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TOWARDS FURTHER UNDERSTANDING THE MECHANISM OF BIOSORPTION THROUGH VARIOUS BIOSORBENTS

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ABSTRACT: Biosorption is a cure to atmosphere containing heavy metals and also an alternative to physical and chemical methods used for heavy metal removal. The main aim of this review is to highlight the various mechanisms of biosorption by using various biosorbent *viz.* microorganisms, no-living biomass, herbal plants for the removal of heavy metals. Industrialization, globalization has provided an ease to humans, but every comfort has its own disadvantages, in this case, it will increase heavy metal pollution which threatens the life of human, animals, and plants. Bio sorption used bio sorbent to remove heavy metal or sorbate and is economical. Nowadays, the natural biological material is used to remove heavy metals like vegetable waste and fruit peels. Herbal medicinal plants are also used to remove heavy metals.

INTRODUCTION: Rapid modernization, industrialization, and urbanization are the main roots due to which heavy metal pollution is on its peak. There is a huge growth in human potentials with the development in science and technology because heavy metals pollution increases, which threatens humans, animals, plants and aquatic life. Heavy metal pollution disturbs our ecosystem's balance, affects our normal lifestyles, and gives rise to human illnesses. Heavy metals are important in various industries and in many technologies also, but pollution due to them is one of the major concerns because heavy metals are non-degradable and get accumulated in nature [1, 2]. Some heavy metals are required in little amount by plants and living organisms like copper, zinc but in spite of that their excessive level in body is dangerous due to their toxic and accumulative nature [3]. Various physical and chemical methods like ion exchange, ultra filtration and chemical precipitation etc are used to remove heavy metals to neutralize its adverse effects from the environment, but these methods do not provide the best results and are very costly. Inevitably these are replaced with biological methods like biosorption and bioaccumulation, which are productive in heavy metal removal from the contaminated environment.

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In these methods, the biological material is used, which adsorb the heavy metal ions on their surface. Bioaccumulation occurs with living cells and is an active process, while biosorption occurs with dead biomass and is a passive process [4]. This review focuses on the aptitude of different biological materials in the removal of heavy metals to decontaminate the environment by using the biosorption method.

Heavy Metals: Heavy metals are available naturally on the earth. Heavy metals can be defined as metals with high density, atomic weight, atomic number or metals whose density is higher than water [5, 6]. Heavy metals are introduced into the environment firstly by natural sources, which involve volcanic emissions, forest fires, geysers, etc. and secondly, by anthropogenic sources, which involves mining, smelting, painting industries, leather tanning, metal manufacturing paints, coating industries, tanneries, printing, pesticides, battery manufacturing, petroleum refining, agricultural runoff, *etc*. Heavy metals are present in low concentrations on earth, but due to human activities, their concentration increases as they have a tendency of amass in the environment. Due to their non-degradable property, they are considered to be harmful to live organisms. Heavy metals are classified as essential and non-essential heavy metals [7]. The name of essential and non-essential heavy metals is mention in **Table 1.**

TABLE 1: TYPE AND NAME OF HEAVY METALS 7

Type of heavy metals	Name of heavy metals
Essential heavy metals	Copper, Zinc, Nickel
Non-essential heavy metals	Lead, Mercury, Cadmium, Arsenic Chromium

Essential heavy metals are required in little amount in the body and are involved in various biological functions like oxidation and reduction processes, but their higher concentration in the body is toxic to human health [7, 8]. Essential heavy metals are also required in small quantities in plants. Lack of supply of essential heavy metals in the body may cause many deficiency diseases or syndromes. Non–essential heavy metals, as the name indicates, have no role; they only exert toxic effects when present in the environment in low concentration [9]. Non-essential heavy metals are highly dangerous, and their low concentration in the body has adverse effects. Some non-essential heavy metals may cause death like mercury, or some of them may cause cancer like arsenic which is categorized as carcinogenic [10]. Non-essential heavy metals are proven hazardous in nature and are entirely debar in food for human consumption [11]. In this review, hazardous effects of non-essential elements are reported along with some essential heavy metals like copper, zinc, nickel, which are harmful at higher concentrations.

Cadmium: Products containing cadmium are unable to recycle. The main source of cadmium subjection is smoking cigarettes. Elements of cadmium are also found in rechargeable batteries of nickel-cadmium. In humans, through food, cadmium enters in body [12]. Cadmium is a cumulative poison, and the human body has less endurance for cadmium. Cadmium pollution also affects plants and is considered the main pollutant by the US Environmental Protection Agency (USEPA). The effects of cadmium exposure on human health can be seen in kidney damage, high blood pressure, bone fractions, knocking down red blood cells, etc. The permissible limit for cadmium as given by World Health Organization (WHO) is 0.01 mg/L [12, 13].

Mercury: The primary source of subjection to mercury in humans is *via* food. Especially people eating fish or seafood get mainly affected because fish or seafood are contaminated with methyl mercury [12]. Methyl mercury is a bioaccumulative environmental toxicant [14]. Bioaccumulative environmental toxicants are persistent; they have high resistance to degradation, high motility in the environment, high toxicity, a high order of bioaccumulation, and biomagnifications with a long retention period [15]. Methyl mercury is formed in the aquatic environment.

It is biomagnified in aquatic food chains because it is not readily eliminated from aquatic organisms. The concentration of methyl mercury increases at every step in the food chain [16. 17]. It is used in fluorescent lights, batteries, and polyvinyl chloride. In a high fish consumption population, mercury enters blood vessels through the stomach and intestine and causes neurological damage [12]. Extreme exposure to mercury may cause kidney effects, respiratory failure, brain, heart, immune system, and death. Mercury exists in metallic mercury, organic mercury & inorganic mercury compounds, where metallic mercury cause health effects when breathed as vapor and absorbed through the lungs. Silver-colored dental fillings also release mercury vapor which contains 50% mercury by weight. The permissible limit set by EPA (Environmental Pollution Agency) for mercury is 0.002 mg/L or 2 ppb.

Lead: Exposure of lead (Pb) in the human body is mainly caused by air and food. The main mode of lead subjection is the emission of lead from petrol. Emission of lead inhaled by human maycause gastro intestinal problems. The use of lead-based paints, lead-arsenate pesticides are also other sources. Lead in food enter by using glazed food container and may leach in the food chain of human. Lead causes neurotoxic effects and may reduce blood level [12]. Accumulation of lead occurs mainly in bones, kidney, muscles, brain and cause many health issues like nervous disorders, kidney disorders, anemia, and even death. The permissible limit of Pb in drinking water set by WHO is 0.05 mg/L [13].

Arsenic: In its inorganic form, arsenic is highly toxic. Arsenic enters the food chain by using contaminated water in drinking, in preparation of food, and in crops cultivation. When groundwater levels drop, naturally occurring arsenic enter through certain rock formations. Industrially arsenic is used AS an alloying agent in the processing of glass, textiles, paper, metal adhesives, wood preservatives, and ammunition. Arsenic is also used in the hide tanning process in pesticides and feed additives *etc.*

Persons eating tobacco also get affected by arsenic as tobacco plants get arsenic naturally from soil. Symptoms showing arsenic present in the human body are diarrhea, blood vomiting, blood in urine, hair fall, stomach pain, muscle cramping, *etc.* [18]. Organs that get affected by arsenic poisoning are the skin, lungs, kidneys, and liver. During long-term exposure, arsenic might cause prostate, lung, skin, bladder, kidney cancer. The effect in the skin may also cause other skin lesions such as hyperkeratosis and change in pigmentation like darkening, discoloration, redness, and swelling. Arsenic in drinking water's permissible limit set as per Indian Standard (IS 3025) is 0.05 mg/L [12, 19, 20].

Chromium: Chromium (Cr) exists in many oxidation states, but its hexavalent ion form is more toxic as it is more soluble and mobile in this stage [13]. Hexavalent chromium (Cr (VI)) compounds are carcinogenic. Chromium is technologically used in metallurgical, chemical and refractory industries. In the metallurgical industry, it exists as an important component of stainless steel. In chemical industry, it is used in chrome plating, leather tanning, paint pigments, wood treatment, *etc.* Cr (VI) is absorbed by the gut, lungs, and skin. Chromium exposure causes asthma, erythema, tracheobronchitis, nasal and sinus cancer. The permissible limit of Cr in drinking water as set by EPA is 100 µg/L.

Nickel: It is an important trace element for the human body [21]. Nickel is used in the metallurgical process, nickel-cadmium batteries, food processing, chemical industries as catalysts and pigments. Nickel and its compounds are known as noxious agents and are hazardous for living organisms [22]. Exposure of nickel in humans causes damage to respiratory, gastrointestinal, immunological, cardiovascular, hepatic, renal damage, *etc.* [13]. The maximum permissible limit for nickel in water is 0.2 mg/L [22].

Zinc: Zinc elements play a dual role for the human body; on the one hand it is important and beneficial at low level, and on the other hand, at an excessive level it causes zinc toxicity. Anthropogenic sources of zinc discharge in the environment by humans are domestic and industrial sewage, combustion of fossil fuels, mining operations, smelting, electroplating, galvanization *etc.* Exposure of zinc at higher level cause fume fever, pneumonitis and it also suppress copper and iron absorption. In solution free, zinc causes harm to plants and aquatic life. AS pollutant in the environment zinc is very hazardous for living organisms. The permissible limit of zinc in surface or groundwater does not exceed 0.01-0.05 mg/L and in drinking water, levels above 3 mg/L is not acceptable [13].

Copper: At low levels of intake, copper is an essential element for humans and animals, but at a higher level of intake, it shows toxicity. Copper is used in mixtures of alloys such as brass & bronze, in the manufacture of sheet metal, pipe, wire, and other metal products, to treat diseases in plants, in chemical industries, AS preservatives in wood, leather, and fabrics, in mining. If intake of copper is high, then it causes liver, kidney damage, and even death. 2 mg/L is the maximum permissible limit for the copper in water [21].

Methods Used for Removal of Heavy Metals: High living standards and an increase in population growth lead to increased use of heavy metals in every part of human life, which in turn increases the emergence of heavy metals as pollutants in the environment [23]. Heavy metals like Cd, Cr, Hg, Pb, AS, Cu, Ni, and Zn are toxic, persistent, and non-biodegradable in nature.

They amass in the environment due to which they enter the food chain of living organisms and cause many adverse health effects like damage to many organs that provide a route for deafness, blindness, infertility, kidney problems, respiratory problems, and even cancer which cause the death of the person. Toxic effects of these heavy metals may also disturb our body systems like circulatory, gastrointestinal, respiratory, and nervous systems [24]. In **Fig. 1,** methods used for heavy metals removal is shown.

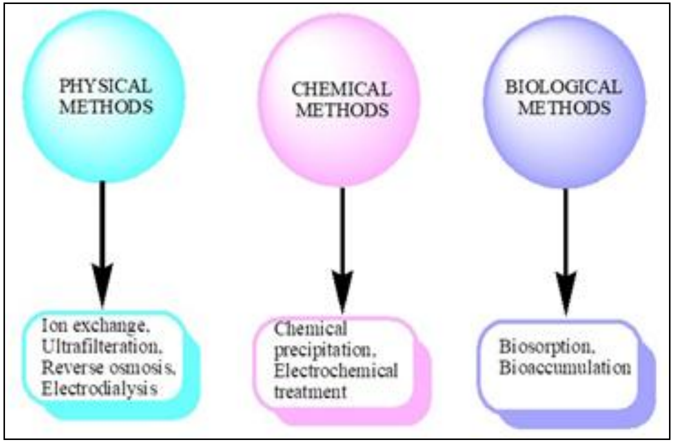

FIG. 1: METHODS OF REMOVAL OF HEAVY METALS

All the mentioned methods in **Fig. 1** are capable of removing heavy metals pollutants from the environment, but apart from this, these methods are linked with various disadvantages like they are costly, labor-exhaustive, lack of selectivity in the treatment process, the requirement of energy, and another high reagent, membrane's fouling, production of toxic slurry and other toxic materials which need careful destruction [7, 10, 24].

These methods are also inefficient in removing heavy metals when their concentration is low in contaminating solution [24]. Because of all these disadvantages, there is a need for a method that is natural and inexpensive in removing heavy metals pollutants.

Biological Methods: Biosorption appears as an environmentally friendly substitute for these methods. Bio sorption has many advantages over these methods. In these biological methods, biomass like bacteria, fungi, algae, agriculture, vegetables & fruits, industrial waste, and herbal plant parts is used.

AS bio sorbent for heavy metal removal as these bio sorbents has functional groups on their outer layer which sequester the metal ions. Recovery of bio sorbents is possible after desorption of metals from bio sorbents, and these bio sorbents can be used back, which makes this method economical. For excessive removal of heavy metal pollutants, chemically and physically modified bio sorbents can be used [24].

Biosorption: Biosorption is a natural, cheap, fast, and environment-friendly treatment technology [2, 25]. For the removal of heavy metals, a process called biosorption is used.

This is a physiochemical process. Biosorption can be defined as the efficiency of biological material to accumulate heavy metals from liquid phase through metabolically mediated (by using energy in form of ATP)or physiochemical pathways (without using energy/ATP), or it is a characteristic of some particular living and non-living biomass which bind and reduce metals from even dilute solutions [7, 10].

This method has many advantages over conventional methods *viz* less expensive, recovery of biological material, high efficiency, complete removal of metals, no nutrient requirement. Additionally, it is highly selective for specific metals uptake. Their removal, minimization of accumulated sludge or reduced volume of production of toxic materials, low operating cost, desorption of metals attached to biomass is easy and cheaper, production of bio sorbent is cheap, and its effectiveness over a broad range of conditions like pH, temperature, metallic ion concentration, *etc.* [2, 7, 10, 25].

Bio Sorption Process Involve Two Phases: solid phase (bio sorbent/biological material); Liquid phase (solvent) in which the dissolved contaminants/metals (sorbate) are present which is to be sorbed.

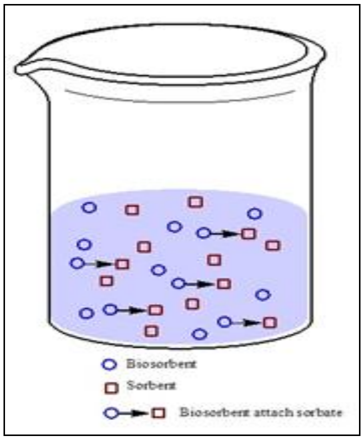

FIG. 2: DIFFERENT PHASES OF BIOSORPTION IN SOLUTION

As biosorbent has a higher binding affinity towards sorbate so these metals get attracted and bound on the surface of bio sorbent by a different mechanism. This binding process of metals and bio sorbent continues until equilibrium is established between bound sorbate and bio sorbent and its left-over portion in solvent **Fig. 2.** The intensity of bio sorbent affinity for the sorbate determines bio sorbent distribution between solid and liquid phase [10].

Potential Bio Sorbent Materials: To remove heavy metals, a large sort of biome is accessible in nature [25]. Enormous groups of bio sorbent are present like algae (microalgae, seaweeds, macroalgae) fungi, bacteria, yeasts which are mainly used due to their great affinity and good accomplishment [2, 25]. Ideal bio sorbent used should possess featured like; easily regeneration /reusability, non-toxic nature, availability, high metal-binding capacity, large scale production, no or minimal use of hard chemicals [2]. Sometimes wastes from agriculture and industrial operations are also used as bio sorbent in heavy metal removal. For example, solid residues of the olive mill, available waste mycelia from fermentation process [26], activated mud or slurry obtained from sewage treatment plants [27], biosolids [28], submerged aquatic plants are used for the process [29]. Some types of bio sorbent are specific for the removal of certain heavy metals, while some types of bio sorbent have a wide range of binding, collecting, and removing most of the heavy metals with no peculiar activity. Some bio sorbents used are particular isolated species of microorganisms, and some bio sorbents are processed to some extent to improve their biosorption characteristic [10]. An inexpensive source of biomass is also a good bio sorbent which is easily available in good amount in ocean seaweeds or algae [2].

Mechanism of Biosorption: The binding mechanism of sorbate onto bio sorbate or biological material is a complicated process [25]. Microbes are organisms that have the capacity of bearing unfavorable conditions [3]. Depending on the composition of microbial biomass, it is clear that there are many ways of metal uptake by cell [10]. The proficiency of microorganisms' example fungi, yeasts, bacteria and algae to remove heavy metals or promote their transfiguration into less toxic form has enchant the notice of many biotechnologists and scientists for many years [3]. There are many mechanisms of biosorption by which these toxic heavy metals can be removed, and all are not clearly understood [10]. The main two mechanisms which are involved are metabolism dependent and metabolism independent [7, 10]. The metabolism-dependent process involves an active defense system, which occurs in active biomass or living biological material [l7]. It is also known ASAS bioaccumulation. This process is relatively similar to biosorption and occurs in two steps; first, attachment of metal ions at the cell surface and the second step involves the active defense system in which transportation of metal ions occurs into cells, but bioaccumulation is a relatively slow process than biosorption [2].

Metabolism dependent process involves various mechanisms. Chelation which is the bonding of ions and molecules to metal ions, includes the formation of two or more different coordinate bonds between a multidentate ligand and a single central atom [7, 30]. Physical adsorption is a type of adsorption that occurs on the surface. In this, adsorbate from gas, liquid or dissolved solid is attached on the surface of the adsorbent, and a layer of adsorbate is formed on the surface of the adsorbent. It can be of two types of physisorption adsorbate attached to the adsorbent surface by weak Vander Waals forces. Another one is chemisorptions, in which strong covalent bonds formed on the surface adsorbate and adsorbent [7].

Precipitation is the creation of solid from solution or the emergence of insoluble solid from a solution. The insoluble solid that emerges from liquid solution is called precipitate and the chemical due to this solid form is called precipitant [7, 31].

Precipitate also emerges as a suspension. Precipitates are formed when two soluble salts react in solution to form one or more insoluble products. Complexation combines individual atom groups, ions or molecules to create one large ion or molecule. One atom or ion is the focal point or central point of the complex. This central atom contains a vacant electron orbital that causes bonding with other ions or any unshared electrons. Bio sorption may involve any one of these processes or combinations of all these processes [7]. Suppose biosorption (binding of metals to the cell surface of biological material) occurs through metabolism dependent process. In that case, it involves energy use through ATP consumption Fig. 3 7. In this process, the uptake of metal ions involves the immobilization of metals through ligands present on cell walls like carboxyl, carboxyl, and phosphoryl, sulfhydryl, and hydroxyl groups [7, 25]. The other factors that can affect metal uptake were the composition of the cell wall of bio sorbent, medium or solvent conditions, heavy metal or sorbent nature [7]. The ligand plays a key role in the biosorption of metals [25]. The living biological material uptake process involves adsorption to the cell wall and entering into cytoplasm [7].

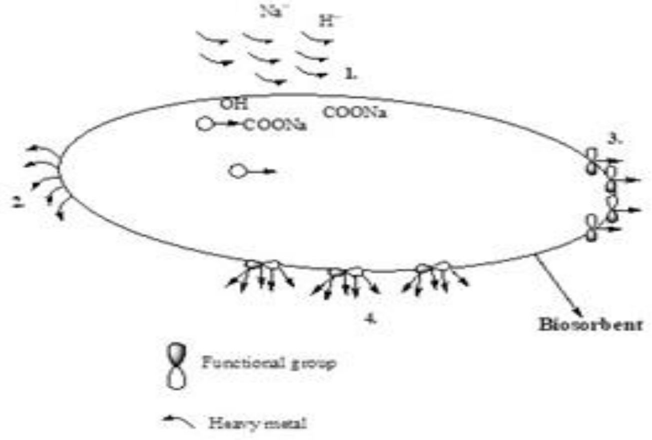

FIG. 3: SCHEMATIC REPRESENTATION OF THE DIFFERENT MECHANISMS OF BIOSORPTION INVOLVED IN THE REMOVAL OF HEAVY METAL POLLUTANTS BY USING BIOSORBENT: 1. ION-EXCHANGE METHOD, 2. PHYSICAL ADSORPTION, 3. ELECTROSTATIC ATTRACTION, 4. COMPLEXATION AND CHELATION

Metabolism independent occurs between metals and functional groups present on the surface of the cell of dead biomass [7]. This process depends on physiochemical interaction between sorbate and bio sorbent functional groups present on the surface of microbial biomass. This is based on physical adsorption, ion exchange, and chemical sorption which are independent of cell's metabolism. The cell surface of microbial biomass has a different composition of proteins, lipids, polysaccharides, alginates and many other metal-binding functional groups such as amino, phosphate, sulphate, carboxyl, *etc*. Metabolism independent biosorption process is relatively fast and reversible in comparison to bioaccumulation [10]. Biosorption mechanism is also divided on the basis of location where sorbate (metal ion) is found **Fig. 4** [10].

Mainly biosorption mechanism depends on the type of microbial biomass used for the removal of toxic metals. Cell wall composition of each microbial mass is different *viz*. alginate in algae, peptidoglycan in bacteria and chitin in fungi. Therefore, dissimilarity in the presence of functional groups on the surface of the cell wall of microorganisms is accountable for the difference in the mechanism. The mechanism of heavy metal ion adsorption by biological material is complex and involves different mechanisms [2].

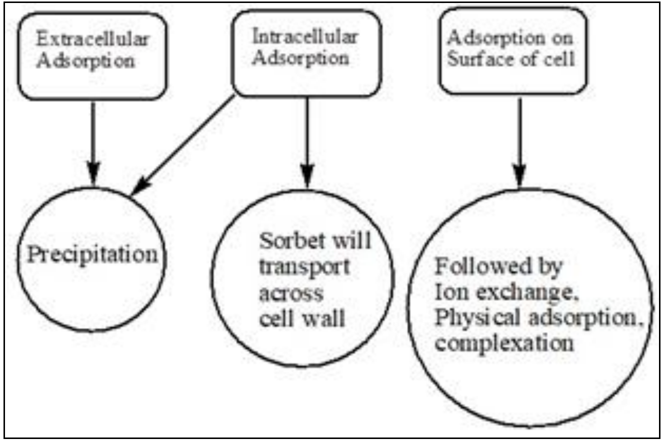

FIG. 4: BIOSORPTION MECHANISM ON BASIS OF LOCATION

Factors Affecting Bio Sorption: Factors that can influence biosorption are pH, temperature, metal ion concentration, the dose of bio sorbent, competing for metal ions, functional group ions, and time of contact [2, 10]. These factors which influence biosorption can be divided as physical and chemical. Chemical properties include molecular weight, ionic radius, the oxidation state of metal ions and structure of microbial cell surface, *etc*. Physical properties include process specifications like pH, temperature, sorbate concentration, biological material, contact time, dose, *etc*. [25]. From all these factors, pH is the most significant factor [2, 10, 25]. It influences redox reactions, the chemistry of solutions, precipitation, hydrolysis, and competition of metallic ions and affinity [10, 25]. For different biological materials or bio sorbent value of optimum pH varied. Protonation and deprotonation of functional group influence medium, which affects the capacity of biosorption [2].

Temperature is also an important factor affecting the biosorption process. An increase or decrease in temperature changes thermodynamic factors, which cause variation in biosorption capacity. For example, with an increase in temperature biosorption of lead by algae was found to be increase but same condition cause a decrease in biosorption of iron, manganese and zinc. Sometimes increase in temperature or very high temperature denature the structure of biological material [2]. In a range of 20-35 °C it appears that temperature does not influence biosorption performances [10]. Time of contact affects the biosorption process. Increasing contact time up to optimum time increases biosorption, but after some time, due to saturation of all binding sites, it becomes constant once equilibrium position establishes. For different biomass, time of contact is also different [2]. In biosorption, more than one ion is present during metal ion removal, and the presence of one metal ion influences the removal of another metal ion like uranium uptake by Rhizopus arrhizus is influenced by the presence of iron and zinc ions [10, 32].

Types of Bio Sorbents Fig. 5: Bio sorbents can be living or non-living biomass. Many of them can be used, which are given below:

Algae: Algae has received great attention for heavy metals removal due to its low cost, good performance, and large quantities. Various algae have been reported as bio sorbents *viz*. marine brown macroalgae, marine algae, marine red macroalgae, and freshwater green macroalgae **Table 2.** The report suggests that all brown algae have good biosorption capacity due to the presence of alginates in their cell walls [3, 7, 33, 38].

Bacteria: It has been reported that the capability of bacterias to accumulate metallic elements. Various researchers have revealed that inactive/dead bacterias biomass can passively bind metal ions via various physicochemical mechanisms **Table 3** [3, 7, 39, 51].

Fungi: Fungus is considered AS bio sorbent due to its high adsorption capabilities. A key role in the adsorption of heavy metals by fungus is due to the number of functional groups on its surface. Various fungus is reported as bio sorbent in the removal of metal ions **Table 4** [3, 7, 57, 61].

Yeasts: Yeasts play very promising bio sorbents as they offer large availability, rapid kinetics of adsorption, high surface to volume ratio, desorption, and low cost. Various yeasts are reported as bio sorbent in the removal of metal ions **Table 5.**

TABLE 2: ALGAL SPECIES USED AND THEIR BIOSORPTION CAPACITY RESULTS OBTAINED FROM THE LITERATURE [3, 7, 33, 38]

Metal ion	Algal Species	Biosorption capacity [mg/g] or % removal
Cadmium	*Sargassum species* *(S. tenerrimum*	84.7
	Fucus vesiculosus	114.9
	Pithophora spp.	95
	Ulva lactuca sp.	43.02
Arsenic	*Spirogyra hyalina*	9.8
Chromium	*Chlorella miniata*	34.60
Lead	*Spirogyra sp.*	140
	Fucus vesiculosus	1.04
	Calotropis procera	22.8
Copper	*Spirulina platensis*	67.93
	Fucus vesiculosus	70.9
	Sargassum filipendula	14.5

Zinc	*Sargassum muticum*	34.10
	Ascophyllum nodosum	53.2
Mercury	*Sargassum sp.*	14.8
	Cladophora fascicularis	20
	Spirogyra hyaline	39.2
Iron	*Sargassum sp.*	14.6
Nickel	*Sargassum sp.*	26.1
	Fucus vesiculosus	0.80
	Ascophyllum nodosum	50

TABLE 3: BACTERIAL SPECIES USED AND THEIR BIOSORPTION CAPACITY [3, 7, 39, 56].

Metal ion	Bacterial species	Metal uptake [mg/gm] or % removal
Cadmium	*Enterobacter cloacae*	58.9%
	Actinomycetes sp.	32.63
	Pseudomonas sp.	278
	Pseudomonas aeruginosa	42.4
	Pseudomonas putida	8.0
	Stenotrophomonas maltophilia	0.12
Chromium	*Micrococcus sp.*	92%
	Pseudomonas aeruginosa Enterobacter cloacae	1.07
	Bacillus licheniformis Staphylococcus saprophyticus	55.8
	Zoogloea ramigera	95% or 69.4
	Staphylococcus xylosus	24.1
	Bacillus megaterium	27.5
	Aeromonas caviae	143
	Pseudomonas sp.	30.7
	Pseudomonas fluorescence	284.4
		95
		40.8
Copper	*Bacillus licheniformis*	32%
	Bacillus cereus Stenotrophomonas maltophilia	50.32
	Geobacillus thermodenitrificans	0.57
	Bacillus subtilis	51
	Pseudomonas putida	20.8
	Micrococcus luteus	6.6
	Streptomyces coelicolor	33.5
	Arthrobacter sp.	66.7
	Enterobacter sp. J1	17.87
	Bacillus jeotgali	32.5
		222.2
Mercury	*Enterobacter cloacae*	43.23
	Bacillus sp.	7.9
Nickel	*Actinomycetes sp.*	36.55
	Micrococcus sp.	90%
	Bacillus thuringiensis	45.9
	E. coli	6.9
	Pseudomonas flouresence	40.8
Zinc	*Pseudomonas aeruginosa*	1.33
	Geobacillus thermodenitrificans	18
	Aphanothece halophytica	133.0
	Pseudomonas putida	6.9
	Streptomyces rimosus	30.0

TABLE 4: FUNGAL SPECIES USED AND THEIR BIOSORPTION CAPACITY [3, 7, 57, 75]

Metal ion	Fungal species	Metal uptake [mg/gm] or % removal
Lead	*Aspergillus niger*	34.4
	Penicillium chrysogenum	204
	Rhizopus nigricans	80.8
	Pleurotus ostreatus	4.84
	Trichoderma longibrachiatum	71

	Penicillium digitatum	0.090
	Rhizopus arrhizus	0.439-0.332
Copper	*Aspergillus niger*	28.7
	Penicillium chrysogenum	92.0
	Pleurotus ostreatus	4.0
	Fomes fasciatus	32.2
	Rhizopus arrhizus	0.738
	Fusarium flocciferum	0.629-0.944
	Penicillium digitatum	0.230
Chromium	*Penicillium purpurogenum*	36.5
	Aspergillus niger	16.39
	Pleurotus ostreatus	1.97
	Trichoderma viride	4.66
	Penicillium canescens	34.8
	Rhizopus arrhizus	1.118-1.427
Nickel	*Penicillium chrysogenum*	260
	Aspergillus niger	7.69
	Rhizopus arrhizus	1.274
	Penicillium digitatum	0.250
Mercury	*Aspergillus flavus*	95.3%
	Aspergillus fumigates	95.3%
Cadmium	*Penicillium simpliccium*	52.50
	Aspergillus cristatus	23.2
	Aspergillus niger	13
	Hydrilla verticillata	15
	Fusarium flocciferum	1.708
	Rhizopus arrhizus	0.240
	Penicillium chrysogenum	0.347
	Penicillium digitatum	0.100
Arsenic	*Penicillium chrysogenum*	24.5
Zinc	*Penicillium simpliccium*	65.60
	Aspergillus oryaze	0.270
	Trichoderma reesii	0.240
	Rhizopus arrhizus	0.195
	Penicillium chrysogenum	0.095
Iron	*Rhizopus arrhizus*	0.622-0.931
	Penicillium digitatum	0.270

TABLE 5: YEAST SPECIES USED AND THEIR BIOSORPTION CAPACITY [7, 72, 75]

Metal ion	Yeast species	Metal uptake[mg/gm] or % removal
Cadmium	*Saccharomyces cerevisiae*	12.3
	Aureobasidium pullulans	4.1
	Pichia fermentans	6.7
	R. rubra	0.8
	H. anomala	33.3
	Streptomyces species K33	70
	K. fragilis	≈90
Chromium	*Saccharomyces cerevisiae*	55.3%
	Candida utilis	28
Copper	*Saccharomyces cerevisiae*	29.9
	Candida pelliculosa	95.04%
	Schizosaccharomyces pombe	74.85
Lead	*Mucor rouxii*	17.13
Mercury	*Saccharomyces cerevisiae*	76.2
Nickel	*Saccharomyces cerevisiae*	14.1
Zinc	*Saccharomyces cerevisiae*	11.8

TABLE 6: NON-LIVING MATERIAL USED AND THEIR BIOSORPTION CAPACITY [76, 83]

Metal ion	Non-living material used	Metal uptake[mg/gm] or % removal
Lead	Orange peel	46.61

	Cucumber peel	28.25/87.12%
	Biomatrix of rice husk	0.28
Copper	Orange peel	92
	Biomatrix of rice husk	0.172
Chromium	Citrus peel	250
	Banana peel	95%
Nickel	Pomegranate peel	52
Mercury	Biomatrix of rice husk	0.18
Cobalt	Biomatrix of rice husk	0.162
Cadmium	Biomatrix of rice husk	0.149
Zinc	Biomatrix of rice husk	0.124
Chromium [III]	Biomatrix of rice husk [at pH 2.0]	1.0 mmol/gm

Non-Living Organic Biomass: The use of non-living organic biomass is becoming more and more attractive. Various bio-wastes are used as bio sorbent for the removal of heavy metal ions **Table 6.**

Bio Sorption Through Plant Biomass /Natural Adsorbents Herbal Plants: Heavy metal removal is also done by using natural adsorbents or using herbal plants. Plant biomass used is *Moringa oleifera*, *Hemidesmus indicus*, *Azadirachta indica*, *Ficus Religiosa*, and many more for biosorption of different metals. Natural adsorbents adsorb different metals according to their adsorbing capacity. These natural adsorbents are more used in recent research studies as they are easily available, low in cost, and do not require much processing (**Table 7** [84].

TABLE 7: BIO SORPTION THROUGH PLANT BIOMASS /NATURAL ADSORBENTS [84, 88]

Metal ion	Plant Name	Plant part used
Lead	*Hemidesmus Indicus*	Root
	Azadirachta indica	Bark
	Moringa Oleifera	Leaves
Zinc	*Hemidesmus Indicus*	Root
Chromium	*Hemidesmus Indicus*	Root
	Azadirachta Indica	Bark
Nickel	*Ficus Religiosa*	Leaves
Cadmium	*Ludwigia stolonifera*	Roots, Leaves, Floating roots

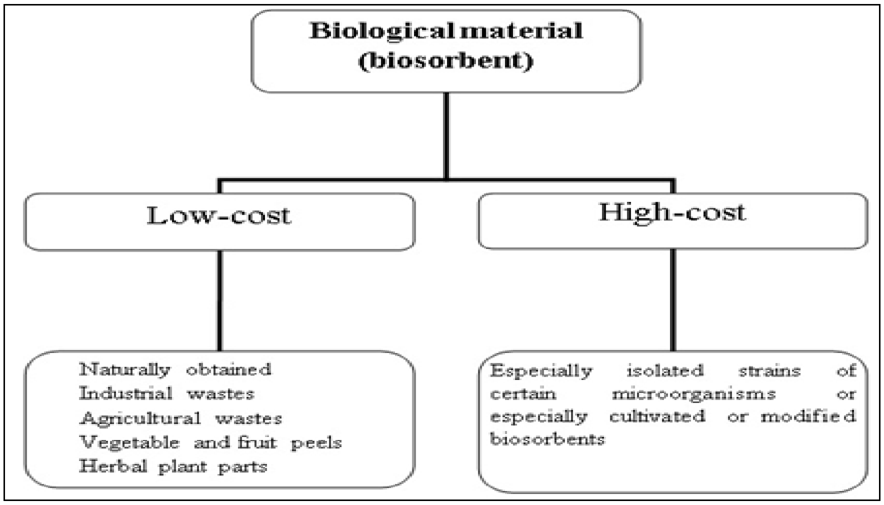

FIG. 5: GENERAL SCHEME SHOWING VARIOUS KINDS OF BIO SORBENTS USED IN BIOSORPTION PROCESS

Bio Sorption through Immobilized Cells: Small particles of biological mass have low density and rigidity and poor mechanical strength, but immobilization of biological mass provides the biomass with good rigidity, appropriate size, mechanical strength, increase necessary pore size for more metal uptake. Immobilization of biomass is done by various techniques like adsorption on inert supports, cell cross-linking, entrapment in a polymeric matrix, or covalent bonds in vector compound [10]. Adsorption on inert supports was carried out in fungal biomass Rhizopus arrhizus, bacterium [10, 89] Citrobacter spp. *etc* [10, 90]. Support materials are introduced before sterilization and inoculated in starter culture and left for a period of time in culture. A layer of biological mass is clearly visible on support materials [10]. Activated carbon [10, 91], polyurethane foam cubes, and coconut fibers [10, 92] are used as support materials on biological biomass. Covalent bonds in vector compounds are mainly used for algal immobilization [10, 93]. The most commonly used cross linkers in cell cross-linking are formaldehyde, divinyl sulfone, formaldehyde-urea mixtures, and glutaric dialdehyde [10]. Formation of stable cellular mass is done by the addition of cross-linkers. This technique is successful in the immobilization of algae [10, 94]. Polymers used in entrapment in polymeric matrices are calcium alginate [10, 95, 96], polyacrylamide [10, 97, 98], polysulfone [10, 92, 99], polyethyleneimine [10, 100]. Biological mass obtained in the form of gel particles after immobilization from calcium alginate and polyacrylamide. Biological mass obtained from polysulfone and polyethyleneimine are the strongest [10].

Desorption of Metals from Bio Sorbents /Recovery of Metals: The most important steps after bio sorption of heavy metals is recovery of metals or desorption of heavy metals from biological mass [7]. Desorbing agents or process of recovery varies according to metals and bio sorbents. Choosing desorbing agent during recovery is crucial because it should not or least harm the physical properties of bio sorbent such as its metal-binding affinity. This property of bio sorbent should be maintained in its original state [7]. For effective reuse, there must be no change in metal uptake to restore the biological mass near its original conditions [10]. The various agents used for desorption are mentioned in **Table 8.**

TABLE 8: ELUANTS USED FOR DESORPTION OF METALS FROM BIO SORBENTS [7, 10]

Category of desorbing agents	Name of desorbing eluants
Organic acids	Acetic acid, Citric acid Formic acid
Mineral acids	Sulphuric acid solution, Nitric acid solution, Hydrochloric acid solution
Chloride salts	Sodium chloride, Potassium chloride, Calcium chloride
Carbonate salts	Sodium bicarbonate, Potassium carbonate, Potassium bicarbonate
Alkali	Sodium hydroxide, Ammonium hydroxide
Thiocyanate salts	Potassium Thiocyanate
Other eluants	Deionized distilled water, Tris buffer [pH7.0], Phosphate buffer[pH7.5], Bicarbonate buffer[pH9.8]
Complexing agents	Thiosulphate, EDTA Na_2EDTA
Mineral Salts	Calcium nitrate, Magnesium nitrate, Ammonium nitrate, Ammonium sulphate, Potassium nitrate

Different metals show low or high desorbing capacity for different agents; like chloride salts and mineral acids are more efficient in desorbing cadmium ions than carbonate salts. Solution of $CaCl_2$ along with HCl is a best desorbing agent for cobalt. Chromium is best desorbed by NaOH then distilled water and HCl. Lead is efficiently desorbed by Na_2 EDTA then HNO_3 or Ca (NO_3) [2]. Some desorbing agents like $CaCl_2$, strong acids, alkali, Thiocyanate salts cause cell and cell wall damage of algal bio sorbents [101].

Bio Sorption Kinetics: The most important thing to be observed before the kinetics study is the quality of biological mass or bio sorbent, which includes firstly, in how much quantity metal ion attracted towards biological mass and secondly, on how much extent metal ions are retained on biological mass in immobilized form. In how much quantity metal uptake is done by biological mass is calculated by analyzing the initial quantity of metal in medium to that of remained metal in the medium after biosorption process completed [7]. This is given by formula:

$$Q = (V\ (Ci - Ce))/M$$

Where q is the amount of metal bio sorbed by biological mass in (mg/g); V is Volume of metal solution in (L); Ci is initial concentration of metal in (mg/L); Ce is the concentration of metal after bio sorption or at equilibrium in (mg/L); M is mass of adsorbent. Sorption capacity of biological mass must be considered before studying bio sorption kinetics of any heavy metal.

Biosorption isotherm should be studied for this. Parameters like pH, temperature, ionic strength are kept constant while metal concentration is varied when isotherm plots are studied. But pH is a confusing parameter in some research paper as it is believed that pH of the medium changes during whole process of biosorption. This biosorption isotherm is the plot of metal uptake (q) against equilibrium solute concentration in solution (Cf) [7]. Two models describe this biosorption isotherm and these two models are greatly used to describe the state of equilibrium for adsorption of metal ions during an experiment. These two models are Freundlich and Langmuir [7].

Freundlich Model: In 1909, Freundlich expressed an empirical equation for explaining the isotherm and it is a non-linear model. It is given by the formula:

Q(e) = KFC e^(1/n) F

Where qe is the amount of metal ion removed (mg/gm); Ce is the equilibrium concentration of metal(mg/L); K and n are Freundlich constants. The value of n more than 1.0 indicates sorption of metals is favorable physical process. It is described by the continual connection between adsorbed molecules and involves single layer sorption of metal ions with active sites [7]. Langmuir model: In 1916 Irving Langmuir published a model isotherm for gases or liquid adsorbed to solids. It is a semi-empirical isotherm and based on four assumptions, the Adsorbent surface is uniform that is all active sites are equal; molecules that are adsorbed do not interact; all adsorption follows the same mechanism; during maximum adsorption only, single layer is formed that is adsorbate molecules do not deposit on other adsorbed molecule only on the free surface of adsorbent adsorbate adsorb. It is given by the formula:

Qe = q(max) (b.C_e)/(1 + bCe)

Where qe is the amount of metal ion removed (mg/gm); Ce is the equilibrium concentration of metal (mg/L); bis Langmuir constants related to affinity; qmax is the maximum metal uptake under given conditions (mg/gm). It also involves single-layer sorption of metal ions with active sites but do not involve a continual connection between adsorbed molecules [7].

CONCLUSION: Biosorption is a cost-effective, environment-friendly, and useful method than any other method for the removal of potentially toxic metals from the environment. It is a very useful method for removing such heavy metals pollutants that are dangerous for the human body at low concentrations. Different studies illuminate various bio sorbents and their mechanism of metal uptake. During the regeneration of bio sorbents for further use, care should be taken that such chemicals should not be used to harm the bio sorbents. During wastewater treatment, one bio sorbent having an affinity for different metals or multiple bio sorbents having different specificity for different metals can be used simultaneously; this will reduce time and steps in the removal of pollutants in the biosorption method. Precautions must be taken during the removal of specific metals as their removal can be hindered by the presence of other contaminants. While using biosorption to treat wastewater, safe bio sorbents like vegetables, fruits, herbal plant parts, or agricultural waste should be used in place of poisonous bio sorbents.

Besides using microorganisms as biomass, agricultural waste can be used it helps in reducing waste from the environment means utilization of biosorption method in waste management. For more development of biosorption, further investigations and studies should be carried out in the direction of the use of low-cost bio sorbents with a maximum affinity of metal removal, but attention to be paid that no bio sorbent should be commercially exploited for money-making ease of biosorption. Steps must be taken to increase the use of biosorption on a large scale in industries to increase the impact of biosorption in society. More work should be done to increase the use of herbal plant parts as biosorbent for the biosorption method.

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