97.55**	**95.88**	**97.43**	**95.44**	**97.30**	**97.06**	**97.20**	**97.23**	**97.07**

KEY:

A: unsterilized bagasse only; B: sterilised bagasse only; C: sterilised bagasse + *Trichophyton* sp.; D: sterilised bagasse + *Aspergillus flavus*; E: sterilised bagasse + *Trichophyton* sp. and *Aspergillus flavus*; Ni: Nickel; Hg: Mercury

APPENDIX IV: Analysis of Variance (ANOVA) for the Percentage Removal of Nickel Ions by Experimental Sorbents at 95% Confidence Interval

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	121.153	4	30.288	8.531	.000
Within Groups	88.759	25	3.550		
Total	209.912	29			

p=0.000

APPENDIX V: Analysis of Variance (ANOVA) for the Percentage Removal of Mercury Ions by Experimental Sorbents at 95% Confidence Interval

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.840	4	.210	.047	.996
Within Groups	112.572	25	4.503		
Total	113.412	29			

p=0.996

APPENDIX VI: Analysis of Variance (ANOVA) for the Adsorption of Nickel Ions by Experimental Sorbents at 95% Confidence Interval

Mean value	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.082	4	.020	2.370	.080
Within Groups	.216	25	.009		
Total	.298	29			

p=0.080

APPENDIX VII: Analysis of Variance (ANOVA) for the Adsorption of Mercury Ions by Experimental Sorbents at 95% Confidence Interval

	Sum of Squares	df	Mean Squares	F	Sig.
Between Groups	287.518	4	71.879	.109	.978
Within Groups	16541.651	25	661.666		
Total	16829.169	29			

$p=0.978$