REMOVAL OF HEAVY METALS FROM REFINERY EFFLUENT USING SAWDUST INOCULATED WITH *ASPERGILLUS NIGER* AND *PENICILLIUM* SPECIES

BY

FATIMAT AHUOIZA MOHAMMED-JAMIU

DEPARTMENT OF MICROBIOLGY,

FACULTY OF LIFE SCIENCES,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

JULY, 2017

REMOVAL OF HEAVY METALS FROM REFINERY EFFLUENT USING SAWDUST INOCULATED WITH *ASPERGILLUS NIGER* AND *PENICILLIUM* SPECIES

BY

FATIMAT AHUOIZA MOHAMMED-JAMIU, BSC (ABU) 2011

P13SCMC8014

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTEROF SCIENCE DEGREE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLGY,

FACULTY OF LIFE SCIENCES,

AHMADU BELLO UNIVERSITY,

ZARIA

JULY, 2017

DECLARATION

I declare that this work in this dissertation titled "REMOVAL OF HEAVY METALS FROM REFINERY EFFLUENT USING SAWDUST INNOCULATED WITH *ASPERGILLUS NIGER* and*PENICILLIUM* SPECIES" was written and compiled by me in Department of Microbiology, Ahmadu Bello University, Zaria, under the supervision of Prof. D.A Machido and Dr. M.B. Tijjani. It has neither in part or whole, been submitted by me or anybody for this or any other degree at this or any other University.

The information derived from literature has been duly acknowledged in the text and a list of references provided

Fatimat A. Mohammed-Jamiu _____ _____

Signature Date

CERTIFICATION

This dissertation report entitled "REMOVAL OF HEAVY METALS FROM REFINERY EFFLUENT USING SAWDUST INNOCULATED WITH *ASPERGILLUS NIGER*and*PENICILLIUM* SPECIES" by FATIMAT A. MOHAMMED-JAMIU meet the regulations governing the award of M.Sc. Microbiology of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. D.A Machido	_____	_____
Department of Microbiology,	Signature	Date
Faculty of Life Sciences,		
Ahmadu Bello University, Zaria		
(Chairman, Supervisory Committee)		
Dr. M.B. Tijjani	_____	_____
Department of Microbiology,	Signature	Date
Faculty of Life Sciences,		
Ahmadu Bello University, Zaria		
(Member, Supervisory Committee)		
Prof. I.O Abdullahi	_____	_____
Head,	Signature	Date
Department of Microbiology,		
Faculty of Life Sciences,		
Ahmadu Bello University, Zaria		
Prof. S.Z. Abubakar	_____	_____
Dean,	Signature	Date
School of Postgraduate Studies,		
Ahmadu Bello University, Zaria		

DEDICATION

This work is dedicated to:

Almighty Allah (SWT) for His overwhelming sustenance no matter what. He is indeed GOD for all. To my parent my backbone, the most influential figure in my academics and my way of life. You are an amazing and ever willing people. To my loving brothers and sisters you are wonderful.

ACKNOWLEDGEMENTS

My sincere and profound gratitude goes to God Almighty, who has in His infinite mercies and guidance given me the wisdom, inspiration, courage and strength to complete this work.

I am greatly indebted in appreciation to my able supervisors Prof. D.AMachido and Dr. M.B. Tijjani for their useful advice, coaching, suggestions, inputs, contributions, review and positive criticisms to the content of this work. I am privileged to have tapped from your wealth of knowledge.

I also wish to thank Prof. I.O. Abdullahi, Prof. S.E. Yakubu and Prof O.S Olonitola for their fatherly advice, words of encouragement and concern and Dr. E.E. Ella for his constant supply of information. Indeed you all have been my supporting block and guiding light.

My thanks and appreciations go to Dr. H.M. Raji, Prof. S.A. Ado, Dr. S.L. Afegbua, Mrs M.A. Shuaibu, Mallam Shitu, Mr John Onu and other staff of the Department of Microbiology, Ahmadu Bello University, Zaria. May God reward you all enormously for your priceless contributions and support throughout the course of this study.

With utmost gratitude, I whole-heartedly thank my parents, Alhaji Ovoko Muhammed-Jamiu and Hajiya Sefiya Mohammed-Jamiu for their inestimable love, prayers and words of wisdom. Special thanks go to my loving brothers and sisters. I say a very big thank you for the concern, support and care you have shown to me throughout the tenure of this program. May Allah put me in a position that I can show how grateful I am to have you people around me; I have gotten a wonderful family.

My appreciation goes to my friends and colleagues, Toheeb Afolayan, Fatima Abba Shehu, Murjanatu Usman, Valeri Ekoja, Mrs Mercy Sule-Otori, Hassana Rilwan, Rukayyah Imrana,Hafsat Abdullahi mohammed, Murjanatu Abdullahi, Aisha Dadan-Garba, Aisha Usman, Zainab Azeez, Linda Ameh, Hussaini Ibrahim, AbdulGafar Aliyu and Bishir Musa for their encouragements, contributions, help and collaboration; it has been academically rewarding and fun knowing and working with you all.

Thank you and God bless you all and your family. Remain blessed and safe.

ABSTRACT

This study was done to evaluate the effectiveness of white and brown wood sawdust alone and when enhanced with *Aspergillus niger* and *Penicillium* species in removing lead, nickel and cadmium ions from effluent of Kadunarefinery. Sawdust obtained from wood industry in Sabon-gari, Zaria was sun dried and ground to moderately fine particle size using a pestle and mortar and then evaluated for proximate content. The ability of the sawdusts to support the growth of the organisms was also studied. The saw dusts used proved to serve as source of carbon as it was decomposed by the organisms (weight after inoculation was 291.1g and after 14 days, it became 255.25g with percentage decomposition of 12.31%) for white wood and for brown wood (weight after inoculationwas 279.05g and after 14 days, it was 246.58g with percentage decomposition of 11.64%). Evaluation of the physicochemical properties of the effluent revealed a high COD, E.C, Sulphate, Phosphate, Pb, Ni and Cd above the permissible limit set by NESREA. The adsorption properties were studied by packing five batches of 100g each of the processed sawdust in separate aluminum columns measuring 5cm in diameter x 25cm in depth to provide substrate medium for growth and accumulation of mycellial biomass of the test fungi. Four out of the five columns were sterilized and a fifth left unsterilized. Three of the sterilized packed columns were inoculated with single and co-culture of the test fungi isolated from refinery effluents (in a previous study). The unsterilized and one of the sterilized packed columns were left un-inoculated to serve as the controls for the experiment. All the columns were incubated aerobically under ambient laboratory conditions for 14 days to obtain the experimental sorbents. One liter (1000ml) of the sterilized refinery effluent was poured into each of the experimental sorption columns and allowed to stand for one hour. 20 milliliters (20ml) of the residual effluent were drawn from each of the set-up at hourly intervals for six hours. The residual concentrations

of the target heavy metal ion in the filtrates were determined using Atomic absorption spectrophotometer. The percentage removal of the targeted metal ions, amounts adsorbed per gram of the sorbent was calculated as well as the relative affinity using Langmuir isotherm. The percentage removal of lead, nickel and cadmium ions was found to increase with increase in time until the second hour in white wood and first hour in brown wood where there was no further increase in adsorption despite the time increment. Irrespective of the wood used the average percentage removal of the metal ions were found to be: Pb (96.27%), Ni (74.31%) and Cd (85.19%). The percentage removal capacity of white wood was found to be 86.77% and brown wood was 80.11%. There was statistical significant difference between the two sorbents in the removal of each metal ion ($P<0.05$). Lead was found to have the highest amount adsorbed followed by Nickel and least Cadmium. Relative affinity was in the order of Pb<Ni<Cd. Wood sawdust can be used effectively as an adsorbent without chemical treatment or enhancement with organism to achieve the reduction of Pb^{2+}, Ni^{2+} and Cd^{2+} from industrial wastewater as it has been shown that under no inoculation condition over 70% adsorption was recorded for all the metals tested.

TABLE OF CONTENTS

Cover page……i

Fly leaf……ii

Title page……iii

Declaration……..iv

Certification……v

Dedication…….....vi

Acknowledgement……vii

Abstract…….....viii

Table of Contents……x

List of Tables……xv

List of Figures……...xvi

List of Plates……...xvii

List of Appendices……xviii

CHAPTER ONE

1.0 INTRODUCTION……1

1.1 Background of the study……..1

1.2 Statement of Research Problem……...4

1.3 Justification……5

1.4 Aim of the Study……7

1.5 Specific Objectives of the Study……....7

CHAPTER TWO

2.0 LITERATURE REVIEW……8

2.1 Industrial Effluents/Wastes……..8

2.2 Components of Industrial Waste……..8

2.3 Sources of Industrial Wastes……9

2.4 Characteristics of Industrial Effluent……10

2.4.1 Biochemical oxygen demand……10

2.4.2 Dissolved oxygen…… .10

2.4.3 Chemical oxygen demand……..11

2.4.4 Electrical conductivity……...11

2.4.5 pH……...12

2.4.6 Temperature……...12

2.4.7 Phosphorus……12

2.4.8 Nitrogen and Sulphur……13

2.4.9 Oil and grease……13

2.4.10 Color……..14

2.4.11 Turbidity……14

2.4.12 Metals……15

2.5 Impacts of Heavy Metals on Human Health……16

2.6 Impacts of heavy metals on surface and ground water……19

2.7 Processes for heavy metal removal……20

2.7.1 Chemical methods……..21

2.7.2 Physical method……23

2.7.3 Biological method……..27

2.8 Cellulosic Biomasses as potential source of Biosorbents …….34

2.9.1 **Modification of Biosorbents/Biomass …….....35**

2.9.2 Drying……35

2.9.3 Size reduction……36

2.9.4 Autoclaving and vacuum drying……36

2.9.5 Treatment with chemicals……..36

2.9.6 Mechanical disruption……37

2.10 Fate of Adsorbent after Bio-sorption…….37

2.11 Advantages of Metal Uptake by Dead Cells……..38

2.12 Fungi…….39

2.12.1 *Aspergillus niger*……....40

2.12.2 *Penicillium* spp……..40

2.12.3 Metal Ion Accumulation by *Aspergillus niger* and *Penicillium* species…….....41

2.12.1 Advantages of fungal bioosorbent over others.……...…….....43

CHAPTERTHREE

3.0 MATERIALS AND METHODS.....44

3.1 Study Site.....44

3.2 Collection and handling of sample.....45

3.2.1 Sawdust sample.....45

3.2.2 Refinery Effluent Sample.....45

3.2.3 Fungal isolates.....45

3.3 Determination of Proximate and lignocellulosic composition of the sawdust sample.....46
3.3.1 Determination of Moisture content……...47
3.3.2 Determination of ash content……47
3.3.3 Determination of protein content……...47
3.3.4 Determination of lipid content……...48
3.3.5 Determination of carbohydrate content……49
3.3.6 Determination of cellulose content……49
3.3.7 Holocellulose content……49
3.3.8 Hemicellulose content……50
3.3.9 Lignin content.....50
3.4 Determination of Physico-chemical properties of the refinery effluent.....50
3.4.1 pH, Temperature, Electrical conductivity and Total dissolved solids……...51
3.4.2 Dissolved oxygen and Biological oxygen demand……51
3.4.3 Determination of Nitrate content.....51
3.4.4 Determination of Sulphate content.....52
3.4.5 Determination of Phosphate content….....52
3.4.6 Determination of Oil and grease content ……53
3.4.7 Determination of Heavy metal content of the effluent…….53
3.5 Determination of the capacity of sawdust to support the growth of *A. niger*and *Penicillium* sp.....54
3.5.1 Preparation of Fungal Spore Inoculum……..54
3.5.2 Preparation of the Sorbent and Inoculation……...54
3.6 Determination of the Effect of Inoculation on the Capacity of Sawdust to Remove Heavy Metal……...55

3.6.1 Preparation of experimental sorbents…….55

3.6.2 The Sorption process…….55

3.6.3 Determination of sorption performance…….56

3.7 Statistical Analysis……...58

CHAPTER FOUR

4.0 RESULTS…….59

4.1 Percentage proximate composition of sawdust used……59

4.2 Physicochemical properties of the effluent from Kaduna State……59

4.3 Ability of the sawdusts biomass to support the growth of *A. niger* and *Penicillium* species……62

4.4 Efficiency of the treatments in the removal of metal ions……65

4.5 Effect of contact time on the removal of metal ion by the experimental sorbents….68

4.6 Heavy metal ions uptake by both white and brown wood sorbent…….75

4.7 Relative affinity of each metal ion to the sorbents……78

4.8 Sorbents performance comparison……78

CHAPTER FIVE

5.0 DISCUSSION…….....82

CHAPTER SIX

6.0 CONCLUSIONAND RECOMMENDATIONS……89

6.1 Conclusions……..89

6.2 Recommendations……91

REFERENCES.....92

APPENDICES.....111

LIST OF TABLES

Table	Title	Page
4.1	Proximate and lignocellulosic component of the wood saw dust samples obtained from Sabon-gari Zaria	60
4.2	Physicochemical properties of the effluent analysed from Kaduna refinery	61
4.3	Ability of the saw dusts biomass to support the growth of *Aspergillus niger* and *Penicillium* species	.63
4.4	performance comparison of the experimental sorbents	.81

LIST OF FIGURES

Figure Title Page

4.1 Percentage removal of Pb, Ni and Cd ions by white sawdust sorbents after six hour's……66

4.2 Percentage removal of Pb, Ni and Cd ions by brown sawdust sorbents after six hours……...67

4.3 Effect of contact time on lead removal by white sawdust sorbent over six hour's period……..……...69

4.4 Effect of contact time on lead removal by brown sawdust sorbent over six hour's period……..……...70

4.5 Effect of contact time on nickel removal by white sawdust sorbent over six hour's period……..…….....71

4.6 Effect of contact time on nickel removal by brown sawdust sorbent over six hour's period……..……..72

4.7 Effect of contact time on cadmium removal by white sawdust sorbent over six hour's period……..……..73

4.8 Effect of contact time on cadmium removal by brown sawdust sorbent over six hour's period……..……..74

4.9 Uptake of Pb, Ni and Cd ions by white sorbents after six hours ……76

4.10 Uptake of Pb, Ni and Cd ions by brown sorbents after six hours ……77

4.11 Relative affinity of white sawdust sorbent for the metal ions ……..79

4.12 Relative affinity of brown sawdust sorbent for the metal ions ……80

LIST OF PLATES

Plate	Title	Page
4.1	Map of Southern Kaduna refinery showing the study site	44
4.2	*Aspergillus niger* grown on sorbent from white wood	64
4.3	*Penicillium species* grown on sorbent from white wood	64
4.4	A*spergillus. niger* grown on sorbent from brown wood	64
4.5	*Penicillium species* grown on sorbent from brown wood	64

LIST OF APPENDICES

Appendix	Title	Page
I	Sulphate standard curve	111
II	Phosphate standard curve	111
III	Experimental setup used for sorption process	112
IV	Site of sample collection at Kadune Refinery and Petrochemical Company	112
V	Pure culture of *Aspergillus niger* used in biosorption of heavymetals	113
VI	Pure culture of *Penicillium* species used in biosorption of heavymetals	113
VII	Microscopic view of *Aspergillus niger* and *Penicillium* species at x40 magnification	114
VIII	Sawdust samples used in biosorption of heavy metals	114
IX	Statistical analysis of percentage removal of heavy metal ions in white wood	115
X	Statistical analysis of percentage removal of heavy metal ions in brown wood	115

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

It has long been established that, waste effluents from petroleum refineries are heavily contaminated with a wide range of toxic organic compounds (Zhu *et al.*, 2001; Bako *et al.*, 2002; Vanhamme *et al.*, 2003) and heavy metals (Beddri and Ismail, 2007; Marcus and Ekpete, 2014). The literature is also replete with evidence of the harmful impacts of these toxic pollutants on the environment and human health (Varsha *et al*., 2010; Mudhoo *et al*., 2011; Ramzan *etal*., 2011; Silins and Johan 2011; Tokar, *et al*., 2011; Marcus *et al*., 2013; Ho *et al*., 2014). The known toxic pollutant metals include lead, chromium, cadmium, copper, nickel, zinc, arsenic and mercury (Hamza-Izzeldin*et al*., 2013). These metal ions are toxic to humans, aquatic flora and fauna. Lead, cadmium and nickel may be accumulated in the human body causing different diseases such as erythrocyte destruction, muscular cramps, renal degradation, pulmonary fibrosis, skeletal deformity, diarrhea, dermatitis and encephalopathy. They also suppress plant and animal life, damage aquatic life and kill microorganisms (Okerentugba and Ezeronye, 2003; Alao *et al*., 2010).

Many of the conventional treatment technologies such as chemical precipitation, chemical oxidation, solidification, ultrafiltration, flocculation, electrolyte extraction, dilution, sedimentation, evaporation, reverse osmosis, neutralization and membrane separation have been used for removal of metal ions from solution (Yan and Viraraghauan, 2005; Ahluwalia and Goyal, 2007; Amany *et al*., 2015). However the application of such methods is limited because of economical or technical constraints. The methods have also been reported to be inefficient in

the removal of heavy metals from effluent (Bishnoi and Garima, 2004; Iqbal *et al*., 2005; Otukunefor and Obiukwu, 2005; Ezzouhri *etal*., 2009).

For these reasons, the past two decades have witnessed continuous and consistent search for efficient, cost effective and eco-friendly means of treating waste and effluents from petroleum related industries prior to their release into the environment (Kapoor *et al*., 1999; Park *et al*., 2006; Demirbas, 2008; Khashimova *et al*., 2008; Farooq *et al*., 2010). The outcome of such researchestablished adsorption as the most effective and versatile technique for heavy metals removal even at very low concentration (Rao *et al*., 2010; Dhokpande and Kaware, 2013; Mohammed *et al*., 2015). However, the high price of adsorbents (usually activated carbon) is the major problem for industrial applications. Biological methods such as biosorptionor bioaccumulation strategies for the removal of metalsions may provide an attractive alternative to existing technologies(Volesky and Holan, 1995; Preetha and Viuthagiri, 2005; Wuyep *et al*., 2007; Dhankhar and Hooda, 2011).

As compared to conventional methods for removing toxic metals from industrial effluents, the biosorption process offers: 1) low operating cost 2) minimization of the volume of chemical and/or biological sludge to be disposed of 3) high efficiency in detoxification of very diluted effluents 4) little or no nutrient requirements 5) high selectivity 6) ease of operation 7) easy regeneration and recycling ability of adsorbent and 8) possibility of metal recovery (Ouki and Neufeld, 1997; Pavasant *et al*., 2006; Vilar *et al*., 2007;Rao *et al*., 2010; Dhankhar and Hooda, 2011). These advantages have served as the primary incentives for developing full-scale biosorption processes to clean up heavy metal pollution (Kratochvil and Volesky, 1998; Ahluwalia and Goyal, 2007).

Consequently, the focus of studies on heavy metal adsorption became centered on natural/organic materials which are available in vast amount as well as certain waste products from industrial and agricultural operations (Monser and Adhoum, 2010). Example of such materials include sawdust, gridish coal, crushed coconut shell, rice husk, groundnut husk, banana peel, waste tyre rubber, mango peel, wheat bran, sugarcane bagasse, cassava peel (Nasri and Garima, 2004; Singh *et al*., 2006; Memon *et al*., 2007), Also various algae, bacteria, fungi and other biomass have been tested (Ansari and Malik, 2007; Kumar *et al*., 2008; Lesmana *et al*., 2009). Based upon the metal binding capacity of these various biological materials, biosorption are employed to separate heavy metals from wastewater (Vilar *et al*., 2007; Lesmana *et al*., 2009). Of the several biomasses tested, those of fungal origin have been of particular interest and therefore, the subject of most research studies (Sudha and Abraham, 1998; Fernandes and Nazareth, 1999; Tang and Cheng, 2003; Rao and Bhargavi, 2013). Thus, many research efforts were directed at assessing the potential of extremely large pool of readily available and inexpensive biomaterials for use as sorbents for removal of heavy metals from industrial effluents. To date, a good number of biomass types have been tested for their metal binding capabilities under various conditions (Bishnoi and Garima, 2004; Sharma *etal*., 2006; Ahluwalia and Goyal, 2007).

However, the major challenge for the biosorption field remains the selection of the most promising types of biomass from the wide range available (Kratochvil and Volesky, 1998). In practice, the biosorbent derived from biomass is packed in sorption columns which provide an effective device for the continuous removal of heavy metals (Kratochvil and Volesky, 1998). The operation commences by loading the sorbent material whereby a metal bearing effluent is passed through the packed-bed and the heavy metals are taken up from the liquid onto sorbent

surface (Kratochvil and Volesky, 1998; Rajesh and Anju, 2011). The nature of biological surfaces is such that differentfunctional groupssuch as carboxyl, sulfurhydryl, phenolic, hydroxyl and amino groups (Zafar *et al*., 2007; Grassi *et al*., 2012) form complexes with metal ions resulting in chemical complexation as an uptake mechanism (Huang *et al*., 1991). Metal uptake can also be due to physical sorption or bioaccumulation. Fungi are known to be important decomposers and plant symbionts/mycorrhizas (Gadd and Sayer, 2000), It is conceivable that partial decomposition of saw dust by fungi could enhance its capacity to bind and remove heavy metal ions from solution. This enhancement could be brought about by (1) generating additional reactive functional groups on the surfaces of the saw dust particles and (2) providing substrate for the growth and accumulation of fungal biomass which would serve as additional sorbent.

The effort of this research is focused at developing an industrially viable, efficient, cost effective and eco-friendly means of detoxifying waste effluents from heavy metals.

1.2Statement of Research Problem

Industries such as tanneries, textile factories, petroleum refineries and other petrochemical industries in Nigeria have been reported to produce large volumes of effluents that contain a wide range of toxic heavy metals (Emoyan *et al*., 2005; Idise *et al*., 2010), often at much higher levels than those considered dangerous to the environment and human health(Ezeonuegbu *et al*., 2014; Machido *et al*., 2015). According to Emoyan *et al*., (2005), toxic effluents from petroleum refineries are dischargedinto the environment without prior effective treatment. The contamination of soils, surface and underground water bodies (Amar *et al*., 1993; Adewuyi and Olowo, 2012) lend strong support to this assertion and this is a serious cause for concern to both environmental and public health authorities.

Heavy metals pose significant risk to human health and natural ecosystem (Okerentungba and Ezeronye, 2003; Alao *etal*., 2010). They contaminate the food resources, environment and water bodies thereby leading to scarcity of clean water and pollution of soils.They cause a range of diseases in humans such as prenatal mortality, mental retardation in children, asthma, dermatitis, gastrointestinal disorder, kidney and liver damages and bone defects (Dhankhar and Hooda, 2011). They retard plant growth when use for irrigation and cause death of aquatic organisms.

According to WHO (2006), the metals of most concern are mercury, lead, cadmium, nickel, copper and chromium due to their high toxicity. In Zamfara State outbreak of lead poisoning was recorded in six villages in June, 2010 which leads to death of many ducks initially and finally death of children, infertility in men and loss of pregnancies. After investigation, high levels of lead was found in blood of cases

The conventional methods used for the treatment of such wastes have been shown to be inefficient as a result of incomplete metal removal especially at low concentrations (Nasri and Garima 2004; Iqbal *et al*., 2005; Otukunefor and Obiukwu 2005; Ezzouri *et al*., 2009). In addition, these methods are costly (high reagents used and energy requirements) and above all, less eco-friendly due to the release of toxic sludge (Nasri and Garima 2004; Rajesh and Anju, 2011). There is therefore, the need to search for more efficient, cheaper and more environmentally compatible technology by which toxic heavy metal ions could be removed from waste effluents prior to their release into the environment.

1.3 Justification for the Research

Reports of several earlier investigations have indicated the potential of various biomass in the removal of heavy metal ions from dilute solutions as are found in waste effluents from industries

(Kratochvil and Volesky, 1998; Singh *et al*., 2006; Memon *et al*., 2007; Amana *et al*., 2008, Wang and Chen, 2009; Hardiject *et al*., 2010). There is therefore the need to investigate the efficiency of one (saw dust) of the many available biomasses as sorbent for the removal of heavy metal ions from refinery waste effluents.

Utilization of this source of biomass as sorbent in the production of low cost adsorbent would render them from being nuisance (waste) to practically usable material (adsorbent) (Babel and Kurniawan, 2003) "from waste to wealth". Furthermore, the reuse/recycling of sawdust could serve as a viable and eco-friendly alternative for adoption of effective solid waste management "a remediation strategy".

The increasing discharge of waste due to industrialization and urbanization has led to release of approximately 2.4 million tons of liquid effluents containing heavy metals and other toxic materials per annum globally (Burgess and Almeida, 2012). The bioaccumulation nature of metals on living things and its resultant pollution and health hazards has become one of the major environmental problems. Their threat in the environment is multiplied by their toxicity effect even at low concentration (Kang *et al*., 2007; Dhankhar and Hooda, 2011). Hence their removal is necessitated to meet ever-changing legislative standard.

Many fungi are capable of degrading and utilizing cellulose and hemicellulose as carbon and energy source (Cullen and Kersten, 2004). Also *Aspergillus niger* and *Penicillium* species are mostly found in wastewater or water containing organic waste and has been confirmed to resist and grow in presence of Pb, Ni and Cd and are capable of removing the same metals ions from refinery effluents by Ezeonuegbu *et al*., (2014) and Machido *et al*., (2014). It is thought that, the partial decomposition of saw dust by *Aspergillus niger* and *Penicillium* species could enhance its

capacity to bind and remove heavy metal ions from solution. The accumulated fungi mycelia on the surface of the saw dust would serve as an additional sorbent.

Furthermore, fungi are aerobic and can grow under environmentally stressed conditions such as low pH and poor nutrient status.Finally, fungi can easily be transported and produce in large quantity (Obire and Putheti, 2009).

1.4 Aim

This study was aimed at assessing the capacity of sorption columns packed with sawdust inoculated with *Aspergillus niger* and *Penicillium* speciesseparately and as co-cultures in the removal of Pb^{2+}, Ni^{2+} and Cd^{2+} from refinery effluent.

1.5 Objectives

The objectives of the study were to:

1. determine the proximate composition of the saw dustbiomass.
2. determine the physico-chemical properties of the refinery effluents.
3. determine the capacity of the saw dust to support the growth of *Aspergillus niger* and *Penicillium* species.
4. determine the effect of inoculation with *Aspergillus niger* and *Penicillium* species separately and as co-culture on the capacity of sawduststo remove $Pb^{2+,}$ Ni^{2+} and Cd^{2+} from the effluent.
5. compare the removal capacities of the two sawdust biomass used.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Industrial Effluent

Industrial effluents/wastes are liquid waste that has been (partially) treated and they are basicallyone of the major causes of environmental pollution. Petroleum refinery and petrochemical industries are most desirable for national development and improved quality of life; the unwholesome and environmentally unacceptablepollution effects (Nwaichi and Warrigbani, 2013) of the wastefrom these industries are causes for worry. This isbecause in the process of converting crude oil intopetroleum products (liquefied petroleum gas,Naphtha, Kerosene, Diesel oil and residual oil) andpetrochemical products (polypropylene andpolyethylene), wastes of different kinds aregenerated. These wastes are released to theenvironment. Petroleum refinery effluents are complex matrices of organic pollutants discharged from refinery industries containing varying degree of pollutants such as heavy metals and hydrocarbons (Lee *et al*., 1999). Industrial wastewater is one of the important pollution of fresh water environment. Huge amounts of industrial wastewater discharged into rivers, lakes, estuary, and other fresh water bodies results in serious pollution problem in the water bodies and causes negative effects to the eco-system and humans.

2.2 Components of Industrial Effluent

Physically, wastewater is usually characterized by a gray colour, musty odour and a solid content and the suspended solids can lead to the development of sludge deposits and anaerobic conditions when discharged into the receiving environment. Chemically, wastewater is composed of organic (insecticides, pesticides, volatile organic compound, carbohydrates, proteins, fats, oils, phenols, surfactants) and inorganic compounds (heavy metals, nitrogen,

phosphorus, sulfur, chlorides) as well as various gases(Suleimanov, 1995; Dhankhar and Hooda, 2011; Nwaichi, *et al*., 2013). Hydrogen sulfide, methane, ammonia, oxygen, carbon dioxide are common dissolved gases in wastewater. The first three result from the decomposition of organic matter present in the wastewater.

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

2.3 Sources of Industrial Effluents

Industrialization is considered the cornerstone of development strategies due to its significant contribution to the economic growth and human welfare (Odesiri-Eruteyan *et al*., 2017). Unfortunately, the rapid increase in Industrial activities such as mining, painting, car manufacturing, refineries, plastic manufacturing, textile, metal plating, paper and pulp, tanneries, and agricultural activities like intense use of fertilizer and fungicidehas led to increase discharge of varying degrees of pollutants into the environment (Kang *et al*., 2007; Jonathan*et al*., 2008; Odesiri-Eruteyan *et al*., 2017). These industries produce their own kind of effluent with peculiar contaminants such as biological oxygen demand, chemical oxygen demand, solids, chlorinated organic compound, mineral oils, heavy metals, organic/inorganic matter, solids, benzene, toluene and phenol (Ahmed *et al*., 2011).Other industrial activities like photo processing, printing, dry cleaning and car repairing produce their own waste containing pollutants such as silver, copper, zinc, solvent waste ink and dyes (Al-rub,2006; Aksu and Acikel 2007).Wastewater discharges from these industries are the major component of water pollution contributing to oxygen demand

and nutrient loading of the water bodies, promoting toxic algal blooms and leading to a destabilized aquatic ecosystem (Odesiri-Eruteyan *et al*., 2017).

2.4 Characteristics of Industrial Waste/Effluent

2.4.1 Biochemical oxygen demand (BOD)

BOD is the amount of oxygen consumed during oxidation of organic contents of the waste waters. When a biodegradable organic waste is discharged into the receiving water body (stream, river and lake) oxygen dissolved in the water is depleted due to its consumption by microorganism to oxidize the organic matter. (Clair *et al.,* 2003;Walakira, 2011; Odesiri-Eruteyan *et al*., 2017). The more biodegradable a waste is, the more rapid is the rate of its oxidation and the corresponding consumption of oxygen. Because of this relationship and its significance to water quality (dissolved oxygen levels in water), the organic content of waste water is usually measured in terms of the biological oxygen demand. High concentration of the organic matter of wastewater leads to increase in rate of its oxidation and inturn the more the consumption of the dissolved oxygen (Walakira, 2011).

2.4.2 Dissolved oxygen (DO)

The minimum amount of dissolved oxygen necessary for normal life in an aquatic environment is about 2mg/L (Attiogbe *et al.,* 2007), and the discharge of high organic matter containing waste waters into water bodies results in the excess consumption of oxygen by the bacteria. This is in an attempt to oxidise the effluent, thus depleting oxygen from the water faster than it dissolves back into the water from the air (Attiogbe *et al*., 2007). This problem leads to the inadequate maintenance of higher life forms. In addition, oxygen availability is important because the end products of chemical and biochemical reactions in anaerobic systems often produce aesthetically

displeasing colours, tastes and odours in water (Attiogbe *et al*., 2007).The decomposition of the organic materials by micro-organisms in the effluents leads to lowering of the level of dissolved oxygen, which in turn inhibits the growth or cause the death of the aquatic organisms (Onuegbu, 2008).

2.4.3 Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is the measure of the total quantity of oxygen required to oxidize all organic materials of a sample into carbon dioxide and water with a strong chemical oxidant under acidic conditions (Sinyanbola *et al*., 2011). It does not differentiate between biologically available and inert organic matter. COD values are always greater than BOD values, but a COD measurement is concluded within a short period while BOD takes longer time (BOD_5). In a study conducted by Chris-Otubor and Olorunfemi (2015),reported similar high BOD and COD of 13.1mg/L and 75.6 mg/L respectively. Similarly, Siyanbola *et al*. (2011) reported COD range of 615-1254.50mg/L from all effluents analyzed.

2.4.4 Electrical conductivity (EC)

The conductivity of the effluent is a function of the concentration of soluble ionic salts present in the wastewater (Morrison *et al*., 2001) and is also a measure of total dissolved solids which determines the quality of water. If an increase in conductivity or TDS is observed, it suggests dissolution of inorganic ions such as sodium, chloride and calcium ions (Mosley *et al*., 2004). Release of waste water with high levels of these ions renders the receiving water unhealthy for drinking and other domestic activities, reduces crop yield when use for irrigation and unattractive for recreational activity (Nadia, 2006). Electrical conductivity can serve an indicator of water quality problems.

2.4.5 pH

pH can be defined as the negative of the logarithm to the base 10 of the hydrogen ion concentration. The intensity of acidity or alkalinity of a sample is measured on a pH scale which actually indicates the concentration of hydrogen ion present. pH levels could affect the solubility of essential elements such as aluminum, iron, boron and manganese (Akan*et al*., 2009) therefore the availability of these substances to aquatic organisms is affected. High algal concentration of water varies with the pH. pH as high as 10 is obtained when an alga uses carbon dioxide during the day for photosynthesis and the pH drops when the alga respires and produces carbon dioxide (Salequzzaman *et al*., 2008). pH changes disrupt the ecological balance of the aquatic system and excessive acidity can result in the release of hydrogen sulfide. Practically all phases of water supply and wastewater treatment are pH dependent. Highly acidic or basic wastewater would kill aquatic life and also enhance toxicity of heavy metals.

2.4.6 Temperature

The temperature of the ready to discharge effluent is an important parameter for aquatic habitat present in the receiving water. At high temperature, the rate of dissolution of atmospheric oxygen in the water is usually low and this affects the sustainability of the aquatic habitats due to reduction in the level of the dissolved oxygen (Ezenuegbu *et al*., 2014). When the temperature of a ready to discharge effluent is high it affects the isolation, growth, and reproduction of fungi since most fungi are mesophilic in nature (Margesin and Schinner, 1997).

2.4.7 Phosphorus

Phosphorus is normally found as phosphate in nature and it serves as nutrients for both plants and animal in aquatic environments. Pure phosphorus (white) can therefore be discharged into

surface waters when it is used in industries to make other chemicals and this can be a serious danger to health as it is very poisonous (Mosley *et al*., 2004). Apart from its poisonous nature, addition of phosphorus may cause aquatic biological productivity to increase, resulting in low level of dissolved oxygen and eutrophication of lakes and rivers (Perrry *et al*., 2007).Also phosphorus addition leads to increase in COD and BOD level.

2.4.8 Nitrogen and sulphur

The nitrogen and sulphur components of the effluent are highly toxic and are represented in the form of ammonia and hydrogen sulphide (H_2S), respectively (Altas and Büyükgüngör, 2008). In aqueous form, H_2S exists in equilibrium with bisulphide (HS-) and sulphide (S_2-), and the latter sulphide is the most reduced form (Altas and Büyükgüngör, 2008). Sulphide has a high oxygen demand of 2molO_2/l mol S_2- thus contributing significantly to oxygen depletion (Poulton *et al*., 2002). This results in mass fish mortality when the threshold limit exceeds 0.5 mg/L for freshwater or saltwater fish (Altas and Büyükgüngör, 2008). Low oxygen level also leads to death of aerobic organisms and anaerobic degradation of the components of the effluent would results. The end product of anaerobic degradation leads to a general decline in the ecological and aesthetic qualities of the water body (Attiogbe *et al*., 2007; Walakira, 2011).

2.4.9 Oil and grease

Oil and grease forms a surface film on the river and can coat plants and animals reducing oxygenation from the atmosphere above. The film of oil affects the transmission of light through the water thereby disturbing the process of photosynthesis in the aquatic plants. In animals, oil coating can destroy the insulating properties of fur and feathers. Oils render the receiving streams unattractive for domestic uses, irrigation, shoreline deposits leading to environmental degradation and interfere with self-purification of the stream (Chris-Otubor, 2015). Oil bio-

accumulate in higher animals and further enters the food chains.Oil and grease are sticky in nature; they tend to aggregate, clogging drain pipes and sewer lines, causing unpleasant odours and corroding sewer lines under anaerobic conditions (Xu and Zhu, 2004). They also interfere with unit operations in municipal wastewater treatment plants because they float as a layer on top of the water. They also stick onto pipes and walls consequently blocking strainers and filters (Xu and Zhu, 2004).

2.4.10 Colour

Coloured effluents are discharge from industries such as pulp and paper mills, tanneries, textile dye-houses and palm oil mills. This coloured effluents/wastewater may have resulted from the presence of natural metallic ions, plankton, weeds, substances like dyes from dying units and other industrial wastes (Kanu and Achi, 2011). When these coloured effluents are discharged into water bodies it makes the water unsuitable for both general and industrial application (Agoyi, 2002; Odesiri-Eruteyan *et al*., 2017).

2.4.11 Turbidity

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through samples (Walakira, 2011). Turbid effluent reduces visibility of aquatic animals and also prevents the penetration of sunlight when discharged into water bodies (Walakira, 2011; Odesiri-Eruteyan *et al*., 2017). Turbidity is due to the presence of suspended matter such as silt, clay, plankton and other microscopic organisms causing cloudiness. The higher the intensity of scattered light the higher the turbidity (Walakira, 2011; Odesiri-Eruteyan *et al*., 2017). High turbidity level affects fish feeding and growth. High turbidity also supports high number of foreign microbiota in the water body, accelerating microbial pollution.

2.4.12 Metals

Metals are elements, compounds or an alloy that is usually hard, opaque, shiny and has a good thermal conductivity. They are generally malleable, fusible and ductile. Metals can be categorized as essential, toxic heavy, radionuclides, and semi metals (Chan and Wang, 2007). They are discharged in effluents from electroplating, refinery, tannery, textile, plastic manufacturing industries.

1. Essential metals: include zinc, nickel, molebdenum, potassium and magnesium. They are important for metabolism because they form integral part of metabolic and biochemical process, serve as catalyst for cellular function, regulate osmotic pressure and provide vital cofactors for metalloproteins and enzymes (Bruins *et al*., 2000).
2. Radionuclides are isotopes of unstable nucleus characterized by number of protons and neutrons in the nucleus. They are important in nuclear medicine procedures but destructive to cells (Chen and Wang, 2007). Examples are uranium, thallium and radium.
3. Semi metals are metalloids that share some properties of metals and that of non-metals depending on the element it is reacting with. For instance, boron when reacting with sodium acts as non-metal and acts a metal when reacting with fluorine. Other examples are selenium, arsenic, and silicon.
4. Toxic heavy metals include lead, cadmium, chromium, mercury and other essential metals that have no biological function in the body and are highly toxic to humans (arsenic, silver, nickel). The term “heavy metal” refers to any chemical element that has a relatively high density and is toxic or poisonous even at low concentrations; they cannot be degraded nor destroyed (Oladoye and Adewuyi, 2014). Among these

toxic heavy metals, lead, cadmium and mercury are referred to as "big three" by Volesky and Holan (1995) and Volesky (1994) and are in the lime light due to their major impact on the environment. According to WHO (2006) among the most toxic heavy metals are lead, nickel and cadmium, mercury and chromium and are referred to as "priority pollutants". Heavy metals are dangerous because they tend to bio accumulate, building up in the fatty tissues of animals in a food chain. Bioaccumulation means increase in concentration of a chemical in a biological organism over time, compared to the concentration in the environment (Ezenuegbu *et al*., 2014).

2.5 Impacts of Heavy Metals on Human Health

Heavy metals are emitted into the environment both in elemental and compound (organic and inorganic) forms by both natural and anthropogenic means and anthropogenic source is the major cause of emission specifically in mining operations (Oladoye and Adewuyi *et al*, 2006). Contamination of soils, groundwater, sediments, surface water and air with hazardous and toxic chemicals poses significant problems for both human health and the environment (Ansari and Malik, 2007). Dietary intake of heavy metals even at low concentrations can be very harmful because they bio-accumulate. The harmful effects of heavy metals to the body when consumed above the bio-recommended limits are referred to as biotoxic effects of heavy metals.

In view of the human health implications, each metal exerts different effects and symptoms. For instance, Minor zinc exposure causes irritability, muscular stiffness, loss of appetite and nausea are common (Bhattacharya *et al*., 2006) and its deficiency causes anaemia and retardation of growth and development. Also high levels of zinc in the body cause depression, lethargy, neurological signs and increased thirst(Babel and Kurniawan, 2003; Barakat, 2011). Meanwhile

zinc is considered to be relatively non-toxic; however, excess amount can cause system dysfunctions that result in impairment of growth and reproduction (INECAR, 2000;Oladoye and Adewuyi, 2014). Zinc is a 'masculine' element that balances copper in the body and is essential for male reproductive activity (Oladoye and Adewuyi, 2014).

Chromium (Martins *et al*., 2006), on the other hand, can cause cancer in the digestive tract and lungs (Kiran *et al*., 2007). Low-level exposure to chromium can irritate the skin and cause ulceration. Long-term can cause kidney and liver damage and damage to circulatory and nerve tissue. Chromium has been found to often accumulate in aquatic life, increasing the chances of being exposed to high levels of chromium through fish consumption.

Manganese (Mn) triggers neurotoxicity, low haemoglobin levels and gastrointestinal accumulation (Parvathi *et al*., 2007).

Copper consumption in high doses brings about serious toxicological concerns since it can be deposited in the brain, skin, liver, pancreas, and myocardium (Vijayaraghavan and Velan, 2006) and initiates intestinal distress, liver and kidney malfunctioning, nausea, vomiting and anemia (Al-Rub *et al*., 2006).

Lead (Pb) has been cited as one of the three most toxic heavy metals that have latent long-term negative impacts on health. Lead is known to be one of the most poisonous environmental contaminants. It can enter human body through inhalation, ingestion or skin contact and may accumulate in bones, brain, kidney and muscles causing severe damage to kidney, nervous and reproductive system (Oladoye and Adewuyi, 2014). A notably serious effect of lead toxicity is its tetratogenic effect. Lead causes anemia, encephalopathy, hepatitis and nephritic syndrome (Deng *et al*., 2006) and sometimes even death. Lead affects children by leading to the poor development

of the grey matter of the brain, thereby resulting in poor intelligence quotient (IQ) (Deng *et al*., 2006; Oladoye and Adewuyi, 2014; Chris-Otubor *et al*., 2015) Owing to the hazardous effects of Pb(II) it is essential to check waste streams containing Pb(II) before being discharged into the water resources. The maximum permissible limit assigned by World Health Organization (WHO) for Pb(II) in drinking water is 0.05 mg/L (Seolatto *et al*., 2012; Teoh *et al*., 2013).

Cadmium is toxic at extremely low levels and also exposes human health to severe risks, as it can provoke cancer, kidney damage, mucous membrane destruction, and itai-itai disease, as well as affect the production of progesterone and testosterone (Johannes *et al*., 2006). Cadmium is also associated with bone defects; osteomalacia, osteoporosis and spontaneous fractures, increase blood pressure and myocardic dysfunctions. Depending on severity of exposure, the symptoms of effects include nausea, vomiting, abdominal cramps, dyspnea and muscular weakness. Severe exposure may result in pulmonary odema and death (European Union, 2002; Johannes, 2006; Oladoye and Adewuyi, 2014). High exposure to cadmium can lead to obstructive lung disease, cadmium pneumonitis. It is characterized by chest pain, cough with foamy and bloody sputum and death of the lining of the lung tissues because of excessive accumulation of watery fluids while long term exposure will result to renal dysfunction characterized by tabular proteinuria (Oladoye and Adewuyi, 2014).

Though nickel is essential as it serves as micro-nutrients and used for redox processes to stabilize molecules and regulation of osmotic pressure(Subudhi and Kar, 2008)but their presence exceeding its critical level might bring about serious lungs and kidney problems aside from gastrointestinal distress, pulmonary fibrosis, prenatal mortality, nasal sinus, cancer of lungs and skin dermatitis (Borba *etal*., 2006).

Mercury has been identified for its nervous system deterioration, including protoplasm poisoning (Alluri *et al*., 2007). Mercury is toxic and has no known function in human biochemistry and physiology. Inorganic forms of mercury cause spontaneous abortion, congenital malformation and gastro-intestinal disorders like corrosive esophagitis. Organic forms of mercury (monomethyle and dimethyle mercury) present erethism (an abnormal irritation or sensitivity of an organ or body part to stimulation), gingivitis, neurological disorders, stomatitis, acrodynia (pink disease, characterized by rash and desquamation of the hands and feet) (Ferner, 2001).

Arsenic acts to coagulate protein, forms complexes with coenzymes and inhibits the production of adenosine triphosphate (ATP) during respiration (Institute of Environmental Conservation and Research, 2000). Its toxicity presents a disorder which is often confused with Guillain-Barre syndrome, an anti-immune disorder that occurs when the body's immune system mistakenly attacks part of the Peripheral Nervous System, resulting in nerve inflammation that causes muscle weakness (Kantor, 2006). They also cause cardiovascular, gastrointestinal, haematological and reproductive malfunctioning (Dhankhar and Hooda, 2011). Death can result from exposure to high level of Arsenic (Oguwuegbu and Ijeoma, 2003; United States Department of Labour, 2004).

2.6 Impacts of Heavy Metals on Surface and Ground Water

The quality of water both surface and ground water is of paramount concern due to the place and importance of water to life. All organisms contain it, some live in it, some consume it. Water is used for many purposes such as irrigation, domestic activities, industrial use and recreational functions. Plants and animals require water that is moderately pure and they cannot survive if their water is loaded with toxic chemicals or harmful micro-organisms (Odesiri-Eruteyan *et al*., 2017). As a result of ineffectiveness of purification systems, waste water may become seriously

dangerous,leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem (Nwaichi *et al*., 2013). Heavy metals are considered to be one of the most hazardous water contaminants; their presence in the wastewater has brought about more environmental concerns due to their toxicity even at low concentrations (Al-Rub, 2006; Kang *et al*., 2007). Generally, metals are emitted during their mining and processing activities (Lenntech Water Treatment and Air Purification, 2004). These metals leached out and in slopy areas, are carried by acid water downstream or carried into water bodies by run-off water. Through river or streams, the metals are transported as either dissolved species in water or as in integral part of suspended sediments. They may be stored in river bed sediments or seep into the underground water thereby contaminating water from underground sources like wells. The degree of contamination depends on nearness of the well to the industry. Wells near mining sites have been reported to contain heavy metals above permissible drinking water criteria (Peplow, 1999).

2.7 Processes for Heavy Metal Removal

Various treatment technologies have been developed for the purification of water and wastewater contaminated by heavy metals. The most commonly used methods for the removal of metal ions from industrial effluents (Mohan and Singh, 2002; Yan and Viraraghauan, 2005; Ahluwalia and Goyal, 2007; Dhankhar and Hooda, 2011) include: chemical precipitation, ion exchange, solvent extraction, chemical oxidation, chemical reduction, cementation, neutralization, coagulation, flocculation, sedimentation, evaporation,electro-dialysis, reverse osmosis, membrane separation, dilution, filtration, flotation, air stripping, steam stripping, adsorption and soil flushing/washing chelation. All these methods can be classified under chemical and physical methods of

wastewater treatment. They have their own setbacks because of high cost and disposal problems.Biological processes such as bio-sorption and phyto-remediation have also been used to treat heavy metal contamination.

2.7.1 Chemical Methods

1. *Chemical precipitation*

The most widely used method to remove heavy metals from waste water is chemical precipitation that involves the formation of insoluble compounds of coagulants which are then filtered out. Coagulants such as alum, lime, iron salts are used to precipitate metals. Chemical precipitation can be hydroxide precipitation which involves the use of calcium or sodium hydroxide to precipitate metals or sulphide precipitation and carbonate precipitation which uses sodium and calcium carbonates to precipitate metals. Chemical precipitation is cheap and easy, but the process results in development of large amount of chemical sludge. This requires to be disposed of after further treatment.Other disadvantages include slow metal precipitation, poor settling and long-term environmental impacts of sludge disposal (Aziz *et al*., 2008) and ineffective when metal ion concentration is as low as 1-100mg/L (Lodeiro *et al*., 2006). These disadvantages make this method unsuitable for use (Babak *et al*., 2012).

2. *Ion Exchange*

This method of waste water treatment is like a displacement reaction that involves the use of an ion exchange resin where metal ions from dilute solutions are exchanged with ions held by electrostatic forces. It is a non-specific electrostatic mechanism of metal binding. Commonly used matrices for ion exchange are synthetic organic ion exchanger. This method is expensive especially when treating large amount of wastewater containing heavy metal in low

concentration (Dhankhar and Hooda, 2011) and cannot handle concentrated metal solutions as the matrix gets easily fouled by organics and other solids in the waste water. Moreover ion exchange is non-selective and is highly sensitive to pH of the solution (Lin *et al*., 2008).

3. *Chemical reduction*

This process involves reduction of toxic heavy metals into less toxic form. For example hexavalent chromium can be reduced to trivalent chromium using electro-chemical units (Wang and Chen, 2009).

4.*Coagulation/ flocculation*

Coagulation is the destabilization of colloids by neutralizing the forces that keep them apart. Many coagulants are widely used in the conventional wastewater treatment processes such as alum, ferrous sulfate and ferric chloride. These are mixed rapidly then stirred to encourage formation of floc prior to settling.This results in the effective removal of wastewater particulates and impurities by charge neutralization of particles and by enmeshment of the impurities on the formed amorphous metal hydroxide precipitates (Jiang and jia, 2015). The coagulants role here is to destabilize the colloidal suspension by reducing the attractive forces, thereby lowering the energy barrier and enabling particles to aggregate. Coagulation is one of the most important methods for wastewater treatment, but the main objects of coagulation are only the hydrophobic colloids and suspended particles. In order to remove both soluble heavy metal and insoluble substances efficiently by coagulation, sodium xanthogenate group was grafted to polyethyleneimine. A number of coagulation mechanisms including charge neutralization, double layer compression, bridging and sweep, have been postulated dependent on the physical and chemical properties of the solution, pollutant and coagulant.

Flocculation is the agglomeration of the destabilized particles by chemical joining and bridging. Once suspended particles are flocculated into larger particles, they can usually be removed or separated by filtration, straining or floatation. Today many kinds of flocculants, such as polyferric sulfate (PFS) and polyacrylamide (PAM), are widely used in the treatment of wastewater (Chekli *et al*., 2017). However, it is nearly impracticable to remove heavy metals very well from wastewater directly by these current flocculants. Generally, coagulation/flocculation cannot treat the heavy metal wastewater completely (Tripathy and De, 2006). Therefore, coagulation/flocculation must be followed by other treatment techniques (Jiang and jia, 2015; Chekli *et al*., 2017).

5.*Cementation*

Cementation is used as a general term to describe the process whereby a metal is precipitated from a solution of its salt by another electropositive metal by spontaneous electrochemical reduction to its elemental metallic state, with consequent oxidation of a sacrificial metal for the recovery of more noble dissolved metal species present in aqueous solutions (Nassef and El-Taweel, 2015). The main disadvantage of cementation is excess sacrificial metal consumption.

2.7.2 Physical Methods

1. *Ultra filtration*

Ultrafiltration involves the use of selective membrane with specific electrodes to concentrate ions. Ultra filtration includes the reverse osmosis and electro dialysis and it is the commonly used physical method of heavy metal removal from waste water. In reverse osmosis pressure difference is employed to begin the transport of solvent across a semi permeable membrane while in electro dialysis current applied to electrode initiates ion migration through a selective

permeable membrane (Volesky, 2001). The shortcomings of this method are the little efficacy and formation of secondary waste that needs further processing (Ahluwalia and Goyal, 2007).

2. *Reverse osmosis*

Reverse osmosis is a process in which heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by dissolved solids in waste water. The disadvantage of this method is that it is costly (Volesky, 2001).

3. *Electro-dialysis*

Electro-dialysis involves the removal of heavy metals through a semi-permeable ion selective membrane. Application of an electrical potential between the two electrodes causes a migration of cations and anions towards respective electrodes. Due to the alternate spacing of cation and anion permeable membranes, cells of concentrated and dilute salts are formed. The setback here lies in the limited lifetime before membrane fouling occurs and also in the formation of metal hydroxide which clog the membrane and energy consumption. The process is relatively expensive (Mohammedi *et al*., 2005).

4. *Adsorption*

It is a bulk transfer process which involves the gathering/amassing/retention/accumulation of substances at the interface of two phases, which could be solid-liquid, gas-liquid and gas-solid interface. The substance being adsorbed/ gathered is known as the sorbate and adsorbing material is called the adsorbent. The properties of adsorbents are specific and their capability to trap any sorbate from wastewater depends on their constituents (Grassi *et al*., 2012). Adsorption becomes one of the most popular methods for the removal of pollutants from effluents since proper design of the adsorption process would produce high quality treated effluents. This process provides an

attractive alternative for treatment of contaminated water, especially if the adsorbent is inexpensive and does not require an additional pre-treatment step before application (Satya, 2012).The importance of adsorption in environmental pollution control has been well highlighted in literature. Khashimova *et al*. (2008) reported that the use of adsorption for separation of pollutants from mixtures has been increasing continuously. About nine thousand and fifty eight (9058) articles on adsorption have been published on adsorption materials. Among these are Raji et al., (1997); Shukla *et al*. (2002); Agarwal *et al*. (2006);Oke *et al*. (2008), Solimon *et al*. (2011),and Omeiza (2011).

Coconut copra meal is a by-product of coconut oil production,and is characterized by the presence of functional groups such asalcohols and carboxylic acids, on its surface. This material has beenemployed as a biosorbent for cadmium removal from wastewaterHo and Ofomaja, (2006). The initial pH of the solution and cadmium concentration, inparticular, significantly affects cadmium sorption onto copra meal.

Wang *et al*. (2006)conceived the possibility ofusing rice bran as an alternative adsorbent for zinc removal fromsynthetic wastewater. They also studied the influence of parameterssuch as pH, temperature, particle size and adsorption time onsorption capacity. The results underscore the importance of pH onzinc sorption capacity, while to a certain extent; the capacity alsorelies upon sorbent size.

The use of *Saccharomuces cerevisiae* in adsorption was compiled by Wang and Chen, (2006). The result revealed the use of this yeast depends largely on pH, initial metal ion concentration, presence of other ligands in solution and biomass concentration. Sawdust been cheap and easily available has been used either as such or treated with chemical to remove metals from

wastewater and the study was focused on the adsorption mechanisms, influencing factors and favorable conditions for different sawdust materials (Shukla *et al*., 2002).

Researchers have also investigated A*zadirachta indica* (neem) leaf as an adsorbent to remove lead, cadmium and chromium ions while varying the pH, agitation time, adsorbent dose and temperature. The result revealed low neem leaf (1.6g/dm^3) could remove 87% of chromium during a 300 minutes period of time. The optimum pH range was found to be 4.5-7.5 (Babu and Gupta, 2008).

Tamarindus indica was also worked on byAgarwal *et al*. (2006) to remove Cr (VI). He concluded that the removal of this metal significantly reduced at increased pH and enhanced with rising temperature.

Principle of adsorption

All adsorption processes are dependent on solid-liquid equilibria and on mass transfer rates (Subramani and Sindhu, 2012). Most adsorbents are highly porous materials, and adsorption takes place primarily on the walls of the pores or at a specific inside the particle. The overall rate of adsorption is controlled by the rate of diffusion of solute within the capillary pores of the adsorbent and varies with square root of the contact time with the adsorbent (Krachovil *et al*., 1998). The adsorption operation can be batch, semi-batch or continuous (Dhankhar and Hoode, 2011).

Types of adsorption process

There are two mechanisms of bio-sorption, namely:

Physicsorption occurs when the attraction of adsorbent and the adsorbed molecules are brought about by weak van der waals forces (physical nature). The process is reversible and occurs lower

or close to the critical temperature of the adsorbed material. Here there is no need of activation energy. The decrease of b value with temperature rise signifies the exothermicity of the process (Shaker, 2007; Mohammed *et al*., 2015)

Chemisorption on the other hand is brought about by strong chemical bonding between the solid and the adsorbed substance and it occurs at the monolayer of the adsorbed molecules. It is also called activated adsorption and it is irreversible. In chemical adsorption, the bonding between heavy metals and active sites of the biosorbent becomes stronger at high temperature in contrast to physical adsorption which weakens. Under favorable conditions both mechanisms can occur simultaneously or alternatively (Mohammed *et al*., 2015).

2.7.3 Biological Methods

1. *Phyto-remediation method*

This method involves the use of growing plants to reduce heavy metal pollution and other hazardous chemicals in soil, air groundwater or wastewater. It refers to the natural ability of certain plants called hyperaccumulators to bioaccumulte, degrade or render harmless contaminants in the environments (Reichenauer and Germida, 2008). There are various ways in which plants can be used to treat contamination by heavy metals namely: phyto-stabilization, phyto-extraction and rhizo-filtration. This method has advantage of relatively low cost and wide public acceptance (Schnoor, 1997) but has disadvantage of taking longer to accomplish than other treatment.

Phyto-stabilization: Also known as phyto-sequestration can involve absorption by roots, adsorption to the root surfaces or the production of biochemical by plants that are released into

the soil or wastewater around the roots and can sequester, precipitate or immobilize nearby contaminants (Mendez and Maier, 2008).

Phyto-extraction involves the take-up of contaminants by plants through their roots and store them in the tissues of the stem or leaves. The contaminants are not necessarily degraded but are removed from the environment when the plants are harvested (Reichenauer. and Germida, 2008).

Rhizo-filtration: this process involves filtering of toxic substances through a mass of roots. Here the pollutants remain absorbed in or adsorbed to the roots (Reichenauer. and Germida, 2008).

2.*Bio-sorption*

Biosorption is process that involves binding of selected metal ions/pollutants on to a certain biological material and can be grouped into two (Lesmana *et al*., 2009; Dhankhar and Hooda, 2011; Mohammed *et al*., 2015) namely: Metabolism dependent bio-sorption (bioaccumulation) which occurs when heavy metals are transported across the cell membrane in a living cell (algae, fungi and bacteria) that yields intracellular accumulation that is, metabolically mediated metal uptake. The disadvantage here is that it requires large area and is constrained by sensitivity towards diurnal variation (Dhankhar and Hooda, 2011).

Non-metabolism dependent, here the metal uptake is by physico-chemical interaction between the metals and the functional groups present on the microbial cell surface. This process includes mechanism such as physical adsorption, ion exchange, precipitation and surface complexation, which is not dependent on the cell's metabolism (Gadd, 2009). In general non-metabolism dependent biosorption, can be defined as the passive sequestering of metal ions by inexpensive metabolically inactive biomass (dead biomass) from waste streams by purely physico-chemical

pathways (Mohammed *et al*., 2015). It is considered to be relatively rapid and reversible process (Febrianto *et al*., 2009; Naja and Volesky, 2011).

Principle of bio-sorption

Bio-sorbents are prepared from the naturally abundant and/or waste biomass of algae, moss, fungi, or bacteria which is inactivated and usually pretreated by washing with acids and/or bases before final drying and granulation (Fourest and Roux, 1994; Kratochvil and Volesky, 1998). While simple cutting and/or grinding of the dry biomass may yield stable biosorbent particles (Kratochvil and Volesky, 1997; Kratochvil and Volesky, 1998), some types of biomass have to be to either immobilized in a synthetic polymer matrix (Greene and Bedell, 1990; Jeffers and Corwin, 1993) and/or grafted on an inorganic support material such as silica (Bedell and Darnall, 1990) in order to yield particles with the required mechanical properties.

The bio-sorbent particles can then be packed in sorption columns which are perhaps the most effective device for the continuous removal of heavy metals. Biosorption columns operate on cycles which consist of loading, regeneration, and rinsing (Kratochvil and Volesky, 1997). When the metal sorption capacity of the bio-sorbent is exhausted the column is taken out of operation. Its bed can then be regenerated with solutions of acids and/or hydroxides. The regeneration produces small volumes of heavy metal concentrates suitable for conventional metal recovery processes (Kratochvil and Volesky, 1998). The cycle ends with rinsing and/or backwashing of the bed with water to remove the remains of the regenerants and suspended solids captured in the column. In order to make the bio-sorption process truly continuous, pairs of columns are employed in parallel so that during the regeneration and rinsing of one of the columns the other is being loaded with heavy metals.

Evaluation of equilibrium binding of metals in bio-sorption

Bio-sorption process involves a solid phase (sorbent) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, e.g. metal ions). Due to the higher 'affinity' of the sorbent for the sorbate species the latter is attracted into the solid and bound there by different mechanisms. This process takes place until equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in solution (at a residual, final or equilibrium concentration C_f). The degree of the sorbent 'affinity' for the sorbate determines its distribution between the solid and liquid phases. The quality of the sorbent material is judged according to how much sorbate it can attract and retain in an 'immobilized' form. The determination of the metal uptake (q) by the biosorbent is most often based on the material balance of the sorption system: sorbate which 'disappeared' from the solution must be in the solid. The sorption uptake qcan be expressed in different units depending on the purpose of the exercise: for examplemgof metal sorbed per gramof the (dry) sorbent material (basis for engineering process mass balance calculations), mmol/g or mequiv/g(used when stoichiometry and/or mechanism is considered).

Advantages of bio-sorption process(Amuda *et al*., 2007)

- **i.** Efficiency: Sorption process is considered very effective as it is capable of removing heavy metals even at low concentration.
- **ii.** Cheap: the cost of adsorbent is low since they are often made from locally, abundantly and easily available materials and most times are by-products of industrial operation.
- **iii.** Regenerative: sorbent material can be reused after regeneration.

iv. Metal Selective: the metal adsorbing performance of different types of biomass can be more or less selective on different metals.

v. No Sludge Development: unlike other methods of wastewater treatment, there is no chemical sludge to be disposed of in adsorption.

vi. Metal Recovery: adsorption entails desorption process which separates the sorbate (metal) from the adsorbent material.

Applications of bio-sorption

Remediation/Purification by adsorption is perhaps the most researched upon and growing known application. Wine clarification is still accomplished by the use of adsorbents and exhausts of industrial plants can be ripped off undesired components by adsorption. Adsorption can be applied to both gaseous and liquid separations (Wilkins and Yang, 1996).

i. Recovery of valuable solvent vapours from dilute mixtures of air or other gases

ii. Removal of moisture dissolved in gasoline.

iii. Removal of toxic gases and objectionable odours from industrial gases for air pollution control and deodorization of air.

iv. Decolourization of petroleum products and aqueous sugar solutions

Factors influencing efficiency of bio-sorption Process

The efficiency of biosorption process is limited by a number of factors such as:

A. Dose of the adsorbent: The sorption of heavy metals onto any biomass is attributed to their chemical constituents, which are mainly proteins, carbohydrates and phenolic compounds, since they contain functional groups such as carboxyl, hydroxyls, sulfhydryl, amides and amines, to which metal ions are attached (Choi and Yun, 2006; Grassi *et al*.,

2012). Therefore,increase in biomass concentration leads to increase in amount of solute biosorbed, because of increased surface area of biosorbent, which inturn increases the number of binding sites (Naja *et al*., 2005; Dhankhar and Hooda, 2011).

B. Composition of the effluent (interfering substances): A majority of industrial effluents contain more than one toxic heavy metal. Consequently, biosorption in columns involves competitive ion exchange whereby several toxic heavy metals compete for a limited number of binding sites on a biosorbent. From theory and practice of ion exchange the species which first breaks through the column of biosorbent bed is the one with the lowest affinity towards the resin. While the cations of Fe^{2+} can potentially compete with cations of toxic metals for the binding sites in the biomass, Fe (III) is usually present in the form of suspended solids (SS). Presence of inorganic elements such as Ca^{2+}, Mg^{2+} also has an important effect on adsorption of heavy metals from wastewater(Reya *et al*., 2012). Calcium ion complexes with the carbon, which increases the adsorption of Ca^{2+}. Many other divalent cations (Cu^{2+}, Zn^{2+}, Ag^{2+}) act in similar way but calcium ion has special affinity because of its ubiquitous distribution in natural water. On the other hand light metals (alkali and alkali earth metal) bind less to biomass than heavy metals and this favors biosorption of heavy metals when present together in an effluent with heavy metals (Reya *et al*., 2012).

C. pH: the number of adsorption sites on carbon is pH dependent. pH of wastewater can change the charge on heavy metals in turn affects adsorption, q_e value increases as pH value decrease. As pH reduces, the number of negative sites reduces which prevents the access of positively charges metal ions from binding (Reya *et al*., 2012). As the pH becomes more acidic, metal cations have to compete for available negatively charged

sites. The effect of pH on sorption of metal ion is principally the result of changes in the net proton charge on particles. As pH increase the amount of hydrogen ion decrease and the electrostatic attraction of the sorbent for a metal ion are enhanced. Extreme pH values damages the structure of the biosorbent. For example (Ozer and Ozer, 2003) exploredshowed the optimum pH, range for biosorption of different metals by *S. cerevisae*. They found that the optimum ph value for lead and nickel ion is 5.0. Optimum pH range for effective biosorption is 3-6 (Volesky and Naja, 2007).

D. Temperature: temperature affects the stability of metal ions in solution or stability of complexes formed with metal. Nevertheless biosorption of some metals such as Uranium and copper may take place without restrictions in a wide temperature range (Ahalya *et al*., 2003). Higher temperatures usually enhance sorption as a result of increased surface activity and kinetic energy of the solute but physical damage to the biosorbent material can be seen with high temperatures and this would lead to low sorption capacity on the biosorbent (Mameri *etal*., 1999). Adsorption reactions are normally exothermic; thus the extent of adsorption generally increases with decreasing temperature (Grassi *et al*., 2012). Low temperature, however, affects living cell systems. However temperature between 20^{o}C and 35^{o}C has less effect on bio-sorption performance (Aksu, 1992; Veglio and Beolchini, 1997).

E. Nature and initial concentration of the sorbate (targeted metals) in the liquid phase (effluent): initial metal concentration is a significant factor that influences adsorption onto an adsorbent. Literature has it that themore the initial concentration, the longer the contact time that would be required for the removal of the metal contaminants. However, this does not imply that equal initial concentration for different metals means they will be

equally removed from the solution (Ngoh, 2006). More so, the solubility of the solute greatly influences the sorption equilibrium. In general, an inverse relationship can be expected between the extent of adsorption of a solute and its solubility in the solvent where the adsorption takes place. Molecular size is also relevant as it relates to the rate of uptake of organic solutes through the pores of the adsorbent material if the rate is controlled by intra-particle transport. In this case the reaction will generally proceed more rapidly with decrease of sorbate molecule.

F. Residence time: generally the biosorption capacity and the metal removal efficiency of sorption process become higher at prolonged contact time. However it is important to optimize the contact time to enable establish maximum time for removal of any pollutant in real practice (Dhankhar and Hooda, 2011).

2.8 Cellulosic Biomasses as potential source of Biosorbents.

Agricultural by-products are reported to be composed mostly of lignin, hemicellulose, cellulose and simple sugars as well as other polar functional group containing compounds which include alcohols, aldehydes, ketones, carboxyl, sulfhydryl, phosphates, ethers and phenols (Ofomaja and Ho, 2007; Naja and Volesky, 2011; Mohammed *et al*., 2015). In addition, cellulosic materials tend to be organized with systems of interconnecting pores thus providing a high surface area per unit mass (Bai and Abraham, 2001). These properties confer on cellulosic biomasses the ability to bind heavy metals ions through replacement of hydrogen ions with metal ions in solution or by donation of an electron pair from these groups to form complexes with metal ions in solution (Rishipal, 2002;Choi and Yun, 2006;Sud *et al*., 2008; Soliman *et al*. 2011**).**Thus, many research efforts were directed at assessing the potential of extremely large pool of readily available and inexpensive biomaterials for use as sorbents for removal of heavy metal ions from industrial

effluents. To date, a good number of biomass types have been tested for their metal binding capabilities under various conditions (Bishnoi and Garima, 2004; Sharma *etal*., 2006; Ahluwalia and Goyal 2007). However, the major challenge for the biosorption field remains the selection of the most promising types of biomass from the wide range of type available (Kratochvil and Volesky, 1998).

Bio-materials such as sawdust (Aydin *et al*., 2004), neem leaf (Sharma and Bhattacharyya, 2004; Babu and Gupta 2008; Goutam, 2009), neem oil cake (Rao and Khan, 2007), and rice bran (Wang *et al*., 2006) have been tested for their metal binding capacities. Similarly, coconut copra meal (Ho and Ofomaja, 2006; Hardiject *et al*., 2010) crushed coconut shell, and coconut fiber (Igwe *et al*., 2008), rice husk (Bishnoi *et al*., 2003) and rice hull(Tang *et al*., 2003)have been investigated along with groundnut husk, banana peel, mango peel, wheat bran, sugarcane bagasse, cassava peel (Nasir and Garima, 2004; Singh *et al*., 2006; Memon *et al*., 2007) with all given a promising result.The outcome ofthese studies have led to development of low-cost biosorbents from agricultural wastes (Park *et al*., 2006; Demirbas, 2008; Lesmana *et al*., 2009).

2.9 Modification methods of Biomass

Once decision has been made to use a certain type of bio-sorbent due to its cheap nature and availability,the next decision may involve whether and how to modify that material to improve its sorption efficiency. There are various treatments or modifications namely:

2.9.1 Drying

Drying condition can impact a positive sorptive capacity to sorbent but excessive drying tends to shrink the material leading to closing up of the pores (Stone and Scallan, 1996, Rocha *et al*.,

2006). Hubbe *et al*. (2011) reported a decrease in surface area by a factor of 100 and this is as a result of collapsing of the mesopores during drying.

2.9.2 Size reduction

In principle, a more finely-ground sample of any adsorbent is expected to adsorb more metal ions from solutions due to increase in surface area compared to coarse particles (Ajmal *et al*., 1998; Blazquez *et al*., 2005). But the energy, time, effort and expense of size reduction is not justified as it was observed in the study carried out by Ajmal *et al*. (1998) that the size reduction of sawdust material from 500 to 100 micrometer gave an increase in sorption capacity by only a factor of about 2. In addition one can anticipate a great difficulty in later separation of very fine particle size of sawdust from the water phase and a problem of clogging of filters (Martin *et al*., 2011).

2.9.3 Autoclaving and vacuum drying

Autoclaving helps to increase the sorption capacity of an adsorbent as it kills the cells and open the internal surfaces, making material more accessible. This was demonstrated in the work carried out by (Merrin *et al*., 1998; Kapoor *et al*., 1999;Deepa *et al*., 2006).

2.9.4 Treatment with chemicals

Pretreatment of biomaterials with chemicals modifies the surface groups either by removing or masking the groups or by exposing more metal-binding sites. Acids such as hydrochloric acid, nitrc acid, sulphuric acid and tartaric acid (Babu and Gupta, 2008; Martin-Lara *et al*., 2008), alkalies (sodium hydroxide, sodium carbonate, calcium hydroxide (Southichak *et al*., 2006; Khormaei *et al*., 2007), detergents (Tan and cheng, 2003; Vasudevan *et al*., 2003), oxidizing agent (hydrogen peroxide) and organic chemicals (formaldehyde, ethylenediamine, methanol) have be used in treatment of biomass. The mechanism of alkaline addition has been thought to

involve saponification of various ester groups increasing the numbers of carboxylate groups on the treated surfaces (Reddad *et al*., 2002e; Li *et al*., 2008) and increase metal uptake capacity significantly, whereas acid treatment has no influence on metal biosorption.

2.9.5 Mechanical disruption

Some researchers have found that the amount of metals adsorbed increased with increasing agitation during batch testing (Ahalya *et al*., 2005; Hubbe *et al*., 2011) but the mechanism is unclear.Specifically speaking, chemical activation methods are not favorable, as the main advantage gained by using biomass as a biosorbent and/or alternative adsorbent, lies in its environmentally friendly (waste for waste treatment) and cost effective nature and this would be lost as chemicals need to be purchased and used to convert the biomass. Furthermore, unused chemicals entail more serious problems and commonly necessitate expensive waste treatment facilities (Lesmana *et al*., 2009).

In view of this, deep drying of the sorbent was avoided, appropriate size reduction was done and the sorbent was further autoclaved (Blazquez *et al*., 2005; Deepa *et al*., 2006; Rocha *et al*., 2006).

2.10 Fate of Adsorbent after Bio-sorption

It is important for both environmental and economic reasons to consider the fate of any exhausted biosorbent because the success of the process depends on it. Any used sorbent can undergo the following processes:

i. Regeneration: biosorbent can be regenerated by treatment with acid solution Chang *et al*., 1997) though the use of alkaline solution or brine has also been investigated (Hubbe *et al*., 2011). The function of the chemical is to remove the metal ions from the sorbent back into the solution. The resultant metal concentrated solution can either be disposed

of or further processed as a source of valuable metals or inorganic compounds (Cui and Zhang, 2008).

ii. Incineration: The metal-containing biomass can be incinerated so that the metal content can be concentrated in the ash (Dhankhar and Hooda, 2011; Hubbe *et al*., 2011).

iii. Landfills: Considering the disadvantage of using chemicals to neutralize the used sorbent which has environmental consequence and energy required to dry the used biosorbent before incinerating; the next best option is landfilling (Hubbe *et al*., 2011). Also here there is possibility of the metal breakdown in the soil or subjected to rainfall and begin to leach to contaminate ground water (Dhankhar and Hooda, 2011; Hubbe *et al*., 2011). Therefore the used sorbent should be considered as having served its purpose since it has been used multiple times in the remediation of metal polluted effluents, thereby adding value to their waste as well as solving their own disposal problems.

2.11 Advantages of Metal Uptake by Dead Cells

Dead biomass in industrial applications offers certain advantages over living cells. Systems using living cells are likely to be more sensitive to metal ion concentration (toxicity effects) and adverse operating condition (pH and temperature). Constant nutrient supply is required for systems using living cells and recovery of metals and regeneration of biosorbent is more complicated for living cells (Hubbe *et al*., 2011). Dead biomass can be procured from industrial source as a waste product from fermentation processes, of agricultural operation, chemical and pharmaceutical industries, food and beverage industry (Bishnoi and Garima, 2005). As noted by

Ahluawalia and Goyal (2005b), dead biomass has many potential advantages including insensitive to growth condition or toxins, easier handling, easier storage and easier disposal.

2.12 Fungi

Fungi - are eukaryotic chemoorganotrophic micro-organisms that require inorganic nitrogen in the form of ammonium salt or nitrates as source of nitrogen. For some species trace elements such as phosphorus, potassium, molybdenum, iron, zinc and copper are needed for growth. They obtain their food from organic matter as saprophytes or by feeding on living hosts as parasites (Dhankhar and Hoode, 2011). Fungi include the molds and yeasts. Fungi with diverse metabolic adaptability have shown resistance to several heavy metals. They immobilize these metal ions by linking them with their cell walls (Vankar *et al.*, 2008). Moreover, many among them have the capability of sequestering metal ions from aqueous solution. Fungal biomass contains chitin within its cell walls (Ahluwalia and Goyal, 2005b), chitosans, and glucans and also contain proteins, lipids, and other polysaccharides. Yeast cell walls consist mainly of glucans and an outer layer of mannoprotein. The amino groups within the chitin may have the potential to bind certain metal ion species in a way different from other kinds of biomass (Bishnoi and Garima, 2005).For example, Pal *et al*., (2006) screened and tested fungi from *Aspergillus*, *Mortierella*, *Paecilomyces*, *Penicillium*, *Pythium*, *Rhizopus* and the *Trichoderma* genus, which are isolated from serpentine soil of Andaman (India) for cobalt-resistance. They have isolated a total of thirty eight (38) fungi, and later selected eleven potential fungi for the biosorption of Co (II) from solution. Their experimental results showed that fungi from a natural metal-percolated environment were equally efficient in biosorbing Co (II) ions.

2.12.1 *Aspergillusniger*

Aspergillus niger is a filamentous ascomycete fungus and one of the most common species in the genus *Aspergillus*. This fungus is commercially and economically essential in fermentation for citric acid and gluconic acid production due to its efficiency and high yield outcome. They are common contaminant in cultures but also known to cause disease in debilitated and immune compromised individuals (Pelczar, 1982). The utilization of this fungus to sequester toxic heavy metals from solution has been reported by Dursun, (2006).

On PDA, surface is velvety to cottony. The culture is wooly, at first white to yellow then finally black. The reverse gives white or yellow (Larone, 2002). Microscopically, hyphae are septate (2-8 micrometer in diameter) with unbranched long conidiophores arising from the foot cell. The conidia arise from the phialides and are borne in chains (Beneke and Rogers, 1980) and the head forms a radiate structure.

2.12.2 *Penicillium* species

Some species cause rot or other spoilage of fruits, grains and grasses. Others are used in the ripening of cheese. Some also are used in industrial fermentations and one of the best-known antibiotics (penicillin) is produced by *P. notatum* and *P. chrysogenum*. *Penicilium* species have known to cause keratitis (inflammation of the cornea), respiratory and urinary disease (Pelczar, 1982; Larone, 2002).

Colony morphology: fast growing, at first the surface is white, velvety to very powdery due to abundance of conidia and bluish green with a white border. Reverse is usually white but may be red or brown.Microscopically, there is the presence of septatehyphae that have secondary branches known as metulae. On the metulae are phialides that bear unbranched chains of smooth

or rough, round conidia (2.5-5 micrometer in diameter). The entire structure forms the characteristic "penicillus" or brush like appearance. Conidia smooth or rough, one celled. Species are separated on the basis of variations in branches (metulae) of the conidiophores, conidia and colonial characteristics (Beneke and Rogers, 1980).

2.12.3 Metal Ion Accumulation by *Aspergillus niger* and *Penicillium* species

The living cells of *Aspergillus*, *Penicillium*, *Mucor* and even *Fusarium* has been shown to biosorb metal ions (Merrin *et al*., 1998). *Aspergillus* species has been used by(Baik *et al*., 2002; Barros *et al*., 2005; Dursun, 2006; Garni *et al*., 2009)to sequester nickel, cadmium and lead respectively. While Ridvan *et al*. (2003); Loukidou *etal*. (2003) used *Penicillium* species in the sorption of nickel, lead and cadmium ions.The mechanisms of metal ion accumulation can be classified as:

a. Cell-surface sorption: this occurs by physicochemical interaction between the metal in aqueous solution and the functional groups on the microbial cell wall. This type of sorption is not dependent on cell's metabolism. i.e., does not require energy and is relatively fast and may be reversible (Ahalya *et al*., 2003)
b. Intracellular accumulation: this type of accumulation is often associated with an active defence system of fungi, which reacts in the presence of toxic metals. When metal ions gain entrance into the cells, they may be preferential within specific organelles and or bound proteins such as metallothionin (Veglio and Beolchini, 1997). This process may take place only in living cells
c. Extracellular accumulation:here the metal uptake may take place both in solution and on the surface of the cell wall and the process can either be metabolically depend if the

organism produces compounds that favor the precipitation or may be metabolically independent if it occurs after a chemical interaction between the metal and the cell surface.

The combination of all the mechanisms each functioning independently contributes to overall metal accumulation. Metal ion accumulation by living cells is a function of pH of the metal solution, cell age, contact time, concentration of biomass and temperature.

pH: biosorption of metal ions strongly depends on pH. The biosorption of heavy metals are inhibited below pH 3 and found to increase with ph from acidic (2) to basic range (7) (Tan and Cheng, 2003). The change in sorption capacity with ph can be explained on the basis of proton-competitive adsorption reaction (Huang *et al*., 1991). At low ph, all binding sites are protonated which prevents the access of positively charged metal ions and complete desorption of linked metal ions (Reya *et al*., 2012). Extreme ph values may damage the structure of the biosorbent, cell deformation and reduction in sorption capacity. Higher ph significantly reduces solubility of metals and thus impede biosorption since most metals are found as the optimum pH range 3-6 (Kratochvil *et al*., 1998; Volesky and Naja, 2007).

Cell age: biosorption of metal ions is greatly influenced by the age of the cell. Increased in biosorption has been observed during the lag period or early stages of growth and declines as cultures reached stationary phase. Volesky and May (1995) observed that 24 hours old cultures of baker's yeast biosorb 2.6 times more uranium than 24 hour cultures.

Concentration of biomass: high biomass concentration is very effective at absorbing various metals while lower biomass concentration leads to a higher intake of specific metal and interference between binding sites (Naja *et al*., 2005). Fourest and Roux (1994) invalidated this

hypothesis attributing the responsibility of the specific uptake decrease due to metal concentration shortage in solution. Hence, this factor needs to be considered in any application of microbial biomass as biosorbent (Das *et al*., 2008).

Temperature: temperature affects the stability of the metal in solution, the configuration of the cell wall or stability of complex cells with bound metal. In general, temperature has less influence than other factors, especially if it is between 20 and 30^{o}C.

2.12.4 Advantages of fungal biosorbent over other biomass (Kapoor and Viraraghavan, 1995, Dhankhar and Hoode, 2011).

Fungal biomass has received much attention as biosorbent because of the following:

i. High percentage of cell wall material (Polysaccharides, Chitin), which increases the variety of functional groups involved in metal binding and thus increases the sequestration ability of fungi.
ii. Fungus is easily cultivated in large scale as it has short multiplication cycle
iii. It can easily be cultivated using unsophisticated fermentation techniques and inexpensive growth media.
iv. The yield of biomass obtained from the growth of fungi is usually much.
v. Fungal biomass is easily available as industrial waste products

CHAPTER THREE

3.0 Materials and Methods

3.1 Study site

Kaduna Refinery and Petrochemical Company (KRPC) located in Chikun local government area of Kaduna state Nigeria was used for this study. The refinery occupies an area of 2.9 square kilometers and is located on an undulating land about 700 meters above sea level(Ezeonuegbu *et al*., 2014). This elevation is equivalent to about seventeen meters (17m) higher relative to Romi River.

Study Site Map:

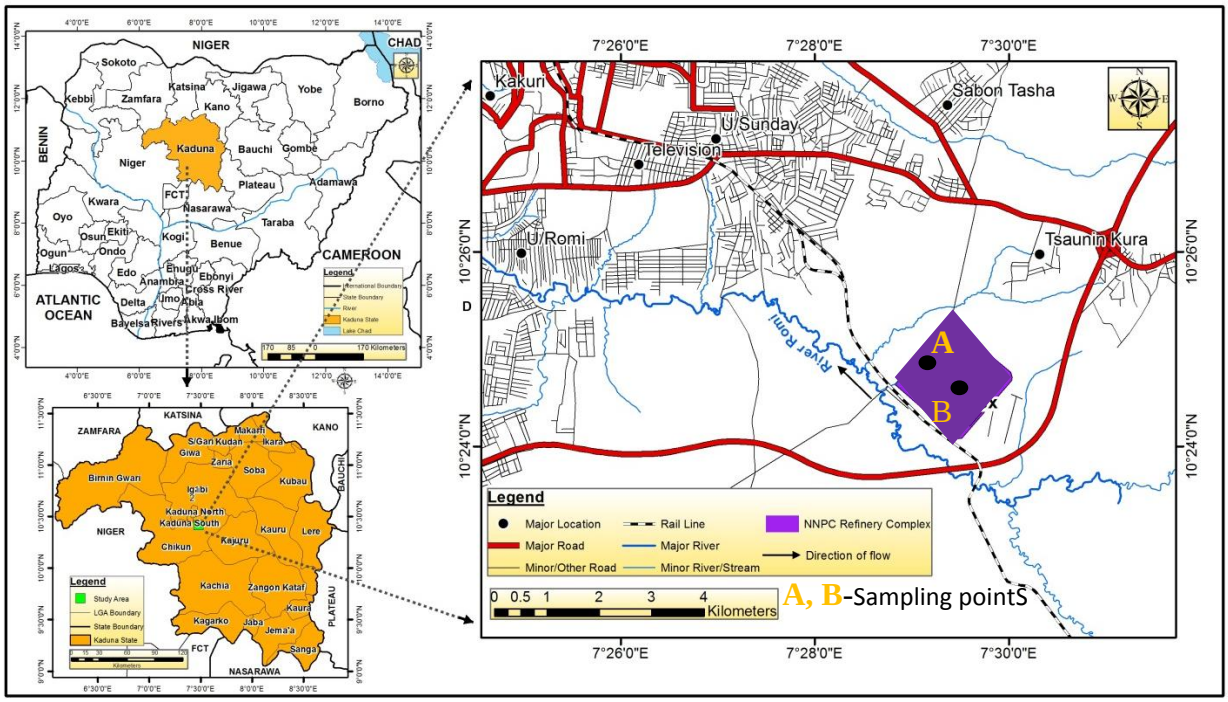


Plate 4.1: Map of the study area (Southern Kaduna) showing the location of the study site(KRPC)

Source: Modified from Ezeonuegbu *et al*.(2014).

3.2 Collection and Handling of Samples

3.2.1 *Saw dust sample*

Two (2.0kg) each of fresh sawdust sample derived from white and brown wood were collected in separate polythene bag from Sabon-gari Zaria and transported to the Department of Microbiology, Ahmadu Bello University, Zaria. The samples thus obtained weresun dried for 72 hours, crushedwith pestle and mortar and sieved witha kitchen sieve to obtain a more uniform particle sizes and stored in clean polythene bags prior to analysis.

3.2.2 *Refinery Effluent Sample*

Ten litres (10L) of the refinery effluent sample was collected into a clean plastic container from waste flow channel of the Kaduna Refining and Petrochemical Company (KRPC). This was done by carefully dipping a wide-mouthed plastic container into the pond to fetch the effluent. The sample collected was transported immediately to the Department of Microbiology, Ahmadu Bello University, Zaria for further analysis.

3.2.3 *Fungal Isolates*

Isolates of *Aspergillus niger* and *Penicillium* sp was obtained from the Department of Microbiology ABU, Zaria. These fungi has earliar been isolated and confirmed to resist and grow in broth charged with 5 to 15µg/ml of Pb, Ni and Cd and capable of removing the same metals ions from refinery effluents by Ezeonuegbu *et al*.(2014) and Machido *et al*.(2014).The isolates were reactivated and re-authenticated following standard procedures (by culture and microscopy) and identification was achieved using an appropriate taxonomic guide of Mycology (Barnett and Hunter, 1999; Hakeem and Bhatnagar, 2010; Thippaswamy *et al.,* 2012).

Reactivation was carried out by aseptically preparing fresh plates of PDA in accordance to manufacturer's specifications and instructions. This was followed by inoculation of the isolates on the PDA plates aseptically, and incubating at room temperature in a dark cupboard for a period of seven days. Re-authentication was carried out by slide culture method using lactophenol cotton blue inoculum preparation. A sterilized scalpelwas used to cut the aseptically prepared PDA and placed on the glass slides. This step was followed by inoculation of the test isolates.Sterile cover slip was placed on the surface of the culture medium at the point of inoculation. The inoculated glass slides were placed in sterilized glass petri dishes containing sterile cotton wool wetted with distilled water. The petri dishes were covered and incubated in a dark cupboard for growth to occur (Machido *et al*., 2014). After the occurrence of growth, the cover slips were carefully removed; a drop of lactophenol cotton blue solution was added on the growth surface of each and placed on sterile glass slides inverted and mounted on a microscope and viewed at ×10 and ×40 objective lens. Microscopic characteristicssuch as presence or absence of septation, sexual reproductive structures, presence of foot cells and chlamydospores was observed and recordedand compared with those on mycology atlas (Atlas and Philip, 2005). The authenticated isolates were sub-cultured on fresh Potato Dextrose Agar (PDA) slants until required.

3.3. Determination of Proximate Composition of Saw dust Samples

Proximate composition of the sawdust was carried out following standard procedures of proximate analysis using Association of Analytical Chemist (AOAC, 2010) method and these were done at the Department of AnimalScience, Institute of Agricultural Research., A.B.U, Zaria.

3.3.1 *Determination of moisture content*

Crucibles used were washed, dried in an oven at 100^{o}C for 1hour then cooled in a desiccator and weighed (W1). Two grams of the sawdust sample was weighed into the crucibles and weighed as W2 before and after drying at 100^{o}C to a constant weight W3.

$$\% \text{ Moisture content} = (W2-W3)/ W2-W1 \times 100$$

3.3.2 *Determination of ash content of the sawdust*

Crucibles were cleaned and dried in an oven then cooled in the desiccator and weighed as W1. Two grams of the sawdust was placed in the crucibles and weighed (W2). The crucibles and the sample were transferred into the muffle furnace set at 550^{o}C for 2 hours until a white or light grey was obtained. The sample was removed and cooled followed by weighing (W3).

$$\% \text{ Ash content} = (W3 - W1) / (W2 - W1) \times 100$$

3.3.3 *Determination of protein content*

Two gram (2g) of the sample was weighed into Kjeldahl's flask and 5g of anhydrous sodium sulphate was added together with 25ml sulphuric acid and few boiling chips. The content was heated in the fume chamber until the solution was clear. The clear solution was cooled and transferred to 250mL volumetric flask and volume made up with distilled water. Distillation was carried out in a clean Kjedahl apparatus. Five millilitre of 2% boric acid and 2 drops of methyl red indicator was poured into 100mL conical flask and placed under the condenser. Five millitre of the digest was pipetted into the apparatus through the small funnel on the distillation unit. The digest was washed down with distilled water and followed by addition of 5mL of 60% sodium

hydroxide solution. This was distilled until the volume of distillate reached 100mL. The blank was titrated in the same way and the time value for the samples was obtained.

$$\% \text{ Nitrogen} = V_S \times V_B \times N \text{ acid} \times 0.04 \times 100$$

Where;

V_S is volume in mL of the acid required to titrate sample

V_B is volume of the acid required to titrate the blank

N acid is normality of acid 0.1N

W is weight of sample in gram

NB: most proteins contain about 16% Nitrogen so that 16mg nitrogen=100mg protein and 1mg nitrogen=6.25mg protein. Therefore, protein (%) = N x 6.25(standard number or conversion factor for protein).

3.3.4 *Determination of lipid (fat) content*

Two grams (2g) of the sawdust was weighed into thimble and sealed with cotton wool (W2) then fitted into the extraction tube of the soxhlet extractor. The soxhlet extractor apparatus was assembled with the round bottom flask containing 250mL petroleum ether and then allowed to reflux for 6 hours after which the thimble was removed with care and the petroleum ether was collected into a container for re-use; after the flask was freed from ether it was dried and weighed (W3). The weight of empty filter paper (thimbles) was noted as W1

$$\% \text{ Fat content} = (W2 - W3) / W2 - W1 \times 100$$

3.3.5 *Determination of carbohydrate content*

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

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

3.3.6 *Determination of cellulose content*

The cellulose content was determined following method described by Badu*et al*. (2011). Two grams of the sawdust sample was weighed and transferred to 250mL beaker. One hundred millilitre of 17.5% of sodium hydroxide solution was added and stirred for 30 minutes at 25^{o}C. The content of the beaker was filtered and washed with 25mL of 9.5% sodium hydroxide solution and 20mL portions of 100mL distilled water. The residue obtained was again washed with distilled water and 40ml of 10% acetic acid and lastly rinsed with 1L distilled water. The residue was dried at 105^{o}C for 24hoursto constant weight.

3.3.7 *Determination of holocellulose content*

Holocellulose content of the effluent was determined in accordance with the method described by Badu*et al*. (2011). 180mL distilled water, 8.6g sodium chloride and 6mL ethanoic acid was added to 2g of the sawdust. The mixture was digested in 250mL conical flask under reflux at 70^{o}C for 3 hours. After cooling, the preparation was filtered and the residue be washed with 20mL portions of the 100mL distilled water. Residue obtained was dried in an oven at 105^{o}C for 24hours which was then cooled and finally weighed to obtain the holocellulose content of the sawdust.

3.3.8 *Determination of the hemicellulose content*

On the basis of solubility in 17.5% sodium hydroxide solution, holocellulose is subdivided into insoluble cellulose and the soluble hemicellulose. To calculate the hemicellulose weight of the sawdust, the cellulose weight was subtracted from the holocellulose weight present (Badu *et al*., 2011).

3.3.9 *Determination of lignin content*

To one gram of the sawdust sample, 14ml of cold 72% sulfuric acid was added and stirred. The mixture was left to stand for 2 hours after which it was washed in a liter conical flask and diluted to 3% sulfuric acid. The resultant mixture was again boiled under reflux for 4 hours. The insoluble material was allowed to settle and filtered. The residue was washed, dried for 2 hours, cooled and weighed to give the lignin content (Templeton and Ehrman, 1995).

3.4 Determination of Physicochemical Properties of Refinery Effluent

Physicochemical analysis was carried out in accordance with the methods suggested by American Public Health Association, (2012) to know the natural conditions of samples and extent of pollution of the effluent. The parameters for the analysis include; Temperature, pH, Electrical Conductivity (EC), Total dissolved solids (TDS), Dissolved Oxygen (DO), Oil and Grease, Sulphate (SO_4^{2-}), Nitrate (NO_3^{2-}) and Phosphate (PO_3^{2-}) while the heavy metal ion (Lead, Nickel, and Cadmium) content of the effluent was determined using fast sequential atomic absorption spectrophotometer model AA240FS.

3.4.1 *Determination of pH, Temperature, Electrical conductivity and Total dissolved solids*

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

3.4.2 *Determination of Dissolved oxygen and Biological oxygen demand*

Dissolved oxygen (DO) and biological oxygen demand (BOD) was determined using HANNA instrument(H198130, Denver, USA). Dissolved oxygen was determined by inserting the HANNA instrument into the sample at the point of collection while the BOD was carried out after the sample was transferred into a BOD bottle and left to stand for five days at room temperature ($28\pm2^{o}C$). The instrument was then inserted into the incubated sample and read. Initial DO was subtracted from the final value got after incubation to obtain the BOD(Radojavic and Bashkin, 1999).

3.4.3 *Determination of Nitrate content by colorimetric method*

This was carried out by the method described by APHA (2012). Ten milliliter of the sample (effluent) was pipetted into a distillation flask and one gram of Dawada Alloy was added. The whole content was the distilled into a 20ml two percent boric acid under the condenser. About 50mls of the distillate was collected and few drops of mix indicator was added. Totration was done with 0.025N $H_2SO_4$from green to purple.The nitrate concentration in percentage was calculated using the formula:

Nitrate (NO_3) = 0.014 x 0.025 x 1000/ Volume of the effluent taken

3.4.4 *Determination of Sulphate content usingturbidity method*

One hundred millilitres (100mL) of the sample was dispensed into Erlenmeyer flask, 5 ml conditioning reagent was added and mixed using a magnetic stirrer. A spoonful of barium chloride crystals was added while the solution was still being stirred. The turbidity was measured at 30seconds after stirring at interval of 4 minutes using turbidity meter (HACH 2100N Turbidimeter, USA Turbidimeter). A blank was also made with no barium chloride added. The sulphate concentration was calculated using the formula:

Sulphate (SO_4) = Weight of sulphate(mg) x 1000/ Volume of sample taken (mL)

The sample was estimated by comparing the turbidity reading with a standard curvepreviously prepared (Appendix I).

3.4.5 *Determinationof Phosphate contentby Bray and Kurtz one method*

One drop (0.05ml) of phenolphthalein indicator was added to 100ml of the sample. Strong acid solution (hydrochloric acid) was added drop wise when the colour turned pink, to discharge the color.One hundred millilitre (100mL) of treated sample was poured into a flask and 4.0mL of molybdate reagent was added and mixed thoroughly. Ten drops (0.5mL) of stannous chloride reagent was added. After 10 minutes, the color was measured photometrically at 690nm and compared with the standard curve. Distilled water was used as blank. Calculation was carried out using the formula:

Phosphate (PO_4)=Absorbanceof phosphate x Volume of sample (mL) x Gradient (4.310)

3.4.6. *Determination of Oil and grease content*

Oil and grease was analysed using the Partition – Gravimetric method. Fifty millilitre of each sample was acidified to pH 2.0 using 5ml of hydrochloric acid and shaken vigorously for three minutes. The sample was transferred into a separating funnel held by a retort stand, and then 15ml of hexane was used to carefully rinse the beaker before pouring the sample into the funnel which separated the content into layers of oil, grease and water. The water being of higher density was collected below, leaving oil and solvent at the surface. Twenty grams of anhydrous sodium sulphate was added to the filter cone and the emulsified solvent was collected. Extraction was done twice using 15ml of the organic solvent in each process. An additional 20ml of solvent was used to wash the filter paper and evaporation was carried out using water bath until all the solvent had evaporated. The flask was transferred to desiccators before weighing. The oil and grease content was determined as follows:

(A-B) x 1000ml of oil and grease/ Volume of sample (oil + grease) (mg/l)

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

3.4.7 *Determination of Heavy Metal content of the effluent*

The concentrations of heavy metal ions (lead, nickel and cadmium) in the effluent sample ware analyzed using the fast sequential atomic absorption spectrophotometer (Varian Technologies, USA) following the modified method of APHA (2012). Briefly, the instrument's settings and operational conditions were carried out in accordance with manufacturer's specifications by calibrating with analytical grade metal standard stock solution. Five millilitre (5ml) of each sample was digested in a beaker by adding 37.5ml of nitric acid and 12.5ml of hydrochloric acid, heated to almost dryness and topped up to 50ml with distilled water. The digested sample was

filtered to remove any insoluble materials that could clog the atomizer. The filtrate was then analyzed for heavy metals using the Atomic AbsorptionSpectrophotometer.

3.5 Determination of the Capacity of Saw dust to Support the Growth of *Aspergillus niger* and *Penicillium* species

3.5.1 *Preparation of Fungal Spore Inoculum*

This was done following modified method of Bekada *et al*. (2008) and Bekker *et al*. (2009). Using a sterile needle, colonies of *A. niger* and *Penicillium* species grown on potato dextrose agar slant for 7 days at 25^{o}C wereaseptically inoculated in a freshly prepared potato dextrose broth. The preparation was allowed standing in a rotary shaker for 72 hours at 25^{o}C. The spore suspensions of the isolate were stored at 4^{o}C in the refrigerator untilrequired for further use.

3.5.2 *Preparation of the Sorbent and Inoculation*

Twenty grams (20g) batches of sawdust sample were placed in large glass Petri dishes and supplemented with 200ppm of Ammonium nitrate, Magnesium sulphate and Potassium hydrogen phosphate as sources of nitrogen, sulphur and phosphorusrespectively which are essential requirements for growth and accumulation of the test fungi with the sawdust as carbon source (Cooney, 1984). The Petri dishes and their contents were sterilized by autoclave at 121^{o}C under 15lb/sq. inch for 15mins. 50ml of the spore suspension of *A. niger* and *Penicillium* species prepared were aseptically decanted into each of duplicate plates of the freshly sterilized sawdust. All inoculated plates were incubated aerobically in a cupboard under ambient laboratory conditions. The inoculated culture media were continuously monitored for appearance of fungal growth for 14 days. After growth, the cultures were then dried and weighed using the top loading balance to obtain the dry weight of the partially digested sawdust.

3.6 Determinationof the Effect of Selected Fungal Isolates on the Capacity of Sawdust to Adsorb Heavy Metals

3.6.1 *Preparation of experimental sorbents*

One hundred grams (100g) of sawdust was packed into each five (5) sorption columns measuring 5.0cm in diameter x 50cm depth (AppendixIII). This was followed by supplemental addition of the salts to each of the columns as in 3.5.2 and labeled as in (Appendix III).

Column B-E was sterilized by autoclaving at 121^{o}C under 15lb/sq. inch for 15minutes leaving column A. Column B was left un-inoculated. Column C, D and E were inoculated with the spore suspension of *A. niger*, *Penicillium* species and mixture of the two fungi respectively. Columns A and B served as the control against which the sorption performances of the experimental sorbents in column C, D and E were assessed. All the setups were left to stand for two weeks for the fungi to interact with the sawdust and obtain experimental sorbents.

3.6.2 The sorption process

The metal bearing effluent sample to be used was sterilized at 121^{o}C for 15minutes, this is important to make sure the effluent is freed from all indigenous organisms. One litre (1L) of the sterilized refinery effluent was poured in an up-flow mode to each of the columns and the various set-ups were left to stand for one hour (Kratochvil and Volesky, 1998).Twenty millilitre (20ml) of filtrate was collected from each sorption column in sterile and well labeled plastic sample bottles.The residual concentrations of Pb, Ni and Cd in the filtrates were then determined using Atomic Absorption Spectrophotometer(model 9100 x Philips, England).This procedurewas repeated at hourly intervals for 6 hours.

3.6.3 Determination of sorption performance

The performance of any potential sorbent can be assessed using several yard sticks, these includes (Adie *et al*., 2012; mohammed *et al*., 2015):

a. Percentage of heavy metal removal.
b. Amount of metal sorbed or adsorbed.
c. Langmuirian equation.

Percentage and amount of metal removed:

The concentration of the effluent solution was determined to serve as the initial concentration. Residual metal ion concentration in the filtrate (after adsorption) in each column was also measured by atomic adsorption spectrophotometry (AAS). The percentage of metal ion removed (R_{metal} %) from solution is expressed as equation (2.1)

$$Rmetal(\%) = \frac{Ci - Cf}{Ci} \times 100 \qquad Eq\ 2.1$$

While the amount of lead (II), cadmium (II) and nickel(II) adsorbed Q (mg/g) at time (t) can be expressed using the general equation of adsorption (Eq 2.2) (Feng lin *et al*., 2013; Azouaou-Naima *et al*., 2014).

$$Qt = \frac{(Ci - Cf)}{M} V \qquad Eq\ 2.2$$

Where:

Q_e (mg/g) is the amount of adsorbate adsorbed per unit weight of adsorbent at equilibrium,

C_i and C_f are the initial and final concentrations (mg/l) of metal ion in solution,

V is the volume (L) of the effluent solution

M is the mass (g) of the adsorbent.

Fitting obtained Experimental Data to Langmuir equation:

One of the initial models for the adsorption of a species onto a simple surface was put forth by Irving Langmuir in 1916. Langmuir adsorption which is most widely used suggests that the sorption occurs on the surface of the solid that is made up of elementary sites, each of which can adsorb one sorbate molecule, that is, a surface consist of a given number of equivalent sites where a species can physically or chemically stick.The Langmuir adsorption equation which shows the intensity of sorption process is expressed in equation (2.3) from which the values b was determined (Rao *et al*., 2009).

$$\mathbf{Q = qmax(bcf)/(1 + bcf)} \qquad \mathbf{Eq\ 2.3}$$

Re-arranging the equation

$$\mathbf{b = \left(\frac{Q}{qmax} \times Cf\right) - (Q \times Cf)} \qquad \boldsymbol{Eq\ 2.4}$$

Where Q = quantity of metal ion adsorbed at time t,

q_{max} = maximum adsorption

C_f= is the final concentration of metal ion at time t.

3.7 StatisticalAnalysis

Data obtained from the study were statistically analyzed using one way Analysis of Variance (ANOVA) where necessary using SPSS Version 20.0. The means of data analyzed were ranked and compared. Results were presented in tables, charts and graphs as appropriate.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Proximate Composition of Saw dust

The percentage proximate and the lignocellulosic composition of the sorbents used are presented in (Table 4.1). The resultsrevealed that white wood sawdust has higher composition of crude protein (7.31%), fats (0.35%), carbohydrate (62.51%), cellulose (47.45%) and hemicellulose (22.12%) content but lower lignin (7%), ash (3.39%) and moisture (4.92%) contentcompared to brown wood with lowerpercentage composition of cellulose (44.48%), hemicellulose (19.80%), fat (0.22%), crude protein (5%), carbohydrate (54.76%) content and a higher composition of moisture (6.04%), ash (5.41%) and lignin (11.40%) content (Table 4.2).

4.2 Physicochemical Properties of the Effluent from Kaduna Refinery

In order to determine the physicochemical properties of the refinery effluent,physical parameters such as (EC, TDS, turbidity and temperature) and chemical parameters (COD, DO, BOD, phosphate, nitrate, sulphate, cadmium, lead and nickel) were analysed (Table 4.2). Among the physical and chemical parameters analysed only the COD (874.1mg/L and phosphate (33.7mg/L) werefound to be higher than the permissible limit given by National Environmental Standards and Regulations Enforcement Agency, (NESREA, 2007) (2100mg/L and 5.0mg/L) respectively. Similarly, the concentration of the targeted metal ions analyzed (Pb(1.587mg/L), Ni (0.2mg/L) and Cd (0.073mg/L)] were all found to be above the allowable limits set by NESREA, (2007)(0.01,0.07 and 0.03mg/L) respectively.

Table 4.1: Proximate and lignocellulosic component of the wood saw dustsamples obtained from Sabon-gari Zaria.

Parameters	Percentage composition (%)	
	White wood sawdust	Brown wood sawdust
Moisture content	4.92±0.02	6.04±0.02
Ash	3.39±0.04	5.41±0.01
Crude fibre	21.52±0.16	28.57±0.08
Crude fat	0.35±0.04	0.22±0.01
Crude protein	7.31±0.00	5.0±0.05
Carbohydrate	62.51±0.00	54.76±0.00
Cellulose	47.45±0.15	44.48±0.04
Hemicellulose	22.12±0.01	19.80±0.2
Lignin	7.00±0.06	11.40±0.1

Mean ±SE; ANOVA

Table 4.2: Physicochemical properties of theeffluentanalysed from Kaduna refinery

Parameters	Values	Permissible Limit (NESREA, 2007)
Physical		
Temperature (^{o}C)	29.6±0.19	40
Electrical conductivity (µS/cm)	717±1.5	900
Total dissolved solids (mg/L)	258±1.5	2100
Turbidity (NTU)	111±2.3	500
Chemical		
Ph	8.83±0.01	6.0-9.0
Biological oxygen demand (mg/L)	1.2±0.21	30
Dissolved oxygen (mg/L)	2.9±0.06	5
Chemical oxygen demand(mg/L)	874.1±0.58	250
Oil and grease (mg/L)	3.7±0.12	10
Phosphate (mg/L)	33.7±0.00	5
Nitrate (mg/L)	1.67±0.01	20
Sulphate (mg/L)	73.2±0.12	500
Heavy metal concentration		
Lead (mg/L)	1.587±0.00	0.01
Nickel (mg/L)	0.2±0.00	0.07
Cadmium (mg/L)	0.073±0.00	0.03

Mean ±SE; ANOVA

NESREA-National Environmental Standards and Regulations Enforcement Agency(2007)

µS/cm - Micro siemens per centimeter

mg/L- Milligram per liter

NTU-Neplometry turbidity unit

4.3 Ability of the saw dust biomassto support the growth of*Aspergillus niger* and *Penicillium* species.

Ability of the saw dusts biomass to support the growth of *Aspergillus niger* and *Penicillium* species by serving as source of carbon and energyis shown in Table 4.3as the percentage decomposition of the sorbent by the organismsand also demonstrated as visible growth of each on the sorbent in (PlatesI-IV). Theweight of the sorbent reduced after inoculation with the organisms and after incubationfor 14 days. The difference in weight of the saw dustsamples were calculated as the percentage of the sawdust decomposedby the organisms. The percentage decomposition of the white sorbent by*A. niger and Penicillium* specieswere obtained as 11.21% and 13.40% respectively, while that of brown wood sorbent by the organismswere 12.55% and 10.73% respectively. The brown wood showed a lower ability to support the growth and probably serve as source of nutrient to *Penicillium*species but a higher ability towards *A.niger* whereas in the white wood,*Penicillium*species displayed higher percentage decomposition.

Table 4.3: Sorbentability to support the growth of *Aspergillus niger* and *Penicillium* species after fourteen days.

Wood sorbent	Organisms	Weight of sample (g)		
		Initial weight (afterinoculation)	Final weight (after 14days of inoculation)	Percentage Decomposed (%)
White wood	*A. niger*	289	256.6	11.21
	Penicillium sp	293.2	253.9	13.40
Brown wood	*A. niger*	278	243.1	12.55
	Penicillium sp	280.1	250.05	10.73

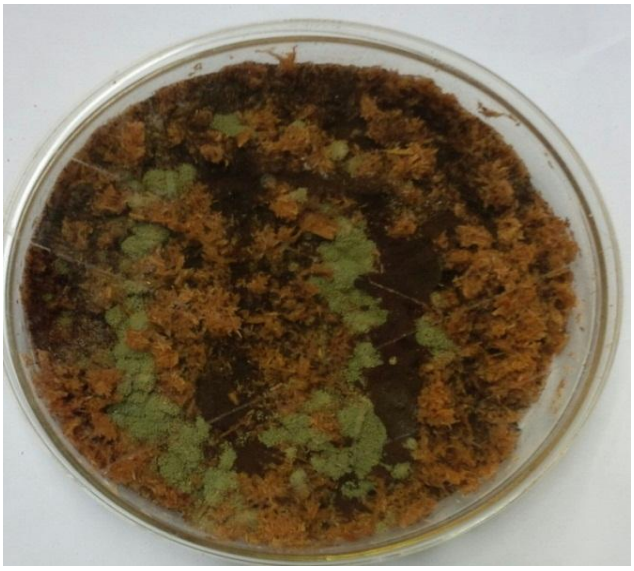


Plate 4.2



Plate 4.3

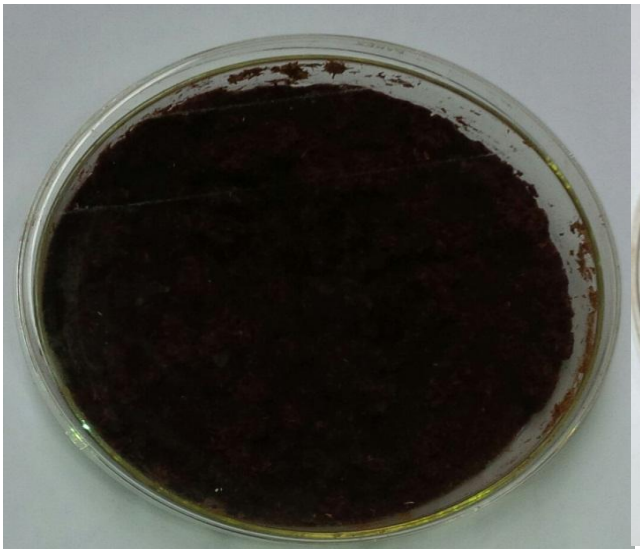


Plate 4.4

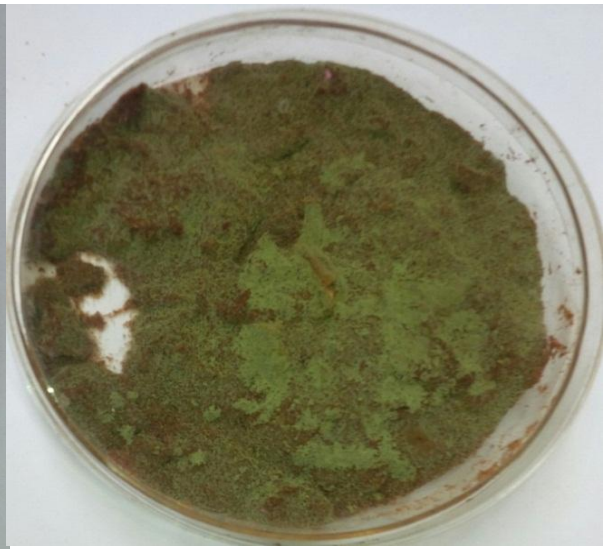


Plate 4.5

Plate 4.2 = *Aspergillus niger* grown on sorbentfromwhite wood

Plate 4.3 = *Penicillium* species grown on sorbent from white wood

Plate 4.4= A*spergillus. niger* grown on sorbent from brown wood

Plate 4.5 = *Penicillium* species grown on sorbent from brown wood

4.4 Efficiency of the treatments in the removal of metal ions

The percentage removal as regards to treatment for both white and brown wood sorbent is depicted in Figures 4.1 and 4.2. Generally, all the treatment gave a high percentage removal of the metal ions of over 60% across both sorbents. Irrespective of the sorbent used, lead was found to have the highest percentage removal followed by cadmium and lastly Nickel. In white wood, treatment B containing only sterilized sawdust had the highest lead removal percentage (98.36%) and the lowest percentage removal was observed with treatment E (96.61%). Also for Nickel treatment B removed highest (84.67%) and the lowest was observed in treatment D (67.72%),while treatment E removed Cd more (90.31%) and the lowest was observed with treatment C (87.19%) (Figure 4.1). On the other hand, brown wood sorbent, the highest percentage removal of Pb was observed in treatment E (94.75%) and lowest removal was in treatment A (92.57%). Ni was removed more in treatment D (70.96%) and less in treatment B (63.94%) while Cd had highest in treatment C (83.19%) and lowest in B (74.39%) (Figure 4.2). The study showed that, the un-inoculated treatments in white wood had the highest removal percentage for the metals (Figure 4.1)compared to the inoculated treatments whereas the reverse is the case with brown wood (Figure 4.2). The trend of efficiency with treatment in white wood sorbent is B>E>A>C>D and brown wood sorbent is D>C>E>A>B. The average percentage removal for Pb, Ni and Cd ions in white wood was found to be 97.5%, 76.2% and 88.8% respectively while for brown wood it was found to be 93.7%, 67.5% and 78.8% respectively. Statistical analysis of the performance of the treatments in removal of each metal ion is statistically not significant ($p>0.05$) (Appendix IV and V).

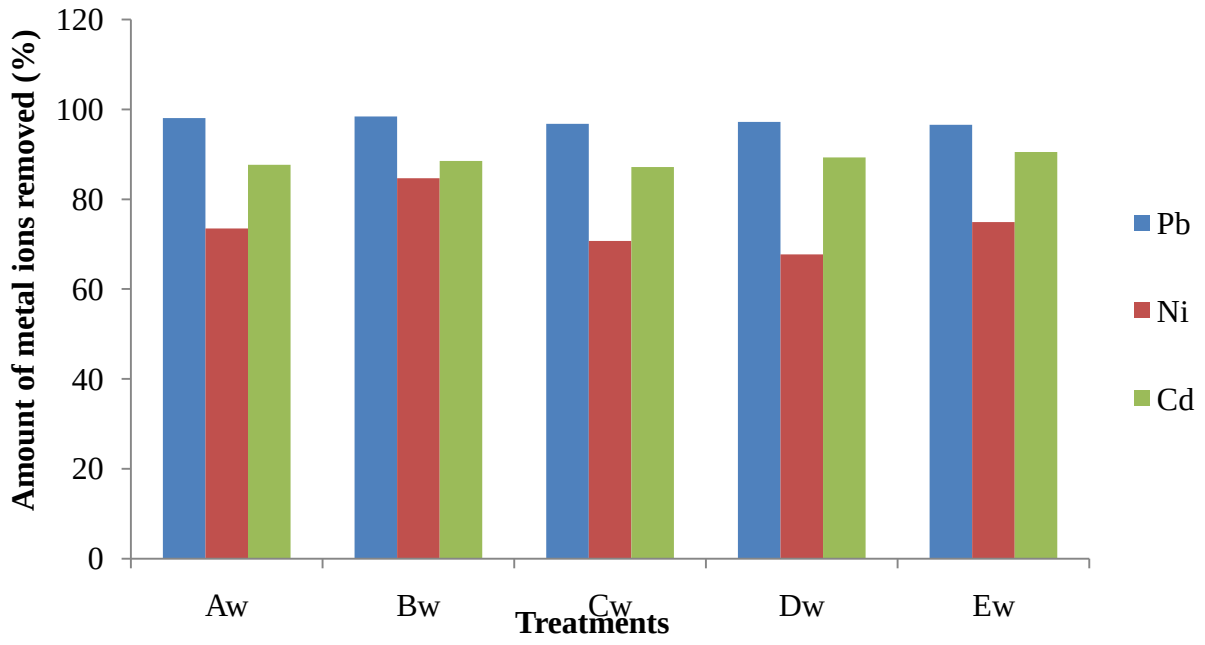


Figure 4.1: Percentage removal of Pb, Ni and Cd ions by white sawdust sorbentsafter six hours

KEY

Aw= Unsterilizedwhite sawdust, Bw = Sterilized whitesawdust, Cw = Sterilized white saw dust inoculated with *A. niger*, Dw = Sterilizedwhiten saw dustinoculated with *Penicillium* species, Ew =Sterilized inoculated with both organisms

.

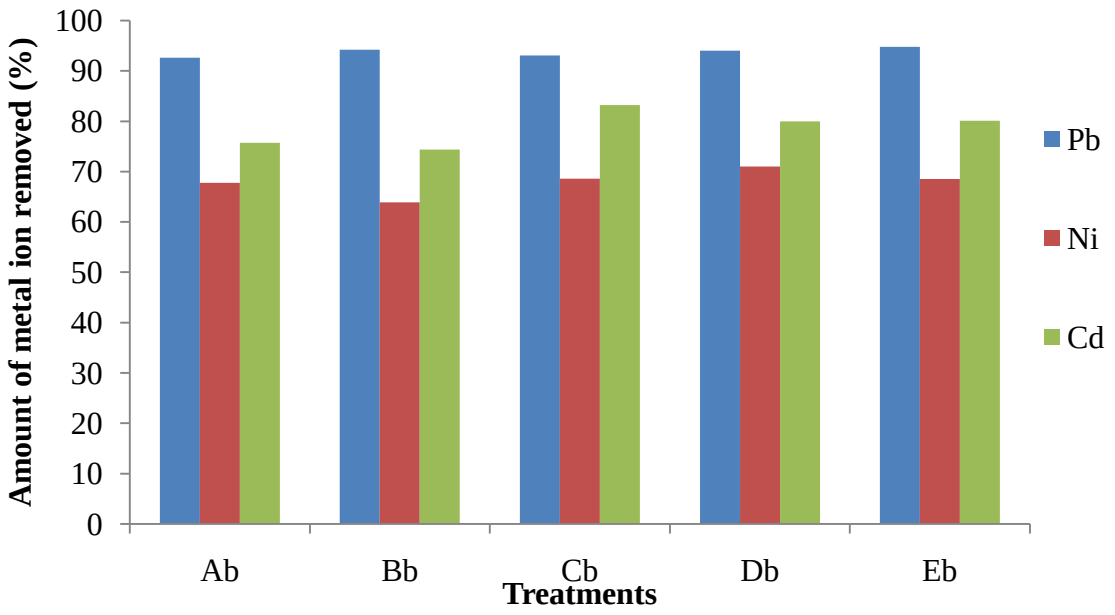


Figure 4.2: Percentage removal of Pb, Ni and Cd ions by brown sawdust sorbents after six hours

KEY

Ab=Unsterilized brown sawdust, Bb= Sterilized brown sawdust, Cb=Sterilized brown sawdust inoculated with *A. niger*, Db=Sterilized brown sawdust inoculated with *Penicillium* species, Eb=Sterilized brown saw dust inoculated with both organisms

4.5 Effect of Contact Time on Removal of Metal ion by Experimental sorbents

Figures4.3-4.8 illustrate the changes in the amount of Pb, Ni and Cd ionsremoved over time by the experimental sorbents.Most of thefigures(Figures 4.3, 4.4, 4.6, 4.8) showed initially a progressive increase in percentage removal of the metal ions until equilibrium time, where further increase in time does not lead to any appreciable increase in their percentage removal.Beyond the equilibrium time, a stationary phase was observed in most of the figures and finally a decrease phase. Figures 4.5 and 4.7 showed an irregular pattern of uptake with time. The maximum removal of Lead was found to be at third hour while that of Nickel and Cadmium was found to be between the second and third hour in white sorbent. On the contrary, in brown sorbent, Pb was maximally removed at the fifth hour, while nickel and cadmium were both removed at the first hour. At equilibrium,the time for the removal of Pb, Ni and Cd was found to be at the second hour, before the first hour and at the second hour respectively in white saw dust sorbent. In the brown sorbent, the equilibrium time of removal was atthe third hour for Pb, while removal for Ni and Cd ion was observed before the first hour.

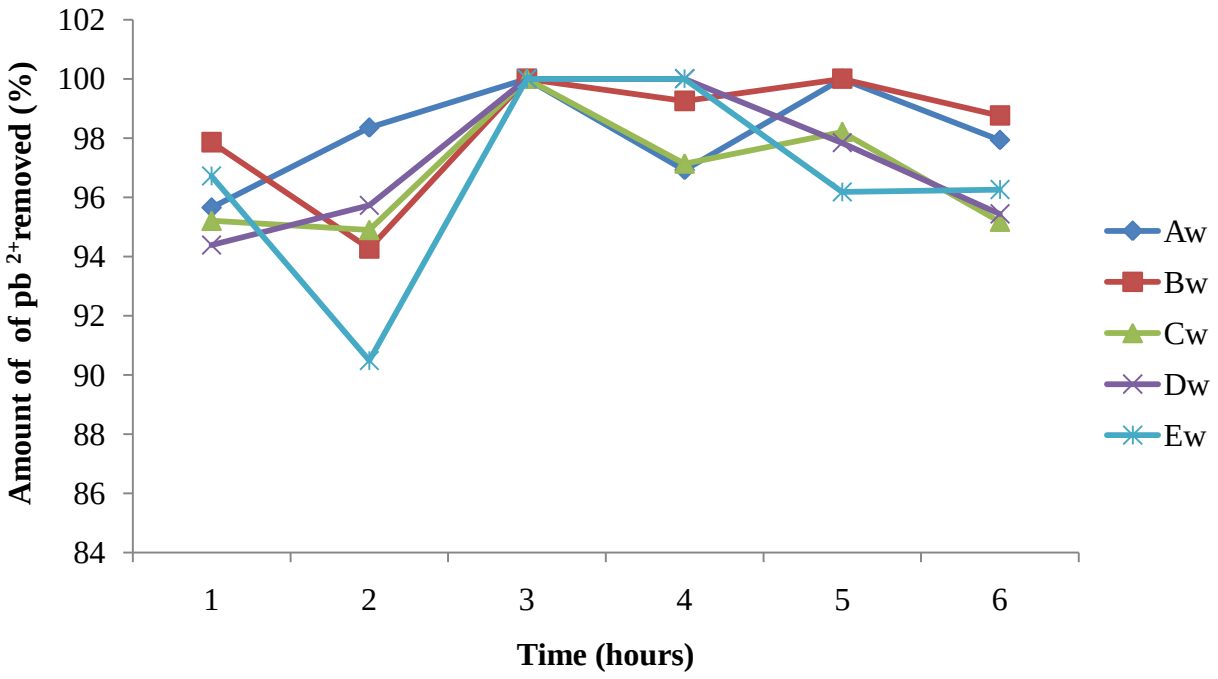


Figure 4.3:Effect of contact time on lead removal by white sawdust sorbentover six hour's period

KEY

Aw=Unsterilized White sawdust, Bw= Sterilized White sawdust, Cw=SterilizedWhite sawdust inoculated with *A. niger*, Dw=SterilizedWhite sawdust inoculated with *Penicillium* species, Ew=Sterilized white sawdust inoculated with both organisms.

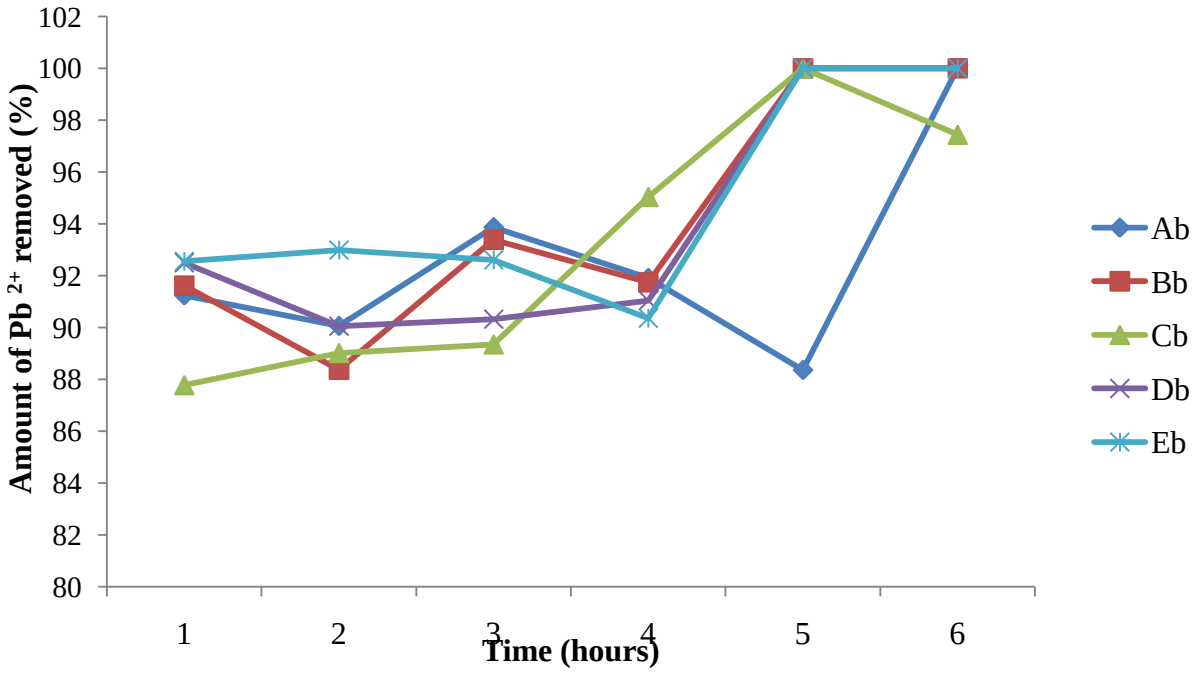


Figure 4.4:Effect of contact time on lead removal by brown sawdust sorbent over six hour's period

KEY

Ab=Unsterilized brown sawdust, Bb= Sterilized brown sawdust, Cb=Sterilized brown sawdust inoculated with *A. niger*, Db=Sterilized brown sawdust inoculated with *Penicillium* species, Eb=Sterilized brown saw dust inoculated with both organisms

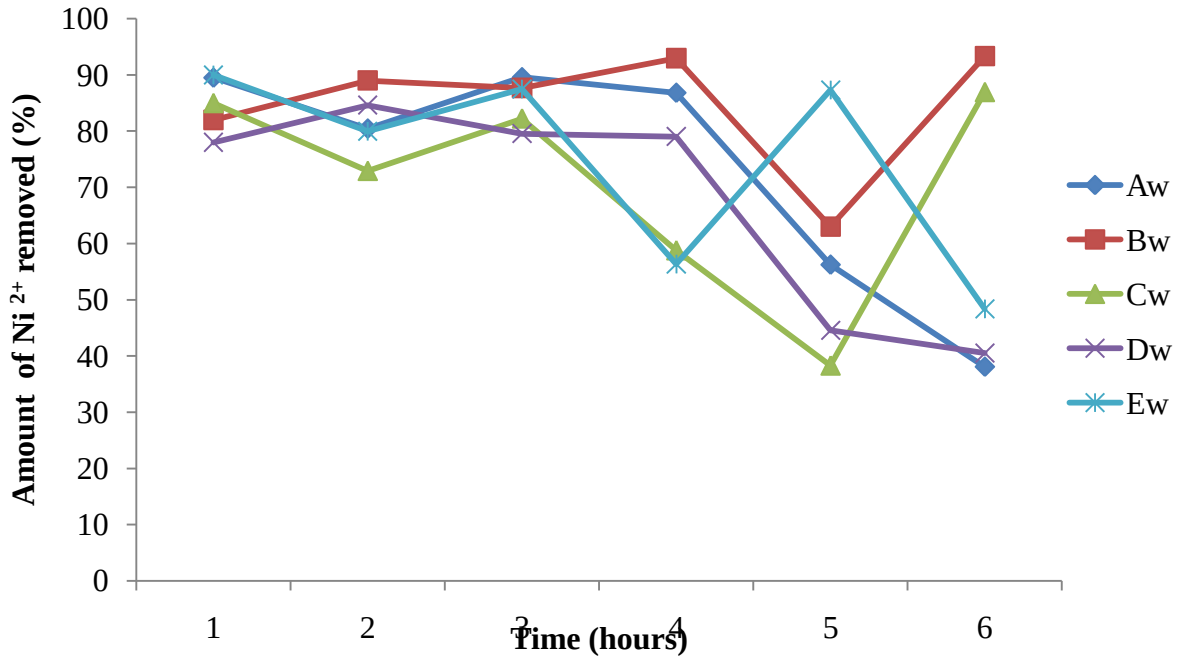


Figure 4.5:Effect of contact time on Nickel removal by white sawdust sorbentover six hour's period

KEY

Aw=Unsterilized White sawdust, Bw= Sterilized White sawdust, Cw=SterilizedWhite sawdust inoculated with *A. niger*, Dw=SterilizedWhite sawdust inoculated with *Penicillium* species, Ew=Sterilized white sawdust inoculated with both organisms.

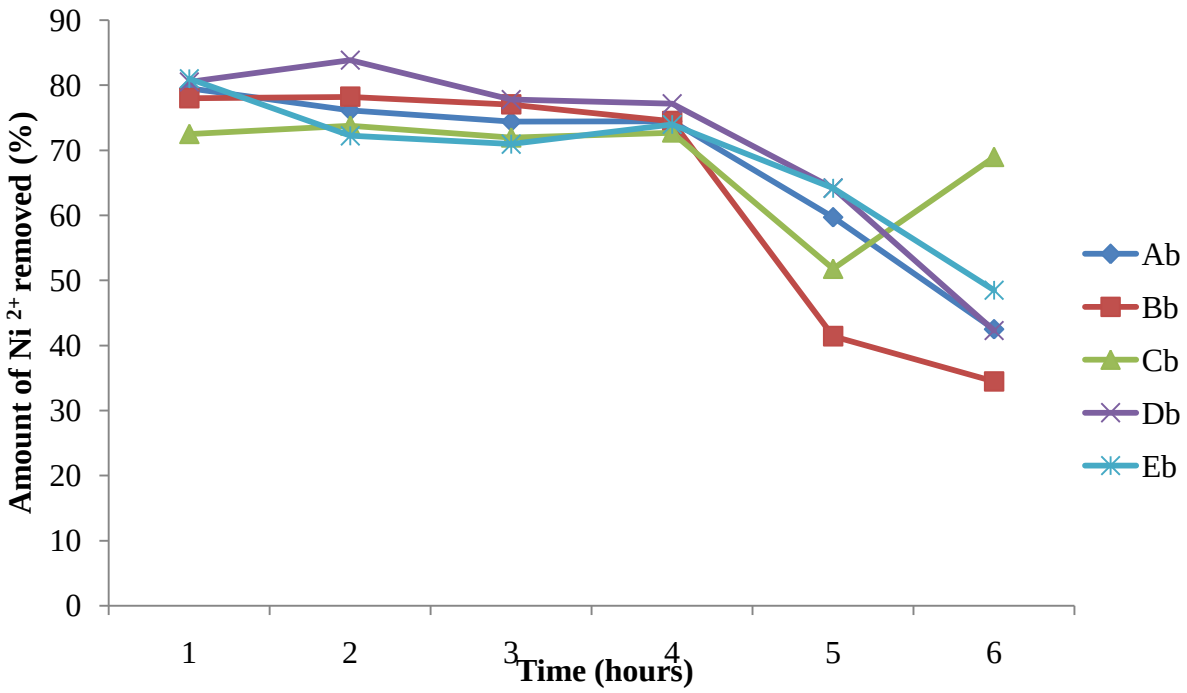


Figure 4.6:Effect of contact time on Nickel removal by brown sawdust sorbentover six hour's period

KEY

Ab=Unsterilized brown sawdust, Bb= Sterilized brown sawdust, Cb =Sterilized brown sawdust inoculated with *A. niger*, Db =Sterilized brown sawdust inoculated with *Penicillium* species, Eb =Sterilized brown saw dust inoculated with both organisms

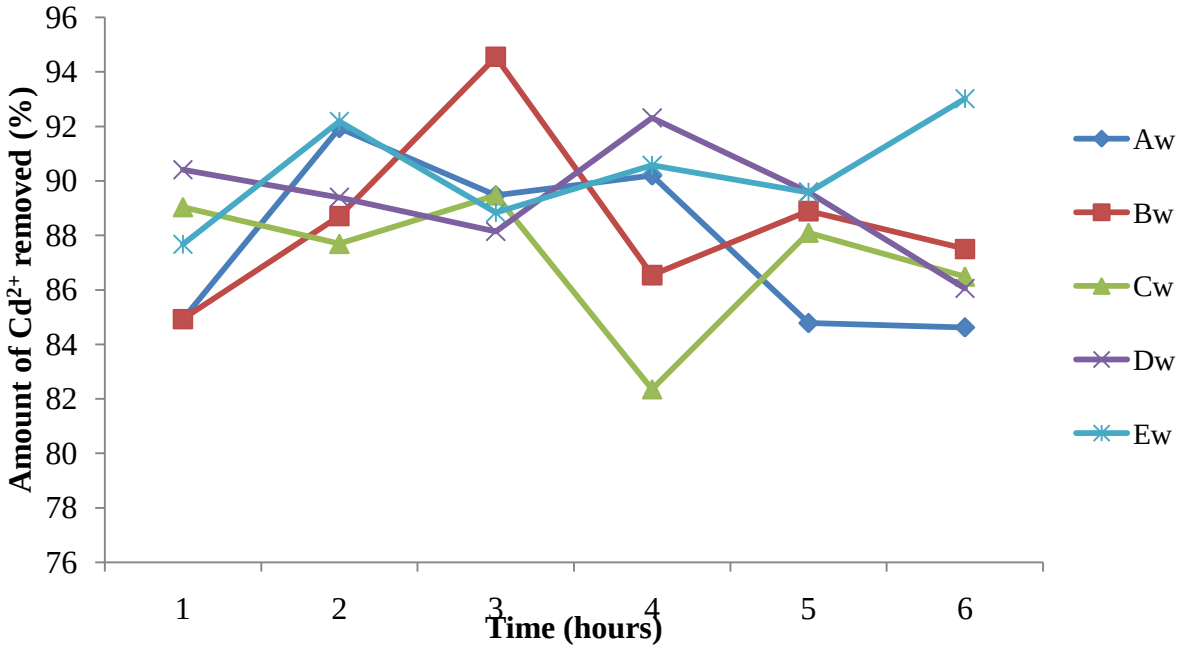


Figure 4.7: Effect of contact time on Cadmium removal by white sawdust sorbentsover six hour's period

KEY

Aw=Unsterilized White sawdust, Bw= Sterilized White sawdust, Cw=SterilizedWhite sawdust inoculated with *A. niger*, Dw=SterilizedWhite sawdust inoculated with *Penicillium* species, Ew=Sterilized white sawdust inoculated with both organisms.

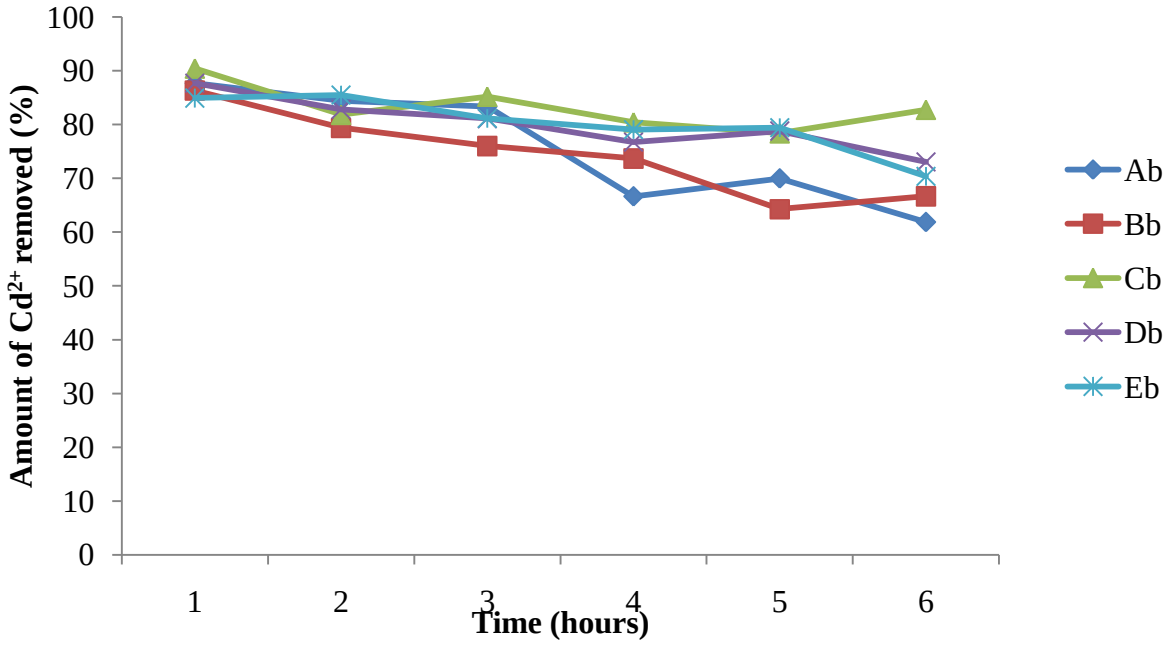


Figure 4.8: Effect of contact time on Cadmium removal by brown sawdust sorbent over six hour's period

KEY

Ab=Unsterilized brown sawdust, Bb= Sterilized brown sawdust, Cb =Sterilized brown sawdust inoculated with *A. niger*, Db =Sterilized brown sawdust inoculated with *Penicillium* species, Eb =Sterilized brown saw dust inoculated with both organisms

4.6 Heavy Metal Ions Uptake by Both White and Brown Wood Sorbent

The amount of Pb^{2+}, Ni^{2+} and Cd^{2+} sorbed in white and brown wood sorbents are presented in Figures 4.9 and4.10.The result revealed that white sorbent had the highest amount of uptake irrespective of the metals compared to brown wood sorbent. In white wood sorbent, treatment A sorbed 1.469mg/g of lead, B-1.47mg/g, C-1.41mg/g, D-1.42mg/g and E-1.39mg/g of lead with treatment B giving the highest uptake and E the least uptake. Ni (treatment A sorbed 0.11mg/g, B= 0.11mg/g, C= 0.08mg/g, D= 0.088mg/g, E=0.097mg/g). Cd (treatment A sorbed 0.048, B= 0.048, C= 0.047, D= 0.051, E= 0.051mg/g) with treatmentC having the lowest adsorption (Figure 4.9). The range of amount sorbed of pb^{2+}, Ni^{2+} and Cd^{2+} was 1.39-1.47mg/g, 0.08-0.11mg/g and 0.047-0.051mg/g respectively. The average uptake of the metal ions across the treatments was Pb 1.43mg, Ni 0.097mg/g and Cd 0.049mg/g in white saw dust sorbent.

However in brown wood Pb (treatment A= 1.18, B= 1.21, C= 1.15, D= 1.21, E= 1.26mg/g), Ni (treatment A= 0.082, B= 0.079, C= 0.069, D= 0.093 and E= 0.078mg/l), Cd (A= 0.038, B= 0.035, C= 0.043, D= 0.04 and E= 0.04mg/l) (Figure 4.10). The range of amount sorbed of Pb^{2+}, Ni^{2+} and Cd^{2+} was 1.15-1.26mg/g, 0.069-0.093mg/g and 0.035-0.043 mg/g respectively and the average uptake of the metals was Pb 1.20mg/g, Ni 0.08mg/g and Cd 0.039mg/g.

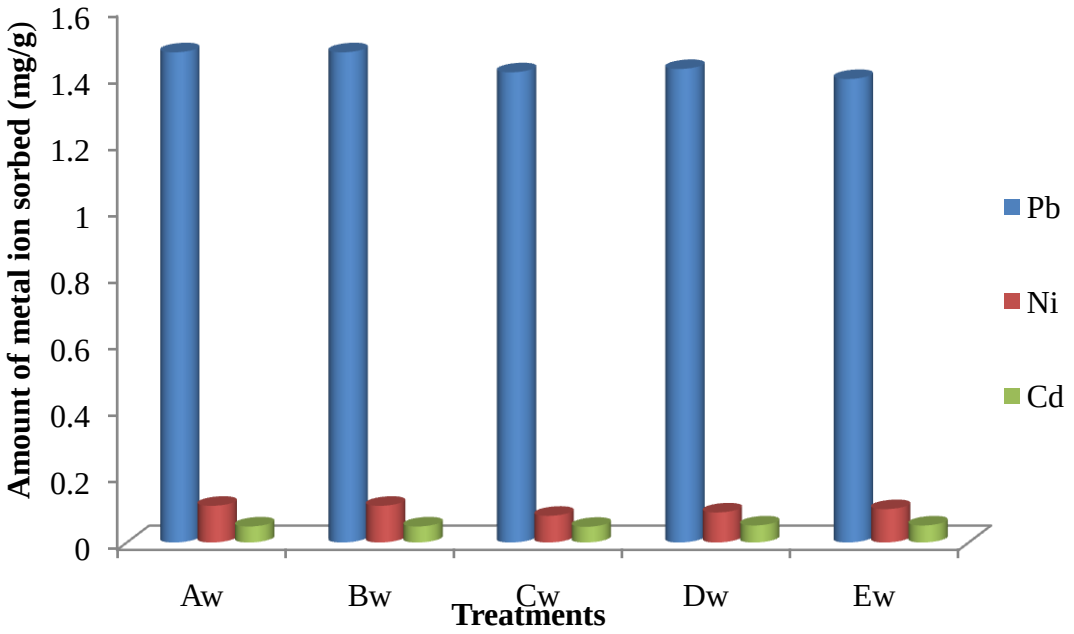


Figure 4.9:Uptake of Pb, Ni and Cd ions by white sorbents after six hours

KEY

Aw=Unsterilized White sawdust, Bw= Sterilized White sawdust, Cw=SterilizedWhite sawdust inoculated with *A. niger*, Dw=SterilizedWhite sawdust inoculated with *Penicillium* species, Ew=Sterilized white sawdust inoculated with both organisms.

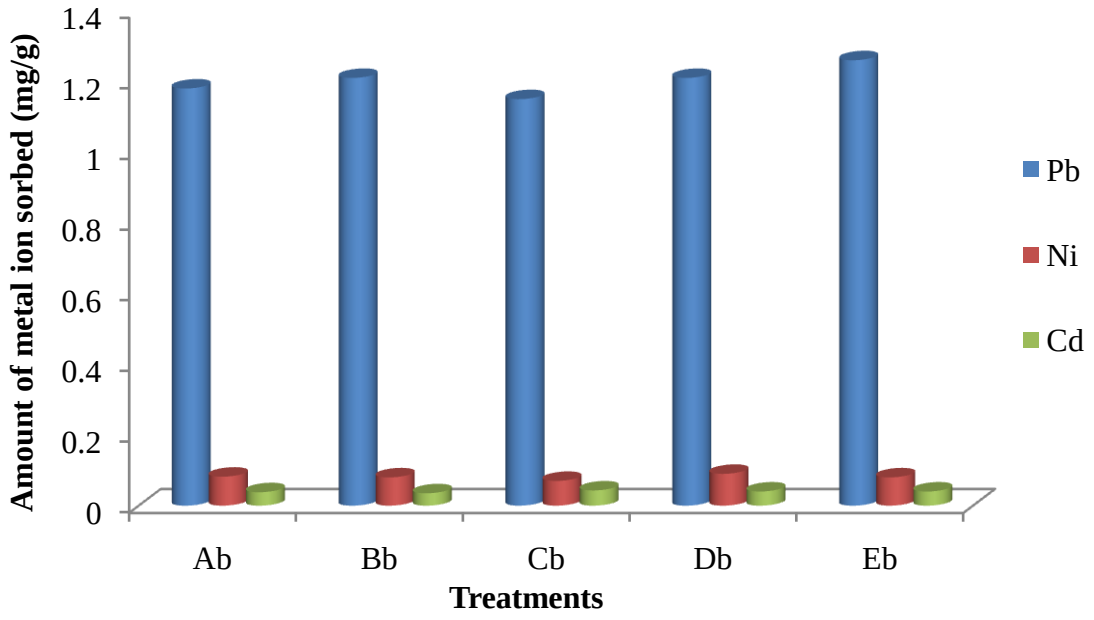


Figure 4.10:Uptake of Pb, Ni and Cd ions by brown sorbents after six hours

KEY

Ab= Unsterilized brown sawdust, Bb= Sterilized brown sawdust, Cb=Sterilized brown sawdust inoculated with *A. nige*, Db=Sterilized brown sawdust inoculated with *Penicillium* species, Eb=Sterilizedbrown sawdust inoculated with both organisms

4.7 Relative Affinity of Each Metal Ion to the Sorbents

Data gathered for the Langmurian coefficient b of each metal ion is as shown in Figures4.11 and 4.12. The results showed that Lead with the highest value of quantity adsorbed (Q) has the lowest langmurian coefficient (b) values across the treatment and between the sorbents followed by Nickel and lastly Cadmium. However, it was observed that the treatments varied in their affinity to the metal ion. For Pb in white wood, treatment A and B had the lowest b value of (0.074) and treatment E had the highest (0.077). For Ni,treatment B had the lowest b value of (0.80) while the highest was observed in treatment C(0.98).For Cd, the highest b value was found in treatment C (2.05) and the Lowest was found in treatment D and E(1.96).Whereas in brown wood, Pb (treatmentC had the highest (0.09)and lowest was observed in treatment E (0.084). Ni (highest was observed in treatmentC=1.09, lowest in treatmentD=0.91) while Cd (lowest and highest b value was observed in treatment C (2.19) and B (2.46). Usually, the lower the b value, the higher the affinity of the sorbent (sawdust) to the sorbate (metal ion).

4.8 Performance Comparison of the experimental Sorbents

Analysis of the performance of white and brown wood sawdust in the removal of the metal ions (Table 4.4) revealed that, all the treatment on the white wood sawdust had a higher percentage removal of all metal ion 86.77% compare to brown wood with percentage removal of 80.11% with p-value less than 0.05 ($P<0.05$).

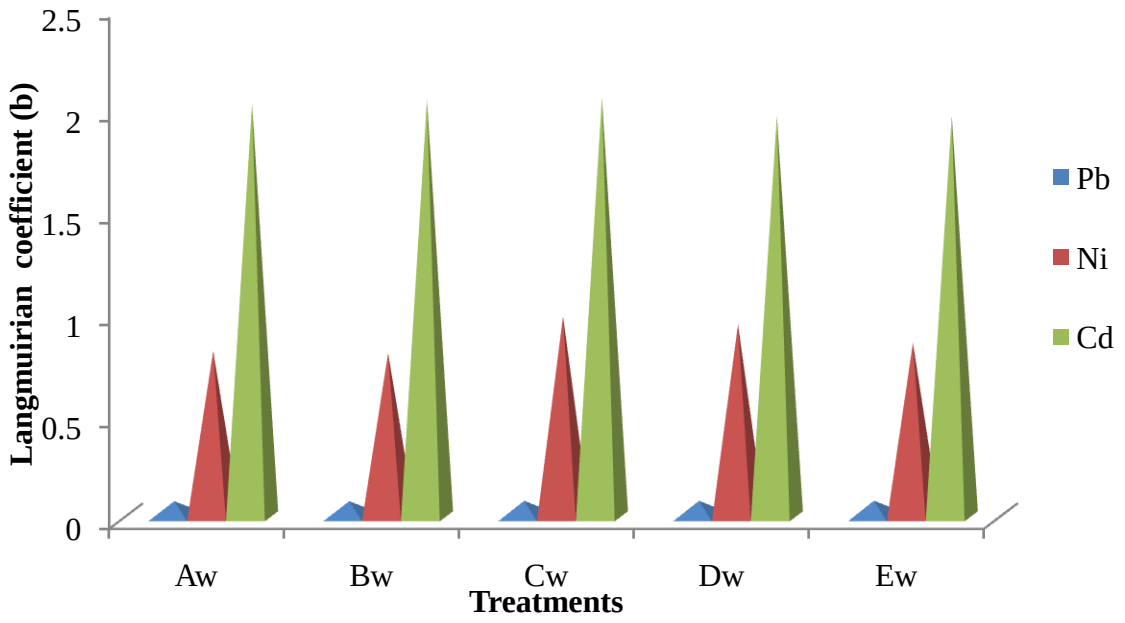


Figure 4.11: Relative affinity of white sawdust sorbent for the metal ions

KEY

Aw=Unsterilized White sawdust, Bw= Sterilized White sawdust, Cw=SterilizedWhite sawdust inoculated with *A. niger*, Dw=SterilizedWhite sawdust inoculated with *Penicillium* species, Ew=Sterilized white sawdust inoculated with both organisms.

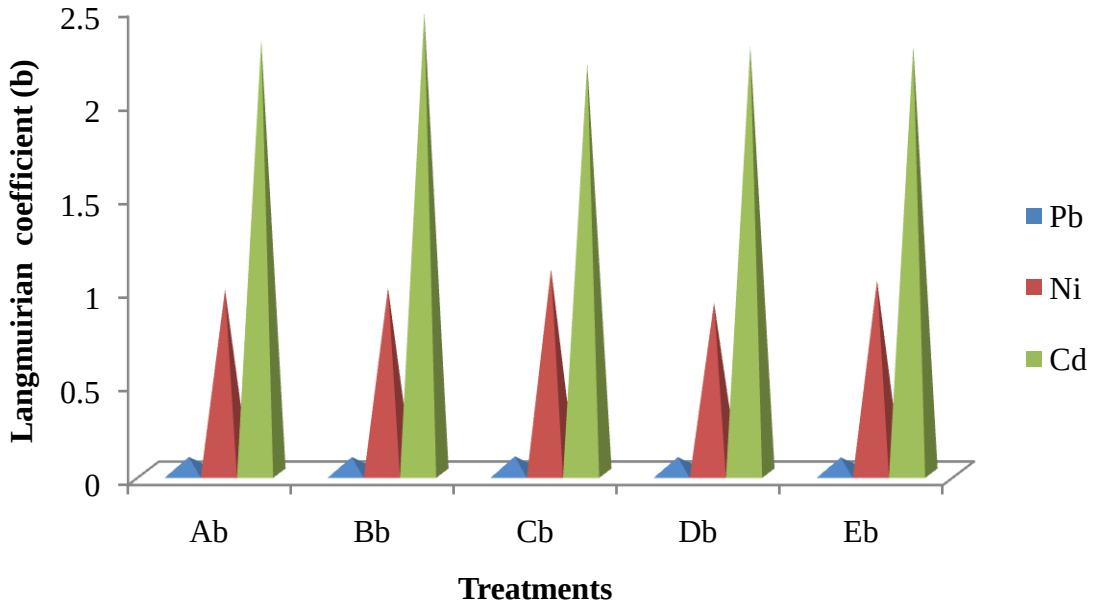


Figure 4.12: Relative affinity of the brown saw dust sorbents for the metal ions

KEY

Ab= Unsterilized brown sawdust, Bb= Sterilized brown sawdust, Cb=Sterilized brown sawdust inoculated with *A. nige*, Db=Sterilized brown sawdust inoculated with *Penicillium* species, Eb=Sterilizedbrown sawdust inoculated with both organisms

Table 4.4: Mean Binding Performance of both White and Brown wood

	White wood	Brown wood
Unsterilized wood	86.42±15.72	78.67±14.53
Sterilized wood	90.52±8.82	77.51±17.72
Sterilized + *A.niger*	84.89±15.21	81.63±11.86
Sterilized + *Penicillium spp*	84.76±16.78	81.66±13.42
Sterilized + both orgs	87.28±13.69	81.09±13.16

P=0.00* F-value 19.12 df=1 Mean ±SD; ANOVA: DMRT

CHAPTER FIVE

5.0 DISCUSSION

Proximate composition of the wood sorbents revealed that, both wood samples (white and brown wood) have components such as cellulose, hemicellulose, lignin and other sugars in varying amounts (Table 4.1). These components contain polar functional group (Naja and Volesky, 2011) that are capable of binding metal ions either by replacement of hydrogen ions with metal ions or by donation of an electron pair from these groups to form complexes with metal ions in solution (Rishipal, 2002).The presence of these components confers on them the potential to support the growth of the fungi and bound metal ions.White wood saw dust was observed to have more of cellulose, hemicellulose, carbohydrate, protein and fat content and lesser moisture, ash and lignin content compared to brown wood saw dust.This difference is expected because the proportion of various constituents of biomass materials can differ greatly among biomass categories and also from species to species (Harman *et al*., 2007). The value for cellulose, hemicellulose and lignin content compares to the work of Hashem *et al*. (2007) that used sawdust treated with polyamine and reported cellulose-50%, hemicellulose-17%and lignin-30%and Soliman *et al*. (2011) that reported ligin-18%, cellulose-45% and hemicellulose-28% from sugarcane bagasse. Our values also agree with the work of Manjunathan and Kaviyarasan, (2011) that reported carbohydrate (58.05%), Protein (25%), fibre (14%), Fat (1.54%), moisture (12.51%) and ash (5.14%). Osakwe, (2014) reported ash to be 4.48% and moisture 7.29%.The moisture content of this study for the two sorbents was found to be low compare to the above report butis favorable. According to (Adie *et al*., 2012), the lower the percentage moisture, the better the yield and quality of the sorbent. The ash content gives an idea of the measure of mineral content such as chloride, magnesium and sodium of the carbon source (Adie *et al*.,

2012).So the result obtained from this study is favorable because the ash serves as interference during the adsorption process, the lower the ash the better the material.

The physical and chemical analysis of the refinery effluent indicated the presence of high level of chemical oxygen demand and phosphate above the allowable limit set by NESREAstandard, (2007). Chemical Oxygen Demand (COD) is a measure of all chemically oxidizable material present in the effluent (Siyanbola *et al*., 2011). The high level of COD could be attributed to the presence of organic and inorganic substances in the effluent resulting from the thermal or catalytic cracking processes in process waste water plant and may also be due to large quantity of non-process waste water (Otukunefor and Obiukwu, 2005). The operation in the treatment plants and treatment plant performance could also cause increase in COD level (Longe and Ogundipe, 2010). The COD value (874.1mg/L) of this study also compares with the range of value (615-1254.50mg/L) reported by Siyanbola *et al*. (2011). Phosphate is an essential nutrient to plant life, but when found in excess quantities, stimulates algal bloom (Igbinosu and Oko, 2009). Release of effluent containing high phosphate level may lead to excessive growth of algae in the receiving water body rendering the water smelly and unattractive.Also when such effluent is consumed, it may lead to hyperphosphatemia in humans. Similarly the high levels of Pb (1.587mg/L), Ni (0.2mg/L) and Cd (0.073mg/L) ionsobserved in this study as against the acceptable limits given by NESREA. This high level could have arisen from the corrosion products of equipment and pipes, chemical additives and catalyst used in petroleum refining processes (Beddri and Ismail, 2007) and could pose significant risk to public health especially when large volume of the effluent is continuously released into the environment. Adverse effects from the discharge of such effluents includes reduction in soil fertility (Ezeonuegbu *et al*., 2014) and neurological dysfunction, Skin dermatitis and pulmonary odema in humans (Borba *etal*.,

2006; Deng *et al*., 2006; Ansari and Malik, 2007). This report agrees with the findings of Ezeonuegbu *et al*.(2014)that also reported high levels of the same metal ions from the same Kaduna refinery (Pb, 0.11, Ni 0.22 and Cd 0.02mg/l).

Other parameters such asDO, BOD, Oil and grease, nitrate, sulphate, pH, EC, TDSand turbidity were all below the set limits by NESREA. The level of sulphate (73.2mg/L) was within the permissible limit which means the effluent when released into the water bodies may be consumed without any poisonous effect and the taking up of oxygen is possible (Chris-Otubor and Olorunfemi, 2015). The DO (2.9mg/L) level indicates that the oxidation may have been used during the treatment process or living organisms are present in the effluent which led to the decline of the dissolved oxygen levels (Chris-Otubor and Olorunfemi, 2015). The low BOD_5(1.2mg/L) of the effluent which was derived from the DO value signifies that the organic matter in the effluent had minimal demand for oxygen (Sharma, 2004). The low nitrate (1.67mg/L) content prevents excessive growth of aquatic plants and algae and also formation of methemoglobinemia if ingested (Addiscott and Benjamin, 2004).These results aresimilar to the report of Ezeonuegbu *et al*., (2014) that reported (BOD 3.10mg/l, DO 2.93mg/l, Oil and grease 0.77mg/l,and temperature 29.53^{o}C) from the same refinery effluent. Electrical conductivity is a measure of salts, ions or impurities present in a sample and the low value of EC (717µS/cm) obtained from this study suggests that the effluent has low concentration of cations and anions from all chemicals used in primary distillation process in refinery industries.When an effluents containing low or moderate level of BOD, DO, Oil and grease, temperature, Ph, sulphate, nitrate and turbidity is released into the surface water bodies, the ability of the water to support aquatic lifeis higher (Odesiri-Eruteyan *et al*., 2017).

The sawdust biomass proved to contain many sugars which are converted into sources of carbon and energy as lignocellulosic components are decomposed and utilize by*A. niger* and *Penicillium*species but theresult revealed that the extent of decomposition of the sorbents by the organisms waslow (Table 4.3) and this could be as a result of the inability of the organisms to effectively degrade the ester and ether linkages between the carbohydrate polymer (Hemicellulose and cellulose) and the aromatic polymer (lignin) of the wood to release the sugars (US Dept of Energy, 2006). The heterogenous structure and irregular arrangement of phenylpropanol polymer of lignin resist chemical or enzymatic degradation to give plant their structural rigidity and help to protect the cellulose and hemicellulose from degradation (Perez *et al*., 2002; Vetayasuporns, 2006). However the white wood sorbent still showed higher ability to support the growth of the organisms than the brown wood probably due its low lignin content and large surface areadue to its higher protein, carbohydrate, and fat content earlierreported (Table 4.1) which is also another source of sugar. This result agrees with the report of Badu *et al*. (2011) that,among the wood species analysed for their mushroom yield, *Triplochiton scleroxylon* which had highest cellulose (46.76%) and lowest lignin (27.55%) gave the highest yield of mushroom (334g) compared to *Ceiba pentandra* with the lowest cellulose (44.79%) and highest lignin content (34.08%) which yielded 193g ofmushroom.

The study of treatment effect on the percentage removal capacity of the sawdust revealed that the un-inoculated columns had the highest percentage removal for lead and nickel in white wood sorbent whereas in brown wood sorbent, the inoculated treatments had the highest percentage removal for all the metals. Cadmium removal was all through favored in the inoculated treatments. The average percentage removal of each metal ion was found to be higher in white wood sorbent than brown wood.The differencecould be attributed to the metal type and overall

net charge of the biomass or the concentration of surface group responsible for adsorption which together facilitates the quick binding and removal of the metal (Dakiky *et al*., 2004; Mahvi *et al*.., 2005b; Farooq *et al*., 2010). This result from white sorbent is similar to the 99% of Pb and 94.5% of Cd reported by Samarghandi *etal*. (2006) while that of brown wood sorbent compares to 94% of Pb and 76% of Ni reported by Mahvi *et al*. (2005b) and 75% of Cd reported also by Nwankwo*et al*. (2014).The difference could be as a result of the difference in sorbents usedand method of treatment of the sorbent. It is worth to emphasize that sorbent used in this work has not been modified chemically which is assumed to increase adsorption rate and may successfully compete with other sorptive materials (Mane *et al*., 2011).

Contact time is an important factor in the process of evaluation of saw dusts potential. The progressive increase in removal of the metals in most figures with time till equilibrium was attained anda later decrease agrees with the findings of Adie *et al*. (2012). This can be explained by the fact that initially, a large number of sorption sites were available and as the sorption process continued, much of the available sites were used up which slowed the process down, later due to exhaustion of remaining sites and repulsive forces developed between solute molecules and bulk phase leads to decrease in the process(Saravanane *et al*., 2002).Figures 4.5 and 4.7 showed an irregular pattern of removal with time and this can be explained by the fact that desorption and sorption may be taking place simultaneously and it could also be due to the inability of the sorbent to continue holding on to the sorbate as changes take place in the medium. Also, the competition among the metal ions for the same binding could result in displacement of already sorbed metal (Mahavi, 2005a).The high percentage removal for the metals over service time is related to the nature of the sorbent and the great accessibility of the binding site present in the sorbents which together affected the time needed to reach equilibrium.

The shorter time of service observed in brown wood is an indication of early material usage due to lesser binding sites (Sai *et al*., 2012). Values obtained as a result of the experiment carried out are relatively higher compared to those of Amuda *et al*. (2007) that reported 30 minutes into the service time for lead adsorption and Mahvi *et al*. (2005b) reported 45 minutes for lead but compared to 70 minutes for cadmium reported by Nwankw *et al*. (2014). However this result is similar to the report of Iyagba and Opete (2009) that attained equilibrium after 2hours.

Uptake of metal ion to biosorbent is characterized initially by physical adsorption, surface precipitation, ionic exchange and chemical reaction with surface sites (Tor-resdey *et al*., 2004) and the rate of uptake is dependent on the availability of the overall electronegative charges on the sorbent which in turn can display several binding sites (Naja and Volesky, 2011). Our investigations revealed that both sorbent had maximum uptake capability for the cationic metal ions, as shown in Figures 4.9 and 4.10 but variation is seen in the amount of metal ion binding to each of the biosorbent. Thisdifference in uptake depends on metal type (Mahavi, 2005) and competition between the cations for the available sites on the sorbent. Parameters such as initial concentration of the adsorbate, presence of other metals and surface area of the adsorbent made comparative investigation of uptake of individual heavy metal ions in the refinery effluent difficult. The present study revealed that the order of adsorption is Pb>Ni>Cd which contradict the works of Soliman *et al*. (2011) and Nwankwo*et al*.(2014) that reported Pb<Cd>Ni. The difference could have resulted from difference in initial metal ion concentration and difference in other components of the effluent used. Difference in cell wall composition between different sorbents, and the intra-group differences, can cause measurable variation in the type and amount of metal ion binding to them (Dhankhar and Hooda, 2011).

Affinity shows the relative interaction between the sorbent to the sorbate and the value of affinity b, is inversely proportional to quantity adsorbed (Q). The lower the b value the higher the affinity of the sorbent to the metal (Kratochvil and Volesky, 1998). The result of affinity of the sorbent for the metal ions (figure 4.11 and 4.12)revealed that lead has the lowest b value suggesting highest affinity, followed by nickel and cadmium has the highest indicating lowest affinity. The decrease in b value signifies the exothermicity of the adsorption process (Shaker, 2007) while the opposite trend indicates that the process requires thermal energy (endothermic) leading to chemisorption (Vilar *et al*., 2008). For 'good' sorbents in general, one must look for a high *Q*and a low value of Langmuir parameter *b*.

The ideal adsorbent should have huge appetite for a broad range of metal ions, binding them quickly, tightly and dependably (Hubbe *et al*., 2011). The maximum ability of any sorbent to sequester metals to its surface depends on the type and net charge of the biomass or the concentration of surface group responsible for the adsorption (Bux and Kasan, 1994; Dakiky, *etal*., 2004). It followed that the higher the biomass electronegativity, the greater the attraction and adsorption of heavy metal cations.The result of the statistics (Table 4.4) revealed that the capacity of the wood were 86.77±1.08 and 80.11±1.08 for the white and brown wood respectively with a P-value of 0.00*. This agrees with ourearlier findings that white wood with the highest negative charge as a result of more proteins, carbohydrate, fats and hemicellulose and less lignin and ash shown in Table 4.1 earlier demonstrated superior bio-sorption clearly shown figures 4.1 and 4.2. However it is evident that both are potential sorbent candidates for treatment of Wastewater containing these metals ions.The result of the experiment agrees with the assertion made by Nasim *et al*., (2004) that wood saw dusts has the potential of removing Pb, Ni and Cd ions from wastewater like many other agricultural waste products.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

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

1. The proximate and lignocellulosic components of the wood sorbent showed that white wood had more carbohydrate, protein, cellulose, hemicellulose and less moisture, ash and lignin compared to brown wood saw dust.
2. Physicochemical analysis of the Kaduna refinery effluent revealed high levels of E.C, COD, Phosphate, Sulphate, Pb, Ni and Cd beyond the permissible limits by FMENV indicating serious waste water quality impairment.
3. The percentage decomposition of 12.31% and 11.49% for white and brown wood sawdust respectively were observed suggesting that the wood sawdust could serve as source of carbon and energy to the fungi.
4. Investigation revealed that the sterilized sorbent without inoculation has the highest percentage removal and uptake capability for Pb^{2+} and Ni^{2+} in white wood sorbent while treatment with both organisms had highest removal for Cd^{2+}ions.
5. In brown wood sawdust, the treatments with inoculation gave the highest removal and uptake of each metal ios.
6. There were statistically insignificant differencesacross the treatments in the removal ofeach metal ion.
7. The equilibration time for the removal of the metal ions in both sorbents was found to be after thefirst hour for Pb and at before the first hour for Ni and Cd.

8. All the treatments in both sorbents showed highest affinity for lead followed by nickel and least affinity for cadmium.
9. White wood sawdust was found have the highest removal and uptake capability for lead, nickel and cadmium ions compare to brown sawdust and the difference was statistical significant ($P<0.05$).

6.2 Recommendations

1) Untreated White wood sawdust is recommended for use as a suitably cheap and economical sorbent for the removal of lead and nickel ions from wastewaters containing them through fixed bed column adsorption technique.
2) Treatment of any wood sawdusts with *Aspergillus niger*is recommended for use in the removal of cadmium ion.
3) Enhancement of brown wood sawdust with *Aspergillus niger* and *Penicillium*species either single or as dual culture is recommended for the maximum uptake of Pb^{2+} and Ni^{2+} and Cd^{2+} ions.
4) Studies on desorption and regeneration of sawdust under different conditions should be investigated to assess its reuse in further cycles and feasibility of recovery of the adsorbed metals.
5) Further research should be aimed atvarying the operational conditions such as adsorbent dose and initial metal ion concentration to ascertain the optimum condition for sorption of Pb, Ni and Cd ions.
6) Regulatory authorities should ensure that industries keep strictly to the recommended standard of the levels of heavy metals in effluent released into the environment.
7) Water from the effluent receiving water body should be thoroughly treated to ensure that the levels of these metals are brought to levels recommended for safe use.

REFERENCES

Addiscott, T.M. and Benjamin, N. (2004). Nitrate and human health. *Soil Use and Management*, **20**(2):98-104.

Adewuyi, G.O. and Olowu, R.A. (2012). Assessment of oil and grease, total petroleum NNPC oil depot in apata, Ibadan metropolis, Nigeria. *International Journal of Review and Revised Applied Sciences*, **13**(1):166-174.

Adie, D.B., Okuofu, C.A., and Osakwe, C. (2012). Comparative analysis of the adsorption of heavy metals in waste waters using *Borassus aethiopum* and *Cocos nucifera*. *InternationalJournal of Applied Science and Technology*, **2**(7):314-322.

Agarwal, G.S., Bhuptawat, H.K., and Chaudhari, S. (2006). Biosorption of aqueous chromium(vi) by *Tamarindus indica* seeds. *BioresourceTechnology*, **97**:949-956.

Agoyi, O.R. (2002). The effect of Kaduna refining and petrochemical company effluent on Romi stream. Bachelor of science degree thesis, Department of Water resources and Environmental Engineering, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Ahalya, N., Ramachandra, T.V, and Kanamadi, R. D. (2003). Biosorption of heavy metals. *Research Journal of Chemistry and Environment*, **7**:71-78.

Ahalya, N., Ramachandra, T.V, and Kanamadi, R. D. (2005). Biosorption of Cr (VI) from aqueous solutions by the husk of Bengal gram. *Electronic Journal of Biotechnology*, **8**:258-264

Ahluwalia S.S. and Goyal, D. (2007). Removal of heavy metals by waste tea leaves from aqueous solution. *Engineering in Life Sciences*, **5**(2):158-162.

Ahluwalia, S.S. and Goyal, D. (2005b). Micobial and Plant derived biomass for removal of heavy metals from wastewater. *Bioresource Technology*,**98**:2243-2257.

Ahmed, G., Miah, M.A., Anawar, H.M., Chowdhury, D.A. and Ahmed, J.U. (2011). Influence of multi-industrial activities on trace metal contamination: an approach towards surface water body in the vicinity of Dhaka Export Processing Zone (DEPZ). *EnvironmentalMonitoring and Assesment*, **112**:1-10.

Ajmal, M., Khan, A.H., Ahmad, S. and Ahmad, A. (1998). Role of sawdust in the removal of copper (ii) from industrial wastes. *Water Resources*, **32**(10):3085-3091

Akan, B.W, Olusegun, O.A. and Adekunle, F.O. (2009). Trace metals levels in fruits juices and carbonated beverages in Nigeria. *Environmental Monitoring Assessment*, **154**(1-4):303-306.

Aksu, Z. (1992). The biosorption of cu (ii) by C vulgaris and Z. ramigera. Environmental Technology, **13**:579-586.

Aksu, Z. and Acikel, U. (2007). Modelling of a single-staged bioseparation process for simultaneous removal of iron(III) and chromium(VI) by using chlorella vulgaris. *Biochemical Engineering Journal*,**4**:229-238.

Alao, O., Arjojoye, O., Ogunlaja, O. and Famuyiwa, A. (2010). Impact assessment of brewery effluent on water quality in Majawe, Ibadan, South Western Nigeria. *Reseacher*, **2**(5):21-28.

Alluri, H.K., Srinivasa, R., Ronda, H.K., Vijaya, S. S., Jayakumar, S.B., Suryanarayana,V. and Venkateshwar, P. (2007). Biosorption: An eco-friendly alternative for heavy metal removal. *African Journal of Biotechnology*. **6**(25):2924-2931.

Al-Rub, F.A.A. (2006). Biosorption of zinc on palm tree leaves: equilibrium, kinetics and thermodynamics studies. *Separation Purification Technolnology*, **41**:3499-3515.

Al-Rub, F.A.A., El-Naas, M.H., Ashour, I. and Al-Marzouqi, M. (2006). Biosorption of copper on *chlorella vulgaris* from single, binary and ternary metal aqueous solutions. *Process Biochemistry*, **41**:457-464.

Altas, L. and Büyükgüngör, H. (2008). Sulfide removal in petroleum refinery wastewater by chemical precipitation. *Journal of HazardousMaterial*, **153**:462-469.

Amana, T., Kazi, A.A., Sabri, M.U. and Banoa, Q. (2008). Potato Peels as Solid Waste for the Removal of Copper (II) from Wastewater/industrial Effluent. *Colloids surf. B: Biointerfaces*, **63**:116-121.

Amany, H., Hameed, A.E, Wedad, E.E., Khadija, A.A., Abou, T. and Mira, H.I (2015). Biosorption of uranium and heavy metals using some local fungi isolated from phosphatic fertilizers. *Annals of Agricultural Sciences*, **60**:345-351.

Amar, M.C., Steve, P.M., Keneneth, E.G., Egbert, R. and Dieter, R.S. (1993). Enumeration of indigenous *Rhizobium leguminosarum biovar Trifolii* in soils previously treated with metal contaminated sewage sludge. *Soil biology and biochemistry*. **25**(3):301-309.

American Public Health Association (APHA) (2012). Standard Methods for the Examination of Water and Wastewater, 22nd edition. APHA/AWWA/WPCF, New York, **5**:24-26.

Amuda, O.S., Ojo, O.I. and Edewor, T.I. (2007). Biosorption of Lead from Industrial Wastewater Using *Chrysophyllum albidum* seed shell. *Bioremediation Journal*, **11**:183-194.

Ansari, M.I and Malik, A. (2007).Biosorption of nickel and cadmium by metal resistantbacterial isolates from agricultural soil irrigated with industrial wastewater. *BioresourceTechnology*,**98**:3149-3153.

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

Argun, M.E. and Dursun, S. (2008). A new approach to modification of natural adsorbent for heavy metal adsorption. *Bioresource Technolnology*, **99**:2516-2527.

Arica, M.Y., Tuzun, I., Yalcin, E., Ince, O. and Bayramoglu, G. (2005). Utilization of native, heat and acid-treated microalgae Chlamydomonas reinhardtii preparations for biosorption of Cr (Vi) ions. *Process Biochemical*, **40**(7):2351-2358

Atlas, R.M. and Philip, J. (2005). Applied Microbial Solutions for Real–World Environmental Cleanup. ASM press, Washington, DC.

Attiogbe, F.K., Glover-Amengor, M. and Nyadziehe, K.T. (2007). Correlating biochemical and chemical oxygen demand of effluents—a case study of selected industries in Kumasi, Ghana. *WestAfrican Journal Applied Ecology*, **11**:110-118.

Aydin, A.H., Bulut, Y. and Yavuz, O. (2004). Acid Dyes Removal using Low Cost Adsorbents. *International Journal of Environmental Pollution*, **21**:97-104.

Aziz, H.A. Adlan, M.N. and Ariffin, K.S. (2008). Heavy metals (Cd, Pb, Zn, Ni, Cu and Cr (III)) removal from water in Malaysia: post treatment by high quality limestone. *Bioresourcetechnology*, **99**:1578-1583.

Azouaou-Naima, Z.S and Hassiba, M. (2014). Removal of lead from aqueous solution unto untreated coffee grounds: a fixed column study. *Chemical Engineering Transaction*, Vol.38.

Babak, L., Supinova, P., Zichova, M., Burdychova, R. and Vitova, E. (2012). Biosorption of Cu, Zn and Pb by thermophilic bacteria- effect of biomass concentration on biosorption capacity. *Acta Universitatis Agriculturae etSilviculturae Mendelianae Brunensis*, **LX(5)**:9-18.

Babel, S. and Kurniawan Ta. (2003). Low cost adsorbents for heavy metals uptake from contaminated water: A review. *Journal of Hazardous Materials*, **97**:219-243.

Babu, B.V. and Gupta, S. (2008). Adsorption of Cr(VI) using activated neem leaves: kinetic studies.*Adsorption*,**14**:85-92.

Badu, M., Slyvester, K.T. and Nathaniel, O.B. (2011). Effects of lignocellulosic in wood used as substrate on the quality and yield of mushrooms. *Food and Nutrition sciences*, **2**:780-784.

Bai, S. and Abraham, T.E. (2001). Biosorption of chromium (VI) from aqueous solution by Rhizopus nigricans. *Bioresource Technology*, **79**:73-81.

Baik, W.Y., Bae, J.H., Cho, K.M. and Hartmeier, W. (2002). Biosorption of heavy metals using whole mold mycelia and parts. *Bioresource Technology*, **81**:167-170.

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

Barakat, M.A. (2011). New Trends in Removing Heavy Metals from Industrial Wastewater-ScienceDirect. *Arabian journal of Chemistry*, **4**(44):361-377.

Barnett, H.L. and Hunter, B.B. (1999). *Illustrated genera of imperfect fungi*. Fourth edition. Prentice Hall Inc.

Barros, L.M., Macedo, G.R., Duarte, M.M.L., Silva, E.P. and Lobato, A.K.C.L. (2005). Biosorption of cadmium using the fungus Aspergillus niger. *Brazilian Journal of ChemicalEngineering*, **22**:319-322.

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

Bedell, G.W. and Darnall, D.W. (1990). Immobilization of nonviable, biosorbent, algal biomass for the recovery of metal ions. In: *Biosorption of Heavy Metals*, B. Volesky, ed. 313-326, Boca Raton, FL: CRC Press.

Bekada, A.M.A., Benakriche, B., Hamadi, k. and Bensoltane, A. (2008). Modelling of Effects of Water activity, pH, and Temperature on the growth rate of *Mucor racemosus* isolated from Camembert Cheese. *World Journal of Agricultural Science*, **4**(6):790-794.

Bekker, T.F., Kaiser, C. and Labuschage, N. (2009). The antifungal activity of potassium silicate and the role of pH against selected plant pathogenic fungi in vitro. *South African JournalPlant Soil*, **26**(1):55-57.

Beneke, E.S., Rogers, A.L. (1980). Medical Mycology Manual. Fourth Edition, pp 11-13.

Bhattacharya, A.K., Mandal, S.N. and Das, S.K. (2006). Adsorption of Zn(II) from aqueous solution by using different adsorbents. *Chemical Engineering Journal*, **123**:43-51.

Biochemical Engineering Journal, **4**:229-238.

Bishnoi,N.R. and Garima, R (2005). Fungus for bioremediation of heavy metals wastewater. *Journal of*Science *and Industrial Resource*, vol **64.**

Bishnoi, N.R and Garima, R. (2004). Fungus- An alternative for bioremediation of heavy metal containing wastewater. A review. *Journal of Scientific and Industrial Research*. **64**:93-100.

Bishnoi, N.R., Bajaj. M., Sharma, N. and Gupta, A. (2003). Adsorption of Cr (VI) on activated rice husk carbon and activated alumina. *Bioresources Technology*, **91**:305-307.

Blazquez, G., Hernainz, F., Calero, M. and Ruiz-Nunez, L.F. (2005). Removal of cadmium ions with olive stones: the effect of some parameters. *Proc. Biochemistry*, **40**(8):2649-2654.

Borba, C.E., Guirardello, R., Silva, E.A., Veit, M.T. and Tavares, C.R.G. (2006). Removal of nickel(II) ions from aqueous solution by biosorption in a fixed bed column:experimental and theoretical breakthrough curves. *Biochemistry Engineering Journal*, **30**:184-191.

Bruins, M.R., Kapil, S. and Oehme, F.W. (2000). Microbial resistance to metals in the environment. *Exocitology and environmental safety*, **45**:198-207.

Burgess, J.E. and Almeida, L.K.S. (2012). Biosorption and bioaccumulation of copper and lead by *Phanaerocheate chrysosporim* and *Pleurotus ostreatus*. Department of Biochemistry, *Microbiology and Biotechnology*, Rhodes University, P.O. Box 94, Grahams Town, 6140, South Africa.

Bux, F. and Kasan, H.C. (1994). Comparism of selected methods for relative assessment of surface charge on waste sludge biomass. *Water SA*, **20**(1):73-76.

Chang, J.S., Law, R., and Chang, C.C. (1997). Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. Water Research, **3**1(7):1651-1658.

Chekli, L., Eripret, C., Park, S.H.,Tabatabai, S.A.A., Vronska, O., Tamburic, B., Kim, J.H. and Shon, H.K. (2017). Coagulation performance and floc characteristics of polytitanium tetrachloride compared with titanium tetrachloride and ferric chloride in algal turbid water. *Separation and Purification Technology*, **175**:99-106.

Chen, C. and Wang, J.L. (2007). Correlating metal ionic characteristics with biosorption capacity using QSAR model, Chemosphere, **69**(10):1610-1616.

Choi, S.B. and Yun, Y.S. (2006). Biosorption of cadmium by various types of dried sludge: an equilibrium study and investigation of mechanisms. *Journal of Hazardous Materials*, B**138**:378-383.

Chris-Otubor, G.O. and Olorunfemi, P. (2015). Evaluation of some industrial effluents in Jos metropolis, Plateau State, Nigeria. *African Journal of Enviromental Science and Technology*, **9**(6):566-572.

Clair, N.S., Perry, L.M. and Gene, F.P. (2003).*Chemistry for Environmental Engineering and Science*, (5th ed.). New York: McGraw–Hill. ISBN 0–07–248066–1

Cooney, J.J. (1984). The fate of petroleum pollutants in fresh water ecosystems. In: *Petroleummicrobiology*, R.M. Atlas, Ed., pp 399-434. Macmillian, New york, NY, USA.

Cui, J.R. and Zhang, L.F. (2008). Metallurgical recovery of metals from electronic waste; A review. *Journal of Hazardous Materials*, **158**(2-3):228-256.

Cullen, D. and Kersten, P.J. (2004). " The Mgota III, *Biochemistry and Molecular Biology*, Springer-verlag, Berlin. 249-273.

Dakiky, M., Khamis, M., Manasora, M., Mareb, M. (2004). Selective adsorption of Chromium (IV) in industrial wastewater using low cost abundantly available adsorbent. *Advanced Environmental Resource*,**6**:533-540.

Das, N., Vimala, R., and Karthika, P. (2008). Biosorption of heavy metals- An overview. *IndianJournal of Biotechnology*, **7**:159-169.

Deepa, K.K., Sathishkumar, M., Binupriya, A.R., Murugesan, G.S., Swaminathan, K. and Yun, S.E. (2006). Sorption of Cr (VI) from dilute solutions and wastewater by live and pretreated biomass of *Aspergillus flavus*. *Chemosphere*, **62**(5):833-840.

Deng, L., Su, Y., Su, H., Wang, X. and Zhu, X. (2006). Biosorption of copper(II) and lead(II) from aqueous solutions by nonliving green algae *Cladophora f ascicularis*: equilibrium, kinetics and environmental effects.*Adsorption,***2**:267-277.

Dermirbas, A., (2008). Heavy metal adsorption onto agro-based waste materials-a review. *Journal of Hazardous Materials*, **157**:220-229.

Dhankhar, R. and Hooda, A. (2011). Fungal biosorption- an alternative to meet the challenges of heavy metal pollution in aqueous solutions. *Journal of Environmental Technology*, **32**:467-491

Dhokpande, S.R. and Kaware, J.P. (2013). Biological methods for heavy metal removal. A Review: *International Journal of Engineering Science and Innovative Technology*, **2**(5):304-309.

Dursun, A.Y. (2006). A comparative study in determination of equilibrium, kinetic and thermodynamic parameters of biosoprption of copper (ii) and lead (ii) ions onto pretreated *Aspergillus niger*. *Biochemical Engineering Journal*, **28**:187-195.

Emoyan, O.O., Ogban, F.E. and Akarah, E. (2005). Evaluation of heavy metals loading of river ijana in ekpan -warri, Nigeria. *Journal of Applied Science and Environmental Management,* **10**(2):121-127.

European Union (2002). Heavy Metals in Wastes, European Commission on Environment.

Ezeonuegbu, B.A., Machido, D.A. and Yakubu, S.E. (2014). Resistance to some Heavy Metals among fungal flora of Raw Refinery Effluent. *Journal of Applied Science EnvironmentalManagement*, **18**:4.

Ezzouhri, L., Castro, E., Moya, M., Espinola, F. and Lairini, K. (2009). Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. *African Journal of Microbiology Research*, **3**(2):35-48.

Farooq, U., Kozinski, J.A., Khan, M.A and Athar, M. (2010). Biosorption of heavy metal ions using wheat based biosorbents-a review of the recent literature. *Bioresource Technology*, **101**:5043-5053.

Febrianto, J., Kosasih, A.N., Sunarso, J., Ju, Y.H., Indraswati, N. and Ismadji, S. (2009). Equilibrum and kinetics studies in adsorption of heavy metals using biosorbent: A summary of recent studies. *Journal of Hazardous Materials*, **162**:616-645.

Feng-lin, M.I., Mohammed, N. and Quratulan, S. (2013). Effect of NaoH and H_2O_2 on degradation of sawdust. *Wulfenia Journal*, **20**:1.

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

Ferner, D.J. (2001). Toxicity, *Heavy Metals. Emed. Journal*, **2**(5):1.

Fourest, E. and Roux, J.C. (1994). Improvement of heavy metal biosorption by mycelial dead biomass (*Rhizopus arrhizus*, *Mucor miehei* and *Penicillium chrysogenum*): pH control and cation activation. *FEMS Microbiology Review*,**14**:325-332.

Gadd, G.M and Sayer, J.A. (2000). Influence of Fungi on Environment Mobility of Metals and Metalloids. In: *Environmental microbe metal interaction* by Derek, R.L. ASM Press, Washington, DC. pp 237-256.

Gadd, G.M. (2009). Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. *Journal of Chemical Technology and Biotechnology*, **84**:13-28.

Gao, H., Liu, Y.G., Zeng, G.M., Xu, W.H., Li, T. and Xia, W.B. (2008) Characterization of Cr(VI) removal from aqueous solutions by a surplus agricultural waste—rice straw, *Journal*of *Hazardous Mater*ials, **150**:446-452.

Garni, S., Ghanem, M. and Bahobail, A.S. (2009). Biosorption characteristics of *Aspergillus fumigatus* in removal of cadmium from an aqueous solution. *African Journal of Biotechnology*, **8**:4163-4172.

Goutam, G. (2009). Fixed bed column study for removal of chromium (vi) from aqeous solution using saw dust. Department of Chemical Engineering, National Institute of Technology, Rourkela.

Grassi, M., Kaykioglu, Belgiorno and Lofrano (2012). Removal of Emerging Contaminants from Water and Wastewater by Adsorption Process. **2**:17-29.

Greene, B. and Bedell, G.W. (1990). Algal gels or immobilized algae for metal recovery. In: *An Introduction to Applied Phycology*, I. Akatsuka, ed. pp. Amsterdam, The Netherlands: SPB Academic Publ.

Hahem, A., Abdel-Halim, E., Maauof, H.A., Ramadan, M.A. and Abo-okeil, A. (2007). Treatment of Sawdust with Polyamine for Wastewater Treatment. *Energy Education Science andTechnology*, **19**(1):45-58.

Hakeem, A.S. and Bhatnagar, B. (2010). Heavy metal reduction of pulp and paper mill effluent by indeginous microbes. *Asian Journal of Experimental Biological Science*, **1**:203.

Hamza Izzeldin A.A., Martincigh Bice S., Ngila J., and Nyamori Vincent O.(2013). Adsorption studiesof aqueous Pb(II) onto a sugarcane bagasse/multi-walled carbon nanotube composite, *Physics and Chemistry of the Earth*, **66**:157-166.

Hardiject, K., Boparai, M., Joseph, D. and Ocarroll, M. (2010). Kinetics and thermodynamics of cadmium ion removal by adsorption onto nano zero valent iron articles. *Journal of HazardousMaterials*, **71**(2):131-140.

Ho, Y.C., Show, K.Y., Guo, X.X., Norli, I., Alkarkhi, F.M. and Morad, N. (2014). Review on industrial discharge and their effects on the Environment. Universiti Tunku Abdulrahaman, Universiti Sains Malasia. pp 1-33.

Ho,Y.S. and Ofomaja, A.E. (2006). Biosorption thermodynamics of cadmium on coconut copra meal as biosorbent. *Biochemical and Engineering Journal*,**30**:117-123.

Huang, C., Huang, C.P. and Morehart, A.L. (1991). Proton competition in Cu(ii) adsorption by fungal mycelia, *Water resource*, **25**:1365-1375.

Hubbe, M.A., Hasan, S.H. and Ducoste, J.J. (2011). Cellulosic substrate for removal of pollutants from aqueous systems: A Review. *Bioresources*, **6**(2):2161-2287

Idise, O.E., Ameh, J.B., Yakubu, S.E. and Okuofu, C.A. (2010). Biodegradation of a refinery effluent treated with organic fertilizer by modified strains of *Bacillus cereus* and *Pseudomonas aeruginosa*. *African journal of Biotechnology*, **9**(22):3298-3302.

Igbinosa, E. and Oko, A (2009). Impacts of discharge wastewater effluents on the physicochemical quantities of a receiving watershed in a typical rural community. *International Journal of Environmental Science Technology*, 6(2):175-182

Igwe, J.C., Abia, A.A. and Ibeh, C.A. (2008). Adsorption kinetics and intraparticulate diffusivities of Hg, As and Pb ions on unmodified and thiolated coconut fiber. *InternationalJournal Environmental Science Technol*., **5**:83-92.

Institute of Environmental Conservation and Research (INECAR, 2000). Position Paper against Mining in Rapu-Rapu, published by inecar, Ateneo De Naga University, Phillippines.

Iqbal,M, Mohd Ikram, A. and Farrukh, A. (2005). Biosorption of Ni, Cr and Cd by metal tolerant Aspergillus niger and Penicillium species using sing and multi-metal solution. *Indian Journal of Experimental Biology*. **44**:73-76.

Iyagba, E.T. and Opete, O.S.E. (2009). Removal of lead and chromium from drill cutting using activated palm kernel shell and husk. *African Journal of Environment*, **3**(7):17-179.

Jeffers, T.H. and Corwin, R.R. (1993). Waste water remediation using immobilized biological extractants. In: *Biohydrometallurgical Technologies, Proceedings of the International Biohydrometallurgy Symposium*, **2**:1-14.

Jiang and Jia, Q (2015).The role of coagulation in water treatment. Current Opinion in Chemical Engineering Nanotechnology. *Separation Engineering*, **8**:36-44.

Johannes Godt, F.S. Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A. and Groneberg, D.A. (2006).The toxicity of cadmium and resulting hazards for human health. *Journal of Occupational Medical Toxicology*, **1**:1-6.

Jonathan, M., Srinivasalu, S., Thangadurai, N., Ayyamperumal, T., Armstrong-altrin, J. and Ram-mohan, V. (2008). Contamination of uppanar river and coastal waters off cuddalore, south coast of India. *Environmental Geology*, **53**(7):1391-1404.

Kang, S., Lee, J. and Kima, K. (2007). Biosorption of Cr (III) and Cr (VI) onto the cell surface of *pseudomonas aeruginosa*. *Biochemical Engineering Journal*,**36**:54-58.

Kantor, D. (2006). Guillian-Barre Syndrome, the Medical Encyclopedia. National Library of Medicine and National institute of Health.

Kanu, I. and Achi, O.K. (2011). Industrial effluents and their impacts on water quality of receiving rivers in Nigeria. *Journal of Applied Technology in Environmental Sanitation*, **1**(1):75-86.

Kapoor, A. and Viraraghavan, T. (1995). Fungal biosorption. An alternative treatment option for heavy metal bearing wastewater-A review. *Bioresource Technology*, **53**:195-206.

Kapoor, A., Viraraghavan, T. and Cullimore, D.R. (1999). Removal of heavy metal using the fungus *Aspergillus niger*. *Bioresource Technology,***70**:95-104.

Khashimova, D., Keil, F.J., Khlomatov, K. (2008). Alkanes in zeolites-molecular simulations, empirical isotherms, mixtures. *Journal of the Universal of Chemical Technology and Metallurgy*, **43**(3):335-344.

Khormaei, M., Nasernejad, B., Edrisi, M. and Eslamzadeh, T. (2007). Copper biosorption from aqueous solutions by sour orange residue. *Journal of Hazardous Materials*, **149**:269-274.

Kiran, B., A. Kaushik, A. C.P. Kaushik, C.P. (2007). Biosorption of Cr(VI) by native isolate of *lyngbya putealis* (hh-15) in the presence of salts. *Journal of Hazardous Materials*, **141**:662-667.

Kratochvil, D. and Volesky, B. (1997). Removal of heavy metals by a new biosorbent. In: *Biotechnology and the Mining Environment, Proceedings of the 13th Biominet Meeting*, Canada SP**97**-(1):41-59.

Kratochvil, D. and Volesky, B. (1998). Advances in the biosorption of heavy metals. *Trends Biotechnoogyl,***16**:291-300.

Kumar, R., Bishnoi, N.R. and Garima, B.K. (2008). Biosorption of chromium(VI) fromaqueous solution and electroplatingwastewater using fungal biomass. *Chemical Engineering Journal*, **135**:202-208.

Larone, D.H. (2002). Identification of Fungi in Culture. In: Medically important fungi. 4thedition. ASM press, Washington DC. 2036-2904. Pp. 67-395.

Lee, C.K., Low, K.S. and P.Y. (1999) Removal of some Organic Dyes by Acid threat Spent Bleaching Earth. *Environmental Technology,* **20**:99-104, 154.

Lesmana, S.O., Febriana, N., Soetaredjo, F.E., Sunarso, J. and Ismadji, S. (2009). Studies on potential applications of biomass for the separation of heavy metals from water and wastewater. *Biochemical Engineering Journal,* **44**:19-41.

Li, X., Tang, Y., Cao, X., Lu, D., Luo, F. and Shao, W. (2008). Preparation and evaluation of orange peel cellulose adsorbents for effective removal of cadmium, zinc, cobalt and nickel. Colloids Surf. A., **317**(1-3):512-521.

Lin, L.C., Li, J.K. and Juang, R.S. (2008). Removal of Cu(II) and Ni(II) from aqueous solutions using batch and fixed-bed ion exchange processes. *Desalination,***225**:249-259.

Liu, D.H.F and Liptak, B.G. (2000). *Wastewater Treatment*, Lewis publishers. Boca Raton, Florida.

Longe, E.O and Ogundipe, A.O. (2010). Assessment of Wastewater discharged impact from a sewage treatment plant on lagoon water, Lagos Nigeria. *Research Journal of Applied Sciences, Engineering and Technology*, **2**(3):274-282.

Louidou, M.X., Matis, K.A., Zouboulis, A.I.and Liakopoulou, K.M. (2003). Removal of As (v) from wastewaterd by chemically modified fungal biomass. *Water Resources*, **37**:4544-4552.

Lenntech Water Treatment and Air Purification (LWTAP, 2004). *Water Treatment*, published by Lenntech, Rotterdamseweg, Netherlands.

Machido, D.A., Yakubu, S.E. and Ezeonuegbu, B.A. (2014). Composition of fungal flora in raw refinery effluent, effluent retention pond and untreated effluent recipient River. *Journal of Applied Science and Environmental Management*, **18**(4):592-596.

Machido, D.A., Yakubu, S.E. and Ezeonuegbu, B.A. (2015). Capacity of isolates of six genera of filamentous fungi to remove Lead, Nickel and Cadmium from refinery effluent. *International Journal of Innovative and Advanced Studies*, **2**(6):27-28.

Mahavi, P. (2005). Use of tea waste as bioabsorbent for removal of heavy metals from wastewater. *Chemosphere***54**:1522-1529.

Mahvi, A.H., Alavi, N. and Maleki, A. (2005b). Application of rice husk and its ash in cd removal from aqueous solution. *Pakistan Journal of Biological Science*, **8**(5):721-725.

Mameri, N., Boudries, N., Addour, L., Belhocine, D., Lounici, H., Grib, H. and Pauss, A. (1999). Batch zinc biosorption by bacterial nonliving *Streptomyces rimosus* biomass. *Water Resources*, **33**:1347-1354.

Mane P. C, Bhosle A. B, Jangam C. M and Vishwakarma C. V. (2011). Bioadsorption of Selenium by Pretreated Algal Biomass.*Advances in applied Science Research*, **2**:207-211.

Manjunathan, J. and Kaviyarasan, V. (2011).Nutrient composition in wild and cultivated edible mushroom, *Lentinus tuberrgium* Tamil Nadu., India. *International Food Research Journal*, **18**:809-811.

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

Marcus, A.C., Okoye, C.O.B. and Ibeto, C.N. (2013). Organic matter and trace metals levels in sediment of bonny river and creeks around Okrika in Rivers State, Nigeria. *International Journal of Physical Science*, **8**(15):652-656.

Margesin, R. and Schinner, F. (1997). Effect of temperature on oil degradation by a psychrotrophic yeast in liquid culture and in soil. *FEMS Microbiology Ecology*, **24**:243-249.

Marshall, W.E, and Johns M.M. (1996). Agricultural by products as metal adsorbents: sorption properties and resistance to mechanical abrasion. *Journal of Chemical Technology and Biotechnonology*, **66**:1192-8.

Martin, A.H., Syed, H.H. and Joel, J.D. (2011). Cellulosic substrates for removal of pollutants from aqueous systems. metal ion sorption: A review, Bioresources, 6(2):2161-2287.

Martin-Lara, M.A., Pagnanelli, F., Mainelli, S., Calero, M. and Toro, L. (2008). Chemical treatment of olive pomace: effect on acid-basic properties and metal biosorption capacity, *Journal of Hazardous Material*, **156**:448-457.

Martins, B.L., Cruz, C.C.V., Luna, A.S. and Henriques, C.A. (2006). Sorption and desorption of Pb2+ ions by dead sargassum sp biomass. *Biochemistry Engineering Journal*,**27**:310-314.

Memon, S.Q., Memon, N., Shaw, S.W., Khuhawar, M.Y. and Bhanger, M.I. (2007). Sawdust – A green economical sorbent for the removal of Cd (II) ions. *Journal of Hazardous Materials,* B**139**:116-121.

Mendez, M.O. and Maier, R.M. (2008). Phytostabilization of mine tailiings in arid and semiarid environments-An emerging remediation technology. *Environmental Health Prospect*, **116**(3):278-361

Merrin, J.S., Sheela, R., Saswathi, N., Prakasham, R.S. and Ramakrishna, S.V. (1998). Biosorption of Cr(iv) using*Rhizopus arrhizus***.** *Indian Journal of Experimental Biology*, **36:**1052-1055.

Mohammadi, T., Mohebb, A., Sadrzadeh, M. and Razmi, A. (2005). Modelling of Metal ion Removal from Wastewater by Electrodialysis. *Sep. Purification Technology*., **41**:73-82.

Mohammed, K, Worku, K. and Sahu, O. (2015). Biochemical Separations and Purification of Heavy Metal from Industrial Waste Water: A Review on Adsorption and Precipitations. Journal of Hydrology and Environment Research, **3**(1):38-46.

Mohan, D. and Singh, K.P. (2002). Single and multiple-component adsorption of cadmium and zinc ions using activated carbon derived from bagasse-an agricultural waste. *Water Resources*, **36**:2304-2318.

Monser, L and Adhoum, N. (2010). Modified activated carbon for the removal of copper, zinc, chromium and cyanide from wastewater. *Separation and Purification Technology*, **26**:137-146.

Morrison, G., Kotoki, O.S., Person, I. and Ekberg, A. (2001). Assessment of the impact of point source pollution from the keiskammahoek sewage treatment plant on the Keiskamma river pH, conductivity, COD and nutrients. *Water SA*, **27**:475-480.

Mosley, L., Sarabjeet, S. and Aalbersberg, B. (2004). Water quality monitoring in pacific island countries. Handbook for water quality managers and laboratories, Public Health Officers, water engineers and suppliers, Environmental protection Agencies and all those orrganisations involved in water quality monitoring (1st Edition). Pp. 30-43. ISSN:1605-4377: SOPAC, The university of the South Pacific. Suva Fiji Islands.

Mudhoo, A., Sharma, S.K., Garg, V.K. and Tseng, C.H. (2011). Arsenic: an overview of applications, health, and environmental concerns and removal processes. Critical *Reviews in Environmental Science and Technology*, **41**:435-519.

Nadia, M.A. (2006). Study on effluents from selected sugar mills in Pakistan: potential environmental, health and economic consequences of an excessive pollution load: sustainable Development Policy Institute. Islamabad, Pakistan.

Naja, G. and Volesky, B. (2011).the mechanism of metal cation biosorption in P. Kotrba et al. (eds). *Microbial biosorption of metals*, Springer science + Business media B.V, **3**:19.

Naja, G., Mustin, C., Volesky, B. and Berthelin, J. (2005). A high resolution titratior: A new approach to studying binding sites of microbial biosorbents. *Water Resources*, **39**:579-588.

Nasim, A.k. Shaliza, I. and Piarapakaran, S. (2004). Elimination of heavy metals from wastewater using agricultural waste as adsorbent. *Malaysian Journal of Sciences*, **23**:43-51.

Nasri, B.R. and Garima, R. (2004). Fungus- An alternative for bioremediation of heavy metal containing wastewater. *A review. Journal of Scientific and IndustrialResearch*. **64**:93-100.

Nassef, E and El-Taweel, Y.A. (2015). Removal of copper from wastewater by cementation from simulated leach liquors. *Journal of Chemical Engineering Process Technology*, 6:214.

National Environmental Standards and Regulations Enforcement Agency (NESREA,2007). Federal water quality standard. NESREA Act. **25**:5-21.

Nwaichi, E.O. and Warrigbani, T.Z (2013). Phenol removal from refinery effluent by Hevea brasilliensis. *Research journal in Engineering and Applied Sciences*, **2**(1):50-53

Nwaichi, E.O., Essien, E.B. and Ugbeyide, E. (2013). Characterization of Warri effluent and its recipient water medium. *International Journal of Pure Applied and Bioscience*, **1**(4):22-27

Nwankwo, O.D., Ewuim, S.C. and Mogbo, T.C. (2014). Comparative study of Bioadsorption of cd and pb from industrial wastewater using melon husk activated with sulphuric acid. *American Journal of Environmental Science Technology*, **3**(6-1):1-8.

Odesiri-Eruteyan E, Obukohwo, F, Uhribo, V. and Asibor, G. (2017). Assessment of the impacts of Refinery Effluent on the Physico-chemical properties of Ubeji Creek, Delta State, Nigeria. *Journal of Environmental and Analytical Toxicology*,**7**:1.

Ofomaja, A.E. and Ho, Y. (2007). Effect of pH on cadmium biosorption by coconut copra meal, *Journalof Hazardous Material*, B **139**:356-362.

Ogwuegbu, M.O. and Ijeoma, M.A. (2003). Effects of Certain Heavy Metals on the Population due to Mineral Exploitation. In: International Conference, Environment and Sustainable Development in Nigeria, University of Ado Ekiti, Ekiti State, Nigeria, **158**:8-10.

Oke, I.A., Olarinoye, N.O. and Adewusi, R.A. (2008). Adsorption kinetics for Arsenic removal by untreated powdered egg-shell from aqueous solutions. *Journal Adsorption Soc*., **144**(1):85-92.

Okerentugba, P.O. and Ezeronye, O.U. (2003). Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. *African Journal of Biotechnology*, **2**(9):288-292.

Oladoye, P.O. and Adewuyi, O.O. (2014).Comparative assessment of lead and zinc in the coastal areas of Niger Delta. *Journal of Research in Environmental Science and Toxicology*, **3**(3):39-45.

Omeiza, F.S. (2011). Equilibrium and kinetics studies of adsorption of Malachite green onto low cost adsorbent. Unpublished M,Sc. thesis department of chemistry, Ahmadu Bello University, Zaria.

Onuegbu, T.U., Okoye, L.O., Dioha, I.J., Okoye, P.A.C., Nwako, P.M. (2008). Treated effluents and sludges samples. Journal Chemical Soc. Nigeria, **33**(1):6-9.

Osakwe, C.E., Ismail, S., Suraj, S. and Adamu, Z. (2014). Adsorption of heavy metals from wastewaters using Adonosia digitata fruit shells and Theobroma cacao pods as adsorbents. A comparative study. American Union Journal, 18(1):11-18.

Otukunefor, T.V. and Obiukwu, C. (2005). Impacts of refinery effluents on the physicochemical properties of a water body in the Niger Delta. *Applied Ecology and EnvironmentalResearch*,**3**(1):61-72.

Ouki, S.K. and Neufeld, R.D. (1997). Use of activated carbon for the recovery of chromium from industrial waste water. *Journal of Chemical Technology and Biotechnol*ogy, **70**:3-8.

Ozer, A and Ozer, D. (2003). Comparative study of biosorption of pb (ii), ni (ii) and cr (vi) ion onto *S. cerevisae:* determination of biosorption heats. *Hazardous Material Biotechnology*, **100**:219-228.

Pal, A., Ghosh, S., and Pau, A.K. (2006). Biosorption of cobalt by fungi from serpentine soilof Andaman, *Bioresource Technol*nology, **97**:1253-1258.

Park, D., Yun, Y., Jo, J.H. and Park, J.M. (2006). Biosorption process treatment of electroplating wastewater containing Cr (VI): Laboratory-scale feasibility Test. *Industrial andengineering chemistry Research*, **45**:5059-5065.

Parvathi, K., Nareshkumar, R. and Nagendran, R. (2007). Biosorption of manganese by *aspergillus niger* and *saccharomyces cerevisiae*. *World Journal of Microbiology Biotechnology*, **23**:671-676.

Pavasant, P., Apiratikul, R., Sungkhum, V., Suthiparinyanont, P., Wattanachira, S. and Marhaba, T.F. (2006). Biosorption of Cu(ii), Pb(ii) and Zn(ii) using dried marine green macroalgae *Caulerpa lentillifera*. *Bioresources Technology*,**97**:2321-2329

Pelczar, M.J. (1982). *Microbiology*. 4th edition. Pp 285-305.

Perry, R.H., Green, D.W. and Maloney, J.O. (2007). Perry's chemical engineers handbook. 7th edition McGraw-Hill:New York.

Poulton, S.W., Krom, M.D., Rijn, J.V. and Raiswell, R. (2002). The use of hydrous iron (III) oxides for the removal of hydrogen sulphide in aqueous systems. *Water Research*. **36**:825-834.

Preetha, B. and Viruthagiri, T. (2005). Biosorption of zinc (II) by Rhizopus arrhizus: equilibrium and kinetic modeling. *African Journal of Biotechnology*. **4**:506-508.

Radojavic, M. and Bashkin,V.N. (1999). Practical Environmental Analysis Cambridge. *The Royal Society of Chemistry.* pp 189-203.

Rajesh, D. and Anju, H. (2011). Fungal biosorption: an alternative to meet the challenges of heavy metals pollution in aqueous solutions. *Journal of Environmental Technology*, **32**:467-491.

Raji, C.A, Manju, G.N. and Anirudhan, T.S. (1997). Removal of heavy metal ions from water using sawdust-based activated carbon. *Indian Journal of Engineering Materials and Sciences*, **4**:254-260.

Ramzan, M., Malik, M.A., Iqbal, Z., Arshad, N., Khan, S.Y. and Arshad, M. (2011). Study of hematological indices in tannery workers exposed to chromium in Sheikhupura (Pakistan). *Toxicology and Industrial Health*, p 19.

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

Rao, K.S., Mohapatra, M., Anand, S. and Venkateswarlu, P. (2010). Review on cadmium removal from aqueous solutions. *International Journal of Engineering, Science and Technology*,**2(**7):81-91.

Rao, M.M., Reddy, D.H.K.K., Venkateswarlu, P., Seshaiah, K., (2009). Removal of mercury from aqueous solutions using activated carbon prepared from agricultural by-product/waste. *Journal Environmental Management*, **90**:634-643.

Rao, R.A.K., and Khan, N.A. (2007). Removal and recovery of Cu(II), Cd(II) and Pb(II) ions from single and multimetal systems by batch and column operation on neem oil cake (NOC), *Seperation and Purification Technology*, **57**:394-402.

Reddad, Z Gerente, C. Andres, Y. and Le-Cloirec, P. (2002e). Adsorption of several metal ions ontoa low-cost biosorbent: kinetic and equilibrium studies. *Environmental Science andTechnology*, **36**(9)-2067-2073.

Reichenauer, T.G. and Germida, J.J. (2008). Phyto-remediation of organic contaminants in soil and ground water. *Chemsuschem*, **1**(8-9):708-725.

Reya, I., Suganya, M., Lakshmi, M.P. and Emilin, R.R. (2012). Biosorption of copper from electroplating effluent and its equilibrium stydies. *International Journal of Pharmaceutical and Biosciences*, **3**(3):875-889.

Ridvan, S., Nalan, Y. and Adil, D. (2003). Biosorption of cadmium, lead, mercury and arsenic ions by fungus *Penicillium pururogenum*. *Separation Science Technology*, **39**:2039-2053.

Rishipal, R.B. (2002). Treatment of organic and inorganic pollutants in municipal wastewater by agricultural by-product based granular activated carbons, M.Sc. Thesis, Department of food science, Louisiana State University.

Rocha, S.C.S., Cavalcante, J.D., Da-Silva, M.G.C. and Pinho, C.G. (2006). Influence of the drying conditions of Sargassum species alga on the bioadsorption of hexavalent chromium. *Environmental Technology*, **27**(9):979-990.

Sai, D.S.R.R., Nageswara, V.R.P., Rajendra, P. and Chitti, N.B. (2012). Sorption of lead (ii) ions from wastewater using *carica papaya* leaf powder. *International Journal of Engineering Scienceand Advanced Technology*, **2**(6):1577-1581.

Salequzzaman, M., Tariqul, I.S.M., Tasnuva, A., Kashem, M.A. and Mahedi Al Masud, M. (2008). Environmental impact of sugar industry- A case study on Kushtis Sugar Mills in Bangladesh: Khulna; Green World Foundation.

Samarghandi, M.R., Nouri, J,. Mesdaghinia, A.R., Mahvi, A.H., Naser, S. and Vaezi, F. (2006). Efficient removal of phenol, lead and cadmium by means of UV/$Ti0_2$/H_20_2 processes. *International Journal of Environmental Science and Technology*, **4**(1):19-25.

Saravanane, R., Sundararaja, T., Sivamurthyreddy, S. (2002). "Efficiency of chemically modified low cost adsorbent for the removal of heavy metals from waste water. A comparative study". *Indian Journal of Environmental Health*, **44**:78-81.

Satya, V.Y. (2012). A review on adsorption of heavy metals from aqueous solution. *Journal of Chemical, Biological and Physical Sciences*, **2**(3):1585-1593.

Schnoor, J. (1997). *Phytoremediation: Ground water Remediation Technologies Analysis.*

Seolatto, A., Silva Filho, C.J. and Mota D.L. (2012). Evaluation of the Efficiency of Biosorption of Lead, Cadmium, and Chromium by the Biomass of Pequi Fruit Skin (Caryocar brasiliense Camb). *Chemical Engineering Transactions*, **27**:73- 78.

Shaker, M.A. (2007). Thermodynamic profile of some heavy metal ions adsorption onto material surfaces. *American Journal of Applied Sciences*, **4**:605-612.

Sharma, A. and Bhattacharyya, K.G. (2004). Adsorption of chromium (vi) on *Azadirachta indica* (neem) leaf powder.*Adsorption*,**10**:327-338.

Sharma, B.K. (2004). Sewage and Sewage treatment. *In: Industrial Chemistry including Chemical Engineering*, **14**:161-165

Sharma, P., Kumari, P., Srivastava, M.M., and Srivastava, S. (2006). Removal of Cd from aqueous system by shelled *Moringa oleifera* seed powder. *Bioresource Technology*, **97**: 299-305.

Shukla, A., Zhang, Y, Dubey, P., Margrave, J.L. and Shukla, S. (2002). The role of sawdust in the removal of unwanted materials from water. *Journal of Hazardous Materials*, **B95**:137-152.

Silins, I. and Johan Högberg, J. (2011). Combined toxic exposures and human health: biomarkers of exposure and effect. *International Journal of Environmental Research & Public Health*, **8**:629-647.

simultaneous removal of iron(III) and chromium(VI) by using chlorella vulgaris,

Singh, K.K., Singh, A.K. and Hassan, S.H. (2006). Low cost bioadsorbent 'wheat bran' for the removal of Cd from waste water. Kinetic and Equilibrium Studies. *Bioresource Technology*, **97**:994-1001.

Siyanbola,T.O., Ajanaku, K.O., James, O.O., Olugbuyiro, J.A.O., and Adekoya, J.O. (2011). Physicochemical parameters of industrial effluents in lagos state, Nigeria. *P&A Science and Technology*, **1**:49-54.

Soliman, E.M., Ahmed, S.A. and Fadl, A.A. (2011). Reactivity of sugarcane bagasse as a natural solid phase extractor for selective removal of Fe(III) and heavy metal ions from natural Water sample. *Arabian Journal of Chemistry*, **4**:63-70.

Southichak, B., Nakano, K., Nomura, M., Chiba, N. and Nishimura, O. (2006). Pb(II) biosorption on reed biosorbent derived from wetland: effect of pretreatment on functional groups. *Water ScienceTechnolnology*. **54**:133–141.

Subramani, T. and Sindhu, S. (2012). Batch study experiments and column analysis for finding out a suitable bio-adsorbent for the removal of heavy metals from electroplating industry effluent. *International Journal of Engineering Research and Applications*, **2**(4):172-184.

Subudhi, E. and Kar, R. N. (2008). *Rhizopus arrhizus* – An efficient fungus for copper effluent treatment. *International Journal of Integrative Biology*, **2**:166-170.Switzerland, 4th edition.

Sud, D. Mahajan, G. and Kaur, M.P.(2008). Agricultural waste material as potential adsorbent for sequestering heavy metal from aqueous solution- a review. *Bioresource Technology*, **99:**6017-6027.

Sudha Bai, R. and Abraham, T.E. (1998). Studies on biosorption of Cr(vi) by dead fungal biomass. *Journal of Science Ind. Research*, **57**:821-824.

Suleimanov, R.A. (1995). Conditions of waste fluid accumulation at petrochemical and processing enterprises and prevention of their harm to water bodies.MadistinaTrudai. *PromyshlennaiaEkologiia*, **12**:31-36.

Tang, P., Lee, C.K., Low, K.S. and Zainal, Z. (2003). Sorption of Cr(VI) and Cu (II) in aqueous solution by ethylenediamine modified rice hull. *Environmental Technology*, **24**:1243-1251.

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

Templeton, D and Ehrman, T.(1995). Determination of Acid-Insoluble Lignin in Biomass. Laboratory Analytical Procedure No. 003, National Renewable Energy Laboratory, Golden, CO.

Teoh, Y, Peng, K. Moonis A. and Choong Thomas, S.Y. (2013). Kinetic and isotherm studies for lead adsorption from aqueous phase on carbon coated monolith. *Chemical Engineering Journal*, **21**:248-255.

Tokar, E.J., Benbrahim-Tallaa, L. and Waalkes, M.P. (2011). Metal ions in human cancer development. *Metal Ions on Life Science*, **8**:375-401.

Tor-resdey, J.L.G., De la Rose, G. and Videa, J.R.P. (2004). Use of phytofiltration techniques in the removal of heavy metals: a review. *Pure Applied Chemistry*, **76**:801-813.

Tripathy, T. and De, B.R. (2006). Flocculation: A new way to treat the wastewater. *In: Journal ofPhysical Sciences*, **10**:93-127.

US Department of Energy Office of Science (2006). Breaking the biological barriers to cellulosic ethanol:A joint Research Agenda. Report from the December 2005 workshop.

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

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

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

Vasudevan, P., Padmavathy, V. and Dhingra, S.C. (2003). Kinetics of biosorption of cadmium on baker's yeast. *Bioresource Technology*, **89**:281-287.

Veglio, F. and Beolchini, F. (1997). Removal of metals by biosorption: A review. *Hydrometallurgy*, **44**:310-316

Vetayasuporn, S. (2006). Oyster mushroom cultivation on different cellulose substrate. *ResearchJournal of Agriculture and Biological Sciences,* **2**(6):548-551.

Vijayaraghavan, P.K. and Velan, M. (2006). Biosorption of copper (II) and cobalt (II) from aqueous solutions by crab shell particles. *Bioresource Technol*ogy, **97**:1411-1419.

Vilar, V.J.P., Botelho, C.M.S. and Boaventura, R.A.R. (2007). Modeling equilibrium and kineticsof metal uptake by algal biomass in continuous stirred and packed bedadsorbers.*Adsorption*,**13**:587-601.

Vilar, V.J.P., Botelho, C.M.S. and Boaventura, R.A.R. (2008). Copper removal by Algae gelidium, agar extraction of algal waste and granulated algal waste: kinetic and equilibrium, *Bioresource Technology*, **99**:750-762.

Volesky, B. (1994). Advance in biosorption of metals: selection of biomass types. *Microbiology Reviews*, **14**(4):291-302.

Volesky, B. (2001). Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy*, **59**(23):203-216.

Volesky, B. and Holan, Z. R. (1995). *Biotechnology Programme*,**11**:235-250.

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

Volesky, B. and Naja, G. (2007). Biosorption Technology: Starting Up an Enterprise. *International Journal of Technology Transfer and Commercialization*, **6**:196-211.

Walakira, P. (2011). Impact of industrial effluents on water quality of receiving streams in Nakawa-Ntinda, Uganda. Research thesis, BSc.Technology-Chemistry.

Wang, J. and Chen, C (2006). Biosorption of heavy metals by *Saccharomyces cerevisae*: a review. *Biotechnology Advances*, **24**:427-451.

Wang, J. and Chen, C (2009). Biosorbents for heavy metals removal and their future. *Biotechnology Advances*, **27**:195-226.

Wang, X.S., Qin, Y. and Li, Z.F. (2006). Biosorption of zinc from aqueous solutions by rice bran: kinetics and equilibrium studies. *SeparationScience Technology*, **41**:747-756.

Wilkins, E. and Yang, Q. (1996). Comparison of the heavy metal removal efficiency of biosorbents and granular activated carbons. *Journal Environmental Science Health*, **31A**:2111.

World Health Organization (WHO, 2006). *Guidelines for drinking water quality*, 3rd edition, WHO Press geneva, Switzerland. p398.

Wuyep, P. A., Chuma, A.G., Awodi, S. and Nok, A. J. (2007). Biosorption of Cr, Mn, Fe, Ni, Cu and Pb metals from petroleum refinery effluent by calcium alginate immobilized mycelia of *Polyporus squamosus. Scientific Research and Essay*, **2**(7):217-221.

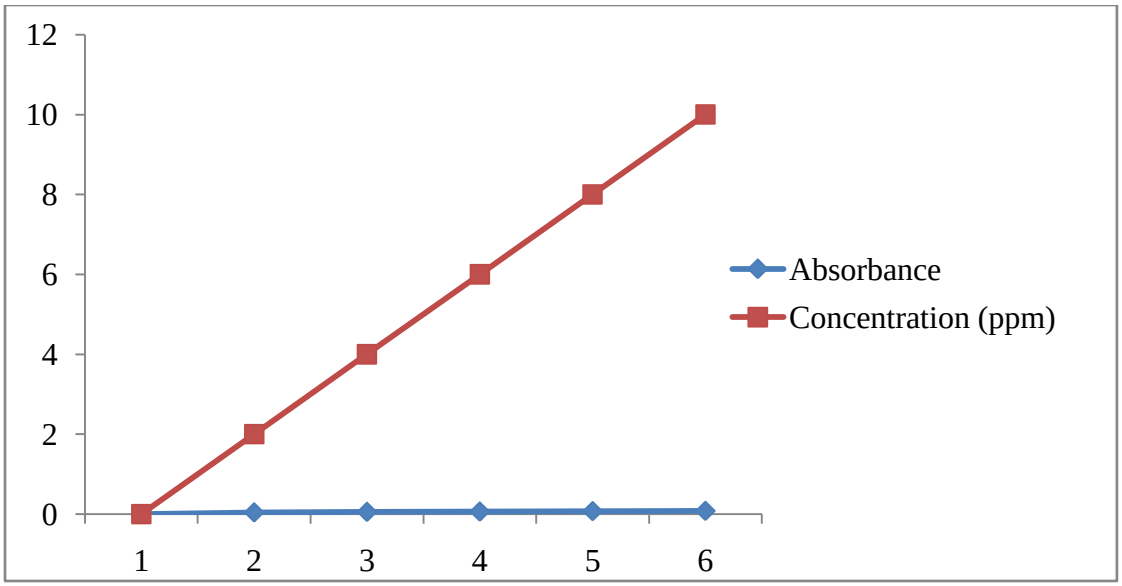
Xu, X., Zhu, X., 2004. Treatment of refectory oily wastewater by electro-coagulation process. *Chemosphere*, **56**:889-894.

Yan, G. and Viraraghauan, T. (2005). Heavy metal removal from aqueous solutions by fungus *Mucor rouxii*, *Water Resource*, pp 37-4486.

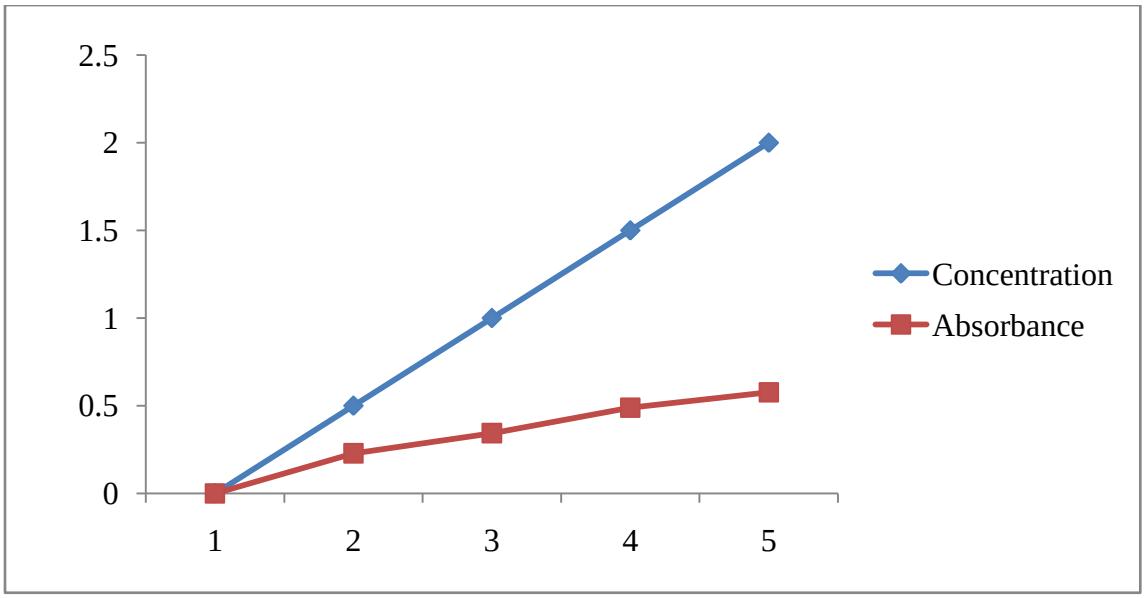
Zafar, S., Aqil, F. and Ahmad, I. (2007). Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. *Bioresource and Technology*, **98**:2557-2561.

Zhu, X., Venosa, A.D., Suidan, M.T. and Lee, K. (2001). Guidelines for the bioremediation of marine shorelines and fresh water wetlands. – In: Annual Report, 1-126. U.S. Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory Land Remediation and Pollution Control Division. 26 W. Martin Luther King Drive, Cincinnati, OH 45268.

Appendix I: Sulphate Standard curve



Appendix II: Phosphate Standard curve



Appendix III: Experimental setup used for sorption process



Appendix IV: Site of sample collection at Kaduna Refinery and Petrochemical Company, Kaduna.



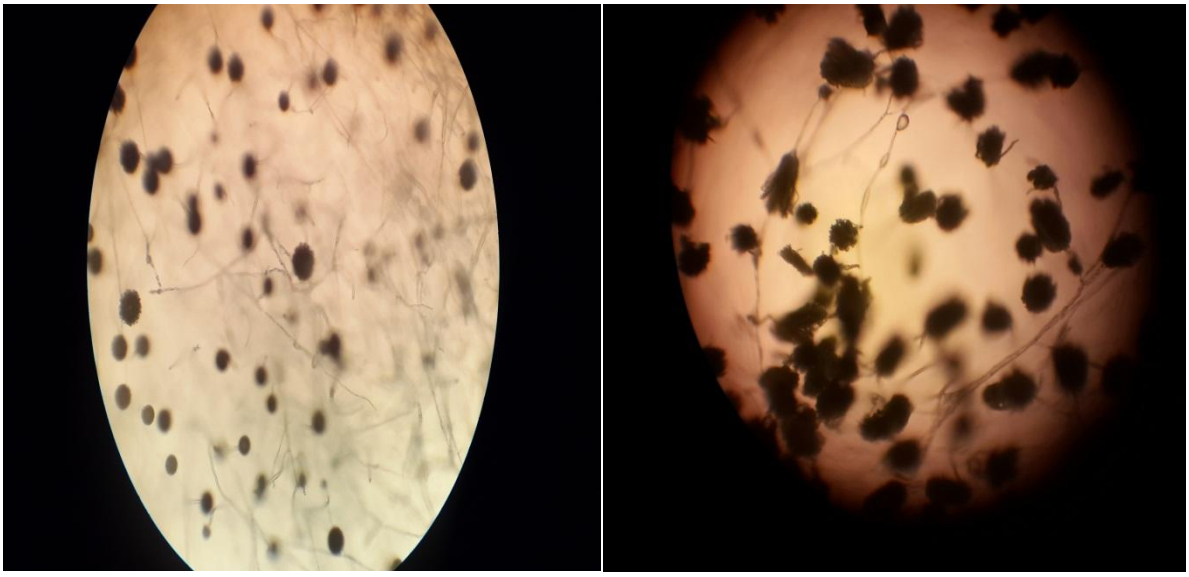
Appendix V: Pure culture of *Aspergillus niger*used in biosorption of heavy metals



Appendix VI: Pure culture of *Penicillium* species used in biosorption of heavy metal

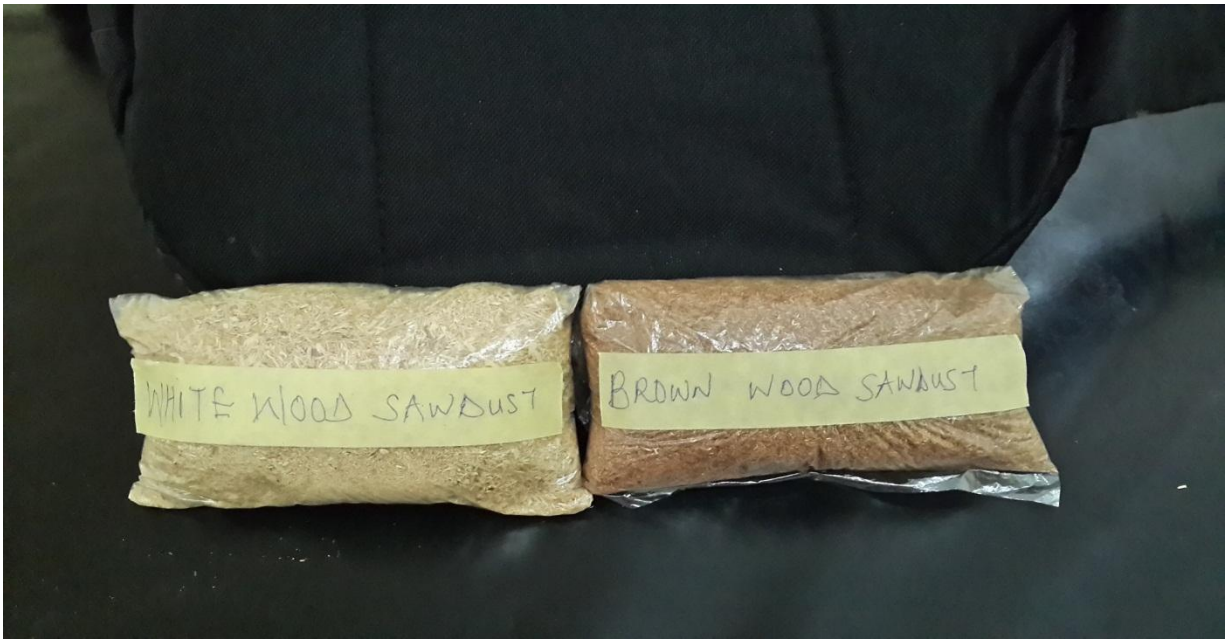


Appendix VII: Microscopic view of *A niger* (a) and *Penicillium* species (b) at x40 magnification



(a) (b)

Appendix VIII: Sawdust samples used in biosorption of heavy metals



Appendix IX: Statistical analysis of percentage removal of heavy metal ions in white wood

	Lead	Nickel	Cadmium
Unsterilized wood	98.14±1.71	73.45±21.41	87.66±3.26
Sterilized wood	98.36±2.16	84.67±11.38	88.52±3.29
Sterilized + *A. niger*	96.77±2.05	70.72±18.96	87.19±2.59
Sterilized + *Penicillium* spp	97.23±2.4	67.72±19.67	89.31±2.11
Sterilized + both orgs.	96.61±3.49	72.93±17.99	90.31±2.03
P-Value	0.34	0.197	0.238

White wood, Mean±SE (Standard Error);ANOVA

Mean with P Value > 0.05 are statistically not significant

Appendix X: Statistical analysis of percentage removal of heavy metal ions in brown wood

	Lead	Nickel	Cadmium
Unsterilized wood	92.57±4.08	67.77±14.13	75.66±10.78
Sterilized wood	94.19±.78	63.94±20.29	74.39±8.14
Sterilized + *Aspergillus niger*	93.11±5.08	68.62±8.40	83.17±4.22
Sterilized + *Penicillium* spp	93.99±4.74	70.96±15.55	80.04±5.06
Sterilized + both orgs.	94.75±4.18	68.46±11.19	80.07±5.47
P-Value	0.67	0.732	0.932

Brown wood, Mean ±SE;ANOVA

Mean with P Value > 0.05 are statistically not significant.