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# Physical, Chemical and Phytoremediation Technique for Removal of Heavy Metals

Sharma S<sup>1</sup>, Rana S<sup>2</sup>, Thakkar A<sup>1</sup>, Baldi A<sup>1</sup>, Murthy RSR<sup>1</sup> and Sharma RK<sup>3</sup><sup>1</sup>Indo Soviet Friendship College of Pharmacy, Moga, Punjab, India<sup>2</sup>Division of CBRN Defence, Institute of Nuclear Medicine and Allied Sciences, Brig S.K Mazumdar Marg, Delhi, India<sup>3</sup>Defence Food Research Laboratory, Siddartha Nagar, Mysuru, India**Corresponding author:** Sharma RK, Defence Food Research Laboratory, Siddartha Nagar, Mysuru 570 011, India, Tel: 0821-2473783, 09449651632; Fax: 0821-2473468; E-mail: rksharmadrl@yahoo.com**Received date:** June 14, 2016; **Accepted date:** July 18, 2016; **Published date:** July 20, 2016**Copyright:** © 2016 Sharma S, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.**Citation:** Sharma S, Rana S, Thakkar A, et al. Physical, Chemical and Phytoremediation Technique for Removal of Heavy Metals. J Heavy Met Toxicity Dis. 2016, 1:2.

## Abstract

Mankind has been using plants and natural products since time immemorial for fighting the menace of heavy metal toxicity both in humans as well as in environment surrounding them. Nearly thirty five metals have been reported to cause occupational or accidental exposure to humans. Amongst these, twenty three are heavy metals. The increasing use of such heavy metals including radionuclides constitutes deleterious health issues. Presence of heavy metals in environment and their subsequent effects on humans down the food chain creates potential health hazard. Therefore removal of heavy metal has been a subject of paramount importance. Results of an exhaustive literature survey of natural and plant based compounds against heavy metal pollution including patents, books and scientific data from globally accepted scientific databases and search engines (Pubmed, Scopus and Web of Science, Sci Finder and Google Scholar), is systematically reviewed. It is conceived that a number of phytochemical agents as well microorganism can act as heavy metal removing agent both from human beings and the environment surrounding. Microbes which are used for the removal of heavy metals from the water bodies include bacteria, fungi, algae and yeast. Some important antioxidants such as flavonoids, pectin and phytic acid are also used for the elimination of the heavy metals from the human body. The present article is an extensive review that will offer a number of strategies and possible mechanisms for the heavy metals removal both from environment as well as from human body.

**Keywords:** Heavy metals; Chelation; Adsorption; Absorption; Bio-sorption; Phytoremediation

## Introduction

Heavy metals are the chemical elements having density greater than 5. Some of these elements called trace elements are a part of our normal diet and are essential for good health and present in human and animal tissue in very low concentration. These trace elements may be essential or non-essential. The important essential elements along with their concentration in blood includes iron (0.06-0.26 mg/l), zinc (4-8 mg/l), cobalt (20 µg/l), copper (0.08-0.45 µg/l), chromium (0.08-0.5 µg/l), manganese (6.7-10.4 µg/l) and molybdenum (5-157 µg/l) [1,2]. Other elements called ultra-trace elements normally comprise less than 1 µg/g of a given organism. Their concentration in blood includes cadmium (0.1-2 µg/l), lead (40-290 µg/l), lithium (0.52-0.64 µg/l), nickel (1.1-4 µg/l), tin (120-140 µg/l) and vanadium (0.1-0.9 µg/l) [3,4]. However chronic exposure of toxic dose of these metals in humans results in various complications in nervous system, respiratory system, renal system, hepatic system as well as reproductive system. Metals are also reported to cause allergies and repeated long-term contact with some metals or their compounds may even prove carcinogenic. Most of the heavy metals are well known toxic and carcinogenic agents and represents a serious threat to the human population and the fauna and flora of the receiving water bodies as they are persistent and non-biodegradable.

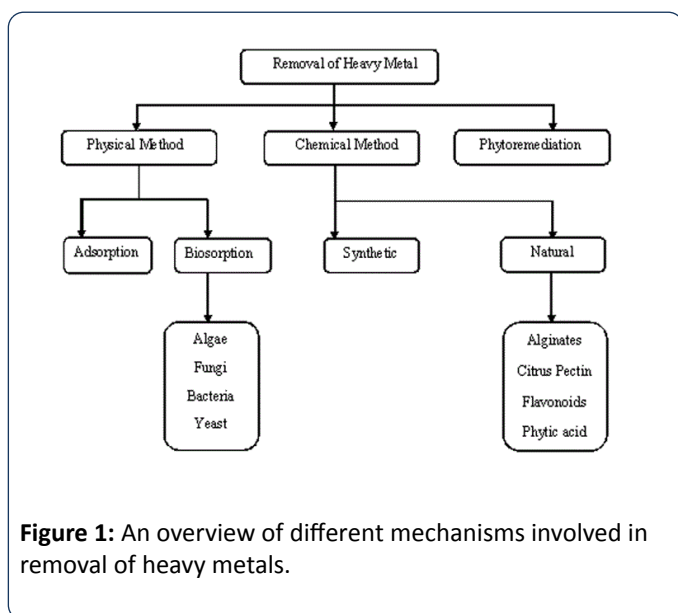
Various agencies around the world take care of diminished quality of life and potential threat to environment associated with exposure to hazardous substances. Agency for Toxic Substances and Disease Registry (ATSDR), a part of U.S. department of health and human services, is the main agency which has compiled a priority list for hazardous substances. Canadian Environment Assessment Agency in Canada is the federal body performing high quality environmental assessment so that potential environmental effect of elements can be prevented. Likewise in India, Ministry of Environment and Forest (MoEF) is the agency for planning, promotion, co-ordination and overseeing of India's environmental and forest

policy and programs. This agency has promulgated "Manufacture, Storage, and Import of Hazardous Chemicals (MSIHC) rules, 1989" under Environment (Protection) Act, 1986 which classify toxic industrial chemicals as high, medium and low risk chemicals.

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues inside the body. The source of heavy metals includes food, water, air, absorption through skin etc. The most common route of exposure for children is ingestion [5]. Less common routes of exposure are during a radiological procedure, from inappropriate dosing or monitoring during intravenous nutrition, from a broken thermometer [6] or from a suicide or homicide attempt. Likewise the source of heavy metals in environment is solid discharge from industry, nuclear power plants, smelting process of various metals, by-product from various process in chemical industry, volcanic eruption, combustion of fossil fuels, pesticides/insecticides etc. [7-9]. The presence of metals in environment is a potential source of toxicity owing to their transport down the food chain and their subsequent bio-magnification. They cannot be destroyed biologically and get transformed into different oxidation states or different organic complex [10,11]. Thus it is pertinent to explore remedy for removal of these toxic heavy metals both from environment as well as from human beings.

## Removal of Heavy Metals: Strategies and Mechanisms

Heavy metal removal may be accomplished by different mechanism. Figure 1 summarizes different possible mechanisms involved in heavy metal removal.



### Physical methods of heavy metal removal

**Adsorption method:** Adsorption is a physicochemical treatment processes which help in effective removal of heavy metals from metals contaminated wastewater and is one of

the most preferred and efficient method. Its major advantage includes effectiveness at both high/low contaminant concentrations, selectivity by employing tailored adsorbents, regeneration ability of used adsorbents and a comparatively low cost. The various adsorbents and corresponding heavy metals adsorbed by them are described in Table 1.

**Table 1:** Summary of modified plant wastes as adsorbents for the removal of heavy metal ions from aqueous solution containing metals.

Adsorbent	Modifying agent(s)	Heavy Metal	Qmax (mg g <sup>-1</sup> )	References
Rice husk	Water washed	Cd(II)	8.58	[79]
	Sodium hydroxide		20.24	
	Sodium bicarbonate		16.18	
	Epichlorohydrin		11.12	
Sawdust (C. deodar wood)	Sod. Hydroxide	Cd(II)	73.62	[80]
	Formaldehyde	Cr(VI)		[81]
	Sulfuric acid	Cu(II)	3.6	[82]
	Sod. Hydroxide	Ni (II)	13.95	[83]
	Sod. Hydroxide	Cu(II)	10.4	[84]
	Formaldehyde in Sulfuric acid	Zn(II)	7	[85]
		Pb(II),	6.92	
		Cd (II)	15.8	
Sawdust (Poplar tree)			9.78	
			9.29	
Sawdust (Pinus sylvestris)				
Groundnut husk	Sulfuric acid followed by silver impregnation	Cr (VI)	11.4	[86]
Wheat bran	Sulfuric acid	Cu (II)	51.5	[87]
	Sulfuric acid	Cd (II)	101	[88]
Banana pith	Nitric acid	Cu (II)	13.46	[89]
Cork powder	Calcium Chloride, Sodium Chloride, Sodium Hydroxide	Cu (II)	15.6	[90]
			19.5	
			18.8	
Corn cob	Nitric acid Citric acid	Cd (II)	19.3	[91]
			55.2	
<i>Azolla filiculoides</i> (aquatic fern)	Hydrogen peroxide–Magnesium chloride	Pb (II)	228	[92]
		Cd(II)	86	
		Cu (II)	62	
		Zn(II)	48	
Sugarcane bagasse	Sodium bicarbonate	Cu (II)	114	[93]
		Pb (II)	196	
		Cd (II)	189	
Sugarbeet pulp	Hydrochloric acid	Cu (II)	0.15	[94]
		Zn (II)	0.18	

Coirpith	ZnCl <sub>2</sub>	Cr(VI) Ni(II) Hg(II) Cd (II)	NA	[95]
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Majority of these adsorbents are chemically modified plant waste substances. The aqueous solution mentioned includes wastes water from different chemical industries and other synthetically made metal solutions for the purpose of study. Chemical pre-treatment of adsorbent results in higher adsorption capacity with respect to unmodified form. This is because pre-treatment causes higher number of active binding sites, better ion exchange properties and formation of new functional groups which have higher capacity of metal uptake. Chemical pre-treatment can be done by numerous chemicals which include mineral and organic acids, bases, oxidizing agent, organic compounds, etc. In one of the work by Gaballah and co-workers bark was studied for its removal efficiency from synthetic solution containing copper [12]. Bark was chemically pre-treated with alkali, acid and organic compound which lead to partial depolymerization of tannins. Pre-treatment helped in efficient removal ability as tannins if present would have increase the biological oxygen demand of the solution and turned the solution brown. A retention of 43 mg of cu/g of dry modified bark was achieved.

Apart from activated/modified plant products, a plethora of other compounds can also act as adsorbents. Some of them include natural zeolite clinoptilolite, montmorillonite clay, activated carbons, sepiolite and kaolin. Natural zeolite clinoptilolite holds great potential for removing heavy metal cation from aqueous solution. In a study by Erdem and coworkers the adsorption behavior of clinoptilolite for Co<sup>2+</sup>, Cu<sup>2+</sup>, Mn<sup>2+</sup> and Zn<sup>2+</sup> was investigated [13]. The batch method was employed using metal concentrations in solution ranging from 100 to 400 mg/l. The adsorption phenomena depend on charge density and hydrated ion diameter and selectivity sequence for adsorption observed was Co<sup>2+</sup>>Cu<sup>2+</sup>> Zn<sup>2+</sup>> Mn<sup>2+</sup>. Thus natural zeolites can be suitably exploited to remove cationic heavy metal species from industrial wastewater. Lin and Juang in one of their batch experiment reported that montmorillonite suitably modified by anionic surfactant sodium dodecyl sulfate can also be used for removal of Cu<sup>2+</sup> and Zn<sup>2+</sup> from aqueous solutions. The removal capacity was relatively higher with respect to raw clay [14]. Sepiolite, a natural fibrous clay mineral, is also used for effective removal of various metal ions from polluted water. In a study by Lazarević and coworkers on natural and acid treated sepiolite, the divalent cation were retained on sepiolite in the order Pb<sup>2+</sup>>Cd<sup>2+</sup>>Sr<sup>2+</sup>. Batch experiments were performed using solutions of Pb(NO<sub>3</sub>)<sub>2</sub>, Cd(NO<sub>3</sub>)<sub>2</sub> and Sr(NO<sub>3</sub>)<sub>2</sub> with a concentration of 0.01 mol/dm<sup>3</sup>, at a ratio sepiolite to electrolyte solution of 0.05 g:25 cm<sup>3</sup>. It was observed that retention of Pb<sup>2+</sup> and Cd<sup>2+</sup> occurred by adsorption and Mg<sup>2+</sup> ion exchange from sepiolite structure while electrostatic forces were main cause for retention of Sr<sup>2+</sup> ions onto the surface of sepiolites [15]. Jiang and coworkers studied kaolinite clay obtained from Longyan, China for Pb(II), Cd(II), Ni(II) and Cu(II) uptake from wastewater. The results were impressive with

maximum adsorption being observed within 30 minutes [16]. Thus adsorption method offers good option for removal of heavy metal in waste water from various industries. However selectivity does exist with regard to adsorbent and metal adsorbed by them. Careful pre-treatment and screening should be done for targeting the metal of choice.

**Biosorption method:** Various techniques have been employed for the treatment of metal bearing industrial effluents like precipitation, ion exchange, membrane and electrochemical technologies etc. However these techniques are expensive, not environment friendly and generally depends on the concentration of the waste. The search for an efficient, eco-friendly, cost effective and biological method for wastewater treatment culminates at biosorption method. The most striking advantage of biosorption method of heavy metal removal is the treatment of large volumes of effluents with low concentrations of biosorbent and no production of toxic secondary compounds. Other advantage includes short operation time. Biosorption essentially involves the passive uptake of metal ions by dead/inactive biological materials or by materials derived from biological sources. It consists of a solid phase (biosorbent) and a liquid phase (solvent, usually water) which contains dissolved species to be sorbed. The basic mechanism involves attraction of sorbent for the sorbate which are subsequently removed by different mechanisms. The biosorption process is affected by factors like status of biomass whether living or dead, type of biomaterial, pH, initial metal ion concentration etc. Biosorption can be attributed to a number of metabolism-independent processes that essentially take place in the cell wall. Important mechanisms involved are complexation, chelation, coordination, ion exchange, precipitation, reduction etc. Temperature does not have any significant effect on biosorption process in the range of 20-35°C [17]. However pH, presence of other metal ion and biomass concentration greatly influences biosorption process. The most prominent effect is of pH as it influences solution chemistry of metal, activity of functional group in the biomass and the competition of metallic ions [18]. A vast array of biological materials, especially bacteria, algae, yeasts and fungi have received increasing attention for heavy metal removal and recovery due to their good performance, low cost and large available quantities. Biosorbents are cheaper, more effective alternatives for the removal of metallic elements, especially heavy metals from aqueous solution.

**Biosorption by algae:** Biosorption by algae requires high metal uptake and selectivity by substrate and suitable mechanical properties. Of all the algae brown algae have been proven to be the most effective and promising. It is their basic biochemical constitution that is responsible for this enhanced performance. More specifically, it is the properties of their cell wall constituents which are chiefly responsible for heavy metal uptake. Biosorption of the metallic cations to the algal cell wall component is essentially a surface process. Carboxyl, amino, sulfhydryl, and sulfonate are the main chemical groups which are involved in metallic cation biosorption. These groups are part of the algal cell wall structural polymers namely, polysaccharides (alginic acid, sulfated polysaccharides), proteins, and peptidoglycans. Ion exchange is one of the main

biosorption mechanisms for heavy metal uptake by algae. However other binding mechanisms like micro-precipitation and complexation are also involved in the process of heavy metal uptake [19]. Table 2 explains some of the main algae used for this purpose and the metal ion biosorbed by them. Results of different batch experiments show the biosorption

capacity of algae to various metals. Therefore biosorption using algae presents an innovative depurative process employing biomaterials which are abundantly present in nature and can be used as a valuable option for treatment of industrial waste water and other heavy metal contaminated water.

**Table 2:** Biosorption by different algae and the corresponding metal sorbed.

Algae	Metal Sorbed	Results	Reference
<i>Spirogyra</i>	Chromium (IV)	Batch experiments at 5 mg/l of initial metal concentration showed removal of $14.7 \times 10^3$ mg metal/kg of dry weight biomass at pH 2.0 in 120 minutes.	[96]
<i>Sargassum</i> sp. (chromophyta)	Copper	Batch experiments using aqueous solution containing copper showed a high metal uptake capacity of 1.48 mmol/g biomass. Experiments were performed using 100 mg of dried biomass added to 25 ml of copper solution in 500 ml polypropylene flasks.	[97]
<i>Lyngbea putealis</i>	Chromium (VI)	82% biosorption of chromium at pH 2-3 and 45°C at initial chromium concentration of 50–60 mg/l of solution	[98]
<i>Sargassum fluitans</i>	Uranium	Uranium sorption capacity observed was 560 mg/g, 330 mg/g and 150 mg/g at pH 4.0, 3.2 and 2.6 respectively.	[99]

**Biosorption by fungi:** The cell wall of fungus can make up 30% or more of its dry and is made mostly of polysaccharides, which constitute about 80% of the dry weight. Fungi can act as efficient bio-sorbent owing to their high percentage of cell wall material, which shows excellent metal binding properties [20]. Fungi have large amounts of chitin, chitosan, glucan and mannan as well as small amount of glycoprotein in their cell walls. These polymers are abundant sources of metal binding ligands like carboxyl, amine, hydroxyl and phosphate groups [21]. Fungal mycelium, the vegetative part of fungus consisting of thread like hyphae, has also been reported for its  $Zn^{2+}$  metal ion biosorption [22]. Use of fungus for biosorption process has many advantages which includes its ease to cultivate at large scale owing to its short multiplication cycle and high yield of biomass. It can be easily grown using unsophisticated fermentation techniques and inexpensive growth media. Fungal biomass is also very easily available as industrial waste products and certainly provides an economic advantage as compared to other biosorbents. Most importantly major fungi used as biosorbent are non-pathogenic and can be easily exploited without any safety concerns.

Biosorption by fungi is affected by many factors each functioning independently which should be taken into

consideration in order to exploit their full potential. Some of the important factors include initial solute concentration, type and nature of biomass, biomass concentration (biosorbent dose/solution volume) in solution and physicochemical factors like pH, temperature and ionic strength. Fungi can be used in many forms as free/immobilized, living/dead, raw/pretreated, lab culture/waste industrial biomass etc. Several studies been done so far have shown excellent potential of fungi as biosorbent particularly for treating industrial waste water full of toxic heavy metals. In one of the finding by Velkova and colleagues, biosorption of Cu (II) onto chemically modified waste mycelium of *Aspergillus awamori* was studied [23]. Maximum biosorption capacity was reached by sodium hydroxide pre-treated waste fungal mycelium at pH 5.0. Table 3 enlists various fungi that have been used in different biosorption experiments. The results discussed of various batch experiments and laboratory investigations proves the potential of fungus for treating metal contaminated waste waters from different sources by selectively using the most optimum biosorbent.

**Table 3:** Biosorption of metals by different fungi species.

Fungi	Metal Sorbed	Important Results	Reference
<i>Penicillin ochrochloron</i>	Copper	Culture studies at pH 2-8 and at copper concentration 5000 ppm in solution showed metal uptake of upto $4.0 \times 10^5$ µg/g dry weight of biomass after 1 day. Experiments with lake water containing metal showed removal and recovery of metal.	[100]
<i>Penicillin chrysogenum</i>	Radium	Culture experiments done using radium at a concentration of 1000 pCi/L showed $5 \times 10^4$ nCi/g radium being biosorbed at pH 7 by the biomass.	[101]
<i>Rhizopus arrhizus</i>	Uranium, Thorium	Rhizopus arrhizus at pH 4 and a maximum metal concentration of 5.5 mg/liter of solution in laboratory experiments exhibited the uranium and thorium biosorptive uptake capacity in excess of 180 mg/g.	[102]



<i>Agaricus macrosporus</i>	Cadmium, Mercury, Copper	<i>Agaricus macrosporus</i> effectively extracted cadmium and mercury from the contaminated substrate. Different experiments at cadmium (10 mg per kg dw) and multisubstrate experiments at cadmium, mercury and lead each at 10 mg kg <sup>-1</sup> showed that fungi biomass effectively extracted metal from the substrate solution containing metals.	[103]
<i>Termitomyces clypeatus</i>	Chromium	Biosorption of chromium from effluents coming from tannery industries was studied using live fungi biomass. The sorption of hexavalent chromium was best obtained at pH 3 and showed prominent reduction in level of metal from the solution.	[104]
<i>Aspergillus parasiticus</i>	Lead	Batch experiments using contaminated lead solution showed biosorption capacity of the fungal biosorbent at $4.02 \times 10^{-4}$ mol g <sup>-1</sup> at pH 5.0 and 20°C in 70 minutes. Regeneration cycles also showed no significant loss of sorption performance during four biosorption-desorption cycles.	[105]
<i>Aspergillus niger</i>	Zinc	Experiments were done both batch wise and at column mode. Results showed that biosorption was function of pH (increasing with increasing pH between 1 to 9), biomass concentration (decreasing at high biomass concentration) and zinc concentration. Pretreatment of biomass with NaOH further increased its biosorption capacity from contaminated metal solution.	[22]
<i>Aspergillus awamori</i>	Copper	Sodium hydroxide and DMSO pre-treatments increased Cu (II) uptake capacity of fungal biomass by 48.20% and 20.05%, respectively. Biosorption experiments were done in 250 ml Erlenmeyer flasks by adding 0.1 g biosorbent to 100 mL metal solution at 20°C.	[23]

**Biosorption by bacteria:** Potent metal biosorbents under the class of bacteria include genre of *Bacillus*, *Pseudomonas* and *Streptomyces*. The bacterial cell wall consists of many functional groups like carboxyl, phosphonate, amine and hydroxyl groups [24,25]. Amongst them, carboxyl groups are abundantly available, negatively charged and actively participate in binding to metal cations. The amine group is also very effective for removing metal ions as it chelates cationic metal ions as well as adsorbs anionic metal species through electrostatic interaction or hydrogen bonding [26,27]. Bacteria are classified into gram positive and gram negative depending on its cell wall composition. Anionic functional groups found in the peptidoglycan, teichoic acids and teichuronic acids of Gram-positive bacteria, and the phospholipids, peptidoglycan and lipopolysaccharides of Gram-negative bacteria are the components primarily responsible for the anionic character and metal-binding capability of their cell wall. Using

potentiometric titrations, metal uptake capacity can be correlated with amount of acidic groups [28]. FT-IR analyses can help to detect nature of binding sites and their involvement during biosorption [29,30]. Table 4 provides basic information to evaluate the possibility of using bacterial biomass for the uptake of metal ions from waste water. This model can be used for employing bacteria for waste water treatment systems. The extent of biosorption depends upon type of metal ions and the bacterial genus as different genus has variable cellular contents. Using bacteria in fine powder form in various batch process helps in quick achievement of equilibrium and improved biosorption capacity due to non-existence of mass transfer resistances. The solution chemistry affects bacterial surface chemistry and metal speciation in the solution. Therefore optimum conditions for biosorption and careful pretreatment of biomass need to be fully understood before full exploitation of bacterial biosorption potential.

**Table 4:** Detailed list of metal biosorption by various bacteria along with their metal uptake capacity. Note: (E)=Experimental uptake, (L)=Uptake predicted by the Langmuir model. NA means not available.

Bacteria	Metal	M=Biomass dosage, teq=Equilibrium time	Uptake (mg/g)	Reference
<i>Bacillus coagulans</i>	Chromium (VI)	M=2 g/l, t <sub>eq</sub> =1 h	39.9 (E) at pH 2.5	[106]
<i>Bacillus licheniformis</i>	Chromium (VI)	M=1 g/l, t <sub>eq</sub> =2 h	69.4 (L) at pH 2.5	[107]
<i>Bacillus megaterium</i>	Chromium (VI)	M=2 g/l, t <sub>eq</sub> =1 h	30.7 (E) at pH 2.5	[106]
<i>Bacillus thuringiensis</i>	Chromium (VI)	M=1 g/l	83.3 (L) at pH 2.0	[108]
<i>Chryseomonas luteola</i>	Chromium (VI)	M=1 g/l, t <sub>eq</sub> =1 h	3.0 (L) at pH 4.0	[109]
<i>Pseudomonas cepacia</i>	Copper	NA	65.3 (L) at pH 7	[110]
<i>Pseudomonas putida</i>	Copper	NA	6.6 (L) at pH 6.0	[111]
<i>SpHaerotilus natans</i>	Copper	M=3 g/l; t <sub>eq</sub> =0.5 h	60 (E) at pH 6.0	[112]
<i>Streptomyces coelicolor</i>	Copper	M=1 g/l; t <sub>eq</sub> =8 h	66.7 (L) at pH 5.0	[113]

<i>Bacillus circulans</i>	Cadmium	M=0.5 g/l; $t_{eq}$ =2 h	26.5 (E) at pH 7.0	[114]
<i>Pseudomonas putida</i>	Cadmium	NA	8.0 (L) at pH 6.0	[111]
<i>Streptomyces rimosus</i>	Cadmium	M=3 g/l	64.9 (L) at pH 8.0	[115]
<i>Corynebacterium glutamicum</i>	Lead	M=5 g/l, $t_{eq}$ =2 h	567.7 (E) at pH 5.0	[116]
<i>Pseudomonas putida</i>	Lead	M=1 g/l, $t_{eq}$ =24 h	270.4 (L) at pH 5.5	[117]
<i>Streptomyces rimosus</i>	Lead	M=3 g/l; $teq$ =3 h	135.0 (L)	[118]
<i>Streptoverticillium cinnamomeum</i>	Lead	M=2 g/l, $teq$ =0.5 h	57.7 (E) at pH 4.0	[119]
<i>Lactobacillus bulgaricus</i>	Lead	M=4.5 g/l.	42.6 mg/gm at pH 6.0	[120]
<i>Bacillus thuringiensis</i>	Nickel	M=1 g/l, $teq$ =8 h	45.9 (L) at pH 6.0	[121]
<i>Streptomyces rimosus</i>	Nickel	M=3 g/l, $teq$ =2 h	32.6 (L) at pH 6.5	[122]
<i>Arthrobacter nicotianae</i> IAM 12342	Thorium	M=0.15 g/l, $teq$ =1 h	75.9 (E) at pH 3.5	[123]
<i>Bacillus licheniformis</i> IAM 111054	Thorium	M=0.15 g/l, $teq$ =1 h	66.1 (E) at pH 3.5	[123]
<i>Bacillus megaterium</i> IAM 1166	Thorium	M=0.15 g/l, $teq$ =1 h	74.0 (E) at pH 3.5	[123]
<i>Bacillus subtilis</i> IAM 1026	Thorium	M=0.15 g/l, $teq$ =1 h	71.9 (E) at pH 3.5	[123]
<i>Corynebacterium equi</i> IAM 1038	Thorium	M=0.15 g/l, $teq$ =1 h	46.9 (E) at pH 3.5	[123]
<i>Pseudomonas</i> sp. (strain MTCC 3087)	Thorium, uranium	M=2 g/l, $teq$ =12 h	Uptake of 43–54% of cell dry weight at pH 4-5	[124]
<i>Citrobacter freundii</i>	Uranium	M=6 g/L	Uptake of 94.68% at pH 6.0	[125]
<i>Arthrobacter nicotianae</i> IAM 12342	Uranium	M=0.15 g/l, $teq$ =1 h	68.8 (E) at pH 3.5	[123]
<i>Bacillus licheniformis</i> IAM 111054	Uranium	M=0.15 g/l, $teq$ =1 h	45.9 (E) at pH 3.5	[123]
<i>Bacillus megaterium</i> IAM 1166	Uranium	M=0.15 g/l, $teq$ =1 h	37.8 (E) at pH 3.5	[123]
<i>Bacillus subtilis</i> IAM 1026	Uranium	M=0.15 g/l, $teq$ =1 h	52.4 (E) at pH 3.5	[123]
<i>Zoogloearea migeri</i> IAM 12136	Uranium	M=0.15 g/l, $teq$ =1 h	49.7 (E) at pH 3.5	[123]

**Biosorption by yeast:** Biosorption by yeast biomass have been studied extensively because of the ease of availability of large amount of waste fungal biomass from various fermentation industries and its amenability to genetic and morphological manipulations. Of all the fungi, biosorption potential of fungi like *Rhizopus*, *Aspergillus*, *Streptoverticillum*, *Phanerochaete* and *Saccharomyces* has been studied the most. Yeast such as *Saccharomyces cerevisiae* is widely used in food and beverage production and is easily cultivated using cheap media. Various batch and culture experiments using yeast have proved the biosorption potential of yeast and its ability to remove metals form contaminated waste waters. Experimental

parameters affecting biosorption process are pH, biosorbent dose, initial metal concentration, contact time and particle size as is the case with other biosorbents. Treatment of biomass with mineral acids causes desorption and help in regenerate ability of biomass. Yeast can accumulate inordinate amount of metals due to production of extracellular yeast glycoproteins. The biosorption mechanisms have been related to different cell wall constituents [31-36]. {Murray, 1975 #85}The major functional groups involved for biosorption are carbonyl, amino groups and methyl groups present in biomass cell surface [37]. Table 5 enlists different experiments using yeast for biosorption of metals and important inference.

**Table 5:** Biosorption by different yeast species and important inferences.

Yeast	Metal sorbed	Results	Reference
<i>Phanerochaete cryosporium</i>	Chromium (VI)	Batch experiments (shake flask condition) using chromium containing wastewater was studied for biosorption capacity of yeast. Maximum biosorption of 63.72% was obtained at pH 2 for acid-treated biomass type at initial concentration of 100 ppm.	[126]
<i>Saccharomyces cerevisiae</i> subsp. <i>uvarum</i>	Mercury	Batch experiments using aqueous solution (20 ml) containing 0.5 mmol/L of $Hg^{2+}$ was incubated with magnetically modified yeast cells at a pH of 7.0 The maximum $Hg^{2+}$ biosorption capacity was 114.6 mg/g at 35°C.	[127]

<i>Candida tropicalis</i> CBL-1	Cadmium	Lab experiments on metal solution at a concentration of 100 mg/l of Cd(II) showed that <i>Candida tropicalis</i> CBL-1 reduced Cd(II) 59%, 64% and 70% from the medium after 48, 96 and 144 h, respectively. Moreover the yeast was also able to remove Cd(II) 46% and 60% from the wastewater containing Cd(II) after 6 and 12 days, respectively.	[128]
<i>Candida tropicalis</i>	Copper	Experiments carried out in culture flasks at different concentration of copper solution showed decrease in uptake capacity with increase in biomass concentration at optimal pH range of 5 to 7. Uptake was reported to be dependent on cell age. Cells at stationary growth phase had highest uptake capacity.	[129]
<i>Saccharomyces cerevisiae</i>	Lead	Entrapment of the biomass in a sol-gel matrix was observed. The yeast cells were homogeneously distributed into the solid matrix and could take up hazardous heavy metals from aqueous solution.  Using inactive biomass, the maximum metal ions uptake at optimum biosorption temperature of 25°C were found to be 270.3, 46.3 and 32.6 mg g <sup>-1</sup> , respectively for Pb(II), Ni(II) and Cr(VI).	[130] [131]
	Copper	<i>Saccharomyces cerevisiae</i> immobilized on sepiolite was able to retain metal when metal solution was passed through the column at pH 8 clearly indicating metal binding capacity of yeast for metal.  Metal uptake capacity of 8.0-8.1 mg/gm for copper from aqueous solution was obtained with formaldehyde cross-linked <i>Saccharomyces cerevisiae</i> in column bioreactors.	[132] [133]
	Cadmium	The adsorption process was pseudo-second-order with respect to metal ion concentration and occurred in four distinct steps.  Biosorption experiments using artificial aqueous solution and pretreated yeast biomass showed maximum metal uptake values (q <sub>max</sub> , mg g <sup>-1</sup> ) at 31.75.	[134] [135]
	Mercury	Yeast cells were successfully used to separate methyl mercury from Hg <sup>2+</sup> using biosorption. Binding of methyl mercury to yeast was independent of solution pH, temperature, incubation time, amount of biomass etc.	[136]
	Nickel	Biosorption experiments using inactive yeast showed maximum Ni <sup>2+</sup> ions uptake of 46.3 mg g <sup>-1</sup> at 25°C.	[131]
	Chromium (VI)	Sorption was exhibited by both intact cell and dehydrated cells with the latter having greater potential at 30°C or 45°C.	[137]
	Uranium	Batch experiments at contact time of 1 h, pH=6.5 and 10 <sup>-1</sup> M UO <sub>2</sub> (CH <sub>3</sub> COO) <sub>2</sub> solution as uranyl source showed the maximum degree of bioaccumulation at 8.75 mmol UO <sub>2</sub> <sup>2+</sup> /g yeast.  Batch experiments using non-living yeast as biosorbent showed an optimum uranium uptake at pH 5 and 100 µm particle size of biomass at adsorbent dose of 10 g/l and initial metal concentration of 100 mg/l. Maximum uptake was observed after contact time of 75 minutes.	[138] [37]

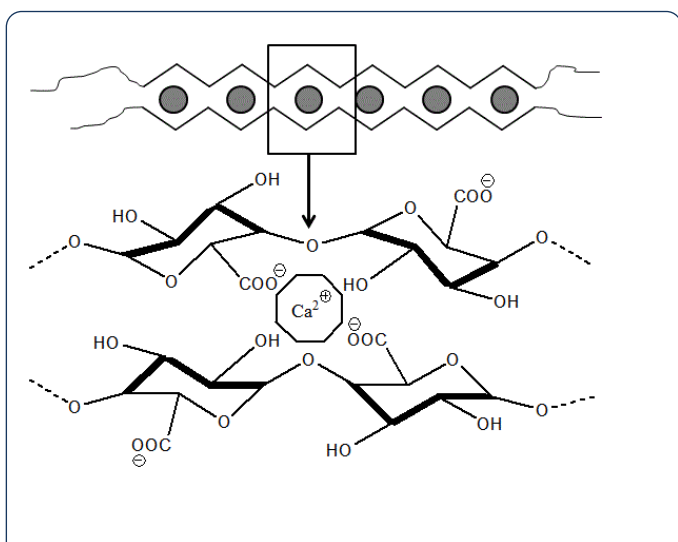
#### Chemical Method of Heavy Metal Removal

Chemical method of removal deals with chelation of heavy metal with suitable natural compound. These natural compounds can be of animal or plant origin such as alginates, citrates, flavonoids and phytic acid.

**Alginates:** Alginate is an anionic polysaccharide found in cell walls of brown algae. Chemically, it is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively. These two units are covalently linked together in different sequences or blocks. Alginates are obtained from sea weeds and bacteria. Seaweeds include the giant kelp *Macrocystis pyrifera*, *Ascophyllum nodosum* and various types of *Laminaria* whereas bacterial source of alginates are *Pseudomonas* and *Azotobacter* genera. Alginates from different sources, collected at different seasons from plants of different age have variable physical property, chemical property and yield [38]. In one of the study by **Tanaka and coworkers**, partially degraded alginates yields products which can better prevent strontium

absorption in the body compared to parent seaweeds [39]. Degraded alginates form relatively non-viscous solutions and are relatively easier to administer with food or in drinking water. Alginates have the capability of reducing body burden of radiostrontium and acts by binding to radioisotope [40]. Binding of radioisotope with alginate is through divalent bonding. Thus alginates are potential candidates for removing or inhibiting heavy metals uptake from the body when taken internally. The 'egg box' model explains the binding of alginates with divalent metal ions [41]. Figure 2 shows a schematic representation of calcium induced gelation of alginate in accordance with 'egg-box' model. The divalent calcium cations (Ca<sup>2+</sup>) binds to guluronate blocks of the alginate chains as they have high affinity to divalent cation. The guluronate blocks of one polymer then form junctions with the guluronate blocks of adjacent polymer via ionic bridges formed between ionized carboxyl groups of the adjacent alginate. Sodium Alginate at a concentration range of 1.4%, 12% and 24% reduces Sr-89 uptake in a constant proportion. In a study reported by Harrison and coworkers,

oral use of commercial jelly containing 1.5 gms of sodium alginate caused two fold reduction of strontium absorption in the body [42]. Such properties of alginates can be attributed to its complexation ability. The binding strength of alkaline earth metals to both polymannuronate and polyguluronate was found to decrease in the order  $Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+}$ . They also interpreted the preferential binding of heavier ions to stereochemical effects [43].

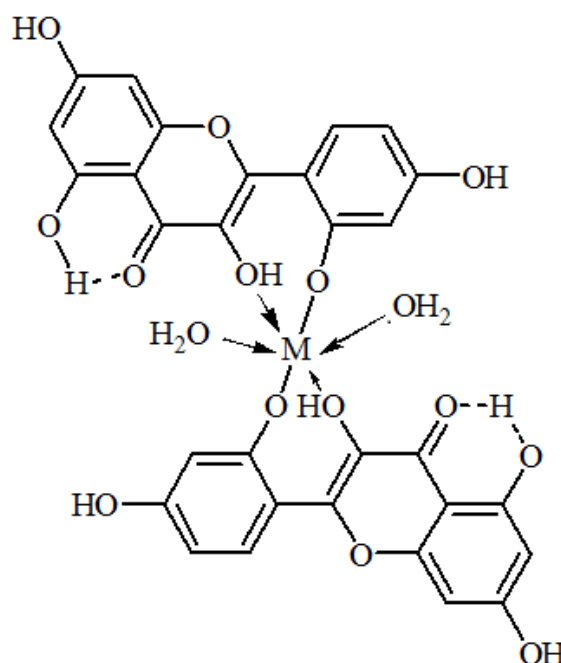


**Figure 2:** 'Egg box model' showing calcium induced gelation of alginates. Here  $Ca^{2+}$  is the calcium ion being held in ionic bridges formed between calcium ions and the ionised carboxyl groups of adjacent alginate chain.

**Citrus pectin:** Citrus pectin is complex polysaccharide derived from the inner peel white pulp of citrus fruit. Its principal monosaccharide is D-galacturonic acid along with some amount of neutral sugars. It has the ability to bind toxic heavy metals and excrete them from the body. Essential minerals are not disturbed during this process [44]. Pectin can bind with Pb, Cu, Co, Ni, Zn, Cd as well as with Ba, Zn, Sr, Mn, Mg [45]. Citrus pectin is used for effective treatment of lead poisoning in children. Clinical study by Zhao and his colleagues on oral administration of modified citrus pectin (MCP) showed effective lowering of lead level in the blood of children between the ages of 5 and 12 years [46]. Another study by Li and co-workers showed that modified pectin cross linked with adipic acid has a rough, porous phase covered with carboxy groups and shows high adsorption capacity. The saturated loading capacity for  $Pb^{2+}$ ,  $Cu^{2+}$  and  $Zn^{2+}$  reached 1.82 mmol/g, 1.794 mmol/g and 0.964 mmol/g, respectively [47]. In another study by Schiewer and Patil on removal of heavy metals from food processing and agriculture industry, it was observed that pectin-rich fruit can be effectively used for removal of dangerous heavy metal cadmium from waste water. They observed that metal uptake got increased with pH, with uptake capacities ranging between 0.5 and 0.9 meq/g of dry peel [48]. Sorption followed second order model. Owing to their low cost and high physical stability, pectin rich fruit can be suitably explored. The modified form of citrus pectin (MCP) consists of approximately 10% rhamnogalacturonan II and is widely known metal cation chelator [49,50]. Oral administration of

modified citrus pectin (MCP) increases total urinary excretion of arsenic and cadmium from body [44]. Pectin has same mechanism of binding as alginate (i.e. 'egg-box' mechanism). According to this, long fiber chains in solution stack together in groups and leads to formation of pockets. Pockets serve as the point where metal cations can form complex with the fibers. This model has been confirmed by X-ray diffraction [51] and NMR spectroscopic analyses [52].

**Flavonoids:** They are a class of plant secondary metabolites that perform a variety of functions such as plant pigment, UV filters and physiological regulator. Different studies by Bukhari and co-workers and Zhou and co-workers found that the antioxidant activity of flavonoids depends mainly on number as well as position of hydroxyl group in the flavonoid structure [53,54]. A number of flavonoids have been shown to have chelation with heavy metal ions particularly with copper and iron. Studies have shown that complex formation can take place between metal (copper and iron) and flavonoids having the stoichiometries of metal:flavonoids 1:1, 1:2, 1:3, 2:2, 2:3 [55]. Optimal pH for complex formation is around 6.0. Flavonoids can also be used as active antidote for heavy metal poisoning *In vivo* [56]. Quercetin forms complex with Al (III) thereby reducing its overload in diet [57]. Fluorescence spectroscopy has shown that quercetin can form 3:1 complex with eight rare earth metals [54]. It is the 3-or 5-hydroxy-pyran-4-one group in B flavonoid ring which plays a principal role in chelating action. Morin forms Pd (II) and Pt (II)-complexes as is shown in Figure 3 [58].



**Figure 3:** Probable structure of M(II)-morin complex. M = Pt(II), Pd(II) and Zinc (II).

The structure is suggested due to the fixation of benzene ring caused by the effect of coordination after the complex



was formed. Malesev and Kuntić showed that benzoyl moiety is basic site for metal chelation by their IR spectroscopy of Pd (II)-quercetin and UO<sub>2</sub>-rutin complexes. The complexes with rutin, morin or 3-hydroxyflavone are quite stable with WO<sub>4</sub><sup>2-</sup> anion in the center with the ligand-metal interaction partly electrostatic [59].

**Phytic Acid:** Phytate (myo-inositol (1, 2, 3, 4, 5, 6) hexakisphosphate) is a natural compound formed during maturation of plant seeds and grains. Structurally phytic acid has 12 replaceable protons in its molecule which gives it tremendous potential of forming complex with positively charged multivalent cations and positively charged proteins [60]. Due to its structure, it can strongly interact with many metals and nonmetals, proteins and starch. The interaction is mainly electrostatic [61-63]. Rimbach and Pallauf reported that bioavailability and toxicity of cadmium was significantly reduced by phytic acid. Similar results were obtained for lead [64]. Phytic acid and iron form insoluble complexes that is not available for absorption under pH conditions of the small intestine [65].

Phytic acid forms variety of salts with metal ions easily and exist as phytate metal ion complex at a certain pH. A change in pH leads to formation of other complex having altered stability. Vohra and coworker reported the order of stability of phytate-metal complex as Cu<sup>2+</sup>>Zn<sup>2+</sup>>Ni<sup>2+</sup>>Co<sup>2+</sup>>Mn<sup>2+</sup>>Ca<sup>2+</sup> at pH 7.4 [66]. Phytic acid immobilized on suitable surface such as poly 4-vinyl pyridine can act as very good adsorbent. The order of metal ion adsorption at pH 6.5 by PVP- Phytic acid complex was Ni<sup>2+</sup>>Zn<sup>2+</sup>>Cu<sup>2+</sup>>Co<sup>2+</sup>> Cd<sup>2+</sup>>Pb<sup>2+</sup> (Tsao et al. 1997). According to International Union of Pure and Applied Chemistry (IUPAC), potentiometry and multinuclear NMR have been the main instrumental techniques used for the determination of stability constants of such complexes [67]. A study by Reinhold and coworkers on phytate rich diet found that phytate has inhibitory effect on Zn absorption [68]. Several subsequent single meal studies clearly showed a negative correlation between presence of phytate or inositol phosphates and zinc absorption in humans [69-72]. In another study by Bohn and coworkers on 20 human volunteers (10 males and 10 females) it was found that fractional magnesium absorption from white bread was significantly impaired by addition of phytic acid. The effect was dose dependent as addition of 1.49 mmol of phytic acid lowered magnesium absorption from 32.5 ± 6.9% to 13.0 ± 6.9% and addition of 0.75 mmol phytic acid lowered magnesium absorption from 32.2 ± 12.0% to 24.0 ± 12.9% [73]. Phytic acid is also known to chelate uranium. A study by Cebrian and his colleagues found that the *In vitro* ability of phytic acid to chelate uranium was 2.0, 2.6 and 16 times higher than that observed for ethidronate, citric acid and diethylenetriaminepenta-acetic acid (DTPA) respectively [74].

## Phytoremediation

The build-up of toxic pollutants such as metals, radionuclides and organic contaminants in soil, sludges, surface water and groundwater by various anthropogenic

activities affects natural resources and causes a major strain on ecosystem. Phytoremediation, also referred to as botanical bioremediation, is the use of green plants for the treatment of soil, water and air pollution [75]. It is an effective *in situ* remediation technology that utilizes the inherent abilities of living plants to cleanse nature. It is an ecologically friendly and solar energy driven clean-up technology. Phytoremediation involves growing plant in a contaminated matrix for a required period of time to remove contaminants from the matrix or to facilitate the immobilization (binding/containment) or degradation of pollutants. The plant can be subsequently harvested, processed or even disposed. Plants have remarkable metabolic and absorption capabilities as well as transport system that can take up nutrients or contaminants selectively from growth matrix, soil or water. The uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing contaminant toxicity are found. The root system owing to their enormous surface area causes absorption and accumulation of water and nutrients essential for growth as well as other non-essential contaminants. It is the genetic adaptation by plants to handle the accumulated pollutants which results in effective contaminant uptake from soil and waste water. Phytoremediation takes advantage of natural plant processes and requires comparatively less equipment and labor than other methods since plants do most of the work. Also, the site can be cleaned up without digging up and hauling soil or pumping groundwater, which saves energy. The widespread plant cover help control soil erosion, reduce noise, and improve overall surrounding air quality. Other advantage includes its low cost, wide spectrum of action against different metals, generation of recyclable plant products and public acceptance. Phytoremediation of land contaminated with inorganic and/or organic pollutants has been a subject of considerable attention and research over the last decade [76-78]. The degradation by-products from plants may be mobilized in groundwater or bio-accumulated in animals. The depth of plant root in soil limits the treatment zone which in most cases is shallow. Climatic factors will also influence its effectiveness. The success of remediation depends on carefully selecting plant community. Introducing new plant species to an area may cause widespread ecological ramifications. Moreover the overall process time is too long taking several years to clean up a site. Phytoremediation can be achieved by different mechanisms that include phytoextraction, phytostabilization, phytotransformation, phytostimulation, phytovolatilization and rhizofiltration.

Phytoextraction involves the uptake of contaminants from contaminated soil or water by plant and their simultaneous translocation to harvestable parts of plant. This follows a complex series of events starting from dissolution of metal, its absorption transport and finally storage. Phytoextraction is an effective *In situ* technique for removing heavy metals from polluted soils and promote long term clean-up of soil or wastewater. Use of hyper accumulators is an important strategy for phytoextraction as they can accumulate inordinate amount of elements within their tissues. A large number of

plants have been used for phytoextraction. Some of them are enlisted in Table 6.

**Table 6:** List of plants used in phytoextraction, their mechanisms and the target metal.

Metal removed	Plant Species	Family	Mechanism for Removal	Reference
Cadmium	<i>Chamomilla recutita</i> and <i>Hypericum perforatum</i> L.	Asteraceae	Secondary metabolites of plant complexes with cadmium forming less toxic organo-metallic complexes.	[139]
Zinc	<i>Brassica juncea</i>	Brassicaceae	Due to production of high biomass of shoot.	[140]
Nickel	<i>Psychotria douarre</i>	Asteraceae	Metal removal attributed to high concentrations of tannins in leaves which functions as a detoxicant for elevated cytoplasmic metal concentrations, in addition to providing defensive benefits.	[141]
Uranium	<i>Brassica chinensis</i> , <i>Brassica juncea</i> , <i>Brassica narinosa</i> , <i>Amaranthus</i> species	Brassicaceae	Chelation	[142]
Thallium	<i>Iberis intermedia</i>	Brassicaceae	Due to high amount of thallium accumulation in the leaves.	[143]
Mercury	<i>Eichhornia crassipes</i>	Potederiaceae	Due to binding of Hg ionically to oxygen ligands in roots, most likely to carboxylate groups and by covalent binding to sulfur groups in shoots.	[143]

Plant root mediates dissolution by secreting phytosiderophores, organic acids, or carboxylates which helps in capturing metal in the rhizosphere and transports it over the cell wall. The transport of metal from root to shoot is regulated by various transporters. For hyperaccumulators, leaves in the shoot system stores maximum amount of heavy metals.

Phytostabilization involves the reduction of mobility of heavy metal in soil through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone of plant. The addition of soil amendments result in decrease of solubility of metals in soil and minimize its leaching to groundwater. Various soil amendments include phosphates minerals (hydroxyapatite, phosphoric acid), iron and manganese oxides, aluminosilicates (bentonite, montmorillonite, zeolites) etc. The net result is that pollutants become less bioavailable and thus human exposure is significantly reduced. In one of the experiments by Blaylock and his colleagues it was observed that phytostabilization may reduce metal leaching by converting metals from a soluble oxidation state to an insoluble oxidation state. This technology does not remove contaminant from its location and thus excludes the need for treatment of secondary waste and further adds to the fertility of soil. The plants which are used for phytostabilization be tolerant to metal and should not accumulate contaminants in above-ground parts which are liable to be consumed by humans or animals.

Phytotransformation is use of plants for transformation of contaminants in sediments, soil and natural water to environmentally more acceptable products. The process involves breakdown of contaminants taken up by plant through various metabolic processes occurring within the plant or breakdown of contaminants in the vicinity of plant by the effect of various compounds such as enzymes produced by the plant. The various complex molecules present as pollutants in the soil or water are degraded into simple molecules which

are simultaneously incorporated into the plant tissue thereby promoting plant growth.

Phytostimulation, also called rhizodegradation, is the plant assisted breakdown of organic contaminants in the soil via enhanced microbial activity in the plant root zone or rhizosphere. The enhanced microbial activity can be attributed to various secretions like sugar, amino acids, carbohydrates and enzymes by the roots. The root system brings oxygen to the rhizosphere thereby ensuring aerobic transformations. Thus microbes help in digestion and breakdown of various pollutants present in the soil. The successful design of phytostimulation experiments requires dense root system and microbes which can degrade the contaminants. However this technique works at low level of pollutants in shallow areas and is a much slower process.

Phytovolatilization is the ability to take up contaminants in the transpiration stream and then transpire volatile contaminants. Thus plants may serve as effective pump-and-treat systems for mobile contaminants including volatile compounds like carbon tetrachloride (CCl<sub>4</sub>) and ethylene dibromide (EDB). Removal of tightly sorbed contaminants from micropores within the soil may be the rate-limiting step for their remediation. Dewatering increases the potential for gas-phase diffusion within the soil. Thus phytovolatilization offers good option for effective removal of volatile contaminants particularly from the soil, surface and water.

## Conclusions

The problem of heavy metal pollution is worsening day-by-day due to human activities. Therefore, the removal of metals from human body and environment becomes a subject of paramount importance. Removal of heavy metals can be done by both physical as well as chemical means. For physical removal, adsorption and bio-sorption are employed

mechanism while chemical removal mainly employs chelation. Adsorption of metal ions using plant waste products presents an effective as well as economical approach for heavy metal removal from aqueous effluent. The stability and adsorption capacity of adsorbent can be enhanced by suitable pretreatment with alkali or acids which causes an increase in active binding sites, better ion exchange properties or may even lead to formation of new functional group that may favor metal ion uptake. Biosorption is also an effective technique used for heavy metal removal from aqueous wastes. For algae biomass, ion exchange is shown to play an important role in metal sequestering mechanisms. For yeast and bacteria, biosorption can be attributed to different cell wall constituents. This technique has been extensively used for treating heavy metal contamination especially in the waste water coming from electroplating, mining and textile bath industries which can contaminate the environment. Another method of heavy metal removal is chelation. Chelation is exhibited by many active principles of plants and animals. Alginates, citrus pectin, phytic acid and flavonoids appear promising in this regard. Majority of these compounds can be used orally after minor modifications for treating heavy metals toxicity as they have the ability to effectively chelate the metal.

The use of plants in metal extraction has also appeared as a promising alternative for the heavy metal removal from water, sludges and soil. The various mechanisms employed include extraction, containment and immobilization, and volatilization etc. However commercial application of phytoremediation is relatively low in spite of large amount of research being done in this field. Selection of appropriate plant species and modification of cultivation condition can help in an enhanced removal of pollutants using plants. Biotechnological interventions can help in creation of new plant species having increased remediation ability.

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