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### RESEARCH ARTICLE

#### A REVIEW ON BIOREMEDIATION OF HEAVY METALS BY MICROBES

Renu Saraswat<sup>1</sup>, Devesh Saraswat<sup>2</sup> and Meenakshi Yadav<sup>3</sup>

1. Associate Professor, Department of Chemistry, Meerut College, Meerut, 250002, India.
2. Lecturer, Department of Chemistry, Eicher School, Faridabad 121001, India.
3. Assistant Professor, Department of Chemistry, Meerut College, Meerut, 250002, India.

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#### Abstract

Over the last few years due to the rapid industrialisation everywhere throughout the world to fulfil the demands of growing population dissemination and mobilization of the poisonous heavy metals in the environment has been expanded to a destructive level. These heavy metals when enter in the food chain show fatal effect on human wellness when present at a slightly higher concentration than that required for regular metabolic process. Contamination caused due to the presence of these toxic heavy metals has become a global issue these days in spite of the fact that the seriousness and contamination level contrast place to place. Therefore the need of safe and effective techniques to eliminate these heavy metals is the requirement of sustainable living. Unlike the organic compounds that degenerate naturally, heavy metals do not break down themselves and therefore get assembled at various important sites. With the increasing awareness for the protection of environment emphasis is more on the development of eco friendly techniques for the decontamination procedures. Bioremediation implies cleanup of the contaminated environment by transforming harmful heavy metals into less harmful form by microorganisms or its enzymes. Hence bioremediation can be described as the 'green' basic technique for the expulsion of heavy metal without creating auxiliary metabolites in the environment. Utilization of microbial frameworks for the biological degradation of heavy metals is most preferred on account of its low expenses and minimum waste producing techniques. Despite of the increasing concentration of these harmful heavy metals microorganisms have adopted a variety mechanisms to adjust themselves to these poisonous heavy metals like lead, mercury, Zinc, nickel etc leading to the immobilization, removal or detoxification of these heavy metals. The present paper specifically focuses on the sources of heavy metals, their poisonous consequences on human health and recent studies made on the bioremediation of these deadly heavy metals using microbes. It also highlights the microbial techniques for the degradation of these deadly heavy metals at a rapid rate from the environment as well as future possibilities and limitations of bioremediation.

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**Corresponding Author:- Renu saraswat**

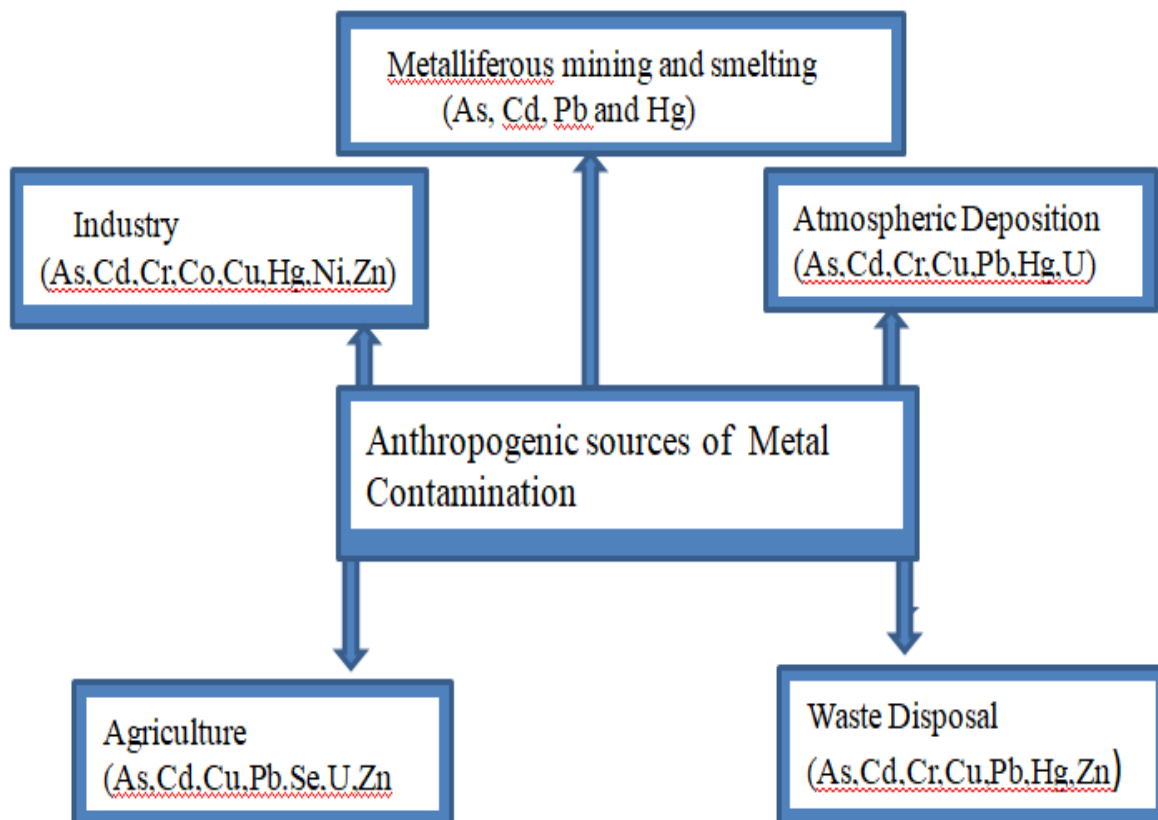
Address:- Associate Professor, Department of Chemistry, Meerut College, Meerut, 250002, India.

### Introduction:-

Heavy metals represent an interesting group of naturally existing inorganic micro-pollutants that are released in the environment by various phenomenon. Heavy metals are important both biologically and industrially but at high concentration in environment they have toxic effects. Metallic elements which have a density more than  $5\text{gm/cm}^3$  are termed as heavy metals. Heavy metals like Fe, Cd, Mn, Hg, Cr and many more are the indicators of human progress. Kennish (1992)<sup>1</sup> classified heavy metals as the metallic elements having atomic weights usually greater than 50. Hence in periodic table the transition elements from Vanadium (Except Sc and Ti) to the metalloid As, from Zirconium (except Y) to Antimony from Lanthanum to Polonium, the lanthanides and the actinides can be defined as heavy metals<sup>2</sup>. In All the major civilization past or present these heavy metals were used in one form or other. we can not imagine of any industry without these metals. Most of the toxic organic pollutants can be destroyed by combustion and can be converted into easily controllable pollutants such as CO, CO<sub>2</sub>, SO<sub>x</sub> and NO<sub>x</sub>, but combustion has no effect on metals. Metals can not be destroyed. These heavy metals exist normally in the earth environment because of the continuous chemical and physical changes occurring due to metamorphic and igneous rocks. Similarly naturally occurring decay of plant and animal waste matter, precipitation or atmospheric accumulation of airborne particles from volcanic eruption, forest fire smoke, wind erosion and oceanic spray also contribute exposure of heavy metals in the environment<sup>1</sup>. It has been found that in the past few years rapid industrialisation throughout the world has increased the concentration of these heavy metals in the environment. Toxic metals mobilize from Industrial activities and Fossil fuel combustion and eventually they are passed into the food chain leading to ecological and severe health problems.

**Table 1:-** Industrial sources of some common heavy metals.

Metals	Industry
Chromium (Cr)	Mining, chrome plating, coolants in industry, As catalyst in tanning of leather, road runoff and making alloys.
Lead (Pb)	Mining, smelting, Lead batteries, electronic-waste, ceramics, bangle industry, ship building, road runoff and in paints industry
Mercury (Hg)	Chlor-alkali plants, fluorescent lights, fluorescent lamps, dental amalgams, thermometers, barometer, electrical appliances, thermal power plants.
Arsenic (As)	Burning of fuel, Alloys, as dopant, arsenical pesticides, herbicide, in electronic industry, thermal power plants and preservatives of wood
Copper (Cu)	Mining, electronic industry, copper plating and smelting operations,
Nickel (Ni)	Metal plating, smelting process, combustion of fossil fuel, electroplating, thermal power plants, battery industry, road runoff.
Cadmium (Cd)	Ni/Cd batteries, e-waste, paint sludge, incinerations and fuel combustion, road runoff.
Zinc (Zn)	Smelting, electroplating, road runoff.



**Fig 1:-** Anthropogenic activities leading to the contamination of soils with heavy metals.

Heavy metals are studied in three categories which include toxic metals (Like Hg, Pb, Zn, Co, etc), precious metals (like Au, Ag, Pt etc) and radionuclides (like U, Th etc)<sup>3,4</sup>. Some of the metals are taken as essential nutrient as they are incorporated in enzymes and cofactors. Non biodegradable nature of heavy metals creates difficulty in their removal from contaminated tissues. Level of toxicity of any of the heavy metal is decided by the dosages consumed by the organism and the time of exposure. Plants are greatly affected by the heavy metal toxicity because their physiological activities gets severely damaged e.g process of respiration, cell division and photosynthesis are affected seriously by the increased level of heavy metals<sup>5</sup>. Moreover due to the oxidative stress generated because of the blockage of enzymes of cytoplasm in plant cells by heavy metal toxicity cell structure can be damaged<sup>6</sup> which affects the growth and metabolism of plants.

Human health may also have detrimental effects by the high concentration of heavy metals due to the ability of these metals to accumulate on living tissues. Some of the common symptoms of the toxic effects of heavy metals are memory loss for a short duration, sense of derealisation, pain in joints, distorted perception, headache, muscular pain, digestive problems, food allergies, vision problems, mental confusion, chronic fatigue, fungal infections, etc. Sometimes the symptoms may be unclear and difficult to analyze<sup>7</sup>.

**Table 2:-** Health damages caused by heavy metal pollutants.

Pollutant	Disorders
Lead	Neurotoxic, anaemia, high blood pressure
Arsenic	Hypo-pigmentation, cancer
Cadmium	Kidney damage, Cancer
Chromium	Cancer, ulceration, nephritis
Nickel	Chronic disorder of lungs and bones
Mercury	Neurotoxic, respiratory disorders
Uranium	Mental retardation, estrogenic effects

Copper	Mucosal irritation , widespread capillary damage, hepatic and renal damage and central nervous system irritation followed by depression
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**Physical and chemical methods:**

Commonly employed physical and chemical methods for the degradation or removal of pollutants rather than completely destroying them only transform their forms<sup>8</sup>. The general methods used in the removal of metal ions from the contaminated soil incorporate ion exchange, reverse osmosis(RO), electrochemical treatment , electro dialysis, ultra filtration , solvent extraction and chemical precipitation<sup>9</sup>. These methods have the disadvantages of incomplete metal removal, requirement of high solvent and production of poisonous waste products<sup>10</sup>. The bioavailability and characteristic properties of heavy metals may be changed by oxidative and reductive reactions but the elemental properties remain as such because of the non decomposable nature of heavy metals.

**Bioremediation:**

Bioremediation is most promising technique used for the elimination of heavy metals from environment that uses inherent biological mechanisms to eliminate or reduce toxicants using microorganisms, plants or their products to reinstate contaminated environments to their original state<sup>11</sup>.

These Biological methods for the removal of heavy metals include the using microbes like algae ,fungi and bacteria, plants (dead or living) and biopolymers and thereby providing safe and suitable way for heavy metal treatment<sup>12</sup>.

Bioremediation may be carried out in two ways in- situ bioremediation and ex- situ bioremediation. In in-situ process the contaminated soil is supplemented with minerals to revive the ability of microbes to degrade pollutants and add new microbes to the environment or improve the native microbes to destroy specific pollutants using genetic engineering<sup>13</sup>. Ex-situ bioremediation involves taking away the contaminated media to the other location for the treatment which is specifically based on depth of contamination ,type and extent of pollution<sup>14</sup>. This method uses nonexpensive techniques, which are of more public acceptance<sup>15</sup>.

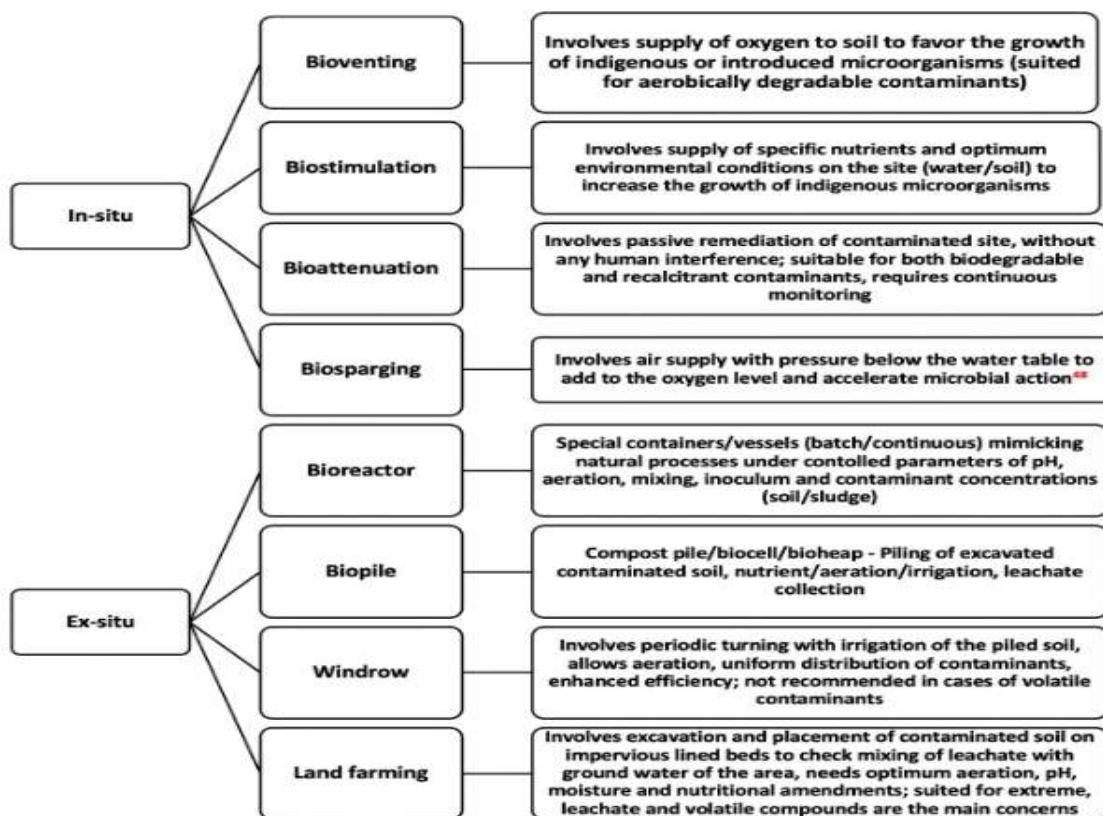


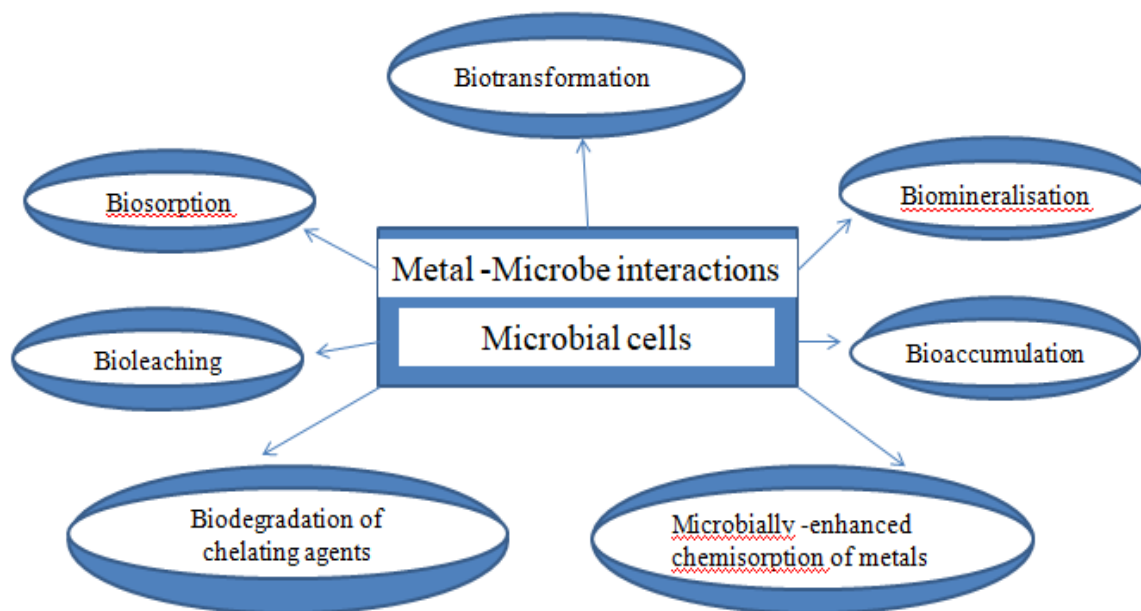
Fig 2:- Types of bioremediation techniques for various pollutants.

**Microbial remediation:**

Microbial remediation is defined as the utilization of microbes to reduce heavy metals in the soil or in the water solution by means of oxidation, reduction, precipitation or absorption of these metals<sup>16</sup>. Microorganisms being the unique owner of different metabolic pathways that makes use of toxicants as a source of energy for various cell activities through respiration, fermentation etc are found to possess an excellent ability to remove heavy metals. Being a significant part of biogeochemical cycle they possess various mechanisms that allow them to convert the soluble and insoluble forms of these heavy metals to less poisonous or non poisonous forms<sup>17</sup>. Due to the small size these microbes possess high surface -volume ratio, which enables them to present a large volume of contact area with the constituents of the soil that contain heavy metals<sup>18</sup>. Microbial remediation incorporated many methods which vary enormously in the mechanism through which microbial cells can remove, immobilize or destroy these heavy metals.

**Mechanism:**

Microorganisms have acquired number of methods for adjustment in the presence of poisonous heavy metals despite of the increased toxicity caused by increased level of pollution. Microbes have been found to possess resistance in polluted as well as non polluted environments<sup>19</sup>. Some of the microbial activities results in the solubilization of metals whereas others reduce their mobilisation in the environment. Microbes can mobilize the metals through a number of processes like chelation (bacteria produce chelating substances known as siderophores which increases the movability and decreases the bioavailability of these heavy metals), methylation and autotrophic and heterotrophic leaching. A number of mechanisms are suggested for immobilization of metals which include biosorption of cell wall, sequestration of heavy metals by intracellular metal binding proteins, precipitation of heavy metals as sulphides or oxalates or by converting the metals to non toxic form. The general techniques of sorption incorporate surface precipitation, chelate formation with organic ligands, chemical adsorption and ion exchange, and oxidation-reduction reactions. Different methods by which microbes act on these heavy metals incorporate bioleaching (process of extracting metals using microbes into its soluble form), biosorption (adsorption of heavy metals on the surface of the cell by physico chemical methods), biomineralization (immobilization of heavy metals by transforming them into minerals by microbes), intracellular aggregation and enzyme catalyzed conversion.



**Fig 3:-** Metal-microbe interactions affecting bioremediation.

**Biosorption:**

The process of sorption and /or complex formation of dissolved metals depends on the chemical activity of microbial biomass or the materials that are derived from various biological origin forms the base of biosorption technique for the removal of heavy metals<sup>20</sup>. Biosorption is the removal of heavy metals, compounds or particulate material from any solution using low cost biological material like dead mass or natural materials with greater

degradative ability<sup>21</sup>. Process of biosorption is considered as metabolically passive as energy is not required in it and the amount of the pollutant that can be removed or destroyed by a sorbent depends on the kinetic equilibrium and composition of sorbents cellular surface<sup>22</sup>. Biosorption technique consists of two steps firstly the metals binds itself to the cell wall ( metabolism independent process) and secondly metal ions are transported through the cell membrane (metabolism dependent). The process of biosorption is considered as reversible . Presence of functional groups like amine, carboxyl, hydroxyl and phosphonate plays a significant role in biosorption<sup>23</sup>. Phosphoric acid anions ,hydroxyl anions and carboxyl anionic groups on the exterior surface of the microbial cell wall present negatively charged reaction sites where the positively charged metal ions are adsorbed by complexing. Sorption power of Gram positive microbes is greater because of the presence of a heavy coating of peptidoglycan which possess greater number of sorption sites. It is found that temperature, ionic strength, pH , presence of other metal and concentration of initial solute play a significant role in the whole process<sup>24</sup>.

#### **Bio-mineralization:**

Microorganisms are capable of producing inorganic compounds such as sulphur which reduces the mobility of many metals and precipitate them. Bio-mineralization is a process by which the living microbes form minerals by transforming the aqueous metal ions into crystalline precipitates thereby removing metal from the solution and thus provides a mean of detoxification as well as biorecovery. Common bio-minerals precipitated by these microbes are oxides , sulphides, oxalate ,sulphates and phosphates. These microbes can also affect the mobility of metals ions since they affect redox potential , pH and so on<sup>25</sup>.

#### **Bio-transformation:**

Microorganisms possess the capability to enzymatically modify metals by different mechanisms like oxidation , reduction ,alkylation and methylation<sup>26</sup>. Mobility of the heavy metal may be decreased indirectly by the production of biosurfactants, pigments or siderophores. It is seen that ferroxamines may form chelates with Ni<sup>++</sup>, Cd<sup>++</sup> and Al<sup>+++</sup><sup>27</sup>. Microbes which are largely Prokaryotes take part in oxidation-reduction reactions and change the activity of heavy metals by changing their valencies thereby affecting the mobilization and toxic effect caused by them<sup>28</sup>. Coryne bacterium and some other microbes can reduce the toxic and water-soluble Cr<sup>6+</sup> into toxic and less water-soluble Cr<sup>3+</sup>. Similarly dead Bacillus licheniformis R08 can cause the reduction of Pb<sup>2+</sup> to Pb<sup>0</sup><sup>29</sup>.

#### **Bio-accumulation:**

Term bioaccumulation may be defined as gradual accumulation of toxic pollutants into the living tissues of an organism from environment .Essential metals are frequently taken by the specific uptake system in the living cell whereas some non essential harmful metals may also be taken up because of improper identification<sup>30</sup>. Then these toxicants are carried into the cell beyond cell membrane and gets accumulated intracellularly. Bioaccumulation depends on the biochemical properties , genetic adaptations ,structural properties, environmental modifications , availability of metal and toxicity<sup>31</sup>. Accumulation of metal is greatly affected by the surface characteristics of microbes whereas metals can also change characteristic properties of surface. Temperature is another factor that affects the accumulation of heavy metals by increasing the rate of reaction. However higher temperature can be lethal for living cells because it destroys bacterial cell membrane<sup>32</sup>.

Therefore different methods employed for the removal of heavy metal using microbes may be summarized as

1. Excretion of metals via efflux transport system
2. Binding and detoxifying the metals inside the cell by sequestering compounds of cytol
3. Adjusting the metals by the release of chelate forming agents into the extracellular environment
4. Converting the metals into less poisonous form by reduction or oxidation
5. Binding large amount of metals by sorption on the cell envelope

#### **Bioremediation of heavy metals using Microorganisms:**

Distinctive type of Microbes like bacteria ,algae and fungi play a significant role in the bioremediation of these heavy metals by following various pathways. A number of methods as well as applications are included in microbial remediation which differ mainly in the method by which these microbial cells can either remove, immobilize or degrade metals.

#### **Bacteria:**

Bacteria are found to be efficient biosorbents of heavy metals because of their ability to grow in diverse environments ,quick growth rate , small size and easy cultivation. Bacteria's have a great tolerance towards heavy

metals through their abilities to adsorb, bioaccumulate and transform metals. The Extracellular polymeric substances (EPS) prepared by nucleic acid, proteins, carbohydrates and lipids plays a significant role in the adsorption of these heavy metal ions. This extracellular polymeric material present on the microbial surface protects the microbes against the poisonous effects of heavy metals by checking their entry into the intracellular environment. Various functional groups like carboxylic, hydroxyl, phosphate and amine are available on the bacterial cell wall<sup>33</sup>. EPS can effectively assemble the heavy metals like Cobalt, mercury, copper and Cadmium<sup>34</sup>. The less toxic reduced state of these heavy metals can be transported inside the bacterial cell wall. On increasing the pH the overall negative charge on the surface of cell increases till the functional groups get deprotonated thereby helping the electrochemical attraction and finally absorption of the metal ion. On increasing the concentration of positive charge interaction of the anions with cell would be more strong because at the lower pH value the functional group gets protonated. The solution chemistry affects metal speciation as well as bacterial surface chemistry. On increasing the pH metal ion in solution gets hydrolysed and the degree of hydrolysis differs with different pH and different metal ions. A summary on the heavy metals removal using bacteria have been given in the table 3.

**Table 3:-** Heavy metal removal by bacterial species.

Bacteria	Heavy metal removed	Reference No.
<i>Pseudomonas aeruginosa</i>	Hg(II)	35
<i>Pseudomonas sp.</i>	Pb(II)	36
<i>Bacillus sp.</i>	Pb(II)	37
<i>Arthrobacter viscosus</i>	Cr(VI)	38
<i>Staphylococcus epidermidis</i>	Cr(VI)	39
<i>Eichhornia spp.</i>	Cu(II)	40
<i>Brevibacterium sp.</i>	Zn(II)	41
<i>Rhodobacter capsulatus</i>	Zn(II)	42
<i>Pseudomonaaeruginosa</i>	Cd(II)	43
<i>Bacillus cereus</i>	Cd(II)	44
<i>Ochrobactrum sp.</i>	Cd(II)	45
<i>Sporosarcina ginsengisoli</i>	As (III)	46

### Fungi:

Fungi are considered as significant part of food web soil that gives nourishment to the other biota that lives in soil. They are important part for the decomposition of waste matter<sup>47</sup>. Fungi can be developed easily, produce high yield of biomass and can easily be manipulated genetically as well as morphologically. Fungi show high resistance to the large amount of heavy metals and simultaneously can accumulate micronutrients (Cu, Zn, Ni, Co and Mn) and nonnutrient metals (Cd, Pb, Hg and Ag). Fungi have exhibited high take up of heavy metals and therefore they found a broader application to adsorb these metals. Cell wall of fungi is composed of chitin, lipids, mineral ions, polysaccharides, polyphosphates, proteins. They can degrade heavy metal ions by extracellular and intracellular precipitation, energetic uptake or by converting the valency of the metal ions. Many fungi can accumulate metals into their spores and mycelium. Outer cell wall of fungi acts as a ligand to eliminate the metal ion by making complex with it<sup>48</sup>. Components of cell wall like polysaccharides, peptides and peptidoglycan are rich in ligands that can bind metal ions (e.g. -OH, -COOH,  $\text{-HPO}_4^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{-RCOO-}$ ,  $\text{R}_2\text{OSO}_3^-$ ,  $\text{-NH}_2$ , and  $\text{-SH}$  where amine group is the most effective one for the uptake of metal as it can bind anionic metals via electrostatic force of attraction and cationic metals via surface complex formation). Fungi have greater surface : volume ratio in comparison to bacteria therefore tolerance level of fungi is more as compared to bacteria<sup>49</sup>. A brief summary of the fungi which are used in the removal of some heavy metals is given in table 4.

**Table 4:-** Heavy metal removal by fungal species.

Fungi	Heavy metal removed	Reference No.
<i>Aspergillus niger</i>	Pb(II)	50
<i>Termitomyces clypeatus</i>	Cr(VI)	51
<i>Saccharomyces cerevisiae</i>	Cu(II)	52
<i>Gloeophyllum sepiarium</i>	Cr(VI)	53
<i>Trichoderma</i>	Cd(II)	54
<i>Penicillium brevicompactum</i>	Co(II)	55

**Algae:**

Algae are the photosynthetic microbes that may be single or multicellular with on roots ,stem or leaves and may grow in freshwater also as in seawater. Algae have been found to possess a potential to absorb the heavy metal ions. Algae have comparatively high binding power and large surface area which attributes to their biosorption capacity. Macroalgae produce metal binding phytochelatins and polyphosphate bodies which are responsible for their high metal tolerance or sequestration of metal ions in storage vacuoles<sup>56</sup>. In all the algae species cell wall is made up of cellulose<sup>57</sup>. Electrostatic attraction and complex formation are among some important properties of cell wall that play a vital part during biosorption. Various functional groups like hydroxyl, carboxyl, carbonyl and amino that are present on the cell wall of algae are mainly responsible for the binding of metal ion on the surface<sup>58</sup>. A summary on the utilization of algae for the removal of heavy metal ions is given in table 5.

**Table 5:-** Heavy metal removal by Algal species.

Algae	Heavy metal removed	Reference No.
Fucus vesiculosus	Pb(II)	59
Sargassum	Cu(II)	60
Spirogyra	Cu(II)	61
Sargassum filipendula	Cd(II)	62
Ulva lactuca	Cr(VI)	63
Oedogonium	As	64

**Limitations of Bioremediation:**

1. Degradation of the pollutant is a strategy used by the microbe to survive for obtaining energy needed for their metabolic reactions. Therefore it is necessary to create certain conditions like introducing oxygen or fertilizer to the polluted soil to enhance the growth of these microbes. This can result in disrupting the life of other pre existing indigenous organisms.
2. Rate of degradation of a pollutant depends on the initial concentration and toxicity of pollutant present to microbe, properties of polluted soil, biodegradability of pollutant and the technique used. Efficacy of bioremediation is restricted where the metal concentration is exceptionally high.
3. The method is restricted to biodegradable compounds only. Method is susceptible to complete degradation. Sometimes the by products left by organism biodegrading the pollutant are found to be more toxic than their parent compounds.
4. Bioremediation methods are exceptionally specific. Achievement of the method are dependent on the adequate amount of nutrients and pollutants, environmental development circumstances and competent microbial populace.
5. In contrast to other techniques bioremediation techniques are much time consuming.

**Future Prospects for Bioremediation:**

Bioremediation is an alternate to the traditional physico-chemical and chemical methods. Genetic engineering has helped in designing the microbes whose genetic code has been altered using by genetic engineering techniques. These kind of scientific techniques are named as recombinant DNA techniques. In this method any acceptable gene can be introduced to produce a particular enzyme which may degrade various contaminants<sup>65</sup>. Taking into consideration the importance of microbes with altered genes in greatly increasing the degradation and detoxification of heavy metal pollutants, more studies should be carried out to enhance their survival when discharged in the environment for bioremediation, because their survivability is currently poor. Factors like temperature, small amount of nutrients and some additional factors that can not be controlled easily, may hinder utilization and effectiveness of the process<sup>66</sup>. Introduced foreign modified strain to the system may cause unmeasurable adverse effect on the natural structural and functional microorganism's community, composition and occurrence. Therefore for complete understanding of the metabolic pathways of these genetically altered microbes used in bioremediation to assure their usefulness and possible side effects during the procedure more research work is required. Taking into consideration that these genetically modified microbes can avoid the use of pathogenic microbes as well as can improve the efficiency of bioremediation process by making use of their functional gene it has a great prospect in future. The capability of the microbes used in bioremediation process to challenge the indigenous microbes is important for the usefulness of bioremediation. Overall more integrated and cross discipline efforts are required for the proper implementation of bioremediation techniques.



**Conclusion:-**

Current paper reviewed the industrial sources, harmful effects of heavy metals on humans health and various techniques employed for the degradation of these heavy metals using different type of microbes. It has revealed the usefulness of bioremediation as a better substitute for the removal of heavy metals from contaminated sites as compared to the physico-chemical methods which are less efficient and expensive due to the amount of energy required. The inherent biological mechanism of microbes permit them to survive beneath heavy metal stress and degrade these heavy metals to less toxic forms. Microbial remediation is considered to be a safe technique because it is dependent on microorganisms that occur naturally in soil and have no harmful effect on the environment as well as people living in that area. The Various mechanisms of degradation of heavy metals using microbes include biosorption, biotransformation and bioaccumulation in which biosorption is considered to be the main degradation mechanism. However for bioremediation the climatic conditions should be favourable. There is a vital need to examine the consequence of various microorganisms in combination against various pollutants for the preservation of natural resources and environment management. Bacteria is one of the greatest vital microbial candidate which needs to be widely explored for the bioremediative ability. Importance of microbes in the bioremediation are immense and need to be explored further.

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