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Bio Synergize: Microbial Synergy Driving Simultaneous Bioremediation and Nanoparticle Synthesis

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Abstract.

Heavy metals even in a very low quantity can be hazardous which can cause significant ecological and health consequences affecting the physiological functioning and structural makeup of all the living things. The elevated level of heavy metals in the environment causes biodiversity loss, environmental pollution, and ecosystem alteration. thus, it is imperative to remove heavy metals from contaminated region through detoxification. Using microorganisms such as fungi, bacteria, algae and yeast is a cost-effective, sustainable, and promising method in order to bioremediate heavy metals. Living organisms can alter contaminants as part of their metabolic processes, breaking them down into less toxic substances. The distinct advantage of microbial remediation is the rate of heavy metal cleanup, which is much faster than phytoremediation. Microorganisms absorb and detoxify heavy metals faster than plants, avoiding the spread of contaminants in the surrounding environment. The ability of microorganisms to execute the detoxification of harmful metal ions by reducing the metal ion into metal nanoparticles makes them appropriate for use in the simultaneous elimination of harmful chemicals from the polluted area and biosynthesis of nanoparticles which holds an immense potential to be used in biomedical and industrial applications. This review focuses the process of detoxifying heavy metals by using microorganisms to synthesize nanoparticles and remediate the affected areas simultaneously.

Keywords: heavy metals, bioremediation, bioaccumulation, synergy, nanoparticles biosynthesis

1. Introduction.

During the last hundred years, industrialization has grown at a tremendous rate. This also increased the requirement for the earth's natural resources; these resources are utilized at a careless rate, which leads to huge contamination of the earth's environment. The biggest contributors to environmental contamination are pesticides and the dumping of industrial waste into water bodies which leads to water pollution. The xenobiotic substances, which are produced by humans, are resistant to natural degradation thus, it’s a matter of great concern because accumulation of the xenobiotic substances can lead to environmental pollution and can affect various life forms. These pollutants are classified as either organic or inorganic pollutants. Some examples of typical organic pollutants are azo dyes, petroleum hydrocarbons, chlorinated phenol, persistent organic pollutants (POPs), insecticides, etc. Heavy metals are inorganic pollutants, which include, lead (Pb), chromium (Cr), mercury (Hg), nickel (Ni), copper (Cu), cadmium (Cd), manganese (Mn), cobalt (Co), iron (Fe) (Pavan Kumar Gautam & Ravindra Kuma, 2022). Metallic elements that have large atomic weights or high atomic numbers, and high densities, are classified as heavy metals. These metallic elements have greater density than 5g/cm^3 (Duruibe et al., 2007). Due to their occurrence in microscopic amounts, heavy metals are often referred to as trace elements. Heavy metals even in a very low quantity can be hazardous and can cause significant ecological and health consequences affecting the physiological functioning and structural makeup of all living things. The heavy metals are quite toxic to various life forms and thus, it is necessary to carry out the detoxification or elimination of the heavy metal from the polluted site. The Conventional physical and chemical method used for removing the pollutants from wastewater can lead to the generation of by-products and secondary pollutants and is thus not a cost-effective and sustainable method for the elimination of heavy metals from the polluted sites (Chibuike & Obiora, 2014). An economical and ecologically sustainable approach needs to be used for the detoxification of heavy metals. Bioremediation is a method that involves utilizing living things like microorganisms and plants, that can break down a broad spectrum of xenobiotics compounds and can convert contaminants into less hazardous forms (Vidali, 2001a). Living organisms can alter contaminants compounds as part of their metabolic processes, breaking them down into less toxic substances. Bioremediation involves phytoremediation which involves the use of plants for the purpose of remediation and micro-remediation involves the use of microorganisms involving fungi, bacteria, and algae. Phytoremediation, despite being a potentially effective and environmentally friendly approach, has several drawbacks and limitations. One of its limitations is that the process takes a lot of time that may require months or years to achieve a substantial reduction in contaminants (Vidali, 2001b). Another safe, effective, and substitute approach for heavy metal eradication is micro remediation. Micro remediation involves the use of fungi, bacteria, and algae for the elimination of heavy metals from the polluted site by converting harmful heavy metals into less harmful forms or immobilizing them making them less toxic(Zhang & Zhang, 2022). Microbes are the preferred choice for this task as they are convenient to handle, cultivate, and implement. Instead of planting lots of plants, microorganisms might be introduced directly to the polluted sites. This eliminates extensive planting and maintenance work, making it practical for the urban environment. The biosynthesis of nanoparticles can be performed using fungi, bacteria, algae, and plants. The biosynthetic process involves the reduction of metal ions into metal nanoparticles. The compounds present in microbes act as stabilizing and reducing agents for the biogenic synthesis of nanoparticles. Due to the high exposure to hazardous chemicals and elevated concentrations of heavy metals, the microorganism has developed some defense mechanisms to survive in such a harsh condition. This review focuses on the synergy of bioremediation of heavy metal by simultaneously synthesizing the nanoparticles using microbes. The microorganism has the ability to detoxify the harmful metal ions by either reducing the metal ion into zero valent state or metal nanoparticles making them suitable to be use for the simultaneous removal of harmful chemicals from wastewater and other contaminated sources and reducing the heavy metal ion into metal nanoparticles.

2. Sources of heavy metal

The earth's crust naturally contains heavy metals. Due to the dramatic growth in the usage of heavy metals, there is an increase in the concentration of metallic components in terrestrial and aquatic ecosystems. Anthropogenic activities including fossil fuel combustion, mining, metal leaching, sewage, oil refinery, lime, industrial waste, inorganic fertilizer, electronic waste, pesticides, and fungicides pesticides are the primary sources of heavy metal pollution (Yadav & Sharma, 2023). Higher quantities of Cr, Ni, Pb, and Cd can be found in inorganic fertilizers, especially in phosphate fertilizers. Cadmium is particularly a dangerous element for plants due to its higher degree of accumulation in leaves, which can be ingested by humans or animals. The excess of Cd causes kidney dysfunction, hypertension, bone fractures, and lung cancer in humans (Kumar Sharma & Agrawal, 2005). Natural sources of heavy metal pollution are soil erosion due to heavy rainfall, volcanic eruptions, comets, metal corrosion, decomposition, and geological weathering. When rocks, minerals, and soil establish a connection with water, atmosphere, and living things, a process known as weathering occurs that breaks them down. It is a site-specific process in which heavy metals are released from the host rock into the surrounding environment by the activity of microorganisms or micro-climatic factors including pressure, ice, water, and heat. (Wen et al., 2021) carried out a study and their findings revealed that weathering causes the release of heavy metals As, Hg, and Tl from the argillaceous sandstone. FTIR and Raman spectroscopy showed that prolonged weathering broke down carbonyl and hydroxyl functional groups, resulting in the release of bound heavy metals from the rocks (Wen et al., 2021). Metals and other substances are taken up as nutrition by the living things from their surroundings. These nutrients and metals are crucial for their development. These nutrients are returned to the environment after their death and decomposition. The most frequent heavy metals present in decomposing organic matter are Hg, Cd, Cr, Pb, As, and Zn (Stefanowicz et al., 2020). Volcanic eruptions are a natural process. It causes particles to be released onto the earth's surface. volcanic ash and pyroclastic material are emitted during eruptions of volcanoes. heavy metals are also found in volcanic ash because volcanoes are created by the metal dissolution from the earth's crust. Cd, Fe, Zn, Pb, Cu, and Cr can be found in volcanic ash (L, 2010). During rainfall, they are frequently transmitted to the environment. Volcanic ash also contains volcanic dust, which is carried by the wind and lands on the ground when it rains. The trace metals that are present in volcanic dust go to the soil, and depending upon temperature, pH, and redox they dissolve in water and get absorbed by the soil particles (Ilyinskaya et al., 2021; Ma et al., 2019).

3. Approaches for the removal of heavy metals.

The elevated level of heavy metals in the environment causes biodiversity loss, environmental pollution, and ecosystem alteration. To keep the environment safer for living organisms, the high concentration of trace elements and heavy metals must be eliminated from polluted water bodies and soil. There are various chemical, biological, and physical methods that can be used for the purpose of remediation. It is possible to eliminate these heavy metals using a variety of techniques, reverse osmosis, membrane technology, oxidation, reduction, filtration, ion exchanges, membrane technology, electrochemical treatment, and evaporation. However, many of these strategies lose their effectiveness at heavy metals concentrations below 100 mg/L (Nourbakhsh et al., 1994). These Physio-chemicals methods are not ecologically friendly and relatively expensive for heavy metals remediation and have other drawbacks also such as inadequate metal removal rates, increased need for energy, and reagents, and the production of hazardous sludge. Additionally, these physio-chemical methods are not suitable for extensive contaminated sites such as industrial waste sites, agriculturally polluted soils, and mining areas due to their high costs.

Hence, ecologically friendly and inexpensive techniques are required for the remediation of metal-contaminated water and soil.

3.1. Biological approach for remediation.

A biological technique called bioremediation in which environmental contaminants are excluded with the assistance of living things including microorganisms and plants which can break down a broad spectrum of xenobiotics compounds and convert contaminants into less hazardous forms (Vidali, 2001a). Living organisms can alter contaminants compounds as part of their metabolic processes, breaking them down into less toxic substances.

Bioremediation has many advantages over physio-chemical techniques, they are less costly when compared to the conventional methods, and they offer a permanent approach that could result in the complete mineralization of contaminants. Physio-chemical methods have a major disadvantage in that they cannot remove contaminants when they are present in a very low concentration, but with the help of bioremediation low concentration contaminants can be treated effectively (Kour et al., 2021).

Phyto-remediation and micro-remediation are the two different terms for bioremediation, in phytoremediation an essential part is played by the plants in the remediation of contaminants. On the other hand, micro remediation is conducted by microorganisms such as fungi, algae, and bacteria.

3.1.1. Phytoremediation

The word “phytoremediation” was introduced in 1991, and is composed of the fusion of two words: Greek phyton (meaning plant) and Latin remediare (meaning remedy). Plants have natural inherent capabilities to absorb, bioaccumulate, preserve, or decompose inorganic or organic chemical compounds (Ali et al., 2013). When compared to other remediation methods, phytoremediation has very low Maintenance and installation costs also applicable for enormous, contaminated area where other alternative remediation methods are neither practical nor economical (Van Aken, 2009). Furthermore, phytoremediation not only cleans up polluted environments but also provides benefits such as biofuel production and carbon sequestration (Doty et al., 2007). (Ranieri et al., 2016) used the plant species *Phragmites Australis* and *Ailanthus Altissima* and removed between 55 to 61% of Cr^{3+} from the water. In both plants, a significant amount of chromium is absorbed by the roots (Ranieri et al., 2016). For heavy metal phytoremediation, hyperaccumulators are most appropriate. Hyperaccumulators are the plant species that have higher efficiency for the metal's accumulation from the soil and storage in their tissues as compared to normal plants (Dinh et al., 2004). They can accumulate concentrations 100 times higher than those of regular plants growing on sites contaminated with metals (Liu et al., 2022). Over 400 species renowned for their exceptional ability to hyperaccumulate metals are from the families Brassicaceae, Asteraceae, Rubiaceae, and Euphobiaceae (McGrath & Zhao, 2003). Hyperaccumulator plants also demonstrate efficient metal transport from roots to shoots. The transport efficiency of non-hyperaccumulators from roots to shoot when compared with the efficiency of hyperaccumulators is less although they accumulate metals in their root in higher concentration. (Robinson et al., 2003) used the rapidly growing hyperaccumulator plant *Berkheya coddii* for the remediation of nickel. They grew the plants hydroponically, and the nutrient solution carries varying concentrations of Ni. To find the concentration of Ni, they employed inductively coupled plasma emission spectroscopy (ICP-AES) and found that the highest Ni concentration was in the leaves and accumulated in a water-soluble state. Ni concentration was greater in the shoot than in the roots (Robinson et al., 2003). Heavy metals accumulation efficiency of plants could be increased by supplying chelators such as EDTA, oxalic acid, and DTPA. Together with the metals in the contaminated soil, they form a complex and enhance the rate of transfer of metals to plant roots and above-ground components. (Ehsan et al., 2014)experimented to investigate, Citric acid effects on the phytoremediation of Cd in *Brassica napus L.* They grow the plants hydroponically and Two weeks later, $CdCl_2$ and citric acid were introduced into the nutrient solution. Plants were then taken out of the solution after 8 weeks. They used flame atomic absorption spectroscopy to measure the amount of Cd in the different parts of the plant and found that, in comparison to plants grown without citric acid, there was a high concentration of Cd in the plant's roots and shoot (Ehsan et al., 2014).

Phytoremediation, despite being a potentially effective and environmentally friendly approach, has several drawbacks and limitations. One of its limitations is that it is a long process that may require months or years to achieve a substantial reduction in contaminants. Also, the selection of suitable plants is important, as not all plants possess the ability to effectively absorb contaminants. It is appropriate for minimal to moderately heavy metal-contaminated regions, since extremely contaminated sites don't promote plant growth and there are concerns regarding the transfer of heavy metals to the food chain if not properly monitored (Vidali, 2001b).

(Bañuelos et al., 2002) carried out various studies, and their findings highlighted that the plants used in the phytoremediation of selenium could transfer Se to animals and insects unintentionally. Insects feeding on these plants have the capacity to accumulate large amounts of selenium. The accumulated high selenium could harm animals and birds that feed on these insects (Bañuelos et al., 2002).

Another safe, effective, and substitute approach for heavy metal eradication is micro remediation, which utilizes fungi, bacteria, and algae to convert harmful heavy metals into less harmful forms or immobilize them making them less toxic. Microbes are the preferred choice for this task as they are convenient to handle, cultivation, and implementation. Microbes are ubiquitous and can survive even in adverse environmental situations such as high pH, temperature, and salt concentration. The microbes are incredibly tiny; their ratio of surface area to volume is high, so their rate of absorption is very high (Zouboulis et al., 2004). They have a rapid multiplication rate and can even work in conditions when extremely low concentrations of heavy metals are found.

The distinct advantage of microbial remediation is the rate of heavy metal cleanup, which is much faster than phytoremediation. Microorganisms absorb and detoxify heavy metals faster than plants, avoiding the spread of contaminants in the surrounding environment. Micro remediation is ideal for cities or small areas to clean polluted water or soil. Instead of planting lots of plants, microorganisms might be introduced directly to the polluted sites. This eliminates extensive planting and maintenance work, making it practical for the urban environment.

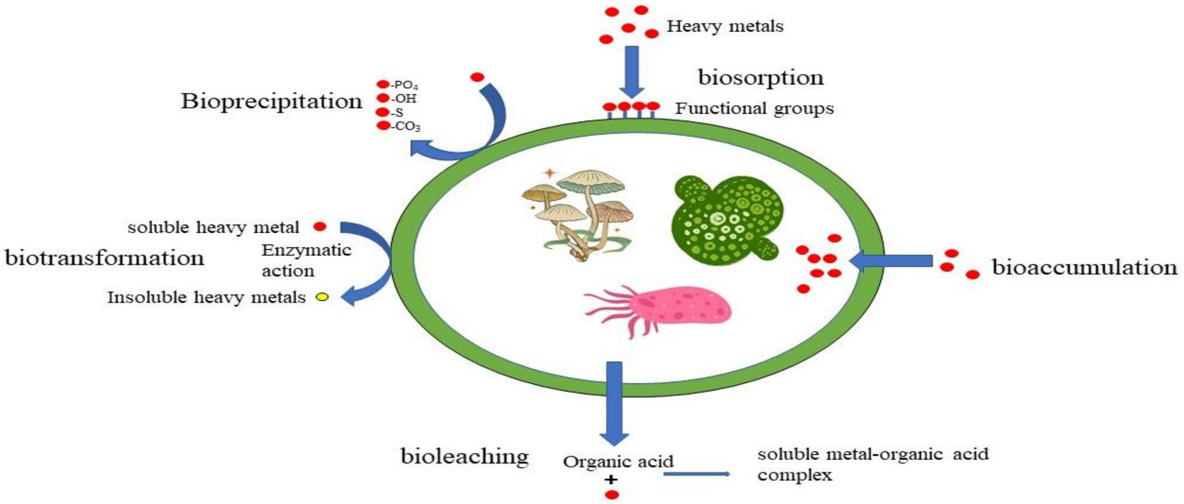

Figure 1. Mechanism of heavy metals remediation in fungi, algae, and bacteria.

3.1.2. Bacteria

Heavy metals also have a toxic effect on bacteria when they are growing in an environment containing high quantity of heavy metals beyond the threshold limit. Bacteria developed distinct mechanisms including biosorption, bioaccumulation, bio-precipitation, and bioleaching to protect themselves against toxic metals, and these metal-resistant bacteria can be utilized as effective bioremediation agents. The first line of defense against heavy metal toxicity is adsorption, in which metal ions bind nonspecifically to the surface of cells. Bacterial cell walls have various types of functional groups, like phosphoryl, sulfate, amino, and carboxyl groups, with which heavy metals interact and get accumulated on the bacterial cell wall's surface. (Musa Jibrin et al., 2020) used *Lysinibacillus fusiformis* 5B for the biosorption of Cr, Ni, Pb, and Cd. Different concentrations of heavy metal-containing culture broths were prepared and inoculated with L. *fusiformis* 5B. For 35 days, inoculated culture broths are kept at 37C^o in a shaker incubator. Atomic absorption spectroscopy was used to quantify the amount of heavy metal biosorption, L. *fusiformis* 5B effectively removed heavy metals from the culture broths and can be used as biosorption agents (Musa Jibrin et al., 2020). Biosorption was observed in both live and dead bacteria cells. dead microbial biomass could be used for bioremediation. (Mohapatra et al., 2019) carried out a study, that used high Pb (II) concentration resistance *Bacillus xiamenensis* PbRPSD202 and analyzed biosorption capacity. Dead and active biomass of bacteria is employed for the elimination of Pb (II) from the aqueous solution. They used the XRD and FTIR techniques to investigate the function of ligands like, hydroxyl, amino, carboxyl, and carbonyl groups present on the bacterial surface in Pb (II) biosorption. Under optimum situations, dead and active biomass of bacteria removed 97.18% and 99.19% lead (II) from the solution (Mohapatra et al., 2019). Utilization of dead biomass of bacteria for bioremediation is becoming more prevalent as no nutrition is required for the bacterial cell's growth, thus reducing the cost of operation. Many bacteria produce extracellular polymeric substances (EPS) that have a major role in the production of biofilms and the adherence of bacteria to different surfaces. The essential components of these substances are polysaccharides, lipids, nucleic acids, and proteins. EPS are engaged in the defense of bacteria against toxic heavy metals, as they consist of various types of anionic and cationic functional groups. These functional groups on EPS interact with the heavy metals, preventing their entry into the bacterial cell, and accumulating them. (Cui et al., 2020) extracted the EPS from *Agrobacterium tumefaciens* F2 that showed effective absorption efficiency for the three heavy metals. Absorption efficiency of Ni^{2+}, Pb^{2+}, Cd^{2+} on EPS was found to be 77.95%, 94.67%, and 94.41% respectively. FTIR analysis showed that polysaccharides are the primary component in the EPS extracted from *Agrobacterium tumefaciens* F2 and have a crucial role in the absorption of Cd^{2+}, Ni^{2+}, and Pb^{2+} ions (Cui et al., 2020). Bacterial cells could alter the ionic state of heavy metals after adsorption, hence lowering their toxicity. Another bioremediation mechanism observed in bacteria is bioaccumulation in which heavy metals are accumulated inside the bacterial cells. When compared to biosorption, bioaccumulation is a more time-consuming and energy-dependent process. (Ahemad & Malik, 2011) isolated 34 bacteria from the heavy metal-contaminated soil, from which pseudomonas isolate SN30, pseudomonas isolate SN7, and pseudomonas isolate SN28 were the zinc-resistant bacteria and shown resistant against Cr^{3+}, Cr^{6}, Hg^{2+}, Cu^{2+} Ni^{2+}, Pb^{2+}, and Cd^{2+}. These bacterial isolates have shown the ability for the accumulation of zinc and copper. When the concentration of copper in the medium is 2.92 mM, bacterial isolates SN30, SN7, and SN28 accumulated 22, 20, and 25 mg/g dry weight of cells. Similarly, when the concentration of zinc is 1.6 mM, zinc resistance bacteria SN30, SN7, and SN28 accumulated 26, 29, and 25mg/g dry weight of cells (Ahemad & Malik, 2011).

3.1.3. Fungi

Fungi are an excellent option for the biological remediation of heavy metals, as they exhibit high-level metal tolerance and impressive wall and intracellular binding of metals. compared to other microorganisms, they are easier to grow, high biomass producers and low-cost media can be used for their growth (White et al., 1995). The Fungal cell wall usually consists of chitin, proteins, polysaccharides (80- 90%), lipids, inorganic ions, and phosphates. These components of fungal cell walls play a crucial part in heavy metal adsorption. There are various negatively charged functional groups like carboxyl, phosphate, amino, sulphate, hydroxyl, and amino groups present on the fungal cell wall. During adsorption, these functional groups bind with various metal cations. (Narolkar et al., 2022) isolated 9 fungal isolates from the metal-polluted site. At 150 ppm of Cr (VI), fungal isolate *Aspergillus candidus* demonstrated an impressive potential for adsorption of 5.49 mg/g and a chromium removal efficacy of 98.75% (Narolkar et al., 2022). (Kurniati et al., 2014) isolated filamentous fungi from the forest soil and investigated the potential of filamentous fungi for mercury bioremediation. *Aspergillus flavus* strain KRP1 exhibited high mercury resistance and demonstrated tolerance up to 100 mg/L. Mercury (II) was eliminated from the PBD media by strain KRP1 via biosorption. After incubating for 7 days, the Removal efficiency of KRP1 in static and shaken conditions is 98.73% and 97.50% in the PBD media containing 10 mg/L mercury (II) (Kurniati et al., 2014). Dead and live fungal biomass could be used as effective bio-sorbents because both have a variety of functional groups on their cell walls which can effectively used for getting rid of harmful heavy metals. (Ranjani, 2019) carried out a study and evaluated the biosorption ability of dead and live biomass of *Rhizopus stolinifer* and *Aspergillus niger* for chromium (VI), isolated from the metal-polluted leather tannery location. *Aspergillus niger* has demonstrated tolerance for chromium (VI) up to 100 ppm and Rhizopus *stolinifer* had a tolerance of up to 1000 ppm. The removal ability of dead and live biomass of *Aspergillus niger* was 45% and 25% and that of Rhizopus *stolinifer* was 83% and 45% at 100 ppm of chromium (VI) (Ranjani, 2019). Fungi exhibit another process by which heavy metals are bioremediated i.e bioaccumulation. It deals with the movement of metals through the cell membrane and accumulation inside the fungal cell. (Dursun et al., 2003) studied the bioaccumulation capability of *Aspergillus niger* and the effect of pH on the growth and bioaccumulation of lead (II), chromium (VI), and copper (II). The ideal pH for the bioaccumulation of lead (II), chromium (VI), and copper (II) and the growth of *Aspergillus niger* were 4.5, 3.5, and 5.0. At concentrations of 100 mg dm^{-3} of lead (II) and copper (II), the maximum accumulation capability of *Aspergillus niger* was 34.4 and 15.6 mg/g, respectively. It was observed that an increase in the concentration of chromium (VI) from 50 to 75, there is no fungal growth, and chromium (VI) uptake occurs. The maximum accumulation capability of *Aspergillus niger* at 50 mg dm^{-3} was 6.6 mg/g (Dursun et al., 2003).

3.1.4. Algae

Algae are quite effective in eliminating heavy metals from polluted environments. The majority of heavy metals are eliminated by algae via biosorption, accumulation, or biotransformation. On the surface of Algal cells, there are a variety of functional moieties present, metal ions bind with these functional groups and get adsorbed at the cell wall. Biosorption is a passive process; dead and living algal biomass can be used as biosorbent. (El-Naggar et al., 2018) used the dried biomass of *Gelidium amansii* for the eradication of lead from the aqueous solution. The FTIR investigation indicated the presence of phosphate, carbonate, methylene, phenolic, and carbonyl groups on the cell wall; these functional groups have a key role in the biosorption of lead. Under optimal conditions, *Gelidium amansii* demonstrated 100% lead removal efficiency from the aqueous solution (El-Naggar et al., 2018). (Shamshad et al., 2016) evaluated the biosorption capacity of *Oedogonium westti* for Cr, Ni, Pb, and Cd. *Oedogonium westti* was cultured in a selected heavy metal-containing aqueous solution for 7 days. The findings of this investigation showed that the adsorption capacity of *Oedogonium westti* for Cr, Ni, Pb, and Cd was 0.620, 0.418, 0.261, and 0.974 mg/g, respectively. Concentrations of metal and pH greatly affect the removal capacity. When pH was 4 and Cr and Cd were present in low concentrations, the removal capacity of *O. westti* was 93% and 95%, respectively. In the case of Ni, increasing the amount of Ni in the aqueous solution also enhanced the removal capacity and reached 87%. The removal capacity for Pb increases with a rise in the pH and metal concentration; at pH 6, the removal capacity for Pb was between 91-96% (Shamshad et al., 2016). Biosorption inhibits the penetration of heavy metals in the algal cell, but when extremely high concentrations of heavy metals exist, they can enter the algal cell. Then algal cells exhibit another mechanism known as bioaccumulation, in which heavy metals are accumulated in the algal cell to mitigate their toxic effects. Bioaccumulation is an energy-dependent and slow process, only living cells can carry out this mechanism. (Pham, 2019) Carried out a study, and their findings revealed that *Scenedesmus sp.* is Able to bioaccumulate Cu. when the amount of Cu in the solution is 2 mg/l, Scenedesmus sp. demonstrated 89.5% Cu removal efficiency within 7 days (Pham, 2019). Similar findings were provided by (Vigara Fernández et al., 2021) that *Chlorella sorokiniana* accumulated 11232 mg/kg of Cd, and demonstrated 65% removal efficiency (Vigara Fernández et al., 2021).

Table 1. Microbial mediated bioremediation of heavy metals.

Type of microorganism used	**Heavy metal to be removed**	**Mechanism of bioremediation used to detoxify the heavy metal**	**Performance of bioremediation**	**Time taken for bioremediation**	**Reference**
Bacteria:					
Arthrobacter viscosus	Cr	Biosorption.	100%	144h	(Hlihor et al., 2017)
EB L14	Cd, Cu, Pb.	Bioaccumulation, biosorption.	75.78%,21.25%, 80.48%.	24h	(Guo et al., 2010)

Bacillus cereus	Hg	Biosorption.	104.4 mg/g.	72h	(Sinha et al., 2012)
Bacillus anthracis PS2010	Co, Zn, Pb, Cd, Cu	bioaccumulation	4.75, 5.22, 6.44, 3.41, 2.03 mg/g.	-	(Sobhy et al., 2014)
Acidithiobacillus ferrooxidans	Fe, Zn, Cu, Pb	Bioleaching	85.42%, 98.73%, 96.44%, 88.90%	28 days	(Rouchalova et al., 2020)
Micrococcus luteus DE2008	Cu, Pb	Biosorption.	408mg/g, 1965mg/g.	12h	(Puyen et al., 2012)
Lactobacillus plantarum MF042018	Cr, Ni	Bioaccumulation	30.2 %, 33.8%	24h	(Ameen et al., 2020)
Variovorax boronicumulans	Zn, Pb, Cd	Bioprecipitation	73.81%, 95.93, 73.81%.	72h	(Jalilvand et al., 2020)
Stenotrophomonas rhizophila	Zn, Pb, Cd	Bioprecipitation	63.91%, 96.25%, 71.3%	72h	(Jalilvand et al., 2020)
Sporosarcina pasteurii	Zn, Pb, Cd	Bioprecipitation	94.83%,98.71%, 97.15%	72h	(Jalilvand et al., 2020)
Pseudomonas fluorescens	Cd	Biosorption	99%	5 min	(Sankarammal et al., 2014)
Oceanobacillus profundus	Pb, Zn	Biosorption	94%, 54%	24h	(Mwandira et al., 2020)
Fungi					

Trichoderma fungus	Cd	Biosorption	78.46%	120 min	(Bazrafshan et al., 2016)
Pleurotus ostreatus	Zn, Ni, Cr, Cu	Biosorption	3.22, 20.40, 10.75, 8.06 mg/g	150 min	(Javaid et al., 2011)
Aspergillus lentulus FJ172995	Ni, Pb, Cu, Cr	Bioaccumulation	42%, 100%, 78%,79%	5 days	(Mishra & Malik, 2012)
Alternaria alternata	Ag, Hg, Cu, Pb	Bioaccumulation	63.1%, 68.5%, 76.4%, 80%	7 days	(Verma et al., 2016)
Komagataella phaffi	Cu, Pb, Cd	Bioaccumulation	21.63, 20.63, 25.23 mg/g.	7 days	(Liaquat et al., 2020)
Fusarium sp. MMT1	Cr	Bioaccumulation ,Biotransformation	100%	72h	(Guria et al., 2014)
Neurospora crassa	Cu, Pb	Biosorption	12.28,49.06 mg/g	15 min	(Kiran et al., 2005)
Neosartorya fischeri	Tl	Biosorption, Bioaccumulation	62.01, 432.91 mg/kg	30 days	(Urík et al., 2010)
Trichoderma gamsii	Cr	Biosorption	89%	420 min	(Kavita & Keharia, 2012)
Algae					
Porphyra leucosticta	Pb, Cd	Biosorption	95%, 75%	120 min	(Ye et al., 2015)
Scenedesmus sp.	Pb	Bioaccumulation	83.5-84.2%	7 days	(Pham et al., 2020)
Spirulina sp.	Mn, Se, Zn,		99.73%,98.83%,		(Mane & Bhosle, 2012)

Spirogyra sp.	Fe, Cr, Cu	Biosorption	79%, 98.93%, 98.3%, 81.2%	7 days	(Mane & Bhosle, 2012)
Botryococusbraunii	Mn, Se, Zn, Fe, Cr, Cu	Biosorption	99.6%, 98.16%, 81.53%, 99.73%, 98.23%, 89.6%	7 days	(Uddin & Lall, 2019)
Spirulina platensis	Cu, Pb, Cd	bioaccumulation	82%, 93%, 89%	90 min	(Al-Homaidan et al., 2014)
Desmodesmus sp.	Cu	Biosorption	90.6%	90 min	(Rugnini L et al., 2018)
Scenedesmus obliquus	Ni, Cu	Biosorption	85%, 94%.	2 days	(Rinanti et al., 2018)
Phacus genera	Cu	Biosorption, Bioaccumulation	65.54% ± 8.04	240 min	(Ahmad et al., 2020)
Spirulina (Arthrospira) platensis	Al, Pb, Ni	Bioaccumulation	64.28%, 79.17%, 66.67%	7 days	(Arunakumara et al., 2008)
Chlorococcum sp.	Pb	Bioaccumulation	188 mg/g	2 days	(Upadhyay et al., 2022)
	As	Bioaccumulation	322.43±2.95µg/g	10 days	

4. Simultaneous bioremediation and nanoparticle synthesis; synergy

Due to an increased exposure to hazardous chemicals and the elevated levels of heavy metals, certain defence mechanisms have been developed by microorganisms in order to survive in such harsh conditions, such as intracellular bioaccumulation, extracellular precipitation, and changes in efflux pumps. The microorganism has the ability to detoxify the harmful metal ions by either reducing the metal ion into a zero-valent state or metal nanoparticles, making them suitable for the simultaneous removal of harmful chemicals from wastewater and other contaminated sources and the biosynthesis of nanoparticles that have a potential to be used in different biomedical applications (Jadoun et al., 2022). The biosynthesis of nanoparticles can be executed using fungi, bacteria, algae, and plants. The microorganism provides a benefit over plant-mediated synthesis of nanoparticles i.e. they can reproduce easily when compared with plants. Microbes in the presence of metal oxides can synthesize metal nanoparticles as well as they can be used for the biological remediation of heavy metals this synergy is due to the capability of microorganisms to tolerate the high concentration of heavy metals because of the presence of intracellular and extracellular proteins that mediate the adsorption and chelation of metal (Gomaa, 2022). The synthesis of metallic nanoparticles can occur either within the microbial cell or using the supernatant of the microbial cell which consists of different proteins, enzymes, and polysaccharides of the microorganism that act as reducing agents and are in charge for converting metal ions into metal nanoparticles.

The intracellular synthesis of nanoparticles involves three steps; trapping of metal ions onto the cell wall of microorganisms, bio-reduction by enzymes present in the cytoplasm, and capping and stabilization by organic molecules within the cell (Fariq et al., 2017). (Salvadori et al., 2014) using the dead biomass *of Rhodotorula mucilaginosa* carried out the intracellular synthesis of Cu nanoparticles. The copper ions were reduced by the dead biomass of red yeast into copper nanoparticles and apart from acting as a reducing agent the dead biomass also contributed in stabilizing the formed copper nanoparticles. The average diameter of the synthesized copper nanoparticles was observed to be 10.5nm and hence they concluded that the dead yeast biomass can be successfully used for the biosynthesis of copper nanoparticles with subsequent bioremediation of wastewater by removing copper from wastewater (Salvadori et al., 2014).

Some enzymes such as NADH-dependent enzymes which are released into the culture medium as extracellular enzymes serve in the metal ion reduction. The proteins, peptides, and other organic molecules found in the supernatant which is released by the microbial cell serve as capping and stabilizing agent for the synthesized nanoparticles (Murali et al., 2023). (Tyagi et al., 2019) used an entomopathogenic fungus, *Beauveria bassiana* for the biosynthesis of Ag nanoparticles. They used the extracellular synthesis approach for manufacturing Ag nanoparticles. The Supernatant was separated from the fungal biomass and in the supernatant silver nitrate was added which was further reduced to Ag nanoparticles. The color change of the solution to dark brown from white color was the first indication that confirmed the synthesis of Ag nanoparticles, further analysis of nanoparticles revealed that their size was in a range of 10-50nm and the Ag nanoparticles exhibit antimicrobial efficacy against different bacterial strains(Tyagi et al., 2019). (Ramanathan et al., 2013) used silver-resistant bacterium, *Morganella morganii* for the biosynthesis of copper nanoparticles and they evaluated the link between the biosynthesis of copper nanoparticles and the resistance that bacteria exert against heavy metal. Bacteria synthesize metalloproteins by utilizing copper, thus copper is a trace element that is utilized by bacteria in several ways however, at high concentrations, Cu (II) is toxic for the cell, and it can induce hydroxyl radical generation which can damage the cell in a different manner and can lead to microbial cell toxicity (Brow et al., 1992). Hence it is necessary for the bacterium to protect itself from the toxic concentration of copper ions and simultaneously must regulate the supply of copper to be utilized for the synthesis of metalloprotein. Further, their results indicated that the copper ions penetrate within the bacterial cell which within the cell associates with the biomolecules and is reduced to copper nanoparticles which are released out of the cells into the medium. Their result also suggested that the Ag^+ ions binding protein, SilE was involved in the uptake of Cu^{2+} ions inside the bacterial cell which further reduced into zero-valent Cu nanoparticles(Ramanathan et al., 2013). Bacteria, fungi, and algae can be effectively used in the bioremediation of heavy metals as well as in the synthesis of metal nanoparticles. Nitrate and silver are the common pollutants that are found in waste from ink, porcelain, and photographic manufacturing industries. (Eltarahony et al., 2016) used *Achromobacter sp.* MMT, which is a bacterial strain for the bioremediation of silver and nitrate pollutants, and due to the presence of periplasmic nitrate reductase enzyme the silver ions were simultaneously reduced to silver nanoparticles. For this purpose, they immobilized the bacteria and nitrate reductase enzyme so that they could withstand the harsh environmental condition of wastewater that was artificially made which contained $AgNO_3$. They observed that the immobilized bacterial cells were found to completely eliminate nitrate within 192 hours and simultaneously reduced Ag^+ ions into Ag nanoparticles that had a size range between 23.26nm and 58.14 nm. The immobilized nitrate reductase enzyme had a lower efficiency in removing the nitrate from wastewater and the synthesized Ag nanoparticles by the enzyme were having a size range between 94.44 nm-172.22 nm(Eltarahony et al., 2016). (Sundararaju et al., 2020) used *Microbacterium sp.* for the removal of heavy metals from the surroundings, *Microbacterium sp.* is a bacterium that exhibits resistance to heavy metal and this bacterium was isolated from electroplating industrial effluent. Cobalt is a heavy metal that is released by some of the industries to the water bodies which can lead to a significant threat to the environment. The bacterium has shown resistance to Co (II) ions and is thus employed for the reduction of Co (II) ions to Cobalt oxide nanoparticles. Co_3O_4 nanoparticles were synthesized extracellularly using the *Microbacterium sp.* The Co_3O_4 nanoparticles were further characterized using different techniques and their analysis revealed their spherical morphology and the size range was observed to be 10–70 nm. These biosynthesized cobalt oxide nanoparticles can be utilized in the field of various industrial and clinical applications(Sundararaju et al., 2020). Phenol is an organic aromatic compound that at high concentrations is a toxic, carcinogenic, mutagenic compound it is released by pharmaceutic, petroleum, and other industries as a byproduct to the waterbodies and it can’t degrade naturally. Thus, an ecologically friendly and economical method is employed for the bioremediation of phenol and tellurite oxyanions by (Hosseini, Lashani, et al., 2023). They used a bacterium, *Lysinibacillus sp.* which has an ability for the bioremediation of phenol and Te oxyanions. The bacteria utilized phenol as a source of carbon and reduced Te oxyanions to Te nanoparticles. The formation of Te nanoparticles was executed using an intracellular synthesis approach where the synthesized Te nanoparticles accumulate within the bacterial cell which was later on separated from the bacteria. The bacterium successfully degraded phenol by using it as a source of carbon and reduced tellurite oxyanions to Te nanoparticles and the formation of a black color of biomass indicated the formation of Te nanoparticles. The nanoparticles characterization indicated that the nanoparticles were observed to be spherical and their size range was observed to be 22 nm-148 nm. Thus, their study concluded that this biological method of bioremediation of these toxic compounds is an effective mechanism for simultaneous bioremediation and nanoparticle synthesis(Hosseini, Lashani, et al., 2023) .

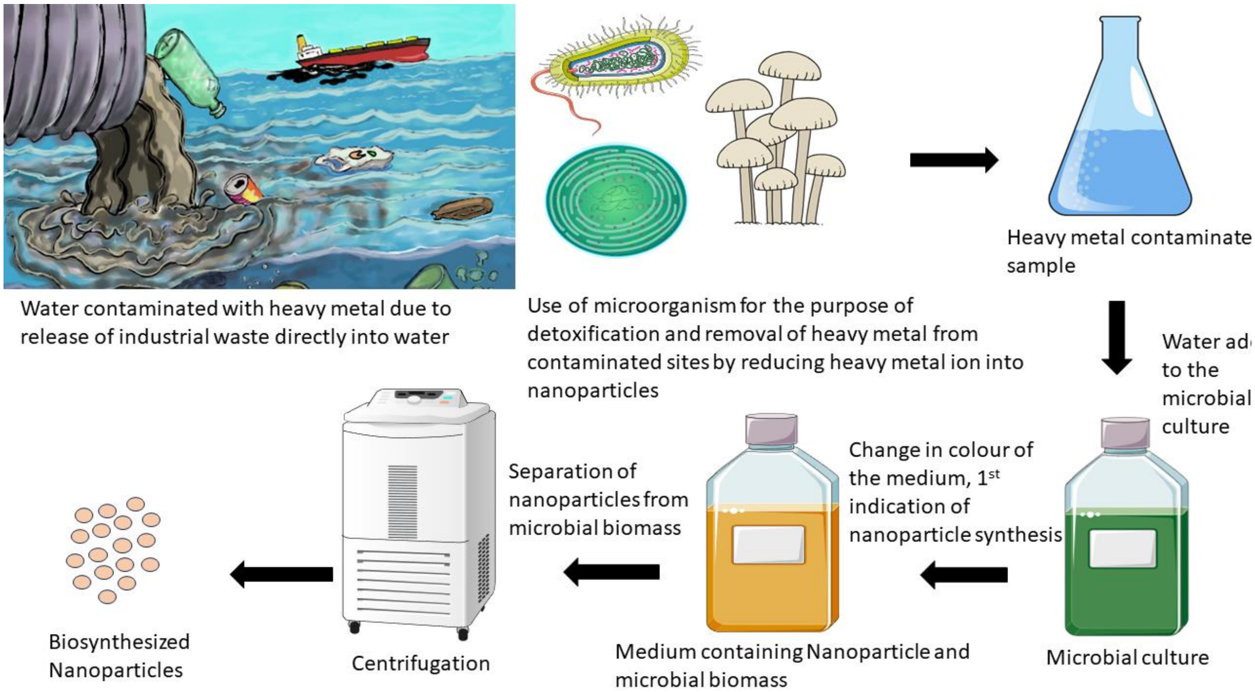

Figure 2. Schematic diagram illustrating simultaneous bioremediation of heavy metals from the contaminated sites and nanoparticle synthesis by reducing the heavy metal ion into nanoparticles

The presence of Cd in water and soil can lead to a significant danger to human life as it is a potent human carcinogen. Cadmium is a heavy metal and is quite toxic and thus it should be removed from the environment in order to prevent its exposure to humans through food and water. (Chakraborty et al., 2018) utilized extracellular polymeric substances released by *Pseudomonas aeruginosa* which is a bacterium isolated from marine areas for the bio-reduction of Cd^{2+}ions to CdS nanoparticles. The Extracellular polymeric substance released by *Pseudomonas aeruginosa* consists of polysaccharides, nucleic acids, proteins, and lipids which are accountable for the reduction of Cd^{2+} ions into CdS nanoparticles. The CdS nanoparticles were further analyzed using various techniques and they were characterized to be spherical and their size was observed to be around 10nm. They concluded that EPS secreted by *Pseudomonas aeruginosa* serves to be a sustainable method for the simultaneous removal of cd ions from the sample and the reduction of these cadmium ions to cadmium sulphide nanoparticles (Chakraborty et al., 2018). (Annamalai et al., 2013) used a biological method for the removal of chromium ions using *Bacillus subtilis.* At first, the biosorption of Cr (VI) was done to Cr (III) and was subsequently reduced to Cr nanoparticles using *Bacillus subtilis*. The manufacturing of Cr nanoparticles was done using an intracellular synthesis approach in which the Cr ions penetrate within the bacterial cell and subsequently inside the cell get reduced to Cr nanoparticles. Their results indicated that at pH 7 maximum chromium ions were removed by

Bacillus subtilis, further, the Cr nanoparticles were characterized to be spherical and their size ranges from 50 nm-78 nm. Thus, they provide sustainable and alternative approach for the detoxifying chromium ions from industrial effluents (Annamalai et al., 2013). Mercury is a heavy metal whose accumulation in the environment can lead to major problems in various life forms. Numerous human activities result in the release of Hg2+ ions into the environment such as the burning of coal, some industrial operations, and mining. Detoxification of Hg^{2+} ions can be done by carrying out the reduction of Hg^{2+} ions into HgS nanoparticles using various microorganisms. (Sathyavathi et al., 2013) used *Bacillus cereus* MRS-1 for the bioremediation of mercury by reducing Hg^{2+} ions into HgS nanoparticles. The major derivative of mercury is $HgCl_2$ which is released into the environment via various human activities. The bacterium has the ability to detoxify mercury chloride into HgS nanoparticles. In this study, $HgCl_2$ was added to *Bacillus cereus* MRS-1 culture, and the HgS nanoparticles were extracellularly precipitated. The biosynthesized HgS nanoparticles were observed to be spherical and their size ranges from 10 nm-100 nm. Thus, the *Bacillus cereus* strain which was isolated from industrial effluent of electroplating can be successfully used for the bioremediation of Hg which is present in the contaminated sites(Sathyavathi et al., 2013). (Chandrakant Vaigankar et al., 2020) carried out the detoxification of Selenite, using the bacterium *Halomonas venusta* which is a salt-tolerant bacterium. The bioremediation of selenite was done by reducing selenite to selenium nanoparticles by the salt-tolerant bacterium. The bacteria carried out the reduction of selenite oxyanions intracellularly and the fabrication of Selenium nanoparticles was first indicated when the color of the medium was changed to brick red from yellow. it was observed that the nanoparticles were spherical in shape and their size was observed in the range of 20 nm-80 nm also hexagonal crystal lattice was exhibited by them. They also evaluated anti-cancer, anti-oxidant, and also their potential against larvicidal mosquitoes (Chandrakant Vaigankar et al., 2020). Selenate and Selenite which are the oxyanions of selenium, these oxyanions are quite toxic at a high concentration to the aquatic and terrestrial life forms. However, some bacteria can withstand a high concentration of selenium ions and can reduce the oxyanions of selenium into selenium nanoparticles.(Pandey et al., 2021) carried out the biosynthesis of selenium nanoparticles in order to eliminate the toxic effect of selenium ions upon various life forms. They cultured *Anabaena sp.* For the biosynthesis of selenium nanoparticles. Sodium selenite was added to the cyanobacterial biomass and the solution was incubated. The nanoparticles were separated as supernatant and cyanobacterial biomass as pellets. Further, the selenium nanoparticles were characterized using different techniques, and the average diameter of the synthesized selenium nanoparticles was observed to be 25nm. The biosynthesized Se nanoparticles exhibited potent anti-bacterial activity and can be employed in the textile industry in the decolorization of dye (Pandey et al., 2021).

(Bedi et al., 2018) carried out the biosynthesis of iron-containing metal nanoparticles via bioremediation of mining waste. A significant amount of the mining waste is produced by the iron ore. They isolated a fungal strain from the mining site containing iron ore tailings *Aspergillus aculeatus*, which able to withstand elevated metal ion concentrations without leading to its cell toxicity and can effectively convert the iron ore into metal nanoparticles. Their results indicated that the iron nanoparticles were highly effective in seed germination as these iron nanoparticles can be uptake by plants as a source of iron. Also, at low concentrations, they positively enhanced root and shoot development, however, they exerted toxic effects at higher concentrations on seedling growth (Bedi et al., 2018). Copper is the most common pollutant found in municipal wastewater, the presence of high concentrations of cupric ions can cause severe toxicity to various life forms. Conventional chemical and physical methods of removing pollutants from wastewater can lead to the generation of by-products and secondary pollutants. (Žvab et al., 2021) utilized the ability of *Chlamydomonas reinhardtii*, a microalga, for the bioremediation of nutrient media which is considered wastewater rich in Cu^{2+} ions. *C. reinhardtii* is used for the bioremediation of cupric ions by simultaneously biosynthesizing Cu nanoparticles. Further characterization of nanoparticles was done and the synthesized nanoparticles were observed to be spherical, and polydisperse and their size was observed to be 10nm. Thus, they effectively carried out the bioremediation of the Cu^{2+} polluted media and evaluated the potential of *Chlamydomonas reinhardtii* for the detoxification of metal ions by reducing it to metal nanoparticles (Žvab et al., 2021). Some natural activities such as the industrial activity, mining, burning of coal, and weathering of rocks can lead to the development of selenite and tellurite oxyanions which are quite toxic for living organisms. Thus, these oxyanions need to be removed from the environment. For this purpose, (Hosseini, Hadian, et al., 2023) carried out the bioremediation of selenite and tellurite oxyanions using *Trichosporon cutaneum* and *Yarrowia lipolytica,* these yeast strains reduced Se and Te ions into Se-Te nanoparticles. The nanoparticles were biosynthesized intracellularly by the yeast. The size of the Se-Te nanoparticles synthesized by *Yarrowia lipolytica* was found in a range of 46 nm-171 nm while the size of nanoparticles biosynthesized by *Trichosporon cutaneum* was observed in a range of 25 nm-53 nm. The analysis of synthesized nanoparticles revealed that the synthesized nanoparticles were covered by carbohydrates, proteins, and lipids and thus they successfully removed the oxyanions from the polluted environment by simultaneously synthesizing Se-Te nanoparticles which can be used in biomedical applications (Hosseini, Hadian, et al., 2023).

5. Future prospect

Research needs to be conducted in order to identify what can be done to improve the rate of biosynthesis of nanoparticles and increase the efficiency of microorganisms to more effectively carry out the bioremediation and detoxification of heavy metals. Additionally, some microorganisms used for simultaneous bioremediation and nanoparticle production could be pathogenic, so they could not be widely employed. Genetic engineering can be used to overcome these challenges. Modification in the genome of organisms can be done to alter the genes, and microorganisms with enhanced bioremediation capacities and less potential to cause diseases can be produced. The majority of these heavy metal remediation techniques are limited to the laboratory scale, employment of simultaneous bioremediation and nanoparticle synthesis at large a scale would be a major challenge. Apart from the biosynthesized nanoparticles have several benefits in different areas however, the nanoparticles can serve to some major health complications if accumulated inside the human body in a high concentration. Thus, apart from the positive applications of nanoparticles the negative impact of nanoparticles on living organisms must also be evaluated.

6. Conclusion

The use of microorganisms such as fungi, yeast, bacteria, and algae is an economical, sustainable, and promising method for the bioremediation, detoxification, or elimination of heavy metals such as copper, cobalt, chromium, tellurite, selenite, cadmium, mercury and other contaminants from the contaminated sites in the environment. Some natural and anthropogenic activities cause these highly toxic heavy metals to release into the surroundings which could pose a significant danger to various life forms, thus it is essential to remove heavy metals from contaminated areas such as wastewater. The use of microorganisms has a dual benefit, they can effectively remove heavy metal from the surroundings by reducing it to nanoparticles, and the biosynthesized nanoparticles due to their smaller size can be employed in various biomedical applications such as antibacterial, anti-oxidant, anti-cancer, etc. thus this dual benefit can largely benefit the society in the removal of contaminants from the contaminated areas and also in the field of medicine, nanotechnology ad industrial applications.

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