ASSESSING THE POTENTIAL OF ASPERGILLUS FLAVUS AND PENICILLIUM SP IN REMOVING MERCURY, ZINC AND CHROMIUM FROM RAW REFINERY EFFLUENTS

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March, 2018

DECLARATION

I hereby declare that the work presented in this dissertation entitled **"Assessing the potential of *Aspergillus flavus and Penicillium* sp in removing Zinc, Mercury and Chromium from raw refinery effluents"** was carried out by me in the Department of Microbiology, Ahmadu Bello University Zaria, Kaduna State, under the supervision of **Prof. D.A. Machido** and **Prof. I.O Abdullahi**. The information derived from literature has been duly acknowledged in the text and a list of references provided. I further declare the work reported has not been submitted and will not be submitted either in part or in full for the award of any other degree or diploma in this institution or any other institute or university.

EGEKWU Stanley _____ _____
MSC/SCl/23410/2012-2013 **Signature** **Date**

CERTIFICATION

This dissertation entitled: **"Assessing the potential of *Aspergillus flavus and Penicillium* sp in removing Zinc, Mercury and Chromium from raw refinery effluents"** by EGEKWU Stanley, meets the regulations governing the award of Master of Science (M.Sc) in Microbiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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Prof. D.A. Machido **Date**
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(Dean, Postgraduate School)

DEDICATION

This work is dedicated to God Almighty for His grace and favour throughout the period of this work. To Him alone be all the glory.

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ABSTRACT

This study was carried out to evaluate the potential of *Aspergillus flavus* and *Penicillium* sp in removing Zinc, Mercury and Chromium from raw refinery effluents. Physicochemical, heavy metal content and heavy metal removal capacity were determined, from the raw refinery effluents from the Kaduna Refinery and Petrochemical Company (KRPC) using standard procedures. Most of the physicochemical and heavy metal content were within the permissible limits set by the Federal Ministry of Environment Nigeria (FEMNV) except temperature (42.7^0C), Conductivity (48.10µS/cm), Chloride (835.5mg/l) and Mercury (0.0024mg/l). Two fungal species; *Aspergillus flavus* and *Penicillium* sp originally isolated from the KRPC were obtained from the Department of Microbiology, Ahmadu Bello University Zaria and re-authenticated by characterization on the basis of their macroscopic and microscopic morphologies using standard taxonomic guides. The isolates were inoculated into 100ml flask containing 50ml of the Potato Dextrose Broth (PDB) supplemented with 5, 10 and 15 µg/ml of Zn, Hg and Cr analytical grade salts, to test for the heavy metal tolerance using dry weight mycelial mat. It was observed that the two fungal species were tolerant and grew in the medium containing 5, 10 and 15ppm of the test heavy metals. The isolates removed substantial amount of Zn, Hg and Cr from the raw refinery effluent. *Aspergillus flavus* was able to remove 74% of Zinc, 85% of Mercury and 81% of Chromium which translated to adsorption of 0.800mg/g of Zinc, 0.530mg/g of Mercury and 0.133mg/g of chromium, after 7days *Penicillum* sp was able to remove 84% of Zinc, 56% of Mercury and 84% of chromium translating to the adsorption of 0.323mg/g of Zinc, 0.413mg/g of Mercury and 0.72mg/g of Chromium, over same period. It was therefore concluded that *Aspergillus flavus* and *Penicillum* sp obtained from effluent polluted environments were tolerant to the heavy metals and are good agents in treatment of refinery effluents and remediation of sites polluted with Zinc, Mercury and Chromium.

TABLE OF CONTENTS

Contents **Pages**

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

Heavy metal contaminants are common features of effluents generated and released by wide range of modern industries (Singh and Sharma 2013) and refinery effluents bearing heavy metals considered to highly toxic (Ezeonuegbu *et al.,* 2014). Heavy metal contaminated wastewater has been identified as one of the most important sources of pollution. Such waste waters are usually generated from a variety of sources such as crude oil producing and refining, other petrochemical industries, metal processing, lubricant and car washing. These sources serve as the major contributors to the problems of heavy metal pollution especially of soil and water environments (Machido, 2015). The discharge of oily wastewater to the environment has potential to cause significant environmental hazard. This is because it contains toxic substances such as petroleum hydrocarbons, phenols, polyaromatic hydrocarbons which are inhibitory to animal and plant growth and also are mutagenic and carcinogenic to human beings (Musa *et al*., 2015).

Beddri and Ismail (2007) reported that waste-water effluents from petroleum refinery and petrochemical plants contain a diverse range of pollutants including heavy metals. The effluents also contain oil and grease,and are characterized by high biochemical oxygen demand (BOD) - bearing materials, suspended solids, dissolved solids, phenols and sulfides.

Heavy metals in refinery effluent mainly originate from the feedstock. Others are from corrosion products of the equipment and pipes, processed chemical additives and from materials like catalysts and other chemicals used in processes downstream of the primary distillation. The most common of these metals are Nickel, Vanadium, Copper, Chromium, Lead, Cadmium, Zinc and Selenium. Mercury has also been found to appear as impurity in natural gas and crude oil (Alao *et al*., 2010).

Heavy metals particularly Zinc, Chromium, Arsenic and Mercury are environmental pollutants threatening the health of human population and natural ecosystem through direct ingestion, inhalation

and indirect by consumption of fish, animals or plants in which the metals have accumulated (Alao *et al*., 2010). Other metals like gold, aluminum, cadmium, silver, lead and mercury are also potentially toxic to living organisms (Kumar *et al*., 2010, Ezeonuogbu *et al* 2014). Toxicity of these metals occurs through the displacement of essential metals from their natural binding site or through ligand interactions.

Methods employed for the removal of organic pollutants from refinery effluents are not applicable when heavy metal ions are at issue because, unlike the organic pollutants, they are non- biodegradable and therefore persist in effluent subjected to conventional treatments (Tran *et al.,* 2015).

Over the years, several approaches including chemical precipitation, chemical oxidation, solidification, ultra filtration, flocculation, electrolyte extraction, dilution, sedimentation, evaporation, reverse osmosis, neutralization and membrane separation have been used for removal of metal ions from solution (Viraraghavan and Yan, 2000).

These methods present disadvantages in that they are capital intensive, may result to incomplete metal removal, high demands of reagents, high energy requirements and generation of toxic sludge or other waste products that require careful disposal (Hemambika *et al*., 2011). With the increase in environmental awareness and legal constraints being imposed on discharge of effluents, the need for alternative cost effective technologies is essential. In this endeavor, biological approach has a great potential for the achievement of this goal and it is economical. Microbial populations in metal polluted environment adapt to toxic concentration of heavy metals and become heavy metal resistant (Prasenjit and Sumathi, 2005). The response of microorganisms towards toxic heavy metals is of special interest with regards to their application in the reclamation of polluted sites (Shankar *et al*., 2007).

Biosorption and bioaccumulation are biological methods of control of heavy metal pollution. These processes involve metal uptake by either non- living (biosorption) or living (bioaccumulation) biomass (Chojnacka *et al*., 2007). Biosorption is influenced by environmental factors like pH, temperature, ion competition, variations in the chemical composition of the cell wall, capsule, and polysaccharides. In any case, metals can be deposited around the cell as phosphates, sulphates and oxides (Giovanni, 2009). These biochemical processes could be an effective solution to the majority of the problems associated with heavy metals released in the environment by industries (Giovanni 2009). They offer many advantages over conventional methods including cost effectiveness, efficiency, minimization of chemicals and biological sludge, requirement of additional nutrients and regeneration of biosorbents with possibility of metal recovery (Ronda *et al*., 2007).

To date, a good number of biomass types have been tested for their metal binding capabilities under various conditions. The outcome of such studies provided proofs that, fungi have great potential in this regards (Ezeonuegbu *et al*., 2014, Machido *et al*., 2015).

Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, extracellular, intracellular precipitation and active uptake. These mechanisms result from complexation and ion exchange reactions between metal ions and groups on their cell walls (Gupta *et al*., 2000; Hemambika *et al.,* 2011). The black molds (Aspergilli) are widely distributed in the environment and are capable of metabolizing enormous variety of substances owing to the large number of diversified enzymes they produce (Angumeenal and Venkappayya, 2004).

The present study is aimed at assessing the potential of *Aspergillus* sp and *Penicillium* sp in the removal of Zinc (Zn), Mercury (Hg) and Chromium (Cr) from refinery effluents released by Kaduna refinery and petrochemical Company (KRPC).

1.1 STATEMENT OF RESEARCH PROBLEMS

The three major petrochemical refineries in Nigeria generate large quantities of effluents that are discharged into natural bodies of water, the quality of which has been widely reported to be tainted as a result (Otokunefor and Obiukwu, 2005; Atubi, 2011). This represents a serious threat to the environment and human health.

Petroleum refineries use relatively large volumes of water, especially for cooling systems. As a result, local studies in Kaduna have also shown that approximately 165,000m^3/ day of untreated liquid effluents containing heavy metals is generated and heavy metals in such effluents often far exceed those permissible for environmental safety (Musa *et al*., 2015). Refineries also generate solid wastes and sludges (ranging from 3 to 5 kg per ton of crude oil processed), 80% of which may be considered hazardous because of the presence of toxic organics and heavy metals. Accidental and intentional discharge of large quantities of pollutants can and do occur as a result of abnormal operation in a refinery and potentially pose a major local environmental hazard (Salleh *et al.,* 2003).

The law under Nigerian constitution (the national effluents and limitation regulation) is currently not upheld, which facilitate indiscriminate discharge from our refineries, because no punishment is meted out to defaulters (Obot *et al*., 2007).

Heavy metals like Zinc, Mercury and Chromium constitute a high proportion of the refinery effluent. Zinc which is considered essential micronutrients is known to become toxic at high concentrations. Mercury is problematic in cryogenic petrochemical facilities and is toxic to humans. Chromium can cause nasal problems and ulcer as well as kidney and liver damage and it has also been found to be carcinogenic leading to depressed growth (Ezeonuegbu, 2014)

The physical and chemical methods currently used to remove heavy metals are not cost effective and often do not restore the environment to its original state. These leaves us with biological method such as adsorption, which is a more effective and sustainable alternative means of restoring the environment to its original state compared to conventional (physical and chemical) methods is adopted (Hussein *et al*., 2004).

1.2 JUSTIFICATION FOR THE STUDY

At a global scale industrial processes generate approximately 2.4 million tons per annum of liquid effluents containing heavy metals and other toxic materials (Veglio and Beolchini, 1997; Bello and Abdullahi, 2016). Local studies in Kaduna have also shown that approximate volume of 165,000m^3/ day of untreated liquid effluents containing heavy metals is generated (Ezeonuogbu, 2014).

Hence, the speedy development, increasing sophistication, arbitrary and unregulated discharge of industrial and urban wastes into the environmental sink has become an issue of global concern (Gupta and Mahaptra, 2003; Strong and Burgess, 2008). Therefore, effort must be out on ground as soon as possible to put a stop to this menace.

The removal and recovery of heavy metals are necessary for the protection of the environment and human health. Conventional methods are ineffective in treatment of low concentration of metal ions

and also generate large quantity of toxic sludge which are to be disposed in further steps. Biological methods such as biosorption and bioaccumulation of heavy metals provide an alternative to conventional (physical and chemical) methods (Hussein *et al*., 2004).

Mycoremediation is a more promising and less expensive way of cleaning up contaminated soil and water (Kamaludeen *et al*., 2003). Mycoremediation through the use of biosorption and bioaccumulation approaches is an emerging technology in which microbes including fungi are used to clean up contaminated soil and water and to remove or stabilize the contaminants which offer a low cost and ecologically valuable means for the mitigation of heavy metal toxicity in the environment (Strong and Burgess, 2008). Many microbial populations including fungi have been identified as superior candidates for metal mycoremediation and major advantages of fungi are its significant metal uptake ability at low anticipated price (Bai and Abraham, 2001). Microbial populations present in metal polluted environment have adapted and become resistant to toxic metal and the presence of heavy metals and their bioavailability exert selective pressure on the microbial community with selective advantage conferred on those organisms that have developed tolerance mechanism to toxic heavy metals (Jaeckle *et al*., 2005).

The cell wall of fungi present a multi-laminated architecture where up to 90% of the dry mass consist of amino and non amino polysaccharides and proteins which offers many functional groups (such as carboxyl, hydroxyl, sulphate, phosphate and amino groups) for binding metal ions. According to Ronda *et al*. (2007), the cell walls of fungi are also rich in glycoproteins such as glycans (β – 1,6- and β- 1,3- linked D- glucose residues), chitosan (β-1,4-linked mannose) and phosphomanans (phosphorylated manans) with various metal binding groups present in the polymers. Heavy metals like Copper, Cadmium, Mercury, Zinc, Manganese, and Lead present in refinery effluent can be

removed by indigenous microbes isolated from the effluents itself (Hakeem and Bhatnagar, 2010). *Aspergillus* and *Penicillium* species accumulate and absorb micronutrient and heavy metals.These organisms are exposed to a lot of health hazards, it is therefore important to remove these toxic heavy metals from waste water before its disposal.

1.3 AIM AND OBJECTIVES

The aim was to assess the potential of *Aspergillus flavus* and *Penicillum* sp in removing Zinc, Mercury and Chromium from raw refinery effluents

1.4 The Specific Objectives were to;

1. Determine the physicochemical properties of the refinery effluent.
2. Re-authenticate the fungi isolates to be used in the study.
3. Determine the tolerance of the fungi isolates to Zinc, Mercury and Chromium invitro.
4. Determine the capacity of the fungi isolates in the removal of zinc, mercury and chromium from the raw refinery effluents.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Heavy Metal: Definition and Toxicity

Heavy metals are generally defined as those whose density exceeds 5g per cubic centimeter (Barakat, 2011). A large number of elements fall into this category. Heavy metals are natural components of the earth's crust which cannot be degraded or destroyed. To a small extent they enter human bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning (Barakat, 2011). Arsenic, cadmium, chromium, copper, nickel, zinc, lead and mercury are of relevance in the environmental context. Arsenic is usually regarded as a hazardous heavy metal even though it is actually a semi-metal. Heavy metals cause serious health effects, including reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death (Barakat, 2011; Ezeonuegbu, 2014). Exposure to some metals, such as mercury and lead, may also cause development of autoimmunity, in which a person's immune system attacks its own cells. This can lead to joint diseases such as rheumatoid arthritis, and diseases of the kidneys, circulatory system, nervous system, and damaging of the fetal brain. At higher doses, heavy metals can cause irreversible brain damage (Ezeonuegbu, 2014)

Wastewater regulations were established to minimize human and environmental exposure to hazardous chemicals. This includes limits on the types and concentration of heavy metals that may be present in the discharged wastewater. The acceptable limits for these heavy metals, established by United States Environmental Protection Agency (USEPA) are summarized in Table 2.1 below (Babel and Kurniawan, 2003; Barakat, 2011)

Table 2.1: The acceptable limits by Federal Ministry of Environment Nigeria for most hazardous heavy metals in refinery wastes

Heavy metal	Toxicities	Acceptable limit(mg/L) FMENV
Zinc	Depression, lethargy, neurological signs and increased thirst	0.80
Chromium	Headache, diarrhea, nausea, vomiting, carcinogenic	0.05
Nickel	Dermatitis, nausea, chronic asthma, coughing, human carcinogen	0.20
Arsenic	Skin manifestations, visceral cancers, vascular disease	0.050
Mercury	Rheumatoid arthritis, and diseases of the kidneys, circulatory system, and nervous system	0.00003
Cadmium	Kidney damage, renal disorder, human carcinogen	0.01
Lead	Damage the fetal brain, diseases of the kidneys, circulatory system, and nervous system	0.006
Copper	Liver damage, Wilson disease, insomnia	0.25

Source: Barakat (2011

2.2 Environmental and Health Risks of Heavy Metals

2.2.1 Chromium

Chromium (Cr) is a naturally occurring element present in the earth's crust, with oxidation states ranging from chromium (II) to chromium (VI) (Jacobs and Testa, 2005). Chromium compounds are stable in the trivalent (Cr(III)) form and occur in nature in this state in ores, such as ferrochromite. The hexavalent (Cr(VI)) form is the second-most stable state (Patlolla *et al.,* 2009**b**). Elemental chromium (Cr(0)) does not occur naturally. Chromium enters into various environmental matrices (air, water, and soil) from a wide variety of natural and anthropogenic sources with the largest release coming from industrial establishments. Industries with the largest contribution to chromium release include metal processing, tannery facilities, chromate production, stainless steel welding, and ferrochrome and chrome pigment production. The increase in the environmental concentrations of chromium has been linked to air and wastewater release of chromium, mainly from metallurgical, refractory, and chemical industries (Barakat, 2011). Chromium released into the environment from anthropogenic activity occurs mainly in the hexavalent form (Cr(VI)) (ATSDR, 2007). Hexavalent chromium (Cr(VI)) is a toxic industrial pollutant that is classified as human carcinogen by several regulatory and non-regulatory agencies (Velma *et al.,* 2009). The health hazard associated with exposure to chromium depends on its oxidation state, ranging from the low toxicity of the metal form to the high toxicity of the hexavalent form.

(Cr(III)) is an essential nutrient that plays a role in glucose, fat and protein metabolism by potentiating the action of insulin (Goya, 2001 and Patlolla *et al.,* 2009**a**). However, occupational exposure has been a major concern because of the high risk of Cr-induced diseases in industrial workers occupationally exposed to Cr(VI) (Agency for Toxic Substance and Disease Registry, 1990). Also,

the general human population and some wildlife may also be at risk. Non-occupational exposure occurs via ingestion of chromium containing food and water whereas occupational exposure occurs via inhalation (Costa, 1997). Major factors governing the toxicity of chromium compounds are oxidation state and solubility. Cr(VI) compounds, which are powerful oxidizing agents and thus tend to be irritating and corrosive, appear to be much more toxic systemically than Cr(III) compounds, given similar amount and solubility (Costa, 1997).

2.2.2 Mercury

Mercury belongs to the transition element series of the periodic table. It is unique in that it exists or is found in nature in three forms (elemental, inorganic, and organic), with each having its own profile of toxicity (Clarkson *et al.,* 2003). At room temperature elemental mercury exists as a liquid which has a high vapour pressure and is released into the environment as mercury vapor. Mercury also exists as a cation with oxidation states of +1 (mercurous) or +2 (mercuric) (Guzzi and Laporta, 2008). Methylmercury is the most frequently encountered compound of the organic form found in the environment, and is formed as a result of the methylation of inorganic (mercuric) forms of mercury by microorganisms found in soil and water (Dopp *et al.,* 2004).

Mercury is a widespread environmental toxicant and pollutant which induces severe alterations in the body tissues and causes a wide range of adverse health effects (Sarkar, 2005). Both humans and animals are exposed to various chemical forms of mercury in the environment. These include elemental mercury vapor (Hg^0), inorganic mercurous (Hg^{+1}), mercuric (Hg^{+2}), and the organic mercury compounds (Zahir *et al.,* 2005). The two most highly absorbed species are elemental mercury (Hg^0) and methyl mercury (MeHg). Since mercury is ubiquitous in the environment,

humans, plants and animals are all unable to avoid exposure to some form of mercury (Holmes *et al.*, 2009).

Mercury is utilized in the electrical industry (switches, thermostats, batteries), dentistry (dental amalgams), and numerous industrial processes including the production of caustic soda, in nuclear reactors, as antifungal agents for wood processing, as a solvent for reactive and precious metal, and as a preservative of pharmaceutical products (Tchounwou *et al.*, 2003).

Humans are exposed to all forms of mercury through accidents, environmental pollution, food contamination, dental care, preventive medical practices, industrial and agricultural operations, and occupational operations (USEPA, 1997**b**). The major sources of chronic, low level mercury exposure are dental amalgams and fish consumption. Mercury enters water as a natural process of off-gassing from the earth's crust and also through industrial pollution (Dopp *et al.*, 2004). Algae and bacteria methylate the mercury entering the waterways. Methyl mercury then makes its way through the food chain into fish, shellfish, and eventually into humans (Sanfeliu *et al.*, 2003).

2.2.3 Lead

Lead is a naturally occurring bluish-gray metal present in small amounts in the earth's crust. Although lead occurs naturally in the environment, anthropogenic activities such as fossil fuels burning, mining, and manufacturing contribute to the release of high concentrations. Lead has many different industrial, agricultural and domestic applications. It is currently used in the production of lead-acid batteries, ammunitions, metal products (solder and pipes), and devices to shield X-rays.

Exposure to lead occurs mainly via inhalation of lead-contaminated dust particles or aerosols, and ingestion of lead-contaminated food, water, and paints (ATSDR, 2007). Lead absorption is influenced by factors such as age and physiological status. In the human body, the greatest percentage

of lead is taken into the kidney, followed by the liver and the other soft tissues such as heart and brain, however, the lead in the skeleton represents the major body fraction (Flora *et al.,* 2006). The nervous system is the most vulnerable target of lead poisoning. Headache, poor attention spam, irritability, loss of memory and dullness are the early symptoms of the effects of lead exposure on the central nervous system (CDC, 2001; ATSDR, 2007). Exposure to lead is of special concern among women particularly during pregnancy. Lead absorbed by the pregnant mother is readily transferred to the developing fetus (Corpas *et al.*, 1995).

2.2.4 Nickel

Nickel may be found in slate, sandstone, clay minerals and basalt. The main nickel source is pentlandite. The element accumulates in sediments and is a part of various biological cycles and may end up in water from both point and non-point sources. Diffuse nickel emissions may stem from power plants, waste incinerators and metal industries. Nickel is directly emitted from various industries through discharge on surface waters. It is applied in alloys for treatment of heavy metal polluted surface water, in nickel-cadmium batteries, as a catalyzer and as a pigment (Ezeonuegbu, 2014)

Small amounts of Nickel are needed by the human body to produce red blood cells, however, in excessive amounts, can become mildly toxic. Short-term overexposure to nickel is not known to cause any health problems, but long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation, Ezeonuegbu, 2014. Nickel can accumulate in aquatic life, but its presence is not magnified along food chains.

Nickel inhalation poses a greater risk than nickel in water. This may cause lung cancer, or nasal tumors. Nickel carcinogenity is probably caused by nickel replacing zinc and magnesium ions on

DNA-polymerase. These observations were mainly made in nickel working employees. Usually only smoking may cause this problem. Many people develop dermatitis upon skin contact with nickel. The same goes for nickel solutions. Nickel compounds may be toxic in high concentrations, but these are often water insoluble, limiting potential harm. For example, nickel tetra carbonyl is water insoluble, but is toxic and carcinogenic nevertheless. Upon intake of higher doses of nickel one usually vomits, resulting in rapid removal from the body (Ezeonuegbu, 2014)

2.2.5 Cadmium

Cadmium is widely distributed in the earth's crust at an average concentration of about 0.1 mg/kg. The highest level of cadmium compounds in the environment is accumulated in sedimentary rocks, and marine phosphates contain about 15 mg cadmium/kg (Sparling, 2016). Cadmium is frequently used in various industrial activities. The major industrial applications of cadmium include the production of alloys, pigments, and batteries (Sparling, 2016). Although the use of cadmium in batteries has shown considerable growth in recent years, its commercial use has declined in developed countries in response to environmental concerns.

The main routes of exposure to cadmium are via inhalation or cigarette smoke, and ingestion of food. Skin absorption is rare. Human exposure to cadmium is possible through a number of several sources including working in primary metal industries, eating contaminated food, smoking cigarettes, and working in cadmium-contaminated work places, with smoking being a major contributor (Internal Agency for Research on Cancer, 1993; Paschal *et al.,* 2000). Other sources of cadmium include emissions from industrial activities, including mining, smelting, and manufacturing of batteries, pigments, stabilizers, and alloys (ATSDR, 2008). Cadmium is also present in trace amounts in certain foods such as leafy vegetables, potatoes, grains and seeds, liver and kidney, and crustaceans and

mollusks (Satarug *et al.,* 2003). In addition, foodstuffs that are rich in cadmium can greatly increase the cadmium concentration in human bodies. Examples are liver, mushrooms, shellfish, mussels, cocoa powder and dried seaweed. An important distribution route is the circulatory system whereas blood vessels are considered to be main stream organs of cadmium toxicity. Chronic inhalation exposure to cadmium particulates is generally associated with changes in pulmonary function and chest radiographs that are consistent with emphysema (Mascagni *et al.,* 2003).

2.2.6 Zinc

Zinc is naturally present in water. The average zinc concentration in seawater is 0.6 - 5 ppb. Rivers generally contain between 5 and 10 ppb zinc. Algae contain 20 - 700 ppm, sea fish and shells contain 3-25 ppm, oysters contain 100-900 ppm and lobsters contain 7 - 50 ppm (Lenntech, 2016).

Elementary zinc does not react with water molecules. The ion does form a protective, water insoluble zinc hydroxide ($Zn(OH)_2$) layer with dissolved hydroxide ions. The solubility of zinc depends on temperature and pH of the water in question. When the pH is fairly neutral, zinc in water is insoluble. Solubility increases with increasing acidity. Above pH 11, solubility also increases. Zinc dissolves in water as $ZnOH^+$ (aq) or Zn^{2+} (aq). Anionic $ZnCO_3$ has a solubility of 0.21 g/L (Lenntech, 2016).

The most significant zinc ores include sphalerite (ZnS) and smithsonite ($ZnCO_3$). These compounds end up in water on locations where zinc ores are found. About three-quarters of the total zinc supply is used in metal form. The remainder is applied as various zinc compounds in various industries. Industrial wastewaters containing zinc stem from galvanic industries, battery production, etc. Zinc compounds are applied for many different purposes. Zinc chloride is applied for parchment production, zinc oxide is a constituent of salves, paints and catalysers, zinc vitriol is applied as a fertilizer, and zinc bacitracine is applied as a growth stimulant in animal husbandry. The larger part

of zinc in wastewater does not stem from point sources. It stems from larger surface waters containing the element (Lenntech, 2016).

.Zinc leaks from zinc pipes and rain pipes, consequential to circulation of carbon rich water. Car tires containing zinc and motor oil from zinc tanks release zinc compounds on roads. Zinc compounds are present in fungicides and insecticides, and consequently end up in water (Lenntech, 2016).

.Zinc was not attributed a water hazard class, because it is not considered a hazard. This however only concerns elementary zinc. Some zinc compounds, such as zinc arsenate and zinc cyanide, may be extremely hazardous. Zinc is a dietary mineral for humans and animals. Still, overdoses may negatively influence human and animal health and over a certain boundary concentration, zinc may even be toxic. Toxicity is low for humans and animals, but phytotoxicity may not be underestimated. Symptoms of high zinc dosage include nausea, vomiting, dizziness, colics, fevers and diarrhoea and mostly occur after intake of 4-8 g of zinc. Intake of 2 g of zinc sulphate at once causes acute toxicity leading to stomach aches and vomiting (Lenntech, 2016).

2.2.7 Arsenic

Arsenic compounds are abundant in the earth's crust. Particles of this element are released during mining, and spread throughout the environment. Arsenic from weathered rocks and soils dissolves in groundwater. Arsenic concentrations in groundwater are particularly high in areas with geothermal activity. In aquatic ecosystems inorganic arsenic derived from rocks such as arsenic trioxide (As_2O_3), orpiment (As_2S_3), arsenopyrite (AsFeS) en realgar (As_4S_4) is most prevalent (Machido *et al* 2015)

Elementary arsenic normally does not react with water in absence of air. It does not react with dry air, but when it comes in contact with moist air a layer is formed. The layer has a bronze colour, and

later develops a black surface. An example of arsenic compounds that reacts strongly with water is orpiment, an amorphous arsenic compound.

In natural water arsenic participates in oxidation and reduction reactions, coagulation and adsorption. Adsorption of arsenic to fine particles in water and precipitation with aluminium or iron hydroxides causes arsenic to enter sediments.

Arsenic related health effects are usually not acute, but mostly encompass cancer, mainly skin cancer. Arsenic may cause low birth weight and spontaneous abortion. Toxicity differs between various arsenic compounds, for example, monomethyl arsenic acid and inorganic arsenide have a higher toxicity level than arsenic choline (Ezeonuegbu, 2014). Acute toxicity is generally higher for inorganic arsenic compounds than for organic arsenic compounds. Oral intake of more than 100 mg is lethal. The lethal dose of arsenic trioxide is 10-180 mg, and for arsenide this is 70-210 mg. Symptoms of acute arsenic poisoning are nausea, vomiting, diarrhoea, cyanosis, cardiac arrhythmia, confusion and hallucinations. Symptoms of chronic arsenic poisoning are less specific. These include depression, numbness, sleeping disorders and headaches (Ezeonuegbu, 2014)

2.3 Industrial Wastewater Sources

Industrial wastewater streams containing heavy metals are produced from different industries. Electroplating and metal surface treatment processes generate significant quantities of wastewaters containing heavy metals (such as cadmium, zinc, lead, chromium, nickel, copper, vanadium, platinum, silver, and titanium) from a variety of applications. These include electroplating, electroless depositions, conversion-coating, anodizing-cleaning, milling, and etching (Barakat, 2011). Another significant source of heavy metals wastes result from printed circuit board (PCB) manufacturing. Tin, lead, and nickel solder plates are the most widely used resistant solder plates.

Other sources for the metal wastes include; the wood processing industry where a chromated copper-arsenate wood treatment produces arsenic-containing wastes; inorganic pigment manufacturing producing pigments that contain chromium compounds and cadmium sulfide; petroleum refining which generates conversion catalysts contaminated with nickel, vanadium, and chromium; and photographic operations producing film with high concentrations of silver and ferrocyanide. All of these generators produce a large quantity of wastewaters, residues, and sludges that can be categorized as hazardous wastes requiring extensive waste treatment (Sorme and Lagerkvist, 2002).

2.4 Conventional Processes for Removal of Heavy Metals

The conventional processes for removing heavy metals from wastewater include many processes such as chemical precipitation, flotation, adsorption, ion exchange, and electrochemical deposition.

2.4.1 Chemical Precipitation

Chemical precipitation is the most widely used for heavy metal removal from inorganic effluent. The mechanism of heavy metal removal by chemical precipitation is presented in the equation below (Wang *et al.*, 2004):

$$M^{2+} + 2(OH)^{-} \leftrightarrow M(OH)_2 \downarrow \quad M2+ + 2(OH)- \leftrightarrow M(OH)2 \downarrow$$

Where M^{2+} and OH^{-} represent the dissolved metal ions and the precipitant, respectively, while $M(OH)_2$ is the insoluble metal hydroxide. Adjustment of pH to the basic conditions (pH 9–11) is the major parameter that significantly improves heavy metal removal by chemical precipitation (Figure 2.1). Lime and limestone are the most commonly employed precipitant agents due to their availability and low-cost in most countries (Mirbagherp and Hosseini, 2004). Lime precipitation can be employed to effectively treat inorganic effluent with a metal concentration of higher than 1000 mg/L. Other

advantages of using lime precipitation include the simplicity of the process, inexpensive equipment requirement, and convenient and safe operations (Machido *et al* 2015). However, chemical precipitation requires a large amount of chemicals to reduce metals to an acceptable level for discharge. Other drawbacks are its excessive sludge production that requires further treatment, slow metal precipitation, poor settling, the aggregation of metal precipitates, and the long-term environmental impacts of sludge disposal (Mirbagherp and Hosseini, 2004).

2.4.2 Ion exchange

Ion exchange is another method used successfully in the industry for the removal of heavy metals from effluent. An ion exchanger is a solid capable of exchanging either cations or anions from the surrounding materials. Commonly used matrices for ion exchange are synthetic organic ion exchange resins. The disadvantage of this method is that it cannot handle concentrated metal solution as the matrix gets easily fouled by organics and other solids in the wastewater. Moreover ion exchange is nonselective and is highly sensitive to the pH of the solution (Machido *et al* 2015)

2.4.3 Electrolytic recovery

Electrolytic recovery or electro-winning is one of the many technologies used to remove metals from process water streams. This process uses electricity to pass a current through an aqueous metal-bearing solution containing a cathode plate and an insoluble anode. Positively charged metallic ions cling to the negatively charged cathodes leaving behind a metal deposit that is strippable and recoverable. A noticeable disadvantage was that corrosion could become a significant limiting factor, where electrodes would frequently have to be replaced (Kurniawan *et al*., 2006; Barakat, 2011).

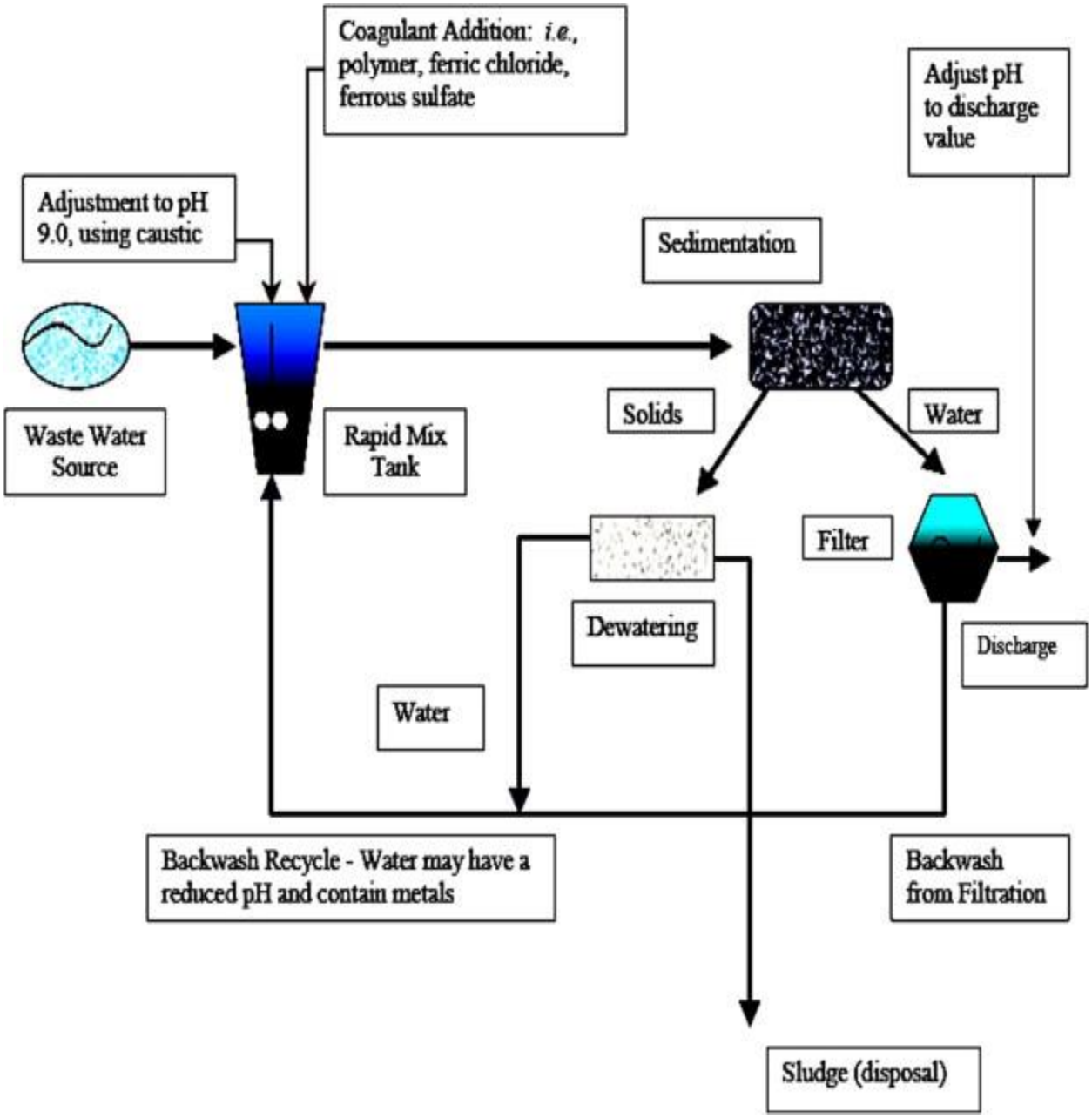

Figure 2.1: Processes of a conventional metals precipitation treatment plant (Wang *et al.*, 2004).

2.4.4 Adsorption on new adsorbents

Sorption is transfer of ions from solution phase to the solid phase, e.g. from water to the soil. Sorption actually describes a group of processes, which includes adsorption and precipitation reactions. Basically, adsorption is a mass transfer process by which a substance is transferred from the liquid phase to the surface of a solid, and becomes bound by physical and/or chemical interactions (Kurniawan and Babel, 2003).

In general, there are three main steps involved in pollutant sorption onto solid sorbent:

(i) The transport of the pollutant from the bulk solution to the sorbent surface.

(ii) Adsorption on the particle surface

(iii) Transport within the sorbent particle.

Technical applicability and cost-effectiveness are the key factors that play major roles in the selection of the most suitable adsorbent to treat inorganic effluent.

2.4.5 Adsorption on modified natural materials

Adsorption has become one of the alternative treatment techniques for wastewater laden with heavy metals. Various low-cost adsorbents, derived from agricultural waste, industrial by-product, natural materials, or modified biopolymers, have been recently developed and applied for the removal of heavy metals from metal-contaminated wastewater.

2.4.5.1 Zeolites

Natural zeolites gained a significant interest, mainly due to their valuable properties as ion exchange capability. Among the most frequently studied natural zeolites, clinoptilolite was shown to have high selectivity for certain heavy metal ions such as Pb, Cd, Zn, and Cu. It was demonstrated that the cation-exchange capability of clinoptilolite depends on the pre-treatment method which improves its ion exchange ability and removal efficiency (Babel and Kurniawan, 2003; Bose *et al*., 2002).

2.4.5.2 Clay–polymer composites

These adsorbents comprise natural clay minerals modified with a polymeric material which when used can significantly improve their capability to remove heavy metals from aqueous solutions as used in the studies of Vengris *et al.*(2001); Sölenera *et al.* (2008) and Abu-Eishah (2008).

2.4.5.3 Phosphate-based adsorbents

Different phosphates such as calcined phosphate at 900 °C, activated phosphate (with nitric acid), and zirconium phosphate have been employed as new adsorbents for removal of heavy metals from aqueous solution (Aklil *et al.*, 2004; Moufliha *et al.*, 2005; Pan *et al.*, 2007). Adsorption capacities of modified natural materials for heavy metals are shown in Table 2.2.

2.4.6 Adsorption on industrial by-products

Industrial by-products such as fly ash, waste iron, iron slags, hydrous titanium oxide, can be chemically modified to enhance its removal performance for metal removal from wastewater.

Several studies have been conducted; Lee *et al.* (2004) studied green sands, another by-product from the iron foundry industry, for Zn(II) removal.

Table 2.2: Adsorption capacities of modified natural materials for heavy metals

Adsorbent	Adsorption capacity (mg/g)						References
	Pb^{2+}	Cd^{2+}	Zn^{2+}	Cu^{2+}	Cr^{6+}	Ni^{2+}	
Zeolite, clinoptilolite	1.6	2.4	0.5	1.64		0.4	Babel and Kurniawan (2003)
Modified zeolite, MMZ	123					8	Nah *et al.* (2006)
HCl-treated clay			63.2	83.3			Vengris *et al.* (2001)
Clay/poly(methoxyethyl)acrylamide	81.02		20.6	29.8		80.9	Sölenera *et al.* (2008)
	85.6						Aklil *et al*. (2004)
Calcined phosphate	155.0						Moufliha *et al*. (2005)
Activated phosphate	4						Pan *et al*. (2007)
Zirconium phosphate	398						Pan *et al*. (2007)

Source: Barakat (2011)

Fly ashes were also investigated as adsorbents for removal of toxic metals. Gupta *et al*. (2003) explored bagasse fly ash, a solid waste from sugar industry, for Cd(II) and Ni(II) removal from synthetic solution at pH ranging from 6.0 to 6.5.

Sawdust treated with 1,5-disodium hydrogen phosphate was used for adsorption of Cr(VI) at pH 2. Iron based sorbents such as ferrosorp plus (and synthetic nanocrystalline akaganeite (Deliyanni *et al.*, 2007) were used for simultaneous removal of heavy metals. Ghosh *et al.* (2003) and Barakat (2005) studied hydrous titanium oxide for adsorption of Cr(VI) and Cu(II), respectively.

2.4.7 Adsorption on modified agriculture and biological wastes (bio-sorption)

A great deal of interest in the research for the removal of heavy metals from industrial effluent has been focused on the use of agricultural by-products as adsorbents. The use of agricultural by-products in bioremediation of heavy metal ions, is known as bio-sorption. This utilizes inactive (non-living) microbial biomass to bind and concentrate heavy metals from waste streams by purely physico-chemical pathways (mainly chelation and adsorption) of uptake (Igwe *et al*., 2005). New resources such as hazelnut shell, rice husk, pecan shells, jackfruit, maize cob or husk can be used as an adsorbent for heavy metal uptake after chemical modification or conversion by heating into activated carbon. Ajmal *et al.* (2000) employed orange peel for Ni(II) removal from simulated wastewater. They found that the maximum metal removal occurred at pH 6.0. The applicability of coconut shell charcoal (CSC) modified with oxidizing agents and/or chitosan for Cr(VI) removal was investigated by Babel and Kurniawan (2003). Cu(II) and Zn(II) removal from real wastewater were studied using pecan shells-activated carbon (Bansode *et al.,* 2003) and potato peels charcoal (Amana *et al.,* 2008). Bishnoi *et al.* (2003) conducted a study on Cr(VI) removal by rice husk-activated carbon from an aqueous solution. They found that the maximum metal removal by rice husk took place at pH 2.0.

Rice hull, containing cellulose, lignin, carbohydrate and silica, was investigated for Cr(VI) removal from simulated solution (Tang *et al.*, 2003). To enhance its metal removal, the adsorbent was modified with ethylenediamine. The maximum Cr(VI) adsorption of 23.4 mg/g was reported to take place at pH 2.

Other type of biosorbents, such as the biomass of marine dried green alga (biological materials) (Fenga and Aldrich, 2004Gupta *et al*., 2006, El-Sikaily *et al.*, 2007; Ahmady-Asbchin *et al*., 2008), were investigated for up-taking of some heavy metals from aqueous solution. Some of the used alga wastes were; *Spirogyra* species (Gupta *et al.*, 2006), *Ecklonia maxima* (Fenga and Aldrich, 2004), *Ulva lactuca* (El-Sikaily *et al*., 2007), *Oedogonium* sp. and *Nostoc* sp. and brown alga *Fucus serratus* (Ahmady-Asbchin *et al*., 2008). On the whole, an acidic pH ranging from 2 to 6 is effective for metal removal by adsorbents from biological wastes. Table 3.3 shows the adsorption capacities of different biosorbents.

2.4.8 Adsorption on modified biopolymers and hydrogels

Biopolymers are industrially attractive because they are capable of lowering transition metal ion concentrations to sub-part per billion concentrations, widely available, and environmentally safe. Another attractive feature of biopolymers is that they possess a number of different functional groups, such as hydroxyl and amines, which increase the efficiency of metal ion uptake and the maximum chemical loading possibility. New polysaccharide-based-materials were described as modified biopolymer adsorbents (derived from chitin, chitosan, and starch) for the removal of heavy metals from the wastewater).

Table 2.3: Adsorption capacities of some agricultural and biological wastes for heavy metals.

Adsorbent	Adsorption capacity (mg/g)						References
	Pb^{2+}	Cd^{2+}	Zn^{2+}	Cu^{2+}	Cr^{6+}	Ni^{2+}	
Maize cope and husk	456	493.7	495.9				Igwe *et al.* (2005)
Orange peel						158	Ajmal *et al.* (2000)
Coconut shell charcoal					3.65		Babel and Kurniawan (2003)
Pecan shells activated carbon			13.9	31.7			Bansode *et al*. (2003)
Rice husk		2.0			0.79		Bishnoi *et al*. (2003)
Modified rice hull					23.4		Tang *et al.* (2003)
Spirogyra (green alga)				133			Gupta *et al*. (2006)
Ecklonia maxima–marine alga	235			90			Fenga and Aldrich (2004)
Ulva lactuca					112.3		El-Sikaily *et al.* (2007)
Oedogonium species	145						Gupta *et al.,* (2006)
Nostoc species	93.5						Gupta *et al.,* (2006)
Bacillus – bacterial biomass	467	85.3	418	381	39.9		Ahluwalia and Goyal (2007)

Source: Barakat (2011).

There are two main ways for preparation of sorbents containing polysaccharides:

(a) Crosslinking reactions, a reaction between the hydroxyl or amino groups of the chains with a coupling agent to form water-insoluble crosslinked networks (gels);

(b) Immobilization of polysaccharides on insoluble supports by coupling or grafting reactions in order to give hybrid or composite materials (Crini, 2005).

Chitin is a naturally abundant mucopolysaccharide extracted from crustacean shells, which are waste products of seafood processing industries. Chitosan, which can be formed by deacetylation of chitin, is the most important derivative of chitin. Chitosan in partially converted crab shell waste is a powerful chelating agent and interacts very efficiently with transition metal ions (Pradhan, *et al* 2005).

The adsorption mechanism of polysaccharide-based-materials is different from those of other conventional adsorbents. These mechanisms are complicated because they implicate the presence of different interactions. Metal complexation by chitosan may thus involve two different mechanisms (chelation versus ion exchange) depending on the pH since this parameter may affect the protonation of the macromolecule (Crini, 2005). Chitosan is characterized by its high percentage of nitrogen, present in the form of amine groups that are responsible for metal ion binding through chelation mechanisms. Amine sites are the main reactive groups for metal ions though hydroxyl groups, especially in the C-3 position, and they may contribute to adsorption.

Hydrogels, which are crosslinked hydrophilic polymers, are capable of expanding their volumes due to their high swelling in water and are widely used in the purification of wastewater. Various hydrogels were synthesized and their adsorption behavior for heavy metals was investigated. Kesenci

et al. (2002) prepared poly(ethyleneglycol dimethacrylate-co-acrylamide) hydrogel beads with the following metals in the order Pb(II) > Cd(II) > Hg(II); Essawy and Ibrahim (2004) prepared poly(vinylpyrrolidone-co-methylacrylate) hydrogel with Cu(II) > Ni(II) > Cd(II); while Barakat and Sahiner (2008) prepared poly(3-acrylamidopropyl)trimethyl ammonium chloride hydrogels for As(V) removal. The removal is basically governed by the water diffusion into the hydrogel, carrying the heavy metals inside especially in the absence of strongly binding sites.

2.4.9 Membrane filtration

Membrane filtration is capable of removing not only suspended solids and organic compounds, but also inorganic contaminants such as heavy metals. Depending on the size of the particle that can be retained, various types of membrane filtration such as ultrafiltration, nanofiltration and reverse osmosis can be employed for heavy metal removal from wastewater.

Ultrafiltration (UF) utilizes permeable membrane to separate heavy metals, macromolecules and suspended solids from inorganic solution on the basis of the pore size (5–20 nm) and molecular weight of the separating compounds (1000–100,000 Da). These unique specialties enable UF to allow the passage of water and low-molecular weight solutes, while retaining the macromolecules, which have a size larger than the pore size of the membrane (Vigneswaran e*t al.*, 2004). UF presents some advantages such as lower driving force and a smaller space requirement due to its high packing density. However, the decrease in UF performance due to membrane fouling has hindered it from a wider application in wastewater treatment. Fouling has many adverse effects on the membrane system such as flux decline, an increase in transmembrane pressure (TMP) and the biodegradation of the membrane materials (Kurniawan *et al*., 2006). These effects result in high operational costs for the membrane system.

Lv *et al.* (2008) investigated amphoteric polybenzimidazole nanofiltration hollow fiber membrane for both cations and anions removal NF membranes perform separation in between those of UF and RO ones. The molecular weight of the solute that is 90% rejected by NF membrane range from 200 to 1000 Da with pore diameters varying from 0.5 to 2 nm (Lv *et al.*, 2008). Polymer-supported ultrafiltration (PSU) technique has been shown to be a promising alternative for the removal of heavy metal ions from industrial effluent (Rether and Schuster, 2003). This method employs water-soluble polymeric ligands to bind metal ions of interest, and the ultrafiltration technique to concentrate the formed macromolecular complexes and produce an effluent, essentially free of the targeted metal ions. Advantages of the PSU technology over ion exchange and solvent extraction are the low-energy requirements involved in ultrafiltration, the very fast reaction kinetics, all aqueous based processing and the high selectivity of separation if selective bonding agents are applied.

2.4.10 Electrodialysis

Electrodialysis (ED) is a membrane separation in which ionized species in the solution are passed through an ion exchange membrane by applying an electric potential. The membranes are thin sheets of plastic materials with either anionic or cationic characteristics. When a solution containing ionic species passes through the cell compartments, the anions migrate toward the anode and the cations toward the cathode, crossing the anion exchange and cation-exchange membranes (Chen, 2004), Figure 2.2 shows the principles of electrodialysis.

Electrodialytic removal of Cd(II) from wastewater sludge, was studied (Barakat, 2011). During the remediation a stirred suspension of wastewater sludge was exposed to an electric dc field. ED process was modeled based on basic electrochemistry rules and copper ion separation experimental data (Mohammadi *et al.*, 2005). The experiments were performed for zinc, lead and chromium ions. It

was found that performance of an ED cell is almost independent on the type of ions and only depends on the operating conditions and the cell structure. In spite of its limitation, ED offers advantages for the treatment of wastewater laden with heavy metals such as the ability to produce a highly concentrated stream for recovery and the rejection of undesirable impurities from water. Moreover, valuable metals such as Cr and Cu can be recovered.

2.4.11 Evaluation of Heavy Metals Removal Processes

In general, physicochemical treatments offer various advantages such as their rapid process, ease of operation and control, flexibility to change of temperature. Unlike in biological system, physico-chemical treatment can accommodate variable input loads and flow such as seasonal flows and complex discharge. Whenever it is required, chemical plants can be modified. In addition, the treatment system requires a lower space and installation cost. Their benefits, however, are outweighed by a number of drawbacks such as their high operational costs due to the chemicals used, high-energy consumption and handling costs for sludge disposal. However, with reduced chemical costs (such as utilizing of low-cost adsorbents) and a feasible sludge disposal, physicochemical treatments have been found as one of the most suitable treatments for inorganic effluent (Kurniawan *et al.*, 2006).

In wastewater systems containing heavy metals with other organic pollutants, the presence of one species usually impedes the removal of the other. For instance, hydrometallurgy, a classical process to recover metals, is inhibited by the presence of organic compounds and a pre-treatment step, to remove or destroy organics, is generally required, pyrometallurgy which is able to decontaminate systems from organic pollutants and recover metals suffers from lack of controllability, demanding extremely high temperatures.

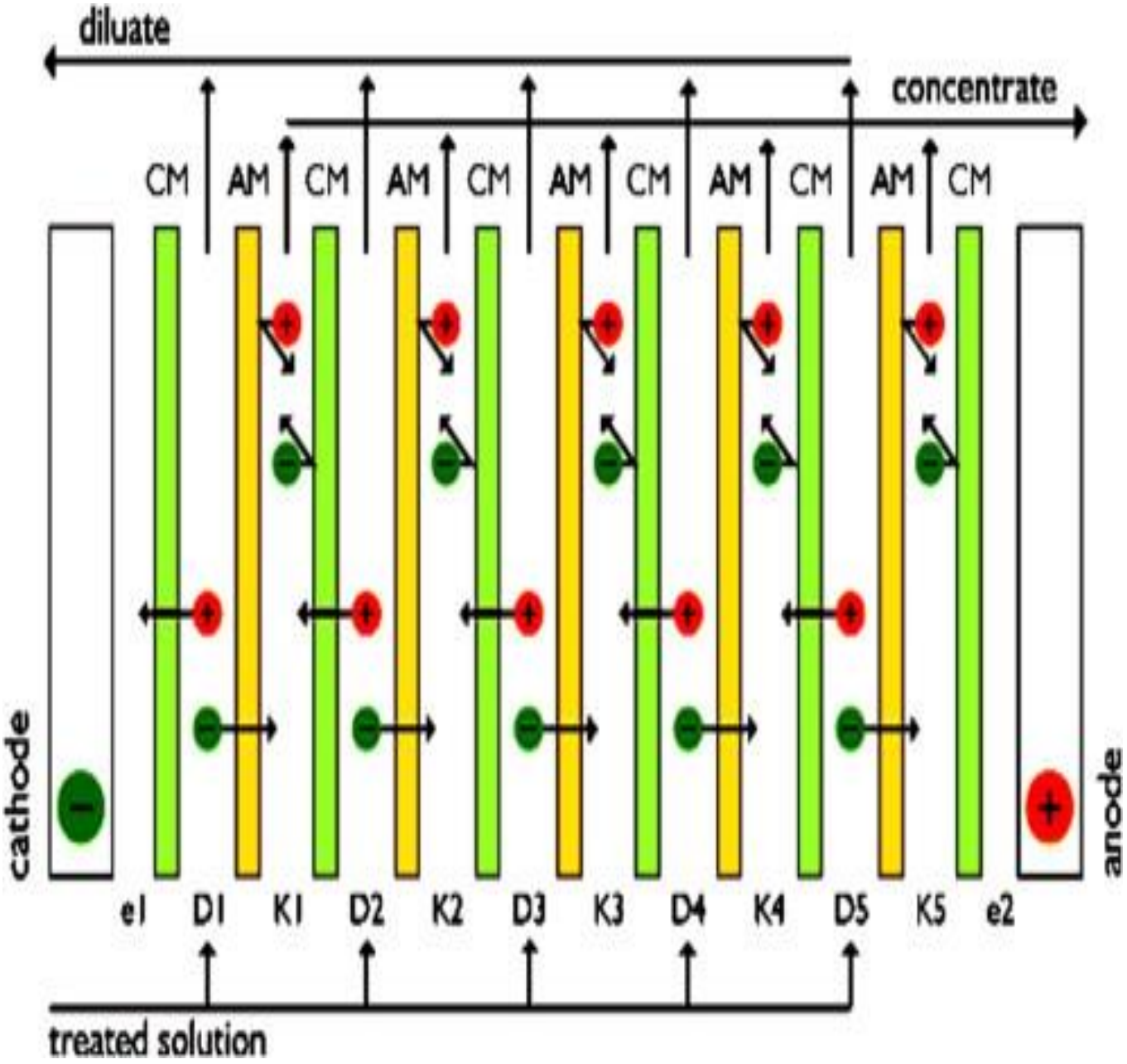

Figure 2.1: Electrodialysis principles (Chen, 2004; Barakat, 2011).

CM – cation-exchange membrane, D – diluate chamber, e1 and e2 – electrode chambers, AM – anion exchange membrane, and K – concentrate chamber.

The most promising methods to treat such complex systems are the photocatalytic ones which consume cheap photons from the UV-near visible region. These photo catalysts serve as electron relays, from the organic substrates to metal ions. Thus, they induce both degradation of organic pollutants and recovery of metals in one-pot systems, operable at traces of the target compounds (less than ppm). Table 2.4 summarizes the main advantages and disadvantages of the various physicochemical treatments described in this review.

2.4.12 The Role of fungi in the Removal of Heavy Metals

The ubiquitous presence of fungi has allowed acclimation to some if not most types of wastes. During the last decade, fungi have been used in the treatment of a wide variety of wastes, wastewaters, and the role of fungi in the bioremediation of various hazardous and toxic compounds in soils and sediments has been established. They are well known to degrade, or cause deterioration to, a wide variety of materials and compounds, processes known as mycodegradation and mycodeterioration, respectively (Arief *et al*., 2008). Polyethylene degradation is a good example of mycodegradation.

Fungi have also demonstrated the ability to remove heavy metals and to degrade, in some cases mineralize, phenols, halogenated phenolic compounds, petroleum hydrocarbons, polycyclic aromatic compounds, and polychlorinated biphenyls (Arief *et al*., 2008).

Table 2.4: Advantages and disadvantages of the physicochemical methods for treatment of heavy metal in wastewater

Treatment method	Advantages	Disadvantages	References
Chemical precipitation	Low capital cost, simple operation	Sludge generation, extra operational cost for sludge disposal	Kurniawan *et al.* (2006)
Adsorption with new adsorbents	Low-cost, easy operating conditions, having wide pH range, high metal-binding capacities	Low selectivity, production of waste products	Babel and Kurniawan (2003); Aklil *et al*. (2004)
Membrane filtration	Small space requirement, low pressure, high separation selectivity	High operational cost due to membrane fouling	Kurniawan *et al*. (2006)
Electrodialysis	High separation selectivity	High operational cost due to membrane fouling and energy consumption	Mohammadi *et al.* (2005)
Photocatalysis	Removal of metals and organic pollutant simultaneously, less harmful by-products	Long duration time, limited applications	Barakat *et al.* (2004)

Source: Barakat, (2011)

The ability of most fungi to produce extracellular enzymes for the assimilation of complex carbohydrates without prior hydrolysis makes possible the degradation of a wide range of pollutants. They also have the advantage of being relatively easy to grow in fermenters, thus being suited for large scale production. Another advantage is the easy separation of fungal biomass by filtration due to its filamentous structure. In comparison to yeasts, filamentous fungi are less sensitive to variations in nutrients, aeration, pH, temperature and have a lower nucleic content in the biomass Ezeonuegbu (2014). In addition, several *Penicillium* strains have been shown to be able to live in saline environments, an advantage of these microorganisms over the others in the bioremediation field. *Penicillium* strains generally are halotolerant microorganisms, able to grow either in the presence or in the absence of salt; those halotolerants that are able to grow, above approximately 15% (w/v) NaCl are considered extremely halotolerant. Hypersaline wastes are generated in several industrial activities, such as chemical manufacture, oil and gas production and waste minimization practices. These wastes, commonly designated as produce waters, are constituted by water containing high concentration of salts, oil, organic acids, heavy metals, and radionuclides (WHO, 1996). Therefore, the ability of halotolerants to remediate pollutants in the presence of salt is useful for biological treatment without damage to the physically sensitive ecosystem.

The morphology and growth characteristics of fungi are responsible for the rapid colonization of substrates. Structural heterogeneity is considered to be one of the most important characteristics of fungal pellets. This feature depends on density of packing and affects limitation of both nutrient and oxygen. Fungi can form or not pellets, depending on several factors such as medium composition, inoculum concentration and preparation, agitation intensity and others (Strater *et al.*, 2010). Tightly

compact pellets are deficient in nutrient and oxygen and generally are hollow in the center, due to autolysis of the mycelium (Leitao, 2009).

Fungal cell walls can act as a cation exchanger due to their negative charge originating from the presence of different functional groups, e.g. carboxylic, phosphate, amine or sulfhydryl, in different wall components (hemicelluloses, pectin, lignin, etc.) (Harvey and McArdle, 2008). The relative importance of each functional group is often difficult to resolve (Tchounwou *et al.,* 2001). Cell walls of fungi are rich in polysaccharides and glycoproteins such as glucans, chitin, mannans and phospho-mannans. These polymers provide abundant sources of metal binding ligands (Leitao, 2009). The cell wall structures of fungi present a multi-laminate architecture where up to 90% of their dry mass consists of amino or non-amino polysaccharides (Patlolla *et al.,* 2009). In general, the fungal cell wall can be considered as a two-phase system consisting of a chitin skeleton framework embedded in an amorphous polysaccharide matrix (Patlolla *et al.,* 2009).

Figure 2.2 shows the schematic representation of the outer layer of fungal cell (Leitao, 2009).

Fungi are present in aquatic sediments, terrestrial habitats and water surfaces and play a significant part in natural remediation of metal and aromatic compounds. Fungi also have advantages over bacteria since fungal hyphae can penetrate contaminated soil, reaching not only heavy metals but also xenobiotic compounds.

Despite of the abundance of such fungi in wastes, penicillia in particular have received little attention in bioremediation and biodegradation studies. Additionally, several studies conducted with different strains of imperfecti fungi, *Penicillium* spp. have demonstrated their ability to degrade different xenobiotic compounds with low co-substrate requirements, and could be potentially interesting for the development of economically feasible processes for pollutant transformation.

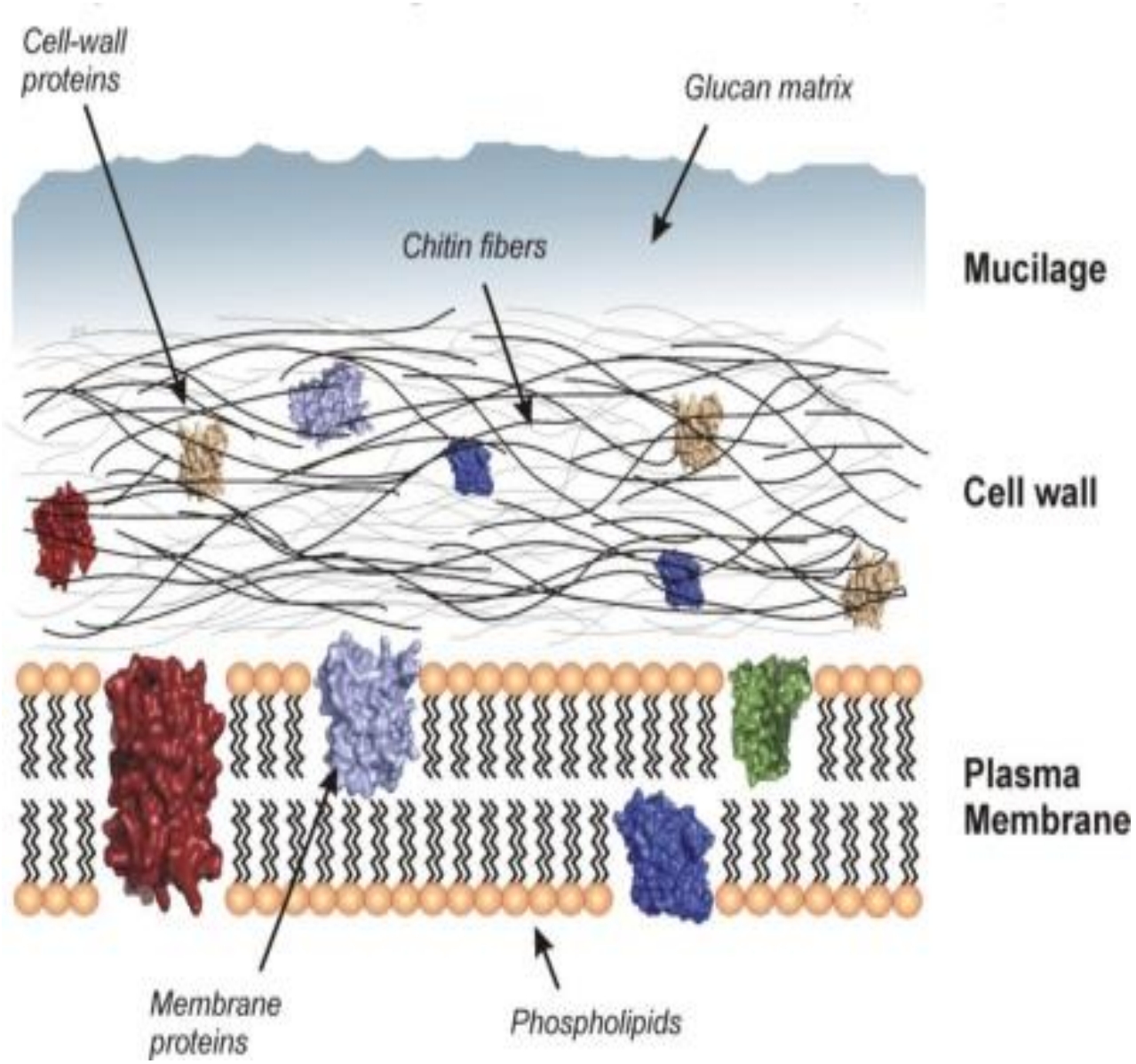

Figure 2.2: The Outer layers of Fungal Cell (Leitao, 2009)

Studies have shown that fungal species are used as sorbents for the effective removal of heavy metal ions from waste water and environment. Arasappan *et al.* (2014) studied the removal and accumulation of Cr and Cu at a higher level by *A. flavus, A. tamarii, A. terreus and A. niger.*

Abdoun- Ouallouche *et al.* (2014), investigated the effect of different pretreatment processes on biosorption capacity of *Rhizopus stolonifer* biomass to remove lead and mercury. The highest metal uptake values (12.10 mg/g and 9.75mg/g for Pb and Hg respectively) were obtained by NaOH treated biomass.

Raja and Bhargavi (2013) also used sodium hydroxide for pretreating *A. niger* and studied lead and nickel biosorption using atomic absorption spectrophotometer. The maximum removal of lead was observed around 75% - 80% at pH 6 -7 with maximum adsorbent dose of 0.2 g/ml. Nickel was observed to have maximum biosorption of around 50%- 60% at pH 5- 8. Das *et al*. (2007) investigated the effect of sodium hydroxide pretreatment on Cd^{2+} biosorption capacity of *Pleurotus florida*. Pretreatment of biomass with NaOH showed maximum increase on biosorption of Cd^{2+} by approximately three times in comparison with living biomass (from 3.21 to 9.76 mg/g). The reason may be due to the removal of surface impurities, rupture of cell membrane and exposure of available binding sites for metal bioadsorption after pretreatment. Chikkhara and Dhankar (2008), also used base treated *A. niger* for Cr(VI) removal and observed 65.28 mg of metal uptake.

The various species of *Penicillium* can colonise many different environments. They are common in soils, in foods, in drinks and in indoor air (Arruti *et al.*, 2010). There are several reasons why *Penicillium* remediation of heavy metal and xenobiotics is important to researchers and practitioners.

Fungi are usually slow growing and often require substrates for cometabolism. *Penicillium simplicissimum* YK is able to degrade polyethylene, with a molecular weight of 400 to 28,000 (Duffus, 2002).

In addition, several *Penicillium* strains have been shown to be able to live in saline environments, an advantage of these microorganisms over the others in the bioremediation field. *Penicillium* strains generally are halotolerant microorganisms, able to grow either in the presence or in the absence of salt; those halotolerants that are able to grow, above approximately 15% (w/v) NaCl are considered extremely halotolerant.

Penicillium digitatum mycelium can accumulate uranium from aqueous solutions of uranyl chloride. Pretreatment of fungal biomass in boiling water or with alcohols, dimethyl sulfoxide, or potassium hydroxide increased the uptake capability to about 10,000 parts per million (dry weight). Meanwhile, formaldehyde killing does not enhance the uranium uptake. Chitin, cellulose and cellulose derivatives from wall fungal interfered in metal ion uptake process. These biopolymers were active in removal of U (VI) (Leitao, 1991).

Penicillium janthinellum F-13 is able to reduce aluminum toxicity and to produce citric acid, but internal or external sequestration of aluminum seems not to be involved in its tolerance to the high aluminum concentrations (Zhang *et al.*, 2002). In *Penicillium simplicissimum*, adsorption of Zn(II) is accompanied by the production of citric acid (Franz *et al.*, 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site:

The study site for this research was Kaduna Refinery and Petrochemical Company (KRPC) located in Romi local government area of Kaduna State Nigeria. As represented in Figure 3.1, the refinery occupies an area of approximately 2.9 square kilometers and is located on an undulating land about 700meters above sea level. This elevation is equivalent to about 17meter higher relative to Romi River Ezeonuegbu (2014). The general direction of flow of the Romi River is south west-north west. The river provides breeding ground for aquatic animals. It is also a source of water for drinking and recreational uses for the public.

3.2 Sample Collection

3.2.1 Sampling Site:

Samples were collected from four main sites for the purpose of this study as represented in the Plate. These include

- Point of Emergence from the refinery (Plate Ia)
- Flow channel 1 after the emergence point (Plate Ib)
- Flow channel 2 before the waste stabilization pond (Plate Ic)
- Wastes Stabilization Pond (Plate Id)

3.3 Collection of Refinery Effluent:

Effluent samples were collected from the various sites using 1litre capacity clean wide mouth plastic, which was first disinfected in the laboratory. A small portion of the sample was used to rinse the bottle before sample was collected. Whilst collecting the sample a small air space is left in the bottle to allow mixing of sample at the time of analysis. The samples were transported using an cooler , to the Department of Microbiology Ahmadu Bello University Zaria for analysis. The schematic diagram in Figure 3.2 clearly shows the sampling points of the raw refinery effluents.

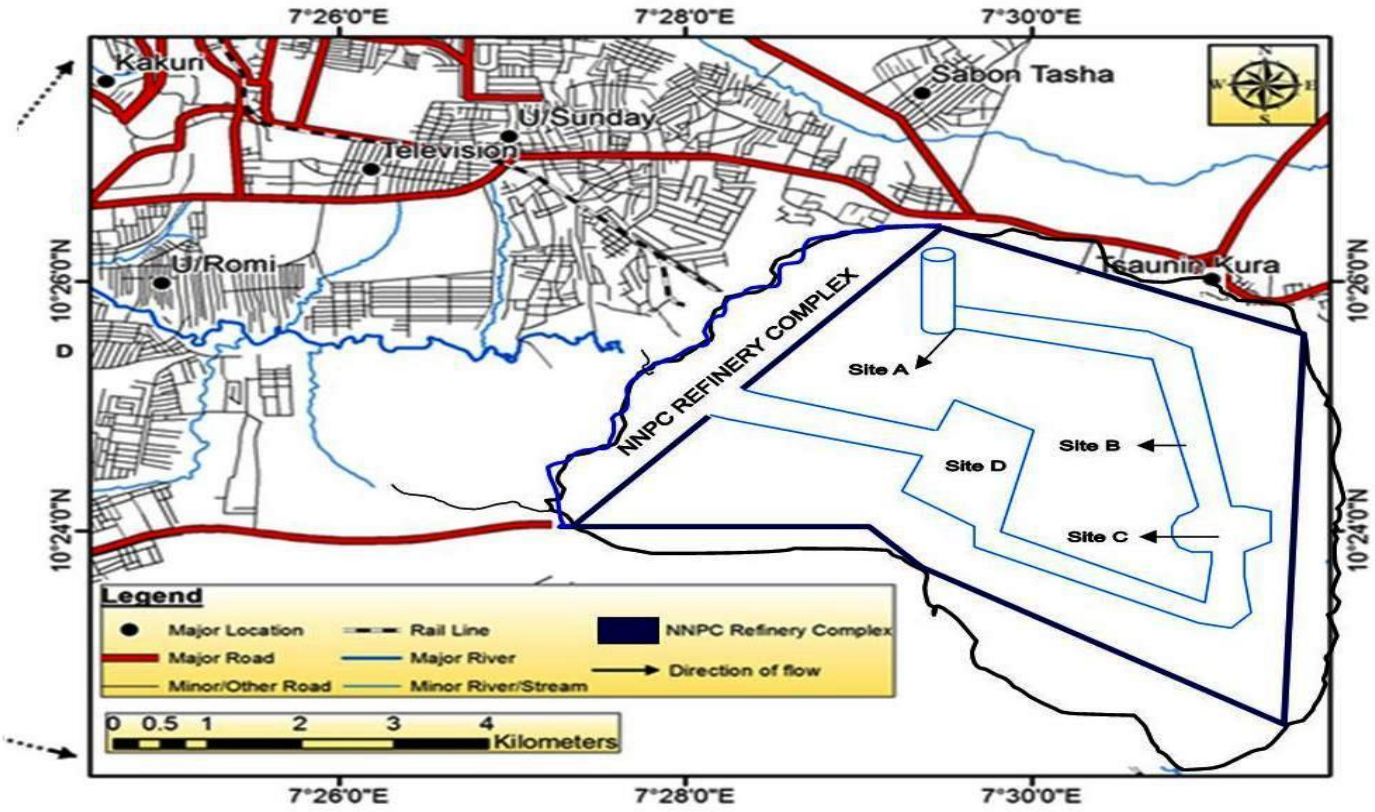

Figure 3.1: Southern part of Kaduna Metropolis showing NNPC Refinery
Source: Modified from the Administrative Map of Kaduna State

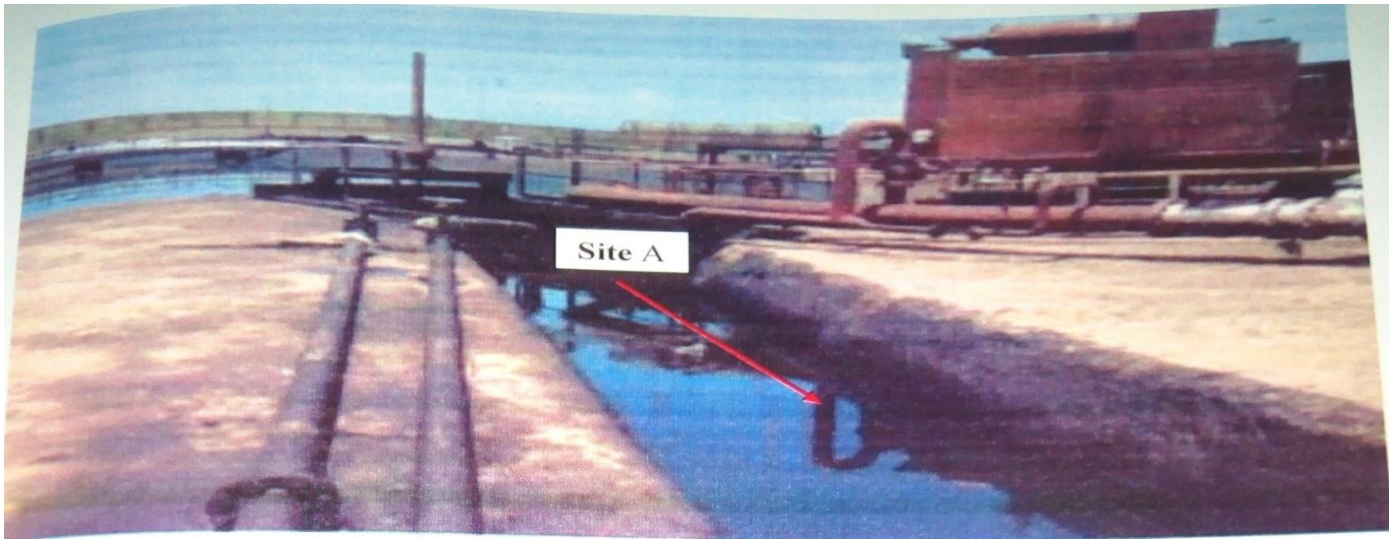

Plate Ia: Raw Refinery Effluent (Site A)

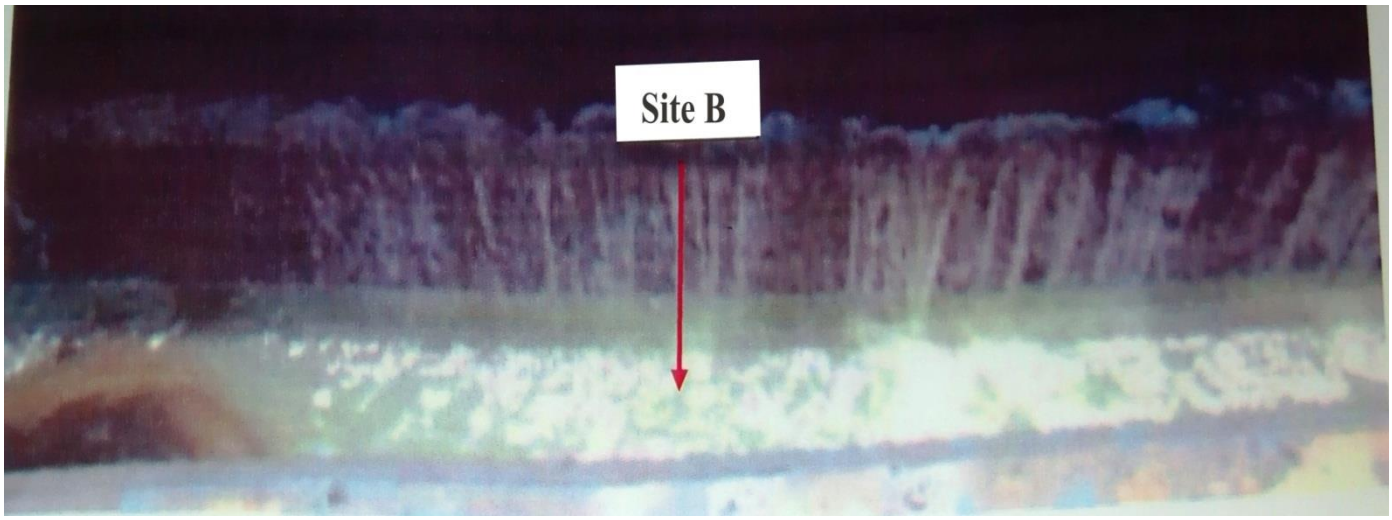

Plate Ib: Effluent in flow channel 1 (Site B)

Plate Ic: Effluent in flow channel 2 and Waste Stabilization Pond (Site C and Site D)

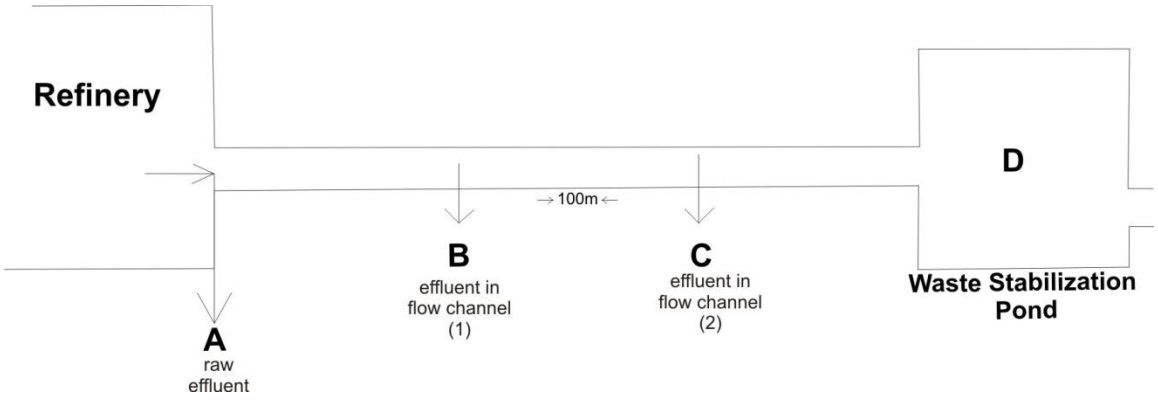

Figure 3.2: Schematic diagram showing the positions of the sampling points relative to the refinery

3.4 Preliminary Analysis of the Raw Refinery Effluents Sample

These were carried out in accordance with the methods suggested by the (APHA, 1995), to determine the natural conditions of the samples, physical and chemical conditions before and after removal of the heavy metals in the effluents.

3.4.1 Determination of raw refinery effluent's pH

The pH was measured using an accurate pH metre (**Perkin-Elmer Spectrophotometer 460. 5 Model made in Germany**) that could detect as low as 0.005 pH unit which is the standard of British standard buffers. The electrodes were rinsed thoroughly first distilled water and immersed in the effluent sample contained in a clean beaker and the reading was recorded.

3.4.2 Determination of raw refinery effluent's temperature

The temperature was measured using mercury- filled Celsius thermometer. The thermometer was immersed in the sample long enough to permit accurate and stable reading and the result is recorded. This was carried out on site during sample collection.

3.4.3 Determination of raw refinery effluent's electrical conductivity

The electrical conductivity of the sample was determined using a conductivity cell (**Perkin-Elmer Spectrophotometer 460. 5 Model made in Germany**). The temperature of the sample was first adjusted to about 20ºC and a portion of the sample was used to wash the conductivity cell and then the samples was filled completely ensuring that no air bubbles adhered to the electrode and the readings were recorded.

3.4.4 Determination of raw refinery effluent's Total Dissolved Solids (TDS)

One hundred millitre (100ml) of the sample was filtered using 0.45mm filter paper and placed in a pre-weighed dish and dried in an oven at 103^0C for 2hours at 103ºC. The dish was allowed to cool

briefly and dried in a desiccator for 1hour 30 minutes. The weight of empty crucible was subtracted from the weight of crucible after drying to give weight of total residue. The total dissolved solids were calculated using the formula:

$$\text{Total dissolved solids (mg/l)} = \frac{\text{weight of total residue} \times 1000}{\text{ml of sample}}$$

3.4.5 Determination of raw refinery effluent's total suspended solids (TSS)

An evaporating dish was weighed empty and 100ml of the sample was dispensed into it, evaporated to dryness in a drying oven at 103^{o}C for 3hours, allowed to cool and dried in a desicator. The dish was reweighed and the total suspended solids were obtained using the formula Machido (2015)

$$\textbf{Total suspended solids (mg/l)} = \frac{\text{mg suspended solid} \times 1000}{\text{Vol. of sample sample evaporated}}$$

3.4.6 Determination of raw refinery effluent's dissolved oxygen (DO)

A glass BOD stopper bottle of 300ml capacity was filled with 250ml of the refinery effluents. Two millilitre (2ml) of manganese sulphate was dispensed into the BOD bottle followed by 2ml of alkali-iodide azide reagent using calibrated pipette. The bottle was stoppered with care to make sure that air was not introduced and the sample was mixed properly by inverting several times and a brownish-orange cloud of precipitate (floc) would appear which was allowed to settle at the bottom.

Two millilitres (2ml) of concentrated sulphuric acid was dispensed into the BOD bottle, stoppered and inverted several times to dissolve the floc. At this point, the sample was "fixed" and stored for 8hours in a cool dark room.

One hundred millilitres (100ml) of the raw refinery effluents was dispensed in 250ml Erlenmeyer's flask and titrated against 0.025N sodium thiosulphate solution until the colour faded to pale yellow.

One millilitre (1m) of the starch solution was added which turned the solution blue. The titration was continued until the solution became clear. The concentration of the dissolved oxygen in the sample was equivalent to the titrant used Ezeonuegbu (2014)

3.4.7 Determination of raw refinery effluents Biochemical oxygen demand (BOD)

Biochemical oxygen demand is the amount of oxygen required by microorganisms to stabilize biochemically decomposable organic matter in water under aerobic condition (Jha, 2006).

The BOD of the sample was determined following the method of APHA (1995).

The BOD bottle was filled with the sample and sealed. The sample was incubated for 5days. The dissolved oxygen content was measured before (DO_1) and after (DO_2) incubation as described in section 3.4.6 of the present study.

The BOD was calculated using the formula:

$$BOD\ (mg/l) = DO_1 - DO_2$$

3.4.8 Determination of Heavy Metal Content of the Effluent

The concentrations of Zn, Hg and Cr in the effluents were determined. This was done by digesting triplicate 5ml batches of the effluent samples using 17.5ml of nitric acid and 12.5ml of hydrochloric acid in a flask. The mixture was heated to near dryness and the volume made up to 50ml with distilled water. The digest was filtered to remove particulate materials that could clog the atomizer. The concentrations of Zn, Hg and Cr in the filtrates were then determined by using Atomic Absorption Spectrophotometer (AA240FS).

3.6 Source Of Fungal Isolates

The two fungal isolates used, were obtained from Department of Microbiology, Faculty of Life Science, Ahmadu Bello University, Zaria. The samples had been isolated from same site and stored in slants before now.

3.6.1 Purification of Fungal Isolates

The fungal isolates preserved in slants, in microbiology laboratory were purified by sub-culturing the isolates on Potato Dextrose Agar (Agar) prepared according to manufacturer's instructions and incubated at ambient temperature. After 5days, the culture's micro-morphological and macro-morphological characteristics such as colour, texture, colour of the reverse side of the fungal isolates were observed and recorded.

For the micro-morphological characteristics, small portion of the growth region was mounted on clean grease free slide containing a drop of lacto phenol blue, covered with a cover slip, and examined using x40 objective lens of a microscope. Characteristics of the sexual reproductive structures, presence or absence of septation, presence of foot cells and chlamydospores were observed and recorded. Each fungal isolate was identified using appropriate taxonomic guide (Thippaswamy *et al*., 2012). The isolated pure cultures were maintained in agar slants and stored in a refrigerator at -8^0c.

3.6.2 Preparation of fungal Inocula

The fungal isolates were grown on Potato dextrose agar slant for 7days at ambient temperature to obtain heavily sporulated cultures. The spores were scraped gently using a sterile inoculation needle under aseptic conditions. Spores suspension of the isolates were obtained by dispensing 15ml of sterile distilled water containing 0.005% Tween 80 into the agar slant and shake properly by vortexing

for 15minutes, to wash off the spores. The spores were diluted with sterile distilled water to obtain a concentration of $6x10^8$spores/ml. These standardized spore suspensions of each isolate were stored for up to 5days at 4 degrees in a refrigerator and used when required.

3.7 Determination of Zinc, Mercury and Chromium Tolerance by the Fungal Isolates:

3.7.1 Preparation of stock solution of heavy metal ions

Stock solutions of Zinc sulphate, mercury sulphate and chromium sulphate were prepared by dissolving 0.1g of each analytical grade salt in separate Erlenmeyer flask containing 500ml of distilled water. The flasks were heated over a hot plate with vigorous shaking to obtain a clear solution of up to 200mg/ml concentrations which was sterilized by autoclaving at 121^oC for 15minutes and used after cooling to room temperature at 25^oC.

3.7.2 Procedure for Determining Heavy Metal Tolerance of Fungal Isolates

Potato Dextrose Broth was prepared following manufacturer's instructions. Fifty (50) ml of the broth was dispensed into 100ml conical flask and the desired concentrations of Zinc, Mercury and Chromium (5mg, 10mg, 15mg) from the stock solution were dispensed into the conical flasks containing the broth in duplicates and sterilized by autoclaving at 121^0c for 15minutes. The preparation was allowed to cool, inoculated with 10^6spores/ml of the isolates, while the control flask was not inoculated and incubated at ambient temperature for 7 days. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of metal that inhibited visible growth of the isolates.

The mycelial mass produced were harvested by filtering the cultures through pre-weighed whatman filter paper (No 1). The filter paper bearing the mycelial mass were dried in an oven at 70^{o}C for 48hours and re-weighed. The yield of the dry mycelial biomass was obtained by subtracting the weight of the filter paper alone from the weight of the filter paper carrying the mycelial biomass.

The tolerance was measured as the growth of the fungi in the presence of heavy metals divided by growth in the same period in the absence of metal (Bello and Abdullahi, 2016).

3.8 Assessment of the capacity of the fungal isolates in removal of Zinc, Mercury and Chromium from the refinery effluents

The capacity of the *Aspergillus* and *Penicillum* species to remove these metals during growth in broth cultures was evaluated by inoculating each isolate into flasks containing 100ml of freshly prepared Potato Dextrose Broth (PDB) charged with sterilized raw effluents in the ratio of 3:1. The control flasks were not inoculated and the set-up was in triplicate. The preparations were incubated in a rotator shaker (120rpm) at ambient temperature for 7 days. The cultures were filtered through pre-weighed filter paper (Whatman No.1) to separate the mycelial mass from the spent broth cultures.

The residual amount of Zn, Hg and Cr in the filtrates was determined using Atomic Absorption Spectrophotometer (AA240FS). The metal removal capacities of the fungal isolates were calculated using the formula (Ezeonuegbu, 2014)

$$\textbf{Metal removal} = \frac{(X - Y)}{X} \times 100$$

Where, X and Y are the initial and final concentrations of the metal ions in the culture filtrates before and after the mycoremediation studies respectively.

The harvested mycelial mass on the filter paper was rinsed repeatedly with distilled water to remove loosely bound metal ions and dried in an oven at 70 degree centigrade for 18 hours. The dry weights of the harvested mycelial biomass was determined using sensitive top loading balance. The metal uptake by the test organisms were calculated using the formula below (Ezeonuegbu *et al.,* 2014).

$$Q = \frac{V(Ci - Cf) \times 1000 \text{ (mg/g)}}{W}$$

Where, **Q** = Amount of metal taken up and accumulated in the fungal biomass (mg/g),

Ci = concentrations of the metal ions in fungal biomass before the experiment,

Cf = concentrations of the metal ions in the fungal biomass after the experiment, **V** = Total working volume, **W** = Dry weight of the fungal biomass harvested.

3.9 Results and Data Analyses:

Results were presented in Statistical analysis, Charts, tables, and graphs and interpreted.

CHAPTER FOUR

RESULTS

4.1 Physicochemical Properties of the refinery effluent

Table 4.1 depicts the physicochemical properties of the effluent samples (raw effluent, effluent flow channel 1, effluent flow channel 2 and waste stabilization pond). The result revealed that temperature of all the sampling sites were not within the permissible limit (40^{o}C) set by the Federal Ministry of Environment, Nigeria (2002). However, temperature of the raw effluent was the highest with a value of 42.7^{0}C and lowest at waste stabilization pond with a value of 30.5^{0}C. The pH of the raw effluent (6.84) were within the permissible limit (6.9) of the FMENV. The lowest pH value (6.20) was noted in the waste stabilization pond.

The Electrical conductivity (EC) of the raw effluent samples were above the permissible limit (2µS/cm) of the FMENV, with the highest (48.10µS/cm) and lowest (23.67µS/cm) observed in raw effluent and waste stabilization pond respectively (Table 4.1).

Total solids (30.41 -35.06mg/l) and Total dissolved solids (TDS) (196.80- 294.0mg/l) were within the permissible limit (500mg/l) recommended by the FMENV. Also, the values of dissolved oxygen, biological oxygen demand, sulphate, phosphate revealed that they were within the permissible limits set by the FMENV as shown in Table 4.1).

Table 4.1: Physicochemical properties of the refinery effluent

PARAMETERS	RAW EFFLUENT	EFC1	EFC2	WSP	FMENV
Temperature (^{0}C)	**42.7**	32.6	30.8	30.5	40
PH	6.84	6.44	6.32	6.20	6.0-6.9
Conductivity (µS/cm)	**48.10**	29.40	23.94	23.67	2.0
Turbidity (NTU)	8.69	5.81	2.83	2.63	10
Total Solid (mg/l)	35.06	33.01	30.91	30.41	50
TDS (mg/l)	294.0	265.10	256.40	196.80	500
Disolved oxygen (mg/l)	1.24	1.18	1.02	0.82	10
BOD (mg/l)	4.10	3.30	2.70	2.50	10
COD(mg/l)	880	680	280	125	1000
Chloride (mg/l)	**835.5**	609.7	477.0	255.2	250
Sulphate (mg/l)	28.0	8	4.00	4.00	50
Phosphate(mg/l)	25.7	2.3	1.5	0.4	50
Zinc (mg/l)	0.0059	0.0045	0.0040	0.0014	0.100
Mercury (mg/l)	**0.0024**	0.0020	0.0019	0.0016	0.001
Chromium (mg/l)	0.0222	0.0196	0.0163	0.0105	0.03

EFC1=Effluent flow channel 1, EFC2= Effluent flow channel 2, WSP=Waste Stabilization pond, BOD=Biological Oxygen Demand, TDS=Total Dissolved solids, COD=Chemical Oxygen Demand, FEMNV=Federal Ministry of Environment, Nigeria, µS/cm=Micro siemens per centimeter, mg/l=Milligram per liter. Values are expressed as means±SE (Standard error of mean) NTU; Nephlometric Turbidity Unit.

Chloride values of all the effluent samples were above the acceptable limit (250mg/l) of FMENV with the highest (835.5mg/l) and lowest (255.2mg/l) observed in raw effluent and waste stabilization pond respectively.

The table also shows the heavy metal contents of the refinery effluents. The concentrations of chromium at the sampled sites were within the acceptable limit (0.03mg/l) of the FEMNV with raw effluent sample having the highest value (0.022mg/l) and waste stabilization pond having the lowest (0.0105mg/l) of chromium. Zinc concentrations at the three sampling points; raw effluent (0.0059mg/l), effluent in flow channel 1 (0.0045mg/l), effluent in flow channel 2 (0.0040mg/l) and waste stabilization pond (0.0014mg/l) were within the standard limit (0.01mg/l) of the FEMNV. Mercury concentration of the study site revealed that raw effluent (0.0024mg/l), effluent in flow channel 1 (0.0020mg/l), effluent in flow channel 2 (0.0019mg/l) and waste stabilization pond (0.0016mg/l) were high compared to standard limit (0.001mg/l).

4.2 Cultural and Microscopic Characteristics of the Fungal isolates used for this Study

The fungi used for this study were *Aspergillus flavus* and *Penicillium* sp. as shown in Plate III and Plate IV. The cultural characteristics of *Aspergillus flavus* (Plate 3a) revealed it as green-ash colony with granular surface and a brown reverse coloration on PDA while its microscopic characteristics (Plate 3b) revealed it as septate hyphae, hyaline and coarsely rough conidiophores. radial and biseriate conidial head with its phialides pointing out in all directions. The cultural characteristics of *Penicillium* sp. (Plate 4a), appeared as bluish-green colony, septate hyphae, with branched conidiophores and phialides. Its microscopic features (Plate IVc) showed septate hyphae with branched conidiophores and secondary branches (metulae). The metulae bear flask shaped phialides with unbranched chains of smooth and round conidia.

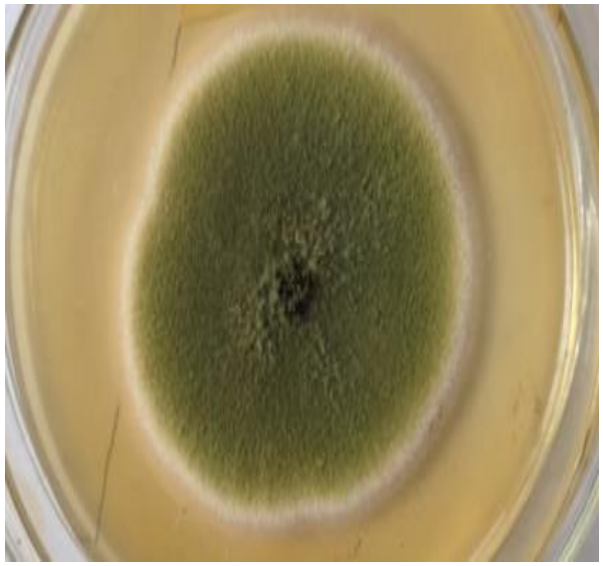

Plate IIIa: Surface Characteristics of *Aspergillus flavus*.

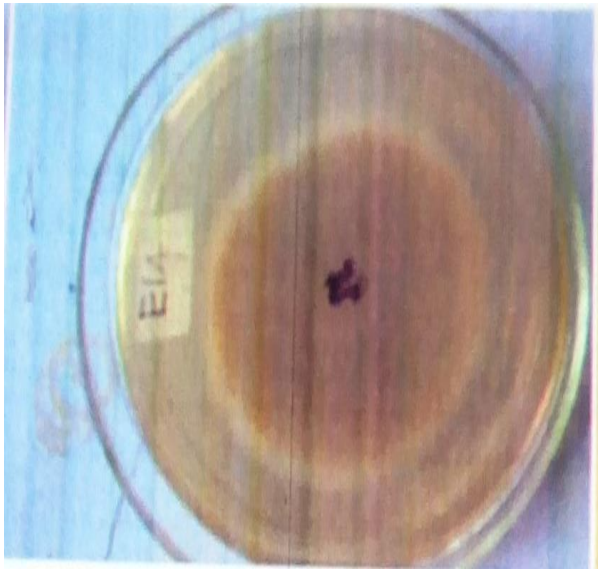

Plate IIIb: Reverse Side of *Aspergillus flavus*.

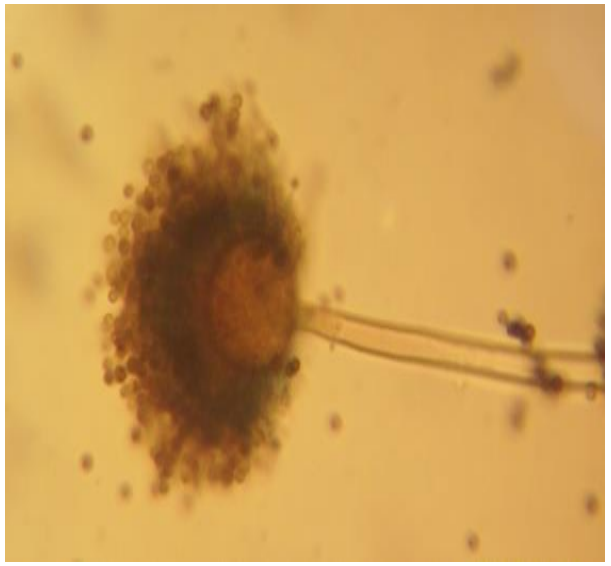

Plate IIIc: Microscopic Characteristics of *Aspergillus flavus*.

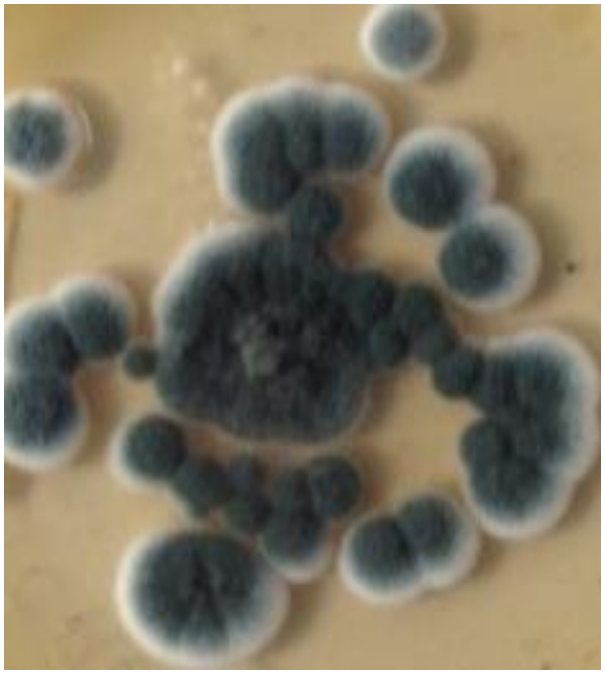

Plate IVa: Surface Characteristics of *Penicillium* sp.

Plate IVb: Reverse Side of *Penicillium* sp.

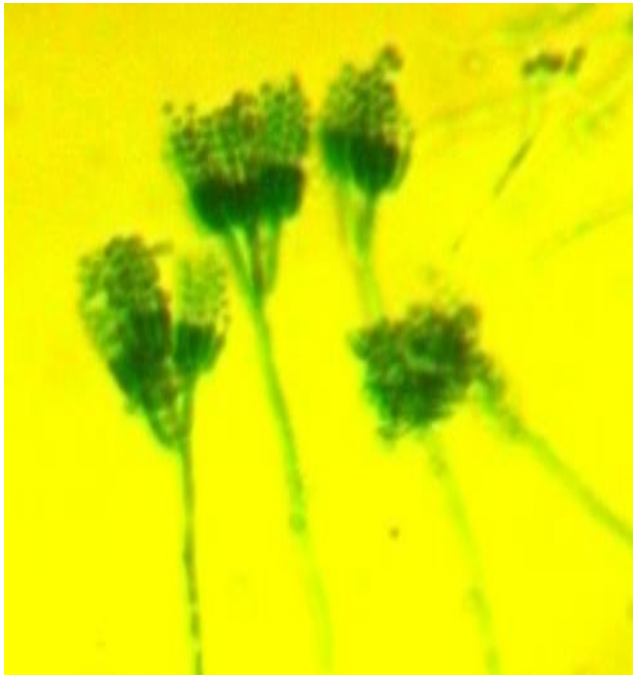

Plate IVc: Microscopic Characteristics of *Penicillium* sp.

4.3 Tolerance of the Test Fungal Isolates to Zinc, Mercury and Chromium

The fungal isolates (*Aspergillus flavus* and *Penicillium* sp.) were subjected to 5mg/l, 10mg/l and 15mg/l of Zinc, Chromium and Mercury salts. They were found to grow in all concentrations of the test heavy metals.

Figure 4.1 shows the biomass yield of *Aspergillus flavus* and *Penicillium* sp. in the presence of 5, 10 and 15mg/l of zinc. The figure revealed that at 5mg/l of zinc, the mean biomass yield of *Aspergillus flavus* and *Penicillium* sp. were 0.37mg and 0.48mg respectively. At 10mg/l of zinc, their biomass values were 0.39mg and 0.45mg respectively. While, at 15mg/l the biomass yield of *Aspergillus flavus* and *Penicillium* sp. were 0.23mg and 0.25mg. Both fungi tolerated and grew in Potato dextrose broth containing 5, 10 and 15mg/l of Zinc ions with the *Penicillium* sp yielding higher mycelial biomass than *Aspergillus flavus.* However the lowest yield of mycelial biomass by the two fungi was recorded when the concentration of zinc ions was 15mg/l (Table 4.1).

Figure 4.2 presents the performance and yields of *Aspergillus flavus* and *Penicillium* sp. at all concentrations of mercury. The biomass yields of *Aspergillus flavus* at 5, 10 and 15mg/l were 0.31mg, 0.39mg and 0.18mg respectively while that of *Penicillium* sp. were 0.58mg, 0.50mg and 0.33mg respectively. *Penicillium* sp. also performed better in mercury than *Aspergillus flavus* in terms of mycelial biomass yield.

Figure 4.3 depicts the biomass yields of the test fungi in the presence of chromium. At 5mg the biomass yields of *Aspergillus flavus* and *Penicillium* sp. were 0.30mg and 0.55mg respectively with *Penicillium* sp. taking up more chromium from the environment. *Aspergillus flavus* and *Penicillium* sp. had equal value (0.49mg) of yields at 10mg/l of

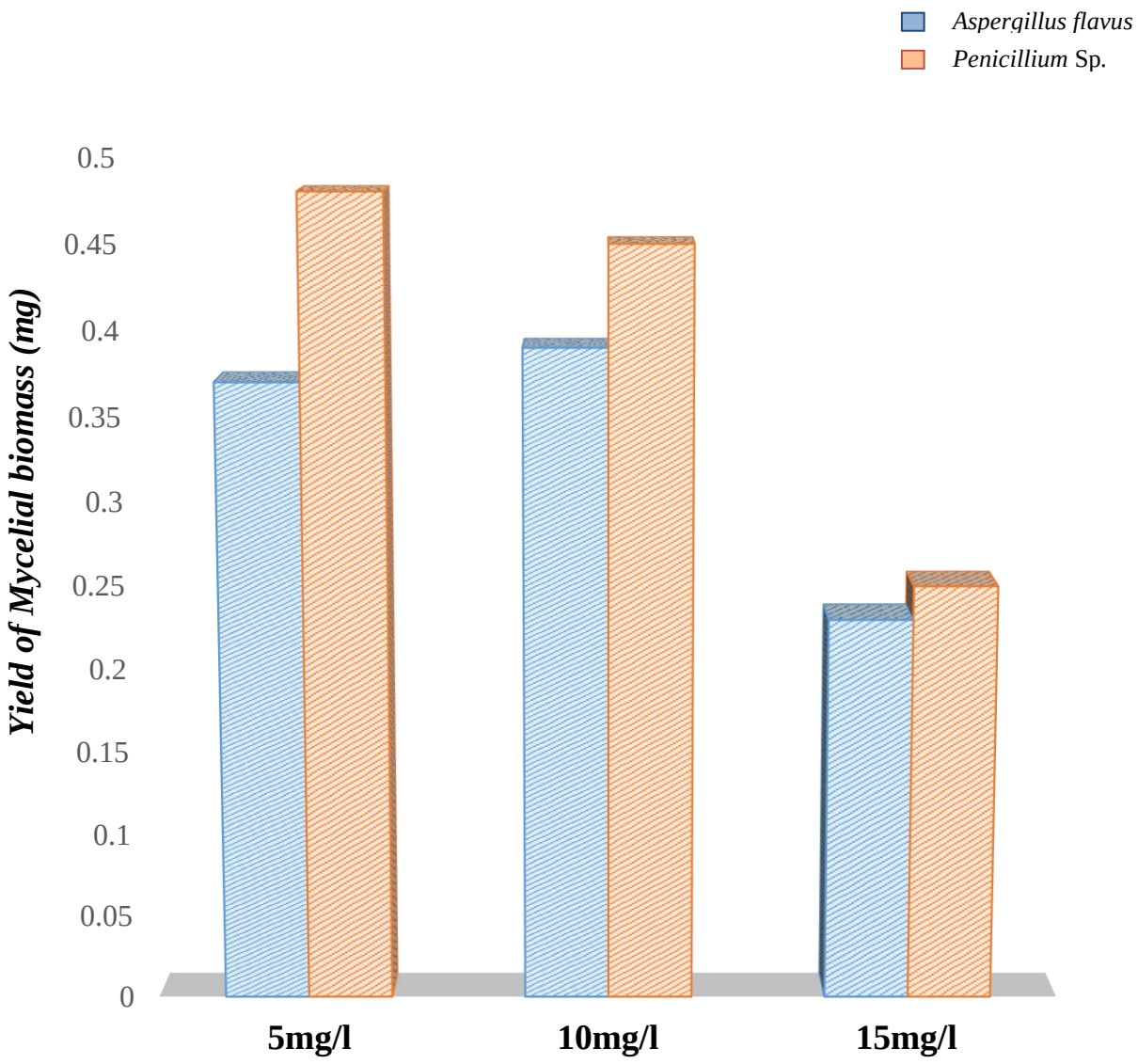

Figure 4.1: Mean Biomass Yield of the Test Fungi to various concentrations of Zinc

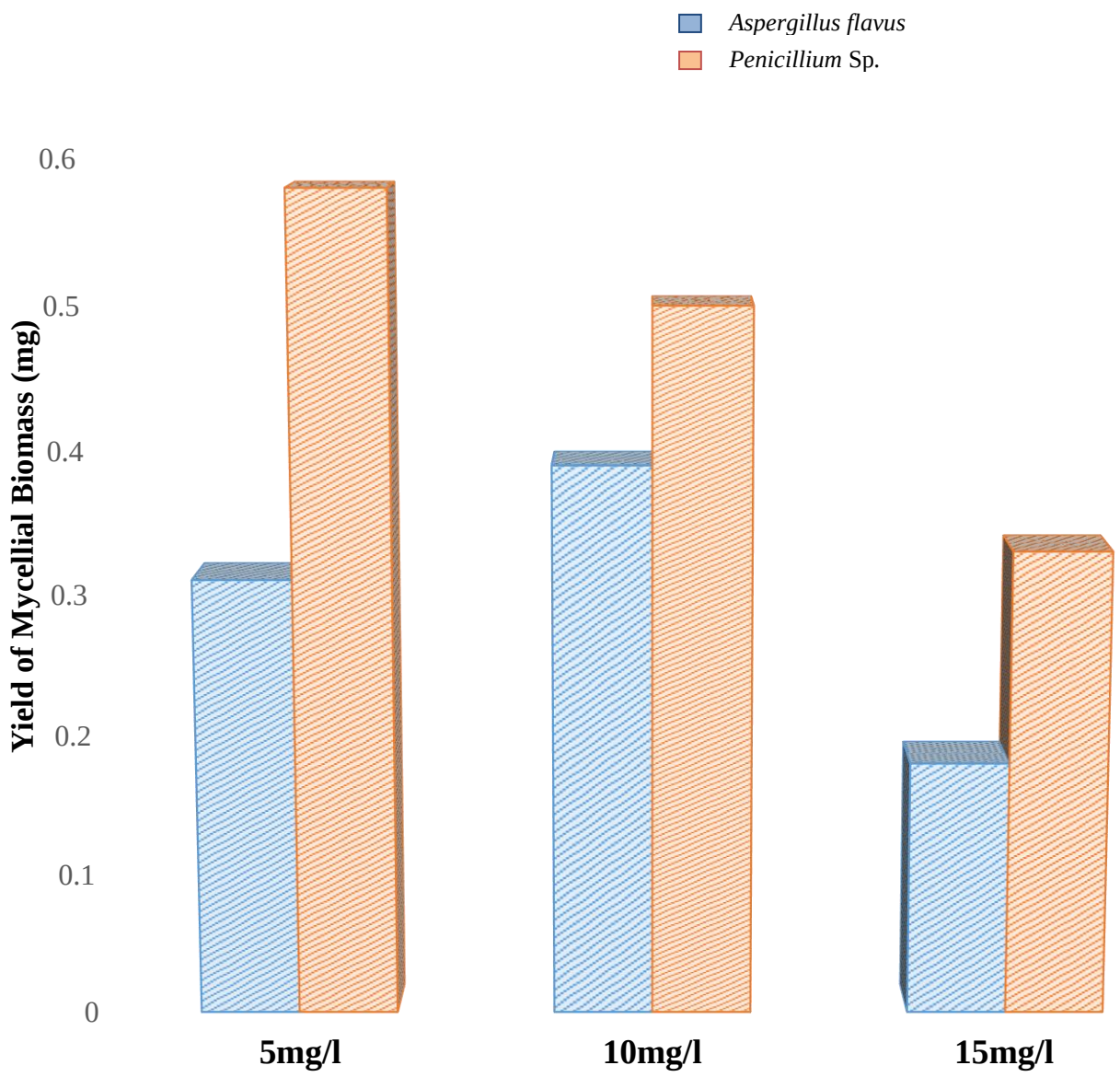

Figure 4.2: Mean Biomass Yield of the Test Fungi to various Concentrations of Mercury ion

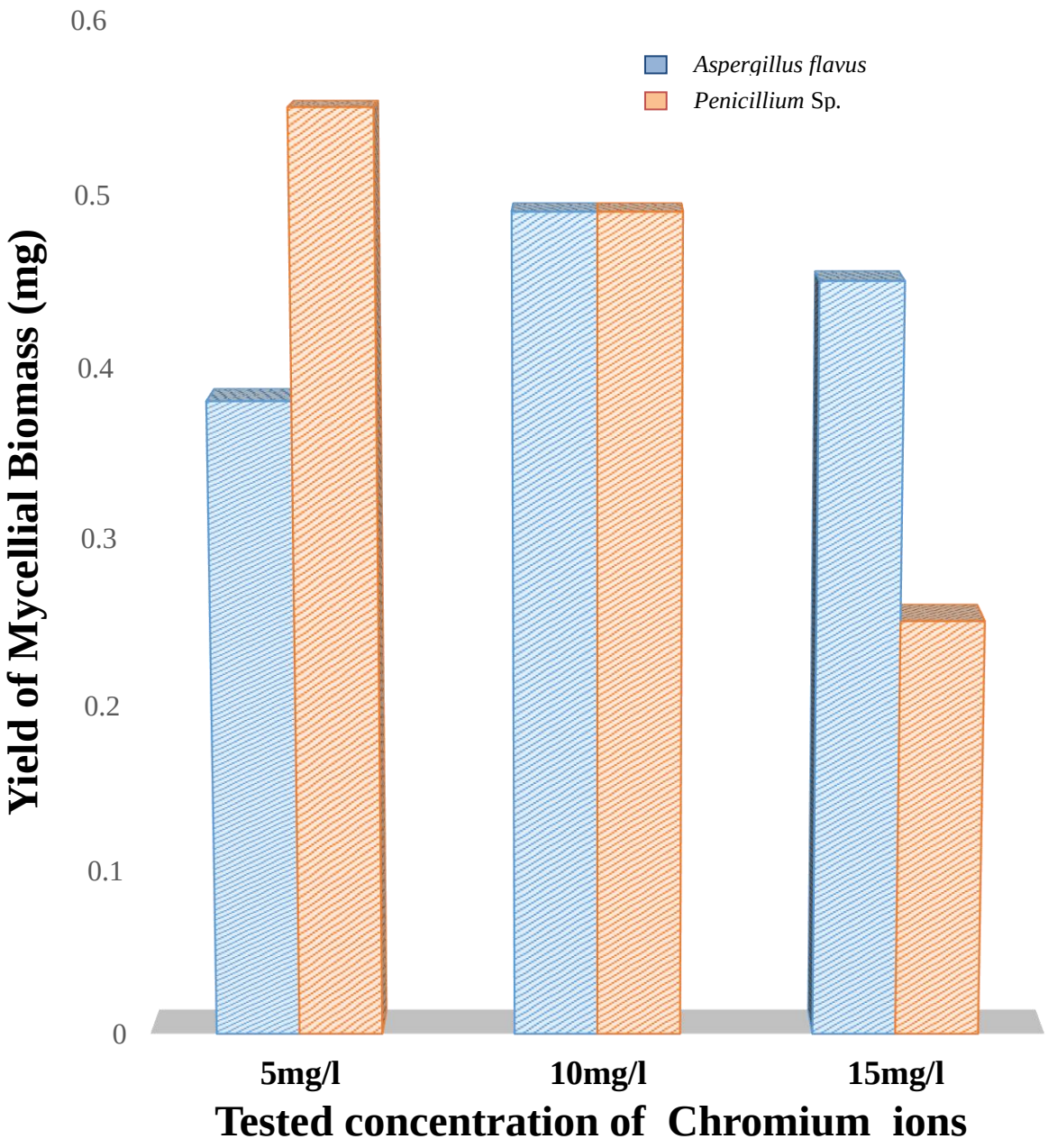

Figure 4.3: Mean Biomass Yield of the Test Fungi to various Concentrations of Chromium

chromium which means in an environment polluted with chromium both penicilium and Aspergillus flavus will be equally effective. At 15mg/l, *Aspergillus flavus* had a yield of 0.45mgand *Penicillium* sp., 0.25mg.

4.4 Performance of the fungi test isolates in removing Zinc, Mercury and Chromium.

Table 4.2 shows the performance of *Aspergillus flavus* in the removal of Zinc, Mercury and Chromium from the raw refinery effluents. The results revealed that the two were effective in the removal zinc, mercury and chromium. *Aspergillus flavus* removed 74% of Zinc, 85% of Mercury and 81% of chromium from the refinery effluent after 7days (Table 4.2). *Penicillium* sp. Was found to remove 84% of Zinc, 56% of Mercury and 84% of Chromium from the refinery effluent over the same period.

However, *Aspergillus flavus* performed better in the removal of mercury with a value of 85% compared to the 56% removal by *Penicillium* sp. (Table 4.2). *Penicillium* sp. Peformed better in the removal of Zn an Cr with an equal value of 84% when compared to *Aspergillus flavus* (Table 4.2).

4.5 Adsorption of Zinc, Mercury and Chromium by *Penicillium* sp. and *Aspergillus flavus.*

Table 4.3 reveals the amount of heavy metal uptake by biomasses of *Aspergillus flavus* and *Penicillium* sp. The result showed that the amount of zinc, mercury and chromium adsorbed by *Aspergillus flavus* were 0.8mg/g, 0.53mg/g and 0.13mg/g respectively from the refinery effluent. *Penicillium* sp. adsorbed 0.32mg/g of zinc, 0.41mg/g of mercury and 0.72mg/g of chromium. However, it was observed that *Aspergillus flavus* adsorbed higher amounts of zinc, mercury than *Penicillium* sp. which showed higher adsorption with chromium compared to the former (Table 4.3).

Table 4.2: Percentage Removal of Heavy Metal by Test Fungi from the Refinery Effluents

Heavy Metal ions	**Amount of heavy metal ions removed (%)**	
	Aspergillus flavus	***Penicillium* sp.**
Zinc	74	84
Mercury	85	56
Chromium	81	84

Table 4.3: Quantities of the Heavy Metals adsorbed from the effluent by the test fungal isolates

HEAVY METALS	**Heavy metal ions adsorbed (mg/g)**	
	Aspergillus flavus	***Penicillium* sp**
Zinc	0.800	0.323
Mercury	0.530	0.413
Chromium	0.133	0.72

CHAPTER FIVE

DISCUSSIONS

5.1 Physicochemical Parameters of the Refinery Effluent.

The physicochemical parameters of the effluent samples revealed that most of the parameters were within the acceptable limit as recommended by FEMNV except for Chloride, temperature, Mercury and conductivity which showed some variations above the limit.

The temperature values obtained in this study were high with the raw refinery effluent having the highest value at 42.7^0C and the waste stabilization pond, the lowest at 30.5^0C. This change in temperature may be as a result of the processes the effluent was still going through whilst being discharged fresh from the refinery and as the reaction reduced, the temperature reduced accordingly. These high temperatures may also be as a result of high sun intensity and the nature of biological activities as these agree with the findings of Ezeonuegbu *et al.* (2014).

In addition, high effluent temperature stress aquatic ecosystem by reducing the ability of water to hold essential dissolved gases like oxygen. The results in agrees with the findings of Adefemi and Awokunmi (2009) who observed similar temperature values.

The electrical conductivity of the effluents samples from the study sites which ranged from 23.67µS/cm to 48.10µS/cm were higher than the acceptable limit set by the FEMNV with the raw effluent having the highest conductivity. This high value could be due to high organics from various chemicals used in downstream processes and primary distillation in refinery plants which was also recorded by Ezeonuegbu *et al*. (2014)

The total solids (TS) and total dissolved solids (TDS) of the effluent sample showed that the highest TS and TDS value were observed in raw effluent with a value of 35.06mg/l and 294mg/l respectively and may be an indication of high sediments in the effluent samples analyzed.

These properties reduce light penetration into the aquatic environment, decreases photosynthetic activities and dissolved oxygen level of waste water. These findings also agrees with those of Ezeonuegbu *et al.* (2014).

The pH of the effluent ranged from 6.20 to 6.84. The lowest was observed in the waste stabilization pond. However the pH of all the sampling points were slightly acidic. This low pH may be attributed to chemicals used during treatment processes and acidification of the oil well and this agrees with the finding of Thippeswamy *et al.* (2012) and Ezeonuegbu *et al.* (2014).

Acidic effluent discharge could 'be very carcinogenic and hazardous to the environment. It adversely affects plants, animals and humans. Acidic pH also increases the concentration and toxicity of some dissolved heavy metals, changes the permeability of soil which results in the pollution of underground water resources (Atubi, 2011; Ezeonuegbu *et al.* 2014).

Chloride contents of the raw effluent at 835.5mg/l were higher than the acceptable limit of FEMNV which is 250mg/l and this may be as a result of sodium hydroxide addition to the effluent to remove harmful substances. Chlorides are formed through the bearing of sodium hydroxide and are inorganic compounds resulting from the combination of chlorine gas with metals (Kumar and Avinash, 2012). Chlorides may get into surface water from several sources including rocks, agricultural run-off, oil well waste and waste water from waste treatment plants. Chlorides can corrode metals and affect the taste of food. It can contaminate aquatic food chain, freshwater, streams and lakes (Kumar and Avinash, 2012).

5.2 Heavy Metal Content of the raw refinery effluents.

Although the heavy metal contents of the effluent were within the acceptable limits of the

FEMNV (0.001) except for mercury (0.0024) which showed a higher value. These high levels of Zn, Hg and Cr observed in these sampled sites may have originated from chemical additives and catalysts, corrosion of equipment and pipes which agrees with the findings of Beddri and Ismail (2007) and Ezeonuegbu *et al*. (2014).

The high levels of Zn, Hg and Cr in the studied sites could pose serious dangers to public health especially in situations where large volume of inadequately treated effluent is continuously released into the environment on a regular basis (Machido *et al.*, 2015). Where the effluent is released into the soil environment, the content of plant nutrient could be affected resulting in low soil fertility. In addition, the poisonous effect of ingesting these metals by aquatic animals, including fishes and their entrance into the food chain cannot be over emphasized. This will in turn cause neurological defects, body organ dysfunctions and anaemia (Ayotamuno *et al*., 2002).

5.3 Tolerance of fungal test isolates to Zinc, Mercury and Chromium.

Aspergillus flavus and *Penicillium* sp. showed high tolerance at the concentrations of 5mg/l, 10mg/l and 15mg/l of the different metal ions giving good mycelial biomass yield at all concentrations of the test heavy metals. These may have been as a result of continuous metal exposure of the fungi to the effluent, leading to the establishment of a resistant and tolerant microbial population (Gadd, 1990), this also agrees with the findings by Machido *et al* (2015).

Heavy metals affect microorganisms in the natural environment by reducing the number and diversity of microbes and selecting a metal resistant population. The tolerance and the resistance of the isolates depended much more on the fungi tested than on the sites of their

isolation. This variation may be explained by the development of tolerance or adaptation of the fungi to heavy metals Ezeonuegbu *et al.,* (2014).

To survive and grow at the tested concentrations of Zn, Hg and Cr, the test isolates may have developed mechanisms by which the toxicity of the metals was circumvented (Machido *et al.,* 2015). Several of such mechanisms have been reported to be employed by fungi growing in environments containing elevated levels of heavy metals. This includes metal exclusion by permeable barriers, intracellular sequestration or accumulation and enzymatic modification of the metal ions to less toxic form (Carroll *et al.,* 1997; Keong *et al.,* 2003).

Fungi are reported to possess specific genes for resistance to heavy metal ions. These genes encode the synthesis of metal binding proteins such as metallothionein and siderophores (Mosa *et al.,* 2016). *Aspergillus flavus* and *Penicillium* sp. were resistant to the metals tested, which may make them promising candidates for further investigations regarding their ability to remove metals form contaminated environments. The results obtained from this present study are very promising as a starting point for a potential application of these microorganisms in metal bioremediation. The isolated fungi can be used in agricultural soils, which can be polluted with heavy metals as a consequence of the incorporation of amendments such as sewage sludge or industrial/municipal solid/liquid wastes.

5.4 Performance of *Aspergillus flavus* and *Penicillium* sp in removing Zinc, Mercury and Chromium from raw refinery effluents .

Aspergillus flavus removed 74% of zinc, 85% of mercury and 81% of chromium from the refinery effluent while *Penicillium* sp. removed 84% of zinc, 56% of mercury and 84% of chromium from the refinery effluent after 7days (Table 4.2).

The high rate of metal removal and adsorption by the two fungal isolates could emanate from their massive growth and enzyme production responses during their growth phases. This could be supported by the reports of Bogan and Lamar (1996), which showed that extracellular ligninolytic enzymes of white rot fungi were produced in response to their growth phases.

Microbial detoxification of metal ions is achieved by several mechanisms including regulation of uptake, transformation into less toxic forms, and intracellular immobilization (Akpe *et al*., 2015; Ezeonuegbu *et al*., 2014; Machido *et al.,* 2015).

Microorganisms inhabiting in metal polluted soils have evolved various strategies to resist metal stress. One of the microbial processes as a bioremediation tool to remove heavy metals from the environment is adsorption (Zolgharnein *et al*, 2007), which is an active process dependent upon metabolic energy of microorganisms to trap and utilize energy from the heavy metals (Rani and Goel, 2009). Therefore, efficient Zinc uptake by growing cells of *Aspergillus* sp isolated from industrial waste has been reported (Sharma *et al.,* 2003). This process could be successfully employed for complete removal of Zinc from real industrial effluents.

Varied mechanisms of metal adsorption result in different localization of the accumulated product. These variations in the mechanism arise out of the toxicity of the metal concerned as well as the environmental conditions to which the microbial strain was exposed. Also, resistance of microorganism to heavy metals is often conferred by products of genes situated

on plasmids (Silver *et al.*, 2001), rendering genetic manipulation for strain improvement easy and feasible. Often, multiple-metal resistance is associated with the same plasmid containing metallothioneins (Malik, 2004).

In addition, the cell wall of fungi present a multi-laminated architecture where up to 90% of the dry mass consist of amino and non-amino polysaccharides and proteins which offers many functional groups (such as carboxyl, hydroxyl, sulphate, phosphate and amino groups) for binding metal ions (Kumar *et al*., 2011). According to Ronda *et al*., (2007), the cell walls of fungi are also rich in glycoproteins such as glycans (β – 1,6- and β- 1,3- linked D- glucose residues), chitosan (β-1,4-linked mannose) and phosphomanans (phosphorylated manans) with various metal binding groups present in the polymers.

The heavy metal removal obtained in this study revealed that the *Aspergillus flavus* and *Penicillium* sp were able to remove Zn, Hg and Cr at different rates. This observation agrees with earlier reports made by Ezeonuegbu *et al.,* (2014) and Machido *et al.* (2015). These observations could be attributed to the important role fungi play in detoxification and removal of heavy metals from contaminated sites through physicochemical and biological mechanisms (Machido *et al.*, 2015).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.0 CONCLUSION

Most of the physicochemical properties of the raw refinery effluent tested were within the acceptable limits set by the Federal Ministry of Environment Nigeria (FEMNV), except for Chloride and Mercury ion contents of the effluent, which were higher than the acceptable limit.

Aspergillus flavus and *Penicillium* sp. when purified revealed features, cultural and microscopic characteristics they are known for as seen in the results.

Aspergillus flavus and *Penicillium* sp. showed resistance to the various concentrations of Zinc, mercury and chromium. *Penicillium* sp. gave better tolerance at 5, 10 and 15mg/l in zinc and mercury than *Aspergillus flavus* which showed better tolerance at 10 and 15mg/l in chromium *Aspergillus flavus* and *Penicillium* sp. removed and adsorbed significant amount of zinc, mercury and Chromium from the effluent. Which means the two fungi isolates can be employed in the remediation of refinery effluents, water bodies and general industrial waste contaminated with Zinc, Mercury and chromium.

6.1 RECOMMENDATION

It is recommended that;

- Regulatory authorities should continuously ensure strict complete adherence to recommended standard of the levels of heavy metals in effluent released into the environment.
- Fungal isolates like *Penicillium* sp and *Aspergillus flavus* with proven mycoremediation capacity to adsorb or remove zinc, mercury and Chromium, should
- be employed in the bioremediation of environments contaminated with the above mentioned heavy metals
- Managers of industries should be educated on the importance of adopting biological approach in the treatment of industrial effluents before releasing it into the immediate environment.

6.2 CONTRIBUTION TO KNOWLEDGE

- Most of the physicochemical properties of effluents generated in KRPC were within the permissible limits set by the Federal Ministry of Environment, except Temperature **(42.7^{0}c)**, Mercury **(0.0024mg/l)**, Choride **(835.5mg/l)** and Conductivity **(48.10mg/l)**.
- Both *Aspergillus flavus* and *Penicillum* sp are tolerant to Zinc, Mercury and Chromium at the concentrations of 5mg/l, 10mg/l and 15mg/l with *Penicillum* sp having a higher mycelial biomass yield in Zinc and Mercury with an average of 0.44mg in all concentrations while *Aspergillus flavus* had a higher mycelial biomass yield in Chromium with an average of 0.46mg in all concentrations.
- The fungi species removed the heavy metals in the refinery effluents with *Aspergillus flavus* removing 74% of zinc, 85% of mercury and 81% of chromium from the refinery effluent while *Penicillium* sp. was found to remove 84% of zinc, 56% of mercury and 84% of chromium from the refinery effluent.

- The *Aspergillus flavus* adsorbed 0.800mg/g of Zinc, 0.530mg/g of Mercury and 0.133mg/g of Chromium, while *Penicillium* sp adsorbed 0.323mg/g of Zinc, 0.413mg/g of Mercury and 0.72mg/g of Chromium from the refinery effluent.

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