FUNCTIONALIZATION OF BIOPOLYMERS FOR THE TREATMENT OF HEAVY METALS FROM AQUEOUS MEDIUM

A THESIS SUBMITTED TO THE

CENTRAL DEPARTMENT OF CHEMISTRY

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

NEPAL

FOR THE AWARD OF

DOCTOR OF PHILOSOPHY

IN CHEMISTRY

BY

JAGJIT KOUR (KARKI)

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RECOMMENDATION

This is to recommend that **JAGJIT KOUR (KARKI)** has carried out research entitled **" FUNCTIONALIZATION OF BIOPOLYMERS FOR THE TREATMENT OF HEAVY METALS FROM AQUEOUS MEDIUM** ″ for the award of Doctor of Philosophy (Ph.D.) in **CHEMISTRY** under our supervision. To our knowledge, this work has not been submitted for any other degree. She has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.

Dr. Kedar Nath Ghimire
Supervisor
Professor
Central Department of Chemistry
Tribhuvan University
Kirtipur, Kathmandu,
Nepal

--
Dr. Megh Raj Pokhrel
Co-Supervisor
Professor
Central Department of Chemistry
Tribhuvan University
Kirtipur, Kathmandu,
Nepal

LETTER OF APPROVAL

On the recommendation of Prof. Dr. Kedar Nath Ghimire and Prof. Dr. Megh Raj Pokhrel, this Ph. D. thesis submitted by Jagjit Kour (Karki)**,** entitled **"FUNCTIONALIZATION OF BIOPOLYMERS FOR THE TREATMENT OF HEAVY METALS FROM AQUEOUS MEDIUM"** is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U.

……………………………………….
Dr. Megh Raj Pokhrel.
Professor,
Head,
Central Department of Chemistry,
Tribhuvan University
Kirtipur, Kathmandu

Nepal

Date: 19/11/2014

DECLARATON

Thesis entitled **"FUNCTIONALIZATION OF BIOPOLYMERS FOR THE TREATMENT OF HEAVY METALS FROM AQUEOUS MEDIUM"** which is being submitted to the Central Department of Chemistry, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Prof. Dr. Kedar Nath Ghimire Central Department of Chemistry, Tribhuvan University and co-supervised by Prof. Dr. Megh Raj Pokhrel.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

Jagjit Kour (Karki)

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Jagjit Kour (Karki)

Ph. D. Scholar

Central Department of Chemistry

Kirtipur, Kathmandu, Nepal

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ABSTRACT

The industrial discharge of heavy metals into waters' course is one of the major pollution problems affecting water quality and human health. Therefore, they must be removed prior to their discharge into waste streams. For this novel cause several types of treatment methods are in use. Among them use of biowaste is emerging as the best method. Therefore different types of agricultural by-products have been explored for their efficiency in removing heavy metal ions from aqueous solution. In this regard a biomass of *Desmostachya bipinnata* (DB) was investigated as the potential bio-adsorbent for the removal of Cd(II), Pb(II), Cu(II) and Zn(II) from wastewater. DB is a sacrificial herb in the south Asian region particular to Hindu religion with medicinal value. It is found to be very effective in the phyto-accumulation of heavy metals from soil as well as from polluted water. This is the first adsorption studies ever performed with DB as the novel bio-sorbent in the removal of heavy metal ions.

Nitrogen functional groups were grafted onto the polymeric backbone of DB with hydrazine monohydrate, dimethylamine and ethylenediamine respectively, for the better adsorption. The characterization of biopolymer was done using SEM, DRFTIR, elemental analysis, zeta potential, XRD and TG/DTA. The characterization results of biopolymer revealed to indicate proper functionalization with nitrogen groups.

The effect of pH, initial concentration and contact time of the metal solution were monitored by batch method. The maximum adsorption capacities determined for Cd(II), Pb(II) Cu(II) and Zn(II) were 76.8 mg/g, 130.4 mg/g, 72.1 mg/g and 58.16 mg/g, respectively, at their optimum pH. The equilibrium data were analyzed using Langmuir and Freundlich isotherm models. Langmuir isotherm model fitted well and the rate of adsorption followed the pseudo second order kinetic. The experiments on regeneration and reuse of biopolymers were conducted with batch method. The results indicated that more than 90% of heavy metals were regenerated and the biopolymers were used for six successive cycles with 90% efficiency.

Thus, the adsorbents derived from DB can be used as an effective, efficient, low cost and environment friendly material for the removal of Cd(II), Pb(II), Cu(II) and Zn(II) from the aqueous medium.

Keywords: Desmostachya bipinnata, Biosorption, Heavy metals, Batch experiment, Pseudo- second order kinetic model, Functionalization, Low cost, Pollution, Biosorbent.

LIST OF ACRONYMS AND ABBREVIATIONS

ADD	Associated Diseases/Disorders
Cd (II)	Cadmium (II)
Cu (II)	Copper (II)
DB	*Desmostachya bipinnata*
DMSO	Dimethyl sulphoxide
DRFTIR	Diffuse Reflectance Fourier Transform Infrared spectroscopy
DTA	Differential Thermal Analysis
EDTA	Ethylene diamine tetra acetic acid
FAO	Food and Agriculture Organization
FDB	Funtionalized *Desmostachya bipinnata*
FDB-Dm	Funtionalized *Desmostachya bipinnata* with Dimethylamine
FDB-E	Funtionalized *Desmostachya bipinnata* with Ethylenediamine
FDB-H	Funtionalized *Desmostachya bipinnata* with Hydrazine
g	gram
HEPES	2-[4-(2-hydroxyethyl)-1-piperazinyl] ethane sulphonic acid
ICP-AES	Inductively coupled plasma atomic emission spectrophotometer
L	Litre
m	meter
mg	milligram
mL	milliliter
mm	millimeter
Pb (II)	Lead (II)
pH	Power of hydrogen ion concentration
RDB	Raw *Desmostachya bipinnata*
rpm	Rotation per minute
SAM	Subsequent Addition Method
SEM	Scanning Electron Microscopy
TGA	Thermo Gravimetric Analysis
UNEP	United Nations Environmental Program

UNO	United Nations Organization
WWTPs	Waste Water Treatment Plants
Zn (II)	Zinc (II)
µg	microgram

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CHAPTER 1

1. INTRODUCTION

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed (Gupta *et al.,* 2000). To a small extent they enter our bodies *via* food, drinking water and air. As trace elements, some heavy metals (*e.g.* copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning (Alluri *et al.*, 2007). Heavy metal poisoning could result, for instance, from drinking-water contamination (*e.g.* lead pipes), high ambient air concentrations near emission sources, or intake *via* the food chain.

There are several ways of metal poisoning; one of them is a process of bioaccumulation. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living organisms any times they are taken up and stored faster, then they are broken down (metabolized) or excreted. Heavy metals can enter water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater.

Although there is no clear definition of what a heavy metal is, density is in most cases taken to be the defining factor. Heavy metals are thus commonly defined as those having a specific density of more than 5 g/cm^3. It is a general collective term which represents a group of metals and metalloids having density higher than 5gm/ cm^3 and atomic weights between 63.5 and 200.6. Some of the examples of heavy metals are antimony, bismuth, cerium, chromium, cadmium, copper, iron, lead, manganese, zinc, tin and so forth (Volesky, B. 1990a; Chen *et al.* 2012).

According to the importance of metals to human beings, they can be divided into three groups (Igwe and Abia, 2006).

The 1st group includes essential light metals like Na, Ca, Mg, K *etc*, which are essential to sustain biological growth of living beings. The 2nd group covers essential heavy metal including Mn, Fe, Co, Cu, Zn, Mo *etc*. They are present in our bodies in much lower levels and essential for our growth, development and reproduction. The 3rd group comprises toxic heavy metals. It includes Cd, Hg, Pb, As, Ni, Cr (VI) *etc*. They are non essential heavy metals. Their presence in our body in concentrations below a certain value do not show any negative effect, but, when their concentration exceeds toxic levels then they become harmful (Chen, 2012).

Environmentally, the main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO.

Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries, though emissions have declined in most developed countries over the last 100 years.

Emissions of heavy metals to the environment occur *via* a wide range of processes and pathways, including to the air (*e.g*. during combustion, extraction and processing), to surface water (*via* runoff and releases from storage and transport) and to the soil (and hence into ground water and crops). Atmospheric emissions tend to be of greatest concern in terms of human health, both because of the quantities involved and the widespread dispersion and potential for exposure that often ensues.

Lead emissions are mainly related to road transport and thus most uniformly distributed over space. Cadmium emissions are primarily associated with non-ferrous metallurgy and fuel combustion, whereas the spatial distribution of anthropogenic mercury emissions reflects mainly the level of coal consumption in different regions.

The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in a high flux of metallic substances in aquatic environment. At the same time, rise in food production has increased the use of chemical fertilizers, pesticides, herbicides, fungicides and other agrochemicals in agriculture. These biologically active chemicals are used for weed and pest control, but their chemical activity extends beyond their limit, causing serious problems in the environment (http://water.usgs.gov/edu/wuww.html).

Sewage and industrial wastewater is commonly used for irrigating agricultural fields in developing countries (Singh *et al.*, 2004; Sharma *et al*., 2007; Pandey *et al*., 2008; Nath *et al.,* 2009; Nagajyothi *et al.,* 2009). Continuous use of wastewater leads to the enrichment of soil with essential macro and micronutrients (Dass and Kaul, 1992; Kanan *et al.,* 2005), which are beneficial for the growth and metabolism of the plants at lower concentrations but become toxic beyond the permissible limit. Several micronutrients are also heavy metals and known to produce undesirable effects on plants at higher concentrations (Kocak *et al.,* 2005). Accumulation of toxic heavy metals can lead to stress conditions in the plant system by interfering with the metabolic activities and physiological functioning of the plants. Heavy metals are known to cause membrane damage, structural disorganization of organelles, impairment in the physiological functioning of the plants and ultimately growth retardation (Kimbrough *et al.,* 1999; Chien and Kao, 2000; Long *et al.,* 2003; Zhang *et al.,* 2002). These metals are of great concern because they are non-degradable and therefore persistent.

Environmental contamination with metal ions represents a potential threat to human, animals and plants. Pollution by metal ions has become a major issue throughout many countries (Matlock *et al.*, 2001) because the contents of metal ions in potable water and wastewater often exceed the permissible sanitary standards. The general distribution of heavy metals in industries are given in Table 1.

In view of our research study under consideration, it would be better to highlight the sources and associated symptoms of the following metals.

Cadmium

Cadmium metal sources: Cadmium is contained in cigarette smoke, tobacco, coffee, gasoline, steel cooking pans, metal pipes, tap water, rubber, fertilizers, dental alloys, batteries, candy, refined cereals, colas, copper refineries, fungicides, refined grains, plastics, marijuana, evaporated milk, motor oil, oysters, paint, pesticides, processed foods, soft drinks, pharmaceutical and recreational drugs (Dojlido and Best, 1993).

Associated symptoms: Headaches, heart problems, anemia, hypertension. Associated diseases/disorders: Mental retardation, kidney diseases, bronchitis, cancer (bladder, esophagus, larynx, lung, mouth, pharynx, prostate and stomach). The most severe form of Cd toxicity in human is 'itai-itai', a disease characterized by excruciating pain in the bone. Other health implication of Cd in human includes kidney dysfunction, emphysema, and testicular atropy (Kadirvelu and Namasivayam, 2000). Chronic exposure in high levels results in death. The maximum permissible limit of Cd in drinking water is 0.003 mg/L according to WHO standard.

Lead

Lead metal sources: Lead is found in dyes, gasoline, lead based paint, old plumbing, pottery, insecticides, tobacco smoke, textiles, scrap metal, automobile exhaust, canned fruit juices, car batteries, crayons, hair coloring, air pollution, mascara, and smelting of lead (Dojlido and Best, 1993). Associated symptoms: Abdominal pain, aching muscles and bones, blue-black line near gums, clumsiness, confusion, constipation and/or diarrhea, emotional instability, headaches, high blood pressure, insomnia, drowsiness, loss of appetite, metallic taste, muscle weakness, nervousness, queasiness, nausea and weight loss (Kadirvelu and Namasivayam, 2000). Associated diseases/disorders (ADD): Alcoholism, anemia, and gout damage the kidney, liver and reproductive system, basic cellular processes and brain functions. Pb forms complexes with oxo-groups in enzyme to affect virtually all steps in the process of hemoglobin synthesis and porphyrin metabolism (Ademorati, 1996). Toxic levels of Pb in man have been associated with encephalopathy, seizures and, mental retardation (Scumann, 1990; Naseem and Tahir, 2001). According to WHO guidline for drinking water, the accepted limit of Pb is 0.01 mg/L (WHO 2000).

Copper

Copper metal sources: Copper is found in mineral supplements, hard water, hot tubs and swimming pool chemicals, cookware, the birth control pill and other estrogen medications like birth control side effects (http://www.drugnews.net/yaz), copper IUD's, dental materials, brackets and jewelry, cooking utensils, piping, fungicides, industrial emissions, insecticides, oysters, chocolate, refrigerator ice makers, some city water, and well water. Associated symptoms: Anemia, bitter taste, deposits in corneas of the eyes, depression, diarrhea, digestive disorders, eczema, excitability, hardening of the skin, high blood pressure, hyperactivity, insomnia, nasal irritation, and nausea. Associated diseases/disorders: Arthritis, atherosclerosis, kidney problems, liver dysfunction, mental disturbances, schizophrenia, and tuberculosis. Excessive intake is extremely toxic to living organism and usually known to deposit in brain, skin, pancreas and mycocardium (Davis *et al.*, 2000). Copper does essential work in animal metabolism. But the excessive ingestion of copper brings about vomiting, cramps, convulsions, or even death (Paulino *et al.*, 2006). The maximum acceptable Cu in drinking water in WHO standard is 2 mg/L.

Zinc

Zinc metal sources: Zn is a trace element essential for human health. Zinc and its products are used in the preparation of certain alloys, principally for galvanizing iron; more than 50% of metallic zinc goes into galvanizing steel. It is used in anticorrosion coatings of steel and iron, in electrical devices, in tyre and rubber industries. It is used for the negative plates in some electric batteries and for roofing and gutters in building construction. Zinc oxide is used as a white pigment in watercolors or paints, and as an activator in the rubber industry. As a pigment zinc is used in plastics, cosmetics, photocopier paper, wallpaper and printing inks (Dojlido and Best, 1993). Zinc is an essential element to man, being a cofactor of many enzyme systems (Ukhum *et al.*, 2005). It is important for the physiological functions of living tissue and regulates many biochemical processes. Associated symptom and diseases/disorders: However, too much zinc can cause eminent health problems such as stomach cramps, skin irritations, vomiting, nausea and anemia (Oyaro *et al.*, 2007).

It has been shown that Zinc and Copper comparatively inhabit Cadmium uptake by cells. The recommended daily intake of Zinc is between 4 to 16 mg depending on age, sex and physiological state.

Toxicity of heavy metals is aversely threatening. Their toxicity lies in the fact that most metallic ions bind with cysteine, while some have affinity for histidine, consequently causing protein denaturation (Sigel, 1992).

The toxicity of heavy metals depends on a number of factors. Specific symptomatology varies according to the metal in question, the total dose absorbed, and whether the exposure was acute or chronic. The age of the person can also influence toxicity. For example, young children are more susceptible to the effects of lead exposure because they absorb several times the percent ingested compared with adults and because their brains are more plastic and even brief exposures may influence developmental processes (http://www.patient.co.uk/health/food-poisoning-in-adults).

All living cells possess some mechanism to debar metallic ions from damaging their systems. They have metalloproteins, a sulfur rich protein to remove the former from their cells, containing cysteine residues which bind the ions to the proteins. Normally this metal bonded protein is useless for the organism but in many there is no possibility for its elimination. Thus the accumulation is unavoidable. When these levels cross detrimental concentrations, the cell eventually dies.

Heavy metals form precipitate with anions like CO_3^{--}, SO_4^{--}, S^{--}, Po_4^{---}**,** *etc.* which accumulate in our body and cause toxicity. They may adsorb onto the organic functional group of enzymes, which detach the essential metals. It alters the biological activities of the enzymes. Some of the heavy metals may cause redox reaction in the tissue which changes the chemistry of basic elements like carbon, *e.g.*, Cr (VI) causes oxidation of carbon in tissues of our body (Singh *et al.*, 2011).

Metals are also emerging as an important class of carcinogens. The mechanism(s) involved in metal carcinogenesis (Kasprzak, 1995) is elusive, partly because of the complex nature of metals' interactions in biological systems. Many including carcinogenic metals, follow the metabolic pathways of similar essential metals (Bal and Kasprzak, 2002). This is probably the result of similar binding preferences between carcinogenic metals and nutritionally essential metals.

The Liver and Kidneys as well as the Gallbladder and Lymphatic system are major "filters" of the blood system, removing toxic wastes and debris. The kidneys especially aid in ridding the body of excess "acids" while rebalancing critical pH. If these "filters" are damaged or continuously unclean, our blood will remain laden with debris and heavy metals that could travel to our brain and cause a stroke, or become part of the cholesterol plaque hardening your arteries and veins, or become "stones" painfully lodged in our kidneys. The clean and efficient functioning of key blood filtering organs is critically essential for life. Heavy metals may also be endocrine disrupters that interfere with the functions of hormones in the human body, especially those controlling growth and reproduction (http://emedicine.medscape.com/article/814960-overview).

The route of exposure is also important. Elemental mercury is relatively inert in the gastrointestinal tract and also poorly absorbed through intact skin, yet inhaled or injected elemental mercury may have disastrous effects. These metals often enter into urban wastewater, which on being used in irrigation, results in their accumulation in surface soils. After saturation, surface soils release these contaminants into groundwater for plant uptake. Wastewater irrigation further increases the heavy metal content of soils (Singh *et al.*, 2006).

To avoid health hazards, it is essential to remove these toxic heavy metals from wastewater before disposal (Nur Sudhada, 2009). The metal ions do not undergo biodegradation and many of them are soluble in aqueous solutions, therefore they become more available for living systems and accumulate in the environment (Igwe, 2006). They are conservative pollutants that are not subject to bacterial attack or other break down or degradation process and are permanent addition to the marine environment. As a result of this, their concentration often exceeds the permissible levels normally found in soil, waterways and sediments and they find their way up the food pyramid.

Some of the main sources of heavy metal toxicity occur through consumption of contaminated water, consuming fish contaminated in mercury, mercury contamination from badly fitted dental fillings, poisoning from vaccinations containing thimerosal.

People who are at risk from heavy metal toxicity are those who work in factories without proper detoxification precautions, exposure to metals by living in old houses and peeling paints, consuming water in areas with high arsenic levels, consuming food that is not organic.(http://www.patientsmedical.com/healthaz/heavymetaltoxicity/causes.aspx)

Our health and many of our activities are dependent on the health of aquatic ecosystems. Most of the water that we drink is taken from lakes or rivers. If the lake or the river system is unhealthy, the water may be unsafe to drink or unsuitable for industry, agriculture, or recreation-even after treatment. Uses of aquatic ecosystems are impaired when these systems are unhealthy. The main reason is the wastewater run-off and its contamination of freshwater sources.

Wastewater regulations were established to minimize human and environmental exposure to hazardous chemicals. This includes limits on the types and concentration of heavy metals that may be present therein. The increasing demand for the better quality life is the main driving force for the sound management of metals in wastewater.

Table 1: General Distribution of Heavy Metals in Different Industrial Effluents

Industries	Hg	As	Cd	Pb	Cu	Fe	Ag	Mn	Ni	Cr	Se	Ti	Zn
General Industry and Mining				*	*	*		*		*			*
Paper Products	*			*	*				*	*		*	*
Plating			*	*	*				*	*			*
Paint Product				*						*		*	
Photographic							*			*			
Pipe Corrosion				*	*								
Electronics							*				*		
Tanning		*		*						*			
Fertilizers	*		*	*	*	*		*	*	*			*

The contamination of water is slowly emerging to be the biggest threat to aquatic species. Water is one of the most important resources for sustaining the environment and life on earth. With the growing population there is increased pressure on water as a resource.

Effluents from industrial processes represent one of the most important sources of heavy metal pollution of our water resources. If these discharges are emitted without treatment, they may have an adverse impact on the environment and consequently on human health.

Underground reservoirs are an important source of water supply for many people. Human use wells to tap renewable and nonrenewable aquifers which quench their thirsts and water crops.

Wastewater is any water that has been adversely affected in quality by anthropogenic influence. Municipal wastewater is usually conveyed in a combined sewer or sanitary sewer, and treated at a wastewater treatment plant. Treated wastewater is discharged into receiving water *via* an effluent sewer. Wastewaters generated in areas without access to centralized sewer systems rely on-site wastewater systems. These typically comprise a septic tank, drain field, and optionally an on-site treatment unit.

Around 90% of wastewater produced globally remains untreated, causing widespread water pollution, especially in low-income countries. Increasingly, agriculture is using untreated wastewater for irrigation. Cities provide lucrative markets for fresh produce, so are attractive to farmers. However, because agriculture has to compete for increasingly scarce water resources with industry and municipal users, there is often no alternative for farmers but to use water polluted with urban waste directly to water their crops (http://water.me.vccs.edu).

There can be significant health hazards related to using the water in this way. Wastewater from cities can contain a mixture of chemical and biological pollutants. In low-income countries, there are often high levels of pathogens from excreta, while in emerging nations, where industrial development is outpacing environmental regulation; there are increasing risks from inorganic and organic chemicals. The WHO, in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the United Nations Environmental Program (UNEP), has developed guidelines for safe use of wastewater.

(http://www.fao.org/docrep/w2598e/w2598e07.htm#pesticides%20and%20water%20quality%20in%20the%20developing%20countries)

Treated wastewater can be reused as drinking water, in industry (cooling towers), in artificial recharge of aquifers, in agriculture (70 percent of Israel's irrigated agriculture is based on highly purified wastewater) and in the rehabilitation of natural ecosystems.

There are numerous processes that can be used to clean up wastewater depending on the type and extent of contamination. There are two basic approaches to use the waste in the water as a resource (such as constructed wetlands) or strictly as pollution (such as the majority of today's treatment plants). Most wastewater is treated in industrial-scale energy intensive wastewater treatment plants (WWTPs) which include physical, chemical and biological treatment processes.

Wherever treatment takes place, a diverse range of technologies is used to purify water. Treatment technologies are selected and applied using several determining factors including water source, type of contaminant, and cost.

Awareness of these facts has generated research and development in the field of waste water management; detoxification and quality improvement has become imperative in eco-technologies.

Enumerated here are some conventional surface water treatments:

Coagulation and flocculation practices, dissolved air flotation systems, flocculation-chlorination system, filtration, firect filtration, flow sand filtration, fio-sand filtration, fiatomaceous earth filtration, fag and cartridge filters, feramic filters, fembrane water treatment systems, electrodialysis, reverse osmosis and nanofiltration, disinfection systems, chlorination, ozone, ultraviolet, adsorption systems, ion exchange, activated carbon, air stripping systems – aeration, packed tower systems, tray aeration systems, diffused aeration systems, solar water treatments, solar distillation, solar disinfection, (George *et al.* 2003). All these techniques show some drawback or the other for long term large scale decontamination. Their main disadvantages are:

Incomplete removal of toxins/ large sludge leftovers/ expensive equipment and monitoring units/ high energy and pressure requirements/ generation of toxic waste/ difficult separation.

This has caused a paradigm shift to bioremediation where treatment of waste or pollutants occurs by the use of microorganisms that break down the undesirable contaminants.

Historically, it has been around since 1900s, when Arden and Lockett discovered how bacterial cultures were capable of removing nitrogen and phosphorous from raw sewage when mixed in an aeration tank. This came to be famously known as the “activated sludge process”, structured around the concept of bioaccumulation and still in use today. Its primary role in wastewater management suffered a setback when in the ‘70s further discoveries led to the revelation of metallic sequestering characteristics present in dead biomass, causing the spotlight to fall on biosorption.

SORPTION is a combo process involving both adsorption and absorption and is the primary method employed for this research.

BIOSORPTION, simply put, is a physiochemical process that occurs naturally in certain biomass (plant materials and animal waste), which allows it to passively concentrate and bind contaminants to its cellular surface. It involves the uptake of heavy metal ions from aqeous media by biosorbent or the adsorbing biomass. This type of sorption is 2-fold: metal is usually uptaken onto surface of cell, being followed by bio-accumulation inside cell due to metal uptake matabolisms (Igwe and Abia 2006).

The salient features of biosorption have thus been seen to be as:

Metabolically passive, determined by equilibrium, amount of effluents adsorbed depends on kinetic equilibrium and composition of the sorbents cellular surface, contaminants adsorbed into cell structure, reversible process, biomass can be recycled and reused or discarded, fast rate of adsorption and higher concentrations and green eco-alternative to man-made ion-exchange resins.

The major advantages of biosorption over conventional treatment methods include (Kratochvil and Volesky, 1998):

- High efficiency
- Minimisation of chemical and biological sludge
- No additional nutrient requirement
- Regeneration of biosorbent
- Possibility of metal recovery
- Use of naturally abundant renewable biomaterials that can be cheaply produced
- Ability to treat large volumes of wastewater due to rapid kinetics
- High selectivity in terms of removal and recovery of specific heavy metals Ability to handle multiple heavy metals and mixed wastes

High affinity, reducing residual metals to below 1 ppb in many cases

- Less need for additional expensive reagents which typically cause disposal and space problems
- Operation over a wide range of physiochemical conditions including temperature, pH, and presence of other ions (including Ca^{2+} and Mg^{2+})
- Relatively low capital investment and low operational cost
- Greatly reduced volume of hazardous waste produced

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases.

During biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups present on the biomaterial. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, (*i.e.,* non-metabolism dependent) is relatively rapid and can be reversible (Kuyucak and Volesky, 1990).

Physical adsorption: In this category, physical adsorption takes place with the help of van der Waals' forces. Kuyucak and Volesky (1990) hypothesized that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomasses of algae, fungi and yeasts takes place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells. Electrostatic interactions have been demonstrated to be responsible for copper biosorption by bacterium Zoogloea ramigera and alga Chiarella vulgaris (Aksu *et al.,* 1991), for chromium biosorption by fungi Ganoderma lucidum and Aspergillus niger. There are two principal modes of adsorption of molecules on surfaces, namely physical adsorption (physisorption) and chemical adsorption (chemisorption). The basis of distinction is the nature of the bonding between the molecule and the surface.

Chemical Adsorption: a chemical bond, involving substantial rearrangement of electron density, is formed between the adsorbate and substrate. The nature of this bond may lie anywhere between the extremes of virtually complete ionic or complete covalent character. There is a possibility that the molecules can decay.

Four interaction forces are important in the biosorption mechanism:

- London-Forces (molecular forces between two anti - polar substance)

- Keesom-Forces (forces from the interaction between permanent dipoles)

- Debey-Forces (by induced dipoles)

- Chemical interaction forces (charge-transfer-interaction)

Bioaccumulation occurs by absorbing contaminants which are transferred onto and within the cellular surface. Both bioaccumulation and biosorption occur naturally in all living organisms, however, in a controlled experiment conducted on living and dead strains of bacillus sphaericus it was found that the biosorption of chromium ions was 13–20% higher in dead cells than living cells.

In terms of environmental remediation, biosorption is preferable to bioaccumulation because it occurs at a faster rate and can produce higher concentrations. Since metals are bound onto the cellular surface, biosorption is a reversible process whereas bioaccumulation is only partially reversible.

Biomass must come from nature, even if as a waste material. It is broadly classified under Bacteria, Algae, Fungi, Yeasts and Agri-wastes. Agri-wastes include protein based animal fibres or cellulose based plant fibres, bear amino/ carboxylic or hydroxyl functional groups, which play the pivotal role in metal binding. The cell wall structures in algae, fungi, bacteria and agri-waste act as ion-exchangers to bind and concentrate heavy metals from even dilute aqueous solutions effectively. These biomass types serve as basis for the biosorption process, and have clearly been seen to accumulate 25% of their dry weight in deposited heavy metals. (Mc Gill University, Montreal http://biosorption.mcgill.ca/whatis.htm). Different species have different sorption characters, being influenced by external factors like pH balance, interaction between different metal-ions, concentration of biomass, temperature and pressure, *etc.* Activated carbon has played a legendary role in the biosorption bracket, but here too it had to be manufactured and was expensive.

Experiments have been carried out widely and successfully with alternatives as activated sludge, coconut husk, crustacean shells, fruit peels, sea weed, almond shells, microorganisms like algae and fungi, bacteria, to name only a few. While their advantages have been aforementioned, a major withdrawing factor of adsorption technology is the accumulation and transfer of pollution load from the aqueous to adsorbent phase. The pollutants may not be eliminated totally but transferred. Some chemi-sorption processes (photocatalysis, electrochemical oxidation, *etc.*) have also known to be destructive techniques. (Theodora Velegraki, Technical University of Crete http://www.researchgate.net/publication/234013556_). But this has conspicuously not been so in the case of biomaterials. In fact the best bet has been placed on a process which combines two: adsorption and biodegradation.

Many industrial effluents contain toxic metals that must be removed. Removal can be accomplished with biosorption techniques. It is an alternative to using man-made ion-exchange resins, which cost ten times more than biosorbents. The cost is so much less, because the biosorbents used are often waste from farms or they are very easy to regenerate, as is the case with seaweed and other unharvested biomass.

Industrial biosorption is often done by using sorption columns. Effluent containing heavy metal ions is fed into a column from the top. The biosorbents adsorb the contaminants and let the ion-free effluent to exit the column at the bottom. The process can be reversed to collect a highly concentrated solution of metal contaminants. The biosorbents can then be re-used or discarded and replaced.

A large quantity of materials has been investigated as biosorbents for the removal of metals or organics extensively. The tested biosorbents can be basically classified into the following categories: bacteria (*e.g.* Bacillus subtillis), fungi (*e.g.* Rhizopus arrhizus), yeast (*e.g.*, Saccharomyces cerevisiae), algae, industrial wastes (*e.g.*, S. cerevisiae waste biomass from fermentation and food industry), agricultural wastes (e.g. corn core) sugarcan bagassage, apple waste (Homagai *et al*. 2010, Homagai *et al*. 2008/2009) and other polysaccharide materials, *etc*. (Vijayaraghavan and Yun, 2008). The role of some groups of microorganisms have been well reviewed, such as bacteria, fungal, yeast, algae, *etc.*

These tested biomasses have been reported to bind a variety of heavy metals to different extents (Gupta *et al.*, 2000). Some potential biomaterials with high metal binding capacity have been identified in part. Some types of biosorbents are used in binding and collecting the majority of heavy metals with no specific priority, while others can even be specific for certain types of metals (Volesky and Holan, 1995).

Some waste mycelia are available in large quantities for the removal of heavy metals (Kapoor and Viraraghavan, 1995; Wang and Chen, 2006). Seaweeds from the oceans produced in copious quantities are another inexpensive source of biomass. Marine algae, especially brown algae such as Sargasso seaweed was investigated for metal removal (Davis *et al.*, 2003c). Abundant natural materials, particularly cellulosic nature, have been suggested as potential biosorbents for the removal of heavy metals. For economical reasons, recently other low-cost biosorbents, such as agricultural wastes are of interest (Bailey *et al.*, 1999). It is necessary to know the mechanism by which biological materials accumulate metals.

Microbial cells contain a large number of metal binding sites called ligands. Biomass cell wall contains polysaccharides proteins and lipids which offer functional groups to bind metal ions. The mechanism by which biological materials remove metals is not completely understood. The performance of the biosorbent depends on the ionic state of the biomass. The uptake of metals by biomass is a two step process, the cells of biomass contains proteins and polysaccharides which offer a lot of binding site for heavy metals. The first step is the stoichiometric interaction between the cell components and the metal ions. The second step is the accumulation of heavy metal on the binding sites.

Removal of heavy metals from aqueous solutions using plants and plant derived biomass is a viable option when cost of removal is a major criterion. Large numbers of works have been performed with various plant based biomass. Eicchornia crassipes, sawdust, bael fruit, wheat straw have been reviewed as biosorbents.

Microbial biomass

Microbe’s posses a large number of polysaccharides in their cell wall which helps in accumulation of metal ions. The mechanism is similar to that of ion exchange method of removing heavy metal (Murugavelh S. & Vinothkumar, 2010). The method is use ful since the cost of biomass is cheaper and can be regenerated easily.

Bacteria

Bacteria may uptake and accumulate a significant amount of metal ions resulting in the transfer of metal ions to a matrix. Suspensions of dead biomass of actinomycetes from industrial fermentation were mixed with waste water. The biosorption of Cd occurred due to negatively charged sites in the bacterial cells (Carlos *et al.*, 2009). Arthrobacter sp was protanated with 0.1 N HCl and titrated with 0.1 N NaOH. Biosorption tests were performed for Cu and Cd adsorption at pH 4 and 5. Subsequent addition method (SAM) was employed. The equilibrium time for each metal was calculated and found to be 30 min.

Fungal biomass

Cu uptake by R. oryzae was studied in flask containing 25 mL of Cu solution in an environmental shaker at 150 rpm. The performance of biomass was maximum at pH 4- 6. Alkali treatment (NaOH) of the biomass resulted in higher amount of Cu removal as alkali neutralized the protons in the native biomass making more binding sites available for Cu ions (Butter *et al*., 2009). Nonliving waste biomass of A. niger attached to wheat bran was used as biosorbent for removal of Zn and Cu from aqueous solution and metal uptake was found to be a function of the pH, initial metal concentration and biomass loading. Alkali treatment of A. niger biomass was found to sequester Cd, Cu and Zn effectively to 10% of its weight. Neurospora, Fusarium and Penicillium were also studied but A. niger gave better results in removal of Cd, Cu and Zn. Dry cells of R. arrhius were used in removal of Fe, Pb and Cd ions from waste water. Higher adsorption rate and adsorption capacity was obtained in a batch method. The maximum adsorption obtained was 100-150mg /L at pH 5 and 30°C (Murugavelh and Vinothkumar, 2010)**.**

Reed

Reed contains lignin and cellulose as the major cellular component which has the ability to adsorb heavy metal ions from metal solutions. Bounheng *et al*., 2006, studied the use of reeds as biosorbent for removal of heavy metals. powdered reed was treated with acid and alkali. 0.05g of reed sorbent so prepared was treated with metal solution at a pH range of 2-3 and for 3 h in a shake flask. Plasma absorption spectroscopic studies showed 64% removal of heavy metals (Southichak *et al.* 2006).

Saw dust

Saw dust was used for the chromium adsorption. The Batch experiment was performed with various concentration of saw dust to flasks containing chromium solutions (1000 mgL^{-1}) at 25°C. The concentration of the chromium is determined by spectrometric method. (Naiya *et al*., 2009) reported 42.52 mg/ g adsorption of chromium at pH one. Various adsorption isotherms were also studied and among them Langmuir isotherm gave the best fit.

Sunflower stem waste

Sunflower stem was sundried and ground in ball mill and washed with distilled water, oven dried at 60°C and sieved to 300 µm. The powdered sunflower stem was treated with water followed by formaldehyde. 0.2 mg of biosorbent / 50 mL of chromium solution (1000 mg/L) at pH -2 was taken in a flask for batch experiment. The solution was centrifuged at 400 rpm for 10 min and the chromium concentration in the supernatant solution was studied using atomic absorption spectroscopy. SEM studies revealed Sunflower stem has large surface area for binding of large number of metal ions (Jain *et al.,* 2009).

Bael fruit

Bael fruit was dried to 110°C and reduced to size range of 600-800 µm. Chemical activation was done with 88% ortho phosphoric acid. Batch kinetic study was performed for pH 1-8 and various chromium concentrations (50-125mg/L) with a predetermined time of 240 min in an orbital shaker. The batch studies were conducted by varying one parameter at a time. The samples were filtered and analyzed in a flame absorption spectrophotometer. Maximum removal of chromium was obtained at pH 2 and the removal efficiency decreased 91.9 % to 85.9 % with increase in initial concentration of chromium from 50-125 mg /L (Srinath *et al.,* 2002).

Wheat straw

Cd and Cu removal by wheat straw was studied with different pH from 4- 7 and varying metal concentrations. 60 % removal of Cd (II) and Cu (II) respectively was reported with the increase in pH from 4 to 7, temperature also was studied as a significant parameter. Experimental studies revealed maximum removal at 25 to 30°C. Langmuir and Freundlich isotherm suited well for Cd. The study proposes wheat straw as a low cost biosorbent for heavy metals removal (Anandkumar *et al*., 2009).

The Kush

Enter the *Desmostachya bipinnata,* commonly known as Kush grass (Figure 1) in Nepal and India, big Cordgrass in USA and Halfa in Australia. In South Asia it bears an exalted mythological pedigree and religious status. Hindu rituals use it as an integral part of their processes. It holds a respectable position in Buddhism as well. Its name signifies the sense of “acuteness”, as its edges are long and sharp, sword like. It is a symbol for discriminating wisdom.

Figure 1: Kush Plant (*Desmostachya bipinnata)*

The inspiration to use the Kush as a novel alternative came epiphanously on discovering a chance article which explained how a man had planted it in an industrial area and it had shown significant absorption of cadmium as well as having high penetrating capacity, enough to absorb x-rays. A little speculation thus led to empirical research.

A major experimental research was carried out by the University of Zabol, Iran, where the grass was tested for its duress and sustainability in such testing conditions as those of the Sistan Plain in the Middle-East. It was concluded categorically that the subject, which reproduced through rhizomes, was suitable for rehabilitation of desertified rangelands and such hyper arid regions, also being used as nutritious fodder for cattle because of its high protein content, 7.56% during vegetative growth (Fakhireh *et al.,* 2012).

Two minor researches have also been brought to light in association with Kush:

1. Phytochemical screening: dry metabolites present in the plant having medicinal value and as such have been used in Ayurveda.

2. Biological screening: bioactive plant compounds have been known to have curative effects on jaundice, cancer and sexual diseases.

It has been made use of in broad spectrum pharmacology providing its active therapeutic value. It fulfills all the requirements of a cheap biosorbent, abundant in nature, free availability, renewability, low economic value. Most man-made synthetic ion exchange resins are marketed for about 30-50 US$/ kg. Biosorbents are worth around 3-5 US$/ kg. Here, in this unharvested lignocellulosic material, we have a virtually free of cost alternative. Its pretreatment significantly enhances its sorption capacity.

A rhizomatous perennial of arid areas, this monotypic (single one of its kind) genus grows in tall tufts in an extensive system of erect and spiky clusters. Stout robust stems branch out from the base, covered with tiny sheaths. The rigid and herbaceous monocot stems or culms have smooth knots, while the spikes are epliptic-oblong and attached directly to the base or sessile. The coarse, tough, narrow leaves grow to a length of 50 cm, almost 3-19 mm in width. The leaf blades are flat, long and acuminate.

Kush, being a lignocellulosic material. It contains cellulose (27.37%), hemicelluloses (35.04%), and lignin (7.64%) as major constituent, which is shown in Table.3.This lignocellulosic material is rich in large amount of easily accessible hydroxyl groups and other variety of functional groups which may be involved in the attachment of amino groups on the surface of biomaterials and make suitable for metal adsorption. The structures of cellulose, hemicelluloses and lignin is shown in Figure 2.

The Kush grass grows frequently in dry open wastelands, sand dunes, inland brackish wetlands, salt affected wastelands and abandoned agro-fields subject to periodic disturbances such as burning, cutting, grazing, degradation. It thrives in hot, dry conditions, becoming invasive and forming tussocks. It has also been found to occur commonly in rice bunds and wheat fields and timberlands as a weed.

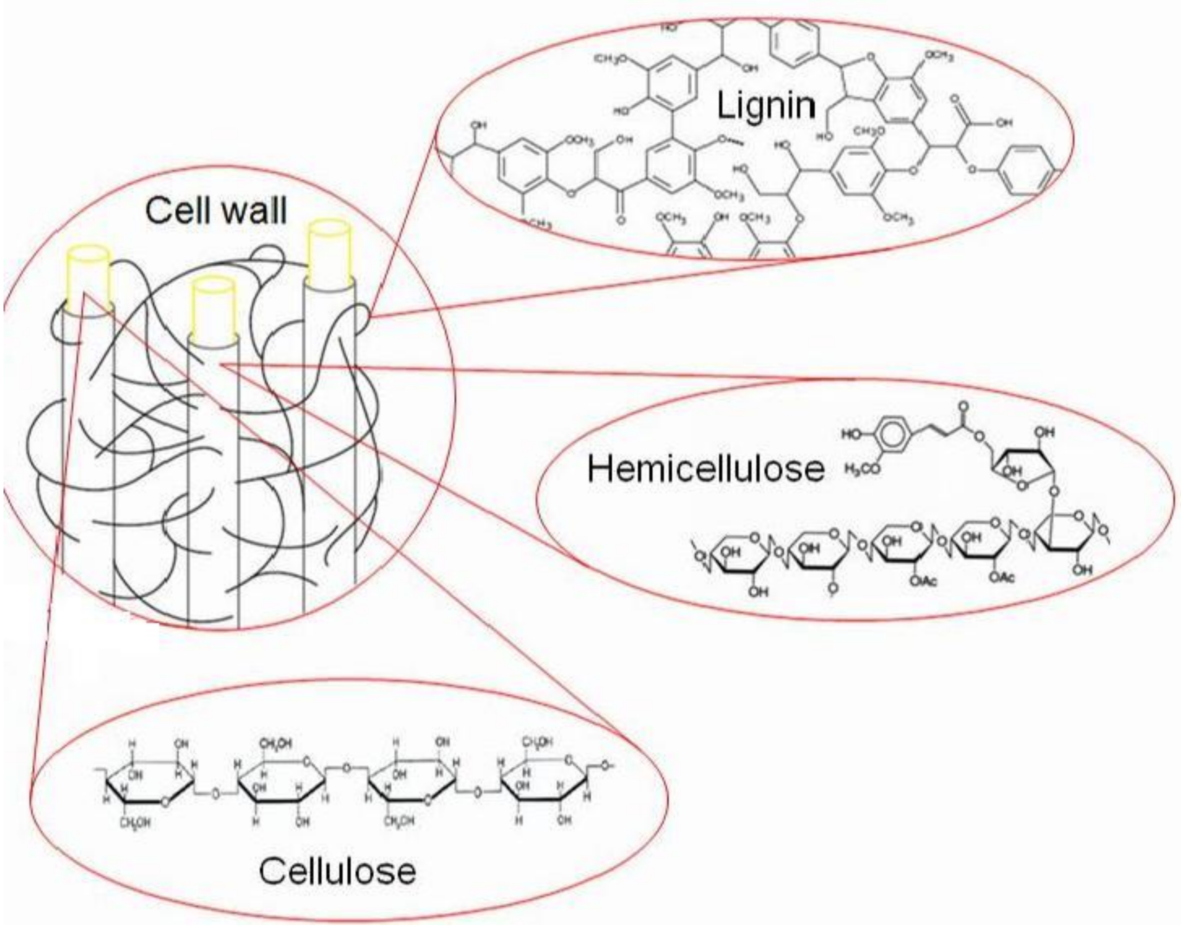

Figure 2: Structures of cellulose, hemicelluloses and lignin

It has been found abundantly distributed in large parts of Africa, the middle-East, Asia, as far as Thailand, growing as a perennial herb in arid and sandy areas. It lives also commonly in brackish waters, by lakes and river banks and river mouths in tufts or tussocks, as clumps.

Being hardy and tensile in nature, it has high drought tolerance and thus withstands rainfall as low as 54 mm, but even so surviving rain above 1000 mm. This dual nature allows it to grow dominantly in soils with restrictive water penetration, and with its immunity and resistance to saline, alkaline, sodic (containing sodium), calcareous (containing calcium) soils, it is naturally adaptable to desert conditions, aridity, irrigation channels as well as brackish water areas.

An aggressive rhizome, it has been known to bind sand excellently, as well as invade the environment as a serious infestation, difficult to eradicate. As such, it is known to be a noxious weed in fruit orchards as in Israel.

Desmostachya bipinnata has been known to have no obvious malefic effects on biodiversity. It combines well with other plants in growth. Its high tolerance to hostile environments and invasive nature, local mobility, sustainance against mutilation, burning and varied soil content have made it plant dominant in saline areas, making it a true halophyte. It is a hardy survivor, whose roots go deep to find water.

Its propagation is vegetative from underground rhizomes, water dispersal and wind dispersion. High winds blow away its aerial parts as tumble weed, scattering it all over. Manually cut roots can regenerate elsewhere in new locations; its propagules (like the spore, seeds or cutting that help in reproduction) remain active over a year.

It flowers and fruits from May to July and matures between August to October. Containment is manual weeding or through chemical processes of dalapon and paraquat application; its known enemies are the insects like Heiroglyphus banian and Belenois gp, which feed on it and even lay eggs. Worldwide, the following uses of the humble Kush grass have been experimented with and noted

-it has been known to be an excellent sand binder, thus as a soil conservation agent; and fibre yarn in Sudan

- culms have been used to thatch huts, make brooms and thick hardy ropes.

- the pulp which constitutes 35% of biomass, has been made into paper

- young shoots with a protein content of 6.75% provide good fodder for livestock in the Middle East and Sub Sahara

- serves as potential forage crops on saline/sandy/marshy wastelands

- under refined conditions along with other plant species, it has been pivotal in reclaiming saline soils

- has empirically shown to have high conductivity for transmitting phonetic vibrations

- is known to absorb 60% radiation

- it has shown in various studies to be a calcium channel blocker, therefore is now proposed as a candidate for adsorption process

- nutrient content and productivity, physiochemical and bio properties of sodic soils has been shown to improve in its presence along with other agro forestry species

- in ayurveda and other traditional medicine, it is used as a diuretic, to cure dysentry and menorrhagia, vitiated pitta, urino-genital disorders, even used as a mild stimulant

- the ancient text, Atri Samhita mentions its virtues as relieving perspiration, burning, skin disorders, jaundice etc..

- contemporarily, it has found novel use as in being made into baskets, flip flops, mats and shoes

Certainly the merits of this humble weed- cereal outweigh its demerits. Its wide availability and low cost source should make it more appealing as a viable option in sophisticated scientific projects in the developing world, where fund resources are scarce. It is time to be aware and alert of its potentials and launch innovative experiments to make use of what nature has provide us with so bountifully and freely (The Kush Seed Society, ancientcerealgrains.org, www.cabi.org).

This is the first ever formal experimental research carried out on the Kush plant, in Nepal.

1.2 Rational

Water is the most precocious source from nature for living beings, and life is not possible without water. Water is being scarce in most of the countries and there is no alternative of water. This precocious source from nature is being polluted each and every day due to various developmental activities. The problem is most serious in developing countries, especially heavy metal pollution, since most of them are not aware of its effect on environment and human health. On the other hand the treatment technique is not affordable. Hence people in developing countries are always living at risk. So there is a dare need of cost effective new techonologies which helps to lower the concentration of pollution in the environment within the level allowed by law. With the awareness in people and development in technologies several schemes and technologies have been adopted, which can be seen else were in the literature. Among them, removal of toxic heavy metal ions from aqueous streams by the use of agricultural by-product as biosorbent is an innovative and promising technology. These materials are economic and ecofriendly due to their unique chemical composition ,easy availability, renewable, low in cost and more efficient, which are the most viable option for heavy metal remediation especially in the developing countries where cost is the main issue for the removal of heavy metal ions in trace amount present in the aqueous solution. These promising agricultural materials are used for the removal of metal ions either in their raw form or after some physical or chemical modification.

The present research work deals with the preparation of biomaterials with the use of three different modifying reagents and utilized in the removal of heavy metal ions.

Depending on the nature of complexity of the waste water, more than one process has to be coupled with the other. However, synthetic materials are quite often used in this respect to cope the stringent environmental regulations. The main rational behind this study is to develop the environmental benign materials that could be cheap and can be easily incenerated without releasing dioxane to the environment as it happens with the synthetic materials.

1.3 Objectives

Recently attention has been drawn towards the biomaterials which are by products or the waste materials from agriculture and foresty. Scattered research has already been done on the different types of biosorbents for the removal of metal ions. Agricultural materials with cellulose, hemicelluloses and lignin contain variety of functional groups which facilitates metal binding process. The major advantage of biosorption over conventional treatment methods includes: low cost, high efficiency, minimization of chemical or biological sludge, regeneration of biosorbents

The major objective of this research work is to explore the biopolymer which can be the by-product or biowaste with low cost, easily available, environmental friendly. The prepared biopolymer must be cost effective and efficient with high loading capacity comparaed to commercially available adsorbent. To meet these objectives different aspects of *Desmostachya bipinnata* are discussed as:

1. Feasibility of *Desmostachya bipinnata* for removing metal ions from aqueous media.
2. Characterization of biopolymers with Elemental analysis, SEM, DRFTIR, Zeta potential, TG/DTA, XRD to confirm the introduction of Nitrogen functionalized groups for the adsorption of heavy metals.
3. Perform the batch experiments at optimum pH to investigate the maximum loading capacities of the biopolymers for metal ions.
4. Define practicality of various isotherm models for the best fit isotherm equation,
5. Determine the kinetic for explaining nature of adsorption process.
6. Discuss various controlling factors, physio-chemical, affecting the process.

7. Perform experiments on adsorption and regeneration to prove the viability of *Desmostachya bipinnata* as progressively undeteriorable even as many as six experimental cycles later.

8. To ensure the applicability of functionalized biopolymer in the treatment of effluents from different industries containing traces of heavy metals.

CHAPTER 2

2. LITERATURE REVIEW

There is a growing concern in the development of bioadsorbents as the alternative to the synthetic chelating resins. Many research works are already in progress regarding how biomaterials can be made more effective (Babel & Kurniawan, 2003). Although various procedures have been used to create a suitable chelating medium onto the polymeric surface of biomaterials (Chergui *et al.* 2007; Kumar & Bandyopadhyay, 2006) very little attention has been given to the functionalization of biomaterials. Removal of toxic heavy metals from waste water is one of the most important environmental issues. Several physicochemical methods have been developed for the removal of heavy metals from wastewater. Conventional methods are limited by technical and economic barriers, especially when concentration of metals in waste water is low (Witek-Krowiak *et al.*, 2011). On the other hand, biosorption has been recognized as a promising technology as an alternative to the conventional processes for the treatment of wastewater at trace levels of metal contaminants in industrial wastewater (Thirumavalavan *et al.,* 2010; Witek-Krowiak *et al.,* 2011 ; Alluri *et al.*, 2007). In recent years, materials of biological origin have attracted many researchers. These include agricultural byproducts, fruit peels, forestry, algal biomass, fungal biomass, and fishery products (Deng & Ting 2005; Yun *et al.*, 2001; Khoo & Ting 2001). These by-products contain mainly polysaccharides like cellulose, hemi cellulose, lignin and pectin with good metal binding capacity and adsorption efficiency. Adsorption of heavy metals onto raw biosorbent has been found to be less effective owing to their limited exposed functional sites. These capacities were increased by the suitable chemical modifications like, saponification, carboxylation, amination, xanthation and phosphorylation. Functional groups like carboxylate, hydroxyl, sulphate, phosphate, amide, and amine groups onto the biosorbents have been reported to be responsible for appreciable metal binding (Alluri *et al.*, 2007; Yun *et al.*, 2001; Tan and Cheng 2003).

The most commonly used natural biosorbents are totally renewable low cost and their use and handling involve no additional risks (Ahmed 2011; Chen *et al.* 2010; Bl´azquez *et al*.

2005). Among various types of biosorbents, few of them are reviewed as, Ghimire *et al.* (2002) studied the adsorptive behavior of phosphorylated orange waste loaded with Fe (III) for arsenic removal. The loading capacity for Fe (III) on phosphorylated orange gel was 67.57 mg/g higher than that of gel prepared from the cellulose 53.61 mg/g and found that arsenic removal efficiency was higher on the phosphorylated orange gel due to higher uptake capacity of gel on Fe (III) which in turn has more ligand exchange capacity. The uptake of Fe (III) was dependent on pH and adsorption was taken place according to Langmuir isotherm and concluded that the adsorbent prepared from seaweed and apple waste revealed to be simple, cost effective and promising for the removal and recovery of metal ions from the aqueous medium.

Ghimire *et al.* (2008) investigated the adsorption of metal ions onto cross linked seaweed, Laminaria japonica. The adsorbent exhibited excellent selectivity towards several divalent and trivalent metal ions. The maximum adsorption capacity for Pb (II), Cd (II) and Fe (III) was found to be 280 mg/g, 123 mg/g and 85.45 mg/g respectively, and was pH dependent. The experimental data was well fitted according to Langmuir adsorption isotherm. The prepared gel was found to be applicable not only for cationic metal ions but also had shown its potential ability for the adsorption of anionic metal ions by loading the gel with Fe (III) prior to its adsorption.

Hawari *et al.* (2006) investigated the feasibility of anaerobic granules as biosorbent for Pb (II) and Cd (II) removal from aqueous solution. Different ionic forms of anaerobic biomass were evaluated in terms of metal binding capacity. The Ca-biomass was found to have the highest uptake capacity. The pH of the solution affected metal uptake. Over the pH range of 4-5.5, pH relating effect was not significant. The uptake was very fast as nearly 75% of total metal uptake was completed within the first 5 minutes of contact. The Langmuir isotherm model showed the good fitting, and maximum metal uptake capacity was found to be 255 mg/g for Pb (II) and 60mg/g for Cd (II).

Jha *et al.* (1988) investigated the utilization of chitosan for cadmium removal. It was demonstrated that an adsorption capacity of 5.39 mg of Cd (II) of chitosan was achieved at a pH range of 4.0-8.3 and that the presence of ethylenediamine tetra acetic acid (EDTA) significantly decreased the cadmium removal by chitosan since EDTA, a stronger chelating agent than chitosan, suppressed the metal uptake by chitosan. It was also reported that in the presence of EDTA, the affinity of Cd (II) for the amino groups was drastically reduced since the EDTA masked the presence of Cd (II) in aqueous solution, causing their removal from the solution to become difficult.

Parajuli *et al.* (2005) studied the adsorption of Fe (III), Cd (II) and Pb (II) on cross linked lignocatecol and optimum pH was found to be less than 5. Maximum adsorption capacity of the adsorbent for Fe (III), Cd (II) and Pb (II) were found to be 75.39, 129.2 and 370.5 mg/g respectively. They also found that the equilibrium data for the adsorption of Pb (II) follows the Langmuir isotherm.

Pavasant *et al.* (2006) evaluated the sorption of Cd (II), Cu (II) and Pb (II) on a dried green macroalga Caulerpa lentillifera in a binary component system and found that presence of secondary metal ion reduced the total sorption capacity of biomass material. They obtained the sorption capacity of Pb (II) was 0.081 mol/kg in single component system which was reduced to 0.071 mol/kg in the presence of Cu (II). Sorption of Pb (II) was found to be significantly decreased in comparison of the sorption of Cu (II) and Cd (II) metal.

Reddad *et al.* (2002) studied the efficiency of adsorbent prepared from sugar beet pulp for the removal of Pb (II), Zn (II) and Cd (II) from aqueous solution. They found the metal removal was due to carboxylic function in lignin of sugar beet pulp. The maximum sorption capacity of Pb (II) and Zn (II) at pH 4 and Cd (II) at pH 6 were 73.76, 17.77 and 24.3 mg/g respectively. The adsorption fit well with Langmuir and Freundlich model. From the comparative kinetic study, pseudo-first and pseudo-second order equation seems to be best described for the adsorption of metal ions.

Schmuhl *et al*. (2001) investigated the cross linking effect of chitosan for the removal of Cr (VI). It was found that non cross linked chitosan has the potential to adsorb 30 mg more of Cr (VI) /g of chitosan. This is consistent with the fact that cross linking reduces the adsorption capacity of chitosan, but this loss of capacity may be necessary to ensure the stability of chitosan.

Sharma and Bhattacharya, (2004) used powdered neem leaf as biosorbent to extract Cr (VI) from aqueous solution. The adsorption was carried out with batch experiment using different concentrations of metal ions, the adsorbent doses, pH, agitation time and temperature. The suitability criteria of the adsorbent were checked by the Langmuir and Freundlich isotherms.

Activated Azadiractha indica was used as a biosorbent (Babu and Gupta, 2008) for the removal of heavy metal from aqueous solution. They observed that the adsorption capacity could be enhanced by modifying the physical structure and surface chemistry of the neem leaf *via* an activation. Sharma & Bhattacharya (2004) investigated that neem leaf is a good adsorbent for cadmium, from simulated wastewater. The increase in the removal efficiency (8.8-70 %) with a rise of pH from 4-7 was due to the considerable effect of pH upon the removal percentage.

Han *et al.* (2006) performed sorption experiment with chaff in a fixed bed column. Chaff is an agricultural by-product containing fiber and protein along with some functional groups like carboxylates, hydroxyls and amidogens and mainly given to livestock. Several operation variables, represented by pH, flow rate, influent concentration of solution and co-existing ions were varied to reveal its effect on the removal performance. In addition, adsorption and desorption cycles of chaff were tried to arrive at a conclusion that their reuse would be feasible.

Another study has also included pectin, an anionic plant cell wall polysaccharide based on alpha-(1-4) linked *D*-galacturonic acid, which is commercially available by extraction from pectin-rich sugar-beet pulp, apple pomace and citrus peels (Schiewer & Patil, 2008). Some degree of similarities exists between pectin structures and alginate, denoting its potential as an alternative sorbent. They compared Cd (II) uptake capacities and stability of several pectin rich fruit wastes. Citrus peels were identified as the most superior alternative adsorbent for its high metal uptake in conjunction with physical stability.

Coconut copra meal is a by-product of coconut oil production and is characterized by the presence of alcohols and carboxylic acids as functional groups on its surface. This by-product has been employed as a biosorbent in the removal of cadmium from wastewater

(Ho & OfoMaja, 2006). The initial pH of the solution and metal ion concentration plays the major role on cadmium sorption.

Pino *et al.* (2006) examined the adsorption capacity of green coconut shell powder on heavy metals. Coconut cell contains lignin and cellulose, which bears acidic functional groups like carboxylic and phenolic groups. The sorption capacities of coconut shell for cadium, chromium and arsenic were studied at different initial metal ion concentration and pH values in batch mode. The obtained experimental data for each metal were also evaluated and fitted with Langmuir and Freundlich isotherms, while the kinetic data were interpreted with pseudo-first and second model.

Rice bran is a by-product of rice milling process which has been used as an adsorbent for zinc removal from synthetic wastewater (Wang *et al.* 2006). They also studied the influence of the parameters such as pH, temperature, particle size and time on sorption capacity. The results showed the importance of pH on zinc sorption capacity while to a certain extent the capacity also relies upon sorbent size.

The biosorbent prepared from Srilankan tea was used for the removal of Cu (II) and Pb (II) from wastewater by (Amarasinghe & Williams, 2007). They confirmed the high potential adsorption capacity of tea waste-based granular activated carbon.

Wheat bran as an agricultural by-product has been employed as a sorbent for detoxification of two heavy metal ions, Cu (II) and Zn (II) from aqueous solution, (Dupont *et al.* 2005). The metallic cations were found to be attached in a complexation reaction to the lignin and fatty acid containing functional groups like alcohol, ketone and carboxylic acid within lignocellulosic substrate of wheat bran.

Alluri, *et al*. (2007) opine biosorption outperforms its predecessors being eco friendly, cost - effective, highly efficient, minimising sludge, allowing for possible metal recovery and regeneration of biosorbents. Dilute mineral acids were used to remove heavy metals from biomass; organic acids and complexing agents can be used for metal elution without affecting biosorbents.

Rao & Khan (2009) investigated Neem Oil biowaste to remove Cu (II) and Cd (II) from aqueous solutions. As a low cost biodegradable, freely found biosorbent, a viable option. Chemisorption efficiency is increased by temperature increase, using pseudo-second order model.

Witek-Krowiak, *et al.* (2011) have explained that peanut shells hold significant potential (being cheap biomass) in removing Cu (II) and Cr (III) ions from wastewater under optimum conditions of pH 5, 20 ^{0}C temperature, contact time 1h, with maximum biosorption capacity of 25.39 mg/g.

Deng, *et al.* (2005) used modified biomass of P. chrysogenum for sorption of Cu (II) and Cd (II), by copolymerising it with AAC. It was proven to be very successful, as grafting effective groups on fungal biomass can result in high sorption capacity biosorbent. Adsorption of the two metals on the biomass was spontaneous, endothermic and complex.

Ahmed, *et al*. (2011) used batch and fix bed column techniques to extract Fe (III) and Cu (II) by using wood sawdust. This is a benign ligno-cellulosic, natural carbo-polymer, inexpensive material containing oxygen, and showed high affinity and selectivity for both iron and cadmium as opposed to other sorbents.

Ayyappan *et al.* (2005) conducted batch adsorption studies to check feasibility of using carbon from agri-waste materials like pith/ bagasse/ sawdust, for wastewater purification. Pith stood the best candidate in the process to remove Pb (II) as metal removal reached 100% within 120 minutes of contact time. Desorption of Pb (II) was achieved by eluting with 0.1 M HNO_3 with 96 % metal recovery; carbon regenerated by washing simply with calcium chloride.

Qiu *et al.* (2009) reviewed a wide variety of adsorption kinetic models and studied the boundary conditions closely for improvement upon adsorption of kinetic modelling, significant to assay an adsorbent in the environmental remediation of wastewater decontamination.

Othman *et al.* (2011) have explained that maleic acid treated *Tamrix articulata* wastes show high potential for Cd (II) removal. The process is spontaneous, exothermic with maximum adsorption at pH 4 in 2 h, with 195.5 mg/g being adsorbed.

Sud *et al.* (2008) have studied various inexpensive and efficient biosorbents in batch experiments. Cellulosic agro-waste are low cost, renewable, have multiple reuses, can be modified for higher efficiency, all for industry-scale applications.

Buasri, *et al.* (2012) modified corn cob to heavy metal ion biosorbent for removal of Zn (II). It was first modified with phosphoric acid. Chemisorption was seen to control the rate, while other conditions like solution concentration/ biomass concentration and temperature affect the biosorption process. Corn cob was found to adsorb 79.21 mg of Zn (II) per 1 g biomass.

Paudyal *et al.* (2013) nominated dried orange juice residue (DOJR- sold commonly as cattle feed in Japan) as a viable adsorbent. It was first converted into fluoride adsorption gel. Spectroscopic analysis confirmed Zr (IV) ions and fluoride efficiently adsorbed on DOJR. Fluoride was desorbed using an alkalin sodium hydroxide solution for its regeneration.

Jia *et al.* (2002) oxidized the commercially available coconut shell derived active carbon, with nitric acid and ammonia treatment. Pyridinic surface groups in active carbon act as adsorption sites for transition of metallic ions in water by co-ordination.

Tsioptsias *et al.* (2010) prepared cellulose acetate ions oxide composite nanofibres *via* electrospining, which showed high thermal stability compared to pure cellulose acetate. These can be used in high degree separation methods like filtration and even has bio-medical applications.

Vinodhini & Das (2010) treated tannery effluents with neem sawdust to remove Cr (VI) in a fixed bed column. It has shown to have high capacity for adsorbing Cr (VI). The process depended upon bed height and flow rate, data being well fitted with BDST.

Moussavi & Khosravi (2010) have successfully introduced a novel adsorbent - pistachio hull powder, PHP- widely & freely available as agro-waste, to treat cyanide containing synthetic liquid streams. PHP was successful in removing high concentration of cyanide ions: 200 mg/L in a relatively short contact time with low adsorbent amount.

Chen *et al.* (2010) have run kinetic equilibrium studies on modified wheat straw - MWS for removal of Cr VI in an endothermic, high temperature situation. It was seen to be an ion-exchange based on mean free energy of adsorption. A large number of positively charged amino groups were found in MWS from spectrum analysis.

Deng *et al.* (2003) used hydrolysis in a sodium hydroxide solution to modify the surface of polyacrylonitrile fibre - PANF. This modified HPANF was effective in adsorbing copper ions from aqueous media. It possessed conjugated sequences of -C=N- on the surface. The amount of copper ions adsorbed increased with solution pH values in the range of 2-6, because of electrostatic interaction between Cu and HPANF. Surface modification can improve the performance of the adsorbent.

Miretzzky *et al.* (2008) investigated the technical feasibility of Nopal cladodes - Opuntia streptacantha, for extracting Pb^{2+}. Nopal biomass effectively removed > 90% Pb from the solution. The kinetics was rapid and monolayer, showing 86.5% in first 7 min of contact time, at pH 5, and biomass concentration 2.5 g.

The feasibility of using bio-waste obtained from fruit juice industry for the removal of toxic heavy metals, Hg (II), Pb (II), Cd (II), Cu (II), Zn (II) and Ni (II) from wastewater was explored by Senthil kumaar *et al.* (2000). Fruit residue and phosphate fruit residues were separately used as biosorbents in which latter showed higher adsorption capacity. The pH of the solution was identified as the most important variable influencing metal adsorption on biosorbent.

Adsorption behaviour of Ni (II), Zn (II), Cd (II), and Cr (III) on untreated and phosphate-treated rice husk was studied by Ajmal *et al.* (2003). The adsorption of Ni (II) and Cd (II) were found to be greater when phosphate treated rice husk was used as an adsorbent.

The potential of papaya wood waste to treat wastewater contaminated with heavy metals Cd (II), Cu (II), and Zn (II) were studied by Saeed *et al.* (2005). Sorption was most efficient at pH 5 during contact time of 60 min. Metal ion sorption was found to increase as the ratio of metal solution to the biomass quantity decreased. The affinity of papaya wood to sorbe metals was in the order of Cu (II) > Cd (II) > Zn (II).

Removal and recovery of Pb (II) from single and multi metal (Cd, Cu, Ni and Zn) solutions by black gram husk was reported by Saeed *et al.* (2005). Complete desorption of metals in single and multi metal solution was achieved with 0.1 M HCl solution in both shake flask and fixed bed column studies.

The capacity of raw rice bran to remove Cr and Ni from aqueous solution was investigated by Oliveira *et al.* (2005). The sorption equilibrium was modelled using the Langmuir and Freundlich equation. The possibility of utilization of the rice bran for sorption of Cu ions from aqueous solution was also studied by Wang and Qin (2004). The experimental results were fitted to the Langmuir, Freundlich, Temkin and Redlich-Peterson isotherms to obtain the characteristic parameters of each model. Except Freundlich, the sorption data fitted well in other three isotherms models.

Hossain and Kumita (2005) studied the dynamic characteristics of Cr (VI) sorption using black tea leaves as biosorbent. Batch experiments were conducted to evaluate the effect of Cr (VI). Experimental and calculated kinetics data for equilibrium were expressed by Langmuir model on the adsorption rate.

The potential to remove Cr (VI) from aqueous solution through biosorption using the husk of Bengal gram (Cicer arientinum) was investigated in batch experiments by Ahalya *et al.* (2005). The results showed 99.9% removal of Cr from 10 mg/L Cr solution. The biomass required at saturation was1g/mg. The adsorption data fitted well with the Langmuir and Freundlich isotherm models.

Vijayaragavan *et al.* (2006) investigated the biosorption of Cu (II) and Co (II) by crab shell. At optimum particle size (0.767 mm), biosorbent dosage (5 g/L) and initial (pH 6), the adsorption recorded were 243.9 mg/g and 322.6 mg/g, of Cu and Co respectively according to Langmuir model.

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

Garg *et al*. (2007) studied the Cr(VI) adsorption from aqueous solution onto different agricultural wastes: sugar cane bagasse, maize corn cob and jatropha oil cake under different experimental conditions (adsorbent dose, Cr(VI) initial concentration, pH, contact time) FTIR spectra of the adsorbents were recorded before and after the adsorption process to explore number and position of the functional groups available for binding of chromiun ions. SEM studies of the adsorbent were recorded to study their morphology. Adsorption data of each system fitted Langmuir and Freundlich isotherms.

Jin *et al.* (2002) studied the removal of lead from aqueous solution with chitosan / PVA (poly vinyl alcohol) hydrogel beads in batch mode at different pH values from 2.0 to 7.6. Lead adsorption on chitosan / PVA beads was found to be strongly pH-dependent and displayed a maximum uptake capacity at pH 4.0 and minimum at pH 6.4. Zeta potential study indicated that chitosan / PVA beads possessed positive zeta potentials at pH < 6.3 and negative zeta potentials at pH > 6.3. Hence, adsorption occurred even though the interaction between lead and chitosan / PVA beads was electrostatically repulsive at pH < 6.3. Complexation, ion exchange, and electrostatic interaction are all believed to play a role in lead adsorption on chitosan / PVA beads, but the relative importance of each of these mechanisms varied with solution. FT-IR and X-ray photoelectron spectroscopy spectra suggested that lead adsorption was mainly through interactions with the *N*- atoms in chitosan in the pH range studied.

Tee *et al. (*1988) used commercially available waste tea leaves for the adsorption of metal ions. The effect of adsorption depends on pH, ionic strength, metal concentration, substrate concentration, and the presence of interfering ions and surfactants. Among the various types of surfactants studied, cetyl trimethylammonium bromide severely hindered the metal uptake while Triton X l00 interfered mildly. Initial increase in uptake of Pb (II) and Zn (II) were observed in the presence of small amount of sodium dodecyl sulphate. The enhancement effect decreased as the anionic surfactant concentration increased. Adsorption capacities of tea leaves were found to be 0.38, 0.28 and 0.18 mmole/ g for Pb, Cd and Zn respectively. Column experiments revealed near 100% efficiency for metal removal of as much as 100 ppm Pb (II) ion at pH 6, using 1.8 g waste tea leaves and flow rate of 25 mL / min. The relative affinities of metal ions towards waste tea leaves was in the order of Pb > Cd > Zn.

Dhakal *et al*. (2005) investigated the adsorption behavior of Pb (II), Zn (II) and Cu (II) on crosslinked pectic and alginic acids, and their amide derivatives. The selectivity order for metal ion uptake is Pb (II) > Cu (II) > Zn (II). The removal of Pb (II) ion was investigated using batch and column tests and compared with that for the commercially available weakly acidic cation exchange DIAIONWK resins. The experimental results indicated much better separation performance of modified acidic polysaccharides than the commercial resins. It suggests their applications in the separation and purification of Pb (II) ion.

Homagai *et al.* (2011) studied the removal efficiency of five different heavy metal ions (Cd, Pb, Ni, Zn and Cu) from aqueous medium using charred xanthated sugarcane bagasse. It was found to have significant adsorption capacity which is more than that of various biosorbents available in literatures. The high adsorption capacity as well as fast kinetics suggested that it can be used as potential adsorbent for the removal of heavy metals from aqueous medium.

Rashid *et al.* (2009) studied the phytoaccumulation of cadmium and zinc in *Desmostachya bipinnata* (DB) growing in industrially polluted soil. He found small amount of cadmium and zinc was accumulated in DB, as well as having high penetrating capacity, enough to absorb X-rays. His concept was to use the plant itself as a whole entity for the accumulation of heavy metals, which was very insignificant in quantity, while our work is devoted to use the natural polysachharide for developing functional biosorbent by suitable chemical modification and to investigate the bio-adsorption properties of DB.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Chemicals

Standard stock solutions (1000mg/L) of Cd, Pb, Cu, and Zn were prepared in Millipore milli-Q deionised water from their nitrate and sulphate salts, respectively. All the working solutions of different concentrations were prepared by diluting standard stock solution with 0.1M nitric acid. In all the working solutions 0.1M HEPES (2-[4-(2-Hydroxymethyl)-1-piperazinyl] ethanesulphonic acid) was used as the buffering agent (Ghimire et al. 2007). The pH of the working solutions was adjusted by treating with dilute nitric acid and sodium hydroxide solutions. All the reagents and chemicals used in this study were of Fisher scientific analytical grade.

3.2 Preparation of Biopolymers

A typical herb, *Desmostachya bipinnata* (DB), was collected from the southern part of Nepal. It was sun dried, cut into small pieces, and then washed with tap water and finally with distilled water. After drying, the material was powdered with the mechanical grinder and made to pass through 212 μm sieve.

About 200 g powdered raw biopolymer was treated with concentrated sulphuric acid and left for 24 h. Acid treatment of such biopolymers create a suitable environment for its ring opening (Morrison and Byod, 1994). Then the biopolymer was washed with deionized water to remove excess acid and any other soluble low molecular weight substances until neutrality. The biopolymer was air and sun dried then finally dried in an oven at 70°C for 24 h.

3.2.1 Functionalization of biopolymer with Hydrazine monohydrate

Thirt grams of Charred *Desmostachya bipinnata* (CDB) was taken in a round bottom flask, treated with distilled water, and kept for swelling overnight. Then hydrazine monohydrate was added drop wise at room temperature and heated for 4 h at 80°C using a water-bath. The mixture was cooled to room temperature and thoroughly washed with distilled water and finally with methanol. It was dried in an oven at 70°C for 24 h. This final product is referred to as Functionalized *Desmostachya bipinnata* with Hydrazine monohydrate (FDB-H) and is ready for adsorption experiments as shown in Figure 3 (a) and 3 (b).

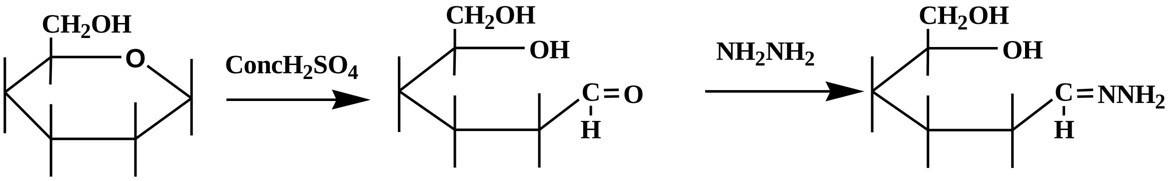

Figure 3(a): Proposed ring opening of monomeric unit of cellulose in *D. bipinnata* followed by functionalization with hydrazine monohydrate.

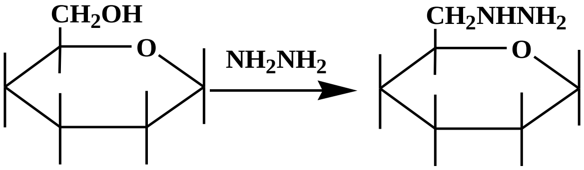

Figure 3(b): Proposed monomeric unit of cellulose in *D. bipinnata* followed by functionalization with Hydrazine monohydrate.

3.2.2 Functionalization of biopolymer with Dimethylamine

Thirty grams of Charred *Desmostachya bipinnata* (CDB) was treated with thionyl chloride in ice cold condition in presence of pyridine and heated on a water bath at 70 °C for one and half hour. The mixture was cooled and washed with distilled water till neutral pH was attained, then finally with propanol and left to air dry. This resulting biopolymer was taken in three necked round bottom flask along with dimethylamine in presence of sodium carbonate and dimethyl sulphoxide (DMSO). The mixture was heated for 8 h at 70 °C, cooled, filtered and washed with 0.1 M hydrochloric acid, then with water till neutral pH and finally with propanol. The biopolymer was air-dried and then oven dried for 24 h at 70°C. This final product is referred as Functionalized *D. bipinnata* with Dimethylamine (FDB-Dm) as shown in Figure 4 (a).

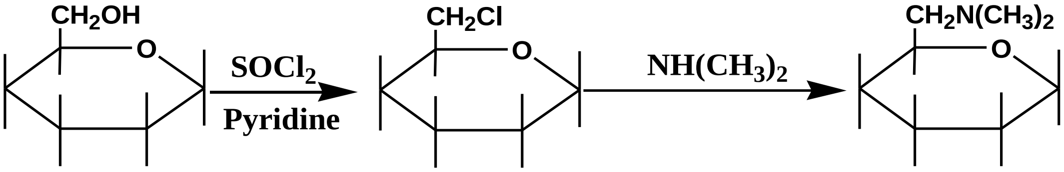

Figure 4(a): Proposed monomeric unit of cellulose in *D. bipinnata* followed by functionalization with dimethylamine

3.2.3 Functionalization of biopolymer with Ethylenediamine

Thirty grams of Charred *Desmostachya bipinnata* (CDB) was treated with thionyl chloride in on ice cold condition in presence of pyridine and heated on a water bath at 70 °C for one and half hour. The mixture was cooled and washed with distilled water till neutral pH, then finally with propanol and left to air dry. This resulting biopolymer was taken in three necked round bottom flask along with ethylenediamine, dimethyl sulphoxide (DMSO) and sodium carbonate. The mixture was heated for 8 h at 70°C, cooled, filtered and washed with 0.1 M hydrochloric acid, then with water till attainment of neutral pH and finally with propanol. The biopolymer was air-dried and then oven dried for 24 h at 70 °C. This final product is referred as Functionalized *D. bipinnata* with ethylenediamine (FDB-E) as shown in Figure 4(b).

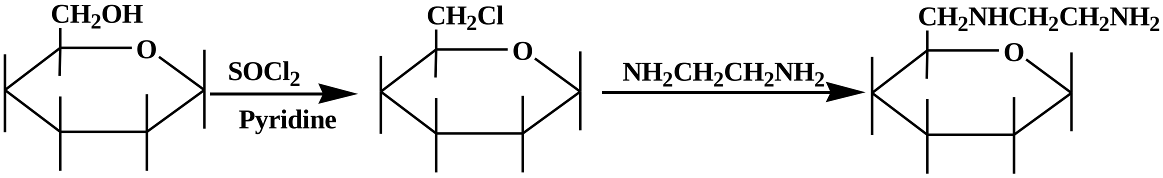

Figure 4 (b): Proposed monomeric unit of cellulose in *D. bipinnata* followed by functionalization with ethylenediamine.

3.3 pH study

pH is one of the important parameters for the sorption of metal ions onto biopolymer. It affects the surface charge and degree of ionization of biopolymer. Batch adsorption tests for different metal ions removal were performed by keeping the concentration of metal ions constant (50 mg/L) as the function of pH (pH = 1, 2, 3, 4, 5, 6, and 7).

All investigations were performed in 50 mL, Erlenmeyer flasks taking 25 mg of three types of dried functionalized biopolymers (FDB-H, FDB-Dm and FDB-E) with 20 mL of synthetic metal ions solutions. The pH of respective metal ions were maintained by treating with dilute nitric acid and sodium hydroxide solutions and flasks were agitated in a shaker at lab temperature at 150 rpm for 24 h to attain equilibrium. After 24 h, the solutions were filtered through cellulose-free filter paper and their equilibrium concentrations were measured using (ICP-AES) inductively coupled plasma atomic emission spectrophotometer (SPECTRO, Analytical Instrument, Kleve, German). The adsorbed amount of metal ions were calculated from the decrease in the metal ions concentration from which percentage adsorption (A%) were calculated (Cochrane *et al.* 2006) as shown in equation (1).

$$A\ \% = \frac{C_i - C_e}{C_i} \times 100 \qquad (1)$$

where C_i and C_e represent initial equilibrium concentrations of metal ion (mg/L) in the aqueous solution.

3.4 Zeta potential

Zeta potential analysis is a technique for determining the surface charge of particles in solution (colloids). Particles have a surface charge that attracts a thin layer of ions of opposite charge to the particle surface. This double layer of ions travels with particle as it diffuses throughout the solution (Figure 5). The electric potential at the boundary of the double layer is known as the zeta potential of the particles and has values that typically range from +100 mV to -100 mV. The magnitude of the zeta potential is predictive of the colloidal stability. Particles with zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. Dispersions with a low zeta potential value will eventually aggregate due to Van Der Waal inter-particle attractions.

Zeta Potential is an important tool for understanding the state of the particle surface and predicting the long term stability of the particle. Zeta Potential analysis is used to determine if a surface modification to the particle has been successful or if a processing step has modified the particle surface. The pH_{pzc} measurement were carried out at different pH ranging from 1- 12. A volume of 20 mL of distilled water was taken in separate flasks and pH was adjusted with the help of 0.1 M nitric acid and sodium hydroxide, respectively. 25 mg of biopolymers were added to each flask and shaken at 150 rpm and allowed to equilibrate for 2 h. Then the mixtures were filtered and the optimum pHs as well as the zeta potential of the solutions were measured using pH meter (Hanna instrument).

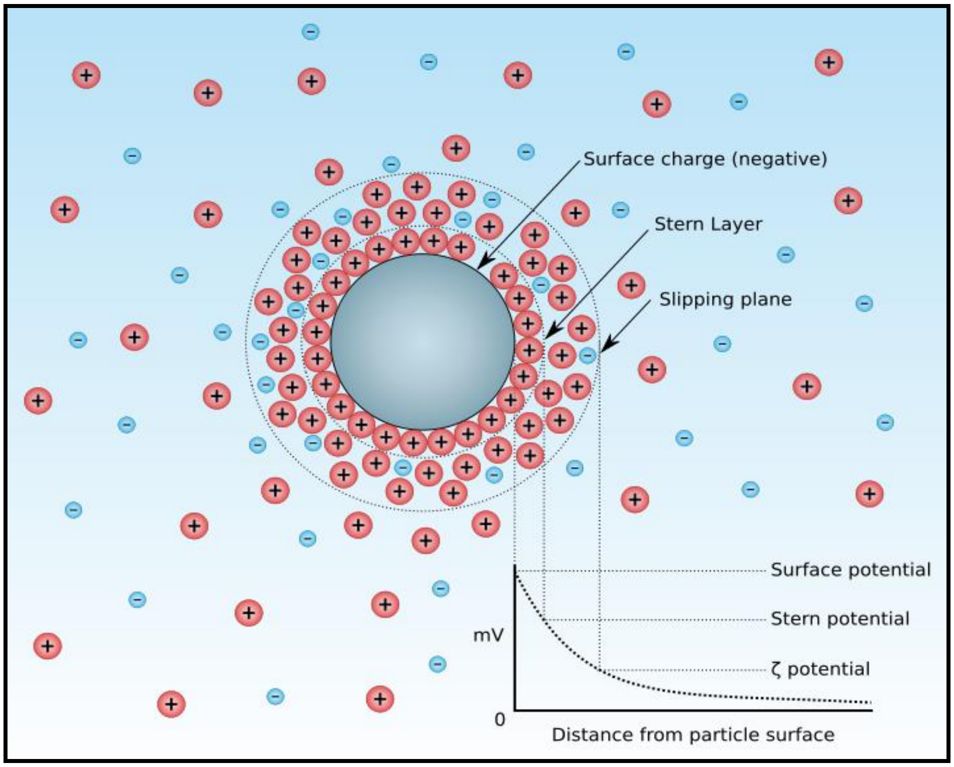

Figure 5 (a): Schematic representation of Zeta potential [McNaught and Wilkinson , 1997].

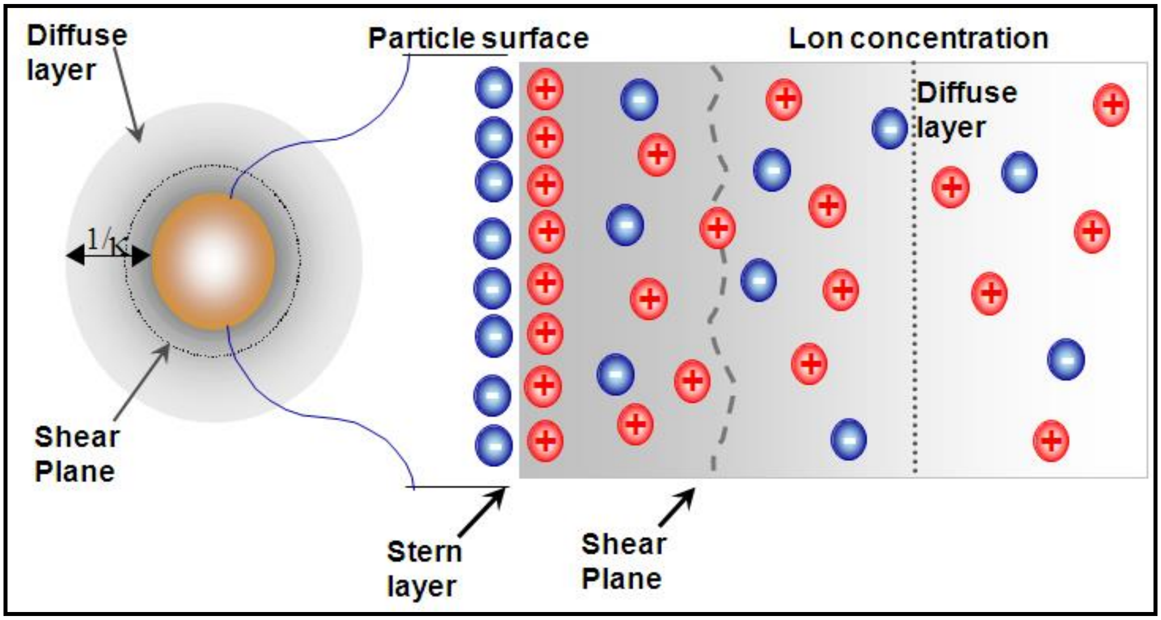

Figure 5 (b): Detail representation of Zeta potential

3.5 Adsorption isotherm study

The metal uptake capacities of three types of functionalized *Desmostachya bipinnata* (FDB-H, FDB-Dm and FDB-E) for Cd (II), Pb (II),Cu (II), and Zn (II) were measured by batch experiment taking 25 mg of biopolymers together with 20 mL of synthetic metal ions solutions of different concentration ranging from 25, 50, 100, 200, 400,600, 800 and 1000 mg/L respectively, at their optimum pH (6, 4, 5, and 6 for Cd, Pb, Cu and, Zn, respectively) in a 50 ml Erlenmeyer flask. The flasks were shaken in a shaker at lab temperature at 150 rpm for 24 h to attain the equilibrium. After attaining equilibrium, the solutions were filtered then initial and equilibrium concentrations of the respective metal ions were measured using ICP-AES (SPECTRO, Analytical Instrument, Kleve, German).

The sorption capacity of metal ions (q in mg/g) is the concentration of the metal ions onto the biopolymer, which can be calculated from the mass balance principle as shown in equation (2).

$$q = \frac{C_i - C_e}{W} \times V \qquad (2)$$

where W and V represent weight of the sobent (mg) and volume of solution (L).

3.6 Kinetic studies

Kinetic studies of sorption are helpful to find the rate at which the metals are removed from aqueous solution. The effects of contact time on to sorption of metal ions were studied from 5 min to 360 min (5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min, respectively). The experiments were carried out taking 25 mg biopolymer along with 20 mL of respective metal ions solutions of 50 mg/L at their optimum pH in 14 different Erlenmeyer flasks. All the flasks were shaken in a shaker for the desired time interval and at the end of each contact time, the solution was filtered, then their concentrations were measured using ICP-AES. The amount of metal adsorbed at time (t) was calculated from the mass balance between initial concentration and concentration at time (t) to analyze the sorption rate of metal ions onto the biopolymer.

3.7 Desorption and Regeneration Study

Biosorption process is considered as an alternative to expensive method in the removal of heavy metal ions from aqueous solution. For this reason the regeneration of biopolymer is considered to be of prime importance. The metal ions are allowed to adsorbed as well as desorbed in order to reuse the biopolymer. The sorption capacity must be maintained after the treatment with eluting reagent and subsequently the metal ions adsorbed by the biopolymer should be easily released under suitable condition (Kong *et al.*, 2014 and Wang *et al*., 2007).

Desorption study was carried out by taking 100 mg functionalized biopolymer (FDB-H, FDB-Dm, FDB-E) with 100 mg/L of 80 mL Pb(II) metal ion solution at its optimum pH in a flask and shaken for 24 h at 150 rpm. After attaining equilibrium, the solution was filtered and concentration was measured. The biopolymer at this condition is the metal loaded biopolymer. It was dried in an oven at 70°C for 24 h. This metal loaded biopolymer was used for the desorption study.

For desorption study, three different types of eluting reagents like 0.1 M sodium hydroxide, 0.1 M hydrochloric acid and 0.1 M nitric acid were used. 25 mg metal loaded biopolymer together with 20 mL of 0.1 M sodium hydroxide, hydrochloric acid and nitric acid were taken in separate flasks and shaken for 3 h. Then, the solutions were filtered and their concentrations were measured. The exhausted biopolymer was regenerated by washing with distilled water and finally dried in an air oven at 70 °C for 24 h. This regenerated biopolymer was used for next five adsorption / desorption experiments to evaluate the efficiency and reusability of biopolymers.

3.8 Analysis

3.8.1 Elemental Analysis

The percentage amount of Carbon (C), Nitrogen (N) and Sulphur (S) in raw and functionalized biopolymers was measured using Elementar Vario Macro elemental analysis instrument. The samples were heated to a temperature of 1000^0C and approximately 25 mg of each type of biopolymer was placed inside a silver capsule and was dropped ino the furnace where it was completely combusted. This instrument relies upon infrared detection to measure the weight percent of carbon, nitrogen and sulphur. The C, N and S contents of the samples were determined from the quantities of CO_2, H_2O and NO_2 produced by the combustion of the dried solid in oxygen (Iqubal *et al*. 2009).

3.8.2 Chemical analysis (Boehm titration)

The chemical analysis was carried out using Boehm titration to find the amount of acidic and basic functional sites present on the surface of the raw as well as functionalized biopolymers. This method involves neutralization of surface acidic sites with base (NaOH) and surface basic sites with acid (HCl). The total acidic sites can be calculated from the amount of base consumed by the biopolymer in neutralization reaction between the biopolymer and base. In the same way, the basic sites can also be calculated from the amount of acid consumed by biopolymer in neutralization reaction between the biopolymer and acid.

In this method 200 mg of raw and functionalized biopolymer were agitated with 50 mL of 0.02 N sodiumhydroxid and 0.02 N hydrochloric acid for 24 h in separate flasks. The mixture was filtered and 5mL of filtrate was back titrated with 0.02 N HCl or 0.02 N NaOH using methyl orange as an indicator to determine the volume of base and acid consumed. On the basis of volume of acid or base consumed by the definite weight of biopolymer, the amount of functional group per gram biopolymer is calculated according to the Boehm procedure.

3.8.3 Scanning Electron Microscope (SEM) Analysis

The surface morphologies of raw and functionalized biopolymers were analysed with the help of S-3000 N Scanning Electron Microscope (SEM) of HITACHI, Japan. SEM is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nm. Specimens can be observed in high vacuum, in low vacuum, (in environmental SEM) in wet conditions and at a wide range of cryogenic or elevated temperatures.

The most common mode of detection is by secondary electrons emitted by atoms excited by the electron beam. The number of secondary electrons is a function of the angle between the surface and the beam. On a flat surface, the plume of secondary electrons is mostly contained by the sample, but on a tilted surface, the plume is partially exposed and more electrons are emitted. By scanning the sample and detecting the secondary electrons, an image displaying the tilt of the surface is created.

3.8.4 Diffuse Reflectance Fourier Transform Infrared (DRFTIR) Spectroscopic Analysis

The types of functional group present on the *Dismostachya bipinnata* were analyzed using diffuse reflectance FTIR spectroscopy (DRFTIR) (Harrick scientific corporation) at frequency range of 4000 to 500 cm^{-1}. Infrared absorption spectroscopy is the method used to determine the structures of molecules with the molecules' characteristic absorption of infrared radiation. Infrared spectrum is molecular vibrational spectrum. When exposed to infrared radiation, sample molecules selectively absorb radiation of specific wavelengths which causes the change of dipole moment of sample molecules. Consequently, the vibrational energy levels of sample molecules transfer from ground state to excited state. The frequency of the absorption peak is determined by the vibrational energy gap. The number of absorption peaks is related to the number of vibrational freedom of the molecule. The intensity of absorption peak is related to the change of dipole moment and the possibility of the transition of energy levels. Therefore, by analyzing the infrared spectrum, one can readily obtain abundant information related to the structure of a molecule.

3.8.5 Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) Analysis

The concentrations of the metal ions were measured using (ICP-AES) inductively coupled plasma atomic emission spectrophotometer (SPECTRO, Analytical Instrument, Kleve, German).

The SPECTRO CIROS VISION is an automatic optical emission spectrometer providing simultaneous measurements. It uses inductively-coupled plasma excitation and a semiconductor-based detector system for quantitative and semiquantitative analysis of liquids. The liquid sample is nebulized and fed into the plasma as an aerosol. The high temperature of the plasma (6000-8000 K) evaporates the sample. The molecules contained in the sample dissociate into atoms. The atoms are excited and partly ionized. The excited atoms and ions emit an element-specific radiation. A transfer optics feeds this radiation into the optical system. The emitted radiation is diffracted into its spectral components in the optical system. The intensity is measured using semiconductor detectors. After processing the measuring signals in the unit, the measured element intensities are evaluated by the Smart Analyzer software.

Methods are set up prior to measuring. Calibration functions for each element to be determined are stored in these methods. Concentrations are calculated from the measured intensities, using these methods. The concentrations are displayed on the screen. They are also printed on a printer (can be activated as required) or saved in a database.

In order to determine the dissolved metals and non-metals the water samples were filtered through a membrane filter (pore size: 0.45 m) and acidified with 65 % (m/V) nitric acid in supra-pure quality (Merck, Germany) to $pH < 2$.

It is possible to prepare calibration samples from single element standard solutions (typically in a concentration of 1000 mg/L) or from multiple element standard solutions.

3.8.6 Thermal Gravimetric Analysis (TG/ DTA)

Thermal gravimetric Analysis (TGA) measures (TG /DTA6200, Japan) the masses of samples as they are heated and cooled through standard firing programs. TGA analysis is similar to DTA analysis. In the analysis of TGA, the increase, decrease or constancy of mass of biosorbent at each temperature in the firing program indicates the presence or absence of reactions and nature of each reaction that takes place. For example, phase changes occur without change of mass, some decomposition reactions are accompanied by loss of weight, and oxidation reactions are accompanied by gain of weight. TGA analysis requires little samples (about 5-7 mg), which must be dried thoroughly before performing the analysis.

3.8.7 X-RayDiffraction Analysis (XRD)

X-ray diffraction is a versatile analytical technique for analysizing different types of crystalline solids. These materials may be powders, single crystals, multilayer thin films, sheets, fibers, or irregular shapes, depending on the desired measurement. X-Ray diffractometers (X- Ray diffractometer, Rigaku, Japan) are most often used to determine the molecular structure of new materials. Power difftactometers are routinely used for phase identification and quantitative phase analysis but can be configured for many applications, including variable temperature studies, texture and stress analysis, grazing incidence diffraction and reflectometry. The theoretical basis of X- Ray diffraction stands on Bragg's equation given by;

$$\mathbf{n\lambda = 2d\ sin\theta}$$

where, n = order of reflection(n=1, 2, 3….,) λ = the wave length, d = distance between parallel lattice planes, θ = angle of incident beam and a lattice plane, known as Bragg angle. When the path length in the crystal (2d sin θ) is a multiple of the wave length, constructive interference occurs and diffracted intensity is obtained.

3.8.8 pH meter

The pH of the sample solution was measured with pH meter (Hanna instrument). It shows the acidic, neutral or basic nature of the samples under measurement.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Elemental Analysis

Elemental analysis shows the percentage composition of elements present in the raw *D. bipinnata*. Nitrogen, carbon and sulphur were found to be 1.13 %, 41.27 % and 0.56 % in RDB. After functionalization with hydrazine monohydrate, dimethylamine and ethylenediamine a change in the percentage composition of subsequent element was observed as shown in Table 2. Increase in percentage of nitrogen after functionalization further supports the fact that introduction of nitrogen functional group onto the *D. bipinnata* has taken place.

Table 2: Elemental analysis of *D. bipinnata* before and after functionalization

Biopolymers	Nitrogen %	Carbon %	Sulphur %
RDB	1.13	41.27	0.56
FDB-H	7.56	48.91	1.06
FDB-Dm	5.42	52.12	2.31
FDB-E	5.49	51.47	2.46

Table 3: Major constituents in the Biopolymer *D. bipinnata*

Biopolymers	Cellulose %	Hemicellulose %	Lignin %
RDB	27.36	35.04	7.64
FDB-H	5.31	2.08	50.27
FDB-Dm	5.83	3.86	71.05
FDB-E	3.22	4.77	37.49

4.2 Chemical Analysis (Boehm titration)

The presence of acidic and basic functional sites on the surface of biopolymers were analysed chemically with the help of Boehm titration. The acidic functional sites on the surface of raw biopolymer were 1.9 m eq /gm. After functionalization the values obtained were 1.0 m eq /gm and 1.3 m eq /g in FDB-H and FDB-Dm respectively.Similarly, the basic functional sites were 1.1 m eq /g in RDB, where as the value increased to 3.8 m eq /g and 3.5 m eq /g in case of FDB-H and FDB- Dm, respectively which is also shown in table 4. The increase in the value of basic functional sites also suggests the introduction of *N*-functional groups onto the functionalized biopolymers.

Table 4: Results of Boehm titration

Biopolymers	**Volume consumed in mL**		**Functional group (m.eq/g)**	
	NaOH	**HCl**	**Acidic**	**Basic**
RDB	19	11	1.9	1.1
FDB-H	10	38	1.0	3.8
FDB-D_m	13	35	1.3	3.5

4.3 Scanning Electron Microscope (SEM) Analysis

The surface morphologies of raw biopolymer and functionalized biopolymer were studied with the help of scanning electron microscope (SEM). The SEM images of biopolymers before functionalization revealed clearly smooth and even surface as shown in Figure 6(a) and 6(b) for RDB at low and high magnifications. After the functionalization in all three types of biopolymers, the prominent changes on their surfaces like uneven porous structure, rough and corroded surface morphology were clearly observed as shown in Figure 7(a) and (b) for FDB-H, Figure 8(a) and (b) for FDB-Dm, and Figure 9(a) and (b) for FDB-E as compared to the raw biopolymer. The introduction of the nitrogen functional group on the surface of the biopolymer can therefore be expected to change the material’s properties and thus affect the metal ion adsorption.

The surface morphologies of metal loaded (Pb) functionalized biopolymers; (FDB-H and FDB-Dm) were also studied using scanning electron microscope (SEM). The rough, corroded and porous surface of the biopolymers were changed in to even and smooth surface with bright images indicating the adsorption of metal ions onto functionalized biopolymers as shown in Figure 10 for FDB-H (Pb) and Figure 11 for FDB-Dm (Pb), respectively.

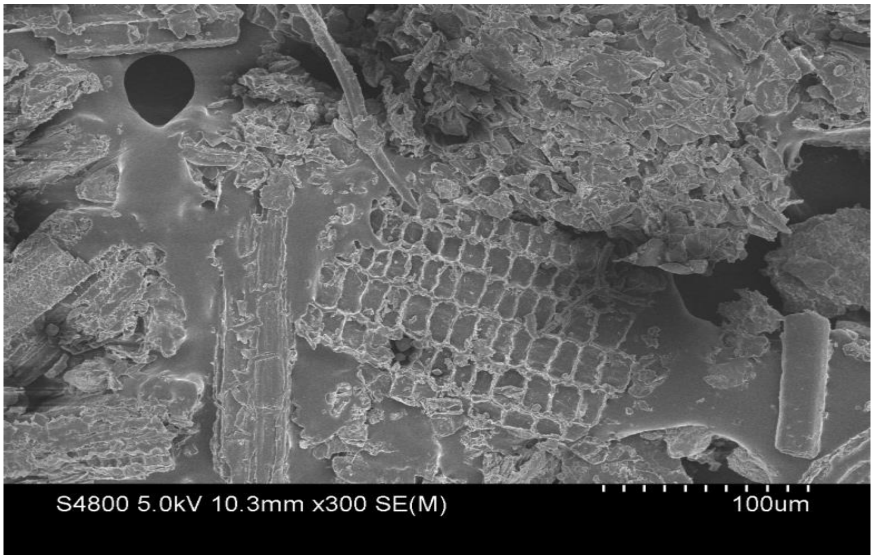

Figure 6(a): SEM of Raw *D. bipinnata* (RDB) with low magnification

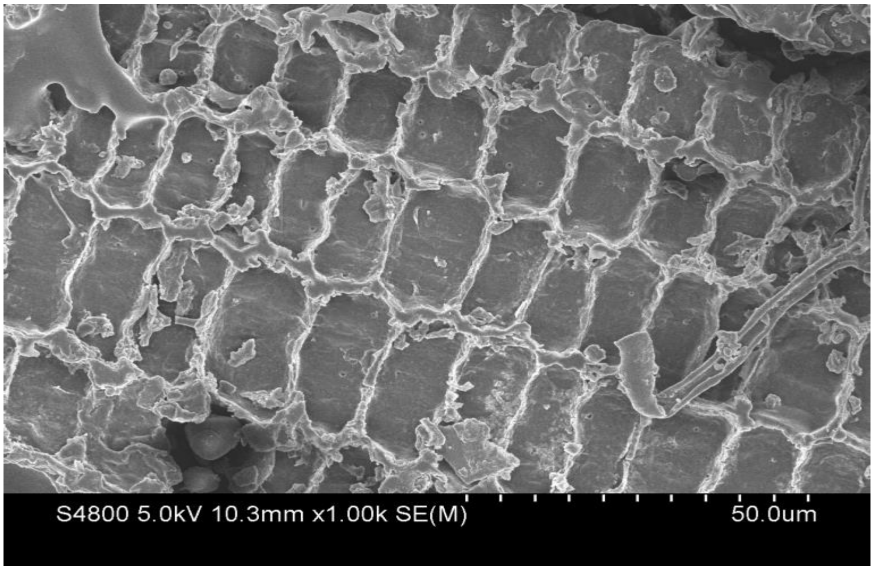

Figure 6(b): SEM of Raw *D. bipinnata* (RDB) with high magnification

Figure 7(a): SEM of functionalized *D. bipinnata* (FDB-H) with low magnification

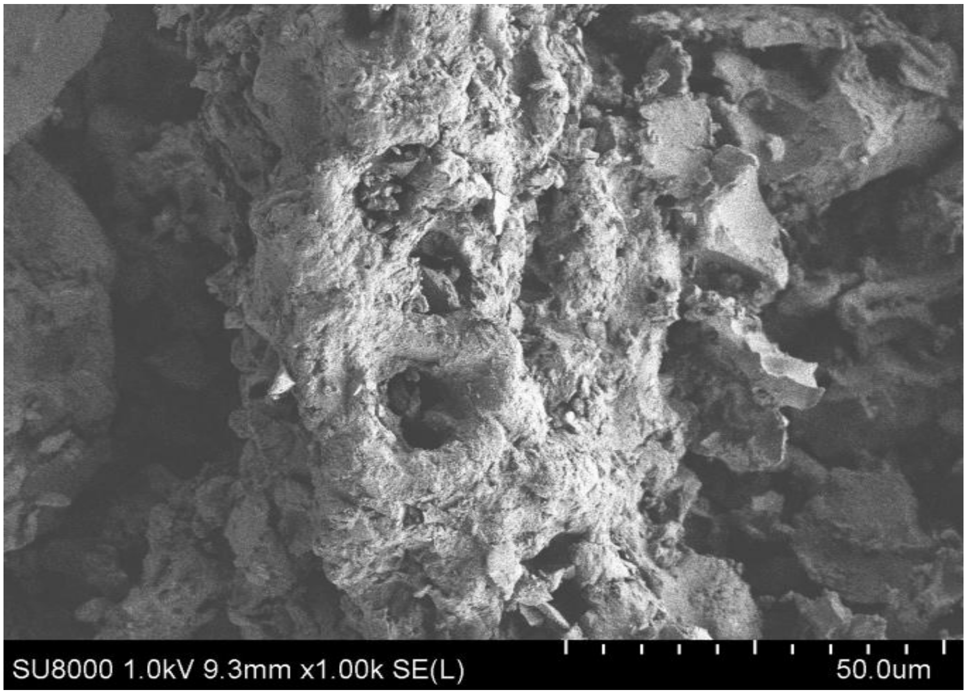

Figure 7(b): SEM of functionalized *D. bipinnata* (FDB-H) with high magnification

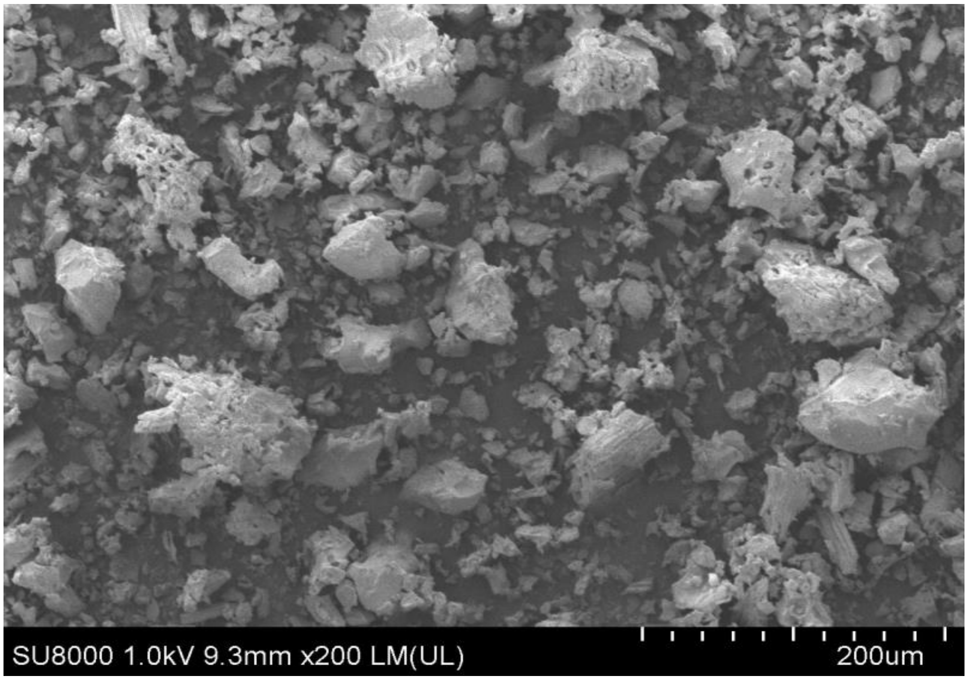

Figure 8(a): SEM of functionalized *D. bipinnata* (FDB-Dm) with low magnification

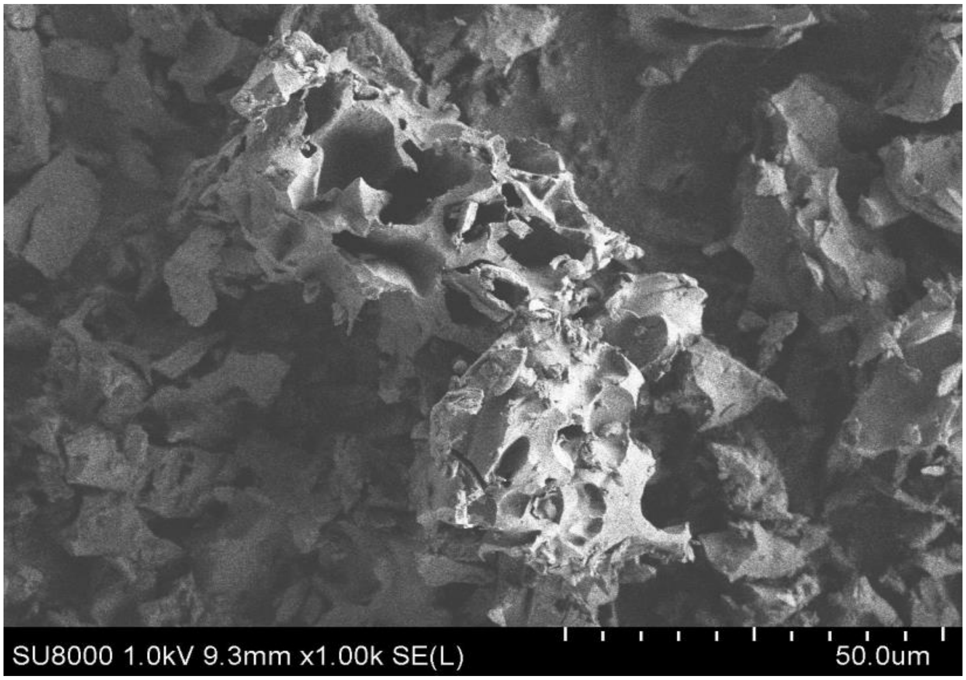

Figure 8(b): SEM of functionalized *D. bipinnata* (FDB-Dm) with high magnification

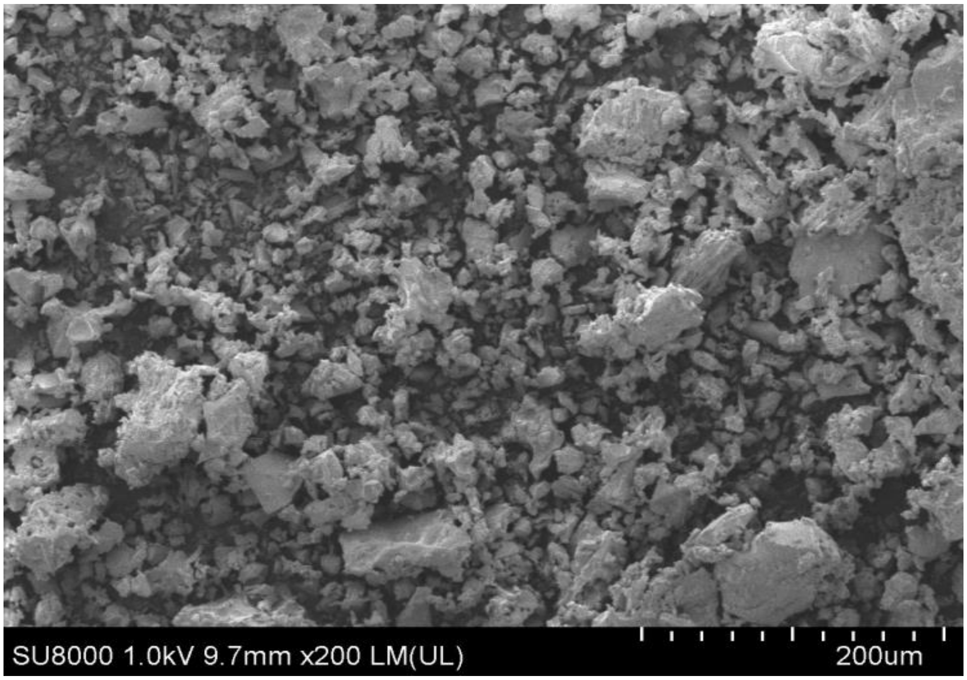

Figure 9(a): SEM of functionalized *D. bipinnata* (FDB-E) with low magnification

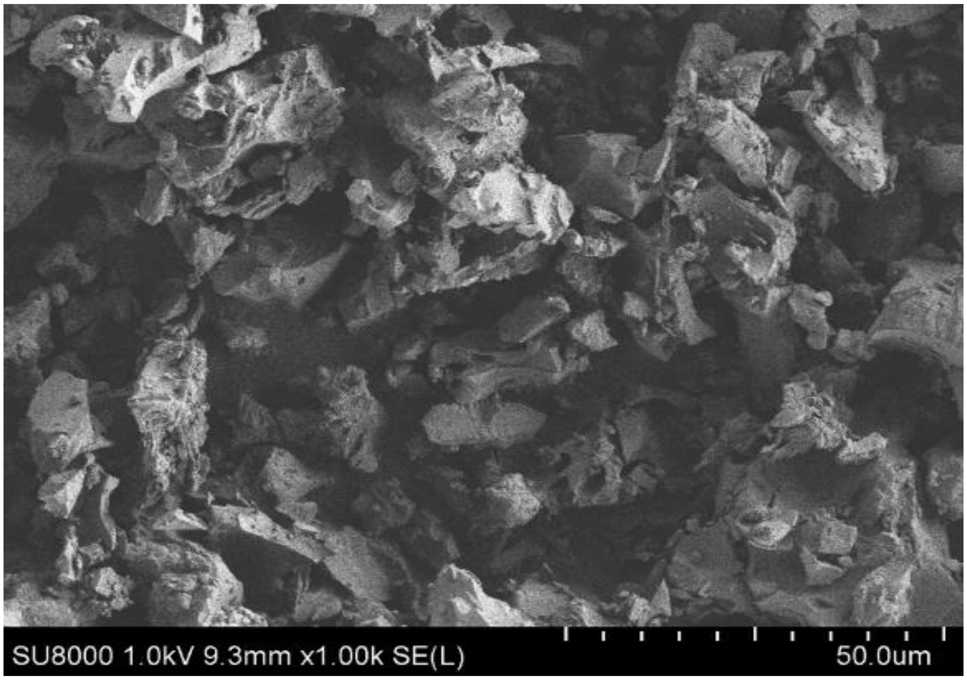

Figure 9(b): SEM of functionalized *D. bipinnata* (FDB-E) with high magnification

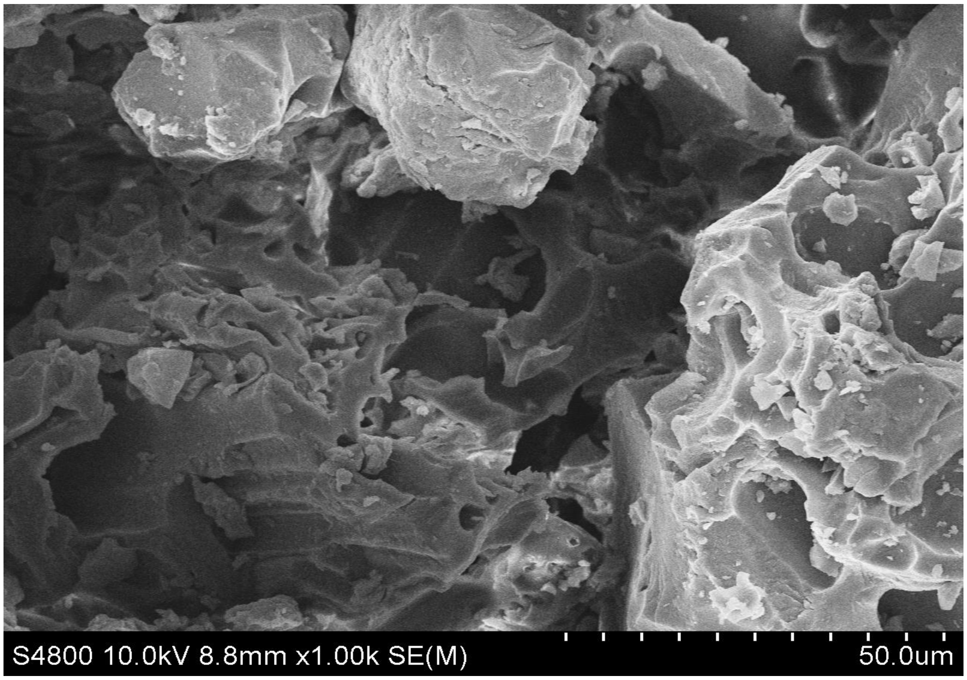

Figure 10: SEM of metal loaded (Pb) FDB-H

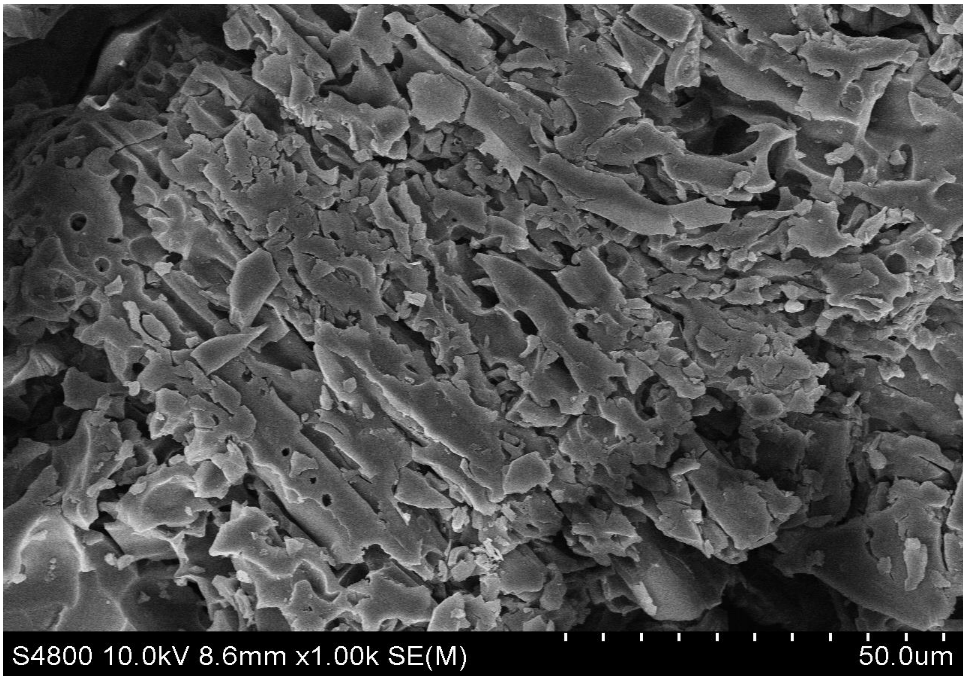

Figure 11: SEM of metal loaded (Pb) FDB-Dm

4.4 Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRFTIR) Analysis

DRFTIR spectra have been a useful tool in identifying the presence of certain functional groups in a molecule (Jin and Bai, 2002).

The spectrum of the raw biopolymer (RDB) was observed to be complex due to the numerous and multifarious functional groups on the surface of the biopolymer. The types of functional group present on the *D. bipinnata* were analyzed using diffuse reflectance infrared spectroscopy (DRFTIR, Harrick scientific corporation). The intense sharp adsorption peak around 3411 cm^{-1} in Figure 12 for RDB can be assigned to the *O–H* stretching vibration due to inter and intramolecular hydrogen bonding of polymeric compounds like alcohols, phenols, and carboxylic acids as in cellulose, hemicelluloses and lignin, indicating the presence of free hydroxyl groups on the biopolymer surface. The peak around 2926 cm^{-1} corresponds to *C–H* stretching in CH_2 and CH_3 groups. Similarly, the peak around 1736 cm^{-1} corresponds to carbonyl *C=O* stretching vibration as well as stretching vibration of bond due to non ionic carboxylic groups like $–COOH$ and $–COOCH_3$. The peak around 1426–1377 cm^{-1} is due to *O–H* bending vibration and 1169–1082 cm^{-1} was due to *C–O* stretching vibration of alcohol. The weak peak at 931 cm^{-1} attributed to the glycoside bonds in the polysaccharide structure (Silverstein *et al.*, 1981; Shriner *et al.,* 1998).

A sharp difference in DRFTIR spectra were noticed after the functionalization of biopolymers with hydrazine monohydrate, dimethylamine and ethylenediamine respectively, as shown in Figure 12. The intense sharp peak at around 3411 cm^{-1} in RDB has been shifted to around 3500 cm^{-1} as a broad peak in FDB-H where as in case of FDB-Dm and FDB-E the peak is shifted at around 3480 cm^{-1}, which may be due to the overlapping of the hydroxyl group with amine group. The peak at around 1639 cm^{-1} in all three types of functionalized biopolymers is due to *N-H* bending vibration of amines. Peaks on the ranges of 1600cm^{-1} to 1100 cm^{-1} in RDB were distinctly changed in all three types of functionalized biopolymers. This is also an alternative proof for the introduction of *N*- functional group onto the surface of biopolymers.

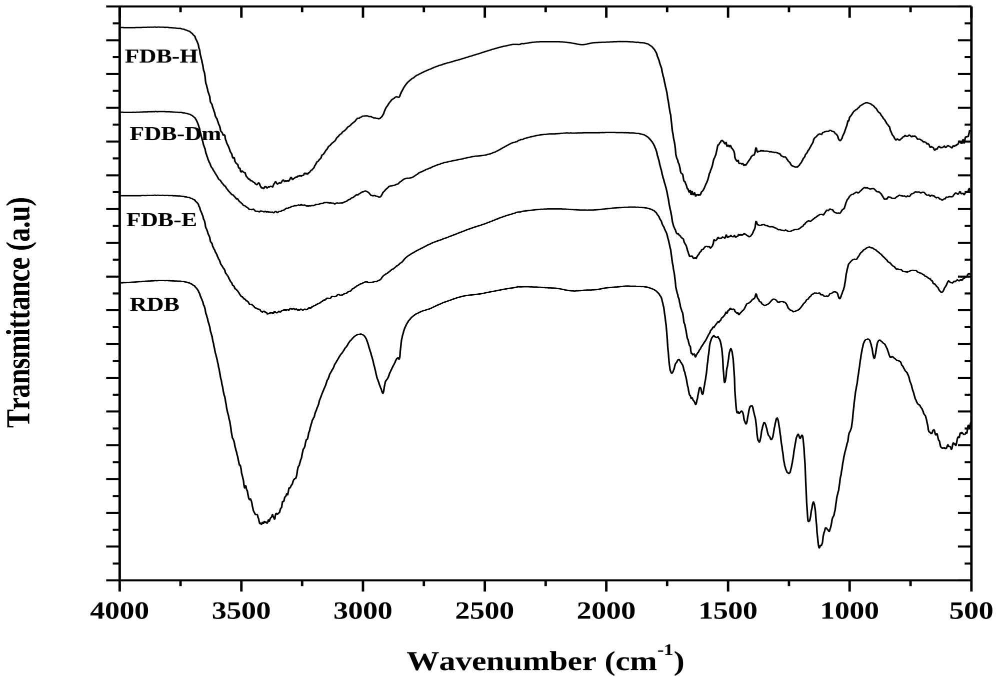

Figure 12: DRFTIR spectra of RDB, FDB-H, FDB-Dm and FDB-E

4.4.1 DRFTIR Analysis of Pb (II) loaded FDB

DRFTIR spectra of Pb loaded FDB-H and FDB-Dm were also analyzed and compared with the spectra of FDB-H and FDB-Dm as shown in Figure 13(a) and Figur 13(b). The peaks were broaden and shifted at the lower wave number in both biopolymers after Pb (II) metal ions was loaded. In the same way there were reduction in transmittance in the Pb (II) loaded biopolymers which is clearly observed from the Figure 13(a) and Figure 13(b). (Peaks at around 2400 cm^{-1} ,2300 cm^{-1} ,1600 cm^{-1}), which also indicates that the *N*-functional groups are involved in the adsorption of lead metal ions (Jin and Bai, 2002).

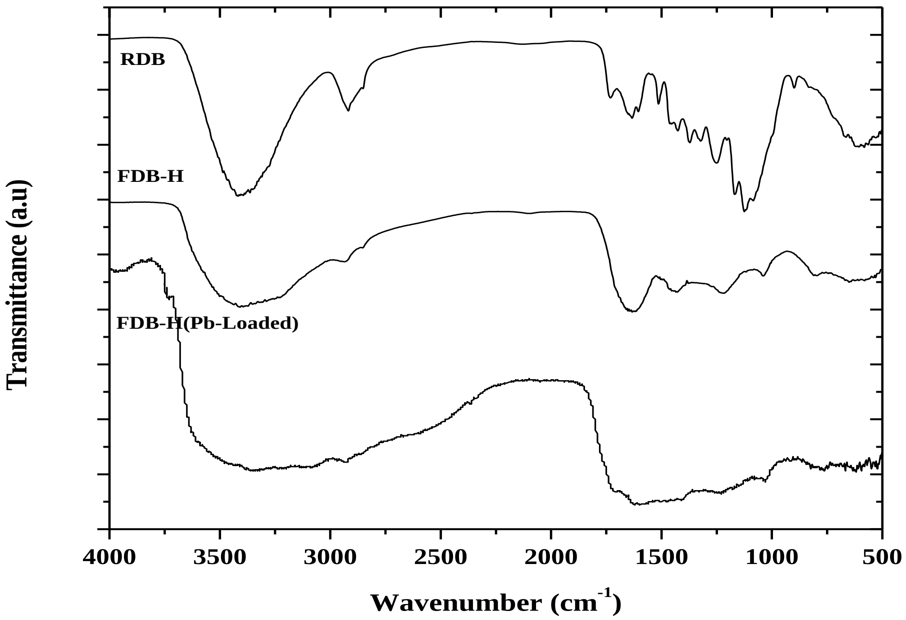

Figure 13 (a): DRFTIR spectra of RDB, FDB-H and (Pb) loaded FDB-H

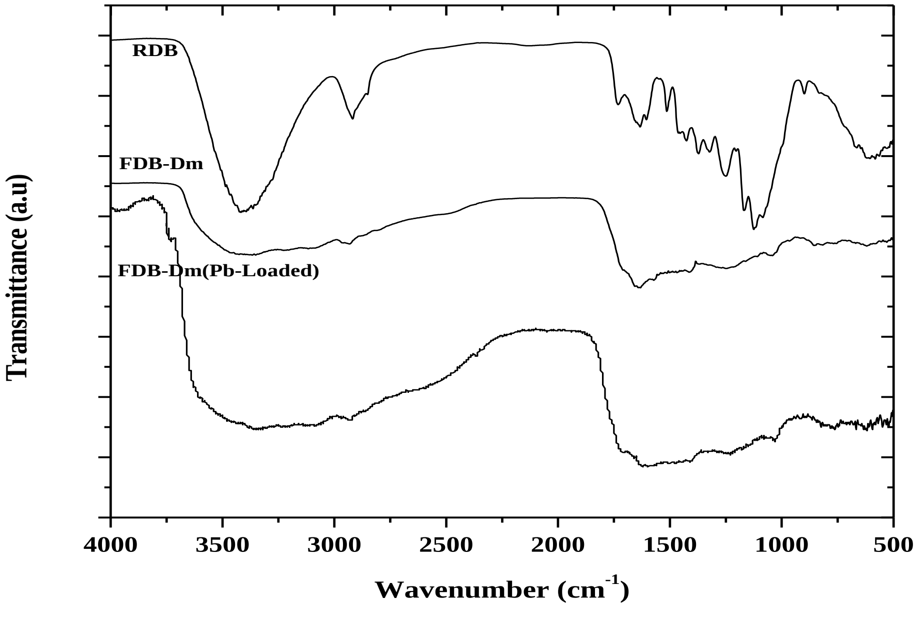

Figure 13 (b): DRFTIR spectra of RDB, FDB-Dm and (Pb) loaded FDB-Dm

4.5 Thermal Gravimetric Analysis (TG/ DTA)

The degradation and thermal stability of RDB and FDB samples (5-6 mg, reference sample Alumina) were compared using thermo-gravimetric analysis (TGA) and differential thermogravimetric analysis (DTA) in a Nitrogen atmosphere using a thermal analyser (TG /DTA6200, Japan) with the increase in temperature of 10 deg min^{-1}.

Raw *D. bipinnata* degraded mainly in two stages such as devolatatilization and solid decomposition. The first stage of decomposition started at around 240°C and finally at 440°C. The first stage was considered for decomposition of lignocellulosic materials and second corresponds to the complete decomposition. Similarly FDB also showed two stages of decomposition at around 270°C and 490°C, respectively, as shown in Figure 14(a) and Figure 14(b). The first stage started at about 120°C for both materials with weight loss of 10-20 % due to loss of physically adsorbed water on the surfaces of the biopolymer. The second stage showed a fast weight loss at 240-490°C respectively. The maximum oxidation reached at around 440°C and 550°C for RDB and FDB respectively. Finally the ash left was less than one mg. The second degradation stage of FDB took place at temperature higher than in RDB, indicating that FDB is thermally more stable than RDB. The DTA curve revealed more accurate difference of the thermal behavior of RDB and FDB which was indicated by sharp peak as shown in Figure 14(a) and 14(b) than the TG curves.

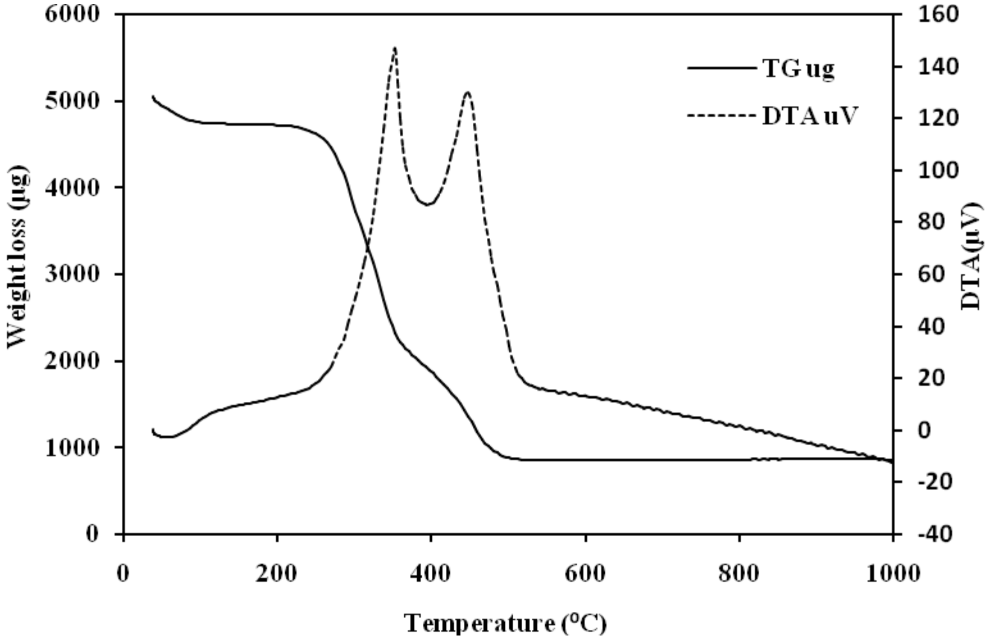

Figure 14 (a): TG and DTA curve for RBD

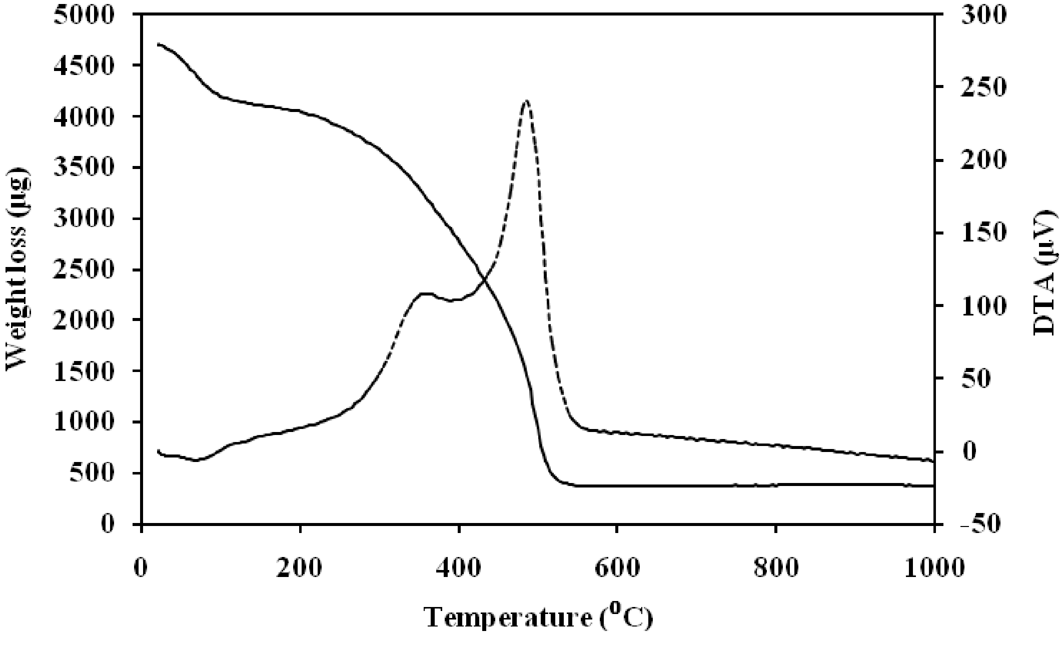

Figure 14 (b): TG and DTA curve for FBD

4.6 X-RayDiffraction Analysis (XRD)

X-ray diffraction spectra of RDB and FDB are shown in Figure 15(a) and (b). The reflection appeared at 2θ = 22°, with less intense peak in FDB as compared to RDB. This indicates the less crystalline nature of the functionalized biopolymers.

The crystallinity of DB polymer could play a restrictive role on metal sorption capacity. The crystallinity parameter of DB is a key parameter in the accessibility to internal sites for both water and metal ions. Many studies have shown that decreasing the crystallinity results in an improvement in metal ion sorption properties (Kurita *et al.*, 1979).

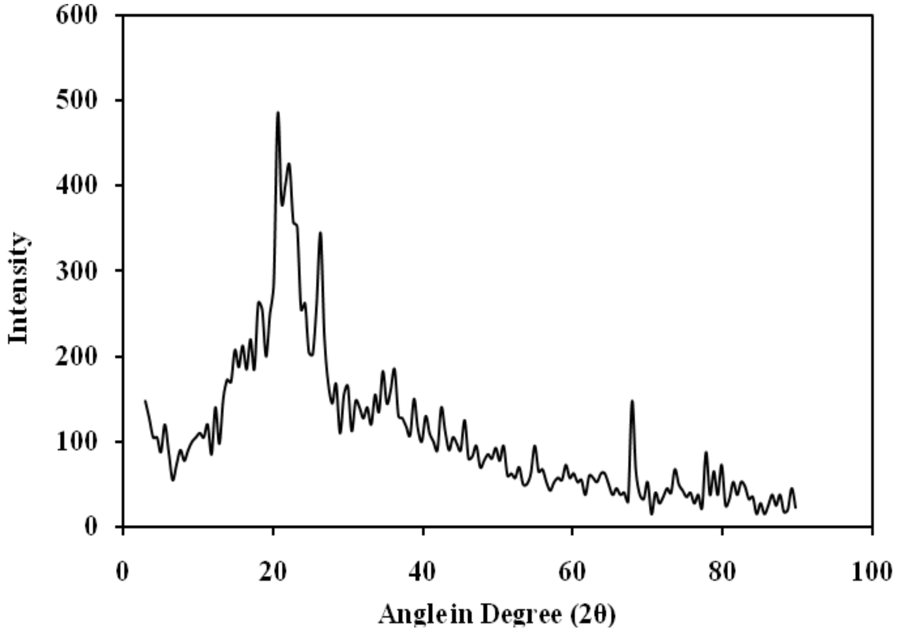

Figure 15(a): XRD spectra of RDB

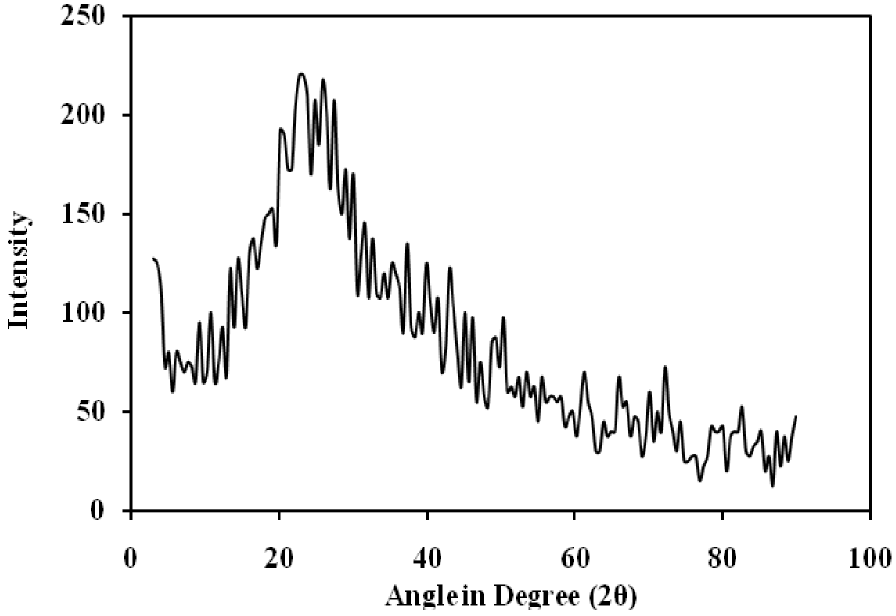

Figure 15(b): XRD spectra of FDB

4.7 pH study

4.7.1 Adsorption of Cd (II), Pb (II), Cu (II), and Zn (II) Metal Ions

The pH of aqueous solution has been considered as one of the major factor, which determines the removal capacity of different metal ions from aqueous solution and eventually decides the surface properties of the biopolymers in terms of ionization of functional groups and surface charge. Variation in pH could change the characteristics as well as the availability of metal ions in solution. At low solution pH, a large quantity of proton (H^+) in solution will compete with the metal ions for binding on the functional groups onto the biopolymer surface. Hence, there would be a decrease in sorption of metal ions. But the sorption of metal ions increase with the increase in pH, due to the decrease in competition between proton (H^+) and metal species for the active sites on the sorbent surface; however, a decrease in sorption will be observed after a certain pH owing to the hydrolysis of metal ions as well as the increased concentration of hydroxyl ions or anionic species in the medium. The maximum sorptions of Cd (II), Pb(II), Cu(II), and Zn(II) metal ions were found at their optimum pH value 6, 4, 5, and 6, respectively, as shown in Figure 16(a), (b) and (c) respectively. The small decrease in the removal of metal ions at higher pH values were due to the formation of hydroxides of the respective metal ions. The micro precipitations of Cd $(OH)_2$, Pb $(OH)_2$, Cu $(OH)_2$, and Zn $(OH)_2$ were observed at pH 7.2, 6.5, 7, and 7.5, respectively, from blank tests (no biopolymer). This may happen due to the decrease in solubility of Cd $(OH)_2$, Pb $(OH)_2$, Cu $(OH)_2$, and Zn $(OH)_2$ metal ions. The optimal pH value 6, 4, 5, and 6 were considered appropriate in all the adsorption experiments for Cd (II), Pb (II), Cu (II), and Zn (II) metal ions as depicted in Figure 16 (a), (b) and (c) respectively.

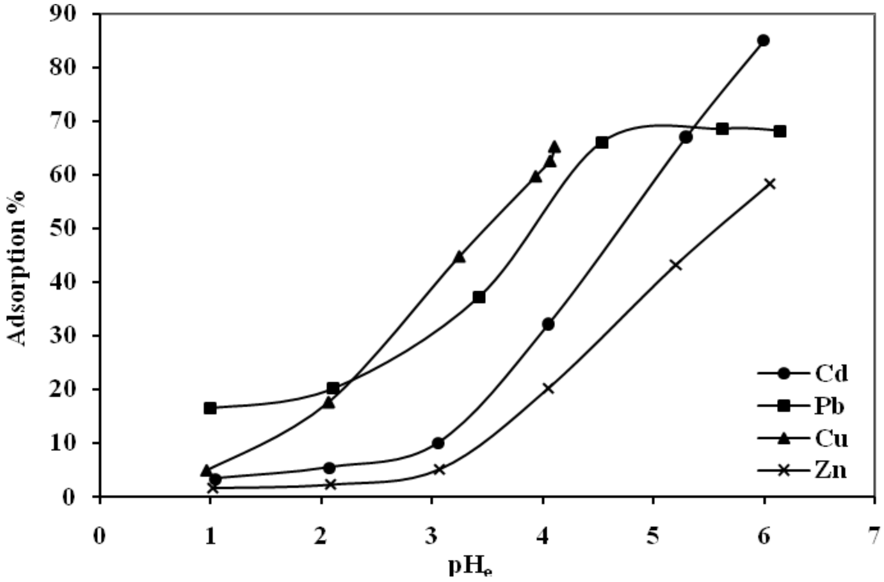

Figure 16(a): Effect of pH on adsorption of metal ions on to FDB-H

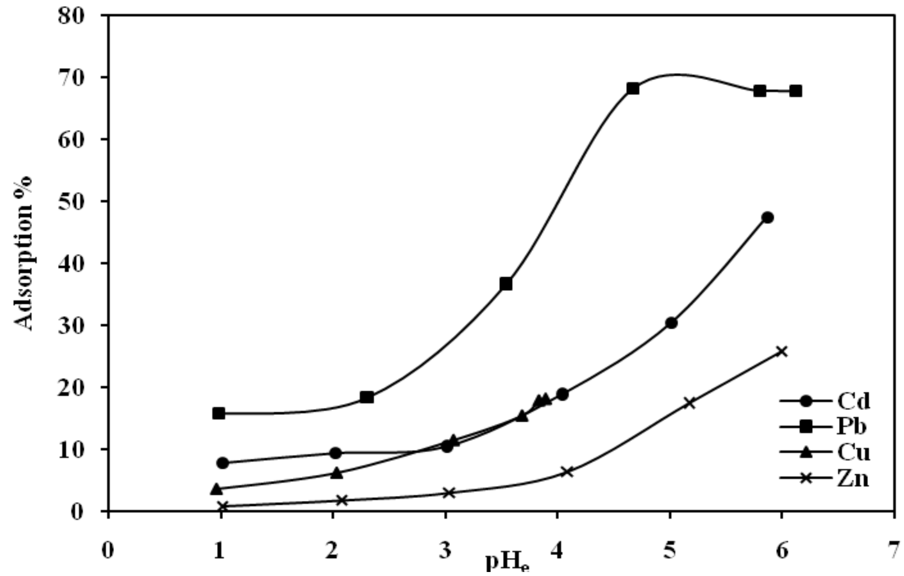

Figure 16(b): Effect of pH on adsorption of metal ions on to FDB-Dm

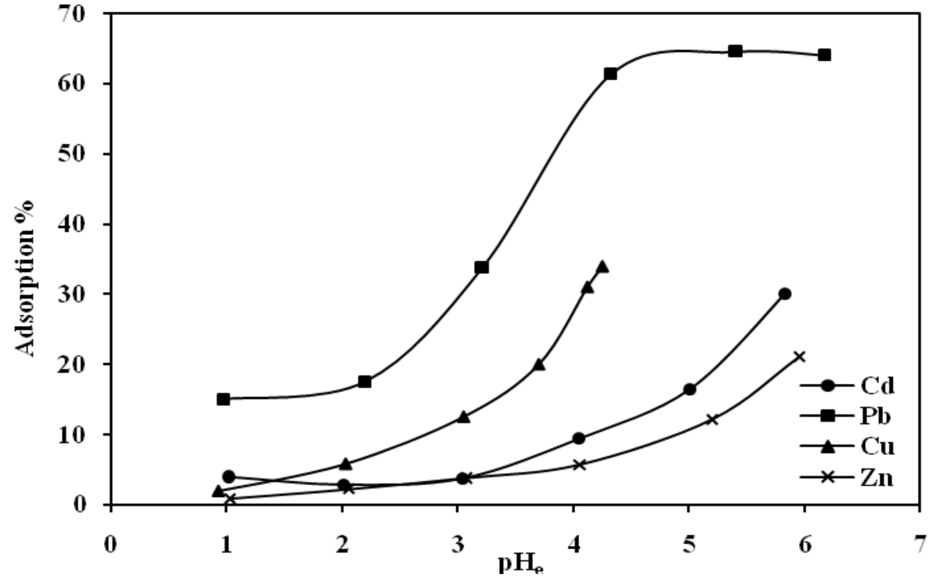

Figure 16 (c): Effect of pH on adsorption of metal ions on to FDB-E

4.8 Zeta Potential

The zeta potential (ZP) is a function of the surface charge which develops when any material is placed in a liquid. It is a very good index of the magnitude of the electrostatic repulsive interaction between particles. The zeta potential is commonly used to predict and control dispersion stability (Deng *et al.,* 2003).

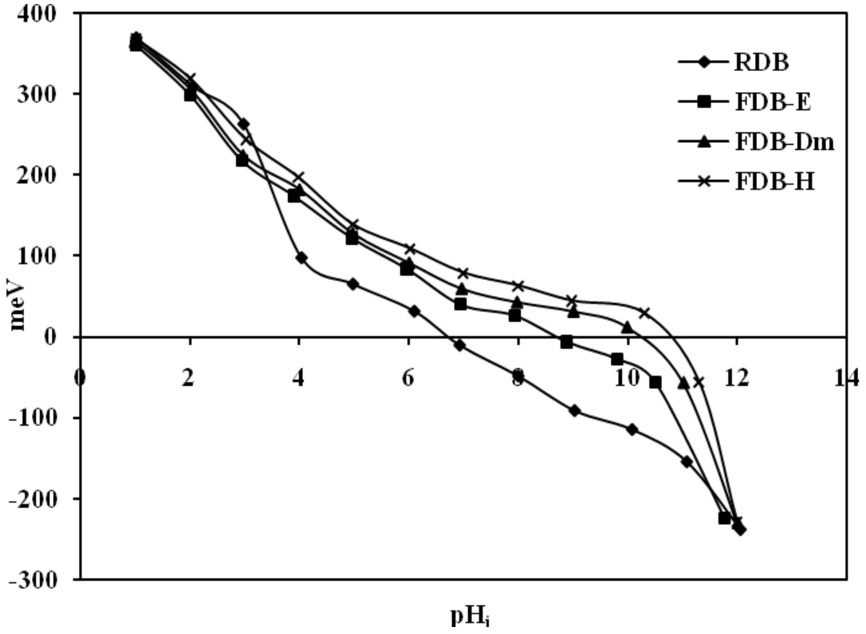

Figure 17: Zeta potential (pH $_{pzc}$) curve of RDB and FDB

The pH $_{pzc}$ is the pH at which the net charge of the surface of biopolymer becomes zero. The zeta potential of RDB in the solution of different pH values are shown in Figure 17. The zeta potential of biopolymer is positive at pH< 6 and negative at pH> 6 as shown in Figure 17, which indicates that the surface of biopolymer becomes positive below 6 while it becomes negative above 6. The pH $_{pzc}$ values had shifted towards higher pH ranges after functionalization of the biopolymer. The pH$_{pzc}$ values for functionalized biopolymers are above pH 9 as shown in Figure 17. The increase in pH$_{pzc}$ values suggests the functionalization with nitrogen functional groups on the surface of biopolymer which may be due to protonation of nitrogen functional group introduced on the surface of biopolymers.

4.9 Adsorption Isotherm Study

Adsorption isotherm is the relationship between the amount of adsorbate adsorbed on the surface of adsorbent and equilibrium concentration of the adsorbate at a certain temperature and other condition. The equilibrium data is formulated into an isotherm model. It is a suitable tool to assess the adsorption capacities in batch and column study. Batch study consists of contacting on adsorbate with a definite quantity of adsorbent in batch stirred system. The mixture is agitated to facilitate the adsorption process.

Different theoretical and empirical models have been proposed to describe the different types of isotherms in batch study (Panday *et al.* 2009). The most widely used isotherm models are as follow.

1. Freundlich isotherm

2. BET isotherm

3. Langmuir isotherm

1. Freundlich isotherm describes the equilibrium on heterogenous surface with a nonuniform distribution of heat of adsorption over the surface and a multilayer sorption to the binding sites located on the surface of the sorbent. It is the earliest known relationship describing the adsorption equation and expresses as shown in equation (3).

$$q_e = \frac{kC_e^{1/n}}{1+bC_e} \qquad (3)$$

And its linearized form is,

$$\ln q_e = \ln k + \frac{1}{n} \ln C_e \qquad (4)$$

where, k = Freundlich constant denoting the relative adsorption capacity ; n = the intensity of adsorption; q_e = maximum adsorption capacity; C_e = equilibrium concentration (mg/L); b = binding constant (L/mg).

2. BET isotherm is based on the assuming the multilayer adsorption ion of the adsorbate on adsorbent surface. As in Langmuir, it assumes that the adsorbent surface is composed of uniform localized sites and adsorption at one site does not affect adsorption at other sites. It also assumes that the energy of adsorption holds the first monolayer but the condensation energy of the adsorbate is responsible for adsorption of successive layer.

$$\frac{C}{\left(C_s - C\right)\frac{x}{m}} = \frac{1}{A\left(x_m\right)} + \frac{A-1}{Ax_m} \qquad (5)$$

where, m = wt of adsorbent (mg)

x_m = amount of solute adsorbed in forming a complete monolayer (mg/g)

Cs = saturation concentration of solute (mg/L)

C = concentration of solute at equilibrium (mg/L)

A = a constant to describe the energy of interaction between the solute and the adsorbent surface

3. Langmuir isotherm (Langmuir, 1916) assumes monolayer coverage of adsorbate over a homogenous adsorbent surface and the sorption of each molecule onto the surface has equal sorption activation energy. It is assumed that once a site is occupied by a metal ion no further sorption can occur. Metal ions are taken up independently on a single type of binding site in such a way that the uptake of the first metal ion does not affect the sorption of the next ion. Langmuir isotherm is expressed by the following equation (6).

$$q_e = \frac{q_{max}\, bC_e}{1 + bC_e} \qquad (6)$$

Adsorption isotherm helps to determine the maximum adsorption capacity of biopolymer for adsorbing different metal ions as well as useful for designing an optimized adsorption process. In present study Langmuir and Freundlich adsorption isotherm were used to evaluate the maximum loading capacity of the functionalized biopolymers (FDB-H, FDB-Dm and FDB-E) for respective metal ions at different metal ions concentrations ranging from 25 to 1000 mg/L as shown in Figure 19(a), (b) and (c) respectively. Batch adsorption experiments were carried out by taking fixed biopolymer dose of 25 mg in 20 mL of respective metal solution at their optimum pH .The solutions were equilibrated for 24 h to attain equilibrium, after attaining equilibrium, the solution were filtered and the concentration of the metal ions were measured. The equilibrium adsorption of metal ions onto the biopolymer could be best described with Langmuir isotherm and the sorption isotherms were evaluated using the most popular linearized Langmuir model represented by the following equation (7).

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m b} \qquad (7)$$

where, q_e = amount of adsorbed metal ions per gram of adsorbent, (mg/g)

$C_{e}=$ residual concentration of metal ions after adsorption, (mg/L)

q_m = maximum adsorption capacity, (mg/g)

$b=$ binding constant, (L /mg)

4.9.1 Adsorption of Pb (II) metal ions onto Raw *D. bipinnata*

Loading capacity of raw *D. bipinnata* was evaluated at pH 4 at different concentrations (50,100, 200, 400 and 600 mg/L) of Pb(II) metal ion solution with fixed dose of raw biopolymer (25 mg) taken in a separate flasks. The mixture was shaken for 24 h to attain equilibrium, filtered and concentration was measured with ICP-AES.

The metal ion sorption was maximum in between 400-600 mg/L and no significant increase in sorption was observed above 600 mg/L. The maximum loading capacity was found to be poor (19 mg/g) indicating that non functionalized biopolymers are not efficient of binding the heavy metals under consideration as shown in Figure 18 (a) and (b). Therefore the raw biopolymer was functionalized with three different types of reagents for better adsorption of metal ions and their loading capacities were evaluated.

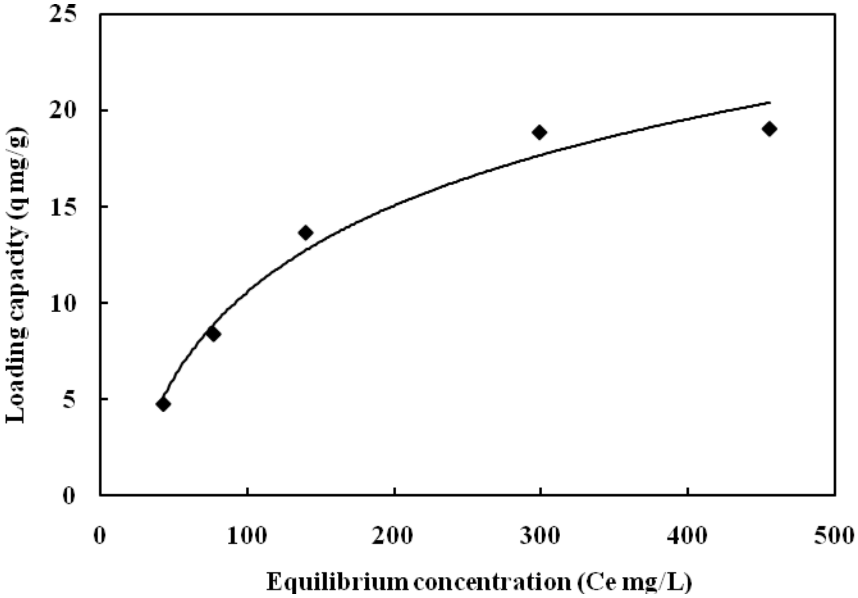

Figure 18 (a): Adsorption Isotherm plot for the Pb (II) metal ions onto RDB

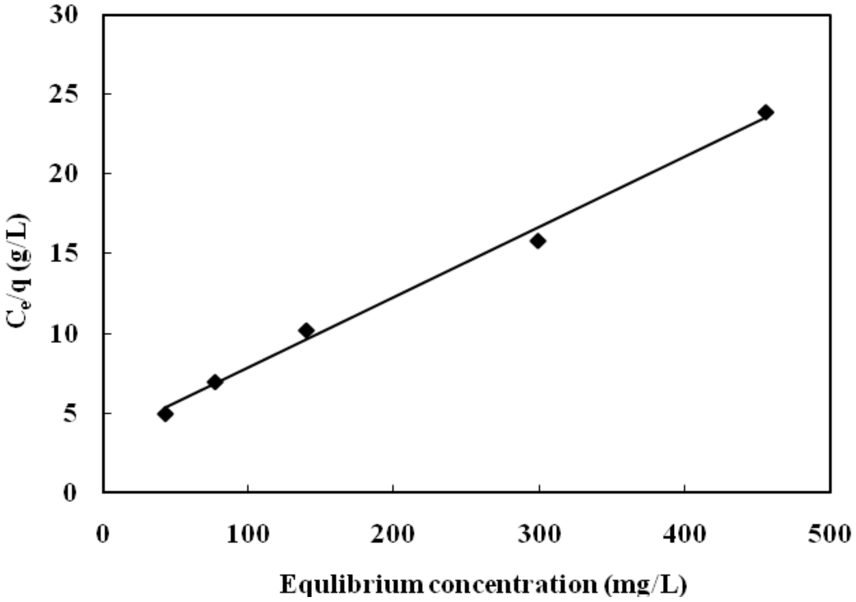

Figure 18(b): Langmuir Isotherm plot for the Pb (II) metal ions onto RDB

The Langmuir constant (q_m and b) were calculated from plots of C_e/q_e versus C_e as shown in Figure 20 (a), (b) and (c) respectively.

The Langmuir constant and its correlation coefficients evaluated from the isotherms for Cd, Pb, Cu, and Zn metal ions are given in Table 5. The correlation coefficient (R^2) of the Langmuir plot is high which suggests that the equilibrium sorption of respective metal ions onto the biopolymer could be best described with the Langmuir isotherm equation. The maximum amount of metal ions adsorbed (q_m) based on experimental data are given in Table 5. On the other hand Freundlich adsorption isotherm did not fit the experimental adsorption data well (result not shown).

The values of *b* are indicative of the affinity of the sorbent by the sorbate, and high values of *b* are associated with high metal adsorbed/ desorbed ratio. The above results are in close agreement with each other suggesting that heavy metals can be quantitatively sequestered onto the functionalized *D. bipinnata* (FDB). Initially the sorption capacity of biopolymer was found to be increased with an increase in the initial concentration of metal ions in solution upto 800 mg/L. In all cases, when the initial concentration exceeded from 600 mg/L to 800 mg/L, the sorption capacity remained almost constant and form a plateau. Initially the active sites on the biopolymer were easily available for the sorption of metal ions, as the concentration increases the active sites on the biopolymers were almost filled by metal ions, and hence the sorption activity on biopolymer is limited. So further increase in initial concentration of metal ions results in no more adsorption, due to unavailability of sorption sites. Therefore, 600 mg/L–800 mg/L was found to be the optimum concentration of metal ions for effective sorption. The maximum loading capacities of the metal ions are given in Table 5. In all three types of functionalized biopolymers, the maximum loading capacity were found to be the highest in Pb(II) ions where as the lowest loading capacity were found in Zn(II) ion in FDB-H and FDB-E and in case of FDB-Dm Cu(II) ion has lowest loading capacity.The highest loading capacity of Pb(II) in all three types of functionalized biopolymers could be due to higher electronegativity and smallest hydrated radius which creates a greater affinity for negatively charged adsorption sites onto FDB. In all cases correlation coefficient (R^2) value was found be 0.99 indicating that the Langmuir isotherm model fitted well as shown in Figure 20(a), (b) and (c) respectively. No information is available in the literature on the isotherm of Cd, Pb, Cu, and Zn adsorption by *D. bipinnata* to compare with our findings.

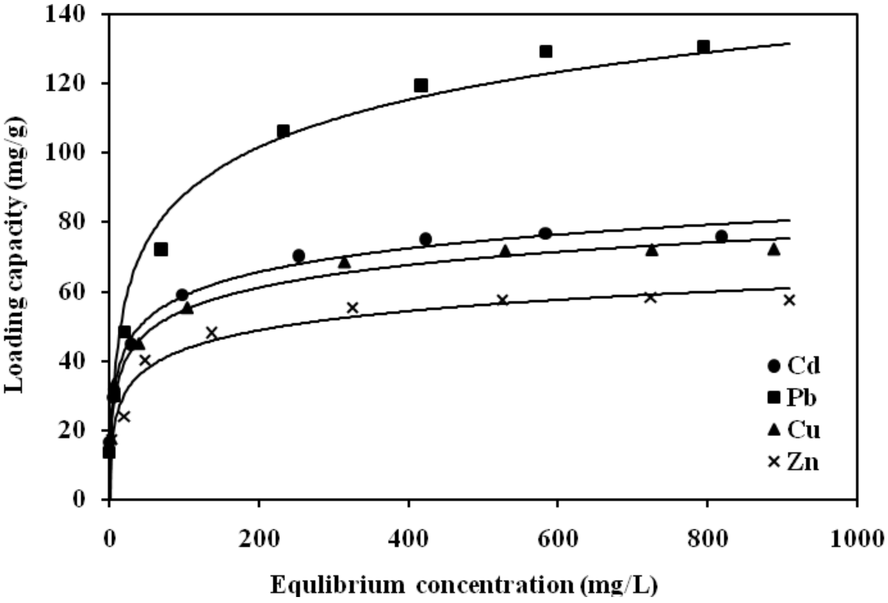

Figure 19(a): Adsorption Isotherm plot for the metal ions onto FDB-H

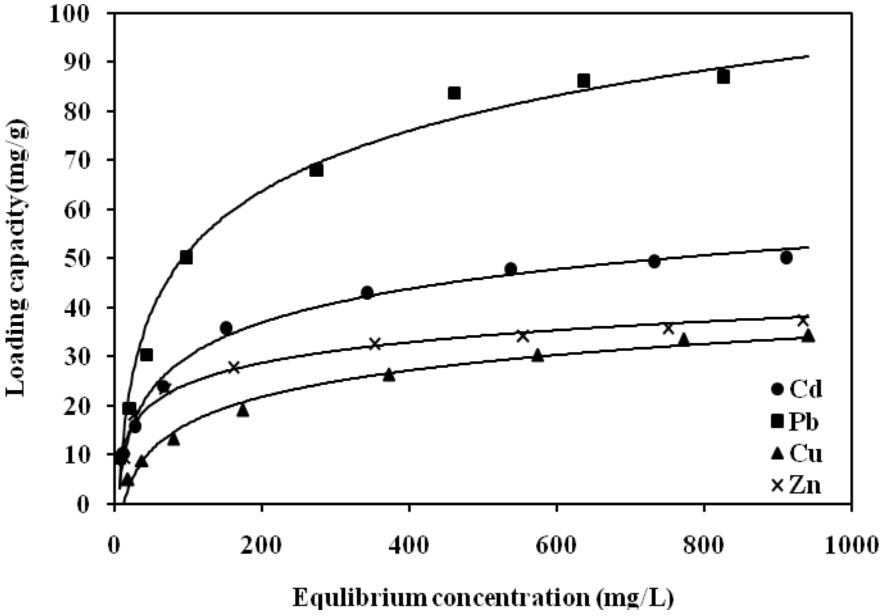

Figure 19(b): Adsorption Isotherm plot for the metal ions onto FDB-Dm

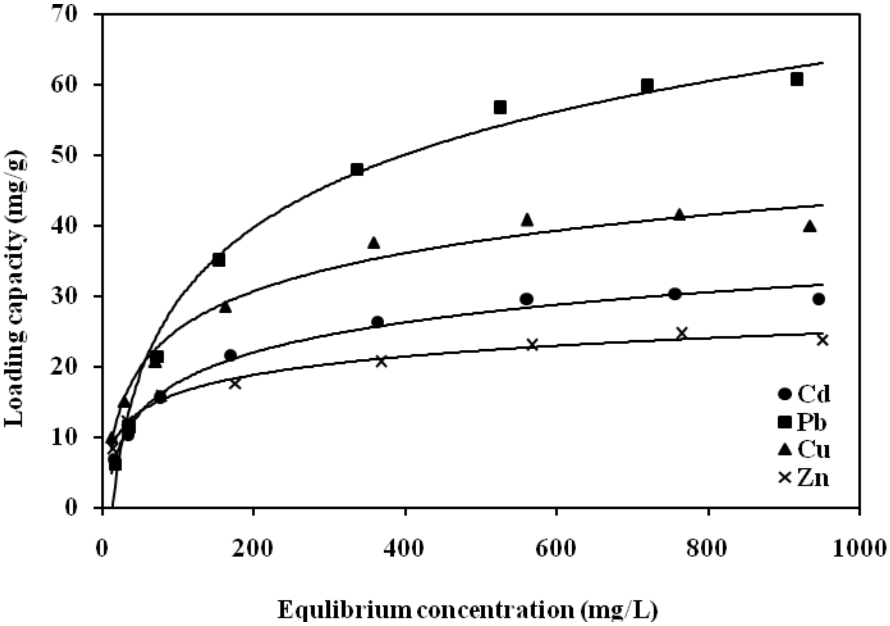

Figure 19(c): Adsorption Isotherm plot for the metal ions onto FDB-E

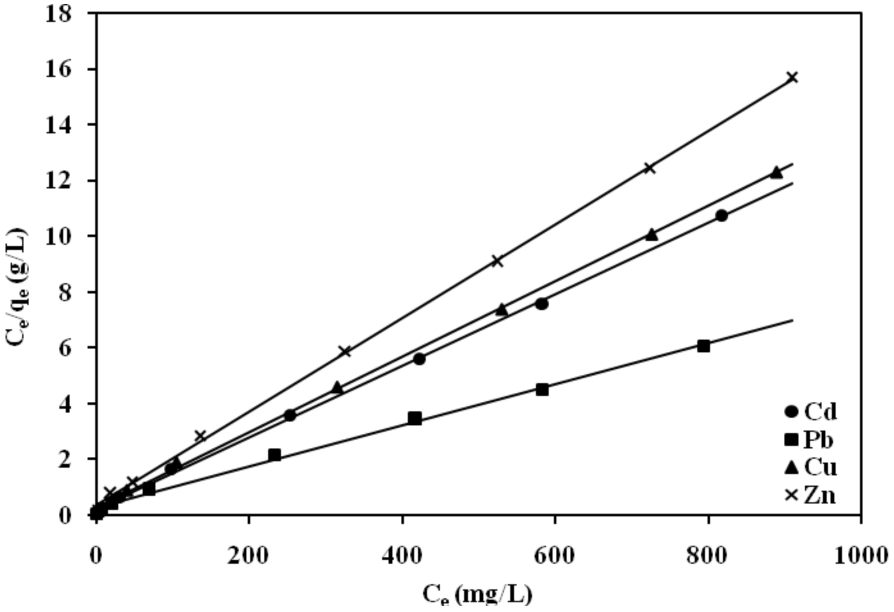

Figure 20(a): Langmuir Isotherm plot for the metal ions onto FDB-H

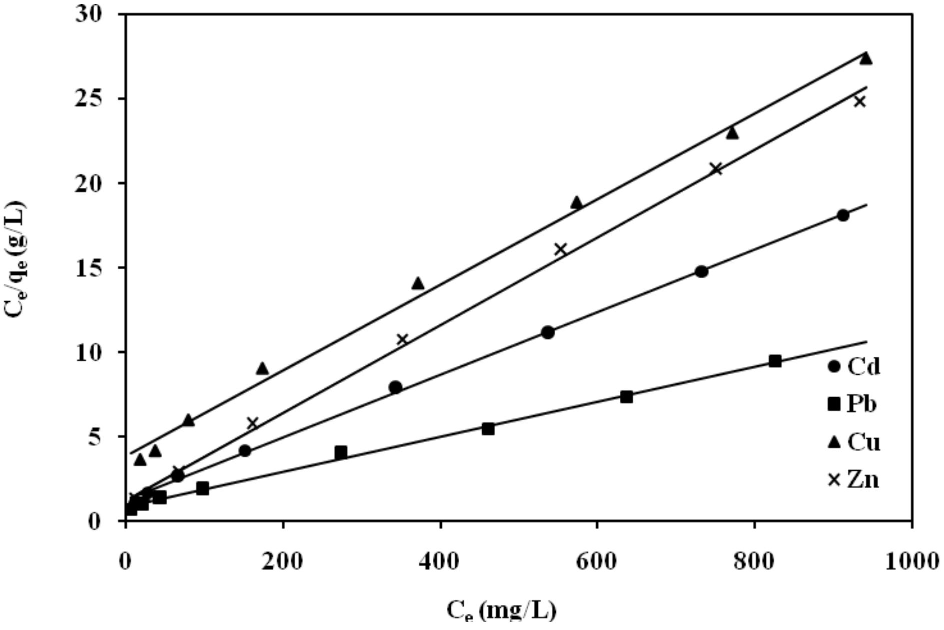

Figure 20(b): Langmuir Isotherm plot for the metal ions onto FDB-Dm

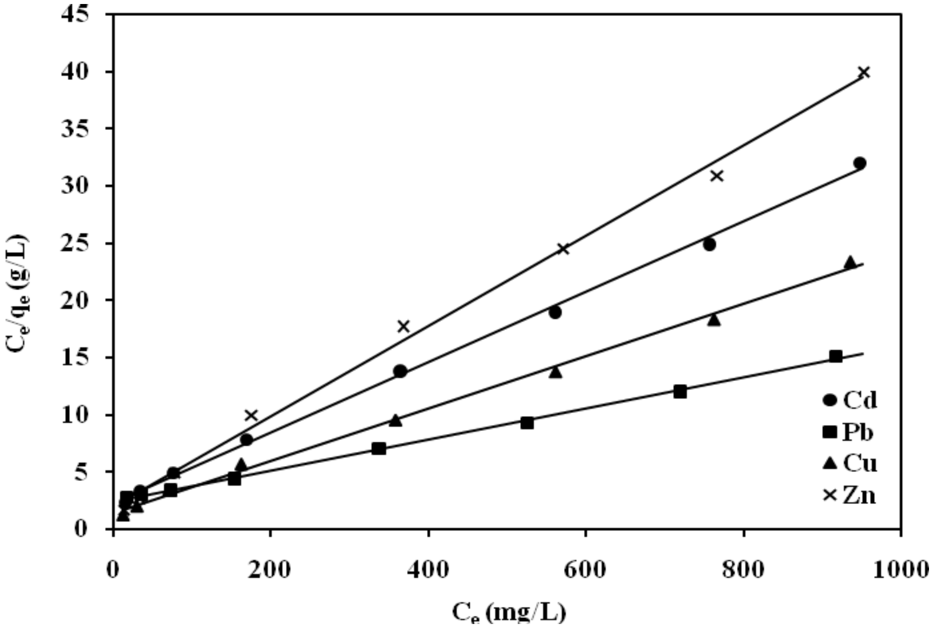

Figure 20(c): Langmuir Isotherm plot for the metal ions onto FDB-E

Table 5: Langmuir adsorption isotherm model parameters and experimental q_{max} of FDB

Biopolymer	**Metal ions**	**q_{exp}(mg/g)**	**q_{graph}(mg/g)**	**b(L/mg)**	**R^2**
FDB-H	**Cd(II)**	76.80	83.33	0.055	0.99
	Pb(II)	130.40	142.86	0.024	0.99
	Cu(II)	72.10	76.92	0.049	0.99
	Zn(II)	58.16	62.50	0.042	0.99

Biopolymer	**Metal ions**	**q_{exp}(mg/g)**	**q_{graph}(mg/g)**	**b(L/mg)**	**R^2**
FDB-Dm	**Cd(II)**	50.40	55.56	0.014	0.99
	Pb(II)	87.20	100.00	0.012	0.99
	Cu(II)	34.40	40.00	0.011	0.99
	Zn(II)	37.60	40.00	0.020	0.99

Biopolymer	**Metal ions**	**q_{exp}(mg/g)**	**q_{graph}(mg/g)**	**b(L/mg)**	**R^2**
FDB-E	**Cd(II)**	29.60	33.33	0.014	0.99
	Pb(II)	60.80	76.92	0.010	0.99
	Cu(II)	40.00	45.45	0.016	0.99
	Zn(II)	23.85	25.64	0.020	0.99

The essential characteristics of the Langmuir isotherm can be expressed by a dimensionless constant called the equilibrium parameter (R_L) expressed by Eq. (8)

$$R_L = \frac{1}{1 + bC_0} \qquad (8)$$

where, b = Langmuir constant

C_0 = Initial metal ion concentration

R_L= Types of isotherm, its value between 0 and 1 indicates the favorable adsorption.

The R_L values calculated from the b values (data not given) for all metal ions at different concentration are between 0 and 1, is also the support for Langmuir model.

4.10 Kinetic study

Sorption kinetics is an important parameter to predict the rate and time at which the pollutant is removed from aqueous solution.

Several kinetic models are described to investigate the reaction order in sorption systems. However, over the past few years, pseudo first- order rate equation and pseudo second-order rate equation have been considered to be among the most appropriate kinetic model.

1. Pseudo- first order rate equation: Lagergren first order rate equation is used to describe the kinetic process of liquid- solid phase adsorption. It is believed to be the earliest model of adsorption rate based on the adsorption capacity and can be expressed as,

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \qquad (9)$$

where q_e and q_t (mg/g) are the adsorption capacities at equilibrium and time t (min), respectively. k_1 (min^{-1}) is the pseudo- first- order rate constant for the kinetic model.

To distinguish kinetic equation based on adsorption capacity from solution concentration, Lagergren's first order rate equation has been called pseudo-first order (Ho and McKay, 1999). In recent year, it has been widely used to describe the adsorption of pollutants from wastewater.

2. Pseudo-second order rate equation: In 1995, (Ho and McKay 1999) described a kinetic process of the adsorption of divalent metal ions onto biopolymer in which the chemical bonding among divalent metal ions and polar functional groups on biopolymer are responsible for the cation exchange capacity of the biopolymer.

Ho's second order rate equation has been called pseudo-second order rate equation to distinguish kinetic equation based on adsorption capacity from concentration of solution (Ho, 2006). This equation has been successfully applied to the adsorption of metal ions from aqueous solution.

$$\frac{dq_t}{dt} = k_2\left(q_e - q_t\right)^2 \qquad (10)$$

In the present study, the sorption kinetics onto FDB (FDB-H.FDB-Dm and FDB-E) were analyzed by taking 50 mg/L initial concentration of respective metal ions at their optimum pH. The sorption kinetics were studied at different time intervals ranging from 5 min to 360 min. The optimum concentrations of metal ions were measured using ICP-AES after shaking for required interval of time for each sample solution. The experimental data showed an increase in adsorption percentage with the increase in time from 5 min to 120 min and then it almost reached a saturation level. As the contact time increased, the active sites on the biopolymers were filled and the sorption rate became slower and reached the plateau as shown in Figure 21(a), (b) and (c) respectively. So the optimum contact time was considered from 100 to 120 min. All the kinetic data of the respective metal ions were tested using the pseudo-first order and pseudo-second order rate equation. In case of the first-order rate equation, data were poorly fitted (data and figure not given) and the Lagergren correlation coefficient (R^2) value was very low.

Therefore, pseudo-second order kinetic model has been used to evaluate the experimental kinetics sorption data given by Ho and McKay.

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \qquad (11)$$

Where, q_t = amount of the adsorption at time t (min), (mg/g)

k_2 = rate constant of the pseudo-second order kinetic adsorption, (g/mg- min)

The straight-line plots of t/q_t versus t, as shown in Figure 22 (a), (b) and (c) respectively, have been used to obtain the rate parameters. Kinetic modeling of the data was studied using pseudo- second order rate equation, and its applicability was tested by linear plot of t/q_t versus t.

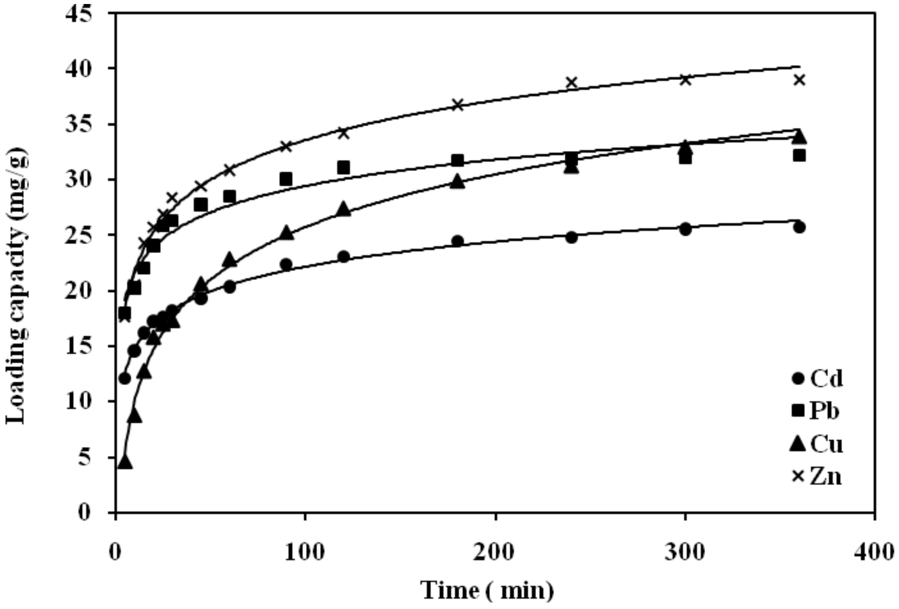

Figure 21(a): Adsorption kinetic plot of metal ions onto FDB-H

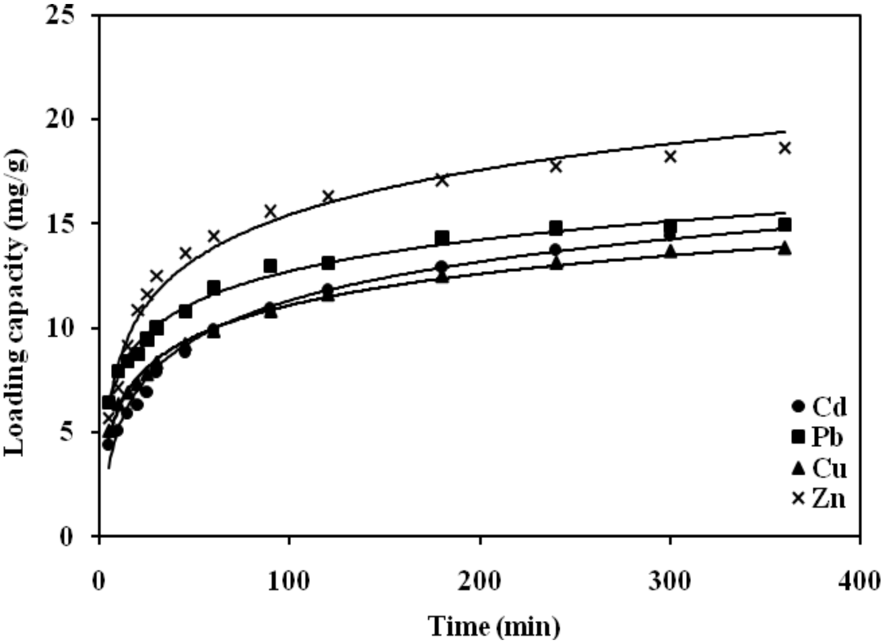

Figure 21(b): Adsorption kinetic plot of metal ions onto FDB-Dm

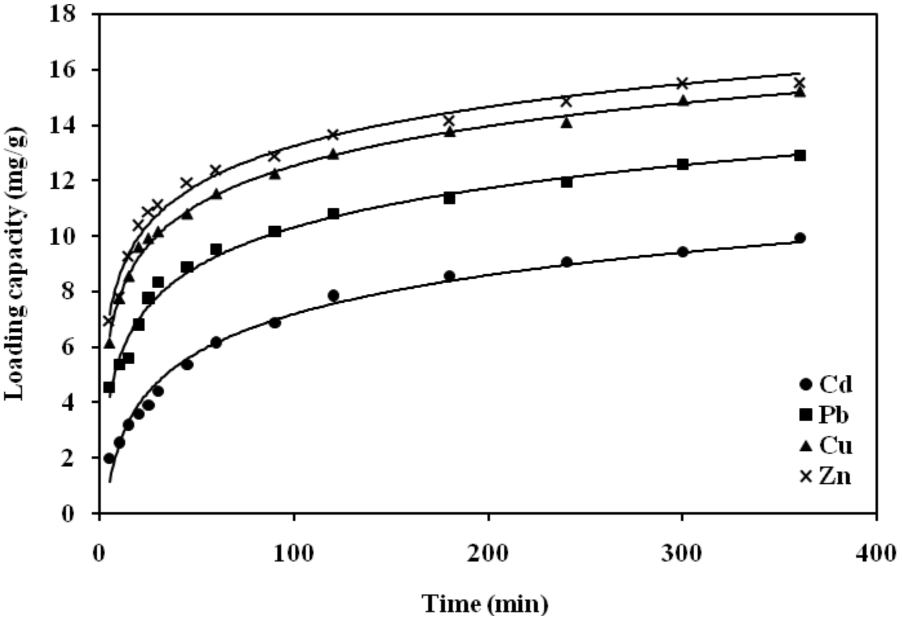

Figure 21(c): Adsorption kinetic plot of metal ions onto FDB-E

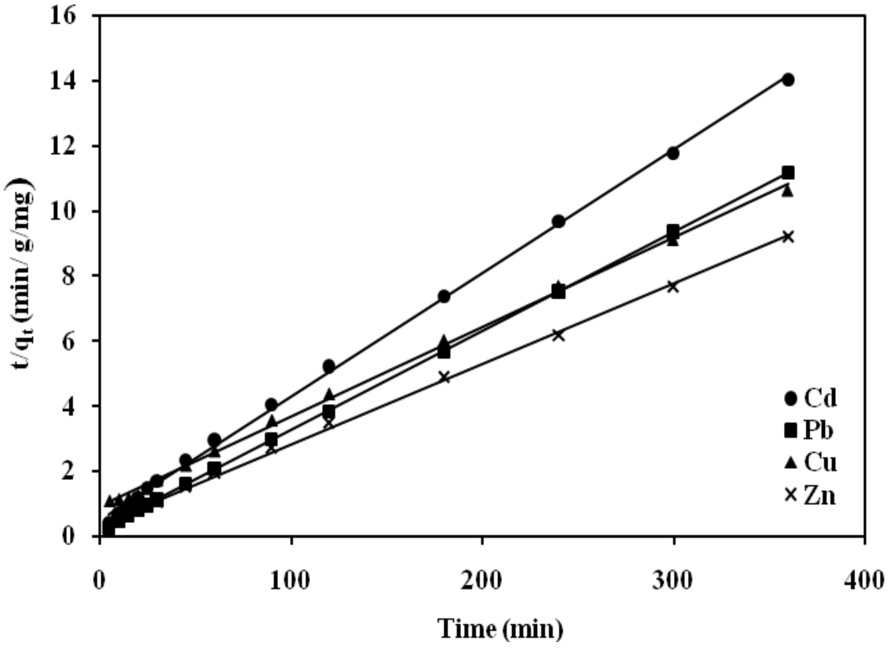

Figure 22(a): Pseudo-second order kinetic plot for FDB-H

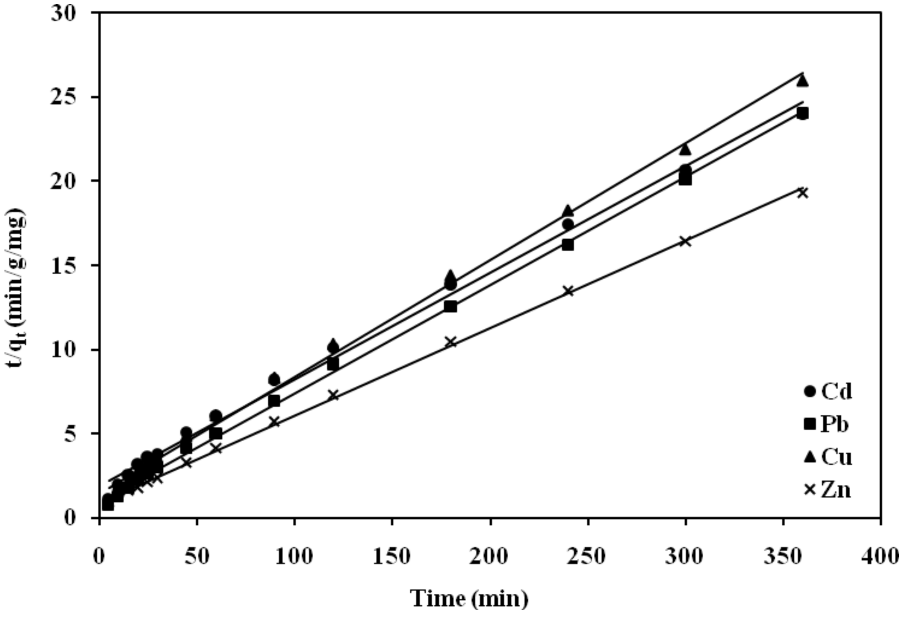

Figure 22 (b): Pseudo-second order kinetic plot for FDB-Dm

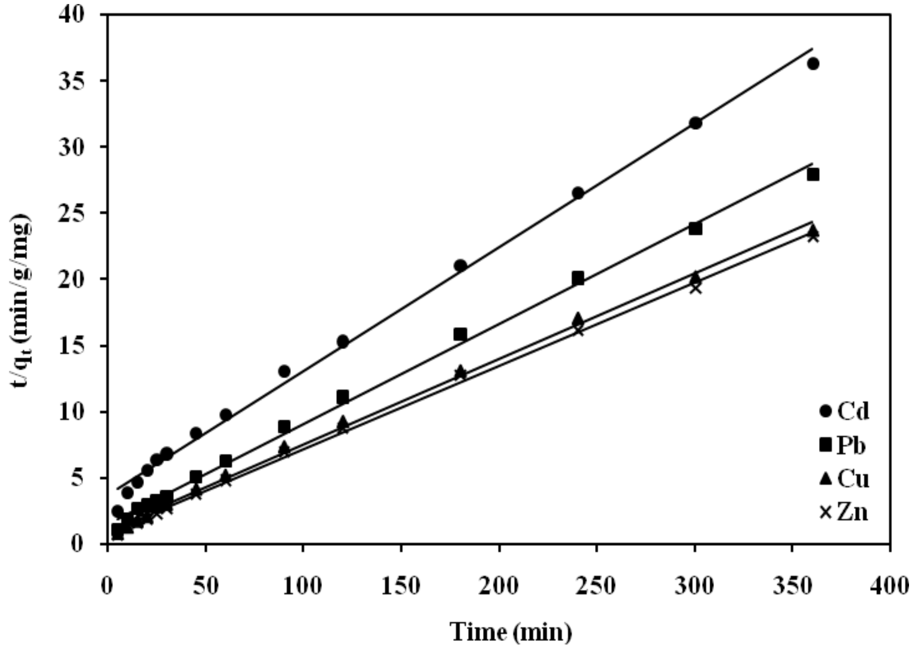

Figure 22(c): Pseudo-second order kinetic plot for FDB-E

The calculated values of k_2, q_e, and R^2 are given in Table 6. The k_2 values for Pb(II) ions are the highest for FDB-H and FDB-Dm, which indicated that Pb(II) ions are adsorbed fastest among other metal ions. In case of FDB-E, Zn(II) ions are adsorbed fastest among other metal ions as it has highest k_2 value. The R^2 values for all metal ions obtained were close to 1.0, which indicates the applicability of the model for the entire sorption process and confirmed the chemisorption of the respective metal ions onto the FDB.

Table 6: Pseudo-second order kinetics for Functionalized Biopolymers

Biopolymer	**Metal ions**	**q_e (mg/g)**	**k_2(g/mg -min)**	**R^2**
FDB-H	**Cd(II)**	25.68	3.16 x10^{-3}	0.99
	Pb(II)	32.22	4.54 x10^{-3}	0.99
	Cu(II)	33.84	0.96 x10^{-3}	0.99
	Zn(II)	39.03	1.98 x 10^{-3}	0.99
Biopolymer	**Metal ions**	**q_e(mg/g)**	**k_2(g/mg -min)**	**R^2**
FDB-Dm	**Cd(II)**	15.00	2.35 x 10^{-3}	0.99
	Pb(II)	14.96	4.59 x 10^{-3}	0.99
	Cu(II)	13.84	3.57 x 10^{-3}	0.99
	Zn(II)	19.64	2.83 x 10^{-3}	0.99
Biopolymer	**Metal ions**	**q_e(mg/g)**	**k_2(g/mg -min)**	**R^2**
FDB-E	**Cd(II)**	9.91	2.75 x 10^{-3}	0.99
	Pb(II)	12.88	3.97 x 10^{-3}	0.99
	Cu(II)	15.20	4.04 x 10^{-3}	0.99
	Zn(II)	15.54	4.87 x 10^{-3}	0.99

4.11 Mechanism of adsorption

Adsorption, precipitation, neutralization, ion-exchange, chelation and complexing reagent are some methods used in the removal of heavy metals from aqueous medium to reduce metal toxicity in the environment. Among these techniques, adsorption using functionalized polymers is generally preferred for heavy metal ion removal due to their availability, high efficiency, easy handling and low cost. A large number of polymers incorporating a variety of functional groups, including carboxylic acid, sulfonic acid, amine, amide, phosphoric acid, have been prepared and their adsorption and analytical properties investigated (Shankar *et al.*, 2007).

One way to remove metals from wastewater is the use of polymeric adsorbent, which consists of different ligands, where metal ions are bound by a coordinate bond. A ligand contains anchoring sites such as nitrogen, oxygen or sulfur atoms obtained either by polymerization of monomer possessing the coordinating site or by a chemical reaction between a polymer and a low moleculer weight compound with coordinating ability (Coleman, 1975; and Kaliyappan and Kannan, 2000).

The sorption mechanism of legnocellulosic materials is different from those of other conventional adsorbents. These mechanisms are complicated because they implicate the presence of different interactions. Metal complexation by *N*-functionalized biopolymer may thus involve two different mechanisms chelation and ion exchange depending on the pH, since this parameter may affect the protonation on the surface of the biopolymer (Crini, 2005; and Jia *et al.*, 2002). The functionalized polymer possesses high percentage of nitrogen atom present in the form of amine groups that are responsible for metal ion binding through chelation mechanism which is represented in Figure 23(a), (b) and (c) respectively. Amine sites are the main reactive groups for metal ions than hydroxyl groups, which contribute to adsorption of metal ions (Jin and Bai, 2002).

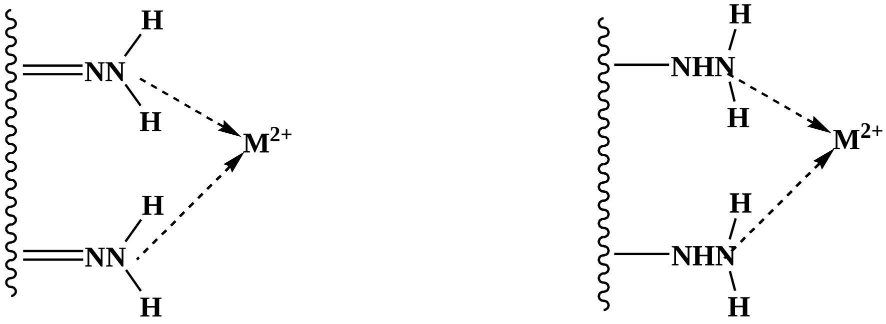

Figure 23(a): Plausible mechanism through complexation onto FDB-H

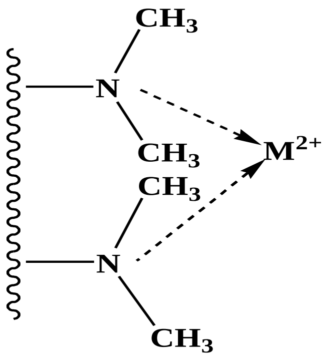

Figure 23(b): Plausible mechanism through complexation onto FDB-Dm

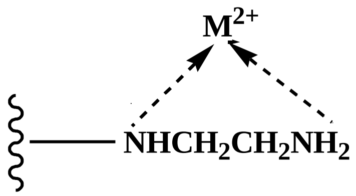

Figure 23(c): Plausible mechanism through complexation onto FDB-E

The other mechanism involved in the sorption of positively charged metal ions is the ion-exchange process between protons and the metal ions from solution. The *N*-atom of amino group has greater tendency to donate lone pair of electrones for sharing with (electropositive species) protons. This protonation reaction can be represented as in equation (a)

$$\text{---}NH_2 \quad + \quad H^+ \longrightarrow \text{---}NH_3^+ \qquad \text{(a)}$$

When metal solution is added to the biopolymer, then metal ions also started to bind with amino group due to sharing of lone pair of electrones from N-atom, which is represented in equation (b).

$$\text{---NH}_2 + M^{2+} \longrightarrow \text{-NH}_2M^{2+} \qquad \text{(b)}$$

The binding of a metal ion to *N*-atom is stronger than the binding of a proton to *N*-atom, because the force of electrostatic attraction between the lone pair of electrons from *N*-atom and divalent metal ion is stronger than that between lone pair of electrons and monovalent proton (Deng and Bai 2003). This difference in binding force causes the compititive sorption of metal ions and proton to the *N*-atom. It is considered as an ion-exchange mechanism and represented in equation (c).

$$\text{---NH}_3^{+} + M^{2+} \longrightarrow \text{---NH}_2M^{2+} + H^{+} \qquad \text{(c)}$$

This amine metal complex then reacts with water and forms metal hydroxide as shown in equation (d). It is due to greater binding force of metal ions with -OH group from water than with *N*-atom of amine group.

$$\text{---NH}_2M^{2+} + H_2O \longrightarrow M(OH)^{+} + \text{---NH}_3^{+} \qquad \text{(d)}$$

As the protonation of the amine groups occur on the surface of functionalized biopolymer, the surface should be positively charged at low pH below 4. Therefore, the electrostatic interactions between the biopolymer and metal ions to be adsorbed were repulsive under the pH studied, since Cd $^{2+}$, $Pb^{2+,}$ Zn^{2+} and Cu^{2+} were the major species in the solutions at pH below 4. At lower pH, the positively charged hydrogen ions may compete with the metal ions for binding with the functional groups on the surface of biopolymer. Once the amine groups were protonated, the strong electrical repulsion prevented the metal ions from being adsorbed onto the surface of biopolymer, resulting in almost no adsorption of the metal ions on the biopolymer. The increase in solution pH decreases the electrical repulsion force, and the metal ions can be easily adsorbed on the biopolymer surface. For all the respective metals, metal up take below pH 4 is not significant, indicating the electrostatic interaction may be no longer the dominant factor for the metal ions approaching the binding sites (Deng S., Ting Y-P., 2005).

4.12 Adsorption of Binary Metal ions Pb(II) and Zn(II)

To understand the nature of metal ions separation of two typical heavy metals, Pb (II) and Zn (II) were considered at the concentration range from 25, 50, 100, 150 and 200 mg/L, respectively, as shown in Figure 24. The experiment was carried out batchwise. It is evident from the experimental results, that the metals ions loaded with the binary mixture are almost in agreement with the sum of the individual loading capacity of the respective metal ions. Alternatively, presence of one metal ions in the aqueous solution interfers the sorption of other metal ions thererby decreasing the loading capacity of both the metal ions. This is simply due to the fact that available functional sites are occupied by the both metal ions in equilibrium (Li *et al*., 2011) . Thus, the available functional sites are most important for sequestering heavy metals from aqueous solution.

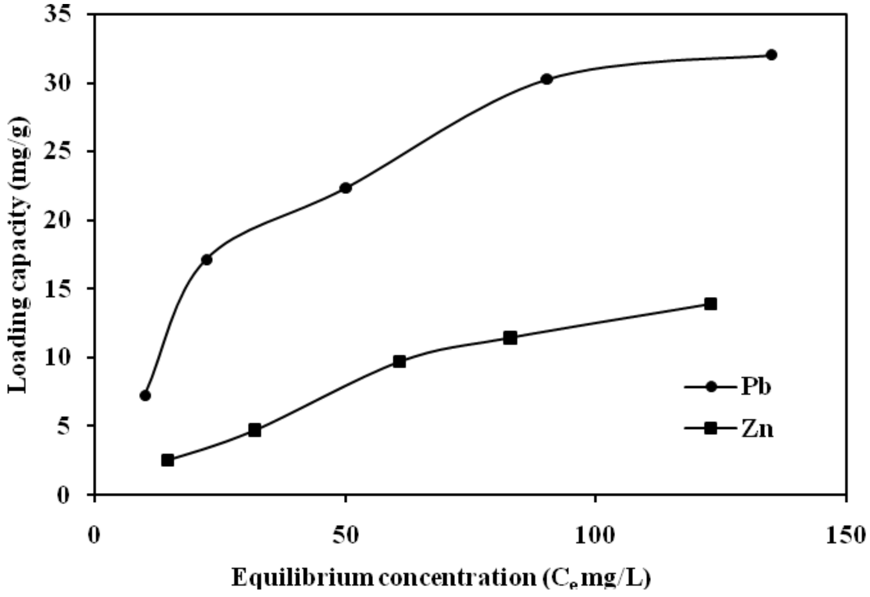

Figure 24: Adsorption isotherm of binary mixture

4.13 Desorption and Regeneration study

Batch desorption experiments were performed to evaluate the efficiency and reusability of all the three functionalized biopolymers. In this study three types of eluting reagents were used (NaOH, HCl and HNO_3). The result indicated that, 0.1 M nitric acid was found to be most effective eluting agent among them releasing the metal ions adsorbed on to the functionalized biopolymer. Figure 25(a), (b) and (c) show the loading capacity (q in mg/g) and the percentage desorption (D %) of the biopolymer for the Pb (II) ions in six cycles. The result revealed that above 90% metal ions were desorbed from the biopolymer. The sorption capacity remained relatively constant in subsequent cycles, the sorption capacity attended was about 90% in fifth cycle, indicating that the metal adsorbed biopolymer can be regenerated completely with 0.1 M nitric acid, and the biopolymer can be repeatedly use for metal ions sorption, there by minimizing the cost for the sorption process.

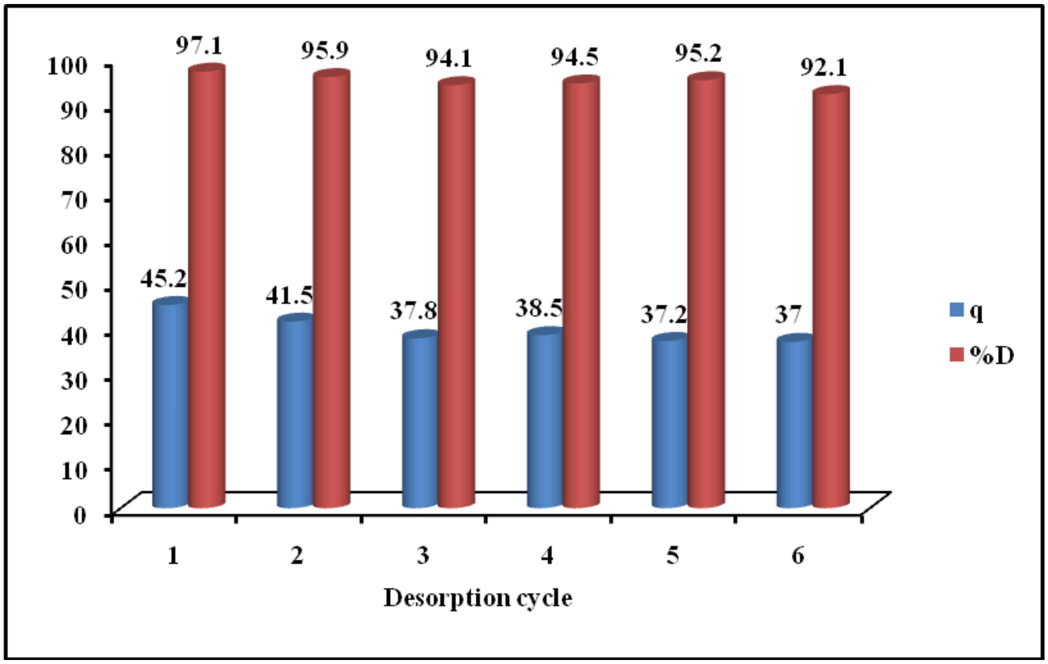

Figure 25(a): Desorption study on FDB-H

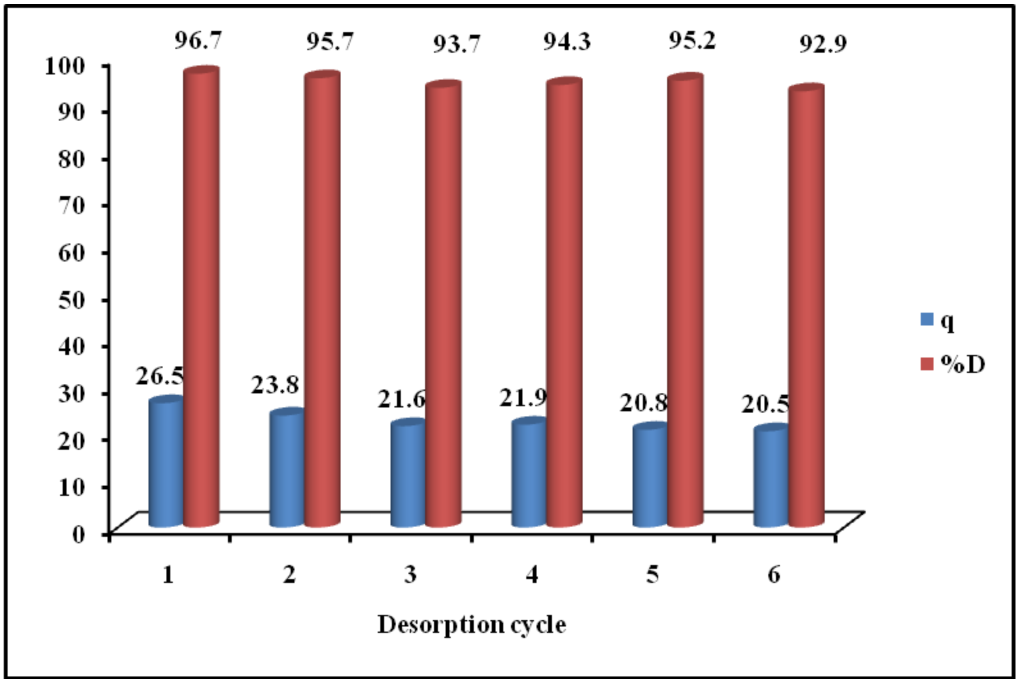

Figure 25(b): Desorption study on FDB-Dm

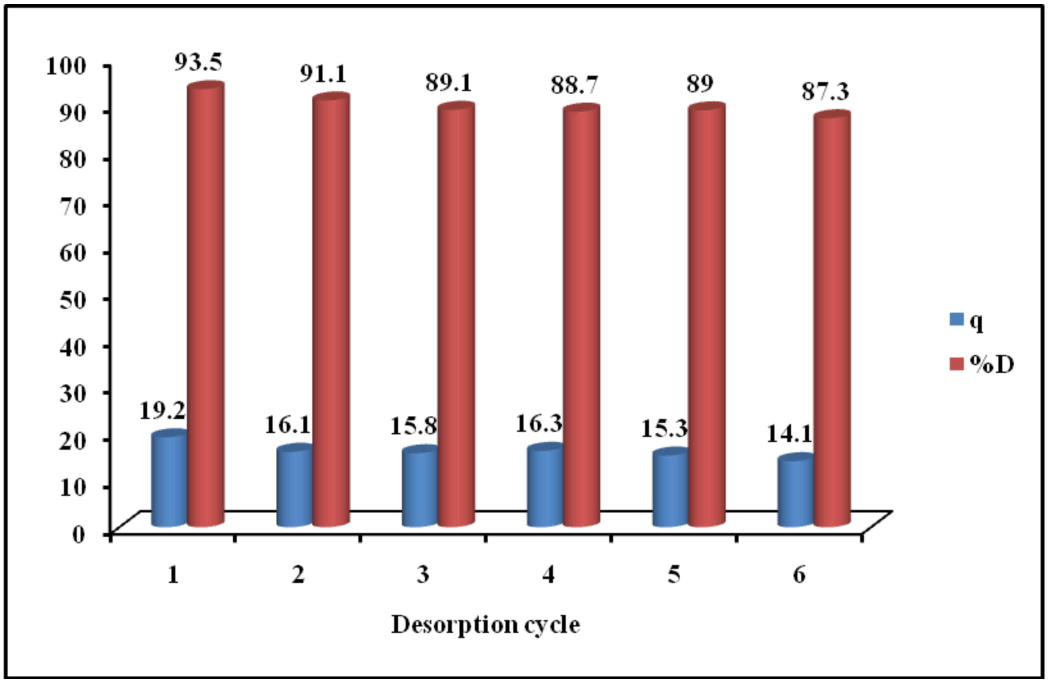

Figure 25(c): Desorption study on FDB-E

Table 7: Comparison of metal loading capacities (mg/g) of different biosorbents for the removal of metal ions reported in the literature.

Adsorbents	**Cd (II)**	**Pb(II)**	**Cu (II)**	**Zn (II)**	**References**
Desmostachya bipinnata	76.80	130.4	72.10	58.16	**this study**
Rice husk	------	120.48	31.85	-----	(Wang *et al.*, 2003b)
Saw dust(Cadrus deodar wood)	73.62	------	-----	------	(Memon *et al.*, 2007)
Saw dust (Poplar tree)	-----	------	13.95	------	(Acar &Eren, 2006)
Saw dust (Poplar tree)	------	------	------	15.8	(Sciban *et al.*, 2006)
Sunflower stalk	42.18	------	29.30	30.73	(Sun and Shi,1998)
Arundo donax L stem	5.7	------	------	------	(Basso *et al.*,2002)
Jute fibres	------	-----	8.40	5.95	(Sukla & Pai, 2005)
Carrot residues	------	-----	32.74	29.61	(Nasernejad *et al.*, 2005)
Azolla filiculoides	86	228	62	48	(Ganji *et al.*, 2005)
Nipah palm shoot biomass	-----	52.86	66.71	-----	(Wankasi *et al.*,2006)
Peanut husk	-----	29.14	10.15	-----	(Li *et al.*, 2006a)

4.14 Comparison of Adsorption Capacities

The metal loading capacities of RDB and FDB-H, FDB-D_m and FBD-E were found to be high in most of the experimental results where as some results are comparable with the capacities of different modified biosorbents given in the literatures as shown in Table 7. Depending upon the loading capacities of the biosorbents, it can be used as an effective and environmental friendly adsorbent in the removal of traces of toxic heavy metals from aqueous medium.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This research work has explored a new biopolymer *Desmostachya bipinnata* (DB) which is, easily available, cost effective, and ecofriendly. The sorption capacity of raw biopolymer was evaluated and found no significant sorption capacity. So the raw biopolymer was functionalized with hydrazine monohydrate, dimethylamine and ethylenediamine to introduce nitrogen functional groups onto the polymeric surface of the biopolymer based on *Desmostachya bipinnata*. It was characterized by elemental analysis, analysis of cellulose, hemicelluloses and lignin, zeta potential, SEM, DRFTIR analysis, TG/DTA and XRD analysis, which suggest the introduction of nitrogen functional groups on to the *D. bipinnata* which were responsible for the quantitative sorption of the respective metal ions from waste water.

The pH studies for the respective metal ions indicated that there were no significant increases in sorption at low pH below 4. As the pH increased adsorption capacities also increased up to their optimum pH values, which were 6, 4, 6 and 5 for Cd, Pb, Zn and Cu respectively. Batch adsorption experiments were performed at their optimum pH to evaluate the loading capacities for the respective metal ions. FDB has excellent adsorption capacities. The maximum loading capacities were found to be 76.8 mg/g, 130.4 mg/g, 72.10 mg/g and 52.16 mg/g for Cd, Pb, Zn and Cu metal ion respectively which were functionalized with hydrazine monohydrate (FDB-H).

Kinetic studies were performed to find out the optimum contact time for the sorption of heavy metal ions onto the functionalized biopolymers. The sorption performances were fitted well with Langmuir model with correlation coefficient R^2 of 0.99 and the rate of sorption followed the pseudo second order kinetics.

The possible reaction mechanism involved onto functionalized biopolymers (FDB-H, FDB-Dm and FDB-E) for the sorption of metal ions were mainly carried through cheleation and also the combination of ion-exchange and electrostatic adsorption.

Adsorption/desorption studies concerning the recycling of functionalized biopolymers were carried out by batch experiment in six cycles to evaluate the efficiency, reusability , cost effectiveness and potential application in the waste water treatment. Three types of eluting reagents (0.1 M nitric acid, 0.1 M hydrochloric acid and 0.1 M sodium hydroxide) were used in this study. Among them 0.1 M nitric acid was found to be the most effective eluting agent releasing the metal ions sorbed on to functionalized biopolymer. The result revealed that above 90% metal ions were desorbed from the biopolymer. The sorption capacity remained relatively constant in subsequent cycles, indicating that the metal adsorbed biopolymer can be regenerated completely with 0.1 M nitric acid, and the biopolymer can be repeatedly used for metal ions sorption. The reuse of functionalized biopolymer indicates the cost effectiveness of the process. Based on the properties and maximum loading capacity for the tested metal ions, the functionalized DB can be an environment friendly, cost effective and efficient biopolymer for the removal of trace amount of heavy metal ions present in aqueous medium.

5.2 RECOMMENDATIONS

The safe and effective waste water treatment with heavy metal ions is always a challenge and dare need for the low cost, effective, efficient and environment friendly treatment techniques.

The legnocellulosic agricultural by-products can be an effective and economic alternative technique for the removal of heavy metal ions from waste water because they are easily available at local level with very low cost and high metal binding capacity. The raw agricultural by-products are cheap as compared to commercially available resins. Under this consideration, biosorption technique for the removal heavy metals using biowaste could be the innovative, economic, and environmental friendly technique, especially in the developing countries where cost is the main issue for the removal of heavy metal ions present in trace amount in the aqueous solution.

Although various techniques are employed in the waste water treatment contaminated with heavy metals. The selection of the most suitable treatment depends upon some basic parameters like pH, initial metal concentration, overall treatment performance compared to other technologies, environmental impacts as well as economic parameters like capital investment and operational costs.

Technical applicability, plant simplicity and cost- effectiveness are the key factors which play major roles in the selection of the most suitable treatment system for the effluents. The factors mentioned above should be taken into consideration while selecting the most effective and inexpensive treatment in order to protect the environment.

This study will also recommend ways to further promote biosorption technology and its application for the removal of heavy metal ions from waste water to acceptable level as provided by environmental law. Finally, the economic viability of producing FDB on an industrial scale needs to be evaluated and assessed more generally in the context of sustainability measures.

CHAPTER 6

6. SUMMARY

Water and soil pollution due to heavy metals is a major environmental problem with significant impact on human health and environment. Heavy metal discharge into water bodies is a major problem especially in developing countries where there are no strict rules for polluting environment. Rapid industrialization causes damage to environment if the waste is not properly treated before their discharge into water bodies. Expensive treatment methods are not acceptable for the people living in developing countries. Therefore biosorption is a potential alternative for the removal of trace level of metal ions from waste water.

The present study is focused on the development of efficient, economically acceptable and environment friendly biopolymers with excellent metal binding capacity to remove heavy metal ions from the waste water. In this study the Kush plant is used as a biopolymer. Kush is a legnocellulosic biomaterial containing cellulose, hemicelluloses and lignin as major components which has different types of functional groups that can bind metals. The raw Kush powder was functionalized with hydrazine monohydrates, dimethylamine and ethylenediamine to introduce nitrogen functional groups onto the surface of biopolymer which enhance the adsorption capacity for the removal of heavy metal ions from waste water.

The functionalized biopolymers were characterized with instrumental analysis, which reveal the successful introduction of Nitrogen functional groups on the surface of biopolymers.

The batch wise experiments were carried out as function of pH, initial concentration and contact time to find the optimum pH for metal ions, maximum loading capacity of the biopolymer and the optimum contact time for maximum sorption of metal ions.

Desorption and regeneration experiments were carried out to evaluate the efficiency and reusability of the biopolymers. Three different types of eluting reagents were used to desorbe the metal ions from biopolymers. Among them 0.1 M nitric acid was found to be most effective eluting agent.

The result revealed that above 90% metal ions were desorbed from the biopolymer. The sorption/desorption capacity remained relatively constant in subsequent cycles, and the biopolymer can be repeatedly use for metal ions sorption, thereby minimizing the cost for the sorption process.

On the basis of maximum loading capacity, contact time and desorption studies the research work suggests that Kush can be used as a potential biopolymer in the removal of heavy metal ions from aqueous medium and the biopolymer can be repeatedly use in adsorption desorption study which will help to minimize the cost for the preparation of biosorbent.

This study will also recommend ways to further promote biosorption technology and its application for the removal of heavy metal ions from waste water to acceptable level.

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ANNEX I

Photographs

Kush Plants

Sun drying of Kush Plants

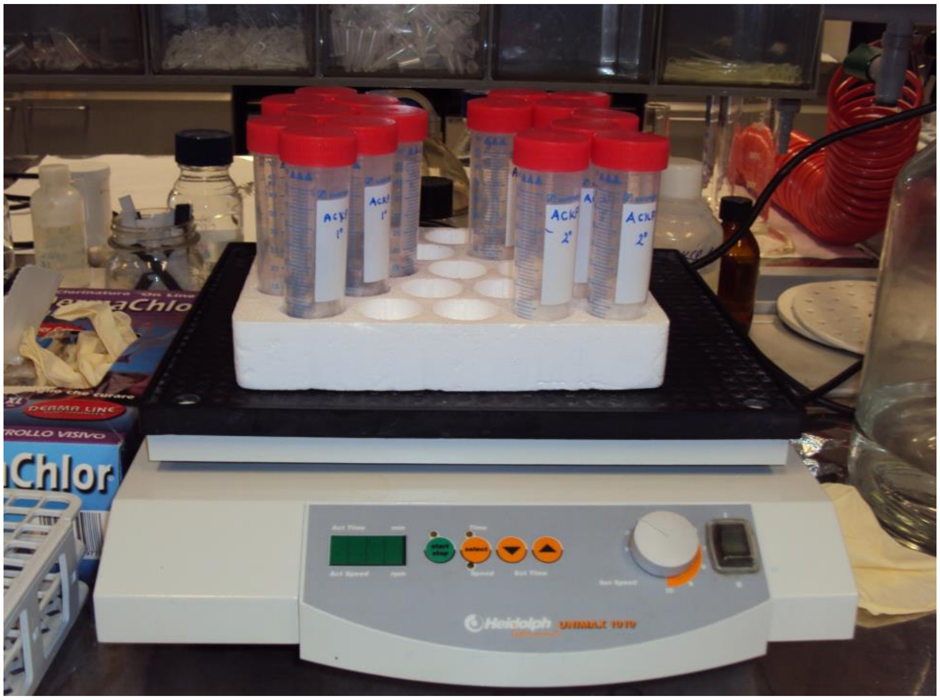
Grinding of Kush plants at CDC, Kirtipur

Seiving of Kush powder at CDC, Kirtipur

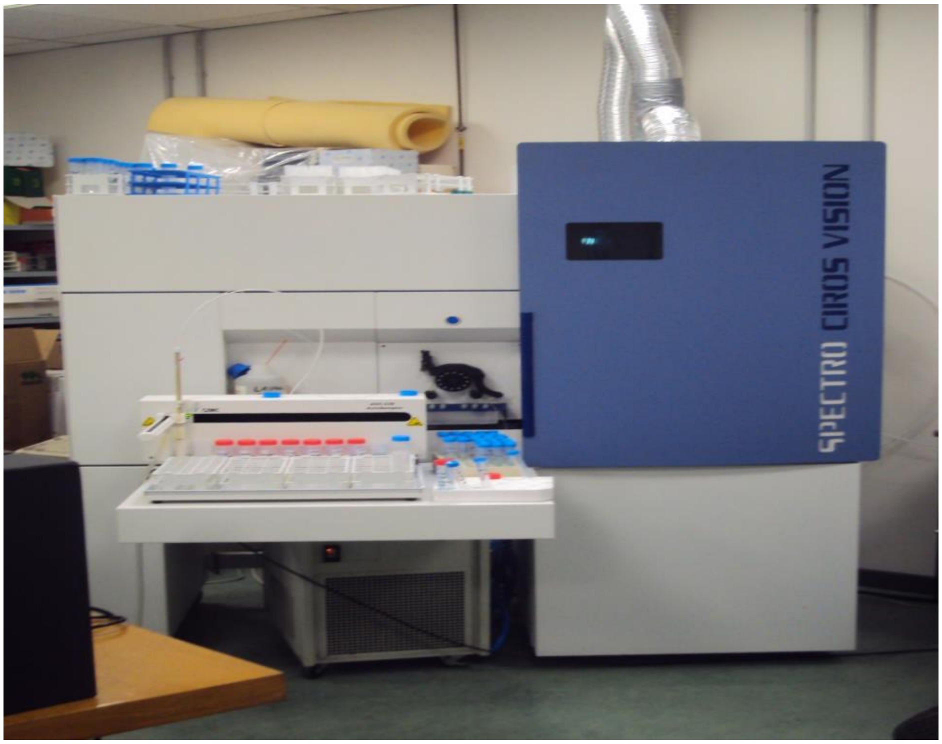
Sample preparation at CDC

Sample solution preparation at CDC

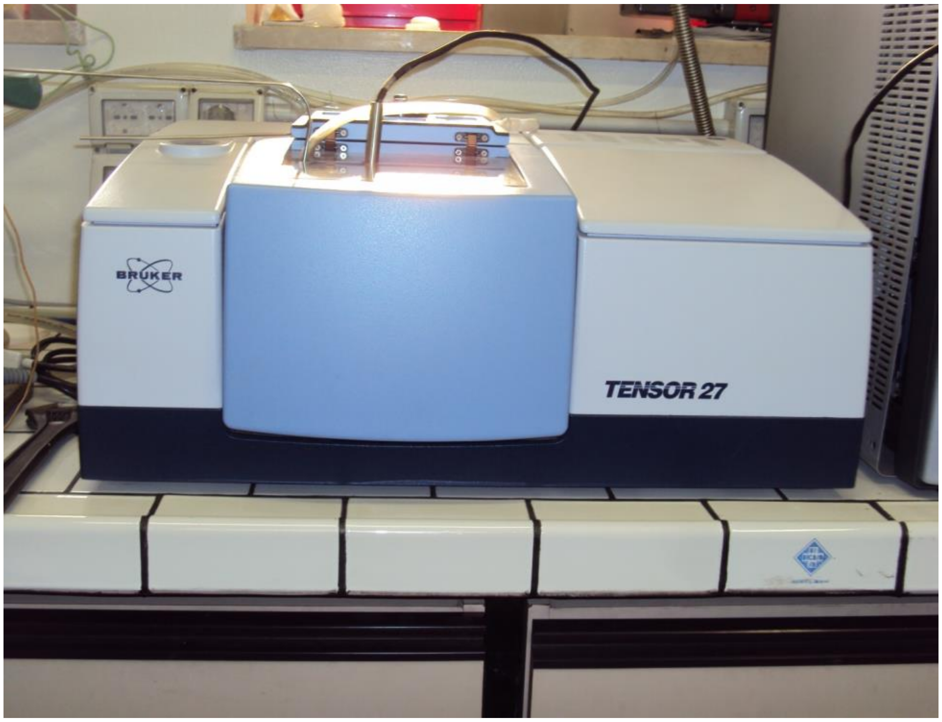
Batch Experiment at Padauva University, Italy



Samples on the shaker for Batch Experiment at Padova University, Italy



Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), Italy



Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRFTIR), Italy

ANNEX II

International Publications:

1. **Jagjit Kour**, Puspa Lal Homagai , Massimo Cagnin , Antonio Masi , Megh Raj Pokhrel and Kedar Nath Ghimire, **(2013).** Adsorption of Cd (II), Cu (II) and Zn (II) From Aqueous Solution onto Nitrogen Functionalized *Desmostachya bipinnata* , *Journal of Chemistry*, Vol. 2013, pp. 1-7.
   DOI.org/10.1155/2013/649142

2. **Jagjit Kour,** Shankar Pandey, Puspa Lal Homagai, Megh Raj Pokhrel and Kedar Nath Ghimire, **(2013).** Adsorptive Removal of Methyl Red from Aqueous Solution onto Charred and Aminated Sugarcane Waste , *Journal of Membrane and Separation Technology,* Vol.2, 63-73.
   E-ISSN: 1929-6037/13

3. Bindra Shrestha, **Jagjit Kour**, Puspa Lal Homagai, Megh Raj Pokhrel, Kedar Nath Ghimire, **(2013)**. Surface Modification of the Biowaste for Purification of Wastewater Contaminated with Toxic Heavy Metals – Lead and Cadmium, *Advances in Chemical Engineering and Science*, Vol.3, No.3, 178-184.
   DOI:10.4236/aces.2013.33022

National Publications:

1. **Jagjit Kour (2014),** Isotherm Study for Adsorption of Heavy Metal Ions from Aqueous Solution, *MECAS TIMES Research Journal*, Vol.2, No.2.

2. **Jagjit Kour (2014),** Water Pollution in Kathmandu Valley: An Overview, *MECAS TIMES Research Journal,* Vol.2, No.1, pp. 57-59

3. **Jagjit Kour (2014),** Study on Characterization of Biosorbent, *MECAS TIMES Research Journal,* Vol.1, No.1.

4. **Jagjit Kour (2014),** The Sacred Kush, *Patan Academic Journal*, Vol.2, No.1, pp. 10- 12.

5. **Jagjit Kour (2014),** Effective Removal of Heavy Metals from Functionalized *Desmostachya bipinnata***,** *Hamro Sampada National Monthly Journal*, Vol.13, No.11, pp.64-68.

6. **Jagjit Kour (2014),** Metal Toxicity, *Hamro Sampada National Monthly Journal*, Vol. 13, No. 9, pp.83-84.

7. **Jagjit Kour (2013),** Adsorptive Removal of Cu (II) Ions from Lignocellulosic Biomaterials, *Peace and Development Research Journal*, Vol.6, No.6.pp.24-30.

8. **Jagjit Kour,** A. Masi, M. Cagnin, P. L. Homagai, M. R. Pokharel and K.N. Ghimire, **(2012),** An Eco-friendly Alternative for the Removal of Cd (II) and Cu (II) from Aqueous Medium, *J. Nepal Chem. Soc,* Vol. 30, (2), pp 37-44.

9. **Jagjit Kour**, P. L. Homagai, M. R. Pokharel and K.N. Ghimire, **(2012),** Adsorptive Separation of Metal Ions with Surface Modified *Desmostachya bipinnata, Nepal Journal of Science and Technology*, NAST, Vol. 13 (1), pp. 101-106.

10. **Jagjit Kour**, Puspalal Homagai, Megha Raj Pokharel and K.N. Ghimire, **(2011)** , Kush- A Potential Biosorbent in the Removal of Cd (II) and Zn (II) from Aqueous Solution, *J. Nepal Chem. Soc,* Vol. 27, pp 107-114.

11. **Jagjit Kour**, **(2010),** Phyto-Chemical studies on Holy plant *Desmostachya bipinnata* (Kush) of Nepalese origin, *Academic View, Journal of Nepal University Teacher's Association,* Vol. 1. pp. 52-55.

Conference Attended and Country Visited:

1. International Conference on Advanced Materials and Nanotechnology for Sustainable Development-2014, November 4-6, Kathmandu, Nepal
2. Bangladesh Chemical Congress 2012, ***BCC-2012***, Dec.07-09, 2012, Dhaka, Bangladesh
3. The Sixth National Conference on Science and Technology, Sept. 25-27, 2012, Kathmandu, Nepal.
4. Kathmandu Symposia on Advanced Materials, ***KaSAM 2012***, May 09-12, 2012, Kathmandu, Nepal.
5. International Conference on Advanced Materials and Nanotechnology for Sustainable Development-2011, October 21-23, Kathmandu, Nepal.
6. World Forum on Advanced Materials ***Polychar 19*** International Conference-March 20-24, 2011, Kathmandu, Nepal.
7. Padova University, Italy, Under Bilateral Exchange Programme between Tribhuvan University, Nepal and Padova University, Italy (*Duration: 28/12/2010 to 8/02/2011*).