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Shalini Srivastava · Pritee Goyal

Novel Biomaterials

Decontamination of Toxic Metals from Wastewater

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Decontamination of Toxic Metals from Wastewater

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Preface

Comprising over 70% of the Earth's surface, water is undeniably the most valuable natural resource existing on our planet. Without this invaluable compound, life on Earth would be non-existent. The vitality of water is mandatory to all forms of life and fundamental for environmental health and management. Due to rapid development and industrialization in many countries, the levels of industrial pollution have been steadily rising. Hence, pollution arising from industrial wastewater is becoming more and more serious in the world. Consequently, the treatment of polluted industrial wastewater remains a topic of global concern since wastewater collected from municipalities, communities, and industries must ultimately be returned to receiving waters or to the land. Water pollution due to toxic heavy metals has been a major cause of concern for environmental engineers. Heavy metals are major pollutants in marine, ground, industrial, and even treated wastewater. Heavy metal pollution represents a significant environmental problem arising from its toxic effects and accumulation throughout the food chain. Lead, arsenic, cadmium, chromium, and nickel are examples of heavy metals/metalloids that have been classified as priority pollutants by the US Environmental Protection Agency. In view of their high toxicity, environment mobility, non-biodegradability, and stability, their removal becomes an absolute necessity. *World Health Organization has recently recognized the health hazards of toxic metals in food chain even at low concentrations*. Over the past few decades, the scientific community has been developing concentrated efforts for the treatment and removal of these toxicants in order to combat this problem. The removal of heavy metals from water at point of use, entry, and for water system is generally accomplished by conventional methods such as electrochemical processes and membrane processes; ion exchange and reverse osmosis are commonly applied to the treatment of industrial effluents. The aforesaid methods have major disadvantages such as high energy requirements, incomplete metal removal, and large quantities of toxic waste sludge that again needs safest disposal. *Research findings have clearly raised strong doubts about the use of conventional methods based on the use of synthetic coagulants for water purification*. Several serious drawbacks, viz., Alzheimer's disease, health problems, carcinogenic effects of alum lime, aluminum sulfate, polyaluminum chloride, polyaluminum silico sulfate, iron hydroxide, iron chloride, soda ash, synthetic polymers,

and the reduction in pH of water resulting from such treatments, have not been appreciated.

Therefore, the search for efficient, eco-friendly, and cost-effective remedies for wastewater treatment has been initiated. In recent years, research attention revolves around the trends of bringing technology into harmony with natural environment and to achieve the goals of protection of the ecosystem from the potentially deleterious effects of human activity. The new emerging terms like COME BACK TO NATURE, GRAY TO GREEN CHEMISTRY, SAFE CHEMISTRY, ENVIRONMENTALLY BENIGN PROCESSES, BENIGN BY DESIGN, ECO-FRIENDLY TECHNIQUES, and SUSTAINABILITY are the new principles guiding the development of next-generation processes and products.

Bioremediation involves processes that reduce overall treatment cost through the application of agricultural residues which are particularly attractive as they lessen reliance on imported water treatment chemicals, negligible transportation requirements, and offer genuine, localized, and appropriate solutions to water quality problems. Regeneration of the biosorbent further increases the cost-effectiveness of the process, thus warranting its future success. Research attention has been paid toward the use of *natural coagulants* to alleviate current problems of decontamination of water. Sorption using plant biomass has emerged as a potential alternative to chemical techniques for the removal and recovery of metal ions from aqueous solutions. A search for low-cost and easily available natural adsorbents for decontamination of metals has led to the investigations of materials of agriculture origin and has become an *area of sustained research*. A detailed survey of the research activities on biosorbents exhibiting potential for the decontamination of toxic metals from water bodies indicates that biomaterials are associated with drawbacks related to sorption efficiency and stability, restricting their commercial use. Sincere efforts toward structural modifications onto the biomaterials leading to the enhancement of binding capacity or selectivity are, therefore, in great demand. A special emphasis is to be paid on chemical modifications resulting in *tailored novel biomaterials* improving its sorption efficiency and environmental stability, making it liable for its commercial use as simple, fast, economical, eco-friendly green technologies for the removal of toxic metals from wastewater particularly for rural and remote areas of the country. The chemist community has to take initiative to tackle this difficult task dealing with environment-friendly issues and technologies for sustainable development of the environment. This book has been written keeping the above points in mind.

Although the bulk of the material is original, every effort has been made to acknowledge material drawn from other sources. We wish to place on record our appreciation to the director, Dayalbagh Educational Institute, Dayalbagh, Agra, for his encouragement extended to us. Our sincere gratitude goes to members of our research group, Prof. Satya Prakash, Prof. L.D. Khemani, Prof. M.M. Srivastav, Ms B.K. Ojeswi, Ms Menka Khoobchandani, Mr. Abhishek Kardam, and Mr. Kumar Rohit Raj, who helped us in the preparation of this book. Authors trust that their

apology will be accepted for any mistakes. The required changes will be included in a later printing or edition.

Agra, India — Dr. Shalini Srivastava
Greater Noida — Dr. Pritee Goyal

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Heavy Metals: Environmental Threat

Heavy metals are important among the toxic pollutants encountered in various ecosystems of the environment. The dissolved metals (particularly heavy metals) escaping into the environment pose a serious health hazard. These metals have been classified as priority pollutants by the US Environmental Protection Agency. Heavy metal pollution in the aquatic system has become a serious threat today and of great environmental concern as they are non-biodegradable and thus persistent. They accumulate in living tissues throughout the food chain which has humans at its top, multiplying the danger. Thus, it is necessary to control presence of heavy metals in the environment (Fig. 1).

From an environmental pollution point of view, metals may be classified into the following categories:

Non-toxic but accessible
Toxic but non-accessible
Toxic and accessible

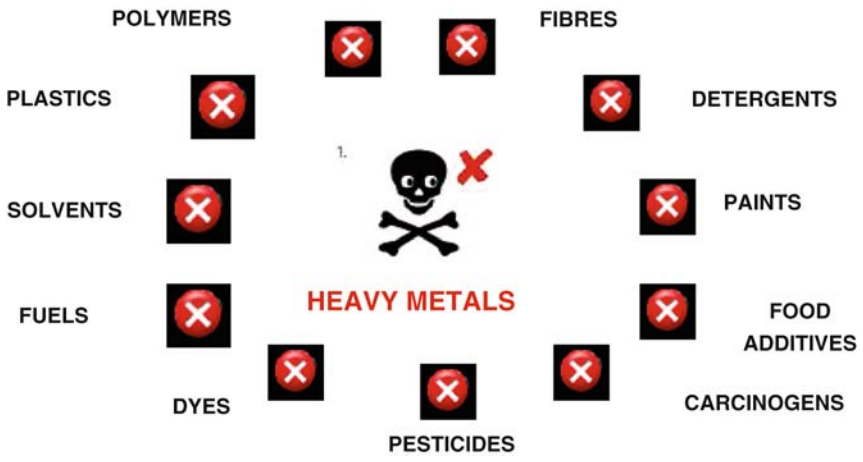

Fig. 1 Pollution: A prize tag of modern society

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It is the third category of potentially toxic and relatively accessible metals that has attracted the keen attention of the researchers. These toxic metals occur in very small quantities in the Earth's crust and hence are called rare metals. These are further arbitrarily subdivided on the basis of their densities. Those having densities less than 5 g/cm^3 are designated as light metals while those having densities more than 5 g/cm^3 are designated as heavy metals. Thus metals like Hg, Cd, Cr, Ni, and Pb are generally known as toxic heavy metals. Surprisingly, even metalloids like Se, As, and Sb are also considered under this category, thereby making the term heavy metals misleading. When considering the environmental impacts of metallic species, Pb, Cd, Cr, Ni, and As are in focus. They can enter the human beings via food chain and often exceed the toxic levels before they produce visible toxic effects. *World Health Organization* has, recently, recognized the health hazards of toxic metals in food chain even at low concentrations. Environmental Protection Agency's "Top 20 Hazardous Substance Priority List" has ranked mercury as 6th, cadmium 7th, chromium 8th, and nickel 13th, while quoting arsenic as the king of poisons.

The toxic and deleterious effects of metals are measured by its dose–response relationship, where response is the sign of an adverse effect (Fig. 2). The curve in Fig. 2 illustrates the relationship between amount and effect (response). Till date, it is debatable at what levels an effect can be considered as catastrophic. The situation continually changes as the sensitivity of measurements increases and very small effects are recognized.

Heavy metals have been reviewed thoroughly in a voluminous manner in the literature. Therefore, important features of target metals like their *physical and chemical properties, environmental sources, environmental concentrations*, and *toxicity* along with *permissible limit* have been presented in a concise manner.

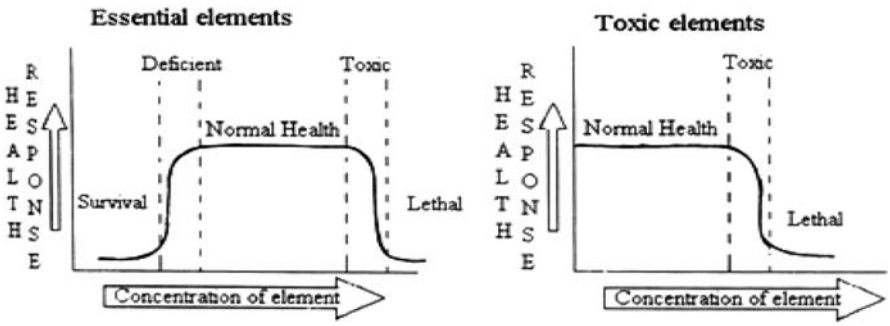
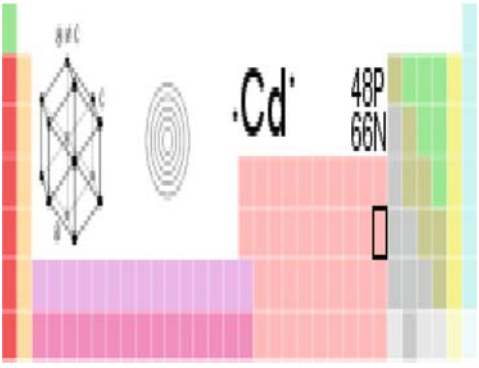


Fig. 2 The relationship between health response and the concentration of the elements – essential elements and toxic elements (Source: Adeloju and Bond, 1985)

Cadmium

Cadmium has been ranked as *seventh* in the Environmental Protection Agency's "Top Hazardous Substance Priority List."

Cadmium was discovered in Germany in 1817 by *Friedrich Strohmeyer*. Cadmium is odorless, tasteless, and chemical analysis is most often required to detect its presence. Remarkable characteristics of cadmium involve its great resistance to corrosion, low melting point, and excellent electrical conductivity because of which it plays a critical role in several cutting-edge technologies such as solar cells. Cadmium is one of the few elements that have no constructive purpose in the human body.



General Properties	
Symbol	Cd
Atomic number	48
Group, period, block	12,5,d
Electronic configuration	[Kr] $4d^{10}5s^2$
Appearance	Silvery grey metallic
Atomic Properties	
Crystal structure	Hexagonal
Electronegativity	1.69
Atomic radius	0.161 nm
Ionic radius	0.097 nm
Covalent radius	0.148 nm
Physical Properties	
Characteristics	Malleable and ductile
Phase	Solid
Density	8.65 g cm^{-3}
Chemical Properties	
Atomic mass	112.41 g/mol^{-1}
Melting Point	321 ^{0}C
Boiling Point	767 ^{0}C
Standard Potential	–0.402 V

Environmental Concentrations

Air

Rural area : 0.1–5 ng/m^3
Urban area : 2–15 ng/m^3
Industrial area : 15–50 ng/m^3

Water

Sea water : ~0.1 mg/L
Fresh water : 1–10 mg/L
Industrial water : 10–100 mg/L

Soil : 3–750 ppm

C A D M I U M

Environmental Sources

Natural

Weathering of rocks
Volcanic eruptions
Fall out

Anthropogenic

Ni-Cd batteries
Pigments
Coatings
Stabilizers
Fertilizers

Government Standards and Guidelines

The Environmental Protection Agency (EPA) allows 5 parts of cadmium per billion parts of drinking water (5 ppb).

The Food and Drug Administration (FDA) limits 15 parts of cadmium per million parts of food color (15 ppm).

The Occupational Safety and Health Administration (OSHA) limits workplace air to 100 micrograms (µg) cadmium per cubic meter (100 µg/m^3).

Itai-Itai disease

Health Effects

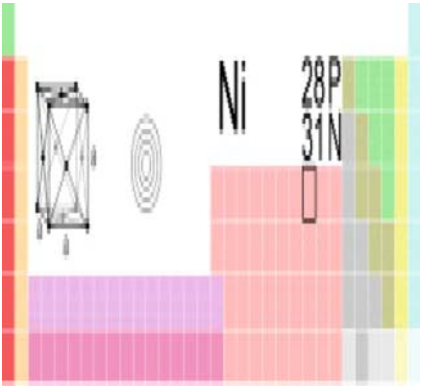
- Itai-Itai
- Muscle cramps
- Fragile bones
- Lungs and Kidney malfunctioning
- Liver injury
- Sensory disturbance
- Carcinogenicity
- Mutagenicity
- Affect cardiovascular system
- High blood pressure

Source: Agency for Toxic Substances and Disease Registry (ATSDR), 2007.

Nickel

Nickel has been ranked as *53rd* in the Environmental Protection Agency's "Top Hazardous Substance Priority List."

Nickel is the world's 24th most abundant transition metal. The element was discovered unintentionally in 1751 by *Baron Axel Frederick Cronstedt*, who extracted it from a mineral called *Niccolite*. Nickel can be combined with other elements such as iron, copper, chromium, and zinc to form alloys. These alloys are used to make coins, jewelry, and items such as valves and heat exchangers. Many nickel compounds dissolve fairly easily in water and have a green color. The most important oxidation state of nickel is +2.



General Properties	
Symbol	Ni
Atomic number	28
Group, period, block	10,4,d
Electronic configuration	[Ar] $3d^8$ $4s^2$
Appearance	silvery metallic
Atomic properties	
Crystal structure	Face centered cubic
Electronegativity	1.91
Atomic radius	.149 nm
Ionic radius	0.069 nm
Covalent radius	0.121 nm
Physical properties	
Characteristics	Lustrous and hard
Phase	Solid
Density	8.9 g cm^{-3}
Chemical properties	
Atomic mass	58.71 g/mol^{-1}
Melting Point	1453^{0}C
Boiling Point	2913^{0}C
Standard Potential	–0.25 V

Environmental Concentrations

Air

Electrolysis sector : 0.4 mg/m^3
Urban area : 17–25 mg/m^3
Industrial area : 120–170 mg/m^3

Water

Sea water : 0.1–20 mg/L
Drinking water : 0.7 mg/L
Industrial water : 1–100mg/L

Soils : 3–1000 ppm

N I C K E L

Environmental Sources

Natural

Weathering of rocks
Volcanic eruptions
Fall out, Meteorites

Anthropogenic

Roasting plants
Smelting plants
Electroplating
Steel industry
Batteries
Oil Hydrogenation

Government Standards and Guidelines

The Environmental Protection Agency (EPA) recommends that drinking water should not contain more than 0.7 milligrams of nickel per liter of water (0.7mg/L).

The Occupational Safety and Health Administration (OSHA) limits workplace air to 1 µg of nickel per cubic meter (1 µg/m^3).

Environmental Toxicity

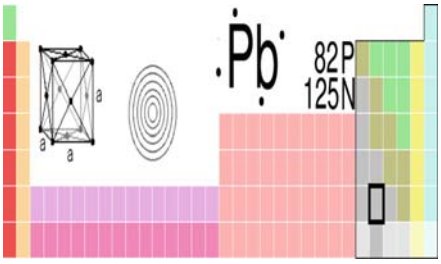
- Dental Prostheses
- Acute poisoning
- Dermatitis
- Asthma
- Respiratory cancer
- Malignant neoplasm
- Lung embolism
- Asthma and bronchitis
- Heart disorders

Source: Agency for Toxic Substances and Disease Registry (ATSDR), 2007.

Lead

> Lead has been ranked as *second* in the Environmental Protection Agency's "Top Hazardous Substance Priority List."

Metallic lead does occur in nature, but it is rare. Lead is usually found in *ore* with *zinc*, *silver*, and (most abundantly) *copper* and is extracted together with these metals. The main lead *mineral* is *galena* (PbS), which contains 86.6% lead. Lead has many *isotopes* but four stable ones. The four stable isotopes are ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb, with ^{204}Pb regarded as primordial Pb while 206, 207, and 208 are formed from the decay of U and Th.



General Properties	
Symbol	Pb
Atomic number	82
Group, period, block	14,6,p
Electronic configuration	[Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$
Appearance	Bluish gray
Atomic properties	
Crystal structure	Cubic face centered
Electronegativity	2.33
Atomic radius	0.180 nm
Ionic radius	0.069 nm
Covalent radius	0.147 nm
Physical properties	
Characteristics	Lustrous and soft
Phase	Solid
Density	11.34 g cm^{-3}
Chemical properties	
Atomic mass	207.20 g/mol^{-1}
Melting Point	327.46^{0}C
Boiling Point	1749^{0}C
Standard Potential	–0.25 V

Environmental Concentrations

Air

Rural area : 0.1-5 ng/m^3
Urban area : 2-15 ng/m^3
Industrial area : 15-50 ng/m^3

Water

Sea water : ~0.1 mg/L
Fresh water : 1-10 mg/L
Industrial water : 10-100 mg/L

Soil : 7–300 ppm

L E A D

Environmental Sources

Natural
Weathering of rocks
Volcanic eruptions
Fall out, Meteorites

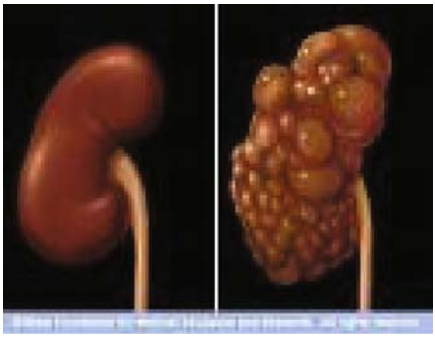
Anthropogenic
Lead-acid batteries,
bullets and shot
Fusible alloys
Paint pigments

Government Standards and Guidelines

The Environmental Protection Agency (EPA) allows 15 parts of lead per billion parts of drinking water (15 ppb).

The Food and Drug Administration (FDA) limits 5 parts of lead per billion parts of food color (5 ppb).

The Occupational Safety and Health Administration (OSHA) limits workplace air to 100 micrograms (µg) cadmium per cubic meter (100 µg/m^3).



Environmental Toxicity

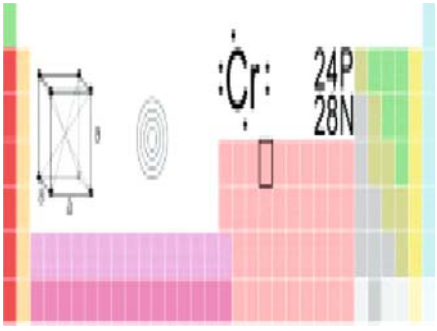
- Blood and brain disorders Nepheropathy
- Colic-like abdominal pains
- Cognitive deficits in children
- Muscle and joint pain Irritability
- Memory or concentration problems
- Swelling of Kidney
-

Source: Agency for Toxic Substances and Disease Registry (ATSDR), 2007.

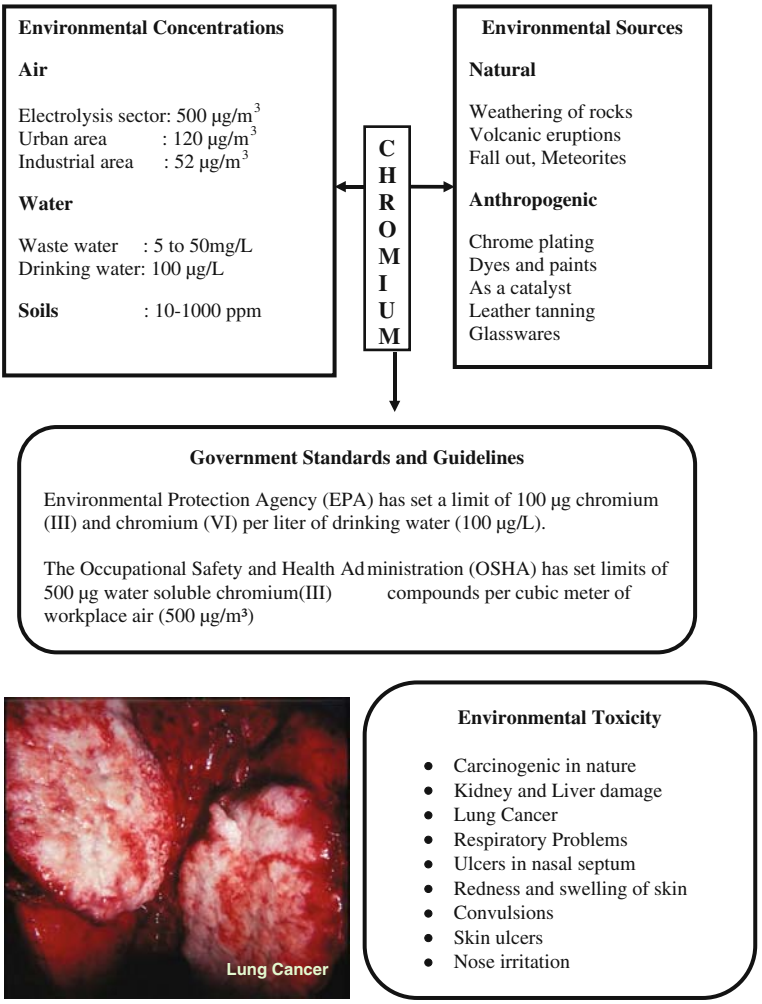
Chromium

Chromium has been ranked as *18th* in the Environmental Protection Agency's "Top Hazardous Substance Priority List."

Chromium was discovered by the French chemist *Nicholas Louis Vauquelin* in 1797. The most common oxidation states of chromium are +2, +3, and +6, with +3 being the most stable. The oxidation states +4 and +5 are relatively rare. Chromium compounds of +6 oxidation states are powerful oxidizing agents. Chrome metal (chromium 0) is the element that makes steel "stainless."



General Properties	
Symbol	Cr
Atomic number	24
Group, period, block	6,4,d
Electronic configuration	[Ar] $3d^4$ $4s^2$
Appearance	Silvery metallic
Atomic properties	
Crystal structure	cubic body centered
Electronegativity	1.6
Atomic radius	.166 nm
Ionic radius	0.061 nm
Covalent radius	0.127 nm
Physical properties	
Characteristics	Lustrous and brittle
Phase	Solid
Density	7.19 g cm^{-3}
Chemical properties	
Atomic mass	51.99 g/mol^{-1}
Melting Point	1907^0C
Boiling Point	2672^0C
Standard Potential	–0.402 V



Source: Agency for Toxic Substances and Disease Registry (ATSDR), 2007.

Detoxification of Metals – Biochelation

Several efforts have been made to detoxify the effect of metals once they are administered in the human body. Chelation is considered the best method used so far. Medicinal treatment of acute and chronic metal toxicity is provided by chelating agents. Chelation is one of the chemical functions that take place in the bodies of almost all living organisms. It is a process by which plants and animals utilize inorganic metals. Chlorophyll, the green matter of plants, is a chelate of magnesium. Hemoglobin, cytochrome C, catalase, and peroxidase are chelators of iron. A host of other metallo-enzymes could be used as examples involving chemical processes. Many of the successful drugs used in the treatment of disease are dependent on chelation processes for their effective therapeutic properties. Chelating agents are organic compounds capable of linking together metal ions to form complex ring-like structure called chelates. Chelate is derived from a Greek word meaning the claws of a lobster and somehow the chelators act in this way. Chelators form complex with the respective (toxic) ion. These complexes reveal a lower toxicity and are more easily eliminated from the body. This chapter focuses on the chemistry of these chelating agents and their pharmacological and toxicological properties. The beneficial and adverse effects including their limitations are briefly mentioned along with the recent developments to ameliorate the problems.

Chemistry of Chelation

Chemical affinity between a metal ion and a complexing agent can be best described based on the hard-soft-acid-base theory. According to the theory, metal ions could be classified into soft, hard, and borderline. Soft metal actions have a large atomic radius and a high number of electrons in the outer shell in contrast to the hard ions. Formation of metal complex involves that the metal actions coordinate or accept free electron pairs that are furnished by electron donor groups from a ligand. Therapeutic chelating agents make use of sulfur, nitrogen, or oxygen as electron donor atoms. The theory suggests that soft metals (sulfur seekers) form the most stable complexes with soft ligands, and hard metals (oxygen seekers) form stable complexes with hard ligands. The interaction between a chelating agent and a toxic metal can be

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expressed in terms of stability constants. Assuming the formation of the simple mononuclear complex only, the equilibrium concentration can be calculated from the law of mass action:

$$\text{Stability constant}, K = [\text{ML}]/[\text{M}][\text{L}]$$

M represents a metal while L indicates a ligand (chelating agent).

A metal with a higher stability constant reacts with the chelating agent with a lower stability value and removes the metal with a lower constant from the complex. Relative concentration also influences this release. Calcium which is readily available in the body binds to EDTA in large quantities if disodium EDTA is administered. An important property of metal complexes is the stereochemistry of the toxic metal ions. Chelating agents tie up all the coordination positions of a metal ion. It should be noted that metal chelating agents usually contain more than one functional group, in order to provide a chemical "claw" to chelate the toxic metal. The link formed between the metal and the chelating agent is of coordinate nature which is generally similar to covalent type but the major difference is that both electrons forming the link are supplied by the binding atom, the resulting compound is called metal complex or coordinate compound. A simplest example in this case is a link formed by proton (hydrogen atom with a positive charge). Besides hydrogen atom there are a number of other atoms which can take part to coordinate complex formations like sodium, magnesium, copper, zinc, and various transition elements such as manganese, iron, and cobalt. Chelation treatment can thus be more completely defined as an equilibrium reaction between a metal ion and a complexing agent, characterized by the formation of more than one bond between the metal ion and a molecule of complexing agent resulting in the formation of a heterocyclic ring structure incorporating the metal ion. Chelation thus is the incorporation of a metal ion into a heterocyclic ring structure.

Characteristics of a Chelating Agent

Ideally the chelating agent should possess the following characteristics:

- Strong affinity for the toxic metal to be chelated
- Ability to chelate with natural chelating groups found in biological system
- Low toxicity
- Ability to penetrate cell membrane to reach site of toxic metal deposit
- Minimal metabolism
- Rapid elimination of metal
- High water solubility

In a typical process, the toxic metal atom incorporates atoms into its coordination sphere which is part of essential molecule having a definite function to perform in the cell. Toxic metals usually do not react with just a single enzyme. They react

with a wide variety of molecules which contain appropriate donor atoms. A given toxic metal is found to have an effect on any part of an organism it reacts with and changes the reactivity of molecules which are critical for the normal functioning of the organism. The clinical use of chelation is to transform the toxic metal complex into a toxic metal complex with the administered chelating agent.

Chelating Agents and Their Properties

Calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$) is a derivative of ethylenediaminetetraacetic acid (EDTA), a synthetic polyamino carboxylic acid. It is a white crystalline solid with a molecular weight of 374.28 and empirical formula $C_{10}H_{21}CaN_2Na_2O_8$. It is a weak tetrabasic acid. This compound is also referred to in the literature as "edetate" and can be found in a variety of cation combinations including calcium, sodium, and zinc. The ability of this compound to form various high-affinity salts has made it useful as a chelator for a variety of metal intoxications.

Sodium 2,3-dimercaprol (BAL) was developed primarily as an antidote to lewisite during World War II and has become rapidly outmoded. The empirical formula of BAL is $C_3H_8OS_2$ and its molecular weight is 124.21. It is an oily, colorless liquid with a pungent, unpleasant smell typical of mercaptans.

Penicillamine (DPA) is D-β,β-dimethyl cysteine and in clinical practice only D-isomer is used although it can exist in two optical forms with dextro and levo configurations as well as in the racemic mixture DL; its empirical formula is $C_5H_{11}NO_2S$ and molecular weight is 149.21. It is available as a hydrochloride salt in capsule, which is a slightly hygroscopic crystalline powder, soluble in water and ethanol.

The one chemical derivative of dimercaprol, which has gained more and more attention these days, is meso 2,3-dimercaptosuccinic acid (DMSA). DMSA is an orally active chelating agent, much less toxic than BAL, and its therapeutic index is about 30 times higher. US FDA had approved this compound in 1991 for the treatment of children whose blood lead concentration was above 45 μg/dL. The empirical formula of DMSA is $C_4H_6O_4S_2$ and its molecular weight is 188.21. It is a weak acid soluble in water.

Sodium 2,3-dimercaptopropane sulfonate (DMPS) was first introduced in the Soviet Union in the 1950s as "Unithiol." Its empirical formula is $C_3H_7O_3S_3Na$ and molecular weight is 210.3.

Diethylenetriamine pentaacetic acid (DTPA) can bind atoms of plutonium and other actinides, thus forming a complex that is quickly excreted from the body. Both zinc and calcium salts of DTPA are generally administered; however, Ca-DTPA is more effective than Zn-DTPA. Its empirical formula is $C_{14}H_{23}N_2O_{10}$ and the molecular weight is 497.4.

Triethylene tetraamine (Trien) is an orally active chelating agent and is used primarily in the treatment of Wilson's disease when patients show immunological intolerance to DPA, the first drug of choice. Like DPA, this chelating agent also

interacts with divalent cations by increasing the excretion of trace elements in the urine, presumably in the chelate form.

Recently some mono and diesters of DMSA especially the higher analogous have been developed and tried against cases of experimental metal poisoning. Among these new chelators, monoesters are found to be effective than DMSA in reducing cadmium and mercury burden. Maximum metal mobilization occurred after treatment with MiADMSA, a C_5 branched chain, alkyl monoester of DMSA.

Deferoxamine is a chelating agent that is synthesized by the actinomycete *Streptomyces pilosus* to extract needed iron from its environment. It has a very high stability constant for Fe(III) and forms a water-soluble complex with iron(III) which is readily excreted in the urine. Its principal use is the control of iron overload in individuals suffering from thalassemia and who must receive blood transfusion during their lifetime. It is very expensive. Its empirical formula is $C_{25}H_{48}N_6O_8$ and molecular weight as a molecule of salt is 656.8.

Metal Detoxification by Chelating Agents

Arsenic

Arsenic compounds have many medical and industrial applications: arsenic-based drugs, dyes, alloys, semiconductors, and pesticides. Inorganic trivalent form of arsenic is regarded as more toxic than pentavalent arsenic. Arsenic was the first xenobiotic to which human liver disease was attributed. Its also affects cardiovascular system and nervous system. Blackfoot disease is also characterized by a progressive loss of circulation in hands and feet. High levels of arsenic may also be found in drinking water. The world's recent and perhaps the worst arsenic contamination of drinking water is in the Indo-Bangladesh region. According to an estimate around 38 million run the risk of poisoning. Chelation therapy has been the basis for the medical treatment of arsenic poisoning. They have been used clinically as antidotes for acute and chronic poisoning. 2,3-Dimercaprol (BAL) has long been the mainstay of chelation therapy of arsenic poisoning due to chemical warfare with gas (AsH_3). After almost 50 years of research we still do not have an effective antidote for arsenic which is almost 10 times more toxic than inorganic arsenic. It has generally been assumed that poisoning by arsenic and inorganic arsenic (III) in rats, mice, and guinea pigs all favor treatment with DMSA and DMPS. DMSA has been tried successfully in animals as well as in few cases of human arsenic poisoning. DMSA has been shown to protect mice due to lethal effects of arsenic. A subcutaneous injection of DMSA provided 80–100% survival of mice injected with sodium arsenite. A significant depletion of arsenic and a significant recovery in the altered biochemical variables of chronic arsenic-exposed rats has been observed. This drug can be effective if given either orally or through the i.p. route. Patients treated with 30 mg/kg DMSA per day for 5 days showed significant increase in arsenic excretion and a marked clinical improvement. It has been recommended that for treating mild

arsenic poisoning an oral dose of 10 mg/kg DMSA thrice a day for 5–7 days may be given followed by two daily doses of 10 mg/kg dose for another 10–14 days, while for severe arsenic poisoning an oral dose of 18 mg/kg thrice a day for the first 5–7 days followed by two doses of the same strength for the next 10–14 days is recommended. An interesting prospective, double blind, randomized controlled trial study conducted on few selected patients from the arsenic-affected West Bengal (India) regions with oral administrations of DMSA suggested that DMSA was not effective in producing any clinical or biochemical benefits or any histopathological improvements of skin lesions. An experimental study recently provided an in vivo evidence of arsenic-induced oxidative stress in a number of major organs of arsenic-exposed rats, and these effects can be mitigated by pharmacological intervention that encompasses combined treatment with *N*-acetyl cysteine and DMSA. DMPS, although known for its antidotal efficacy against mercury, too has been reported to be an effective drug for treating arsenic poisoning. This drug can be administered both orally and intravenously. An oral dose of 100 mg/kg thrice a day for 10–12 days is effective against mild arsenic poisoning while no recommendation for treating chronic arsenic poisoning is available. A maximal intravenous dose of 15 mg/kg DMPS in humans for the treatment of arsenic cardiomyopathy is recommended. In experimental animals i.p. administrations of DMPS increased the lethal dose of sodium arsenite in mice four folds. A quantitative evaluation of three drugs reveals that DMPS is 28 times more effective than BAL in arsenic therapy in mice, while DMSA and DMPS are equally effective.

Cadmium

Cadmium is a widely studied toxic metal and has widespread industrial use. Cigarette smoke is the most significant source of exposure to cadmium among the general population. Cadmium effluent has been linked to the outbreak of itai-itai disease in Japan. Cadmium has been shown to be toxic to numerous organs in rats: liver, kidney, testis, and spleen. Among these kidney is the organ critically affected on long-term exposure and the proximal tubuli are primarily affected. At a later stage irreversible glomerular effects occur. Cadmium was also recently designated a human carcinogen. Cadmium is also known for its ability to produce low molecular weight molecular-binding proteins known as "*Meta Rothionem,*" which is a cysteine-rich protein composed of 60 amino acid units.

Treatment of cadmium intoxification still remains a challenge to the toxicologists. The problem in cadmium chelation is special toxicokinetics, which makes its entry into the intracellular sites and then binds itself to MT. Cadmium bound to MT is slowly released into the blood and then gets deposited in proximal tubular cells of kidneys. In chronic exposure conditions, cadmium is mainly present in intracellular MT and there is very little extracellular cadmium in the body. Therefore an ideal chelating agent for treating cadmium should be able to reach inside the cell easily, chelate cadmium from MT, and increase its excretion without causing tissue damage or increase in the cadmium level of any organ. Large numbers of chelating

agents have been tried in experimental animals. Among all these chelating agents, DTPA was an efficient antidote under certain experimental conditions like injecting chelator and metal together. Oral administration of disodium salts of DTPA reduced intestinal absorption of cadmium and enhanced survival after oral exposure. Jones and his group have developed a number of new chelators capable of mobilizing cadmium. A large number of diethyldithiocarbamates were synthesized and evaluated for their ability to mobilize cadmium. Among the effective compounds are mono alkyl esters or mono alkyl amides of DMSA and amphipathic carbodithioates and *N*-(4-methoxy benzyl)-4-*O*-(D-galactopyranosyl)-D-glucamine carbodithioates. Although most of these new compounds including the most promising ones, monoisoamyl DMSA, are effective they invariably suffer from a number of side effects. Recently, a beneficial role of zinc supplementation during chelation of cadmium by DTPA in rats was reported. Thus search is still on to find a suitable, effective, and safe chelating agent for treating cases of cadmium intoxification. The implication for chelation therapy is important and requires careful examination.

Lead

Exposure to lead via inhalation may occur in certain occupational environments. The addition of alkyl lead compounds to gasoline is also a source of lead exposure via inhalation. The general population is mainly exposed to lead from food (either through the food items or through contamination of food stored in containers partly made of lead such as soldered cans) or pottery glazed or painted with lead. Lead released from water pipes containing lead may contaminate drinking water. Lead and its compounds primarily affect the central and peripheral nervous systems. Severe inorganic lead intoxication may lead to encephalopathy with symptoms such as ataxia, coma, and convulsion. Children are at particular risk to the neurotoxic effects of lead owing to their greater absorption of ingested lead, an immature blood–brain barrier, and a nervous system under development. Lead also inhibits the biosynthesis of heme at several points through interaction with key enzymes, particularly aminolevulinic acid dehydratase (ALAD). Lead has been shown to impair renal mitochondrial function and biosynthesis.

Historically, the drug of choice in treatment of lead toxicity is BDTA. $CaNa_2EDTA$ is recommended in monotherapy for the treatment of children with blood lead levels between 45 and 69 μg/dL. It is also recommended for use in combination with BAL for the treatment of blood lead levels higher than 70 μg/dL. The common practice of treating children with high lead exposure (blood lead levels of 70–100 μg/dL) with a combination of EDTA and BAL is believed to reduce mortality from about 30% to 1 or 2% and is more effective than the treatment with EDTA alone. Treatment with $CaNa_2EDTA$ requires hospitalization, with the resultant increased costs. This chelating agent is usually given by slow, intravenous infusion. Treatment is given for 5 days and after a rest period of 5–7 days, a

5-day treatment course may begin. For children the maximal daily dose is 75 mg/kg divided into two or three doses. The total dose of $CaNa_2EDTA$ per 5-day course should not exceed 500 mg/kg for treating lead poisoning.

US Food and Drug Administration has recently licensed the drug DMSA for reduction of blood lead levels. The recommended initial dose for treating lead poisoning in children is 30 mg/kg/day or 1,050 mg/kg for 5 days in three divided doses. After the initial 5 days the dose is reduced by one-third to 20 mg/kg/day in two divided doses for an additional 2 weeks. For the treatment of lead poisoning the recommended dose is 500 mg–1.5 g/day in four divided doses. The drug may be given for 5 days on an empty stomach. It was reported that EDTA increases the lead content in the brain due to redistribution. DMSA when administered either alone or in combination with EDTA decreases the lead concentration in the brain.

DMPS appeared to be effective at least in reducing the body lead burden. By the parenteral route, treatment of adults may be started with 250 mg DMPS (DMPS must not be mixed with other infusions or administered through intravenous route) and continued with 250 mg every 4 h on the first day. On the second day 250 mg may be given every 6 h. On the following days dosages should be adjusted to the clinical status and the results of the toxicological analysis. In children, 5 mg/kg per single dose should be given. Oral DMPS treatment in adults may be given with initial dose of 100–300 mg and continued with 100 mg every 6–8 h. In case of chronic intoxication 100 mg given three times a day may be sufficient. In children, the oral dosage is 5 mg/kg/day.

Mercury

Mercury and its salts have widespread industrial applications like mercury vapor lamps and electronic apparatus, in pesticides, mercury cells for caustic soda and chlorine production in dental amalgam, antifouling paints, batteries, and catalysts. The main form of mercury of environmental concern is methyl mercury. It is formed through methylation of inorganic mercury by microorganism in aqueous environments. The high concentration of mercury in certain species of fish is a public health concern among fish-eating communities. Primary risk groups are pregnant and nursing women owing to the susceptibility of the developing nervous system of fetus and infants to methyl mercury toxicity.

Human cases of mercury poisoning have been treated with chelating agents like BAL, DPA, DMSA, and DMPS. Due to low toxicity and high efficacy, DMSA and DMPS are currently favored compared to the traditional treatment with BAL followed by DPA administration. Several studies explore antidotes against acute mercury-reducing mercury deposits in various organs and increasing its excretion. The mono alkyl esters of DMSA have also been proved very effective in mobilizing mercury. Several such esters have been found to be more effective than DMSA and DMPS in reducing whole body mercury retention and increasing the urinary mercury excretion. Among these chelators, mono isoamyl DMSA ester has proved

the most effective. However, human uses of these compounds have not yet been undertaken and await vigorous clinical and experimental trials including safety testing. Recommended dose for DMSA has already been discussed above. In case of DMPS, it can be administered parenterally in adults with 250 mg (slow i.v. injection is recommended) and continued with 250 mg every 4 h on the first day. On the second day 250 mg may be given every 6 h. On the following days the doses may be adjusted to the clinical status. In children 5 mg/kg per single dose should be applied. Oral treatments in adults may be started with an initial dose of 100–300 mg and continued with 100 mg every 6 or 8 h; 100 mg every 4 or 2 h may be applied for oral continuation of therapy in severe cases. In case of chronic intoxication 100 mg given three times a day may be sufficient. In children the oral dose is 5 mg/kg/day.

Side Effects of Chelation Therapy and Possible Solutions

The most important problem concerning the medical use of chelating agents is their low therapeutic range, which is mainly due to the inherent toxicity of the chelator itself. Chelation is not specific to toxic ions and is causing disturbances of all biological processes depending on a physiological equilibrium of ions. In general, chelators with the extracellular activity are more toxic and have a lower therapeutic range than those distributed only in the extracellular spaces.

Most of the currently used chelating agents have serious adverse effects. $CaNa_2EDTA$ is a general chelating agent that complexes a wide variety of metal ions and is used clinically. $CaNa_2EDTA$ cannot pass through cellular membranes and therefore is restricted to removing metal ions from their metal complexes in the extracellular fluid. The principal toxic effect of $CaNa_2EDTA$ is on the kidneys. Renal toxicity may be related to the large amounts of chelated metals that pass through the renal tubules in a relatively short period during therapy. Monitoring of zinc status is also recommended during therapy because of massive diuresis of endogenous zinc. Addition of zinc during chelation with $CaNa_2EDTA$ has recently been proved beneficial in experimental animals. Other minor problems with the treatment of $CaNa_2EDTA$ include malaise, fatigue, anorexia, nausea and vomiting, sneezing, glycosuria, anemia, and hypotension. Intramuscular $CaNa_2EDTA$ is also painful. BAL is the first chelating agent to be successfully introduced into clinical practice as an antagonist for cases of acute and chronic metal intoxication. It is also the most toxic of the agents approved for clinical use. One of the consistent responses to dimercaprol is a rise in systolic and diastolic arterial pressures accompanied by tachycardia. Other serious side effects include headache, nausea, vomiting, rhinorrhea, abdominal pain, anxiety, and unrest. Dimercaprol is contraindicated in patients with hepatic insufficiency.

The main disadvantage of treatment with the pencillamine as a chelating agent is that it might cause anaphylactic reaction in patients allergic to penicillin. Prolonged

use of penicillamine may induce several cutaneous lesions, dermatomyositis, adverse effects on collagen, dryness, etc. The hematological effects include leukopenia, aplastic anemia, and agranulocytosis. Renal toxicity is usually as reversible proteinuria while elevation in liver enzymes indicates hepatotoxicity. Children undergoing D-penicillamine therapy should be monitored with blood counts, urinalysis, and serum creatinine every 2–4 weeks.

In comparison to other chelating drugs, treatment with DMSA and DMPS has got less adverse effects. Few adverse effects include mild and transient elevation of SOFT and SGOT, mild abdominal disturbances, and skin reaction possibly of allergic origin.

Recently a number of strategies have been suggested to minimize numerous problems. One of the important solutions could be the use of an adjuvant, viz., essential metals, vitamins, and amino acids. In the first, the effects of zinc and copper supplementation on the safety and efficacy of $CaNa_2EDTA$ in the treatment of lead and cadmium poisoning in experimental animals have been examined. However, it was also suggested that higher dose and prolonged administration of zinc should be avoided.

In the second approach, efficacy of some naturally occurring compounds like vitamin and amino acid were tried both as potential chelating agents and as an adjuvant to conventional chelating agents during experimental lead intoxification. Concomitant administration of thiamine, ascorbic acid, or methionine during experimental lead intoxification has been proved beneficial. A somewhat different class of compounds, the esters of dimercaptosuccinic acid, has also been investigated as agents to mobilize lead from aged intracellular deposits. The efficacy of *S*-adenosyl L-methionine in the prophylaxis of lead poisoning has been demonstrated in both humans and animals. Methionine being a ready source of sulfhydryl group would increase the bioavailability of glutathione, which would provide additional complexing sites for lead poisoning.

Much of the current interest in chelating agents stems from concern about the possible beneficial effects of removal of toxic metals in people with low-level exposure without overt symptoms of toxicity. It is now well established that low-level exposure of lead in early childhood may impair cognitive and behavioral development, with lifetime accumulation of cadmium in liver and kidneys associated with renal tubular dysfunction and hypercalciuria in later life. More recently there have been assertions that mercury vapor released from dental amalgams might be responsible for a spectrum of chronic health problems. Chelation on therapy has long been the only method for reducing body burden of metals resulting from genetic disorders of metal metabolism such as copper accumulation in Wilson's disease, cystine crystal formation in cystinuria, and removal of tissue iron in hemochromatosis. There is still a need for increased research efforts to provide a better understanding of what chelation therapy does and does not do and to identify its risks. In most cases it is not difficult to demonstrate increased excretion of the metal, but in few instances has the clinical efficacy of the treatment been demonstrated with any scientific rigor. Although there may be evidence for the ability of a particular agent to enhance excretion of the metal in question, there is paucity of evidence

that any of the uses of chelation therapy reverses toxicity at the cellular level or prevents progression of the pathology produced by the accumulated metal. Further, there has not been sufficient basic research to elucidate the cell effects and mechanisms of action and effects of the chelating agents on the biokinetics of toxic metals.

Metal Decontamination: Techniques Used So Far

The constantly increasing degree of industrialization and rising standards of living are strongly impacting on the use of available water sources. Controlling heavy metal discharges and removing toxic heavy metals from aqueous solutions have become a challenge for the twenty-first century. The commonly used procedures for removing metal ions from aqueous streams include distillation, ion exchange, reverse osmosis, electrodialysis, precipitation, coagulation, flocculation, and nanofiltration. The basic principle, procedural details, and commercially available instrumentation based on the above phenomenon are described in brief below.

Distillation

Distillation is probably the oldest method of water purification. Water is first heated to boiling. The water vapor rises to a condenser where cooling lowers the temperature so the vapor is condensed, collected, and stored. It removes a broad range of contaminants.

Evaporation

Evaporation is the most common and cost-effective technique for recovery of heavy metals. Evaporation can provide total as well as partial recovery of metals. The application of atmosphere evaporation is used on a wide variety of process effluents from industries.

Chemical Precipitation

Chemical treatment process prior to biological process is widely applicable in the treatment of raw wastewaters. Moreover, various existing conventional primary wastewater plants are shifting toward chemically enhanced treatment to improve

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the quality of the treated effluent and reduce cost. Chemical precipitation is incorporated by raising the pH of wastewater by addition of alkaline chemicals, viz., lime, limestone, caustic soda, soda, ash, and magnesium hydroxide; at alkaline pH most heavy metal sizes range between 0.1 and 100 μm precipitated as metal hydroxides or metal carbonates and separated by gravity clarification or field separation methods.

Flocculation and Coagulation

Flocculation is a process that clarifies the water. Clarification is done by causing a precipitate to form in the water which can be removed using simple physical methods. For water treatment plants using surface water as the source water, coagulation–flocculation is the most commonly used physicochemical process for particle removal and is an essential pretreatment process for sedimentation and filtration (Fig. 1). The three main types of chemical coagulants are (i) inorganic electrolyte, e.g., alum, lime, ferric chloride, anhydrous sulfate; (ii) organic polymers; and (iii) synthetic polyelectrolytes with anionic and cationic functional groups which are often used for precipitation. On addition of coagulant, flocculation occurs and the size of the particle in floc increases by aggregation and settles at a faster rate. Soluble impurities in water can also be partially removed by coagulation. Because the complete removal of impurities requires the separation of aggregates from water treatments, colloidal systems of extremely slow settling velocity are viewed as stabilized systems. The coagulation process in water treatment includes three sequential steps (Fig. 2).

Fig. 1 Coagulation

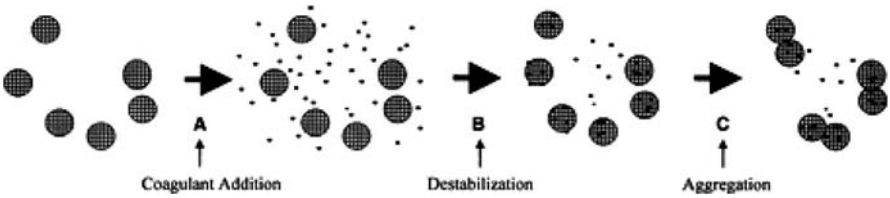

Fig. 2 Schematic illustration of coagulation–flocculation: (**a**) addition of coagulant, (**b**) destabilization of particles, (**c**) aggregation of destabilized particles

Electrocoagulation

Electrocoagulation is based on the formation of the coagulant as the sacrificial anode corrodes due to an applied current while the simultaneous evolution of hydrogen at the cathode allows the pollutant removal by floatation. Chemical reaction occurring in the electrocoagulation process shows that the main reaction occurs at the electrodes which are as follows:

$$\mathrm{Al} \leftrightarrow \mathrm{Al}^{+3} + 3\mathrm{e}^{-}$$
$$2\mathrm{H_2O} + 3\mathrm{e}^{-} \leftrightarrow 3/2\,\mathrm{H_2} + 3\,\mathrm{OH}^{-}$$

In addition, Al^{+3} and OH^{-} ions generated at electrode surfaces react in the bulk media wastewater to form aluminum hydroxide [$Al(OH)_3$] flocs which act as adsorbents for metal ions and eliminate them from the wastewater. The hydroxyl ions which are produced at the cathode increase the pH in the electrolyte bulk liquid and may include co-precipitation of metals in the form of their corresponding hydroxides (Fig. 3). This acts synergistically to remove pollutants from wastewater.

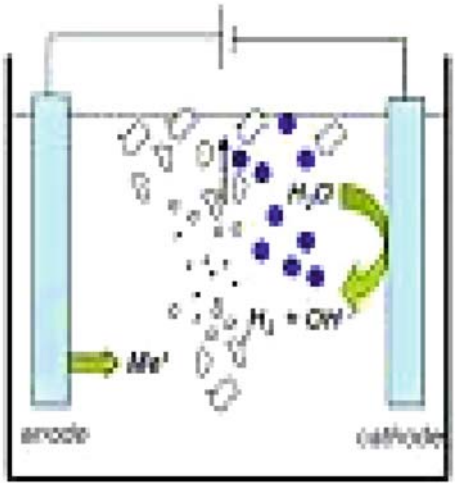

Fig. 3 Electrocoagulation

The efficiency of electrocoagulation is dependent on pH, current density, and metal ion concentration.

Ion Exchange

Ion exchange processes in water treatment have been used primarily for softening. Some of them are used to deionize, disinfect, or scavenge macromolecules from water. Typical ion exchangers are ion exchange resins (functionalized porous or gel polymer), zeolites, montmorillonite, clay, soil humus, and synthetically produced organic resins. The synthetic organic resins are frequently used today because their characteristics can be tailored to specific applications.

Ion exchange is an exchange of *ions* between two *electrolytes* or between an electrolyte *solution* and a *complex* (Fig. 4). In most cases the term is used to denote the processes of purification, separation, decontamination of aqueous and other ion-containing solutions with solid *polymeric* or *mineralic* ion exchangers. Ion exchangers are either cation exchangers that exchange positively charged ions (cations) or anion exchangers that exchange negatively charged ions (anions). There are also amphoteric exchangers that are able to exchange both cations and anions simultaneously. However, the simultaneous exchange of cations and anions can be more efficiently performed in mixed beds that contain a mixture of anion and cation exchange resins or passing the treated solution through several different ion exchange materials.

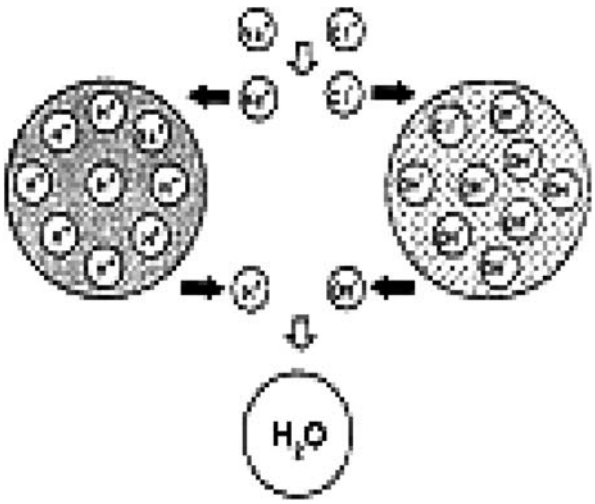

Fig. 4 Ion exchange process

Ion exchangers can be unselective or have binding preferences for certain ions or classes of ions depending on their chemical structure. This can be dependent on the size of the ions, their charge, or their structure.

The commercially available resins used in ion exchange technology for wastewater treatment are listed in Table 1.

Table 1 List of commercially available ion exchange resins

Commercially available ion exchange resins	Activity	Company
Cesium ion exchange resin (CPU)	For liquid tank waste removal	Northwest Laboratories, UK
Resorcinol-formaldehyde ion exchange resin (ReFlX)	For radioactive waste removal	AlChem Laboratories, USA
Macroporous resin (A62MP)	For nitrate removal	Nytrox systems, UK

Membrane Process

A membrane is best defined as a material through which one type of substance can pass more readily than others.

Ultrafiltration

Ultrafiltration (UF) is a type of *membrane filtration* in which hydrostatic pressure forces a liquid against a *semi-permeable membrane*. Suspended solids and solutes of high molecular weight are retained while water and low molecular weight solutes pass through the membrane (Fig. 5). This *separation process* is used in industry and research for purifying and concentrating macromolecular (10^3–10^6 Da) solutions, especially protein solutions. Ultrafiltration is not fundamentally different from *reverse osmosis*, *microfiltration*, or *nanofiltration*, except in terms of the size of the molecules it retains.

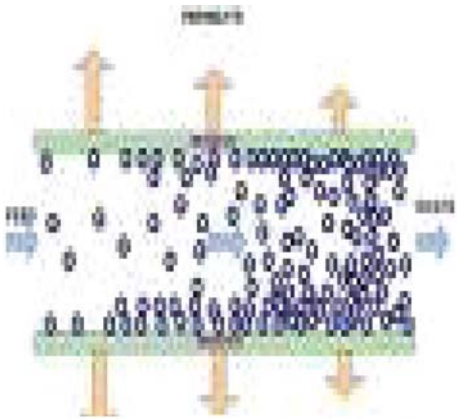

Fig. 5 Ultrafiltration

The UF process is applicable for particles in the molecular range of 0.1–0.01 μm. Ultrafiltration (UF) is a pressure-driven, membrane filtration process that is used to separate and concentrate macromolecules and colloids from wastewater. A fluid is placed under pressure on one side of a perforated membrane of a measured pore size. All materials smaller than the measured pore size pass through the membrane, leaving large contaminants concentrated on the feed side of the membrane. UF is used as a pretreatment step to reverse osmosis (RO) or as a stand-alone process. The UF process cannot separate constituents from water as effectively as RO. However, the two technologies can be used in tandem, with UF removing most of the relatively large constituents of a process stream before RO application selectively removes water from the remaining mixture.

Reverse Osmosis

Reverse osmosis (RO) fills a unique position in the area of water and wastewater treatment. Reverse osmosis (RO) is the most economical method of removing 90–99% of all contaminants. The pore structure of RO membranes is much tighter than UF membranes. RO membranes are capable of rejecting practically all particles, bacteria, and organics of molecular weight >300 Da.

Natural osmosis occurs when solutions with two different concentrations are separated by a semi-permeable membrane (Fig. 6). In water purification systems,

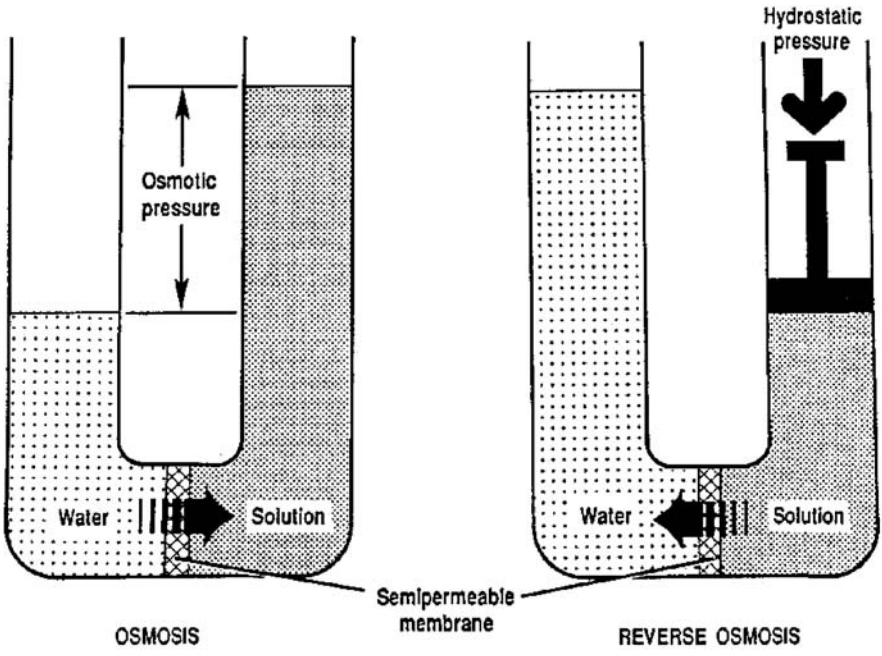

Fig. 6 Reverse osmosis

hydraulic pressure is applied to the concentrated solution to counteract the osmotic pressure. Pure water is driven from the concentrated solution and collected downstream of the membrane. Because RO membranes are very restrictive, they yield very slow flow rates. RO also involves an ionic exclusion process. Only solvent is allowed to pass through the semi-permeable RO membrane, while virtually all ions and dissolved molecules are retained (including salts and sugars). The semi-permeable membrane rejects salts (ions) by a charge phenomena action: the greater the charge, the greater the rejection. Therefore, the membrane rejects nearly all (>99%) strongly ionized polyvalent ions but only 95% of the weakly ionized monovalent ions like sodium (Table 2).

Table 2 List of commercially available reverse osmosis membranes

Commercially available reverse osmosis membranes	Activity company	Reverse osmosis membranes
Cellulose acetate membranes	For salt removal	Biosource Inc., Worcester, UK
Cellulose triacetate membranes	For bacterial removal	Miox Corp., Albuquerque
Thin film composite membranes	For silica removal	Miox Corp., Albuquerque

Electrodialysis

Electrodialysis (ED) is used to transport *salt ions* from one *solution* through *ion-exchange membranes* to another solution under the influence of an applied *electric potential* difference. The membranes are cation or anion selective, which basically means that either positive ions or negative ions will flow through. Cation-selective membranes are polyelectrolytes with negatively charged matter, which reject negatively charged ions and allow positively charged ions to flow through. This is done in a configuration called an electrodialysis cell. The cell consists of a feed (dilute) compartment and a concentrate (brine) compartment formed by an *anion* exchange membrane and a *cation* exchange membrane placed between two *electrodes* (Fig. 7). In almost all practical electrodialysis processes, multiple electrodialysis cells are arranged into a configuration called an electrodialysis stack, with alternating anion and cation exchange membranes forming the multiple electrodialysis cells. Inside an electrodialysis unit, the solutions are separated by alternately arranged anion exchange membranes, permeable only for anions, and cation exchange membranes, permeable only for cations. By this, two kinds of compartments are formed, distinguishing in the membrane type facing the cathode's direction. Applying a current, cations within the dilute move toward the cathode passing the cation exchange membrane facing this side and anions move toward the anode passing the anion exchange membrane. A further transport of these ions, now being in a chamber of the concentrate, is stopped by the respective next membrane. Electrodialysis processes are unique compared to *distillation* techniques and other membrane-based processes in that dissolved species are moved away from the feed stream rather than the reverse.

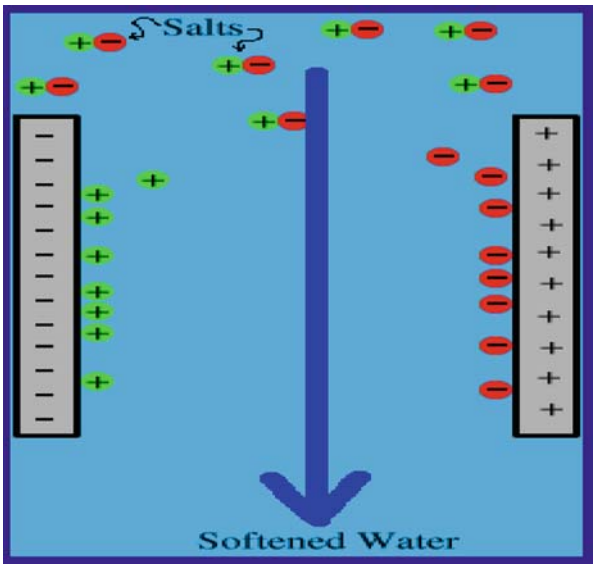

Fig. 7 Electrodialysis

Because the quantity of dissolved species in the feed stream is far less than that of the fluid, electrodialysis offers the practical advantage of much higher feed recovery in many applications.

Nanofiltration

Nanofiltration is a relatively recent membrane *filtration* process used most often with low *total dissolved solids* water such as *surface water* and fresh *groundwater*, with the purpose of softening (*polyvalent cation* removal) and removal of disinfection by-product precursors such as natural *organic* matter and synthetic organic matter. Nanofiltration is also becoming more widely used in *food processing* applications such as *dairy* for simultaneous concentration and partial (*monovalent ion*) demineralization.

Nanofiltration is a pressure-driven separation process. The filtration process takes place on a selective separation layer formed by an organic semi-permeable membrane. The driving force of the separation process is the pressure difference between the feed (retentate) and the filtrate (permeate) side at the separation layer of the membrane. However, because of its selectivity, one or several components of a dissolved mixture are retained by the membrane despite the driving force, while water and substances with a molecular weight <200 Da are able to permeate the semi-permeable separation layer. Because nanofiltration membranes also have selectivity for the charge of the dissolved components, monovalent ions will pass the membrane and divalent and multivalent ions will be rejected.

A nanofiltration filter has a pore size around 0.001 μm. Nanofiltration removes most organic molecules, nearly all viruses, most of the natural organic matter, and a range of salts. Nanofiltration removes divalent ions, which make water hard, so nanofiltration is often used to soften hard water. Heavy metal separation by nanofiltration takes place through combination of charge rejection, solubility diffusion, and sieving. Copper and cadmium were reported to be removed successfully from industrial wastewater by nanofiltration technology. Efficient removal of about 90% and 82–97% of Cu^{+2} and Cd^{+2}, respectively, was observed from wastewater with initial concentration ranging between 25 and 2,000 mg/L (Fig. 8).

Fig. 8 Nanofiltration

Existing Metal Removal Technologies: Demerits

The currently practiced technologies for removal of pollutants from industrial effluents appear to be inadequate, often creating secondary problems with metal-bearing sludge, which are extremely difficult to dispose of. The process cannot precipitate metals to low levels of solubility unless additional treatment reagents are employed, the use of which may significantly add to the volume of sludge. Most authorities consider such treatments to be best performed in specialized environments by trained personnel. Further, research findings have clearly raised strong doubts about the advisability of the use of synthetic coagulants used for metal removal. Their usage leads to several serious demerits concerned with the central nervous system of human beings. For electroplating and finishing industries, the cost involved in the treatment of effluent produced is sometimes prohibitively expensive, especially for the smaller installations, and far outweighs the advantages of recycling and regenerating materials. Present treatment strategies require costly chemical and physical operations and involve a high degree of maintenance and supervision. While wastewater treatments by ion exchange resins are both effective and convenient, they are too expensive to be used by the developing countries and their availability is limited to developed nations of the world. Government closely monitors their handling and disposal, in most instances. Processes like reverse osmosis include many of the small organic molecules that are precursors of *trihalomethane* generated during chlorine disinfection. The *Environmental Protection Agency* (EPA) strictly limits the allowable amounts of these compounds in drinking water based on the possibility that chronic exposure to them could cause cancer.

- Need spent resin replacement
- Pretreatment is required
- Membrane clogging leads to sludge problem
- Need periodic replacement of filters
- Large amounts of water wastage
- Need regular cleaning
- Expensive
- Not recommended for higher concentrations
- Need special handling techniques
- Result in large amounts of sludge

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Hyperaccumulation: A Phytoremedial Approach

The prime requisite of agriculture is soil which serves as a reservoir of nutrients and water for the crops. Unfortunately, all the soil available on this planet is not arable, fertile, and it remains agriculturally unproductive. Land is mainly contaminated with heavy metals like Zn, Pb, Cr, Ni, and Cd. Metal-rich mine tailings, metal smelting, electroplating, gas exhausts, fuel production, downwash from power lines, intensive agriculture, and sludge dumping are the most important human activities that contaminate soils with large quantities of toxic metals. Metals are non-biodegradable and have long biological half-life. Remediation of metals from soil and water has been found to be a difficult and expensive goal. The remediation of heavy metal-contaminated soils can be achieved through various physical and chemical conventional remediation measures. Due to ever-growing number of toxic metal-contaminated sites, the commonly used methods dealing with heavy metal pollution are either extremely costly processes or simple isolation of the contaminated sites. The remediation of large volumes of contaminated sites by conventional technologies has been proved to be very expensive. It has been estimated that the cost of conventional remediation of heavy metal-contaminated sites in the USA alone would exceed $7 billion.

Agricultural plants represent an important pathway for the movement of potentially toxic waste material from source to human beings. A number of wild plants that grow on metal-contaminated sites accumulate large amounts of heavy metals in their plant parts. This property of plants has been given much attention and is being used for reclamation. These tolerance mechanisms could be possibly exploited to remove heavy metal pollutants. Recently the value of metal-accumulating plants for environmental remediation has been fully realized, giving birth to a new eco-friendly technology. The use of plants in environmental remediation has been given various terms like *GREEN REMEDIATION, PHYTOREMEDIATION, BOTANICAL REMEDIATION, HYPERACCUMULATION, and BIOCONCENTRATION*. The development of phytoremediation is based on the earlier science of bio-indicator plants, biogeochemical prospecting, and genetic metal tolerance by plants. Use of plants for cleaning its own support system offers a cheap, renewable, and promising method. Phytoremediation of metal-contaminated sites offers a lower cost method for remediation. The cost of growing a crop is minimal compared to

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those of replacement. Some extracted metals may be recycled for their different values. Phytoremediation involves the use of plants to make contaminant sites also non-toxic. The process of phytoremediation uses one basic concept that plants take the pollutant through the roots. The pollutants can be stored (phytoextraction), volatilized (phytovolatilization), and metabolized by the plant in order to reduce the bioavailability of the heavy metals from soil solution or a situation in which metals become non-mobile and relatively less toxic in the soil environment (phytostabilization).

The present discussion primarily focuses on the phytoextraction aspect of phytoremediation. The term phytoextraction illustrates the potential of growing unusual metal hyper accumulating crops on contaminated soils to remove some metals and provide biomass and an ash, which can be recycled to reduce the cost of remediation. Crops can be harvested as a biomass crop which is air-dried and burned in a biomass power generator. Ash is a high-grade ore, different from the traditional metal ores of commerce. Thus hyperaccumulation by plants offers a new cost-effective approach for remediation. For various metals like Zn and Cd, the value of biomass energy and metal for recycling is found to be a profitable opportunity.

Which Plant?

It remains a debatable question whether hyperaccumulator plants can remove enough metal to decontaminate sites by using simple farming technology, making hay from the biomass, and recycling the metals. Scientists have long wondered how evolutionary processes selected hyperaccumulator plants. The idea of using rare plants which hyperaccumulate metals to selectively remove and recycle excessive metals was introduced by Chaney in 1983 which gained public exposure in 1990. Brooks and Reeves (1997) coined the word *hyperaccumulator* for some plant species. Since then plant hyperaccumulation has been examined in the agricultural–environmental research community as a potential practical and more cost-effective technology for decontamination of metals. The majority of current research in the phytoremediation field revolves around determining which plant works most efficiently in a given application. Not all plant species will metabolize, volatilize, and accumulate pollutants in the same manner. The goal is to ascertain which plants are most effective at remediating a given pollutant. Research has yielded some general guidelines for phytoextraction of plants. The plant must grow quickly and consume large quantities of water in a short time. A good plant would be able to remediate more than one pollutant because pollution rarely occurs as a single compound. There are various expectations from hyperaccumulators. They should have high accumulation rate, ability to accumulate very high levels of the contaminant, ability to accumulate several heavy metals, high biomass production, and resistance to diseases and pests.

A small number of plant species have been identified that not only are capable of growing on metal-contaminated sites but also accumulate metals in high concentration levels. Ernst and Brooks (1974) are credited with bringing this

Table 1 List of plants and pollutants they remediate

Plant	Chemicals
Arabidopsis	Mercury
Bladder campion	Zinc, copper
Brassica family (Indian mustard and broccoli)	Selenium, sulfur, lead, chromium, nickel, zinc, copper, cesium, strontium, cadmium
Buxaceae (boxwood)	Nickel
Compositae family	Cesium, strontium
Euphorbiaceae	Nickel
Tomato plant	Lead, zinc, copper
Trees in the Populus genus (poplar, cottonwood)	Pesticide, atrazine, trichloroethylene, carbon tetrachloride, nitrogen compounds, 2,4,6,-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5 triazine (RDX)
Pennycress	Zinc, cadmium
Sunflower	Cesium, strontium, uranium
Genus Lemna (duckweed)	Explosive wastes
Parrot feather	Explosive wastes
Pondweed, arrowroot, coontail	TNT, RDX

information to a wider research community. Plants have been designated for their hyperaccumulating ability for specific metals ranging from 10 to 100 times higher than normal concentrations in plants. Hyperaccumulators are defined as plants that contain more than 1,000 mg/g of Co, Cu, Cr, Pb, and Ni. Hyperaccumulator species are more clearly defined as those whose leaves contain more than 100 mg/kg Cd or Ni or Cu while >10,000 mg/kg Zn and Mn on dry weight. Plant species, which accumulate over 1% metal, have been called, in general, *Hypermetallophores* for different metals like hypernickelophore, hyperzincophore, hypercadmiophore (Table 1).

Chaney et al. (1983) suggested the possibility of the use of some plant species such as *Arenaria patula* and *Alyssum bertolonii* as hyperaccumulator for phytoextraction of metals from contaminated sites. Baker and Brooks (1989) reported that some *Brassica* species could accumulate unusually high concentrations of toxic metals. *B. juncea* has the highest metal-accumulating ability among the other species tested. This ability may be inherited from some of the wild species in Brassicaceae which grow in metal-rich sites and accumulate large amounts of metals (Table 2). Plant species of Brassicaceae accumulate Pb in roots and its transportation to the shoots (108.3 mg of Pb/mg in the roots and 34.5 mg of Pb/mg in the shoots on dry weight basis are noticed). *B. juncea* has been reported to concentrate Cr^{6+}, Cd, Ni, Zn, and Cu from a substrate containing sulfates and phosphates as fertilizers (Fig. 1). Lee and Chen (1992) considered growing some cut flowers to phytoextract Cd from contaminated sites and the flowers are sold not posing any Cd risk to the purchasers. *B. juncea* is known as phytoaccumulator and provides a method for the removal of metals from contaminated water, sewage, and groundwater (Baker et al. 1994).

Table 2 Lead content of roots and shoots of the plants of Brassicaceae family

Plant species	Mg of Pb/g DW	Weight ± SE
B. juncea (L.)	10.3 ± 2.9	103.5 ± 12.3
B. nigra (L.)	9.4 ± 2.5	106.6 ± 10.7
B. campestris (L.)	7.2 ± 2.2	103.4 ± 7.7
B. carinata (A.)	4.6 ± 2.6	108.9 ± 13.9
B. napus (L.)	3.4 ± 1.0	61.2 ± 11.9
B. oleracea (L.)	0.6 ± 0.2	52.7 ± 3.8

Fig. 1 *B. juncea* (Indian mustard) cadmium hyperaccumulators from contaminated water

Fig. 2 *Thlaspi caerulescens*, a small herb, accumulates large amounts of zinc and cadmium

Other such plant species are *Thlaspi caerulescens* (Fig. 2) and *Alpine pennycress* (Fig. 3) which are known as Zn and Cd hyperaccumulators.

Hydroponically grown *T. caerulescens* plants have been shown to accumulate up to 33,600 mg Zn/kg and 1,140 mg Cd/kg on dryweight basis in shoots from a

Fig. 3 *Alpine pennycress*, a small herb, accumulates large amounts of zinc and cadmium

Table 3 Hyperaccumulation of toxic metal in leaves of *Thlaspi* species

Plant species	Element	Metal levels
T. calaminare	Zn	39,600
T. caerulescens	Cd	1,800
Aeollanthus biformifolius	Cu	13,700
Phyllanthus serpentinus	Ni	38,100
Haumaniastrum robertii	Co	10,200
Astragalus racemosus	Se	14,900
A. racemosus	Mn	11,500

solution containing 650 mg Zn/L and 22 mg Cd/L (Brown et al. 1994, 1995). *T. caerulescens* shows much greater accumulation and tolerance to both Zn and Cd than the other plant species studied (Table 3). *T. rotundifolium* has been reported to hyperaccumulate Pb from contaminated soil.

Hyperaccumulation and Hypertolerance

Hyperaccumulation and hypertolerance of heavy metals by plants are fascinating areas of research with possible important commercial applications. The unique "metal accumulation" and "metal resistance" genes of metal-accumulating plants may directly benefit world agriculture. Combining improved cultivation of these accumulator plants, agronomic management practices to maximize yield and burning the biomass to generate power and recovery of metals from the ash appear to offer an economic technology compared to conventional removal methods. Hyperaccumulation, hypertolerance, and high biomass are important characteristics of phytoremediation (Table 4). The ability to hyperaccumulate and hypertolerate

Table 4 List of plant products having remediation possibilities

Plants	Chemicals	Limits
Pondweed	TNT and RDX	0.016–0.019 mg of TNT/L/day and 0.133–0.291 mg RDX/L/day
Poplars	Nitrates from fertilizers	
Pumpkin vines	Lead	45% of the excess was removed
Halophytes	Salts	Reduced the salt level in the soil by 65%
Pennycress	Hydrocarbons	From TPH concentrations greater than 100 ppm to TPH concentrations less than 10 ppm in less than a year
Poplar trees	Atrazine	Lab: 91% of the atrazine was taken up in 10 days
Indian mustard seedlings	Lead(II), strontium(II), cadmium(II), nickel(II), cesium(I), chromium(IV)	Lab: Concentration in the plant was 100 times the concentration in solution
Sugarbeet cell cultures	Nitroglycerine (GTN)	Lab: From 1.8 mM GTN to undetectable levels in 20 h

the metals is of greater importance than high biomass. The hypertolerance of metals is the key plant characteristic required for hyperaccumulation. Vacuolar compartmentalization among the plant part appears to be the source of hypertolerance of natural hyperaccumulation of plants. It is suggested that yield of a hyperaccumulating crop would be two orders of magnitude higher than that of normal crop. Pot and field studies show that some perennial species grown as a crop can attain as high as 5 t ha^{-1} before breeding to increase the combination of yield and shoot metal concentration (Brown et al. 1995).

Recycling of shoot metals, in commerce, may provide value for the ash from metal hyperaccumulators such that there is no need to pay attention for safe disposal. Comparing biomass ash containing 20–40% Zn for *T. caerulescens* and only 0.5% for *Zea mays,* the former is considered to be a rich ore, while the latter is a phytotoxic waste, requiring disposal. Increase in the yield of a crop could give a linear increase in phytoremediation capacity. But increasing from normal tolerance to hypertolerance increases the potentially important annual removal of the soil contaminant 25–400 fold. Thus, the importance of the domestication of metal hyperaccumulator plants and breeding of improved cultivars has been emphasized. For phytoremediation to be effective, it is necessary to delineate patterns of uptake and limits of tolerance of potential plant species. It is also important to define accumulation patterns at low concentrations of soil metals and to determine phytoextraction coefficient. Phytoextraction coefficient is the ratio between microgram of metal/gram dry weight of shoot and microgram of metal/gram on dry weight basis.

Mechanisms of Hyperaccumulation

Today, research attention has been focused toward the characterization of the mechanisms used by hyperaccumulators to accumulate, translocate, and hypertolerate the metals. Eventually, the cloning and use of the genes to convert high biomass agronomic plants into special phytoremediation cultivars is required (Chaney et al. 1995). Extensive progress has been made in characterizing soil chemistry management which is also needed for phytoremediation. The physiological aspects of plants which hyperaccumulate and hypertolerate metals are equally important. Research has identified a number of soil management practices, which may affect the efficiency of phytoextraction of elements. However, different elements may be affected differently by various environmental factors. Different models have been put forward for the development of practical phytoextraction systems with the prime requisition that a plant must have the ability to translocate an element from roots to shoots at high rates and must be able to tolerate high levels of the elements. Studies have been conducted to evaluate the transfer of both essential and non-essential elements in soil to plants (Cataldo 1988). Source to plant transfer (SPT) processes have been characterized as complex phenomena occurring in multistep processes of adsorption, dissolution, diffusion, and other convection movements (McBride 1994). Adsorption, desorption, and precipitation reactions directly affect the partitioning of trace elements between solid and aqueous phase while complexation and oxidation–reduction affect metal bioavailability. Soil-applied chelators dissolve metals based on the activity of soils and the selectivity of metal-binding capacity of the chelator. Non-target elements can be dissolved and leach down soil profiles, if fields are irrigated. The goal of developing transgenic plants with increased metal-binding capacity is to keep metals in plant roots and thus reduce metal movement to the food chain.

Kramer et al. (1996) found that the chemical forms of Ni found in extracts of leaves of *Alyssum* hyperaccumulators are the chelates with maleate, citrate, and histidine in the xylem exudates. Robinson et al. (1999) tested NTA and EDTA with *Berkheya coddii*, a South African accumulator of Ni. The special property of Indian mustard, which allowed high accumulation of Pb, might be due to its affinity toward EDTA, a complexing agent which does not commonly occur in other species. Chaney (1999) has shown that if one applies strong chelating agents such as EDTA, Pb can be dissolved and the root membranes weakened enough to promote uptake of PbEDTA with the water moving into roots. Transpiration carries the PbEDTA from source into plant shoots. When high Pb levels are reached, the crop plants tested so far are not tolerant of the accumulated Pb, and the growth ceases. *Poaceae* species are observed to secrete mugineic acid, a chelating agent known as phytosiderophore, to solubilize Fe (Nomoto 1996). Raskin (1996) suggested that transgenic plants could be developed to secrete metal-selective ligands into the rhizosphere, which could specifically solubilize elements for phytoremediation. Regulatory control of *phytosiderophore* secretion in barley was induced by Fe deficiency, but not Mn, Zn, and Cu deficiency in contrast to other reports, which indicated that Zn deficiency also induced the biosynthesis and secretion of phytosiderophores.

Metal–organic acid complexation in the soil plant system resulting in the formation of organically bound metal, enhancing its plant accumulation, provides an important dimension to the mechanism of hyperaccumulation of metals. Organic acid released in the rhizosphere has been implicated in several mechanisms for altering the level of ions and the molecules within the rhizosphere. Organic acids being negatively charged species are likely to interact with metal ions forming chelates under a wide range of aqueous and soil conditions (Jones and Darrah 1994). Metals chelated, in aqueous solution, in general, are formed by displacement of one or more weak protons of chelating agent by metallic ion. When a metal and ligands combine, the resulting complex possesses a net charge that can be positive, negative, or neutral (White et al. 1981). Low molecular weight organic ligands (carboxylic and amino acids) have been designated important *phytochelators,* converting metal ions into its organically bound form which is available to the plants (Stevenson and Ardakani 1972). The chelated metal compounds are found to be more soluble than inorganic precipitate and have different rate of mobilization. Srivastava and Prakash (1998, 1999) have pointed out the role of organic acids in the mobilization of metals with special reference to their hyperaccumulation. In a series of pot culture (hydroponic, sand, and soil) experiments, they have highlighted the existence of metal–organic acid interaction in the soil plant system modifying the chemical nature of metals and its subsequent increased uptake in the plants (Srivastava and Sonal 1998, 2000). Hyperaccumulation of metals in the plants has been explained on the basis of the tendency of metals to interact with organic ligands (carboxylic and amino acids), resulting in the formation of organically bound metals which are soluble, mobile, and therefore become plant available (Srivastava and Nigam 2000–2001). Mench and Martin (1991) are of the same opinion and state that metal-solubilizing ability of organic acids is parallel to their metal-binding ability, which in turn is correlated with their dissociation constants. Amino acids are reported to have poor metal-mobilizing capacity compared to carboxylic acids. Lower value of dissociation constant of amino acids implies their low binding or solubilizing ability compared to organic acids.

Transfer of genes through breeding of plants is an onerous task. The most promising approach would be to engineer fast-growing, high biomass-producing crops, especially trees (owing to their large size and extensive root system), to become hyperaccumulators for a wide range of heavy metals. For commercial environmental remediation, which a society can afford, attempts have been made to engineer the plants for accumulating heavy metals in high concentrations and improving the annual rate of phytoextraction. Genetic engineering is used to develop new plants for phytoremediation. The metal hyperaccumulator genes required could be cloned and transferred to high-yielding crops using a direct gene transfer technique involving the combination of yield and metal concentration. One successful example is transfer of microbial mercuric reduction genes to higher plants, which allow plants to reduce mercuric ion to mercury metal which can be evaporated and thus reduce the risk. Methylated mercury is the dangerous from of Hg in the environment. It is lipophilic and biomagnified especially in the aquatic food chains. Plants with both methyl mercury hydrolase and mercuric ion reductase have been developed (Rugh

et al. 1996). Expression in plants of the hmt 1 vacuolar pump for Cd-PCs from fission yeast (Ortiz et al. 1995) has not yet been successful. The modification of gene sequences may be required before its effectiveness can be tested. The expression of MT as the whole protein or the Cd binding domain part of the protein, or a fusion protein with glucuronidase, increased Cd tolerance of plants, but has little effect on Cd transport to shoots (Brandle et al. 1993; Pan et al. 1994). The use of the improved 35S2 promoter has increased the ability of MT to keep Cd in roots (Elmayan 1994). Tests have not yet progressed to field studies, which must be the important measure of success.

A Canadian group has demonstrated that poplar trees genetically engineered with a bacterial *mercuric reductase* gene are capable of removing and vaporizing mercury from contaminated sites. A gene which encodes mercuric ion reductase reduces highly toxic ionic mercury to much less toxic volatile elemental mercury. Yong and his associates were successful in overexpressing *E. coli* gsh 11 gene encoding *glutathione synthetase* in *B. juncea* (Indian mustard) which is most suitable for phytoremediation due to its rapid biomass production. As a result of this overexpression the Cd concentration was 40–90% higher in the transgenic shoots compared to untransformed control shoots. *Arabidopsis thaliana* and wheat are known to contain genes which code for phytochelation. But the expression of this gene is not high in the shoots of all metal-accumulating plants. There is a need to regulate phytochelatin synthase genes so that they are expressed at higher levels in the shoots and leaves. It would prove to be a better approach as it is easier to harvest plant parts above the ground. Attempts were made to engineer the *bacterial mer*, a gene in yellow poplar trees, which release elemental mercury approximately 10 times the rate of untransformed plants. This opened the path for embarking upon the task of reducing the metals via enzymatic process which helps to phytoremediate toxic metals like copper, lead, and chromium. Planting genes from efficient hyperaccumulators into the taller plants like *Thalspi* plants is more advisable which can act as better bioaccumulators because of their long heights.

In general, we can view various advantages and disadvantages of phytoremediation as follows:

Advantages

- Aesthetically pleasing.
- Solar driven.
- Works with metals and slightly hydrophobic compounds, including many organics.
- Stimulate bioremediation in the sites closely associated with the plant root.
- Plants can stimulate microorganisms through the release of nutrients and the transport of oxygen to their roots.
- Relatively inexpensive.
- Even if the plants are contaminated and unusable, the resulting ash is meaningful.

- Having "ground cover on" property reduces exposure risk.
- Planting vegetation on a site also reduces erosion by wind and water.

Disadvantages

- Takes many growing seasons to clean up a site.
- Plants have short roots and they can clean up soil or groundwater near the surface in situ, typically 3–6 ft but cannot remediate deep aquifers without further design work.
- Trees have longer roots and can clean up slightly deeper contamination than plants, typically 10–15 ft, but cannot remediate deep aquifers without further design work.
- Tree's roots grow in the capillary fringe but do not extent deep into the aquifer.
- Plants that absorb toxic materials may contaminate the food chain.
- Vitalization of compounds can transform a groundwater pollution problem to an air pollution problem.
- Returning the water to the earth after aquaculture must be permitted.
- Less efficient for hydrophobic contaminants, which bind tightly to soil.

Phytoremediation, although still in its infancy, may one day become an established environmental cleanup technology. The development of phytoextraction requires an integrated multidisciplinary research effort that combines plant biology, soil chemistry, soil microbiology, and agricultural and environmental engineering. It is possible that accumulated toxic metals may be grown and harvested economically, leaving the soil or water with a greatly reduced level of toxic metal contamination. The use of plants in environmental cleanup may guarantee a greener and cleaner planet for all of us. This method of metal remediation allows effective and persistent remediation at low cost and should be applied for dispersal of the contaminated material at many locations. The commercial strategy of phytoextraction is to concentrate metals from low-grade ores or mine or smelter wastes and the sale of the ash as an alternative metal concentrate. Phytoextraction is more applicable to soils or ores that cannot be economically enriched by traditional mining. Both agronomic management practices and plant genetic abilities need to be optimized to develop commercially useful practices. It is believed that the use of metal hyperaccumulator species would give much lower costs for remediation of metal-contaminated sites than possible with the engineering alternatives. Many more labs are joining this important area of research and more and more new genes are being identified globally to tackle the issue of environmental cleanup. Several agriculturally important crop species still await incorporation of such genes not only for cleaning up the heavy metals but also to extend the area under cultivation. In other words there is need to achieve horizontal growth by planting such genetically engineered heavy metal-tolerant food crops in contaminated sites.

Biosorption: A Promising Green Approach

Unfortunately, the science particularly chemistry, despite numerous contributions to the well-being and progress of humanity, has been blamed for the present ills of the world. In fact, it is not chemistry or science or technology but our past mistakes of increasing only the production without considering the simultaneous generation of large amounts of side products or waste which have underlined us as the culprit. Basically unscientific and careless rapid urbanization, industrialization, and agriculturalization are major threats to the environment. It is not the need of poor but the greed of rich nation, which has been the main cause of environmental degradation of the world.

Chemists, since 1990, have started addressing complicated environmental issues in a safe and an economically profitable manner under various names like Clean Chemistry, Environmentally Benign Chemistry, Sustainable Chemistry, Come Back to Nature, Gray to Green Chemistry, Green Technologies, Eco-friendly Techniques, Green Processes, and more popularly Green Chemistry. Green Chemistry is a special contribution of chemists to the conditions for sustainable development, incorporating an environmentally benign design approach to all aspects of chemical industry. The word Green Chemistry was coined jointly by Prof. Paul T. Anastas and Prof. John C. Warner, which means "The invention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances."

To combine technology with environmental safety is one of the key challenges of the new millennium. There is a global trend of bringing technology into harmony with the natural environment, thus aiming to achieve the goals of protection of ecosystem from the potentially deleterious effects of human activity and finally improving its quality. The magic plants are around and waiting to be discovered and commercialized. They are now recognized and accepted as storehouses of infinite and limitless benefits to human beings. These natural systems are often referred to as *Green Technologies* as they involve naturally occurring plant materials. *Biosorption* is one such important phenomenon, which is based on one of the 12 principles of Green Chemistry, i.e., "Use of renewable resources."

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Prof. Paul T. Anastas

Prof. John C. Warner

Prof. Paul T. Anastas (Green Chemistry Institute, American Chemical Society, Washington) and Prof. John C. Warner (University of Massachusetts, Washington) have given 12 principles of Green Chemistry.

Twelve Principles of Green Chemistry

- Design for energy efficiency
- Less hazardous chemical synthesis
- Use of renewable feedstocks
- Atom economy
- Safer chemistry
- Safer solvents and auxiliaries
- Designing safer chemicals
- Use of selective catalyst
- Reduced derivatives
- Design for degradation
- Prevention is better than cure
- Real-time analysis for pollution prevention

A great deal of attention has been garnered in recent years due to rise in environmental awareness and consequent severity of legislation regarding the removal of toxic metal ions from wastewater. It can occur in both plant and microbial species.

It was only in the late 1990s that a new science, biosorption, that could help to remove and recover heavy metals came into existence. It can efficiently and effectively sequester dissolved metals out of dilute complex solutions with high efficiency and also quickly. Biosorption can be defined as "a non-directed physicochemical interaction that may occur between metal/radionuclide species and microbial cells." Natural materials that are available in large quantities or certain waste from agricultural operations may have potential to be used as low-cost adsorbents, as they represent unused resources that are widely available and

environmentally friendly (Kratochvil and Volesky 1998). Availability is a major factor to be taken into account to select biomass for clean-up purposes (Volesky and Holan 1995). Some biosorbents can bind and remove a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metals. When choosing the biomass for metal biosorption experiments, its origin is a major factor to be taken into account (Keith et al. 1979).

Biomass can come from

- ⋆ industrial waste, which should be free of charge.
- ⋆ plants easily available in large amounts in nature and of quick growth.
- ⋆ agricultural waste products.
- ⋆ organisms of quick growth, especially cultivated for biosorption process.

The biosorption process involves a solid phase (biosorbent) and a liquid phase (solvent, normally water) containing dissolved species to be sorbed (sorbate, metal ions). Due to 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 to the sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between solid and liquid phases. In biosorption, the use of non-living biomaterials containing metal-binding compounds would have the advantage of not requiring tremendous care and maintenance as well as being useful in remediating toxic high levels of contaminants that would otherwise kill live systems (Basso et al. 2002). However, live biological systems work well for low concentrations; they cannot survive the high levels that are found in seriously contaminated areas and industrial effluents (Fourest and Roux 1992).

Biosorption clearly shows that from most perspectives, plants are ideal for environmental cleanup: capital cost is low, ongoing operational costs are minimal, implementation is easy and non-invasive, and public acceptance is high (Veglio and Belochini 2001; Volesky 1999).

- High efficiency
- Minimization of chemical and/or biological sludge
- Regeneration of biosorbent
- Possibility of metal recovery
- Competitive performance
- Heavy metal selectivity

All these show that biosorption is a new and vibrant technology having great potential. To realize this, it will be necessary to understand the various processes

that are involved in it. This may require a multidisciplinary approach and diverse fields of plant biology.

> Biosorption based on metal binding to accumulate heavy metals from waste water through metabolically mediated means is proved to be potential biosorbents
>
> Volesky (1987)

Biosorption: Mechanistic Aspects

The complex structure of plant materials and microorganisms implies that there are many ways for the metal to be taken by the biosorbent. Numerous chemical groups have been suggested to contribute to biosorption metal binding by either whole organisms or molecules. These groups comprise hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, amino, imidazole, phosphonate, and phosphodiester. The importance of any given group for biosorption of certain metals by plant biomass depends on factors such as number of sites in the biosorbent material, the accessibility of the sites, the chemical state of the sites (availability), and affinity between site and metal (Volesky et al. 1999). Adsorption and desorption studies invariably yield important information on the mechanism of metal biosorption. This knowledge is essential for understanding of the biosorption process and it serves as a basis for quantitative stoichiometric considerations, which constitute the foundation for mathematical modeling of the process (Yang and Volesky 2000).

Biosorption Mechanism

Various metal-binding mechanisms have been postulated to be active in biosorption process and are presented in Fig. 1.

Due to the complexity of the biomaterials used, it is possible that at least some of these mechanisms are acting simultaneously to varying degrees, depending on the biosorbent and the solution environment.

Chemisorption

Chemisorption is of three types: *ion exchange, chelation, complexation or coordination*. It is the adsorption in which the forces involved are valence forces of the same kind as those operating in the formation of chemical compounds. Some features, which are useful in recognizing chemisorption, include the following:

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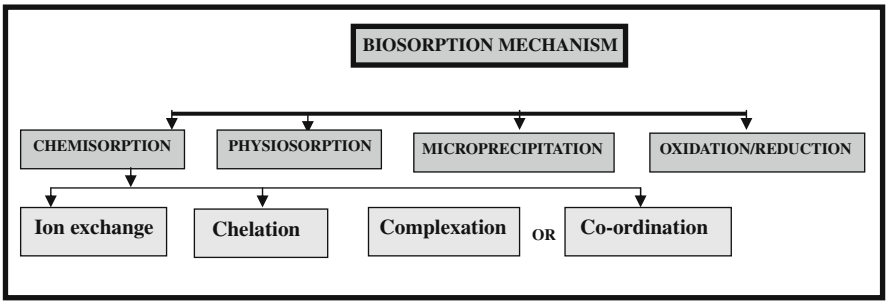


Fig. 1 Mechanism of biosorption

- The phenomenon is characterized by chemical specificity.
- Since the adsorbed molecules are linked to the surface by valence bonds, they will usually occupy certain adsorption sites on the surface and only one layer of chemisorbed molecules is formed (monolayer adsorption).
- The energy of chemisorption is of the same order of magnitude as the energy change in a chemical reaction between a solid and a fluid.
- Chemisorption is irreversible.

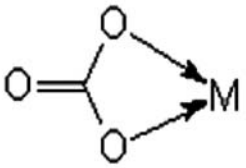
a. *Ion exchange*
Ion exchange is a reversible chemical reaction wherein an ion in a solution is exchanged for a similarly charged ion attached to an immobile solid particle. These solid ion exchange particles are either naturally occurring inorganic zeolites or synthetically produced organic resins. Synthetic organic resins are the predominant type used today because their characteristics can be tailored to specific applications.
Ion exchange reactions are stoichiometric, reversible, and as such they are similar to other solution phase reactions. For example, in the reaction

$$NiSO_4 + Ca(OH)_2 \rightarrow Ni(OH)_2 + CaSO_4$$

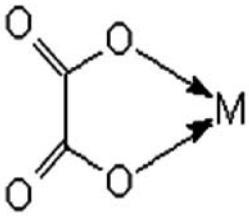
the nickel ions of the nickel sulfate ($NiSO_4$) are exchanged for the calcium ions of the calcium hydroxide [Ca $(OH)_2$] molecule.

b. *Chelation*
The word *chelation* is derived from the Greek word *chele*, which means *claw*, and is defined as the firm binding of a metal ion with an organic molecule (ligand) to form a ring structure. The resulting ring structure protects the mineral from entering into unwanted chemical reactions. Examples include the carbonate (CO_3^{2-}) and oxalate ($C_2O_4^{2-}$) ions:

c. *Coordination (complex formation)*
A coordination complex is any combination of cations with molecules or anions containing free pairs of electrons. Bonding may be electrostatic, covalent, or a



carbonato complex



oxalato complex

combination of both; the metal ion is coordinately bonded to organic molecules. Example of the formation of a coordination compound is

$$Cu^{2+} + 4H_2O \rightarrow [Cu\,(H_2O)]_4^{2+}$$
$$Cu^{2+} + 4Cl^{-} \rightarrow [CuCl_4]^{2-}$$

where coordinate covalent bonds are formed by donation of a pair of electrons from H_2O and Cl^- (Lewis bases) to Cu^{2+} (Lewis acid).

In general, biosorption of toxic metals and radionuclide is based on non-enzymatic processes such as adsorption. Adsorption is due to the non-specific binding of ionic species to polysaccharides and proteins on the cell surface or outside the cell. Bacterial cell walls and envelopes, and the walls of fungi, yeasts, and algae, are efficient metal biosorbents that bind charged groups. The cell walls of gram-positive bacteria bind larger quantities of toxic metals and radionuclide than the envelopes of gram-negative bacteria.

Bacterial sorption of some metals can be described by the linearized Freundlich adsorption equation:

$$\log S = \log K + n \log C$$

where S is the amount of metal absorbed in μmol/g, C is the equilibrium solution concentration in μmol/L, and K and n are the Freundlich constants.

Biomass deriving from several industrial fermentations may provide an economic source of biosorptive materials. Many species have cell walls with high concentrations of chitin, a polymer of *N*-acetyl glucosamine that is an effective biosorbent.

Biosorption uses biomass raw materials that are either abundant (e.g., seaweeds) or wastes from other industrial operations (e.g., fermentation wastes). The metal-sorbing performance of certain types of biomass can be more or less selective for heavy metals, depending on the type of biomass, the mixture in the solution, the type of biomass preparation, and the chemical–physical environment.

It is important to note that the concentration of a specific metal in solution can be reduced either during the sorption uptake by manipulating the properties of the biosorbent or upon desorption during the regeneration cycle of the biosorbent.

Physiosorption

In physiosorption, physiosorbed molecules are fairly free to move around the sample. As more molecules are introduced into the system, the adsorbate molecules tend to form a thin layer that covers the entire adsorbent surface. Some features, which are useful in recognizing physiosorption, include the following:

- The adsorbate molecules are held by comparatively weaker van der Waal's forces, thus resulting in lower activation energy.
- The process is, however, reversible as the substance adsorbed can be recovered from the adsorbent easily on lowering the pressure of the system at the same temperature.
- Physiosorption may extend beyond a monolayer also, since the physical forces can operate at any given distance.
- Physical adsorption is not specific in nature because it involves van der Waal's forces, which exist among the molecules of every two substances.

Physiosorption takes place with the help of van der Waal's forces. Knyucak and Volesky (1987) hypothesized that uranium, cadmium, zinc, copper, and cobalt biosorption by certain plant materials takes place through electrostatic attraction between the metal ions in solution and functional groups present on the cell surface.

Factors affecting biosorption

- Temperature seems not to influence the biosorption performances in the range of 20–35°C.
- pH seems to be the most important parameter in the biosorption process. It affects the solution chemistry of the metals, the activity of the functional groups in the biomass, and the competition of metallic ions.
- Biomass concentration in solution seems to influence the specific uptake. An increase in biomass concentration leads to interference between the binding sites.
- Biosorption is mainly used to treat wastewater where more than one type of metal ions would be present; the removal of one metal may be influenced by the presence of other metals.

Biosorbents Used So Far

Biosorption promises to fulfill the requirements, which are competitive, effective, and economically viable. Efforts have been made to use different forms of inexpensive plant materials for the abatement of toxic metals from the aqueous media. Biosorbents, explored so far in removing toxic metals from water bodies, have been listed in Table 1.

Table 1 List of biosorbents used for metal removal

Biosorbent	Metals removed	References
Wood	Cu(II), Cr(III), As(III)	Clausen (2000)
Fruit peel of orange	Ni(II)	Ajmal et al. (2000)
Crab shell	Cd(II), Zn(II), Ni(II)	An et al. (2001)
Cone biomass	Cr(VI)	Ucun et al. (2002)
Orange juice residue	As(III), As(V)	Ghimire et al. (2002)
Portulaca oleracea	Cd(II), Cr(III), Pb(II), As(III), Ni(II)	Vankar and Tiwari (2002)
Olive pomace	Cd(II), Cu(II), Pb(II)	Pagnanelli et al. (2003)
Lemna minor	Pb(II), Ni(II)	Nicholas et al. (2003)
Chitosan	Cr(VI)	Boddu et al. (2003)
Cassava waste	Cu(II), Zn(II)	Horsfall et al. (2003)
Flyash	As(III)	Nagarnaik et al. (2003)
Waste crab shells	Au(II), Cr(VI), Se(II)	Niu and Volesky (2003)
Sphagnum peat moss	Cd(II), Pb(II), Ni(II)	Rosa et al. (2003)
Water lettuce	As(III), As(V)	Basu et al. (2003)
Cocoa shells	Cd(II), Cr(III), Ni(II)	Meunier et al. (2003)
Date pits	Cd(II)	Banat et al. (2003)
Sugarcane bagasse pith	Cd(II)	Krishnan and Anirudhan (2003)
Turbinaria ornata seaweed	Cr(VI)	Aravindhan et al. (2004)
Hazelnut shell	Cr(VI)	Kobya (2004)
Aquatic moss	Cd(II), Zn(II)	Martins et al. (2004)
Polysaccharides	Pb(II), Hg(II), Cu(II)	Son et al. (2004)
Grape stalk wastes	Ni(II), Cu(II)	Isabel et al. (2004)
Saltbush plant	Cr(III), Cr(VI)	Sawalha et al. (2005)
Jute fibers	Cu(II), Ni(II), Zn(II)	Shukla and Roshan (2005)
Chitosan	Cu(II), Cr(III), As(III)	Kartal and Imamura (2005)
Rice polish	Cd(II)	Singh et al. (2005)

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Table 1 (continued)

Biosorbent	Metals removed	References
Lechuguilla	Cr(III)	Gonzalez et al. (2006)
Rice husk ash	Cd(II), Hg(II)	Kumar and Bandyopadhyay (2006)
Datura innoxia	Pb(II), Ni(II)	Abia and Asuquo (2006)
Maize leaf	Pb(II)	Adesola Babarinde et al. (2006)
Okra waste	Pb(II)	Hashem (2007)
Tamarindus indica	Cr(VI)	Srinivasa (2007)
Cassia fistula	Ni(II)	Hanif et al. (2007)
Juniper bark and wood	Cd(II)	Shin et al. (2007)
Sugarcane bagasse	Cd(II)	Garg U et al. (2008)
Calymperes erosum	Zn(II)	Adesola Babarinde et al. (2008)
C. fistula	Cr(III), Cr(VI)	Abbas et al. (2008)
Rice husk	Ni(II)	Bansal et al. (2009)

Biosorption: Application Strategies

Over the past few years, intensifying research into metal biosorption elucidated the principles of this effective metal removal phenomenon. Biosorption can be cost-effective, particularly in environmental applications where low cost of the metal removal process is most desirable. Some efficient natural biosorbents have been identified that require little modification in their preparation. It is particularly in ecological aspects where biosorption can make a difference due to its anticipated low cost. The application aspect is what makes the research and development work in this novel area exciting and worthwhile. While the biosorption process could be used even with a relatively low degree of understanding of its metal-binding mechanisms, better understanding will make for its more effective and optimized applications. If the biosorption processes were to be used as an alternative in the wastewater treatment scheme, the regeneration of the biosorbent may be crucially important for keeping the process cost down and to open the possibility of recovering the metals extracted from liquid phase. For this process it is desirable to desorb the sorbed metals and to regenerate the biosorbent material for another cycle of application.

Desorption involves the following:

- Yield of the metals in a concentrated form
- Restore the biosorbent close to the original conditions for effective reuse
- Undiminished metal uptake
- No physical change or damage

Extensive "desorption" work may be necessary for assessing whether this is possible and under what conditions. Desorption and sorbent regeneration studies might require somewhat different methodologies. While the regeneration of the biosorbent may be accomplished by washing the metal laden with an appropriate solution, the type and strength of the solution would depend on just how the deposited metal has

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been bound. Dilute solutions of mineral acids (hydrochloric acid, nitric acid, sulfuric acid, and acetic acid) can be used for metal desorption from the biomass (Holan et al. 1993; Pagnanelli et al. 2002).

Due to different affinities of metal ions for the predominant sorption site (under the solution conditions), there will be a certain degree of metal selectivity by the sorbent on the uptake. Similarly, selectivity may be achieved upon the elution–desorption operation (Kratochvil and Volesky 2000). Advantage could be taken of this selectivity on the desorption side of the operation which can contribute to the separation of metals from one another, if desirable.

The overall capacity of sorption process is to concentrate the sorbate metals. This is assessed by expressing a simple overall process parameter, the concentration ratio (CR). Obviously, the higher the CR, the better the overall performance of the sorption process, making the eventual recovery of the metal more feasible as it becomes more concentrated in the small volume of the eluant solution.

A considerable amount of research on biosorbent materials has developed solid basis of knowledge and indicated their enormous potential. The highest priority at the early stage would be the preliminary and approximate assessment of the commercial potential and practicality of application of the new technology based on the family of new biosorbent products. The preliminary assessments that should be carried out simultaneously as part of a better quantitative estimation of this technology are as follows.

Assessment of the Competing Technologies

The current costs and market share of the established conventional processes for metal removal/recovery from dilute solutions or wastewaters have to be summarized or assessed. As the emission standards tighten the conventional methods for metal detoxification are becoming progressively more inadequate or prohibitively costly for use of water treatment. Better and effective metal removal technologies are invariably more costly and often just not feasible for that purpose. The search is on for efficient and particularly cost-effective remedies. Biosorption promises to fulfill the requirements. Its overall performance and process application modes justify a comparison with the other existing techniques.

Assessment of the Market Size

While it is known that the environmental-based market for metal removal/decontamination of metal-containing (industrial) effluents is enormous, the actual figure to support this generally prevailing perception would be most convincing for commercialization. Comparison of costs between the traditional and new technology establishes the feasibility of biosorbent applications and their competitiveness in the marketplace. As the application of biosorption proves cheaper, it is anticipated that new applications, otherwise perhaps not feasible, will significantly increase the size of the current market and scope of potential clients for biosorption technology.

Assessment of Costs of New Biosorbent

Approximate costs of different types of raw biomass need to be ascertained, as well as the costs of processing the biomass into applicable biosorbent materials maintaining their high efficiency. Preliminary technical work needs to be carried out on the processing necessary for biomass formulation into a product suitable for use. Different raw biomass materials would require specific treatment for their optimal formulation into finished ready-to-use products. This part would entail specifically planned small-scale laboratory work resulting in an efficient biosorbent material. The most compelling reasons for using biosorption technology, based on renewable or waste raw materials, are that it is effective and inexpensive. The initial information gathered in preliminary economic feasibility studies leads to the following main conclusions regarding the application of bioremediation technology.

Biosorption Can Be Viewed as

- Water treatment process.
- Significant cost-saving process in comparison with existing competing techniques.
- Effective in terms of technical performance, operational qualities, and chemical properties.
- Commonly usable having low sensitivity to environmental and impurity factors.
- Additional cost reducing because of possible recovery of heavy metals.
- Cost-effective, obviously reinforced by a higher market value of recovered metal and lower cost of biomass.

Designing of Experiments

The biosorption experiments involve the following major steps:

Preparation of Stock Solutions: Analytical grade reagents should be used throughout the experiments. The stock solutions of desired concentrations of metals under study are to be prepared by weighing requisite amount of their parent salts and dissolving them in double distilled water. Working solutions should be freshly prepared from stock solutions for each experimental run. Accuracy of weighing should be carefully kept in mind.

Biosorbent Preparation: The sources of agricultural wastes selected for the study should be identified from authenticated taxonomy division. The biosorbents should be washed repeatedly with water to remove dust and soluble impurities, shade dried, crushed, and finally sieved through copper sieves of different mesh sizes.

Sorption Studies for Single Metal System: Batch experiments of at least three replicates should be performed in clean air-conditioned environmental laboratory using standard practices as a function of particle size, biomaterial dosage, contact time, metal concentration, and pH. After proper pH adjustments, a known quantity of biosorbents is to be added. Finally metal bearing suspensions should be kept under magnetic stirring until the equilibrium conditions are reached. After shaking, suspension is to be allowed to settle down. The residual biomaterial sorbed with metal ions should be filtered using Whatman 42 filter paper. Filtrate is to be collected and subjected for metal ion estimation.

Sorption Studies for Multi-metal System: Multi-metal solution of each metal ion under study should be prepared. After pH adjustments, requisite quantity of biomaterial is to be added and the suspension should be magnetically shaken for 40 min. The residual biomaterial sorbed with metal ions is to be filtered using filter paper, and to the filtrate, metal estimations should be carried out. Percent metal sorption by the sorbent should be computed using the same equation as employed in case of single metal system.

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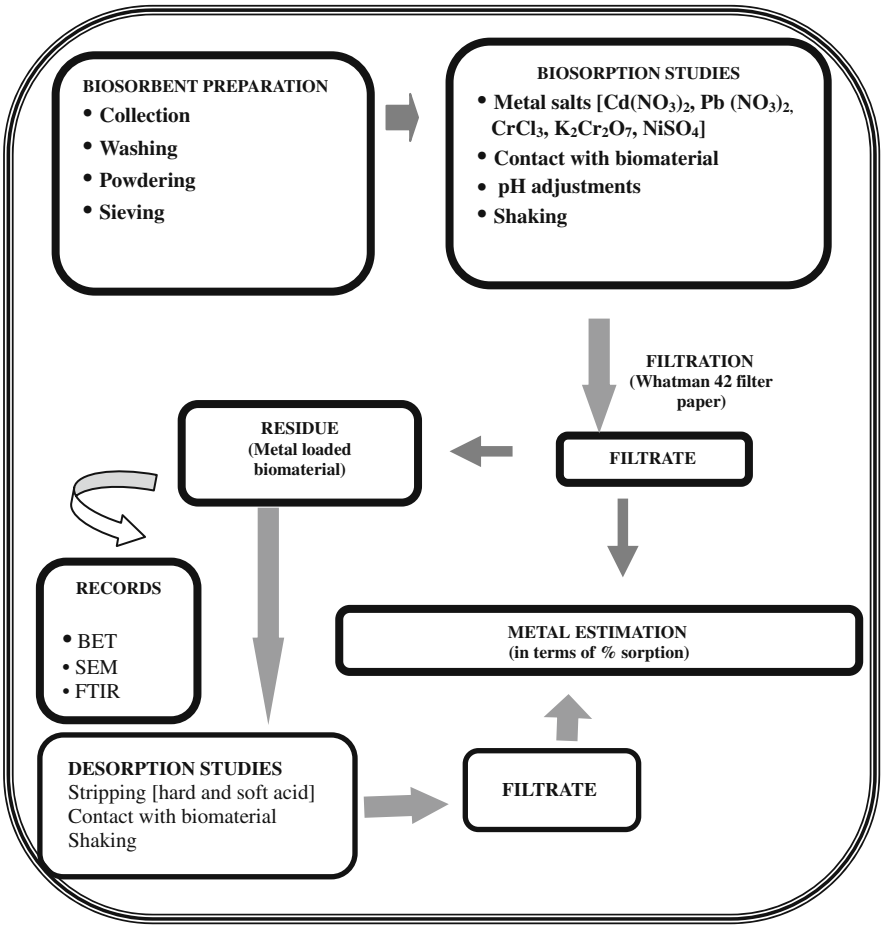
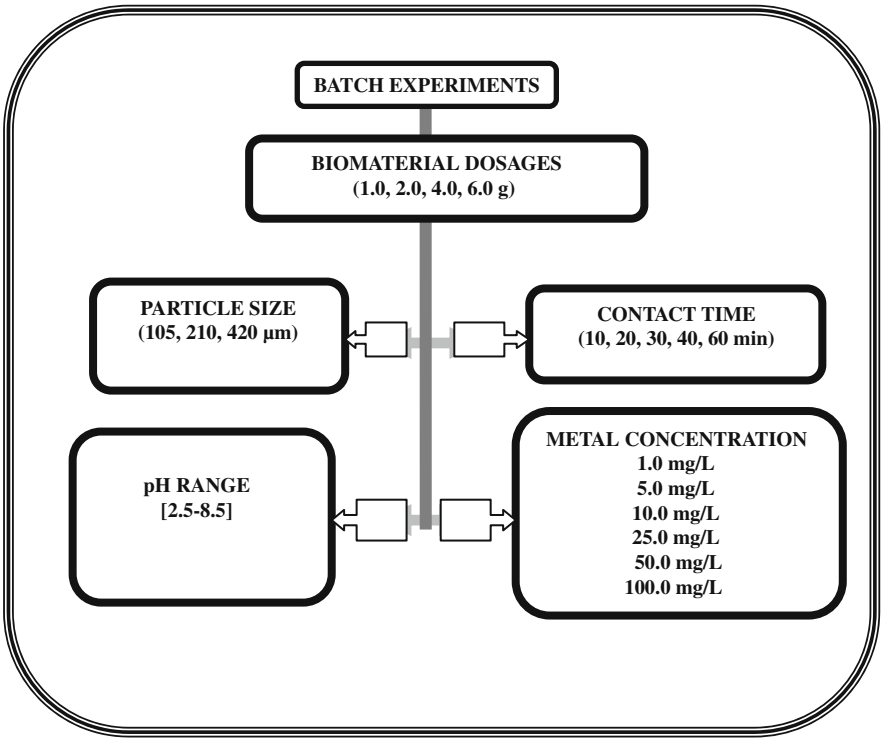


METHODOLOGY AT A GLANCE

Metal Analysis Using Various Instruments

Metal ions are analyzed using various analytical instruments like *atomic absorption spectrometer (FAAS and GFAS), ultraviolet spectrometer, gamma spectrometer,* and *neutron activation analyzer (NAA).*

Some important instruments specifically used for this purpose are discussed below.



EXPERIMENTAL CONDITIONS AT A GLANCE

Instrumentation

Atomic Absorption Spectrometer

The metal is to be estimated by *FLAME ATOMIC ABSORPTION SPECTROMETER* using its cathode lamp.

Atomic absorption spectrometer (AAS) is an efficient method from the viewpoint of chemical analysis. In this method, the element to be analyzed in a sample can be determined quantitatively by measuring the absorbance value at the specific wavelength of the analyte element. In AAS four techniques are commonly used, like

Fig. 1 Atomic absorption spectrometer

flame technique (Fig. 1), *graphite furnace technique, cold vapor technique,* and *hydride generation technique*.

Principle

Atomic absorption spectrometry is based on the principle of measurement of decrease in light intensity from a source when it passes through a vapor layer of the atoms of the analyte element. In atomic absorption spectrometry, a light beam is directed through the flame, into a monochromator, and on to a detector that measures the amount of light absorbed by the atomized element in the flame. Because each metal has its own characteristic absorption wavelength, a source lamp composed of that element is used, which makes the method relatively free from spectral or radiation interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

Sensitivity and Detection Limit

The *sensitivity* of flame atomic absorption spectrometer is defined as the metal concentration that produces absorption of 1% (an absorbance of approximately 0.0044). The *detection limit* is defined as the concentration that produces absorption equivalent to twice the magnitude of the background fluctuation. The detection limit for a given analytical procedure is the concentration that can be detected with a stated statistical certainty:

$$C = K\ \sigma/\mu$$

where C = detection limit; σ = absolute standard deviation; μ = sensitivity; K = a factor, usually taken as 2 or 3, depending on the required statistical certainty and should be stated.

Sensitivity and detection limits vary with the instrument, the element determined, and the technique selected. The most widely used flame for atomic absorption spectrometry is the air–acetylene flame and the nitrous oxide–acetylene flame with premix burners. Flow rate of fuel gas is 2.2 L/min. The metal is to be directly aspirated into the air–acetylene flame of an AAS and measured at a wavelength of 232 nm at a slit width of 0.2 nm.

Neutron Activation Analyzer

Neutron activation analysis (NAA) is an important technique for quantitative multi-element analysis of major, minor, trace, and rare elements (Fig. 2).

Fig. 2 Neutron activation analyzer

Principle of Neutron Activation Analysis

The initial step in neutron activation analysis is irradiating a sample with neutrons in a nuclear reactor or sometimes in other neutron sources. The stable nucleus absorbs one neutron and becomes a radioactive nucleus. The concentration of the stable element of interest in the sample can be measured by detecting the decay of these nuclei.

In NAA, stable nuclide (^{A}Z, the target nucleus) samples undergo neutron capture reactions in a flux of neutrons. The radioactive nuclides produced in this activation process usually decay by emission of a beta particle (β^{-}) and gamma ray(s) with a unique half-life. A high-resolution gamma-ray spectrometer is used to detect these

"delayed" gamma rays in the presence of the artificially induced radioactivity in the sample for both qualitative and quantitative analyses.

NAA Detectors

There are a number of detector types and configurations used in NAA. Most are designed to detect the emitted gamma radiation. The most common types of gamma detectors encountered in NAA are the *gas ionization* type, *scintillation* type, and the *semiconductor* type. Scintillation-type detectors use a radiation-sensitive crystal, most commonly sodium iodide, NaI (TI), which emits light when struck by gamma photons. These detectors have excellent sensitivity and stability and a reasonable resolution. NAA can detect up to 74 elements depending on the experimental procedure.

Detection Limit

Activation analysis determines the total amount of an element in a sample in grams (or micrograms). A certain amount of an element, say arsenic, is needed in the sample for detection. For arsenic, under ideal conditions, 5 ng is required. To determine 5 ppb of arsenic, 1 g of the sample is enough – to determine 0.5 ppb of arsenic, 10 g of sample is necessary, etc. In some cases, it is possible to "push" detection limits by longer irradiations. If a sample is irradiated twice as long, it becomes twice as radioactive, or the detection limit is improved. This is true up to one half-life, or so, of the isotope to be determined. One disadvantage to this approach is the cost of nuclear reactor time.

Doubling the counting time can also improve detection. However, background noise from the environment limits this approach. Doubling the counting time will not help if all this does is double the background noise level.

Usually the detection limit depends on the "other" elements in the sample – the matrix. If an element in the sample becomes radioactive, besides the element of interest, the background noise may be too high to determine the desired element at low levels. This noise does not produce wrong results, just high detection limits. The signal-to-noise ratio will improve with time if the element of interest has a long half-life compared to the element(s) producing the noise.

Accuracy and Precision

Accuracy is how close the determination is to the actual value. Precision is how close replicate determinations are to each other. An analysis may be very precise (duplicates may have the same results), but not very accurate (the real value is much different). In neutron activation analysis, precision usually varies from 2 to 5% of the value obtained – independent of concentration. This precision depends on the background noise level and the concentration of the determined element. Using standards, the accuracy has been determined to be within the precision, provided

"gross" errors have been avoided. Gross errors include wrong sample weight and mathematical mistakes.

For trace element determinations, the accuracy and precision of neutron activation analysis cannot be matched. However, for major constitutions another approach should be considered.

Sensitivity

The sensitivities for NAA are dependent on the irradiation parameters (i.e., neutron flux, irradiation, and decay times), measurement conditions (i.e., measurement time, detector efficiency), nuclear parameters of the elements being measured (i.e., isotope abundance, neutron cross section, half-life, and gamma-ray abundance). The accuracy of an individual NAA determination usually ranges between 1 and 10% of the reported value.

All methods for detection of radioactivity are based on the interactions of the charged particles or the electromagnetic rays with matter traversed. Electromagnetic radiations like X rays and gamma rays lose their energy in a stopping material mainly through three mechanisms, namely (i) photoelectric effect, (ii) Compton scattering, and (iii) pair production. These processes are strongly dependent on the energy of photon and atomic number (Z) of the stopping material. Other effects such as Rayleigh scattering and Thompson scattering are much less important and can be ignored in the detection process (Fig. 3).

Fig. 3 NaI (TI) gamma-ray detector coupled to a 4 K MCA

Photoelectric Effect

In this process, a photon is absorbed in the medium and energy is transformed to one of the electrons (normally tightly bound orbital electron); then the velocity of

that electron will be too high to remain in the orbital resulting in its emission. The difference between the incident photon energy (E_i) of the electron and the binding energy of the photon (E_b) appears as the kinetic energy of the ejected electron (E_e):

$$E_e = E_i - E_b$$

The probability of the emission of the electron is in the order of *K>L>M>N*... electrons if the energy of the photon is high enough. Photoelectric effect is characterized by the total absorption of the photon energy within the medium and is the predominant mode of interaction of low-energy gamma rays.

Compton Scattering

In this process, the photon interacts with an electron that may be loosely bound or free. The incoming photon is deflected through an angle with respect to its original direction and a fraction of its energy is transferred to the electron. The energy of the electron and the scattered photon is

$$E_g = E_o/[1 - E_o(1 - \cos\theta)/mc^2]$$

$$E_e = E_o - E_g$$

where E_o is the energy of the incident photon, E_g is the scattered photon energy, E_e is the scattered electron energy, m is the electron rest mass, and c is the velocity of light. θ is the angle between scattered and incident gamma-ray spectra.

Pair Production

This process involves the complete absorption of a photon in the vicinity of an atomic nucleus with the formation of an electron–positron pair. In accordance with the momentum conservation, this interaction mainly occurs in the field of the nucleus of the absorbent material. Pair production cannot occur when the energy of gamma radiation is less than 1.02 MeV that is equivalent to the rest mass of $e^- - e^+$ pair with zero kinetic energy. The cross section for pair production is also proportional to Z^2. At high energies, where pair production is the predominant process, gamma-ray energies can be best determined by measurement of the total energies of electron–positron pairs. Pair production is always followed by annihilation of the positron, usually with the simultaneous emission of two 0.51 MeV photons. The absorption of quanta by the pair production process is, therefore, always complicated by the appearance of this low-energy secondary radiation with its associated Compton.

In summary, all the three processes produce moving electrons in matter that can be detected directly or can initiate other electronic processes to obtain an electric

charge pulse that represents the initial photon energy. The recorded pulse is proportional to the energy lost in all the three processes. Full energy photopeak results from the complete energy deposition of the photon in the detector by any one or the combination of the three processes mentioned.

Simple Counting System

A basic measurement in many physics experiments is a simple counting of the number of pulses from a detector. In this setup, the analog signal from a detector is amplified by the preamplifier, shaped, and further amplified by the amplifier. The resulting analog signal is then sent through the low-level discriminator that delivers a signal for every input pulse with amplitude greater than the threshold value. The signal is then sent to the timer/scalar that counts each arriving pulse for a giving counting period *T* preset in the timer section. Discriminator serves the dual purpose of excluding low-level noise in analog suit and shaping the accepted signal to a form suitable for the scalar to accept.

General Principle of Detection

In all radiation detectors, deposition of radiation in its volume causes ionization releasing electric charge (in the case of scintillator emitted light is converted to electric charge in photomultiplier tube) and effective collection of this charge, under the applied electric field, forms the basic signal of the interacting radiation. The ionization results in a very low current or voltage pulse that needs proper amplification. The amplified pulses are further processed to obtain the number of radiations (counting) or the energy and the intensity of the radiations (spectrometry). Interaction time is very small, a few nanoseconds in gases and a few picoseconds in solids. These times are so short that the deposition of the radiation energy can be considered instantaneous. The detector acts like a capacitor and the charge collected in the capacitor can be discharged through a resistance giving a voltage pulse.

NaI (TI) Scintillator Detector

Scintillation detectors are based on the conversion of the absorbed energy into light and detection by the use of photomultiplication. Among the inorganic scintillators, NaI, activated with 0.1–0.2%, thallium is, by far, the most widely used. The high density (3.7 g/cm^3) of NaI and the high molecular weight of iodine make this a very efficient gamma-ray detector. Approximately 30 eV of energy deposition in a NaI (TI) crystal is required to produce one light photon, and it takes on an average about 10 photons to release one photoelectron at the photocathode of the multiplier. These photoelectrons are then accelerated by a potential of the order of 100 V to the first dynode where each one produces "*n*" secondary electrons; these

secondary electrons are then similarly accelerated and multiplied n-fold at the second dynode, and so on. With 10 dynodes and with n typically about 3 or 4, the total multiplication factor is n^{10} or of the order of 10^5 or 10^6. Thus a 0.3 MeV gamma ray absorbed in a NaI (TI) crystal might produce 10^4 light photons giving 10^3 photoelectrons and leading eventually to an output pulse of about 10^8 electrons or 1.6×10^{-11} coulomb (C). In an output circuit of about 10^{-10} F capacity this would be a pulse of about 0.16 V requiring further amplification. There is a good correlation between the energy absorbed in the scintillator and the size of the output pulse.

An additional feature in the NaI (TI) spectra is the so-called iodine escape peak about 28 keV. It results from the absorption of gamma ray near the surface of the detector and the subsequent escape of the K–X ray of the iodine. This effect becomes less pronounced with increasing gamma-ray energy because fewer of the initial interactions take place near the surface.

Multi-channel Analyzer

Multi-channel analyzer (MCA) is used widely for pulse height analysis. The basic function of the MCA is to sort out the incoming pulses from the detector according to the pulse height and keep the count of the number of pulses at each pulse height in a multi-channel memory. The content of a large number of channels is known as pulse height spectrum which can be displayed on the visual display unit for monitoring.

MCA works by digitizing the amplitude of the detector pulse heights with a nuclear analog to digital converter (NADC). The MCA then takes this channel address and increments by one of the corresponding memory channel contents whose address is proportional to the digital number. In this way pulses are sorted out according to the height of the analog pulse and the number at each pulse height stored in the corresponding memory locations. As a result of this histogram process, one builds up the pulse height spectrum. The total number of channels into which the voltage range is to be digitized is known as the "conversion gain" and it determines the resolution of the MCA.

Interference in Gamma Counting

There are several factors such as background radiations, sample geometry, and dead time which affect the count rate or counting efficiency of the detector. These factors are as follows.

Background Radiations

Interfering background in gamma spectra originates from either within the sample being counted (Compton produced or due to the presence of other radionuclides) or the environment. The Compton increases if the sample being analyzed has a high

content of high-energy gamma-emitting radioisotopes. For extremely weak samples, the environmental background becomes more significant. This can be reduced using massive shielding generally made of lead.

Sample Geometry

The sample size should be selected as large as possible for maximum efficiency. The sample was distributed uniformly so as to minimize the distance between the sample volume and the detection itself.

Dead Time

Radiation produces a pulse in the detector subsequent to its interaction. While this pulse is being processed, detector is not available for processing the next pulse that might be generated during this interval. Because of the random nature of radioactive decay, there is always some probability that a true event will lose because it occurs too quickly following a preceding event. The minimum time separation required to record two successive events as two separate pulses is usually called the dead time of the counting system. The dead time loss increases with increase in the count rate. The detector should have small dead time so that many counts are not lost just because the detector is still processing an earlier pulse. The percentage dead time is given by

$$\%\,\text{Dead Time} = (\text{True Time} - \text{Live Time}) \times 100/\text{True Time}$$

Energy and Efficiency Calibration

The detector should be calibrated for the gamma-ray energy of a particular isotope using gamma reference standards (Module Disc Type, provided by Electronics Corporation of India Ltd.). Details of gamma reference standards are tabulated below.

Decay characteristics of few radionuclides used for calibration:

GAMMA REFERENCE STANDARDS – R 2300 B			
ISOTOPE	GAMMA ENERGY (MeV)	NOMINAL ATIVITY	HALF-LIFE
Co	0.123	3.0	273 days
Co	1.17; 1.33	1.0	5.3 years
Cs	0.662	1.0	30 years

The gamma-ray intensity (A) is given by the following expression: Net Count Rate (P) = Abundance (A) × Efficiency (E_f) × Disintegration Rate (D), where "P"

is the photo peak area, "E_f" is the efficiency for detection of a gamma ray with energy "E," and "D" is the disintegration rate of the sample. The efficiency of the detector depends on the gamma-ray energy and the detector type, its shape, and size. At low energies, the attenuations of the gamma rays in the window and dead layer are predominant. This effect reduces as the energy of the gamma ray increases. For high-energy gamma ray for a given size, detector efficiency again falls due to greater chance escape of Compton-scattered gamma ray. Thus depending on the type and size of the detector, efficiency peaks at certain energy falls on both sides of this value.

Calculation for Metal Uptake

Metal uptake by biosorbent should be calculated using the mass balance equation for the biosorbent: $q = [V(C_i - C_f)]/W$, where q = metal uptake (mg metal/g dry weight), V = volume of metal-bearing solution contacted (batch) with the biosorbent (L), C_i = initial concentration of metal in solution (mg/L), C_f = final concentration of target seed biomaterial in solution (mg/L), W = dry weight of biosorbent added (g).

Sorption Isotherm

The adsorption capacity and affinity of biosorbent for metal ions are to be determined with two well-known isotherm models.

Freundlich sorption isotherm is given in the form of a linearized equation as follows: $\log q = \log K_f + 1/n \log C_e$, where q is the metal uptake per unit weight of biosorbent, C_e is the equilibrium (residual) concentration of metal ion in solution, K_f and n are the characteristic constants. *Langmuir equation* has the general form as $C_e/q_e = (1/Q_o b) + (C_e/Q_b)$, where C_e is the equilibrium concentration, q_e is the amount of adsorbed metals at equilibrium, Q_o and b are the Langmuir constants related to adsorption capacity and energy of adsorption, respectively. The biosorption capacity (K_f and Q_o) and biosorption intensity/energy ($1/n$ and b) are to be estimated from the slope and intercept of the Freundlich and Langmuir isotherms.

Considerations for Desorption Experiment

Desorption experiments are to be carried out in order to explore the feasibility of recovering the metals and reuse the metal-treated biosorbent for further cycle of sorption. Desorption studies (batch process) are to be conducted to restore the biomaterial as a function of concentration of different desorption reagents: *hard acid* [0.05 M HNO_3 and HCl], *soft acid* [0.5 M citric acid], *base* [0.05 M NaOH], and distilled water. Metal-loaded biosorbent obtained from sorption experiments should be transferred to Erlenmeyer flasks and shaken with 50 mL of each desorption reagent as a function of time (20, 40, 60, and 80 min) at room temperature. At the end of each time interval the suspension should be stirred for 5 min. The suspension is to

be filtered using Whatman 42 filter paper and in the filtrate estimation of metal ion concentration is to be carried out.

The amount of metal ion remaining on the biomaterial as a function of time is to be calculated using the mass balance equation: $q_t = q_e - c_t$ (v/m), where q_t and q_e are the biomaterial phase metal ion concentration (mg/L) and c_t solution phase metal ion concentration (mg/L) at time t (min), respectively.

Statistical Analysis

Batch experiments should be conducted in three replicates ($N = 3$) and data represent the mean value. Correlation coefficient and standard deviations should be calculated using SPSS PC^{+TM} statistical package. For the determination of intergroup mean value differences, each parameter should be subjected to the student-t test for significance level ($p < 0.05$).

Interpretations

Single Metal Sorption

Percentage removal for metal ions is calculated in terms of sorption efficiency of the respective biosorbents under various experimental conditions, viz., biomaterial dosage, contact time, metal concentration, optimum particle size, volume, and pH. The data are to be handled with appropriate statistical treatment and tabulated. The concentration of the removed metals may be represented in terms of μmol, μg, and ppm. The representative tables exhibiting the sorption efficiency of particular biosorbents used for the decontamination of toxic metals from water bodies are given below. The influence of each variable is taken into account for its effect on the sorption phenomenon (Tables 1, 2, 3, 4, and 5).

Results may be incorporated in the following terms.

Effect of Particle Size on Metal Sorption

The particle size is an important factor from the biosorption point of view and has a significant influence on the kinetics of adsorption. Figure 1 explains the role of size of the particle of the biosorbent [*Leucaena leucocephala* (LLSP)] on the sorption phenomenon.

The figure explains that as the size of the particle goes on decreasing there is an increase in the sorption efficiency of the metal ions. The reduction in particle size of the biosorbent results in an increase in surface area. With increased availability of the surface area, more adsorption sites are available for metal removal.

Effect of Contact Time on Metal Sorption

Time-dependent studies offer data about the changes in metal sorption related to time. In these studies, the minimum time necessary for the biomaterial to be in contact with the metal ion is identified. The variation of contact time with biosorbent [*Z. mays* cob powder (ZMCP)] may be interpreted in the following fashion:

S. Srivastava, P. Goyal, *Novel Biomaterials*, Environmental Science and Engineering,
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Table 1 This table explains Cd(II) ion concentration (μM) after adsorption on *L. leucocephala* seed powder (LLSP) as functions of contact time and biomaterial dosage at volume (200 mL), particle size (105 μm), and pH (6.5)

	Time interval (minutes)				
Initial conc. (μM)	10	20	30	40	60
Biomaterial dosage (1.0 g)					
01.77[a]	$1.35\pm0.05^{bx+\Phi}$	$1.20\pm0.05^{x+}$	$1.08\pm0.04^{x+}$	$1.04\pm0.04^{x+}$	$1.04\pm0.03^{xx+}$
08.89	$6.06\pm0.26^{x+}$	$5.39\pm0.23^{x+}$	$4.64\pm0.20^{x+}$	$4.49\pm0.19^{x+}$	$4.46\pm0.19^{xx+}$
17.79	$10.95\pm0.49^{x+}$	$9.57\pm0.43^{x+}$	$8.32\pm0.37^{x+}$	$8.04\pm0.37^{x+}$	$8.02\pm0.37^{xx+}$
44.48	$25.95\pm1.24^{x+}$	$22.45\pm1.14^{x+}$	$20.12\pm0.96^{x+}$	$19.25\pm0.94^{x+}$	$19.19\pm0.94^{xx+}$
88.96	$51.82\pm2.64^{x++}$	$44.81\pm2.42^{x++}$	$40.13\pm2.04^{x++}$	$38.39\pm2.03^{x++}$	$38.27\pm1.99^{xx++}$
177.92	$103.49\pm5.48^{x++}$	$89.42\pm4.92^{x++}$	$80.11\pm4.16^{x++}$	$76.54\pm4.13^{x++}$	$76.47\pm4.05^{xx++}$
Correlation coefficient (r)	0.98	0.97	0.98	0.98	0.98
Biomaterial dosage (2.0 g)					
01.77[a]	$0.97\pm0.04^{\Phi x+}$	$0.81\pm0.03^{x+}$	$0.69\pm0.03^{x+}$	$0.65\pm0.02^{x+}$	$0.65\pm0.02^{xx+}$
08.89	$3.57\pm0.16^{x+}$	$3.04\pm0.13^{x+}$	$2.81\pm0.13^{x+}$	$2.61\pm0.12^{x+}$	$2.61\pm0.12^{xx+}$
17.79	$5.97\pm0.28^{x+}$	$5.05\pm0.23^{x+}$	$4.48\pm0.21^{x+}$	$4.43\pm0.21^{x+}$	$4.41\pm0.2^{xx+}$
44.48	$13.87.68^{x+}$	$12.09\pm0.61^{x+}$	$10.78\pm0.54^{x+}$	$10.33\pm0.53^{x+}$	$10.28\pm0.53^{xx+}$
88.96	$27.47\pm1.40^{x++}$	$24.07\pm1.27^{x++}$	$21.42\pm1.15^{x++}$	$20.58\pm1.13^{x++}$	$20.51\pm1.13^{xx++}$
177.92	$54.69\pm3.01^{x++}$	$48.07\pm2.73^{x++}$	$42.66\pm2.47^{x++}$	$41.02\pm2.33^{x++}$	$40.97\pm2.33^{xx++}$
Correlation coefficient (r)	0.91	0.91	0.92	0.96	0.96
Biomaterial dosage (4.0 g)					
01.77[a]	$0.72\pm0.03^{\Phi x+}$	$0.60\pm0.02^{x+}$	$0.51\pm0.02^{x+}$	$0.42\pm0.02^{x+}$	$0.40\pm0.02^{xx+}$
08.89	$3.18\pm0.15^{x+}$	$2.54\pm0.09^{x+}$	$1.04\pm0.07^{x+}$	$1.15\pm0.05^{x+}$	$1.15\pm0.05^{xx+}$
17.79	$4.75\pm0.24^{x+}$	$3.32\pm0.16^{x+}$	$2.59\pm0.10^{x+}$	$1.20\pm0.06^{x+}$	$1.20\pm0.06^{xx+}$
44.48	$10.31\pm0.53^{x+}$	$6.49\pm0.38^{x+}$	$3.23\pm0.19^{x+}$	$\mathbf{1.22\pm0.07^{x+}}$	$1.19\pm0.07^{xx+}$
88.96	$20.54\pm0.99^{x++}$	$12.81\pm0.67^{x++}$	$6.42\pm0.40^{x++}$	$2.41\pm0.14^{x++}$	$2.34\pm0.14^{xx++}$
177.92	$41.02\pm2.39^{x++}$	$25.40\pm1.62^{x++}$	$12.77\pm0.83^{x++}$	$4.78\pm0.31^{x++}$	$4.64\pm0.29^{xx++}$
Correlation coefficient (r)	0.94	0.96	0.93	0.98	0.96

Table 1 (continued)

Initial conc. (μM)	Time interval (minutes)				
	10	20	30	40	60
Biomaterial dosage (6.0 g)					
01.77[a]	$0.71\pm0.03^{\Phi\Phi x+}$	$0.60\pm0.02^{x+}$	$0.51\pm0.02^{x+}$	$0.40\pm0.01^{x+}$	$0.40\pm0.01^{xx+}$
08.89	$3.16\pm0.15^{x+}$	$2.02\pm0.09^{x+}$	$1.53\pm0.07^{x+}$	$1.13\pm0.05^{x+}$	$1.13\pm0.04^{xx+}$
17.79	$4.73\pm0.24^{x+}$	$3.30\pm0.17^{x+}$	$1.97\pm0.10^{x+}$	$1.15\pm0.06^{x+}$	$1.17\pm0.06^{xx+}$
44.48	$10.34\pm0.52^{x+}$	$6.44\pm0.36^{x+}$	$3.18\pm0.17^{x+}$	$1.24\pm0.07^{x+}$	$1.32\pm0.06^{xx+}$
88.96	$20.44\pm1.08^{x++}$	$12.70\pm0.73^{x++}$	$6.26\pm0.36^{x++}$	$2.25\pm0.13^{x++}$	$2.13\pm0.12^{xx++}$
177.92	$40.76\pm2.19^{x++}$	$25.21\pm1.48^{x++}$	$12.36\pm0.72^{x++}$	$4.43\pm0.27^{x++}$	$4.14\pm0.25^{xx++}$
Correlation coefficient (r)	0.90	0.91	0.92	0.94	0.95

Mean difference [initial Cd(II) loaded (μM) versus Cd(II) (μM)] as functions of time. ^{x}significant ($p < 0.10$), ^{xx}insignificant ($p > 0.10$).
Metal concentration $^{+}$significant ($p < 0.01$), $^{++}$insignificant ($p > 0.01$).
Biomaterial dosage $^{\Phi}$significant ($p < 0.01$), $^{\Phi\Phi}$insignificant ($p > 0.01$).
[a]Number in parenthesis represents metal concentrations in μM.
[b]Standard deviation values of replicate ($N = 3$) determinations.

Table 2 This table explains Cr(VI) ion concentration (μM) after adsorption on *Saraca indica* powder (SILP) as functions of contact time and biomaterial dosage at volume (200 mL), particle size (105 μm), and pH (2.5)

	Time interval (minutes)				
Initial conc. (μM)	10	20	30	40	60
Biomaterial dosage (1.0 g)					
03.84[a]	$3.30\pm0.15^{bx+\Phi}$	$3.23\pm0.15^{x+}$	$3.07\pm0.13^{x+}$	$2.88\pm0.13^{x+}$	$2.88\pm0.13^{xx+}$
19.23	$12.88\pm0.57^{x+}$	$12.11\pm0.56^{x+}$	$11.32\pm0.51^{x+}$	$10.61\pm0.49^{x+}$	$10.61\pm0.49^{xx+}$
38.46	$20.03\pm0.96^{x+}$	$19.26\pm0.92^{x+}$	$17.03\pm0.83^{x+}$	$15.46\pm0.75^{x+}$	$15.42\pm0.74^{xx+}$
96.15	$46.07\pm2.34^{x+}$	$37.81\pm1.92^{x+}$	$36.12\pm1.87^{x+}$	$34.53\pm1.83^{x+}$	$34.46\pm1.82^{xx+}$
192.30	$87.12\pm5.15^{x+}$	$84.31\pm4.21^{x+}$	$67.80\pm4.09^{x+}$	$63.81\pm3.99^{x+}$	$62.68\pm3.98^{xx+}$
384.61	$174.81\pm10.84^{x++}$	$147.5\pm8.72^{x++}$	$137.42\pm8.46^{x++}$	$132.46\pm8.11^{x++}$	$129.26\pm8.09^{xx++}$
Correlation coefficient (*r*)	0.98	0.97	0.98	0.98	0.98
Biomaterial dosage (2.0 g)					
03.84[a]	$2.88\pm0.12^{\Phi x+}$	$2.65\pm0.11^{x+}$	$2.23\pm0.10^{x+}$	$2.03\pm0.09^{x+}$	$2.03\pm0.09^{xx+}$
19.23	$11.12\pm0.52^{x+}$	$9.23\pm0.43^{x+}$	$7.46\pm0.35^{x+}$	$6.42\pm0.30^{x+}$	$6.38\pm0.29^{xx+}$
38.46	$18.97\pm0.91^{x+}$	$14.19\pm0.69^{x+}$	$11.76\pm0.57^{x+}$	$10.66\pm0.52^{x+}$	$10.65\pm0.52^{xx+}$
96.15	$41.26\pm2.02^{x+}$	$26.23\pm1.28^{x+}$	$23.52\pm1.15^{x+}$	$22.33\pm1.09^{x+}$	$21.26\pm1.04^{xx+}$
192.30	$79.38\pm4.20^{x+}$	$49.19\pm2.66^{x+}$	$41.88\pm2.39^{x+}$	$38.38\pm2.35^{x+}$	$37.92\pm2.17^{xx+}$
384.61	$158.51\pm8.83^{x++}$	$98.19\pm5.62^{x++}$	$86.53\pm5.05^{x++}$	$81.57\pm4.87^{x++}$	$80.73\pm4.68^{xx++}$
Correlation coefficient (*r*)	0.91	0.92	0.93	0.93	0.95
Biomaterial dosage (4.0 g)					
03.84[a]	$2.15\pm0.09^{\Phi x+}$	$1.87\pm0.08^{x+}$	$1.69\pm0.07^{x+}$	$1.53\pm0.06^{x+}$	$1.52\pm0.06^{xx+}$
19.23	$6.65\pm0.31^{x+}$	$5.69\pm0.26^{x+}$	$4.76\pm0.21^{x+}$	$4.11\pm0.18^{x+}$	$4.11\pm0.17^{xx+}$
38.46	$7.96\pm0.36^{x+}$	$6.34\pm0.32^{x+}$	$5.39\pm0.27^{x+}$	$4.23\pm0.21^{x+}$	$4.19\pm0.20^{xx+}$
96.15	$17.19\pm0.92^{x+}$	$11.42\pm0.56^{x+}$	$7.42\pm0.41^{x+}$	$41.88\pm0.27^{x+}$	$4.69\pm0.26^{xx+}$
192.30	$34.23\pm1.90^{x+}$	$22.65\pm1.33^{x+}$	$14.69\pm0.86^{x+}$	**$10.23\pm0.54^{x+}$**	$9.96\pm0.52^{xx+}$
384.61	$68.31\pm3.81^{x++}$	$45.11\pm2.66^{x++}$	$29.26\pm1.72^{x++}$	$18.26\pm1.12^{x++}$	$17.76\pm1.10^{xx++}$
Correlation coefficient (*r*)	0.92	0.94	0.98	0.96	0.96

Table 2 (continued)

Initial conc. (μM)	Time interval (minutes)				
	10	20	30	40	60
Biomaterial dosage (6.0 g)					
03.84[a]	$2.11\pm0.09^{\Phi\Phi x+}$	$1.88\pm0.88^{x+}$	$1.65\pm0.07^{x+}$	$1.53\pm0.07^{x+}$	$1.53\pm0.07^{xx+}$
19.23	$6.57\pm0.31^{x+}$	$5.65\pm0.28^{x+}$	$4.61\pm0.23^{x+}$	$4.07\pm0.21^{x+}$	$4.03\pm0.20^{xx+}$
38.46	$7.89\pm0.39^{x+}$	$6.23\pm0.33^{x+}$	$5.19\pm0.25^{x+}$	$4.11\pm0.22^{x+}$	$4.07\pm0.21^{xx+}$
96.15	$16.84\pm0.87^{x+}$	$11.03\pm0.60^{x+}$	$7.01\pm0.38^{x+}$	$4.61\pm0.25^{x+}$	$4.30\pm0.24^{xx+}$
192.30	$33.14\pm1.81^{x+}$	$21.80\pm1.24^{x+}$	$13.69\pm0.78^{x+}$	$8.92\pm0.50^{x+}$	$9.07\pm0.45^{xx+}$
384.61	$66.81\pm3.65^{x++}$	$43.42\pm2.51^{x++}$	$27.11\pm1.57^{x++}$	$17.61\pm1.02^{x++}$	$15.82\pm0.95^{xx++}$
Correlation coefficient (r)	0.90	0.92	0.93	0.95	0.96

Mean difference [initial Cr(VI) loaded (μM) versus soluble Cr(VI) (μM)] as functions of time. ^{x}significant ($p < 0.10$), ^{xx}insignificant ($p > 0.10$).
Metal concentration $^{+}$significant ($p < 0.01$), $^{++}$insignificant ($p > 0.01$).
Biomass dosage $^{\Phi}$significant ($p < 0.01$), $^{\Phi\Phi}$insignificant ($p > 0.01$).
[a]Number in parenthesis represents soluble metal concentrations in μM.
[b]Standard deviation values of replicate ($N = 5$) determinations.

Table 3 This table explains soluble Ni(II) ion concentration (μM) after adsorption on shelled *Moringa oleifera* seeds (SMOS) as functions of contact time and biomass dosage at volume (200 mL), particle size (105 μm), and pH (6.5)

	Time interval (minutes)				
Initial conc. (μM)	10	20	30	40	60
Biomass dosage (2.0 g)					
03.41[a]	$02.73\pm0.15^{bx+\Phi}$	$02.45\pm0.13^{x+}$	$02.28\pm0.12^{x+}$	$02.18\pm0.11^{x+}$	$02.18\pm0.10^{xx+}$
17.06	$11.94\pm0.54^{x+}$	$10.85\pm0.52^{x+}$	$10.20\pm0.51^{x+}$	$09.72\pm0.48^{x+}$	$09.69\pm0.50^{xx+}$
34.12	$22.01\pm1.01^{x+}$	$19.72\pm0.92^{x+}$	$18.08\pm0.90^{x+}$	$17.06\pm0.85^{x+}$	$17.03\pm0.85^{xx+}$
85.32	$48.46\pm2.16^{x+}$	$42.45\pm2.16^{x+}$	$39.21\pm1.96^{x+}$	$36.58\pm1.82^{x+}$	$36.48\pm1.75^{xx+}$
170.64	$96.72\pm4.64^{x++}$	$84.67\pm4.14^{x++}$	$78.25\pm3.91^{x++}$	$72.90\pm3.64^{x++}$	$72.76\pm3.63^{xx++}$
341.29	$193.17\pm9.65^{x++}$	$169.07\pm7.60^{x++}$	$156.00\pm7.02^{x++}$	$145.56\pm6.98^{x++}$	$145.25\pm6.97^{xx++}$
Correlation coefficient (r)	0.96	0.97	0.97	0.98	0.99
Biomass dosage (4.0 g)					
03.41[a]	$01.97\pm0.10^{x+\Phi}$	$01.60\pm0.09^{x+}$	$01.39\pm0.07^{x+}$	$01.33\pm0.07^{x+}$	$01.33\pm0.07^{xx+}$
17.06	$08.49\pm0.45^{x+}$	$06.99\pm0.37^{x+}$	$06.14\pm0.34^{x+}$	$05.76\pm0.29^{x+}$	$05.76\pm0.29^{xx+}$
34.12	$14.94\pm0.79^{x+}$	$12.49\pm0.66^{x+}$	$11.26\pm0.58^{x+}$	$10.22\pm0.52^{x+}$	$10.20\pm0.51^{xx+}$
85.32	$32.80\pm1.73^{x+}$	$26.05\pm1.38^{x+}$	$22.78\pm1.13^{x+}$	$20.78\pm1.07^{x+}$	$20.71\pm1.03^{xx+}$
170.64	$65.49\pm3.53^{x++}$	$52.01\pm2.80^{x++}$	$45.42\pm2.36^{x++}$	$41.39\pm2.06^{x++}$	$41.29\pm2.06^{xx++}$
341.29	$130.71\pm6.79^{x++}$	$103.72\pm5.39^{x++}$	$90.61\pm4.80^{x++}$	$82.55\pm4.29^{x++}$	$82.32\pm4.11^{xx++}$
Correlation coefficient (r)	0.96	0.97	0.95	0.98	0.99

Table 3 (continued)

Initial conc. (μM)	Time interval (minutes)				
	10	20	30	40	60
Biomass dosage (6.0 g)					
03.41	$01.97\pm0.11^{x+\Phi\Phi}$	$01.60\pm0.09^{x+`}$	$01.39\pm0.08^{x+}$	$01.33\pm0.07^{x+}$	$01.33\pm0.08^{xx+}$
17.06	$08.49\pm0.45^{x+}$	$06.99\pm0.37^{x+}$	$06.14\pm0.34^{x+}$	$05.76\pm0.29^{x+}$	$05.76\pm0.29^{xx+}$
34.12	$14.91\pm0.76^{x+}$	$12.45\pm0.67^{x+}$	$11.22\pm0.60^{x+}$	$10.20\pm0.53^{x+}$	$10.17\pm0.52^{xx+}$
85.32	$32.74\pm1.57^{x+}$	$25.98\pm1.35^{x+}$	$22.68\pm1.22^{x+}$	$20.71\pm1.11^{x+}$	$20.64\pm1.07^{xx+}$
170.64	$65.35\pm3.59^{x++}$	$51.80\pm2.84^{x++}$	$45.25\pm2.39^{x++}$	$41.26\pm2.18^{x++}$	$41.12\pm2.17^{xx++}$
341.29	$130.30\pm6.25^{x++}$	$103.31\pm5.16^{x++}$	$90.23\pm4.51^{x++}$	$82.21\pm4.27^{x++}$	$82.01\pm4.26^{xx++}$
Correlation coefficient (r)	0.95	0.95	0.93	0.95	0.96

Mean difference [initial Cr(III) loaded (μM) versus Cr(III) (μM)] as functions of time. ^{x}significant ($p < 0.10$), ^{xx}insignificant ($p > 0.10$).
Metal concentration $^{+}$significant ($p < 0.01$), $^{++}$insignificant ($p > 0.01$).
Biomaterial dosage $^{\Phi}$significant ($p < 0.01$), $^{\Phi\Phi}$insignificant ($p > 0.01$).
[a]Number in parenthesis represents metal concentrations in μM.
[b]Standard deviation values of replicate ($N = 3$) determinations.

Table 4 This table explains Cr(III) ion concentrations (μM) after adsorption on *Zea mays* cob powder (ZMCP) as functions of contact time and biomaterial dosage at volume (200 mL), particle size (105 μm), and pH (6.5)

	Time interval (minutes)				
Initial conc. (μM)	10	20	30	40	60
Biomaterial dosage (1.0 g)					
03.84[a]	$3.34\pm0.15^{bx+\Phi}$	$3.21\pm0.15^{x+}$	$3.09\pm0.13^{x+}$	$2.91\pm0.13^{x+}$	$2.89\pm0.13^{xx+}$
19.23	$12.91\pm0.57^{x+}$	$12.14\pm0.56^{x+}$	$11.35\pm0.51^{x+}$	$10.67\pm0.49^{x+}$	$10.64\pm0.49^{xx+}$
38.46	$20.07\pm0.96^{x+}$	$19.29\pm0.92^{x+}$	$17.09\pm0.83^{x+}$	$15.51\pm0.75^{x+}$	$15.49\pm0.74^{xx+}$
96.15	$46.12\pm2.34^{x+}$	$37.86\pm1.92^{x+}$	$36.19\pm1.87^{x+}$	$34.59\pm1.83^{x+}$	$34.52\pm1.82^{xx+}$
192.30	$92.32\pm5.15^{x++}$	$75.35\pm4.21^{x++}$	$71.84\pm4.09^{x++}$	$68.85\pm3.99^{x++}$	$68.71\pm3.98^{xx++}$
384.61	$183.84\pm10.84^{x++}$	$150.53\pm8.72^{x++}$	$143.46\pm8.46^{x++}$	$137.49\pm8.11^{x++}$	$137.29\pm8.09^{xx++}$
Correlation coefficient (r)	0.98	0.97	0.98	0.98	0.98
Biomaterial dosage (2.0 g)					
03.84[a]	$2.89\pm0.12^{\Phi x+}$	$2.67\pm0.11^{x+}$	$2.28\pm0.10^{x+}$	$2.09\pm0.09^{x+}$	$2.02\pm0.09^{xx+}$
19.23	$11.15\pm0.52^{x+}$	$9.27\pm0.43^{x+}$	$7.49\pm0.35^{x+}$	$6.46\pm0.30^{x+}$	$6.41\pm0.29^{xx+}$
38.46	$19.05\pm0.91^{x+}$	$14.24\pm0.69^{x+}$	$11.77\pm0.57^{x+}$	$10.72\pm0.52^{x+}$	$10.67\pm0.52^{xx+}$
96.15	$41.31\pm2.02^{x+}$	$26.29\pm1.28^{x+}$	$23.59\pm1.15^{x+}$	$22.37\pm1.09^{x+}$	$21.32\pm1.04^{xx+}$
192.30	$82.42\pm4.20^{x++}$	$52.23\pm2.66^{x++}$	$46.92\pm2.39^{x++}$	$44.42\pm2.35^{x++}$	$41.99\pm2.17^{xx++}$
384.61	$164.57\pm8.83^{x++}$	$104.24\pm5.62^{x++}$	$93.56\pm5.05^{x++}$	$88.59\pm4.87^{x++}$	$83.78\pm4.68^{xx++}$
Correlation coefficient (r)	0.95	0.95	0.96	0.97	0.97
Biomaterial dosage (4.0 g)					
03.84[a]	$2.19\pm0.09^{\Phi x+}$	$1.91\pm0.08^{x+}$	$1.72\pm0.07^{x+}$	$1.57\pm0.06^{x+}$	$1.57\pm0.06^{xx+}$
19.23	$6.69\pm0.31^{x+}$	$5.72\pm0.26^{x+}$	$4.81\pm0.21^{x+}$	$4.18\pm0.18^{x+}$	$4.17\pm0.17^{xx+}$
38.46	$8.12\pm0.36^{x+}$	$6.43\pm0.32^{x+}$	$5.42\pm0.27^{x+}$	$4.29\pm0.21^{x+}$	$4.23\pm0.20^{xx+}$
96.15	$17.21\pm0.92^{x+}$	$11.46\pm0.56^{x+}$	$7.46\pm0.41^{x+}$	$\mathbf{4.88\pm0.27^{x+}}$	$4.53\pm0.26^{xx+}$
192.30	$34.27\pm1.90^{x++}$	$22.69\pm1.33^{x++}$	$14.74\pm0.86^{x++}$	$9.28\pm0.54^{x++}$	$12.02\pm0.52^{xx++}$
384.61	$68.36\pm3.81^{x++}$	$45.11\pm2.66^{x++}$	$29.35\pm1.72^{x++}$	$18.32\pm1.12^{x++}$	$24.82\pm1.10^{xx++}$
Correlation coefficient (r)	0.92	0.93	0.96	0.98	0.97

Table 4 (continued)

Initial conc. (μM)	Time interval (minutes)				
	10	20	30	40	60
Biomaterial dosage (6.0 g)					
03.84[a]	$2.16\pm0.09^{\Phi\Phi x+}$	$1.92\pm0.88^{x+}$	$1.69\pm0.07^{x+}$	$1.57\pm0.07^{x+}$	$1.51\pm0.07^{xx+}$
19.23	$6.63\pm0.31^{x+}$	$5.69\pm0.28^{x+}$	$4.64\pm0.23^{x+}$	$4.13\pm0.21^{x+}$	$4.08\pm0.20^{xx+}$
38.46	$7.93\pm0.39^{x+}$	$6.27\pm0.33^{x+}$	$5.24\pm0.25^{x+}$	$4.14\pm0.22^{x+}$	$4.13\pm0.21^{xx+}$
96.15	$16.89\pm0.87^{x+}$	$11.07\pm0.60^{x+}$	$7.07\pm0.38^{x+}$	$4.65\pm0.25^{x+}$	$11.34\pm0.24^{xx+}$
192.30	$33.58\pm1.81^{x++}$	$21.85\pm1.24^{x++}$	$13.73\pm0.78^{x++}$	$8.97\pm0.50^{x++}$	$11.92\pm0.45^{xx++}$
384.61	$66.89\pm3.65^{x++}$	$43.46\pm2.51^{x++}$	$27.19\pm1.57^{x++}$	$17.57\pm1.02^{x++}$	$24.75\pm0.95^{xx++}$
Correlation coefficient (r)	0.90	0.92	0.93	0.95	0.97

Mean difference [initial Cr(III) loaded (μM) versus Cr(III) (μM)] as functions of time. ^{x}significant ($p < 0.10$), ^{xx}insignificant ($p > 0.10$).
Metal concentration $^{+}$significant ($p < 0.01$), $^{++}$insignificant ($p > 0.01$).
Biomaterial dosage $^{\Phi}$significant ($p < 0.01$), $^{\Phi\Phi}$insignificant ($p > 0.01$).
[a]Number in parenthesis represents metal concentrations in μM.
[b]Standard deviation values of replicate ($N = 3$) determinations.

Table 5 This table explains Pb(II) ion concentrations (μM) after adsorption on *S. indica* leaf powder (SILP) as functions of contact time and biomaterial dosage at volume (200 mL), particle size (105 μm), and pH (6.5)

	Time interval (minutes)			
Initial conc. ppm/(μM)	10	20	30	40
Biomass dosage (1.0 g)				
01 (03.84)[a]	$01.24\pm0.12^{bx+\Phi}$	$01.01\pm0.11^{x+}$	$0.45\pm0.09^{x+}$	$0.45\pm0.09^{xx+}$
05 (19.23)	$14.11\pm0.56^{x+}$	$12.38\pm0.49^{x+}$	$10.76\pm0.45^{x+}$	$10.74\pm0.44^{xx+}$
10 (38.46)	$24.69\pm1.02^{x+}$	$21.61\pm0.94^{x+}$	$19.57\pm0.83^{x+}$	$19.54\pm0.82^{xx+}$
25 (96.15)	$55.46\pm2.33^{x+}$	$46.50\pm1.96^{x+}$	$40.96\pm1.76^{x+}$	$40.80\pm1.74^{xx+}$
50 (192.3)	$110.92\pm4.92^{x++}$	$93.01\pm4.84^{x++}$	$81.92\pm3.83^{x++}$	$81.60\pm3.82^{xx++}$
100 (384.61)	$221.82\pm11.3^{x++}$	$118.4\pm9.6^{x++}$	$163.8\pm8.76^{x++}$	$162.7\pm8.74^{xx++}$
Correlation coefficient (*r*)	0.97	0.97	0.98	0.99
Biomass dosage (2.0 g)				
01 (0.96)[a]	$0.51\pm0.03^{bx+\Phi}$	$0.44\pm0.02^{x+}$	$0.37\pm0.02^{x+}$	$0.37\pm0.02^{xx+}$
05 (4.82)	$2.12\pm0.11^{x+}$	$1.75\pm0.09^{x+}$	$1.46\pm0.08^{x+}$	$1.46\pm0.08^{xx+}$
10 (9.65)	$3.56\pm0.23^{x+}$	$2.92\pm0.21^{x+}$	$2.41\pm0.18^{x+}$	$2.36\pm0.14^{xx+}$
25 (24.13)	$7.79\pm0.53^{x+}$	$6.13\pm0.48^{x+}$	**$4.89\pm0.39^{x+}$**	$4.88\pm0.23^{xx+}$
50 (48.26)	$15.54\pm1.19^{x++}$	$12.25\pm1.08^{x++}$	$9.74\pm0.92^{x++}$	$9.60\pm0.78^{xx++}$
100 (96.52)	$31.02\pm2.84^{x++}$	$24.38\pm2.6^{x++}$	$19.4\pm2.43^{x++}$	$19.3\pm2.17^{xx++}$
Correlation coefficient (*r*)	0.97	0.97	0.98	0.99

Table 5 (continued)

Initial conc. ppm/(μM)	Time interval (minutes)			
	10	20	30	40
Biomass dosage (6.0 g)				
01 (0.96)[a]	$0.39\pm0.02^{x+\Phi\Phi}$	$0.30\pm0.02^{x+}$	$0.25\pm0.04^{x+}$	$0.24\pm0.04^{xx+}$
05 (4.82)	$1.46\pm0.08^{x+}$	$1.08\pm0.06^{x+}$	$0.84\pm0.05^{x+}$	$0.81\pm0.05^{xx+}$
10 (9.65)	$2.25\pm0.16^{x+}$	$1.43\pm0.12^{x+}$	$0.88\pm0.07^{x+}$	$0.87\pm0.03^{xx+}$
25 (24.13)	$4.50\pm0.35^{x+}$	$2.45\pm0.24^{x+}$	$1.13\pm0.12^{x+}$	$1.09\pm0.09^{xx+}$
50 (48.26)	$8.96\pm1.01^{x++}$	$4.86\pm0.89^{x++}$	$2.12\pm0.75^{x++}$	$2.09\pm0.64^{xx++}$
100 (96.52)	$17.8\pm2.51^{x++}$	$9.68\pm2.3^{x++}$	$4.21\pm2.18^{x++}$	$4.09\pm1.89^{xx++}$
Correlation coefficient (r)	0.96	0.96	0.98	0.97

Mean difference [initial Pb(II) loaded (μM) versus soluble Pb(II) (μM)] as functions of time. ^{x}significant ($p < 0.05$), ^{xx}insignificant ($p > 0.05$).
Metal concentration $^{+}$significant ($p < 0.05$), $^{++}$insignificant ($p > 0.05$).
Biomass dosage $^{\Phi}$significant ($p < 0.05$), $^{\Phi\Phi}$insignificant ($p > 0.05$).
[a]Number in parenthesis represents soluble metal concentrations in μM.
[b]Standard deviation values of replicate ($N = 3$) determinations.

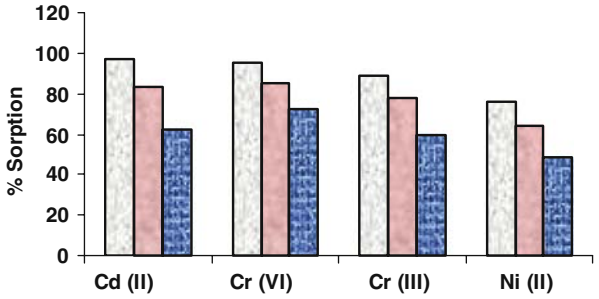

Fig. 1 Effect of particle size on metal sorption at LLSP (Time: 40 min, biomaterial: 4.0 g)

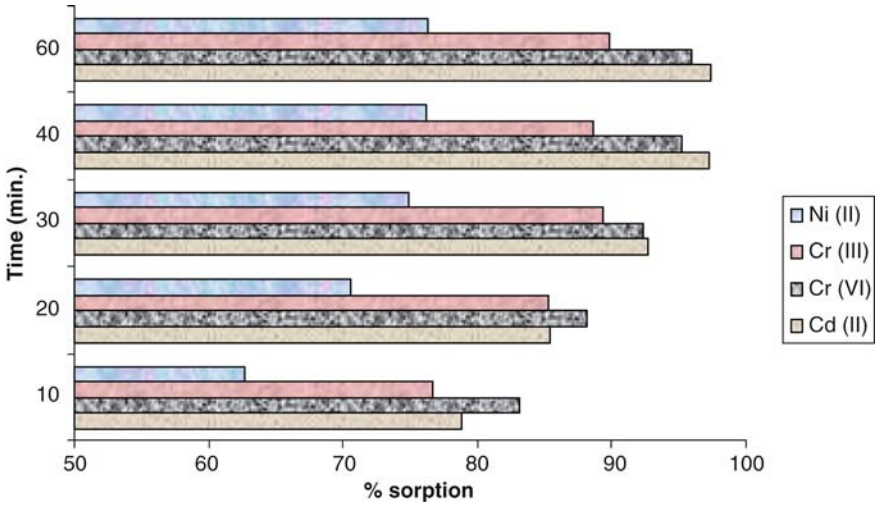

Fig. 2 Effect of contact time on metal sorption at ZMCP (Conc. initial: 25 mg/L for Cd(II), Cr(III), and Ni(II) and 50 mg/L for Cr(VI); biomaterial: 4.0 g)

It has been observed that initially there is increase in sorption with the increase in time and finally attaining a maximum value; however, any further increase in contact time may not result in increase in the sorption efficiency (Fig. 2).

Effect of Biomaterial Dosage on Metal Sorption

The amount of biomaterial seems to influence the extent of uptake of metals. Hence, this factor needs to be taken into consideration in application of any biomaterial as biosorbent. Therefore, sorption efficiency of biosorbents for different metals may be evaluated as a function of biomaterial dosage leading to standardization of optimum amount of biomaterial required. Percentage sorption versus biomaterial dosage indicates, in general, sorption efficiency of different metal ions increased with increase of biomaterial dosage attaining a maximum value; however, further increase in

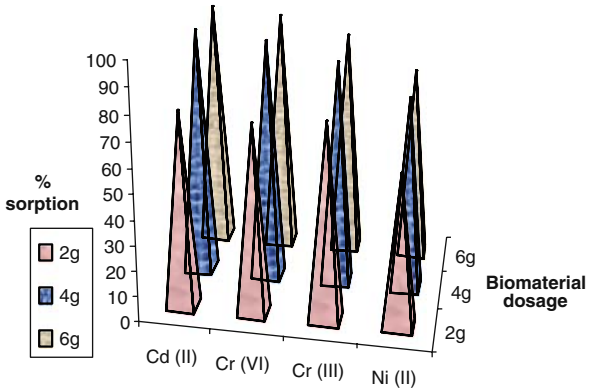

Fig. 3 Effect of biomaterial dosage on metal sorption at (SMOS) (Conc. initial: 25 mg/L for Cd(II), Cr(III), Ni(II) and 50 mg/L for Cr(VI); time: 40 min)

dosage does not result in increase in the sorption efficiency. Figure 3 shows the effect of biomaterial dosage on metal sorption.

The basis of attainment of equilibrium between adsorbate and adsorbent at the existing operating conditions is likely to render the adsorbent incapable of further adsorption.

Effect of Concentration on Metal Sorption

The sorption capacity of the biomaterial at a fixed amount for different metal ions present in the various concentration ranges can be calculated. Percent sorption of biosorbents is to be calculated in each case.

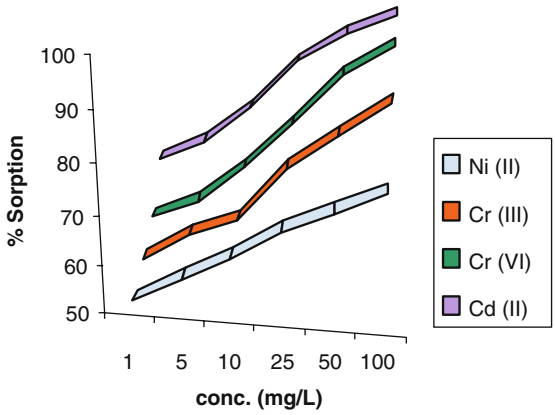

Fig. 4 Effect of metal concentration on metal sorption at SILP (Time: 40 min, biomaterial: 4.0 g)

Biosorption efficiency, in general, increases initially; however, further increase in concentration may not result in the sorption efficiency (Fig. 4).

Although actual wastewater treatment systems have to deal with a mixture of heavy metals, most research activities are single metal sorption oriented and not realistic; therefore, the assessment of the biosorption performance becomes less accurate. Multi-metal biosorption studies are, therefore, particularly important for evaluating the degree of interference with a biosorption process of common metal ions in wastewater.

Mechanistic Aspects of Sorption

The pH condition of the solution is an extremely important parameter in metal biosorption. It governs a series of phenomena like site dissociation, solubility, mobility, and chemistry of the metals ions. pH affects the selectivity of the biomaterial to bind a variety of metals. At different pH values, binding sites are different. Heavy metals tend to bind the biomaterial at acidic pH than the pH at which the metal precipitates in hydroxide form. Record of pH profile becomes necessary since unknown reactions between the metal ions and the biomaterial might occur, modifying the extent of normal metal behavior.

Keeping the above views in mind, pH profile for metal ion binding is to be recorded for each metal. Consider the example of biosorbent [*Ficus religiosa* leaf powder (FRLP)] which may be discussed as follows. The percentage sorption of Cd(II) and Ni(II) on seed biomaterial increases as the pH of the solution increased from 2.5 to 6.5. No significant difference in sorption behavior was noticed with further increase in pH up to 7.5. The pH profile for Cd(II) and Ni(II) sorption on FRLP shows that metal sorption is a function of pH, exhibiting maximum removal efficiency at pH 6.5.

Investigation on pH variation beyond 7.5 yielded an apparent increase in sorption up to pH 8.5, which might be due to precipitation carryover of Cd(II) and Ni(II) starting at pH 7.5 onward. Cd(II) and Ni(II) precipitation is undistinguishable from sorption phenomenon at pH 7.5 (Fig. 5).

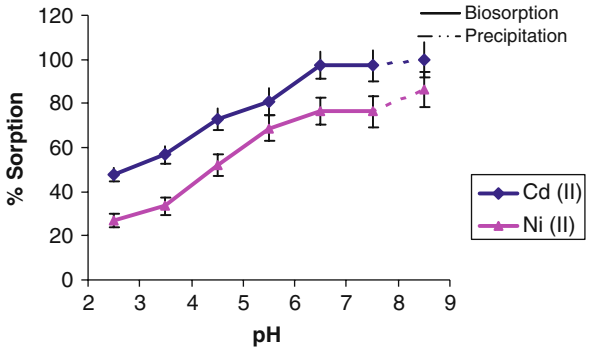

Fig. 5 pH profile and metal ion binding (Conc. initial: 25 mg/L for Cd(II) and Ni(II), biomaterial: 4 g, time: 40 min). Error bar represents standard deviation for three replicates

The biosorption of different oxidation states of metals like Cr(III) and Cr(VI) by sorbent taking the example of *L. leucocephala* seed powder (LLSP) in the pH range 2.5–8.5 has been considered. The biosorption efficiency of Cr(III) increased gradually with rise in pH from 2.5 to 6.5 attaining optimum sorption at 6.5. However, percent sorption is found to be almost constant with further rise in pH up to 7.5.

Increase in pH from 7.5 to 8.5 results in Cr(III) hydroxide precipitation. Anionic metallic species [Cr(VI)] showed high sorption tendency in acidic pH range (2.5–3.5). Further increase in pH from 3.5 to 8.5 resulted in a sharp decreasing trend of sorption (Fig. 6).

A possible mechanism for metal binding to the biosorbent may be designed. The aqueous solution of biosorbents, in general, is a heterogeneous complex mixture having various functional groups: protein, fat, carbohydrate, ash, and high amounts of free amino acids.

Amino acids have been found to constitute a physiologically active group of transporters, working even at low concentrations, which because of ability to interact with metal ions is likely to increase their mobility. These proteinaceous amino acids have a variety of structurally related pH-dependent properties of generating appropriate atmosphere (positively and/or negatively charged sites) for attracting the cationic and anionic species of metal ions.

pH profile and sorption behavior of various metals provide an insight into the mechanistic aspects of sorption process. Maximum sorption of metals (cationic species) is found to be in the pH range 6.5–7.5 for Cd(II), Cr(III), and Ni(II) and 2.5–3.5 for anionic species Cr(VI). Cr(III) exists in cationic forms such as Cr^{3+}, $Cr(OH)^{2+}$ in the pH range 4.0–6.0, whereas Cr(VI) exists in anionic forms such as $Cr_2O_7^{2-}$, $HCr_2O_7^{-}$, $HCrO_4^{-}$, and $CrO4^{2-}$ at low pH values 1.0−4.0.

The majority of amino acids present in biosorbents have isoelectric points in the pH range 4.0–8.0. In this range of pH, over 90% of the amino acid molecules are in ionized state, i.e., they have both positively charged amino groups and negatively

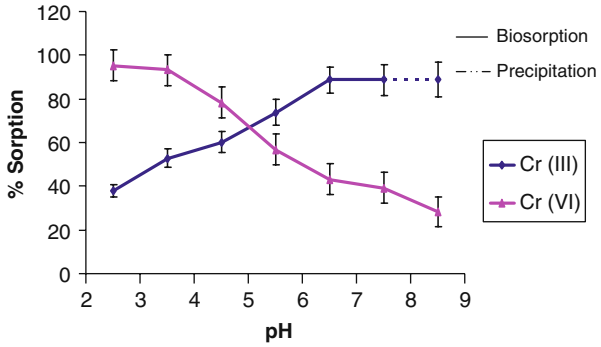

Fig. 6 pH profile and metal ion binding (Conc. initial: 25 mg/L for Cr(III) and Cr(VI), biomaterial: 4 g, time: 40 min). Error bar represents standard deviation for three replicates

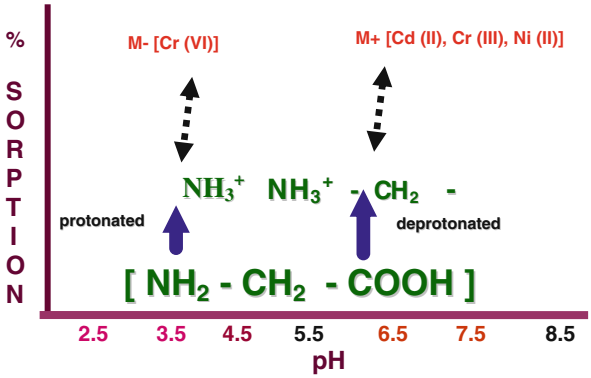

Fig. 7 Amino acid–metal interaction

charged carboxylate ions. Sorption tendency of cationic metallic species is very less at lower pH values. It may be because of lower pH; binding sites (amino acid moieties) in the biomaterial are generally protonated and thus repulsion occurs (Fig. 7).

Sorption of cationic metallic species increases with rise in pH, attaining a plateau around 6.5–7.5. At relatively higher pH (above 4.5), the carboxylic groups are deprotonated and as such are negatively charged. These negatively charged carboxylate ligands are likely to attract the cationic metallic species.

The solution chemistry of Cr(VI) clearly shows its existence as an oxoanion in several stable forms such as $Cr_2O_7^{2-}$, $HCr_2O_7^{-}$, $HCrO_4^{-}$, and CrO_4^{2}. Optimum sorption efficiency of biosorbents for Cr(VI) is found to be at pH 2.5. At lower pH (2.5), the sorbent is positively charged due to protonation of amino groups, while the sorbate, dichromate ion, exists mostly as an anion leading to electrostatic attraction between sorbent and sorbate. This fact results in increased sorption efficiency of biosorbents for anionic metallic species at low pH.

This is in support of the school of thought that metal binding is likely to be caused by interactions with functional groups such as carboxyl and amino groups located on the cell surface of the biosorbent.

In addition to metal–amino acid interactions responsible for biosorption several other hypotheses based on metal–polyphenols, metal–hydroxyl, metal–sulfhydryl, and metal–carbohydrates have also been mentioned in the literature.

Sorption Isotherms and Kinetics

The distribution of metal ions between the biosorbent and the metal solution, when the system is at equilibrium, is of paramount importance in determining the maximum adsorption capacity of the biosorbent toward the metal ions. The adsorption data are to be analyzed in the light of various isotherm models like Freundlich and Langmuir adsorption models. The Freundlich isotherm model proposes a monolayer sorption with a heterogeneous energetic distribution of active sites, accompanied by interactions between adsorbed molecules. The Langmuir isotherm model suggests that uptake occurs on a homogenous surface by monolayer sorption. In addition, the model assumes uniform energies of adsorption onto the surface and no transmigration of the adsorbate.

Freundlich Isotherm

The *Freundlich equation* has the linear form

$$q = k_{\mathrm{f}} C_{\mathrm{e}}^{1/n}$$

and the data were fitted to the logarithmic form of the equation:

$$\log q = \log K_{\mathrm{f}} + 1/n \log C_{\mathrm{e}}$$

where "q" is the uptake of metal per unit weight of biosorbent, "C_{e}" the equilibrium (residual) concentration of metal ion in solution, and "K_{f}" and "n" the characteristic constants. The biosorption capacity [K_{f} (mg/g)] and the biosorption intensity ($1/n$) are to be estimated from the intercept and slope of the Freundlich isotherm, respectively.

Values obtained from sorption experiments of different metals using biosorbents are to be used to calculate the Freundlich parameter. According to the Freundlich model, the maximum biosorption capacity [K_{f} (mg/g)] and biosorption intensity ($1/n$) obtained for all the metals under study depict the higher sorption efficiency of biosorbents for the metals. Representative Freundlich isotherm constants are presented in Tables 1 and 2.

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Table 1 Freundlich isotherm constant for single metal sorption on *Zea mays* cob powder (ZMCP)

	Freundlich constants		Correlation coefficient
Metals	K_f (mg/g)	$1/n$	R^2
Cd(II)	0.78	0.55	0.99
Cr(III)	0.80		0.99
Pb(II)	0.72	0.52	0.99
Cr(VI)	0.67	0.60	0.93
Ni(II)	0.60	0.64	0.96

Table 2 Freundlich isotherm constant for single metal sorption on *Leucaena leucocephala s*eed powder (LLSP)

	Freundlich constants		Correlation coefficient
Metals	K_f (mg/g)	$1/n$	R^2
Cd(II)	3.03	0.16	0.99
Cr(III)	2.93	0.27	0.98
Ni(II)	2.67	0.38	0.97
Cr(VI)	2.98	0.23	0.98

Langmuir Isotherm

The *Langmuir equation* has the linear form of

$$C_e/q_e = (1/Q_o b) + (C_e/Q_b)$$

where "C_e" is the equilibrium concentration, "q_e" is the amount of metals adsorbed at equilibrium, and "Q_o" and "b" are the Langmuir constants related to adsorption capacity (mg/g) and energy of adsorption (L/mg), respectively. "Q_o" and "b" are to be determined from the slope and intercept of the Langmuir isotherm, respectively.

Similarly, the values of biosorption capacity (Q_o (mg/g)) and biosorption energy (b (L/mg)) obtained from Langmuir model for the biosorbents represent their favorable sorption. Magnitude of Freundlich and Langmuir constants showed the easy separation of metal ions from water bodies. Representative Langmuir isotherm constants are presented in Tables 3 and 4.

High values of correlation coefficient (R^2) indicate that the adsorption pattern for the metals of biomass used followed Langmuir and Freundlich isotherms. The linearity of Freundlich (Figs. 1 and 2) and Langmuir plots suggested the formation of homogenous monolayer of metals on the outer surface of the biosorbent (Figs. 3 and 4).

Table 3 Langmuir isotherm constants for single metal sorption on shelled *Moringa oleifera* seeds (SMOS)

	Langmuir constants		Correlation coefficient
Metals	Q_o (mg/g)	b (L/mg)	R^2
Pb(II)	2.98	0.07	0.99
Cd(II)	2.91	0.10	0.99
Cr(III)	2.63	0.24	0.98
Cr(VI)	2.78	0.21	0.97
Ni(II)	2.51	0.36	0.96

Table 4 Langmuir isotherm constants for single metal sorption on *Saraca indica* leaf powder (SILP)

	Langmuir constants		Correlation coefficient
Metals	Q_o (mg/g)	b (L/mg)	R^2
Cd(II)	2.03	0.12	0.99
Pb(II)	2.01	0.09	0.99
Cr(III)	2.73	0.22	0.98
Ni(II)	2.49	0.19	0.97
Cr(VI)	2.78	0.35	0.98

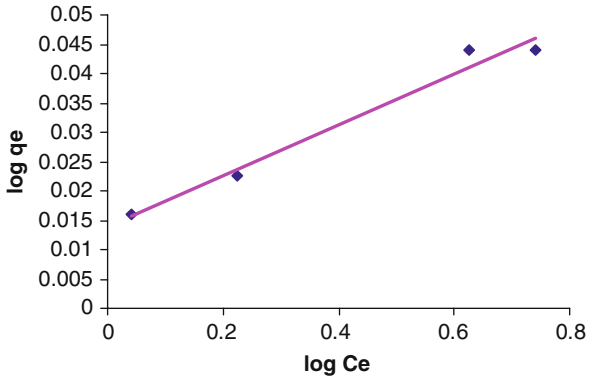

Fig. 1 Freundlich isotherm plot for adsorption of Cr(III) at (FRLP) (Conc. initial: 25 mg/L, biomaterial: 4.0 g, time: 40 min)

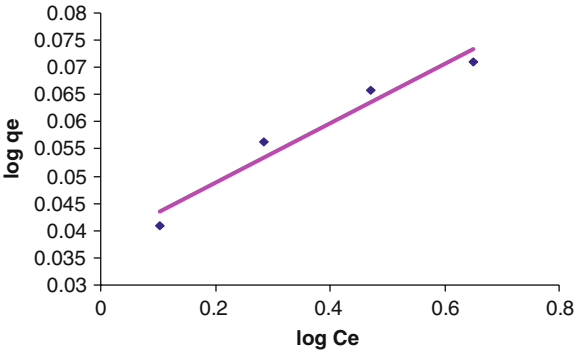

Fig. 2 Freundlich isotherm plot for adsorption of Cr(VI) at (LLSP) (Conc. initial: 25 mg/L, biomaterial: 4.0 g, time: 40 min)

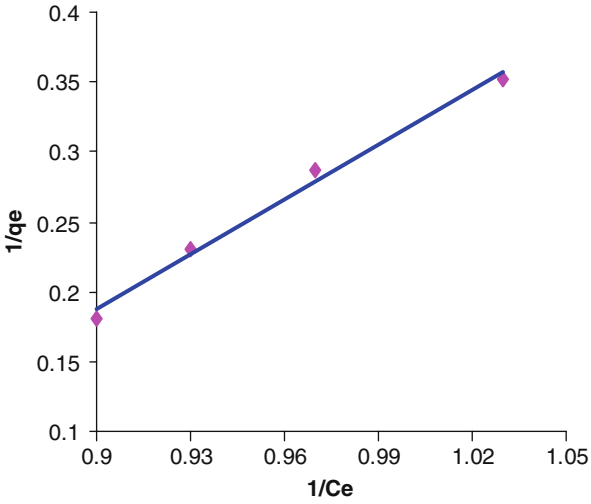

Fig. 3 Langmuir isotherm plot for adsorption of Cr(III) at (SMOS) (Conc. initial: 25 mg/L, biomaterial: 4.0 g, time: 40 min)

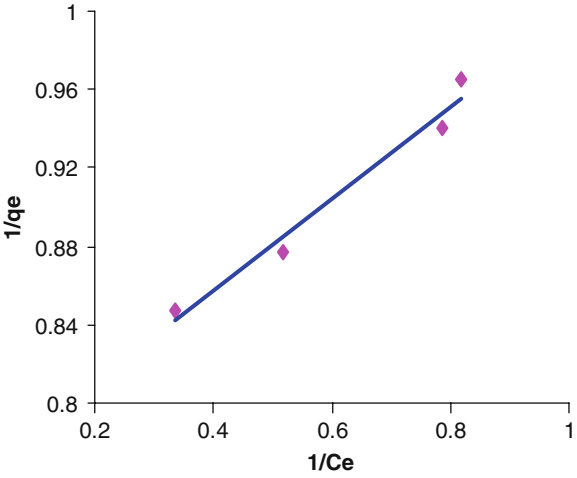

Fig. 4 Langmuir isotherm plot for adsorption of Cr(VI) at (SILP) [(Conc. initial: 25 mg/L, biomaterial: 4.0 g, time: 40 min)]

Reusability of Biomaterial: A Cost-Effective Approach

Regeneration of metal-treated biosorbent is an important aspect of cost-effectiveness of wastewater treatment. In general, desorption behavior of metals from biomaterials is usually carried out by using an appropriate stripping agent. Desorption behavior of metal ions from biosorbents is to be observed after eluting with different stripping agents (soft acid, hard acid, base, and distilled water).

The process becomes more lucrative if the active agent can be regenerated through desorption cycle without destroying the integrity of the cell wall. In order to design the proposed process of sorption to be more economical, attempts are to be made to regenerate the metal-treated biomaterial for its effective reuse. The recyclability of biosorbents with desorption reagents in the acid, basic, and neutral media is considered at varying contact times. Tables 1 and 2 represent desorption of metal ions using *soft acid* (0.5 M citric acid) as eluant as well as sorption of regenerated biomaterial cycles taking the example of ZMCP.

This behavior clearly indicates that the present biosorbents can be used after regeneration for the sorption of metals from wastewater system. Some data related with regeneration of biomass used for desorption of various metals are tabulated.

Tables 3 and 4 represent desorption of metal ions using *hard acid* (0.05 M of hydrochloric and nitric acids) as eluant followed by desorption on regenerated biomaterial.

Table 1 Desorption of single metal (25 mg/L: Pb(II), Cd(II), Cr(III), and Ni(II); 50 mg/L: Cr(VI)) ions from *Zea mays* cob powder (ZMCP) using 0.5 M of citric acid

No. of cycles	Desorption (%)				
	Pb(II)	Cd(II)	Cr(III)	Ni(II)	Cr(VI)
1	52.43	51.03	48.18	53.18	53.53
2	52.47	51.07	48.89	53.28	53.84
3	52.52	51.12	49.13	53.34	54.08
4	53.45	52.45	49.41	53.32	54.23
5	53.73	52.70	49.53	53.49	54.46

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Table 2 Sorption of single metal (25 mg/L: Pb(II), Cd(II), Cr(III), and Ni(II); 50 mg/L: Cr(VI)) ions on regenerated biomaterial

No. of cycles	Sorption (%)				
	Pb(II)	Cd(II)	Cr(III)	Ni(II)	Cr(VI)
1	94.72	92.89	81.02	71.53	86.34
2	95.10	93.67	81.76	72.64	87.73
3	96.03	94.67	82.28	73.71	88.01
4	96.02	94.79	82.39	73.87	88.21
5	74.30	72.76	72.15	63.28	73.01

Table 3 Desorption of single metal (25 mg/L: Pb(II), Cd(II), Cr(III), and Ni(II); 50 mg/L: Cr(VI)) ions from *Z. mays* cob powder (ZMCP) using 0.05 M of hydrochloric acid

No. of cycles	Desorption (%)				
	Pb(II)	Cd(II)	Cr(III)	Ni(II)	Cr(VI)
1	92.45	92.18	92.47	91.21	92.49
2	92.58	92.29	92.69	91.42	92.78
3	94.12	93.27	92.97	91.48	93.21
4	95.03	94.08	93.28	91.94	93.27

Table 4 Sorption of single metal (25 mg/L: Cd(II), Cr(III), and Ni(II); 50 mg/L: Cr(VI)) ions on regenerated biomaterial

No. of cycles	Sorption (%)				
	Pb(II)	Cd(II)	Cr(III)	Ni(II)	Cr(VI)
1	94.35	92.79	83.71	73.61	91.42
2	95.78	93.21	84.42	73.69	91.78
3	96.08	94.81	82..42	73..83	88.19
4	75.21	74.38	72.25	63.35	75.11

In general, extent of metal desorption increases with increase in strength of acids ranging from 0.01 to 0.05 M. The maximum desorption was by hydrochloric acid (0.05 M).

Sorption of metals on regenerated *biosorbents* remained constant initially then started decreasing. A better desorption is achieved with the same strength (0.05 M) of nitric acid as eluant (Tables 5 and 6).

The amount of metal ion remaining on the biomaterial as a function of time was calculated using the mass balance equation $q_t = q_e - c_t$ (v/m), where q_t and q_e are the biomaterial phase metal ion concentration (mg/L) and c_t solution phase metal ion concentration (mg/L) at time t (min).

Desorption in the acidic media for metal ions appeared to be rapid and higher than in basic and neutral media. In the basic media, less than 20% of the metal ions were recovered from the metal-laden biomaterial. Insignificant level (<4%) of

Table 5 Desorption of single metal (25 mg/L: Cd(II), Cr(III), and Ni(II); 50 mg/L: Cr(VI)) ions from *Z. mays* cod powder (ZMCP) using 0.05 M of nitric acid

No. of cycles	Desorption (%)				
	Pb(II)	Cd(II)	Cr(III)	Ni(II)	Cr(VI)
1	98.56	98.23	99.12	98.31	98.56
2	98.89	98.72	99.43	98.76	98.89
3	99.25	99.19	99.61	99.23	99.12
4	99.28	99.22	99.82	99.57	99.35

Table 6 Sorption of single metal (25 mg/L: Cd(II), Cr(III), and Ni(II); 50 mg/L: Cr(VI)) ions on regenerated biomaterial

No. of cycles	Sorption (%)				
	Pb(II)	Cd(II)	Cr(III)	Ni(II)	Cr(VI)
1	94.87	92.85	83.78	73.68	91.48
2	95.12	93.28	84.49	73.73	91.82
3	96.05	94.76	82.42	73.81	88.13
4	76.43	74.43	72.31	63.48	75.23

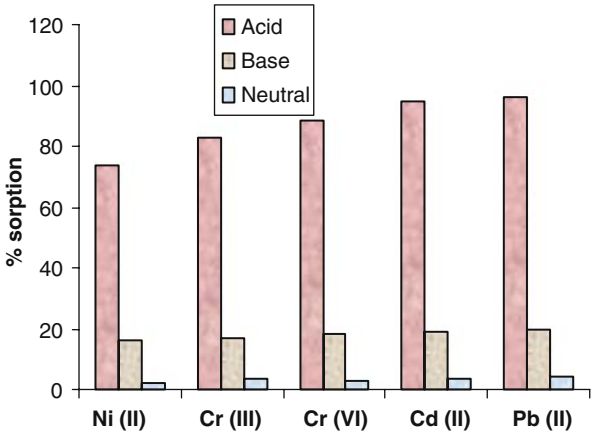

Fig. 1 Effect of desorption media on the recovery of Cd(II), Cr(III), and Ni(II) from metal-loaded SILP

desorption was recorded for distilled water. It is inferred from Fig. 1 that desorption in the acidic media for metal ions using *Saraca indica* leaf powder (SILP) was rapid and higher than in basic and neutral media.

The kinetics of desorption assesses the overall performance of desorbing reagent. The pseudo-first-order kinetics of desorption K_{des} was used to evaluate the release constant. The larger the value of K_{des}, the greater the desorption. The release constant, K_{des}, and value of desorbable fraction (θ) for all metal ions as obtained

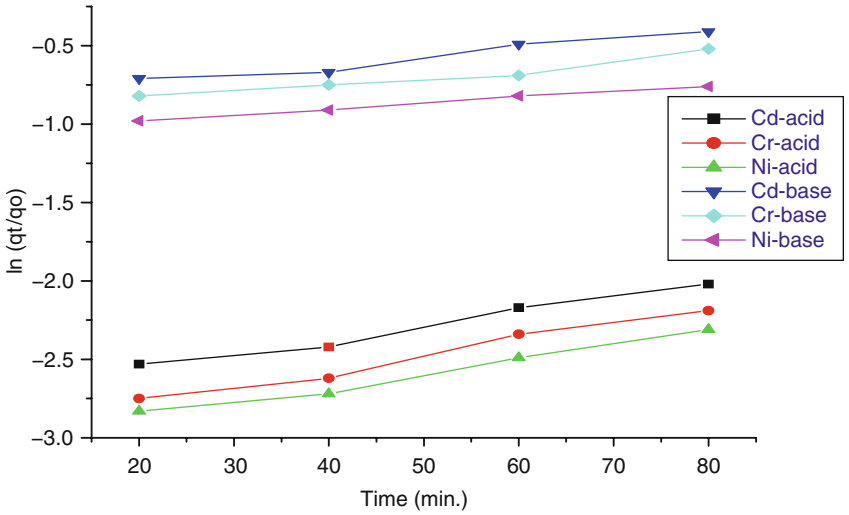

Fig. 2 Pseudo-first-order desorption kinetics on the recovery of metal ions from metal-loaded LLSP

Table 7 Value of release constant (K_{des}) and desorbable fraction (θ) of Cd(II), Ni(II), and Cr(III)

Desorption reagent	Cd(II)		Ni(II)		Cr(III)	
	K_{des}/min	θ	K_{des}/min	θ	K_{des}/min	θ
0.05 M HCl	7.86×10^{-2}	0.89	8.56×10^{-2}	0.78	8.17×10^{-2}	0.83
0.05 M NaOH	3.27×10^{-2}	0.23	2.56×10^{-2}	0.01	3.02×10^{-2}	0.13

from the regression lines (Fig. 2) are presented in Table 7. The release constant of the acid reagent was double than the basic reagent. Among the acid eluants, hard acid [(0.05 M) hydrochloric acid and nitric acid] showed higher desorption, while comparatively low desorption rate was achieved with soft acid (0.5 M citric acid). Sorption of metals on regenerated LLSP biomaterial with hydrochloric acid and nitric acid (0.05 M) remains constant up to three cycles and then started decreasing. Citric acid (0.5 M) elution of the metals resulted in the reusability of the biomaterial for four cycles. It is to think that we should have better desorption by non-eco-friendly hard acid or less with soft green acid but with less desorption rate.

Characterization of Metal–Biomaterial Interaction

Various important techniques used for the characterization of metal–biomaterial interaction responsible for sorption phenomenon are Brunauer–Emmett–Teller technique, Fourier transform infrared spectroscopy, scanning elecron microscopic technique, and X-ray diffraction. The salient features of some of them are explained here.

Biosorption – BET Studies

The surface area of the biosorbent is to be measured using BET surface area analyzer (Fig. 1).

Some important BET areas of biosorbents are listed in Table 1:

Fig. 1 BET surface area analyzer

Scanning Electron Microscopic Analysis (SEM)

The morphological characteristics of biosorbents are to be evaluated using *scanning electron microscope* (Fig. 2).

Scanning electron microscopy is essential in structural analysis and important to any investigation relating to the properties and behavior of materials that involve their microstructure. SEM provides information relating to morphology, phase distribution, and compositional differences that help in the establishment of the

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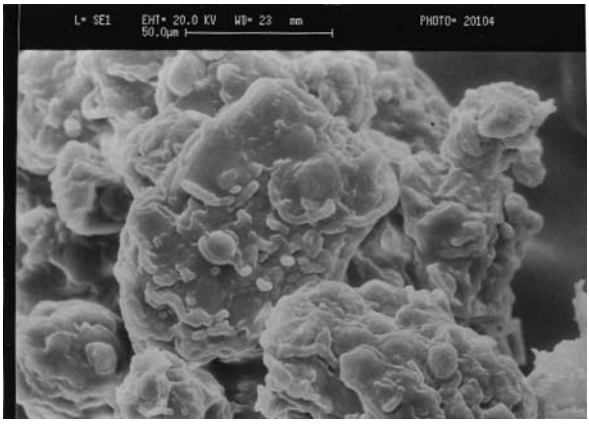
Table 1 BET surface area of different biosorbents

Biosorbents	Surface area (m^2/g)
LLSP	5.17
SMOS	4.01
ZMCP	4.32
SILP	4.96
FRLP	5.01

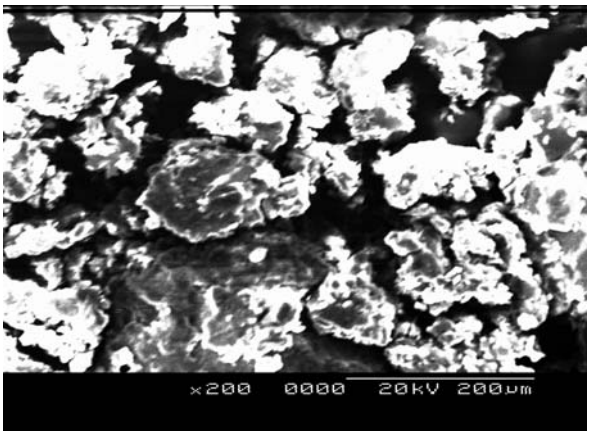
Fig. 2 Scanning electron microscope (SEM)

process. Thus, it is used to confirm the biosorption phenomenon. SEM of untreated (native) and metal-treated biosorbents is to be recorded. Below are the micrographs of some untreated and treated biosorbents.

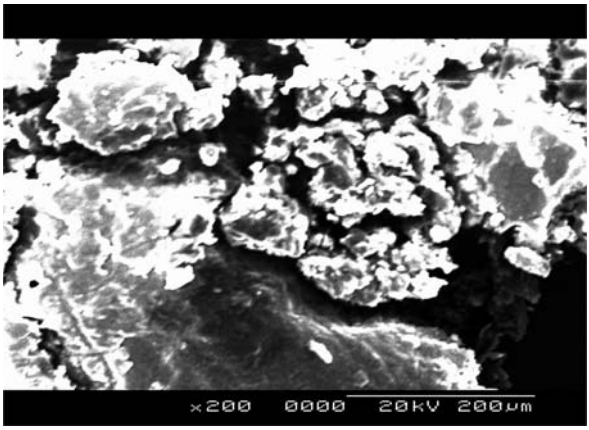
Micrographs 1, 2, and 3 represent the scanning electron micrographs of untreated shelled *Moringa oleifera* seed (SMOS), *Ficus religiosa* leaf powder (FRLP), and *Zea mays* cob powder (ZMCP). Micrographs 4, 5, and 6 depict the SEM of Cd(II), Cr(III), and Ni(II) treated bisorbents, respectively.



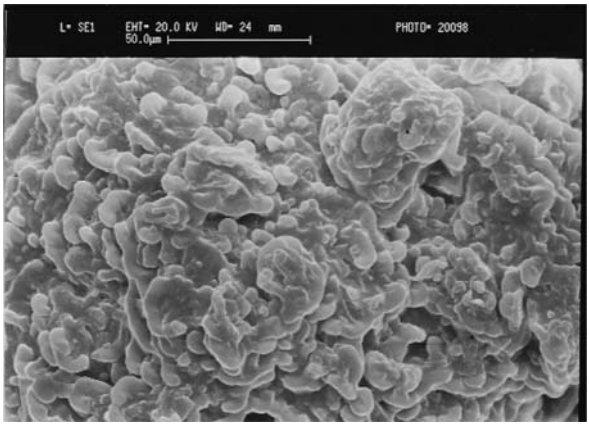
Micrograph 1 Scanning electron micrograph of untreated shelled *M. oleifera* seeds



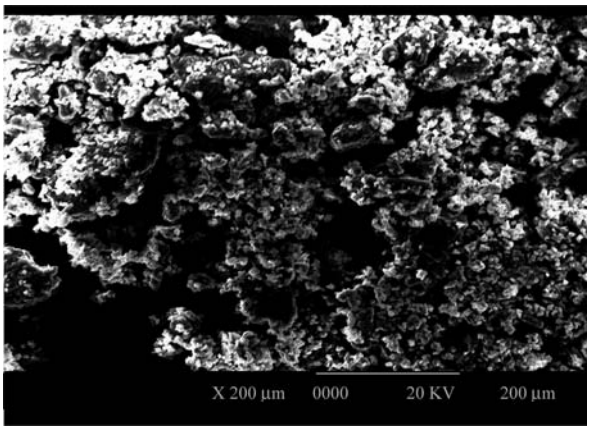
Micrograph 2 Scanning electron micrograph of untreated *F. religiosa* leaf owder (FRLP)



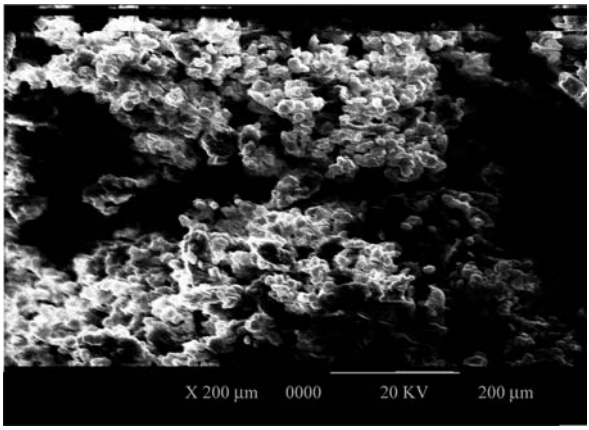
Micrograph 3 Scanning electron micrograph of untreated *Z. mays* cob powder (ZMCP)



Micrograph 4 Scanning electron micrograph of Cd(II)-treated shelled *M. oleifera* seeds (SMOS)



Micrograph 5 Scanning electron micrograph of Ni(II)-treated *F. religiosa* leaf powder (FRLP)



Micrograph 6 Scanning electron micrograph of Ni(II)-treated *Z. manys* cob powder (ZMCP)

The comparison of scanning electron micrographs of native and treated biosorbents indicates aggregation and reduction in pore area of treated biomaterial. It may be ascribed due to liquid phase concentration of metal species at experimental pH, confirming the biosorption phenomenon involving the sorption of metal species on the cellular surface of the present biosorbents.

FTIR Studies

FTIR analysis in solid phase is performed using a Shimadzu 8400 Fourier transform infrared spectroscopy. Spectra of the sorbent before and after metal sorption are to be recorded (Fig. 3).

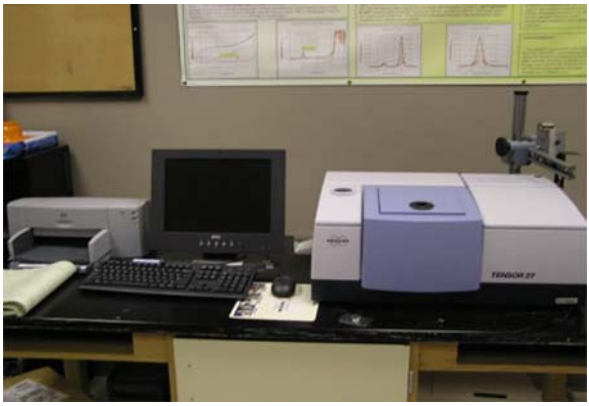
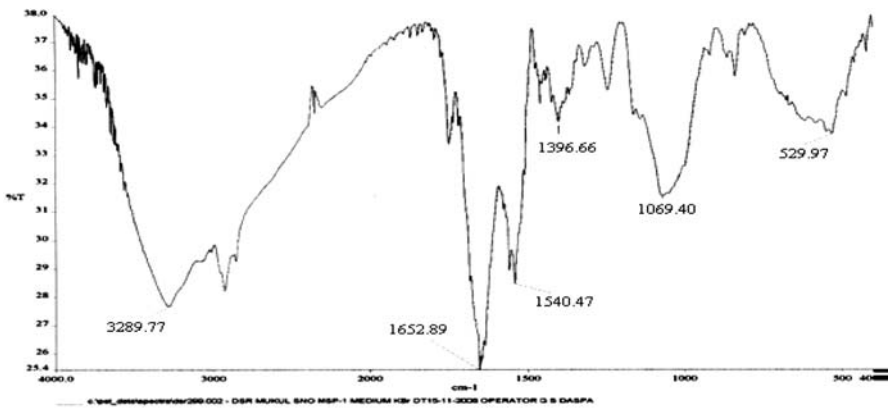


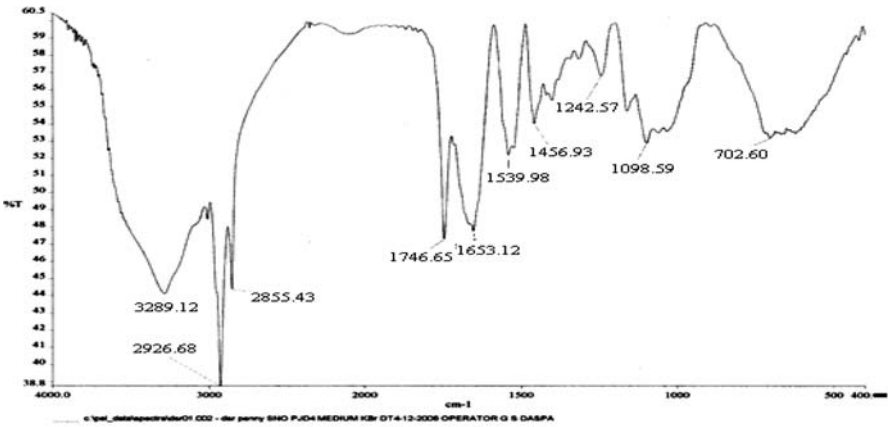
Fig. 3 Fourier transform infrared spectrometer (FTIR)

Fourier transform infrared spectroscopic study reveals the important information about the chemical environment of the biomaterial responsible for sorption. Representative FTIR records of untreated and metal-treated *biosorbents* are presented here.

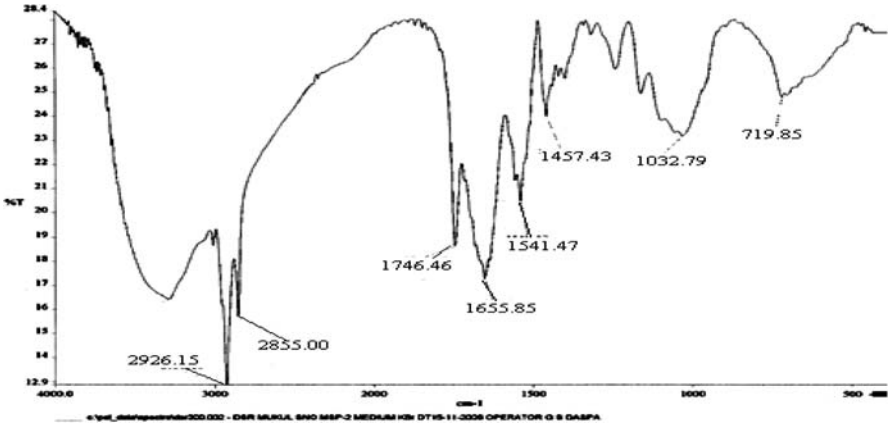
- FTIR records (Spectras 1, 2, and 3) show the presence of various functional groups of amino acid moiety in the *Leucaena leucocephala* seed biomaterial (1,300–30,000 cm^{-1}). The analysis of FTIR records of native and treated samples reflects the complex nature of the biosorbent.
- Spectra 1 explains the FTIR spectra of untreated *L. leucocephala* seed powder showing the presence of amino acid moieties in terms of NH_4^+ and COO^- stretching bands at 3289.77 cm^{-1} and 1396.66 cm^{-1}, respectively.



Spectra 1 FTIR spectra of untreated *L. leucocephala* seed powder (LLSP)



Spectra 2 FTIR spectra of Cd(II)-treated *L. leucocephala* seed powder (LLSP)



Spectra 3 IR spectra of Cr(VI)-treated *L. leucocephala* seed powder (LLSP)

- Spectra 2 represents the FTIR record of Cd(II)-treated LLSP, showing the presence of both amino and carboxylic groups. The characteristic peak shifting of carboxylate ion in untreated sample from [1,396.66 cm^{-1}] to [1,456.43 cm^{-1}] in Cd(II)-treated biomaterial confirms ionic interaction between carboxylic groups of amino acid and the cationic metallic species.
- Spectra 3 displays the FTIR spectra of Cr(VI)-treated LLSP, showing the presence of both carboxylic and amino groups. In the FTIR records of native and Cr(VI)-treated biomaterial, a well-pronounced shift of NH_4^+ in native sample from [3,289.77 cm^{-1}] to [2,926.15 cm^{-1}] in treated sample results. This shifting in peaks corroborates the electrostatic attraction between NH_4^+ of amino acids present in *L. leucocephala* leaf powder and anionic metallic species.

Protein as Possible Bioactive Principle

Active Sites for Sorption

Various mechanisms for the sorption of metal ions onto biomaterials have been discussed in the literature based on the presence of different functional groups like polyphenols, carbohydrates, polypeptide hydroxyl groups, sulfonic acid groups, nitro, carboxyl acid groups, and proteinaceous amino acids. Among the above functional groups, proteinaceous amino acids have been found to play an important role in metal sorption mechanism. The proteinaceous amino acids have a variety of structurally related pH-dependent properties of generating appropriate atmosphere for attracting the cationic and anionic species of metal ions simultaneously. This chapter is devoted to provide experimental proof that carboxyl and amino ligands present in the biomaterial are responsible for metal binding.

The above facts have been established on the basis of the following experiments.

Esterification

The biomaterial was subjected to esterification using standard practices:

$$\text{Biomaterial} - \text{COOH} + \text{CH}_3\text{OH} \overset{(\text{CH}_3\text{O})_3\text{CH}}{\rightarrow} \text{Biomaterial} - \text{COOCH}_3 + \text{H}_2\text{O}$$

The esterification of naturally occurring carboxylic acids resulted in the formation of ester. The esterified biomaterial was monitored for its biosorption efficacy, exhibiting decreasing trend of sorption for cationic metals. The decreasing trend of sorption of the esterified biomass is likely due to blocking of COOH group resulting in the formation of ester linkage (-COOR-) which is not supposed to show any affinity for the attraction of M^+ ion. This fact indirectly highlights the role of COO^- ligand in M^+ binding.

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Propylamination

The biomaterial was subjected to propylamination using standard practices:

$$\text{Biomaterial} - \text{COOH} + \text{CH}_3\text{CH}_2\text{CH}_2\text{NH}_2 \rightarrow \text{Biomaterial} - \text{COONH}_2 + \text{C}_3\text{H}_6$$

Similarly carboxylic acid group was converted into amide by reaction with propylamine. Propylamination of the biomaterial has again resulted in the decrease of sorption efficiency highlighting the role of carboxylic acid moiety for cationic metal sorption. Blocking the carboxylic groups with propylamine, which neutralizes these anions, considerably decreases the cationic metal ion uptake, indicating that negatively charged carboxylic groups play an important role in biosorption due to electrostatic attraction.

The observed decrease in sorption of the cationic metals after neutralizing the carboxylate anion of the biomaterial clearly indicates that negatively charged carboxylate anions play an important role in biosorption phenomenon.

Tables 1, 2, and 3 include the decreasing trend of esterified and propylaminated biomaterial with respect to unmodified representative biomaterial *Leucaena leucocephala seed powder* (*LLSP*) *and Zea mays cob powder* (*ZMCP*).

Table 1 Decrease in sorption efficiency of different chemically modified LLSP in case of single metal solution

Nature of biomaterial	Cd(II)	Cr(III)	Ni(II)
Unmodified (%)	97.25	88.65	76.23
Esterified biomaterial (%)	91.46	81.17	71.67
Propylaminated biomaterial (%)	89.23	73.35	64.78

Table 2 Decrease in sorption efficiency of different chemically modified *Z. mays* cob powder in case of single metal solution

Nature of biomaterial	Cd(II)	Cr(III)	Ni(II)
Unmodified (%)	79.36	76.43	71.98
Esterified biomaterial (%)	71.46	81.17	71.67
Propylaminated biomaterial (%)	89.23	73.35	64.78

Table 3 Enhancement of sorption efficiency of acetylated ZMCP

	Sorption efficiency (%)
Type of biomaterial	Cr(VI)
Unmodified ZMCP	78.12
Acetylated biomaterial	71.76

The role of amino group present in the biomaterial for metal sorption can also be highlighted based on the following chemical reaction:

$$\text{Biomaterial} - NH_2 + (CH_3CO)_2O \rightarrow \text{Biomaterial} - NHCOCH_3 + CH_3COO^- + H^+$$

Acetylation of the biomaterial containing amino groups with acetic anhydride results in the blocking of available amino ligands and decreases the number of positively charged sites on the biomaterial surface. The resulting acetylated biomaterial exhibited decrease of sorption of anionic metal, exhibiting equally important role of amino group in the sorption of anionic metal.

Tables 4 and 5 present the chemical composition of major constituents with special reference to the free and bound amino acids in the representative plant biomasses of shelled *Moringa oleifera* and *L. leucocephala* seed powder.

CELLULAR CONSTITUENTS OF SMOS

Heterogeneous complex mixture having various functional, importantly low molecular weight amino acids	
Crude protein	432.5 g/kg DM
Crude fat	312.0 g/kg DM
Carbohydrate	211.2 g/kg DM
Ash	44.3 g/kg DM

CELLULAR CONSTITUENTS OF LLSP

Heterogeneous complex mixture having various functional, importantly low molecular weight amino acids	
Fat	7.50%
Ash	0.78%
Protein	32.90%
Calcium	0.42%
Phosphorus	0.64%

Sincere efforts have been made to isolate the protein content of the seed biomaterial and to further fractionate the various protein bands with the determination of their molecular weight. It is also reported that major fraction of the protein is likely to be responsible for metal sorption efficacy and considered as bioactive principle.

Table 4 Amino acid composition of *L. leucocephala* seed powder (Padmavathy and Shobha 1987)

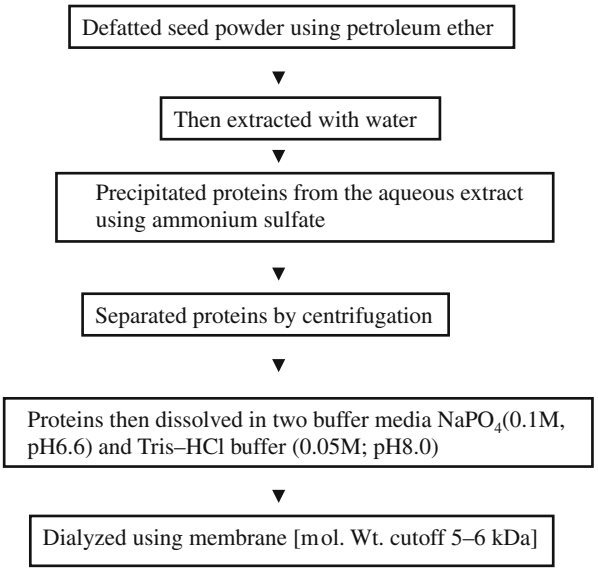
Amino acid	*L. leucocephala* seed powder (g/100 g)
Isoleucine	15.88
Trypsin	15.90
Glutamic acid	11.35
Arginine	6.17
Leucine	4.84
Lysine	4.21
Glycine	3.68
Serine	3.36
Valine	3.02
Phenylalanine	3.02
Alanine	3.00
Proline	2.78
Histidine	2.57
Threonine	2.26
Methionine	0.85
Cystine	0.87
Tyrosine	2.42

Table 5 Amino acid composition of shelled *Moringa* oleifera seed powder (Bachewel et al. 1995)

Amino acid	Shelled *M. oleifera* seed powder (g/100 g)
Isoleucine	12.81
Trypsin	11.56
Glutamic acid	9.15
Arginine	5.27
Leucine	5.54
Lysine	6.11
Glycine	5.18
Serine	2.36
Valine	2.02
Phenylalanine	2.02
Alanine	2.56
Proline	3.13
Histidine	2.98
Threonine	2.53
Methionine	0.81
Cystine	0.76
Tyrosine	2.44

Isolation and Characterization of Protein

Protein content of seed powder (SMOS and LLSP) was isolated using standard practices. The various steps involved in the isolation of protein are the following:



Quantification of Protein

The active proteins are to be bioassayed (Bradford 1976).

Different dilutions of the protein standard (bovine serum albumin) are to be prepared. Protein sample solution is to be taken in a clean dry test tube and mixed with dye reagent (Coomassie brilliant blue solution) and vortex. The solutions are incubated at room temperature. The absorbance is to be measured against blank (dye reagent) at 595 nm using UV spectrophotometer. The concentration of the recovered protein content from dialysis is calculated (Fig. 1).

Fig. 1 UV spectrophotometer

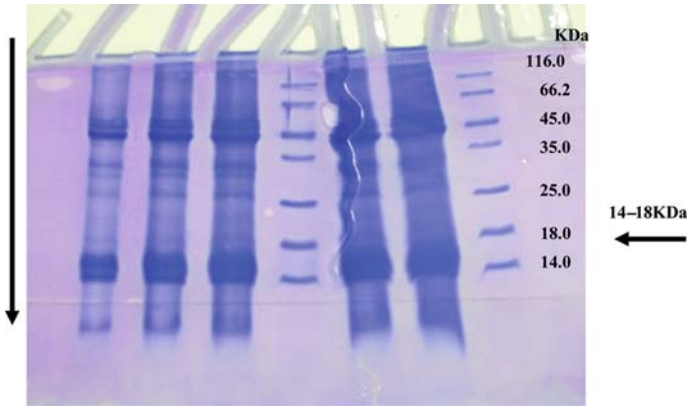
Molecular Weight Determination (Gel Electrophoresis)

Molecular weight of the proteins is to be determined using SDS-PAGE, 15% gels (Laemmli 1970). Active protein, flowthrough, washings, and elutions are to be subjected for gel elctrophoresis. Samples (10 μL) are to be loaded on each well and run on 15% polyacrylamide gels using a Mini Protean II. A wide-range molecular weight marker (10–20% Biomarker, BioRad) is used as a size maker. Polyacrylamide gels (7 × 10 cm) containing 30% acrylamide are to be prepared (Laemmli 1970). The separating gel (15%) contained 30% acrylamide, 0.8% bisacrylamide, 4× Tris–Cl\SDS, pH 8.8, H_2O, 10% ammonium persulfate, and TEMED. The stacking gel (5%) contained 30% acrylamide + 0.2% Bis, 4× Tris buffer (pH 8.8), 4× Tris buffer\SDS (0.5 M), pH 6.8, 10% ammonium persulfate, TEMED, and H_2O. Electrophoresis is to be performed at 30 mA and 150 V per gel until the dye reached the bottom of the gel. After electrophoresis the gels are to be stained in Coomassie Brilliant Blue (contains 0.1% Coomassie brilliant G-250, 0.2% phosphoric acid, 10% ammonium sulfate, and 20% methanol) overnight and destained with a solution containing 1% acetic acid and 1% glycerol.

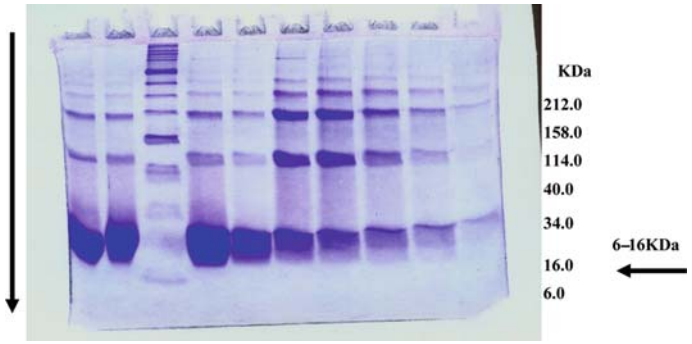
Characterization of Protein

A gel chromatogram of any protein sample represents the presence of prominent protein bands of different intensities which are to be further fractionated in terms of molecular weight.

Representative gel chromatograms of the protein content of the LLSP and SMOS are presented in Photographs 1 and 2.



Photograph 1 Electrophoretic nature and mol. wt. determination of LLSP protein. The *arrow* indicates direction of protein migration



Photograph 2 Electrophoretic nature and mol. wt. determination of SMOS protein. The *arrow* indicates direction of protein migration

The fractions of proteins having different molecular weights are collected, dried, and assayed for sorption efficacy to trace the exact protein content showing maximum sorption efficacy and then predicted as bioactive principle.

Novel Biomaterials – Commercialization Approach

Biomaterials have been found to be associated with drawbacks related to less sorption efficiency and stability, restricting their commercial use (Pagnanelli et al. 2000; Scowronski et al. 2001). Sincere efforts toward structural modifications on the biomaterials leading to the enhancement of binding capacity and selectivity are, therefore, in great demand. A special emphasis is to be paid on green chemical modifications resulting in *tailored biomaterials* improving its sorption efficiency and environmental stability and thus making it liable for its commercial use as simple, fast, economical, eco-friendly green technologies. Attention has been paid by various research groups (Tsezos 1985; Saito et al. 1991; Gardea-Torresdey et al. 1998) to increase the sorption capacity of biomaterials for abatement of different metal ions. Klimmek and Stan (2001) reported that the maximum sorption capacities of the alga *Lyngbya taylorii* could be increased significantly after phosphorylation of the biomass. Bai and Abraham (2002) noted that the sorption ability of the fungus *Rhizopus nigricans* for Cr(VI) is also improved after the introduction of carboxyl and amino groups. The pretreatment of biomass with surfactants and cationic polyelectrolyte and deacetylation treatment of amino groups of chitin are favorable for abatement of metal ions (Tan and Cheng 2003). Pretreatment methods using different kinds of modifying agents such as base solution, organic acid solutions, and oxidizing agents have been used for the purpose of removing soluble organic compounds. Increase in efficiency of metal adsorption has been recently performed by many researches (Taty-Costodes et al. 2003; Gupta et al. 2003; Min et al. 2004; Acar and Eren 2006; Abia et al. 2006; Wankasi et al. 2006; Hannafiah et al. 2006). These chemical modifications in general improved the adsorption capacity of biomaterials probably due to higher number of active binding sites after modification, better ion exchange properties, and formation of new functional groups that favor metal uptake. The semi-synthetic biomaterials are deemed to be good candidates for their commercial use as biomaterials for removing toxic metals from wastewater with high adsorption efficiency.

Natural biomaterials are biodegradable and cannot be used for long-term applications for sorption as sorbent regeneration is necessary for cost-effectiveness. Therefore, constructive efforts toward enhancement of binding capacity and simultaneously increasing stability of sorbents for the improvement in their mechanical strength are the keen goals of the scientific community. Graft co-polymerization is

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relatively a new technique in which one structure moiety is grafted on another structure moiety resulting in the formation of grafted co-polymer with above desired properties. Graft co-polymers are recognized for special properties including high strength, thermal stability, biodegradability, and non-toxicity (Ramakrishna et al. 2005). Many of the graft co-polymers of the present inventions are novel and are ideally suited for sorption. Polymer blends give rise to new polymeric properties. The invention relates to a process for grafting an alkyl acrylate to a polycarbonate, polysaccharides, and other cellulosic materials in the solid phase (Wada et al. 2006). The graft co-polymerization process can be adapted to be carried out in either a batch, semi-batch, or continuous mode. The polymers derived from the unique process of this invention are useful in paints, adhesives, stabilizers, composites, bulk polymers, bulk plastics, bulk elastomers, fabrics, fibers, and fillers for sterilizable packaging, metal coatings, and solidified sorbing surfaces (Zumei and Ramakrishna 2006). Baxo-Xiu et al. (2006) prepared a graft co-polymerization of acrylic acid and acrylamide which was capable of removing Cu(II) from aqueous solutions with high removal efficiency and a regeneration of seven successive cycles without any loss in metal uptake. In another study, Hashem (2006) grafted sunflower stalks with acrylonitrile using $KMnO_4$ as redox initiator. Similarly Shibi and Anirudhan (2002) grafted acrylamide onto banana stalk, which increases the adsorption capacity of the biomaterial from 138 to 210 mg^{-1} for Hg(II). Thus grafting improves the adsorption capacity, selectivity, and stability of the biomaterial by forming many reactive groups on the polymer (Bicak et al. 1999; Liu et al. 2002; Orlando et al. 2002; Guclu et al. 2003; O'Connell et al. 2006).

Recently, Goyal and Srivastava (2009) and Kardam et al. (2009) have modified sorption efficiency, simultaneously increasing regeneration cycle of various agricultural wastes using synthetic modification including graft co-polymerization.

Synthetic Modifications onto Biomaterial to Increase Its Sorption Efficiency for Cationic Metals

Strengthening of Bioactive Functional Group [COO^-]

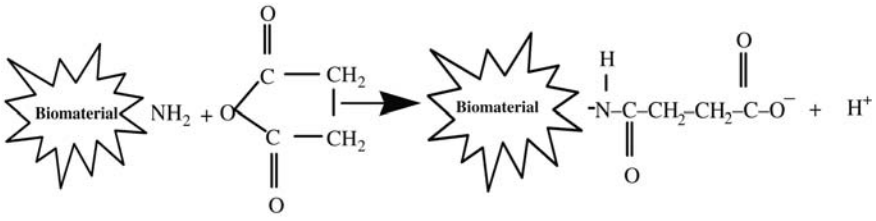
It is inferred that carboxyl ligands are important in the binding of metal ions. Thus increasing the number of such groups should increase the biomaterial binding ability. This is achieved through normal chemical reaction using mono-, di-, and tri-carboxylic acids or anhydrides.

Reaction with Anhydrides

The succination of the amino groups on the biomaterials is to be achieved by washing biomaterials first in HCl to remove any debris, followed by washing in sodium

acetate at pH 8.0. The biomaterials are to be suspended in $NaC_2H_3O_2.H_2O$ at pH 8.0. Succinic anhydride should be added to the suspended biomaterial. An additional succinic anhydride is to be added after 15-min intervals for the next 1½ h (six additions of succinic anhydride to the biomaterial). The biomaterial should be washed with HCl, centrifuged, and washed again with deionized water. Although the amino group is neutralized, it now forms an additional carboxyl group. By the addition of a carboxylate group, there should be an enhancement of metal binding by those metals that bind to carboxyl ligands. Succination of the biomaterial is performed to add a carboxyl group onto the nitrogen ligand. Sorption studies on this modified biomaterial show increase in sorption efficiency. This increased sorption may be ascribed to the addition of carboxylate ion.

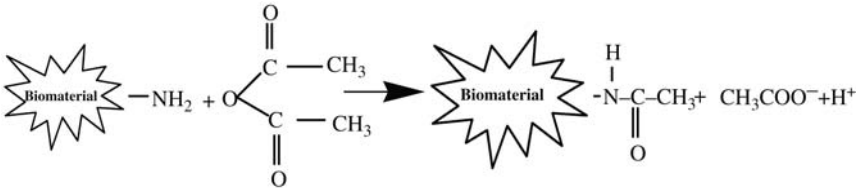
Succination of the biomaterial can be represented, in general, as follows:



Acetylation of amino group on the biomaterials is to be achieved by washing biomaterial first in HCl to remove any debris, followed by washing in sodium phosphate/sodium acetate buffer solution ($Na_3PO_4/NaC_2H_3O_2$) at pH 7.2. The biomaterials should be reacted with acetic anhydride and stirred while maintaining the pH of 7.2 for 1 h. The acetylated biomaterials will be next centrifuged for 5 min at 100.62 g. After removing the supernatant, the biomaterials are to be resuspended in hydroxylamine to remove *O*-acetyl groups. The biomaterials should be washed with HCl to remove any more soluble materials and finally washed with deionized water.

Acetylation of the biomaterial by acetic anhydride blocks the available amino ligands and decreases the number of positively charged sites on the biomaterial surface. This synthetic amendment reduces interference of the amino group, finally resulting in the increase of sorption of cationic metal species.

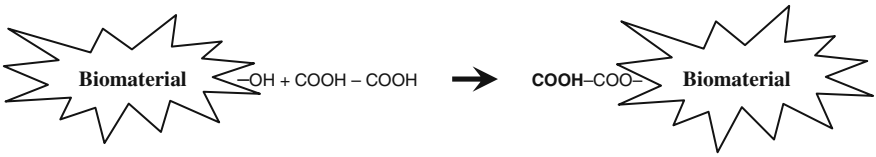
In general, the above fact can be represented as follows:



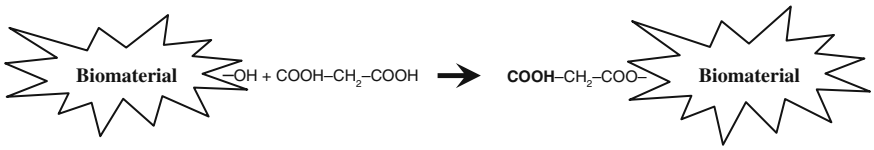
Reactions with Acids

The synthetic modifications of biomaterial (SMOS) powder were carried out with different acid solutions (oxalic acid, succinic acid, malonic acid, tartaric acid, and citric acid) in 1.0 M concentration in the ratio 1:5. Each modified biomaterial was used to monitor for the enhancement in their sorption efficiency for different cationic metal ions.

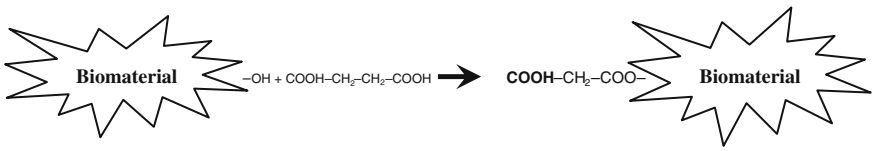
Oxalic Acid Modified SMOS



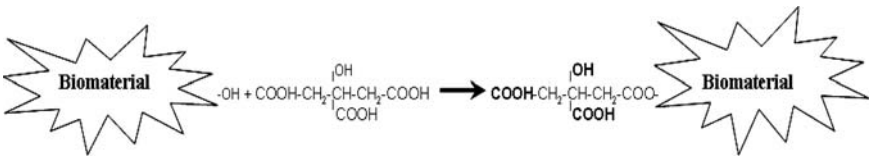
Malonic Acid Modified SMOS



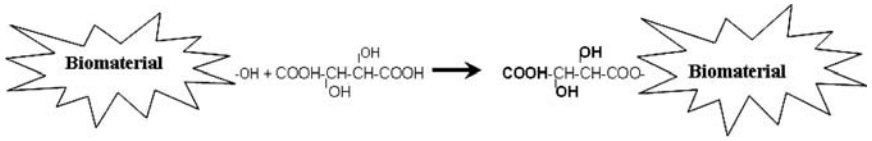
Succinic Acid Modified SMOS



Citric Acid Modified SMOS



Tartaric Acid Modified SMOS



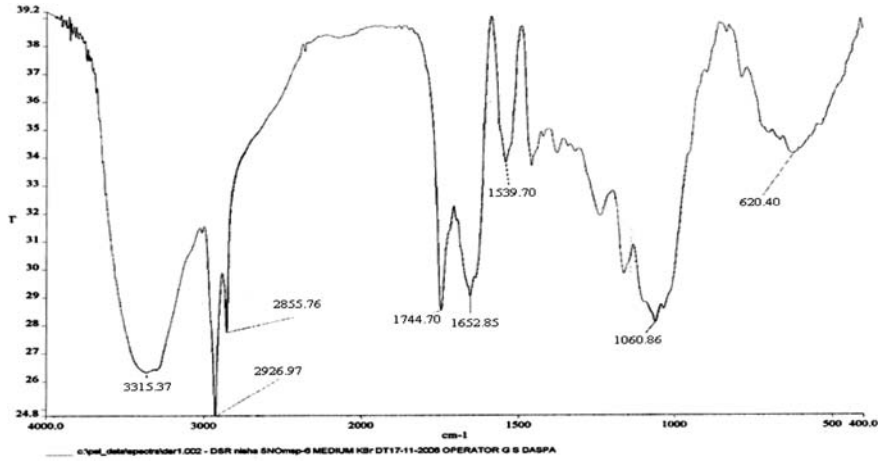
Evidence in Support of Chemical Modifications Occurring on Biomaterials Leading to Enhanced Sorption

FTIR analysis in solid phase in KBr is to be performed using a Fourier transform infrared spectrometer. Spectra of the sorbent before and after modifications should be recorded. Representative IR spectra of untreated and succinated biomaterial show the presence of additional peak of carboxylate ion [1,744.70 cm^{-1}] and conversion of amino to amide group [3,289.77–3,315.37 cm^{-1}] in succinated LLSP (*Leucaena leucocephela* seed powder), confirming succination of the LLSP biomaterial (Spectra 1). The conversion of amino to amide group [3,289.77–3,365.14 cm^{-1}] confirmed the acetylation process (Spectra 2).

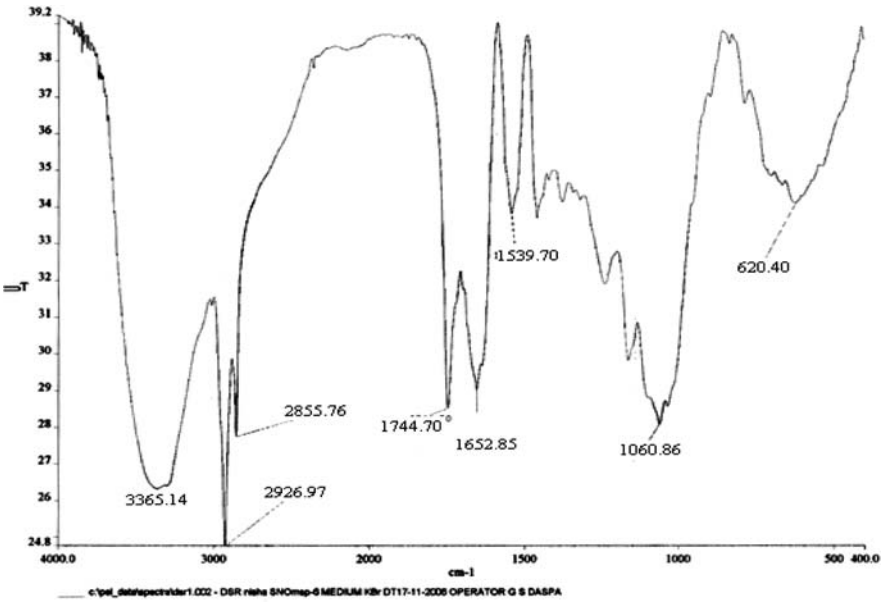
Increase in sorption efficiency and number of regeneration cycles of the chemically modified biomaterial is to be monitored.

Evaluation of Enhanced Sorption Efficiency of Modified Biomaterial

All the modified biomaterials are to be subjected to the assessment of sorption efficiency for cationic metals under previously standardized optimum conditions. The treatment of oxalic, malonic, succinic, tartaric, and citric acids with the biomaterial



Spectra 1 IR spectra of succinated *L. leucocephala* seed powder (LLSP)



Spectra 2 IR spectra of acetylated *L. leucocephala* seed powder (LLSP)

is an esterification process, introducing a carboxyl group to the product (Fig. 1). The introduction of such additional carboxyl ligands (COO^-) may be ascribed to the increased sorption potential for positively charged metal ions. The different chemically modified biomaterials showed the following order of increase in sorption potential for cationic metals in the range of 8–10%.

Fig. 1 Unmodified SMOS and different chemically modified SMOS

Oxalic < Malonic < Succinic < Tartaric < Citric Acid Modified Biomaterial

The order may be explained on the basis of increase in one carboxylic ligand with successive increase in the length of alkyl chain (oxalic acid to succinic acid) which is likely to increase the surface area of the biomaterial and result in successive higher sorption. Further increase in the sorption in citric and tartaric acid modified biomaterial may be described due to increase in the number of carboxylic ligands (tartaric acid –1, citric acid –2) along with other negatively charged bioactive OH groups. Such modification increases the total negative charge on the surface of the biomaterial resulting in further improved sorption.

Graft Co-polymerization

It was inferred that carboxyl ligands are important in the binding of metal ions, thus increase in the number of these groups should increase the biomaterial binding ability. This is achieved through graft co-polymerization of biomaterials using standard polymerization techniques and bulk of COOH groups were introduced at the surface.

For grafting, the biomaterial is to be dispersed in a definite amount of water. Appropriate initiators (ceric ammonium nitrate/ammonium persulfate/potassium persulfate) and nitric acid are to be added slowly to the reaction mixture. Appropriate monomers (acrylic/maleic/itaconic acid) should be added dropwise to the reaction mixture from the dropping funnel. The reaction flask is to be placed in a water bath at 100–85°C for various time periods under stirring by a magnetic stirrer. After a definite time period, the reaction mixture is to be filtered and the polymer should be removed with excess water. The grafted sample is to be dried to a constant weight and used for sorption studies (Figs. 2, 3, and 4). From the increase in weight of the biomaterial, percentage of grafting should be calculated as follows: % grafting $= (W_2 - W_1)/W_1 \times 100$, where W_1 and W_2 denote the weight of native and grafted biomaterial after complete removal of the homo-polymer, respectively.

Graft Co-polymerization with Acrylic Acid

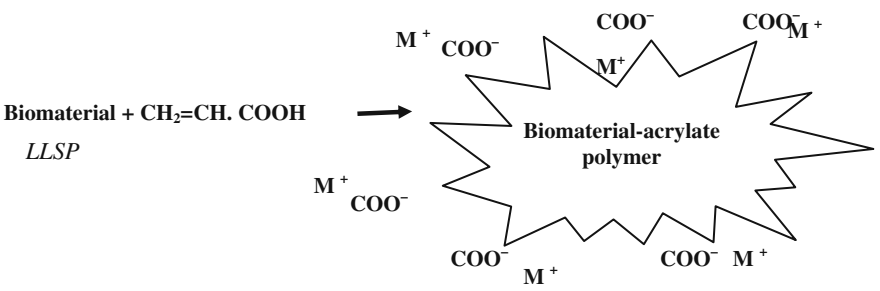
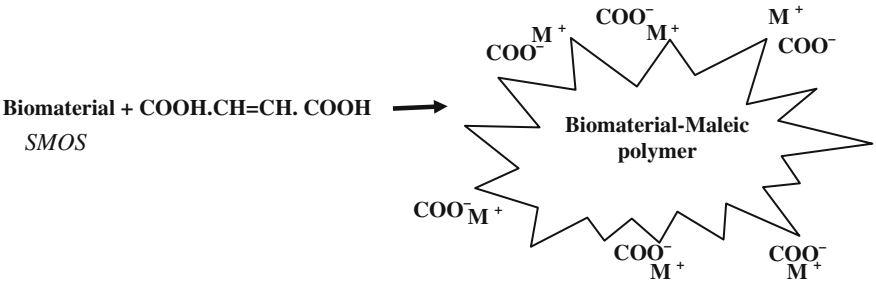


Fig. 2 Acrylic acid-grafted LLSP

Fig. 3 Maleic acid-grafted SMOS

Graft Co-polymerization with Maleic Acid



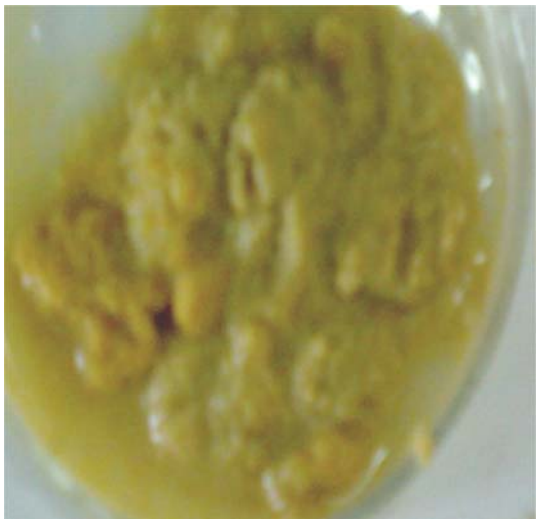
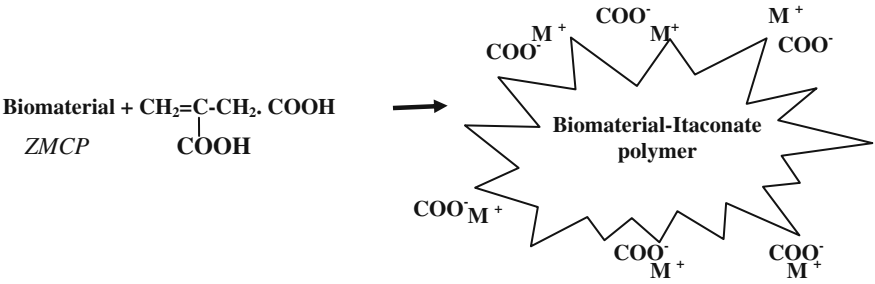


Fig. 4 Itaconic acid-grafted SMOS

Graft Co-polymerization with Itaconic Acid



Graft co-polymerized biomaterials exhibited maximum metal sorption potential. This is mainly due to the bulk of COOH group introduced at the surface. Grafting of the bulk of COOH groups onto the biomaterial becomes the basis of the increase in sorption efficiency in the range 15–20% for the abatement of different metal ions.

Evidence in Support of Improved Environmental Stability of the Biomaterial

The comparison of initial decomposition temperature (IDT) and final decomposition temperature (FDT) of unmodified and graft co-polymerized biomaterial should be estimated with *thermograms*. Thermogravimetric analysis of untreated (native) and

graft co-polymerized biomaterials shows significant differences in the initial decomposition temperature (IDT) and final decomposition temperature (FDT). Upon grafting, temperature of biomaterial (SILP) is found to be increased (IDT: 37.94 to 49.32°C; FDT: 600 to 609.23°C), indicating that grafting with acrylic acid improves the stability of SILP biomaterial (Figs. 5 and 6).

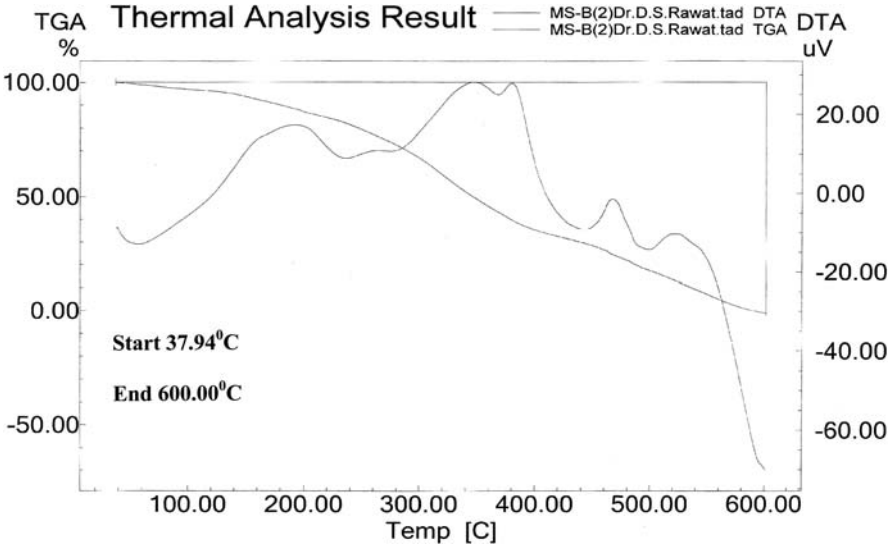


Fig. 5 TGA of ungrafted *Saraca indica* leaf powder (SILP)

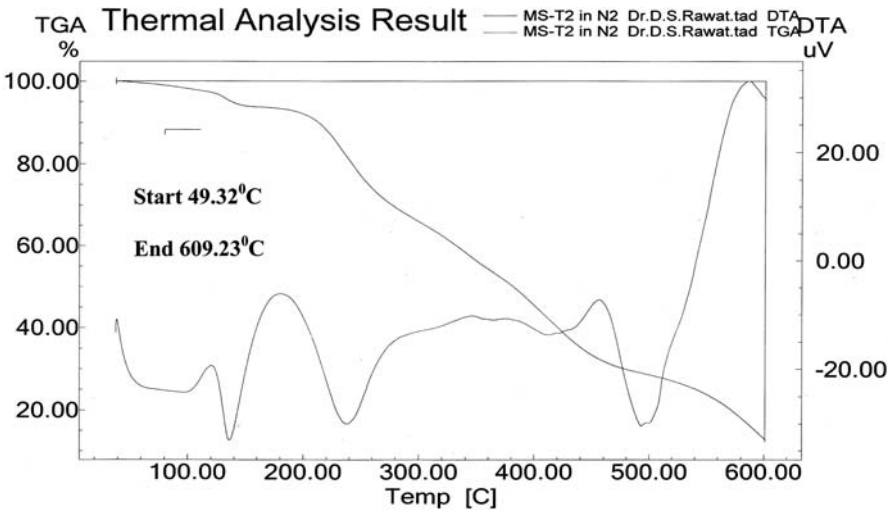


Fig. 6 TGA of acrylic acid-grafted *S. indica* leaf powder (SILP)

Table 1 Sorption of cationic metal ion on regenerated modified (graft co-polymerized) FRLP biomaterial

	% Sorption		
Cycles	Cd(II)	Cr(III)	Ni(II)
1	98.61	89.33	76.93
2	98.20	88.85	76.64
3	98.04	88.65	76.49
4	97.78	88.28	76.26
5	97.55	88.14	75.13
6	97.12	88.01	77.02

Increased stability of the graft co-polymerized biomaterials is to be monitored on the basis of increase in number of regeneration cycles. Table 1 clearly shows that structurally modified biomaterial (polymerized) can be used six times compared to only four times of the unmodified biomaterial (FRLP), exhibiting its increased environmental stability.

Synthetic Modifications onto Biomaterial to Increase Its Sorption Efficiency for Anionic Metals

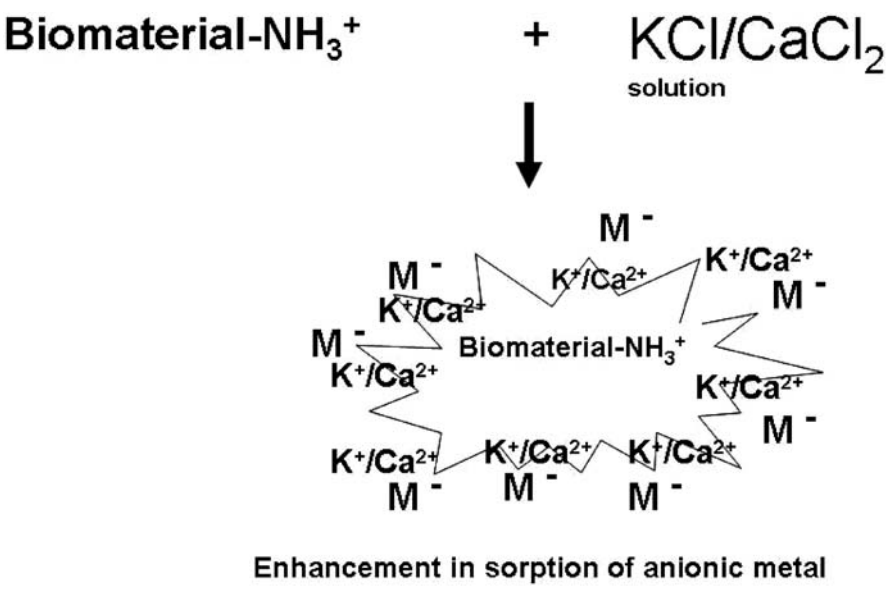
In order to use same biomass with enhanced sorption potential (*ZMCP*) for removing anionic metals also, different synthetic modifications have been carried out. The surface of the biomass has been made anion attracting by modifying with K^+ and Ca^{2+} ions. The surface of the cellulosic biomaterial (most of the time) in contact with water is negatively charged (Koshy et al. 2006). Ionic salts (KCl and $CaCl_2$) when dissolved in polar solvent like water then dissociate into ions (K^+ and Ca^{2+}) and these positive entities get deposited on the surface of biomass attracting negatively charged metal species.

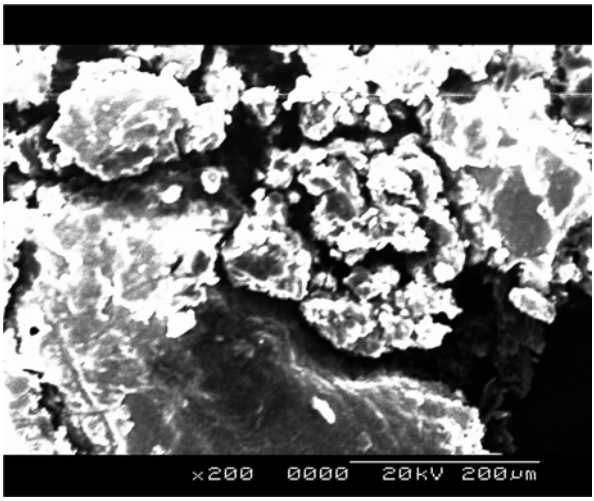
Impregnation of Positively Charged Layer

Experiments have been conducted with different biomaterials like *Zea mays* corn cob, *Moringa oleifera* seed powder and *L. leucocephela* seed powder as follows. A known amount of biomaterial powder was kept in contact with 200 mL solution containing 10.0 g of KCl/$CaCl_2$ for 40 min. The solution was filtered and the solid material obtained was dried at 65°C in the oven. The prepared powder was used for further experiment of sorption.

The formation of positively charged surface on the biomaterials was confirmed on the basis of SEM records (Micrographs 1 and 2).

Tables 2 and 3 provide data of enhanced sorption efficacy of impregnated biomaterials for Cr(VI) ions from water system.

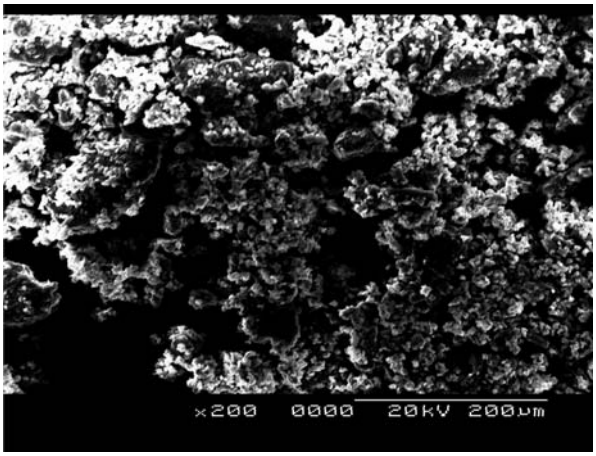




Micrograph 1 Scanning electron micrograph of native ZMCP showing large spherical cluster-type morphology

The data for Cr(VI) fitted well into both Freundlich and Langmuir isotherms. The magnitude of values K_f (0.12) and $1/n$ (0.28), Q_0 (3.91), and b (0.33) for *ZMCP* indicates the successful sorption of anionic metal species Cr(VI) (Figs. 7 and 8).

About 1,500,000 t maize is produced in India annually and the cobs are thrown as waste so the material is easily available practically at no cost. Therefore metal



Micrograph 2 Scanning electron micrograph of modified ZMCP showing dense agglomerated, etched cluster-type morphology

Table 2 Soluble Cr(VI) ion concentration (μM) after adsorption on *Z. mays* cob powder (ZMCP) as functions of contact time and biomass dosage at volume (200 mL), particle size (105 μm), and pH 2.5

	Soluble Cr(VI) concentration on powdered corncob			
Initial conc. mg/L (μM)	Biomass 0.5 g	Biomass 1.0 g	Biomass 2.0 g	Biomass 4.0 g
5 (19.23)	$14.76 \pm 0.69^{+\Phi}$	$12.38 \pm 0.50^{+\Phi}$	$10.92 \pm 0.52^{+\Phi}$	$10.96 \pm 0.56^{+\Phi\Phi}$
10 (38.46)	$25.26 \pm 1.13^{+\Phi}$	$21.11 \pm 1.01^{+\Phi}$	$18.57 \pm 0.77^{+\Phi}$	$18.65 \pm 0.95^{+\Phi\Phi}$
25 (96.15)	$48.34 \pm 2.07^{+\Phi}$	$41.15 \pm 2.01^{+\Phi}$	$36.26 \pm 1.84^{+\Phi}$	$36.34 \pm 2.25^{+\Phi\Phi}$
50 (192.30)	$96.46 \pm 5.11^{+\Phi\Phi}$	$82.07 \pm 4.59^{+\Phi\Phi}$	$72.11 \pm 3.89^{++\Phi}$	$72.26 \pm 3.54^{++\Phi\Phi}$
Correlation coefficient (r)	0.75	0.78	0.80	0.79

Numbers in parenthesis represent soluble metal concentrations in μM, standard deviations ±, mean value difference (initial Cr(III) and Cr(VI) loaded versus soluble Cr(VI) (μM)) as functions of metal concentration; $^{+}$significant ($p < 0.05$), $^{++}$insignificant ($p > 0.05$).
Biomass dosage; $^{\Phi}$significant ($p < 0.05$), $^{\Phi\Phi}$insignificant ($p > 0.05$).

Table 3 Soluble Cr(VI) ion concentration in % sorption efficiency of impregnated shelled *Moringa oleifera* seed (SMOS) powder at volume (200 mL) and particle size (105 μm)

Biomaterial	Cr(VI) (%)
Native SMOS	88.15
Impregnated SMOS	91.09

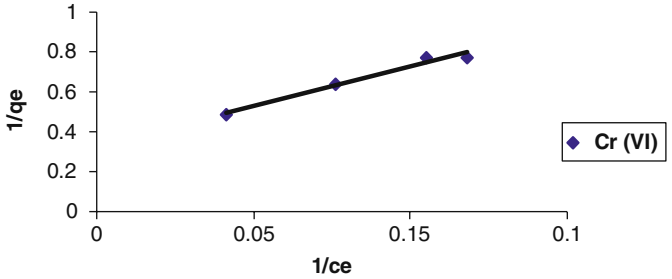


Fig. 7 Langmuir isotherm plot for the adsorption of Cr(VI) ion on impregnated ZMCP

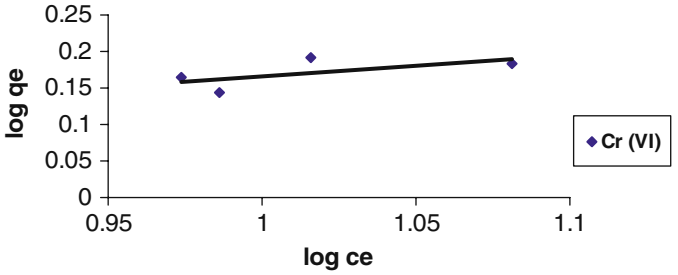


Fig. 8 Freundlich isotherm plot for the adsorption of Cr(VI) ion on impregnated ZMCP

sorption efficacy of modified *ZMCP* for different oxidation states of the metal ions provides a simple, efficient, instantaneous, and highly economical, eco-friendly method for removing toxic heavy metals from water bodies particularly for remote and rural areas.

Suggested Reading

Abbas M, Nadeem R, Zafar MN and Arshad M. (2008). Biosorption of Chromium (III) and Chromium (VI) by untreated and pretreated *Cassia fistula* biomass from aqueous solutions. Water, Air & Soil Pollution, 191, 1–4.

Abdel-Ghani NT and El-Chaghaby GAF. (2007). Influence of operating conditions on the removal of Cu, Zn, Cd and Pb ions from wastewater by adsorption. International Journal of Environmental Science and Technology, 4(4), 451–456.

Abdel-Ghani NT, Hefny M and El-Chaghaby GAF. (2007). Removal of lead from aqueous solution using low cost abundantly available adsorbents. International Journal of Environmental Science and Technology, 4(1), 67–73.

Abia AA and Asuquo ED. (2006). Lead and nickel adsorption kinetics from aqueous metal solutions using chemically modified and unmodified agricultural adsorbents. African Journal of Biotechnology, 5(16), 1475–1482.

Abia AA, Horsfall JR and Didi O. (2003). The use chemically modified and unmodified cassava waste for the removal of Cd, Cu and Zn ions from aqueous solutions. Bioresource Technology, 90, 345–348.

Acar FN and Eren Z. (2006). Removal of Cu(II) ions by activated poplar sawdust (Samsun Clone) from aqueous solutions. Journal of Hazardous Materials, 137, 909–914.

Adesola Babarinde AA, Babalola JO and Sanni RA. (2006). Biosorption of lead ions from aqueous solution by maize leaf. International Journal of Physical Science, 1(1), 23–26.

Adesola Babarinde AA, Oyesiku OO, Oyebamiji Babalola J and Janet OO. (2008). Isothermal and thermodynamic studies of the biosorption of Zn(II) ions by *Calymperes erosum*. Journal of Applied Sciences Research, 4(6), 716–721.

Agency for Toxic Substances and Disease Registry [ATSDR]. (2007). Toxicological profile for cadmium. U.S. Department of Health and Human Services, Public Health Service, Atlanta.

Ahammed MM and Chaudhari M. (1999). A low cost home water filter. Journal of Water Supply: Research and Technology, 48, 263–269.

Ajmal M, Rao AKR, Ahmad R and Ahmad J. (2000). Adsorption studies on *Citrus reticulata* (fruit peel of orange): Removal and recovery of Ni(II) from electroplating water. Journal of Hazardous Material, B79, 117–131.

Ajmal M, Rao AKR, Rais A and Ahmad J. (2003). Adsorption studies on rice husk: Removal and recovery of Cd(II) from wastewater. Bioresource Technology, 86, 147–149.

Ajmal M, Rao RAK and Siddiqui BA. (1996). Studies on removal and recovery of Cr(VI) from electroplating wastes. Water Research, 30(6), 1478–1482.

Akbar MA, Gupta PC. (1985). Proximate composition, tannin and mineral contents of different cultivars and of various plant parts of subabul (*Leucaena leucocephala*). Indian Journal of Animal Sciences, 55(9), 808–812.

Aksu Z, Sag Y and Kutsal T. (1992). The biosorption of copper (II) by *C. vulgaris* and *Z. ramigera*. Environmental Technology, 13, 579–586.

S. Srivastava, P. Goyal, *Novel Biomaterials*, Environmental Science and Engineering,
DOI 10.1007/978-3-642-11329-1, © Springer-Verlag Berlin Heidelberg 2010

An HK, Park BY and Kim DS. (2001). Crab shell for the removal of heavy metals from aqueous solutions. Water Research, 35, 3551–3556.

Aravindhan R, Madhan B, Rao JR and Nair BU. (2004). Recovery and reuse of chromium from tannery wastewaters using *Turbinaria ornata* seaweed. Journal of Chemical Technology and Biotechnology, 79, 1251–1258.

Ashkenazy R, Gottlieb L and Yannai S. (1997). Characterization of acetone washed yeast biomass functional groups involved in lead biosorption. Biotechnology and Bioengineering, 55(1), 10001–10010.

Bai RS and Abraham TE. (2002). Studies on enhancement of Cr(VI) biosorption by chemically modified biomass of *Rhizopus nigricans*. Water Research, 36(5), 1224–1236.

Baig TH, Garcia AE, Tiemann KJ and Gardea-Torresdey JL. (1999). Adsorption of heavy metal ions by the biomass of *Solanum elaeagnifolium* (Silverleaf Night Shade). Proceedings of the 1999 Conference on Hazardous Waste Research, pp. 131–141.

Baker AJM and Brooks RR. (1989). Terrestrial higher plants which hyperaccumulate metal elements. A review of their distribution, ecology and phytochemistry. Biorecovery, 1, 81–126.

Baker AJM, Reeves RD and Hajar ASM. (1994). Heavy metal accumulation and tolerance in British population of metallophyte *Thlaspi carulescens* J & C. Presl (*Brassicaceae*). New Phytol.

Baker AJM, Reeves RD and McGrath SP. (1991). In situ decontamination of heavy metal polluted soils using crops of metal-accumulating plants – A feasibility study. In R.E. Hinchee and R.F. Olfenbuttel (eds.), In Situ Bioreclamation, pp. 600–605. Butterworth Heinemann, Boston.

Banat F, Asheh S-Al and Makhadmeh L. (2003). Kinetics and equilibrium study of cadmium ion sorption onto Date-pits, an agricultural waste. Adsorption Science and Technology, 21, 245–260.

Bao-Xiu Z, Peng W, Tong Z, Chun-yun C, Jing S. (2006). Preparation and adsorption performance of a cellulosic adsorbent resin for copper (II). Journal of Applied Polymer Science, 99, 2951–2956.

Basso MC, Cerrella EG and Cukierman AL. (2002). Activated carbons developed from a rapidly renewable biosource for removal of cadmium and nickel ions from dilute aqueous solutions. Journal of Industrial Engineering and Chemical Research, 41, 180–189.

Basu A, Kumar S and Mukhrjee S. (2003). Arsenic reduction from aqueous environment by water lettuce (*Pistia stratiotes* L.). Indian Journal of Environmental Health, 45, 143–150.

Bicak N and Senkel SF. (1998). Sorbitol-modified poly(N-glycidyl styrene sulfonamide) for removal of boron. Journal of Applied Polymer Science, 68, 2113–2119.

Boddu VM, Abburi K, Talbott J and Smith ED. (2003). Removal of hexavalent chromium from wastewater using a new composite Chitosan biosorbent. Environment Science and Technology, 37, 4449–4456.

Bradford M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72, 248–250.

Brandel JE, Labbe H, Hattori J and Miki BL. (1993). Field performance and heavy metal concentration in transgenic flue-crude tobacco expressing a mammalian metallothionein, glucuronidase gene fusion. Genome, 36, 255–260.

Brewbaker JL. (1987). *Leucaena*: A multipurpose tree genus for tropical agroforestry. In H.A. Steppler and P.K.R. Nair (eds.), Agroforestry: A Decade of Development, pp. 289–323. ICRAF, Nairobi, Kenya.

Brewbaker JL, Hedge N, Hutton EM, Jones RJ, Lowry JB, Moong F and Vanden Beldt R. (1985). *Leucaena* – Forage Production and Use, Vol. 39, pp. 112–117. NFTA, Hawaii.

Brooks RR. (1977). Copper and cobalt uptake by *Haumania* strum species. Plant and Soil, 48, 541–544.

Brooks RR. (1983). Biological Methods of Mineral Mining. Wiley, New York.

Brostlap AC and Schuurmans J. (1998). Kinetics of Valine uptake in tobacco leaf disc. Comparison of wild types the digenic mutant and its monogenic derivatives. Planta, 176, 42–50.

Brown SL, Angle JS, Chaney RL and Baker AJM. (1995). Zinc and cadmium uptake by *Thlaspi caerulescens* grown in nutrient solution. Soil Science Society of America Journal, 59, 125–133.

Brown SL, Chaney RL, Angles JS and Baker AJM. (1994). Zinc and cadmium uptake by *Thlapsi caerulescens* and *Silene vulgaris* in relation to soil metals and soil pH. Journal of Environmental Quality, 23, 1151–1157.

Budavari S, O'Neil MJ, Smith A and Heckelman PE. (1989). Merck Index. Ed. Merck & Company.

Caceres A and Saravia A. (1992). Pharmacological properties of *Moringa oleifera*: Screening for antispasmodic, anti-inflammatory and diuretic activity. Journal of Ethnopharmacology, 36, 233–237.

Cataldo DA, Kristin M and Thomas R. (1988). Organic constituents and complexation of Nickel (II), Iron (III), Cadmium (II) and Plutonium (IV) in soybean xylem exudates. Plant Physiology, 86, 734–739.

Chaney RL. (1983). Plant uptake of inorganic waste constituents. In J.F. Parr, P.B. Marsh and J.M. Kla (eds.), Land Treatment of Hazardous Waste. Noyes Data Crop, pp. 50–76. Park Ridge, NJ.

Chaney RL. (1993). Zinc phytotoxicity. In A.D. Robson (ed.), Zinc in Soil and Plants, pp. 135–150. Kluwer Academic Publ., Dordecht.

Chaney RL, Brown SL, Li YM, Angle JS, Homer FA and Green CE. (1995). Potential use of metal hyperaccumulators. Mining Environmental Management, 3(3), 9–11.

Chaney RI, Hornick SB and Sipora LJ. (1981). Review and preliminary studies of industrial land treatment practices. In Proceedings of Seventh Annual Research Symposium on Land Disposal of Municipal Solid and Hazardous Waste and Resource Recovery, pp. 200–212.

Chaney RL, Kaufman DD, Hornick SB, Parr JF, Sikora LJ, Burge WD and Fisher RH. (1981). Review of information relevant to land treatment of hazardous wastes. Report to EPA Solid and Hazardous Waste Research Division, p. 476.

Chaney RL, Li YM, Angle JS, Baker AJM, Reeves RD, Brown SL, Honer FA, Malik M and Chinm M. (1999). Improving metal hyperaccumulator wild plants to develop commercial phytoextraction system: Approaches and progress. In N. Terry and G.S. Banuelos (eds.), Phytoremediation of Contaminated Soil and Water, pp. 131–160, CRC Press, Boca Raton, FL.

Clausen CA. (2000). Isolating metal tolerant bacteria capable of removing copper, chromium, and arsenic from treated wood. Waste Management and Research, 18, 264–268.

Cobo JM, Castineira M. (1997). Oxidative stress, mitochondrial respiration, and glycemic control: Clues from chronic supplementation with Cr^{3+} or As^{3+} to male Wistar rats. Nutrition, 13, 965–970.

Costa G, Michant JC and Guckert G. (1997). Amino acids exuded from cadmium concentrations. Journal of Plant Nutrition, 20, 883–900.

Cotton FA, Wilkinson G, Murillo CA and Bochmann M. (1999). Advanced Inorganic Chemistry, 6th ed. John Wiley & Sons, New York.

Cunningham SD and Berti WR. (1993). In vitro. Cell Development Biology, 29, 207–212.

Dahot U. (1998). Antimicrobial activity of small protein of *Moringa oleifera* leaves. Journal of Islamic Academy of Sciences, 11, 111–113.

Dangi SY, Jolly CI and Narayanan S. (2002). Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera*. Pharmaceutical Biology, 40, 144–148.

Das BR, Kurup PA, Rao PL and Narasimha. (1957). Antibiotic principle from *Moringa pterygosperma*. VII. Antibacterial activity and chemical structure of compounds related to pterygospermin. Indian Journal of Medical Research, 45, 191–196.

Daxenbichler ME, Spencer FG, Carlson DG, Rose GB, Brinker AM and Powell RG. (1991). Glucosinolate composition of seeds from 297 species of wild plants. Phytochemistry, 30, 2623–2638.

Delvin S. (2002). Amino Acids and Proteins, 1st ed. IVY, New Delhi.

D'Mello JPF and Fraser KW. (1981). The composition of leaf meal from *Leucaena leucocephala*. Tropical Science, 23, 75–82.

Ellman GL. (1959). Tissue sulfhydryl groups. Archive of Biochemistry, 82, 70–77.

Elmayan T and Tepfer M. (1994). Synthesis of a bifunctional metallothionein/glucuronidase fusion protein in transgenic tobacco plants as a means of reducing leaf cadmium levels. Plant Journal, 433–440.

Ernst, WHO. (1974). Schwermetallvegetation der Erde. Fisher. Stuttgart, Germany.
Ezeamuzie IC, Ambedederomo AW, Shode FO and Ekwebelem SC. (1996). Anti-inflammatory effects of *Moringa oleifera* root extract. International Journal of Pharmacognosy, 34, 207–212.
Faisal, M and Hasnain, S. (2004). Microbial conversion of Cr(VI) into Cr(III) in industrial effluent. African Journal of Biotechnology, 3(11), 610–617.
Faizi S. (1994). Pharmacological studies on hypotensive and spasmolytic activities of pure compounds from *Moringa oleifera*. Phytotherapeutic Research, 8, 87–91.
Faizi S, Siddiqi BS, Saleem R, Aftab K, Shaheen F and Gilani AH. (1998). Hypotensive constituents from the pods of *Moringa oleifera*. Planta Medica, 64, 225–228.
Faizi S, Siddiqui BS, Saleem R, Siddiqui S and Aftab K. (1994). Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. Journal of Natural Products, 57, 1256–1261.
Ferguson J. (1990). The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Pergamon Press, Oxford.
Fincher RM and Koerker RM. (1987). Long term survival in acute arsenic encephalopathy. Follow up using newer measures of electrophysiologic parameters. American Journal of Medicine, 82, 549–552.
Flora SJS. (1991). Influence of zinc and copper supplementation during chelation of lead in rats. Human & Experimental Toxicology, 10, 331–336.
Flora SJS. (1999). Arsenic induced oxidative stress and its turn over following combined administration of N-acetylcysteine and meso 2, 3-dimercaptosuccinic acid in rats. Clinical and Experimental Pharmacology and Physiology, 26, 865–869.
Flora SJS and Kumar P. (1993). Biochemical and immunotoxicological evaluation of few metal chelating drugs in rats. Drug Investigation, 5, 269–273.
Flora SJS and Mehta A. (2003). Haematological, hepatic and renal alternations after repeated oral and intraperitoneal administration of monoisoamyl DMSA. Changes in female rats. Journal of Applied Toxicology, 23, 97–102.
Flora SJS and Tandon SK. (1990). Beneficial effects of zinc supplementation during chelation treatment of lead intoxication in rats. Toxicology, 64, 129–139.
Flora SJS and Tandon SK. (1995). Adjuvants for therapeutic chelating drugs in lead intoxication. Trace Elements and Electrolytes, 12, 131–140.
Flora SJS and Tripathi N. (1998). Beneficial effects of combined administration of thiamine, methionine or zinc with few chelating agents in preventing acute cadmium toxicity in mice. Indian Journal of Pharmacology, 30, 209–217.
Flora SJS, Bhattacharya R and Sachan SRS. (1994). Dose dependent effects of zinc supplementation during chelation of lead in rats. Pharmacology & Toxicology, 74, 330–333.
Flora SJS, Bhattacharya R and Vijayaraghvan R. (1995). Combined therapeutic potential of meso 2, 3 dimercaptosuccinic acid and calcium disodium edentate in experimental lead intoxication in rats. Fundamental and Applied Toxicology, 25, 233–240.
Flora SJS, Dhawan M and Tandon SK. (1990). Influence of copper supplementation during chelation of lead. Clinical Chemistry and Enzymology Communications, 3, 97–105.
Flora SJS, Dube SN, Arora U, Kannan GM, Shukla MK and Malhotra PR. (1995). Therapeutic potential of meso 2, 3-dimercaptosuccinic acid and 2, 3-dimercaptopropane 1-sulfonate against chronic arsenic poisoning in rats. Biometals, 8, 111–116.
Flora SJS, Gubrelay U, Kannan GM and Mathur RJ. (1998). Effects of zinc supplementation during chelating agents administration in cadmium intoxication in rats. Journal of Applied Toxicology, 18, 357–362.
Flora SJS, Pant BP, Tripathi N, Kannan GM and Jaiswal DKJ. (1997). Essential metal status during pre-term labour. Journal of Occupational Health, 39, 119–123.
Flora SJS, Singh S and Tandon SK. (1986). Chelation in metal intoxication XVIII: Combined effect of thiamine and calcium disodium versenate on lead toxicity. Life Science, 38, 67–71.
Folkard GK and Sutherland JP. (1996). *Moringa oleifera* a tree and a litany of potential. Agroforestry Today, 8, 5–8.

Folkard GK and Sutherland JP. (2002). Development of a naturally derived coagulant for water and wastewater treatment. Water Supply, 2, 89–94.

Fourest E and Roux J. (1992). Heavy metal biosorption by fungal mycelial by products: Mechanisms and influence of pH. Applied Microbiology and Biotechnology, 37, 399–403.

Gardea-Torresdey JL, Tang L and Salvador JM. (1996). Copper adsorption by esterified and unesterifed fractions of *Sphagnum* peat moss and its different humic substances. Journal of Hazardous Material, 48, 191–206.

Gardea-Torresdey JL, Tiemann KJ, Dokken, K and Gamez, G. (1998). Investigation of metal binding in *Alfalfa* biomass through chemical modification of amino and sulfhydryl Ligands. Conference on Hazardous Waste Research. pp. 111–121.

Garg U, Kaur MP, Jawa GK, Sud D, Garg VK. (2008). Removal of Cadmium (II) from aqueous solutions by adsorption on agricultural waste biomass. Journal of Hazardous Material, 154(1–3), 1149–1157.

Ghimire KN, Inoue K, Makino K and Miyajima T. (2002). Adsorptive removal of arsenic using orange juice residue. Separation Science and Technology, 37, 2785–2799.

Goering HK and Vansoest PJ. (1970). Forage Fiber Analysis. Handbook No. 379, USDA, Washington, DC.

Gonzalez RL, Videa JRP, Rodriguez E, Delgado M and Torresday GJL. (2006). Potential of *Agave lechuguilla* biomass for Cr(III) removal from aqueous solutions: Thermodynamic studies. Bioresource Technology, 97, 178–182.

Goyal P and Srivastava S. (2008a). Metal biosorption equilibria in a ternary aqueous system using agricultural waste Ficus religiosa leaf powder. National Academic of Science Letter, 31(11 & 12), 347–351.

Goyal P and Srivastava S. (2008b). Role of biosorbent *Ficus religiosa* leaf powder (FRLP) in chemical speciation of chromium: A green method for separation. Archives of Environment Protection, 34(2), 35–45.

Goyal P and Srivastava S. (in press). A novel eco-friendly biomaterial *Ficus religiosa* leaf powder (FRLP) for the removal of Ni(II) ion from water bodies. Asian Journal of Water, Environment and Pollution, The Netherlands.

Goyal P, Sharma P, Srivastava S and Srivastava MM. (2007). Potential of *Saraca indica* leaf powder (SILP) for chromium removal from aqueous solution. Archives of Environment Protection, 33(2), 35–44.

Goyal P, Sharma P, Srivastava S and Srivastava MM. (2008). *Saraca indica* leaf powder for decontamination of lead: Removal, recovery, adsorbent characterization and equilibrium modeling. International Journal of Environmental Science & Technology, 5(1), 27–34.

Goyal P, Srivastava MM and Srivastava S. (2007a). Ficus religiosa leaf powder: A green economical biomaterial for the removal of Cd(II) ions from aqueous solution, Journal of Nuclear Agriculture and Biology, 36(1&2), 16–27.

Goyal P, Srivastava MM and Srivastava S. (2007b). Multi metal removal from aqueous system using agricultural waste *Saraca indica* leaf powder. Journal of Environment Biology, 30(1) (in press).

Goyal P, Srivastava MM and Srivastava S. (2009). Characterization of novel *Zea mays* based biomaterial designed for toxic metals biosorption. Journal of Hazardous Materials, 179, 1206–1211.

Guclu G, Gurdag G and Ozgumus S. (2003). Competitive removal of heavy metal ions by cellulose graft copolymers. Journal of Applied Polymer Science, 90, 331–333.

Gupta VK, Jain CK, Ali I, Sharma M and Saini VK. (2003). Removal of cadmium and nickel from waste water using bagasse fly ash – A sugar industry. Water Research, 37, 4038–4044.

Hanif MA, Nadeem R, Zafar MN, Akhtar K and Bhatti HN. (2007). Kinetic studies for Ni(II) biosorption from industrial wastewater by *Cassia fistula* (Golden Shower) biomass. Journal of Hazardous Material, 145(3), 501–505.

Hannafiah MAKM, Ibrahim SC and Yahya MZA. (2006). Equilibrium adsorption study of lead ions onto sodium hydroxide modified lalang (Imperata cylindrical) leaf powder. Journal of Applied Sciences and Research, 2, 1169–1174.

Hasan SH, Ranjan DB, Bhattacharjee N and Talat M. (2008). Optimization of sorption of Pb(II) from water using rice bran. 4th Kuala Lumpur International Conference on Biomedical Engineering, Vol. 21, pp. 879–882.

Hashem A. (2006). Amidoximated sunflower stalks (ASFS) as a new adsorbent for removal of Cu(II) from aqueous solutions. Polymer Plastic Technology and Engineering, 45, 35–42.

Hashem MA. (2007). Adsorption of lead ions from aqueous solution by okra wastes. International Journal of Physical Science, 2(7), 178–184.

Hering JG. (1995). Metal speciation and contamination of soil. CRC, Boca Raton, FL, pp. 59–86.

Homer FA, Morrison RS, Brooks RR, Clemens J and Reeves RD (1991). Comparative studies of nickel, cobalt and copper uptake by some nickel hyperaccumulators of the genus Alyssum. Plant and Soil, 138, 195–205.

Horsfall Jnr M and Spiff AI. (2005). Effect of metal ion concentration on the biosorption of Pb^{+2} and Cd^{+2} by *Caladium bicolor* (wild cocoyam). African Journal of Biotechnology, 4(2), 191–196.

Horsfall Jnr M, Abia AA and Spiff AI. (2003). Removal of Cu(II) and Zn(II) ions from waste water by cassava (Manihot esculenta Cranz) waste biomass. African Journal of Biotechnology, 2, 360–364.

Iqbal M, Saeed A and Akhtar N. (2002). Petiolar felt sheath of palm: A new biosorbent for the removal of heavy metals from contaminated water. Bioresource Technology, 81, 151–153.

Irene MV, Robert, M Gajo T and Rosette CG. (1998). Bioactivity studies on the alkaloid extracts from seeds of *Leucaena leucocephala*. Physiotherapy Research, 11(8), 615–617.

Isabel V, Nuria F, Maria M, Nuria M, Jordi P and Joan S. (2004). Removal of copper and nickel ions from aqueous solutions by grape stalks wastes. Water Research, 38, 992–1002.

Jahn SAA. (1989). Simplified water treatment technologies for rural areas, GATE (GTZ), 1, 37–41.

Jahn SAA. (1991). The traditional domestication of a multipurpose tree *Moringa stenopetala* in Ethiopian Rift valley. Ambio, 20: 244–247.

Johari M and Noor MM. (2001). *Moringa oleifera* seeds as a flocculant in waste sludge treatment. International Journal of Environmental Studies, 58, 185–195.

Johari M and Noor MM. (2002). Effects of oil extraction from *Moringa oleifera* seeds on coagulation of turbid waters. International Journal of Environmental Studies, 59, 243–254.

John J and Narwal SS. (2003). Allelopathic plants *Leucaena leucocephala* (Lam.) de Wit. Allelopathic Journal, 12(1), 13–36.

Jollow DJ, Mitchell JR, Zamppaglione Z and Gillette JR. (1974). Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolites. Pharmacology, 11, 151–154.

Jones RJ. (1979). The value of *Leucaena leucocephala* as a feed for ruminants in the tropics. World Animal Review, 31, 13–23.

Jones DL, Edwards AC, Donachie K and Darrah PR. (1994). Role of proteinaceous amino acids related in root exudates in nutrient acquisition from the rhizosphere. Plant and Soil, 158, 183–192.

Jose TA, Oliveira, Silveria BS, Vasconcelos LM, Cavada BS and Moriera RA. (1999). Compositional and nutritional attributes of seeds from the multipurpose tree *Moringa oleifera* Lam. Journal of the Science of Food and Agriculture, 79, 815–820.

Kalogo Y and Rosillon F. (2000). Effect of water extract of *Moringa oleifera* seeds on the hydrolytic microbial species diversity of UASB reactor treating domestic wastewater. Letters in Applied Microbiology, 31, 259–264.

Kalogo Y and Verstraete W. (2000). Technical feasibility of the treatment of domestic wastewater by CEPS-UASB system. Environmental Technology, 21, 55–65.

Kamer U, Cotter-Howells JD, Charnock JM, Baker AJM and Smith AJC. Free histidine as a metal chelator in plants that accumulate nickel. Nature, 379, 635–638.

Kang BT, Wilson GF and Sipkens L. (1981). Alley cropping maize (*Zea mays* L.) and *Leucaena* (*Leucaena leucocephala* Lam de Wit) in southern Nigeria. Plant and Soil, 63, 167–179.

Kardam A, Goyal P, Arora JK, Raj KR and Srivastava S. (2009). Novel biopolymeric material: Synthesis and characterization for decontamination of cadmium metal from waste water. National Academy of Science Letter, 32(5&6), 179–181.

Kartal SN and Imamura Y. (2005). Removal of Cu(II), Cr(III) and As(III) from CCA-treated wood onto chitin and chitosan. Bioresource Technology, 96, 389–392.
Keith LH and Telliard WA. (1979). Priority pollutants. Environment Science and Technology, 13, 416–424.
Klimmek S, Stan HJ, Wilke A, Bunke G, Buchholz R. (2001). Comaparative analysis of the biosorption of cadmium, lead, nickel and zinc by algae. Environmental Science & Technology, 35(21), 4283–4288.
Kobya, M. (2004). Removal of Chromium (VI) from aqueous solutions by adsorption onto hazelnut shell activated carbon: Kinetics and equilibrium studies. Bioresource Technology, 91, 317–321.
Kratochvil D and Volesky B. (1998). Advances in the biosorption of heavy metals. Trends in Biotechnology, 16, 291–300.
Kratochvil D and Volesky B. (2000). Multicomponent biosorption in fixed beds. Water Research, 34, 3186–3196.
Kratochvil D, Pimentel P and Volesky B. (1998). Removal of trivalent and hexavalent chromium by seaweed biosorbent. Environmental Science Technology, 32, 2693–2698.
Krishnan KA and Anirudhan TS. (2003). Removal of cadmium from aqueous solution by steam activated sulphurised carbon prepared from sugar cane bagasse pith. Water, 29, 1520–1528.
Kumar U and Bandyopadhyay M. (2006). Sorption of cadmium from aqueous solution using retreated rice husk. Bioresource Technology, 97, 104–109.
Kumari P, Sharma P, Srivastava S and Srivastava MM. (2005). Arsenic removal from aqueous system using plant biomass: A bioremedial approach. Journal of Industrial Microbiology and Biotechnology, 32, 521–526.
Kumari P, Sharma P, Srivastava S and Srivastava MM. (2006). Biosorption studies on shelled *Moringa oleifera* Lamarck seed powder: Removal and recovery of arsenic from aqueous system. International Journal of Mineral Processing, 78(3), 131–139.
Kumari P, Sharma P, Srivastava S and Srivastava MM. (2007). Isolation and characterization of protein content of shelled *Moringa oleifera* seeds used for decontamination of arsenic from water bodies. Process Biochemistry, 28(1), 758–765.
Kumari P, Srivastava S and Srivastava MM. (2005). Biosorption studies on shelled *Moringa oleifera* Lamarck seed powder: Removal and recovery of arsenic from aqueous system. International Journal of Mineral Processing, 78, 131–139.
Kuyucak N and Volesky B. (1988). Biosorbents for recovery of metals from industrial solutions. Biotechnology and Letters, 10, 137–142.
Laemmli UK. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680–685.
Lander JJ, Stanley, RJ and Summer HW. (1975). Angiosarcoma of the liver associated with Fowler's solution (potassium arsenite). Gastroenterology, 68, 1582–1586.
Le Quesne PM. (1982). Metal-induced diseases of the nervous system. British Journal of Hospital Medicine, 28, 534–538.
Lee J, Reeves RD, Brooks RR and Jaffer TT. (1977). Isolation and identification of a citric acid in some nickel-accumulating plants. Phytochemistry, 16, 1503–1505.
Lee J, Reeve RD, Brooks R and Jaffer TT. (1978). The relation between nickel and citric acid in some nickel-accumulating plants. Phytochemistry, 17, 1033–1035.
Lerman BB, Ali N and Green D. (1980). Megaloblastic, dyserythropoietic anaemia following arsenic ingestion. Annals of Clinical and Laboratory Science, 10, 515–517.
Lien HC, Tsai TF and Lee YY. (1999). Merkel cell carcinoma and chronic arsenicism. Journal of the American Academy of Dermatology, 41, 641–643.
Limaye DA, Nimbkar AY, Jain R and Ahmed M. (1995). Cardiovascular effects of *Moringa pterygosperma*. Phytotherapy Research, 9, 37–40.
Lin S and Rays GD. (1998). Impact of surface modification on binding affinity distributions of *Datura innoxia* biomass to metal ions. Environmental Science & Technology, 32, 1488–1492.
Liu M, Deng Y, Zhan H, Zhan X. (2002). Adsorption and desorption of Copper (II) from solutions on new spherical cellulose adsorbent. Journal of Applied Polymer Science, 84, 478–485.

Ma JF and Nomoto K. (1996). Effective regulation of Iron acquisition in graminaceous plants. The role of mugineic acids as phytosideres. Plant Physiology, 97, 609–617.

Makkar HPS and Becker K. (1996). Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Animal Feed Science and Technology, 63, 211–228.

Martins JE, Pardo R and Boaventura RAR. (2004). Cadmium (II) and Zinc (II) adsorption by the aquatic moss *Fontinalis antipyretica*: Effect of temperature, pH and water hardness. Water Research, 38, 693–699.

Maruganandan S, Srinivasan K, Tandon SK, Lal J, Chandra S, Raviprakash, Hasan HA, Dewived S, Kukreja AK, Sharma A, Singh IK, Sharma S and Tiwari R. (2001). Anti-inflammatory and analgesic activities of some medicinal plants. Journal of Medicinal and Aromatic Plant Sciences, 22–23 4A-1A, 56–58.

Mazumder DN, Das-Gupta J and Santra A. (1998). Chronic arsenic toxicity in West Bengal – The worse calamity in the world. Journal of the Indian Medical Association, 96, 4–7.

Mazumder UK, Gupta M, Chakrabarti S and Pal D. (1999). Evaluation of hematological and hepatorenal functions of methanolic extract of *Moringa oleifera* Lam. root treated mice. Indian Journal of Experimental Biology, 37, 612–614.

McBride MB. (1994). Environmental chemistry of soils. Oxford University Press, New York.

McConnachie GL. (1993). Water treatment for developing countries using baffled channel hydraulic flocculation. Water Maritime and Energy, 101, 55–61.

McConnachie GL, Evison LM and Folkard GK. (1999). Field trials of appropriate hydraulic flocculation processes. Water Research, 33, 1425–1434.

McCrod JM, Keele BB and Fridovich I. (1976). An enzyme based theory of obligate anaerobiosis; The physiological functions of superoxide dismutase. Proceedings of the National Academy of Sciences USA, 68, 1024–1027.

Mehta A and Flora SJS. (2001). Possible role of metal redistribution, hepatotoxicity and oxidative stress in chelating agents induced hepatic and renal metallothionein in rats. Food and Chemical Toxicology, 39, 1029–1038.

Mench M and Martin E. (1991). Mobilization of cadmium and other metals from two soil by root exudates of *Zea mays*, *Nicotiana tabacum*, *Nicotiana rustica*. Plant and Soil, 132, 187–196.

Meunier N, Laroulandie J, Blais JF and Tyagi RD. (2003). Cocoa shells for heavy metal removal from acidic solutions. Bioresource Technology, 90, 255–263.

Min SH, Han JS, Shin EW and Park JK. (2004). Improvement of cadmium ion removal by base treatment of juniper fiber. Water Research, 38, 1289–1295.

Muyibi SA and Evison LM. (1995a). *Moringa oleifera* seeds for softening hard water. Water Research, 29, 1099–1105.

Muyibi SA and Evison LM. (1995b). Optimizing physical parameters affecting coagulation of turbid water with *Moringa oleifera* seeds. Water Research, 29, 2689–2695.

Muyibi SA and Evison LM. (1996). Coagulation of turbid water and softening of hard water with *Moringa oleifera* seeds. International Journal of Environmental Studies, 49, 247–259.

Muyibi SA and Okuofu CA. (1995). Coagulation of low turbidity surface water with *Moringa oleifera* seeds. International Journal of Environmental Studies, 48, 263–273.

Nagarnaik PB, Bhole AG and Natarajan GS. (2003). Adsorption of arsenic on flyash. Indian Journal of Environmental Health, 45, 1–4.

NAS. (1977). *Leucaena* – Promising forage and tree crop for the tropics. National Academy of Science, Washington, DC.

National Research Council (NRC). (1999). Arsenic in Drinking Water, p. 310. National Academy Press, Washington, DC.

Ndabigengesere A. (1995). Active agents and mechanism of coagulation of turbid water using *Moringa oleifera*. Water Research, 29, 703–710.

Ndabigengesere A and Narasiah KS. (1998). Quality of water treated by coagulation using *Moringa oleifera* seeds. Water Research, 32, 781–791.

Ndabigensere A and Narasiah KS. (1996). Influence of operating parameters on turbidity removal by coagulation with *Moringa oleifera* seeds. Environmental Technology, 17, 1103–1112.

Nicholas R, Steven PK and Claussen K. (2003). Lead and nickel removal using Microspora and Lemna minor. Bioresource Technology, 89, 41–48.

Nigam R, Srivastava S, Prakash S and Srivastava MM. (2000). Effect of organic on the availability of cadmium in wheat. Chemical Speciation and Bioavailability, 12(4), 125–132.

Nigam R, Srivastava S, Prakash S and Srivastava MM. (2001). Cadmium mobilization and plant availability – The impact of organic acids commonly exudates from roots. Plant and Soil, 230, 125–132.

Niu H and Volesky B. (2003). Characteristics of anionic metal species biosorption with waste crab shells. Hydrometallurgy, 71, 209–215.

Nyambati EM, Sollenberger LE, Karue CN and Musimba NKR. (2006). The value of *Acacia brevispica* and *Leucaena leucocephala* seed pods as dry season supplements for calves in dry areas of Kenya. African Journal of Agricultural Research, 1(4), 118–124.

O'Connel DW, Birskinshaw C and O'Dwyer TF. (2006). A chelating cellulose adsorbent for the removal of Cu(II) from aqueous solutions. Journal of Applied Polymer Science, 99(6), 2888–2897.

Olayemi AB. (1994). Studies on traditional water purification using *Moringa oleifera* seeds. IEM Journal, 15, 135–142.

Oliveria JTA and Silveria SB. (1999). Compositional and nutritional attributes of seeds from the multiple purpose tree *Moringa oleifera* Lam. Journal of the Science of Food and Agriculture, 79, 815–820.

Orlando US, Baes A, Nishijima W, Okado M. (2002). Preparation of chelating agents from sugarcane bagasse by microwave radiations as an alternative ecologically benign procedure. Green Chemistry, 4, 555–557.

Ortiz DE, Ruscitti T, McCue KF and Ow DW. (1995). Transport of metal binding peptides by HMTI, a fission Yeast ABC-type vacuolar membrane protein. The Journal of Biological Chemistry, 270, 4721–4728.

Padmavathy P and Shobha S. (1987). Effect of processing on protein quality and mimosine content of Soobabul. Journal of Food Science and Technology, 24, 180–182.

Pagnanelli F, Papini MP, Toro L, Trifoni M and Veglio F. (2000). Biosorption of metal ions on *Arthrobacter* sp.: Biomass characterization and biosorption modeling. Environmental Science and Technology, 34(13), 2773–2778.

Pagnanelli F, Sara M, Veglio F and Luigi T. (2003). Heavy metal removal by olive pomace: Biosorbent characterization and equilibrium modeling. Chemical Engineering and Sciences, 58, 4709–4717.

Pagnanelli F, Toro L and Sara M. (2002). Removal and recovery of Cd(II), Ni(II), Pb(II) and Cr(III) by olive mill residue. Environment Science and Technology, 15, 47–59.

Pan A, Tie F, Duau Z, Yang M, Wang Z, Li L, Chem Z and Ru B. (1993). Domain of human metallothionein I-A can bind to metals in transgenic tobacco plants. Molecular and General Genetics, 242, 666–674.

Parmar H, Patel J, Sudhakar P and Koshi VJ. (2006). Removal of fluoride from water with powdered corn cobs. Journal of Environmental Science & Engineering, 48(2), 135–138.

Parvathi K, Nagendran R and Nareshkumar R. (2007). Lead biosorption onto waste yeast by product, a means to decontaminate effluent generated from battery manufacturing industry. Environment Biotechnology, 10(1), 92–105.

Paul SS. (2000). Detoxification of mimosine and dihydroxypyridone: A review. Agriculture Review, 21(2), 104–109.

Ramakrishna S, Fujihara K, Teo WE, Lim TC and Ma ZW. (2005). World Scientific, New Jersey.

Raskin I. (1996). Plant genetic engineering may help with environmental cleanup. Proceedings of the National Academy of Sciences USA, 93, 3154–3166.

Ravindran V. (1992). Aminoacid composition and protein quality of common Sri Lankan feedstuffs for growing pigs. Journal of National Science Council, Sri Lanka, 20(1), 99–105.

Robinson BH, Brooks RR and Clotheir BE. (1999). Soil amendments affecting nickel and cobalt uptake by *Berkheya coddii*: Potential use for phytomining and phytoremediation. Annals of Botany, 84, 689–694.

Rosa GD, Torresday G, Videa JR, Herrera I and Contreras C. (2003). Use of silica-immobilized humin for heavy metal removal from aqueous solution under flow conditions. Bioresource Technology, 90, 11–17.

Rugh CL, Wilde HD, Stock NM, Thompson DM, Summers AD and Meagher RR. (1996). Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial mer A gene. Proceedings of the National Academy of Sciences USA, 93, 3182–3187.

Sag Y and Kutsal T. (1996). Fully competitive biosorption of Chromium (VI) and Iron (III) ions from binary metal mixtures by *R. arrhizus*: Use of the competitive Langmuir model. Process Biochemistry, 31, 561–579.

Saito K, Iwata H and Furusaki S. (1991). Adsorption characteristics of an immobilized metal affinity membrane. Biotechnology Progress, 7, 412–418.

Salt DE, Blaylock M, Nanda-Kumar PBA, Dushenkov V, Ensley BD and Raskin I. (1995). Phytoremediation: A novel strategy for the removal of toxic elements from the environment using plant. Biotechnology, 13, 468–475.

Sawalha MF, Torresdey GJL, Parsons JG, Saupe G and Peralta-Videa JR. (2005). Determination of adsorption and speciation of chromium species by saltbush (*Atriplex canescens*) biomass using combination of XAS and ICP-OES. Microchemical Journal, 81, 122–132.

Sharma P, Goyal P and Srivastava S. (2007). Biosorption of trivalent and hexavalent form of chromium from aqueous systems using shelled *Moringa oleifera* seed. Chemical Speciation and Bioavailability, 19(4), 175–181.

Sharma P, Kumari P, Srivastava MM and Srivastava S. (2006). Removal of cadmium from aqueous system by shelled *Moringa oleifera* seed powder. Bioresource Technology, 97(2), 299–305.

Sharma P, Kumari P, Srivastava MM and Srivastava S. (2007). Ternary biosorption studies of Cd(II), Cr(III) and Ni(II) on shelled *Moringa oleifera* seeds. Bioresource Technology, 98(2), 474–477.

Shelton HM and Brewbaker JL. (1994). *Leucaena leucocephala* – The most widely used forage tree legume. Cited in http://www.defra.gov.uk

Shibi IG and Anirudhan TS. (2002). Synthesis, characterization and application as a mercury (II) sorbent of banana stalk-polyacrylamide grafted copolymer bearing carboxyl groups. Industrial Engineering and Chemical Research, 41, 5341–5352.

Shin EW, Karthikeyan KG and Tshabalala MA. (2007). Adsorption mechanism of cadmium on juniper bark and wood. Bioresource Technology, 98, 588–594.

Shukla PC, Talpada PM, Desai MC, Desai HB and Meisuriya MI. (1987). Chemical composition and nutritive value of *Leucaena leucocephala* seeds. Indian Journal of Animal Nutrition, 4(3), 207–208.

Shukla SR and Roshan SP. (2005). Adsorption of Cu(II), Ni(II), and Zn(II) on modified jute fibres. Bioresource Technology, 96, 1430–1438.

Singh KK, Rastogi R and Hasan SH. (2005). Removal of cadmium from wastewater using agricultural waste rice polish. Journal of Hazardous Material, 121, (1–3), 51–58.

Singh KK, Talat M and Hasan SH. (2006). Removal of lead from aqueous solutions by agricultural waste maize bran. Bioresource Technology, 97, 2124–2130.

Skowronski T, Pirszel J, Pawlik-Skowronska B. (2001). Heavy metal removal by the waste biomass of *Penicillium chrysogenum*. Water Quality Research Journal of Canada, 36(4), 793–803.

Smith RM and Martell AE. (1976). Critical Stability Constants, Inorganic Complexes. Plenum, New York, pp. 10–95.

Son BC, Park K, Song HS and Yoo YJ. (2004). Selective biosorption of mixed heavy metal ions using polysaccharides. Korean Journal of Chemical Engineering, 21, 1168–1172.

SPSS. (1983). Statistical Package for Social Science Marketing Department, SPSS. North Michigan Avenue, Chicago.

Srinivasa RP, Ajithapriya J, Kachireddy VN, Reddy S and Krishnaiah A. (2007). Biosorption of hexavalent chromium using tamarind (*Tamarindus indica*) fruit shell – A comparative study. Environmental Biotechnology, 10(3), 358–367.

Srivastava S, Nigam R, Prakash S and Srivastava MM. (1998). Studies on plant uptake of chromium in presence of organic acids – Possible role of root exudates. Journal of Nuclear Agricultural and Biology, 27(3), 208–213.

Srivastava S, Nigam R, Prakash S and Srivastava MM. (1999). Mobilisation of trivalent chromium in presence of organic acids: A hydroponic study on wheat plant (*Triticum vulgare*). Bulletin of Environmental Contamination and Toxicology, 63, 524–530.

Srivastava S, Prakash S and Srivastava MM. (1999a). Studies on mobilisation of chromium with acids. BioMetals, 12, 201–207.

Srivastava S, Prakash S and Srivastava MM. (1999b). Chromium mobilization and plant availability – The impact of organic complexating ligands. Plants and Soil, 212, 203–208.

Srivastava S, Prakash S and Srivastava MM. (1999c). Estimation of Cr(III), Cr(VI) and organically bound Cr(III) species using ion exchangers and ^{51}Cr radiotracer. Indian Journal of Chemistry, 38(A), 514–517.

Srivastava S, Shanker K, Prakash S and Srivastava MM. (1999d). Bioavailability of chromium: An interaction aspect. Journal of Environmental Biology, 20, 49–54.

Srivastava S, Srivastava S, Prakash S and Srivastava MM. (1998). Fate of trivalent chromium in presence of organic acids: A hydroponic study on the tomato plant. Chemical Speciation and Bioavailability, 10(4), 147–150.

Tan TW and Cheng P. (2003). Biosorption of heavy metal ions with *Pencillium chrysogenum*. Applied Biochemistry and Biotechnology, 104(2).

Taty-Costodes VC, Faudet H, Portet C, Delacroix A. (2003). Removal of Cd(II) and Pb(II) ions from aqueous solutions by adsorption onto sawdust of *Pinus sylvestris*. Journal of Hazardous Materials, B 105, 121–142.

Tsezos M. (1985). The selective extraction of metals from solution by microorganisms. A brief overview. Canadian Metallurgical Quarterly, 24, 141–144.

Ucun H, Bayhan YK, Kaha Y, Cakici A and Algur OF. (2002). Biosorption of Chromium (VI) from aqueous solution by cone biomass of *Pinus sylvestris*. Bioresource Technology, 85, 155–158.

Vankar PS and Tiwari V. (2002). Phytofiltration of toxic metals ions by *Portulaca oleracea*. Journal of Analytical Chemistry, References Number 2002_AAS001. Cited in Abdul, R. and Shakoori, A.R. (2004). Tolerance and uptake of cadmium and nickel by *Chlorella* sp., isolated from tannery effluents. Pakistan Journal of Zoology, 36, 327–332.

Veglio F and Beolchini F. (2001). Removal of metals by biosorption: A review. Hydrometallurgy, 59, 301–316.

Volesky B. (1987). Biosorbents for metal recovery. Trends in Biotechnology, 5, 96–101.

Volesky B. (1999). Detoxification of metal bearing effluents: Biosorption for the next century. Hydrometallurgy, 44, 301–316.

Volesky B. (2003). Sorption and biosorption. (ISBN 0-9732983-0-8) BV-Sorbex, Inc. St. Lambert (Montreal), Quebec, Canada.

Volesky B and Holan ZR. (1995). Accumulation of cadmium, lead and nickel by fungal and wood biosorbents. Applied Biochemistry and Biotechnology, 53, 133–146.

Volesky B, Kratochvil B and Schiewer S. (1999). Biosorption process for heavy metal removal. In Lovley DR (ed.), Environmental Microbe-Metal Interactions. Chapman and Hall, New York.

Wada Y, Mitomo H, Kasuya K, Nagasawa N, Seko N, Katakai A and Tamada M. (2006). Journal of Applied Polymer Science, 101, 3856–3861.

Wankasi D, Horsfall M, Jr, Spiff AI. (2006). Sorption kinetics of Pb^{2+} and Cu^{2+} ions from aqueous solutions by *Nipah palm* (Nypa fruiticans Wurmb) shoot biomass. Electronic Journal of Biotechnology, 9, 587–592.

White M, Chaney RL and Deker AM. (1981). Metal complexation in xylem fluid. III Electrophoretic evidences. Plant Physiology, 67, 311–315.

Yang J and Volesky B. (2000). Modeling of the uranium-proton ion exchange in biosorption. Environment Science and Technology, 33, 4079.

Zuwei Ma and Ramakrishna S. (2006). Journal of Applied Polymer Science, 101, 3835–3841.

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