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Nanotechnology: Bioremediation of Some Heavy Metals

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***Abstract**: The growing concern over heavy metal pollution has become a pressing issue worldwide, as it poses significant threats to both public health and the environment. To combat this challenge, constant monitoring of heavy metal levels in food, water, and the surroundings is essential, alongside the implementation of effective remediation measures. Nanotechnology has emerged as a potential sustainable solution to address heavy metal contamination in the future, although its practical application for pollutant remediation is still a subject of debate.In recent times, the environmentally friendly synthesis of nanoparticles has gained considerable attention due to its economic and ecological advantages. This green synthesis approach relies on biologically derived materials to produce nanoparticles. The review highlights the health risks associated with heavy metals while also exploring the potential of nanotechnology in bioremediation to combat these contaminants. By harnessing the potential of nano bioremediation and green synthesis, researchers and practitioners can develop sustainable and efficient strategies to tackle heavy metal contamination in soil and water.These innovative approaches hold great promise in minimizing environmental damage and safeguarding human well-being, contributing to a healthier and more sustainable future. By adopting eco-friendly technologies, we can take significant strides toward creating a cleaner and safer environment for generations to come.*

***Keywords:** Heavy metal pollution, Nanotechnology, Bioremediation, Soil and water contamination, Green Synthesis, Environment.*

I. Introduction:

The widespread issue of heavy metal contamination poses a critical environmental concern, particularly with regards to soil pollution [1]. Human activities, such as mining and urban development, have significantly contributed to the escalating levels of heavy metal contamination in soil [2]. Mining waste accumulated in disposal sites can have detrimental effects on surrounding areas, potentially rendering agricultural lands infertile. Additionally, water used in mining operations, including mineral processing and washing, serves as a major source of soil contamination near mining sites. Therefore, the monitoring of soil pollution caused by heavy metal-contaminated water is of utmost importance, especially in metal mines. Moreover, climatic conditions also influence soil contamination in proximity to mining regions. Unanticipated environmental pollution can further aggravate soil pollution in these areas.Agricultural lands irrigated with heavy metal-contaminated water or excessive use of fertilizers pose risks, as heavy metals can impede crop growth and enter the food chain. Addressing these challenges is crucial to mitigate the adverse impacts of heavy metal contamination on both the environment and human health. By implementing effective measures to manage and remediate soil pollution caused by heavy metals, we can work towards creating a safer and healthier environment for present and future generations.

A study conducted by Bhardwaj et al. revealed that the Yamuna River stretch in Delhi, India, is heavily contaminated with several heavy metals, including iron, lead, chromium, cadmium, nickel, zinc, and copper [3]. Similarly, Kumar et al. reported in their study that cadmium and arsenic are the predominant contaminants found in various soils across India [4]. The Agency for Toxic Substances and Disease Registry (ATSDR) has identified arsenic as the most hazardous substance, followed by lead, mercury, and cadmium. Considering the significant environmental risks associated with heavy metals, it becomes crucial to establish and adhere to standard limits for their presence to ensure economic sustainability and viability. Table 1 provides a list of acceptable limits for heavy metals in drinking water.

Table 1. Acceptable limits of heavy metal in drinking water

Heavy Metal	Atomic weight (u)	WHO limits (mg/L)	US-EPA limits (mg/L)	BIS limits (mg/L)
Arsenic (As)	74.92	0.01	0.010	0.01
Lead (Pb)	207.2	0.01	0.015	0.01
Cadmium (Cd)	112.41	0.003	0.005	0.003
Mercury (Hg)	200.59	0.006	0.002	0.001
Chromium (Cr)	51.99	0.05	077.1	0.05
Nickel (Ni)	58.69	0.07	0.1	0.02

*WHO-World Health Organization, US-EPA-U.S Environmental Protection Agency, BIS- Bureau of Indian Standards (BIS).

The remediation of toxic heavy metals typically involves four main approaches: in situ containment, ex-situ containment, in situ treatment, and ex-situ treatment. However, the limitations and inefficiencies associated with conventional and bioremediation methods have led to the exploration of nanotechnology in this field [5]. Nanotechnology has gained significant attention due to its ability to create materials with unique properties at the nanoscale, which are distinct from their bulk counterparts. Leveraging quantum and surface phenomena, nanotechnology enhances existing processes and materials [6]. Nanoparticles possess characteristics such as uniform shape and high surface area to volume ratio, which significantly influence their ability to penetrate cell membranes and facilitate biochemical activities [7]. Additionally, the environmentally friendly nature of biogenic nanoparticles reduces reliance on toxic chemicals, further fueling their widespread use in the removal of heavy metals and metalloids. The present review highlights the impact of heavy metals and metalloids on the environment and human health, and discusses their remediation through the integration of nanotechnology with bioremediation methods.

Understanding the Environmental and Health Effects of Heavy Metals

The Impacts of Heavy Metals on Soil

The contamination of soil with heavy metals arises from various industrial activities, agricultural practices, and improper waste management. These persistent non-biodegradable pollutants pose a significant threat to the ecosystem, persisting in the environment for prolonged periods [8]. Heavy metal presence not only inhibits the biodegradation of organic pollutants but also disrupts the delicate food chain balance. Beyond compromising crop quality and reducing plant yields, heavy metal contamination impacts the size, composition, and activity of microbial communities in the soil. The enzymatic activity of these microorganisms, which is vital for nutrient cycling and soil health, is significantly affected by heavy metal presence. Recognizing and addressing the detrimental consequences of heavy metal contamination on soil ecosystems is crucial [9]. Understanding their effects and implementing effective remediation strategies are essential to preserve soil health and safeguard the overall environmental balance.

The Impacts of Heavy Metals on Water

Heavy metals are known to accumulate in water bodies and soil, originating from various sources such as municipal runoff, industrial activities, and urban areas. Contaminated sewage water contributes to the accumulation of these pollutants in irrigation systems, posing serious problems for both humans and ecosystems [10]. The presence of even trace amounts of heavy metals in water leads to a decrease in dissolved oxygen concentration, negatively affecting aquatic life. Upon release into rivers, heavy metals and metalloids undergo rapid dilution and are transported over long distances. They then accumulate in aquatic organisms and settle in sediments. Changes in pH values, hydrological conditions, and external redox conditions can trigger the release of heavy metals from sediments into the water column, resulting in toxicity to organisms [11]. Due to their toxic nature, ease of enrichment, and resistance to degradation, heavy metals are susceptible to biomagnification and bio-enrichment within the food chain, potentially causing adverse effects on ecosystems and endangering water supplies for humans. Recognizing the accumulation and impacts of heavy metals and metalloids in water and soil is crucial for implementing effective measures to mitigate their adverse effects. Addressing the sources and pathways of contamination is essential to preserve the integrity of ecosystems and ensure the safety of water resources.

Bioremediation

Bioremediation is an effective technique that utilizes biological agents to convert various contaminants into less toxic forms through degradation and mineralization [12]. It relies on microorganisms, nutrients, and environmental parameters to enzymatically attack and detoxify pollutants, ensuring their safe conversion. However, despite its advantages, bioremediation has limitations, including time-consuming degradation and restricted applicability in highly contaminated sites. To address these challenges, integrating nanotechnology with bioremediation offers enhanced adsorption capacities, making it more efficient for remediating contaminated areas [13]. This integration takes advantage of surface effects, small size effects, quantum effects, and macro quantum surface effects, promising effective environmental cleanup.

Nanobioremediation

Nanotechnology offers a promising approach to enhance conventional remediation and bioremediation methods by accelerating the transformation rate of contaminants due to the smaller size of nanoparticles [14]. Integrating nanotechnology with bioremediation can nurture the environment by effectively removing contaminants and advancing the remediation process [15]. This combination, known as nanobioremediation, involves the use of nano-sized particles or materials synthesized by plants, fungi, or bacteria to remove heavy metals, metalloids, organic, and inorganic pollutants from contaminated sites. Key attributes of nanobioremediation include the use of green and clean nanomaterials, providing contaminant removal solutions, and functioning as sensors for environmental agents. The small size and larger surface area of nanoparticles make them effective catalysts for degrading waste and safely treating contaminants without harming the environment [16]. Microorganisms play a crucial role in converting heavy metals and metalloids into non-toxic forms through the mineralization of organic contaminants into carbon dioxide and water. The nano size of nanoparticles enables deeper penetration into contaminated sites, leading to improved results compared to traditional bioremediation methods.

Properties Of Nanoparticles

Nanoparticles are aggregates with dimensions between 1 and 100 nm, possessing unique physicochemical properties compared to bulk materials. They can exist in various geometries such as thin films, wires, rods, and spheres, based on the number of dimensions in which electrons can be confined. Their large surface area to volume ratio due to the smaller size imparts distinct physical and chemical properties. Nanoparticles can be categorized into organic (micelles, fullerenes, dendrimers) and inorganic (ceramic, steel, metal oxide) nanoparticles, with variations in size, shape, and state of size distribution [17]. For nanoparticle synthesis, both chemical and biological methods are utilized. The biological synthesis method is preferable due to its eco-friendly nature, low cost, rapid synthesis, and control over size characteristics. Biological systems can self-organize and synthesize molecules with highly selective properties. The physical properties of nanoparticles depend on size, shape, surface area, solubility, and structure. The increased surface area to volume ratio makes the surface more reactive, and variations in size and shape affect the optical properties. The chemical properties are defined by zeta potential, surface chemistry, photocatalytic properties, and chemical composition [18]. Green nanotechnology approaches use living organisms, microbes, and plants for nanoparticle synthesis. Microbes are of particular interest due to their high tolerance, rapid decontamination, and nanoparticle production capabilities. Biologically generated nanoparticles often exhibit high catalytic reactivity and specific surface area, and the presence of capping agents secreted by microorganisms helps prevent nanoparticle aggregation. Extracellular biosynthesis of nanoparticles eliminates the need for downstream processing and reduces costs.

Synthesis Of Nanoparticles

Nanoparticles can be synthesized through top-down (physical) and bottom-up (chemical and biological) approaches. In the top-down approach, larger particles are broken down systematically into smaller particles to produce nanoparticles, while the bottom-up approach involves the self-assembly of smaller particles. Biological methods have gained attention due to their eco-friendly nature, low cost, and reduced use of toxic chemicals. Biogenic nanoparticles have excellent adsorption, catalytic activity, and environmental benefits, making them ideal for environmental clean-up. Microorganisms can synthesize nanoparticles either intracellularly or extracellularly, with metal ions reduced and converted into non-toxic nanoparticles through enzymatic processes. The synthesis of biogenic nanoparticles is affected by factors such as temperature, pH, pressure, time, particle size, and composition of metabolites. Standardizing green synthesis of nanoparticles can be influenced by the source of bioactive compounds and the type and quality of enzymes secreted by microbes [19].

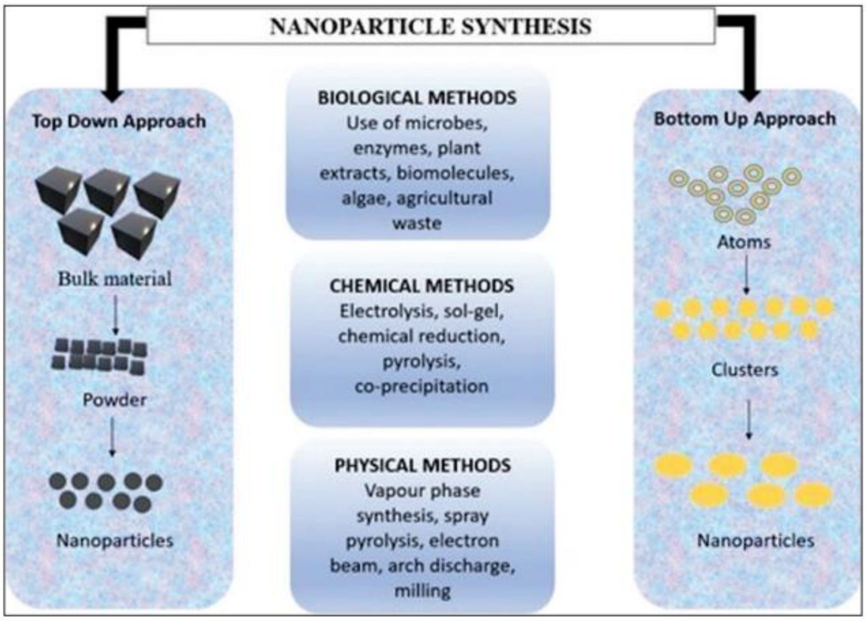

Figure 1: Approaches for nanoparticle Synthesis

Characterization Techniques

Characterization of biogenic nanoparticles is essential to understand their surface area, shape, size, chemical composition, and dispersity. Various techniques are employed for characterization, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier-transform infrared (FTIR) spectrum analysis, X-ray diffraction (XRD), UV–Visible spectrum analysis, energy-dispersive X-ray spectroscopy (EDX), and Brunauer-Emmett Teller (BET). SEM provides high-resolution 3D images for morphological and topographical analysis. UV–Vis spectroscopy quantifies light absorption and scattering, characterizing the optical properties. EDX is used for chemical or elemental analysis. XRD is important for topographic analysis, measuring angles at which X-ray beams are diffracted by crystalline phases. FTIR determines molecular bonds by measuring light absorption between near infrared and far infrared wavelengths. The BET theory helps measure the material's specific surface assimilation of gas molecules, which is crucial for understanding its properties.

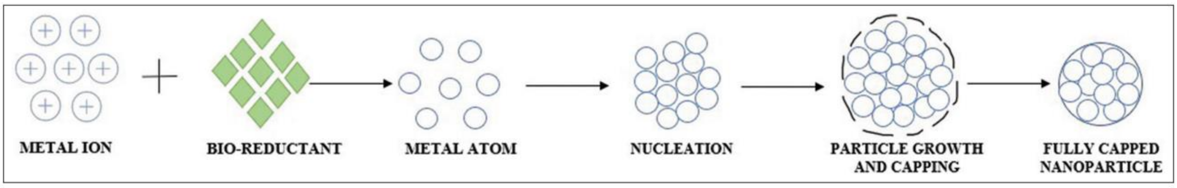

Figure 2. Microbial synthesis of nanoparticle

Nanoparticle Synthesis by Biological Sources

Synthesis of nanoparticles by bacteria

Bacteria possess the ability to bind and concentrate dissolved metal and metalloid ions, converting them into non-toxic nanoparticles. Their diverse and highly adaptable nature makes bacterial synthesis of nanoparticles a promising approach. Bacteria detoxify the immediate cell environment by reducing toxic metal species into metal nanoparticles. During nanoparticle synthesis, bacterial biomolecules act as stabilizing and capping agents. The extracellular synthesis of biogenic nanoparticles is more efficient and easier to extract, allowing for the production of large quantities of pure nanoparticles [20]. Bacteria have been successfully used to synthesize various nanoparticles, such as palladium, titanium, magnetite, gold, and silver, making them suitable for biocatalysis and mineralization purposes. They offer a flexible, cost-effective, and scalable method for large-scale nanoparticle production. Examples of nanoparticles synthesized by bacteria for environmental remediation include biogenic manganese oxide nanoparticles by Pseudomonas putida, silver nanoparticles by

Bacillus cereus, gold nanoparticles by Rhodopseudomonascapsulata, and biogenic selenium nanoparticles by Citrobacter freundii Y9 [21], among others.

Synthesis of nanoparticles by algae

Algae, as hyper-accumulators, have the ability to accumulate heavy metal ions and transform them into more manageable forms through their photoautotrophic, oxygenic, and eukaryotic nature. Algae offer several advantages for nanoparticle synthesis, including their capacity for bioaccumulation of metals, economic viability, ease of handling, and high tolerance. Brown algae, in particular, have cell walls rich in mucilaginous polysaccharides and carbonyl groups that facilitate metal uptake. Algal extracts contain pigments such as chlorophyll, phycobilins, and carotenoids, as well as carbohydrates, minerals, proteins, and antioxidants, which aid in reducing metal ions and stabilizing nanoparticles through capping. In the inorganic nanoparticle synthesis process, the algal extract is mixed with water or organic solvent, boiled for a certain time, and then blended with a metal ion solution while stirring. Nucleation is indicated by a change in color, resulting in the formation of thermodynamically stable nanoparticles with varying shapes and sizes [22]. For instance, the synthesis of silver nanoparticles by Fatima et al. using red algae Portieriahornemannii demonstrated a color change from pink to dark brown. The intracellular synthesis of nanoparticles depends on pathways such as photosynthesis, respiration, and nitrogen fixation, whereas the extracellular synthesis requires pre-treatment, such as washing and blending, and is supported by various metabolites, ions, enzymes, pigments, DNA, RNA, lipids, hormones, and antioxidants. Algae have been used to synthesize nanoparticles for environmental remediation, such as iron nanoparticles by Chlorococcum sp. MM11, gold nanoparticles by Euglena gracilis, and silver nanoparticles by Caulerpa racemosa [23].

Application of Nanotechnology in the Bioremediation of Heavy Metals

Nanotechnology has significantly enhanced the bioremediation of heavy metals, with applications ranging from pollutant control and sensing to the use of nanoparticles for remediation. Notable studies include the formulation of myco-synthesized iron oxide nanoparticles by Chatterjee et al., using Aspergillus niger BSC-1 to successfully remove chromium through adsorption. Keskin et al. developed efficient Lysinibacillus sp. encapsulated nanofibers for the remediation of hexavalent chromium, nickel, and dye [24]. Magnetic iron nanoparticles synthesized using the reducing biomolecule in a living D. radiodurans R1 strain demonstrated exceptional arsenic removal capacity. Plant-based biogenic nanoparticles derived from Aloe vera leaves were highly efficient in adsorption and remediation of arsenic from contaminated water. Additionally, zero-valent silver nanoparticles synthesized by Ficus benjamina leaf extract effectively removed cadmium (Cd^{2+}) [25]. Biogenic iron oxide ferromagnetic nanoparticles functionalized with 3-mercaptopropionic acid proved effective as an adsorbent for nickel removal. Selenium nanoparticles synthesized using bacterium Citrobacter freundii Y9 were successful in removing mercury from groundwater. A nanocomposite of Saccharomyces cerevisiae immobilized on Titania nanopowder, designed by Choudhury et al., demonstrated exceptional effectiveness in removing Cr(VI) from contaminated water [26]. Various other biogenic nanoparticles have been employed in the remediation of heavy metals and metalloids [see Table 2]

Methodologies of Nanobioremediation

Biogenic nanoparticles employ two main mechanisms, adsorption and reduction, to interact with and remediate heavy metals and metalloids. The adsorption mechanism can be classified into physical and chemical adsorption, where physical adsorption relies on permeable structures, and chemical adsorption requires functional groups on the adsorbent's surface for heavy metal remediation through electrostatic attraction or chemical binding forces. Chemical adsorption is considered a more effective remediation method. In simpler terms, heavy metal reduction occurs through two mechanisms: direct reduction by the nanoparticles or first, adsorption onto the nanoparticle surface, followed by reduction to lower valences. Once reduced to less toxic levels, these nanoparticles can be easily biodegraded, increasing the rate of biodegradation [27].

Future Perspective and Challenges

A sustainable and eco-friendly approach to heavy metal removal has emerged through the integration of nanotechnology with biological systems, known as nanobioremediation. This approach offers environmental advantages and addresses various environmental challenges. Nanoparticle technology has a vast potential not only in heavy metal remediation but also in the removal of microplastics, which are highly toxic to marine flora and fauna [28]. The global nanotechnology market is projected to exceed US$125 billion by 2024, and the incorporation of nanotechnologies with biological methods can open new opportunities and boost international trade [12]. However, the commercialization of these technologies remains a challenge, with only about 1% of nanotechnological aspects being commercialized. To make nanobioremediation a game changer at a commercial level, continuous support from researchers and government funding for cost-effective and sustainable production is essential. Additionally, there is a concern about the long-term effects and accumulation of biogenic nanoparticles in the environment and their impact on animals and humans. Nonetheless, it is acknowledged that biogenic nanoparticles have less severe toxic effects compared to chemically synthesized nanoparticles [29].

TABLE 2 Example of nano-bioremediation of heavy metals

Nanoparticle	Biological agent	Class of Bioagent	Contaminant	References
Iron	Chlorococcumsp. MM11	Algae	Hexavalent chromium	[68]
Iron	Deinococcusradiodurans R1	Bacteria	Arsenic	[93]
Silver	Catharanthus roseus	Plant	Chromium and cadmium	[107]
Palladium	Shewanellaloihica PV-4 and Enterococcus faecalis	Bacteria	Hexavalent chromium	[109]
Nano zero valent iron-immobilized calcium alginate beads	Bacillus subtilis, E. coli, and Acinetobacter junii	Bacteria	Hexavalent chromium	[99]
Zinc oxide	E. coli	Bacteria	Lead and Cadmium	[100]
Titanium oxide	Syzygiumcumini	Plant	Lead	[101]
Copper oxide	Extracts of mint leaves (Mentha) and orange peels	Plant	Lead, nickel, and cadmium	[105]
Iron oxide	Enterococcus faecalis	Bacteria	Hexavalent chromium	[106]
Nanoscale zero valent iron	Azadirachta indica and Mentha longifolia	Plant	Nickel and lead	[104]
Filtrate-chitosan	Fusarium solani YMM20	Fungus	Nickel, cadmium, iron, and lead	[108]

II. Conclusion

Over the years, various approaches have been developed to address heavy metal contamination arising from human and industrial activities. Nanobioremediation has emerged as a highly efficient and environmentally friendly solution, revolutionizing the remediation process. Adopting a greener approach has resulted in significant reduction in heavy metal contamination, minimizing toxic effects, and lowering overall cost and remediation time. The mechanism behind microbial synthesis of nanoparticles remains partially understood; however, microorganisms and plants have demonstrated their ability to produce stable nanoparticles with exceptional properties. The use of biomolecules as reducing agents eliminates the need for expensive chemical reductants utilized in physiochemical methods. Biogenic nanoparticles possess a larger surface area, leading to enhanced adsorption capacity. Some biogenic nanoparticles have lipid bilayers that provide stability and improved physiological solubility. Manipulating size and shape can be achieved by adjusting pH, substrate availability, and reaction time. Employing a biological approach for nanoparticle synthesis offers easier implementation, ease of multiplication, uniformity in size, and simple biomass increase. Therefore, the integration of nanotechnology with bioremediation not only enhances efficiency but also provides a safer alternative to conventional techniques.

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