



Bioremediation techniques for heavy metal and metalloid removal from polluted lands: a review

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Abstract

Heavy metal accumulation increases concentrations of toxic contaminants in soil and the environment. They possess momentous warnings to ecosystems throughout the world. Industries and agricultural activities introduce heavy metals/metalloids into the ecosystem which affects agricultural crops and poses various potential health threats to humans and animals by entering the food chain. Therefore, neglecting its deleterious effects on the environment may worsen the current situation. Thus, the need for bioremediation is of paramount importance in the reclamation of polluted soils and lands. The technique involves the use of microorganisms, such as bacteria, fungi, and algae, to lower/remove the toxicity of contaminants in affected regions. It is an environment-friendly, efficient, and cost-effective solution for soil heavy metal and metalloid degradation in both *in situ* and *ex-situ* conditions. Microorganisms can also be genetically modified to be efficient for pollutant degradation. Better alternatives such as nanobioremediation, vermiremediation, and genetically modified microbes have been discussed in detail. These integrated approaches can be more effective than conventional approaches for the restitution of contaminated sites and reducing the toxicity of hazardous compounds. The present review will provide a deep interpretation of the various bioremediation techniques, their merits, and their limitations to the readers. It will also give insights on how these techniques can be implemented as future support to direct the global problem of metal remediation.

Keywords Heavy metal and metalloids · Hazardous compounds · *In situ* or *Ex-situ* bioremediation · Genetically engineered microorganisms (GEMs)

Introduction

The soil is an ecosystem that comprises functions of multiple flora and fauna and promotes living organisms' health by providing food, nutrients, medicines, and immune boosters. However, due to geogenic and anthropogenic activities, there is a substantial increase in soil pollutants. World's Soil Resources report (SWSR) concluded that "soil pollution is the main menace affecting universal soil and environment." Soil pollution is gradually becoming an alarming ambush that contributes to third most important problem in Europe and Eurasia, fourth in North Africa, and fifth in Asia, (FAO and ITPS Report 2015). Different countries, such as China,

India, Pakistan, and Bangladesh, are reported to be severely affected by heavy metals contamination due to effluents discharged from industries, wastewater irrigation, and inappropriate use of chemical pesticides and fertilizers (Bi et al. 2020). In India, heavy metal polluting soil is an emerging constraint for the agricultural sector influencing the country's economy (Kumar et al. 2019). The expanding population, prompt urbanization, and industrialization generate aberrant concentrations of various toxic substances which accumulate in soil and water (Zobrist and Giger 2013; Giudice 2016; Demkova et al. 2019). The natural constituents of soil and water bodies are also altered by menaces produced by the mining of coals and metal ores (Rajput et al. 2017; Barsova et al. 2019; Siddique and Kiani 2020). Global-scale contamination by metals and metalloids has affected plants and animals, most importantly the food chain (Rodríguez-Eugenio et al. 2018). Scientific documentation suggests the role of soil pollution in the degradation of the biosphere and reduction in food security, making it unsafe for consumption by flora and fauna (Kumar et al. 2019; Milicevic et al. 2021).

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Soil pollution can originate new variety of pests and diseases by distressing the equilibrium of ecosystems and give rise to the disappearance of predators or competing species (Jensen and Pedersen 2006; Wang et al. 2020; Borowik et al. 2019; Li 2017; Li et al. 2020). The United Nations Environmental Assembly (UNEA-3) acquires an aspiration for quicker actions to direct and govern soil pollution (<https://sdg.iisd.org/news/unea-3>). This concurrence realized by more than 170 countries shows universal applicability to finding solutions to soil pollution. The absence of novel and effective resources to reduce the challenging task of environmental pollution is a growing and crucial concern all over the world (Ganie et al. 2021). In this review, we describe various potential methods for building an ecosystem that will reduce the toxic effects of metals and metalloids effectively.

Heavy metals are highly emitted contaminants (Rzymiski et al. 2015). Industrial, mining, and anthropogenic activities are the major sources responsible for the widespread dispersal of heavy metals and metalloids in water and soil. The ever-increasing volume of metals and metalloids in the soil is a problem for our sustainable future. Heavy metals, such as Mn, Fe, Co, Ni, are beneficial for living organisms at low concentrations while some heavy metals such as Cd, Pb, and Hg are not essential and are life-threatening even at low concentrations. Thus, the importance of monitoring the concentration of heavy metals and metalloids in the environment becomes a major step toward sustainable remediation (Raffa et al. 2021).

Heavy metals cannot be degraded and conserved in the ecosystem for a very long time. Long-term remediation disobedience causes contamination of sediment and soil and enters the food chain (Ali et al. 2013; Gaur et al. 2014). Heavy metal contamination causes a significant unfavorable impact on human health (Ayangbenro and Babalola 2017; Kumar et al. 2021). Drinking contaminated water and consumption of seafood is the principal source of human exposure to heavy metals, causing high morbidity and mortality worldwide (Jarup 2003; Wasana et al. 2017; Rehman et al. 2018). The antagonistic outcome of it includes the production of reactive species involved in oxidative DNA damage, genotoxicity, mutagenicity, and subsequent health hazards (Genestra 2007; Fu and Xi 2020; Leelapongwattana and Bordeerat 2020). Several diseases like immunodeficiency, osteoporosis, neurodegeneration and multiple organ failures are induced by chronic or acute heavy metal toxicity (Rzymiski et al. 2015). It is also responsible for cancer, mental retardation, cardiovascular diseases, respiratory problems, neurocognitive and behavioral disorders in children (Al Osman et al. 2019; Wang and Shi 2001; Gaur et al. 2014; Kumar et al. 2021). Acute or chronic exposure to females with heavy metals leads to endometriosis, spontaneous abortions, hypotrophy, breast cancer, endometrial cancer, stillbirth, and pre-term deliveries (Rzymiski et al. 2015.) The

adverse effect of heavy metal exposure on the male reproductive system is reported (Wirth and Mijal 2010).

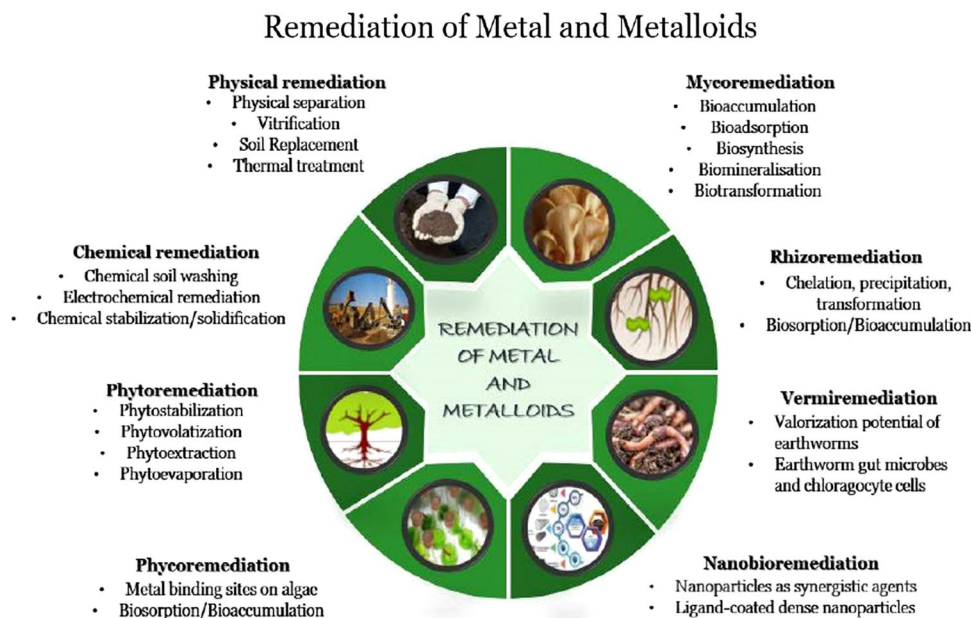
For the persistence of living beings, remediation of these contaminated regions needs attention (Amundson et al. 2015; Kopittke et al. 2019). It is pivotal to evolve a plan of action to combat the heightened problem of heavy metal and metalloid contamination of soil and water that adversely affects flora and fauna (Akhtar and Mannan 2020). Removal of polluted soil is accomplished by science-based remediation methods. To remediate a polluted environment, chemical inactivation or sequestration in the process of the landfill is replaced by microbial degradation or phytoremediation. The microbes or their enzymes transform toxic organic and inorganic compounds into non-toxic or less toxic metabolites (Ndeddy and Babalola 2016; Okoduwa et al. 2017). It is low energy option, cost-effective, environmentally friendly, and a new method to improve and promote the remediation potency (Azubuike et al. 2016; Schenk et al. 2012). To facilitate the prevention of soil quality deterioration and heavy metal pollution, microbial indicators such as microbial abundance, community diversity, structure, and functional activity are monitored. In this paper, we addressed the guideline values for major heavy metals in soil/drinking water, toxicity effects of various metals and metalloids in humans and plants, remediation through physio-chemical and various biological approaches including technology based nanobioremediation, and genetically modified microorganisms specific for heavy metals remediation (Fig. 1).

Sources of metal and metalloids

Major sources of soil pollution are natural and anthropogenic. The low concentration of heavy metals is added into the soil by natural processes ongoing in the earth's crust (Smiljanic et al. 2019). Volcanic explosion, weather beating of soil native material, and mineral dust in the form of aerosols add heavy metal contaminants into the soils innately (Garrett 2000; Artinano et al. 2003; Karl et al. 2007; Ikem et al. 2008). Other sources of heavy metals include disposal of metal waste, gasoline, fertilizers, atmospheric deposition, sewage sludge, animal manures, high tension lines, paper processing plants, microelectronics, wood preservation, wastewater irrigation and spillage of petrochemical (Dhaliwal et al. 2020; Suvarapu and Baek 2017; Mishra 2017). Nuclear tragedy and incomplete combustion of radionuclide metal like lead, thallium, manganese, cadmium, and mercury lead to soil contaminants (Pandit et al. 2020; Shukla et al. 2017; Richards et al. 2020).

Metal corrosion, soil corrosion of metal ions, heavy metal leaching, climatic deposition, and metal evaporation from water resources are other environmental factors for heavy metal pollution (Tchounwou et al. 2012). Another important

Fig. 1 Diagrammatic representation of various remediation techniques involved in removal of metal and metalloids



provenance of soil adulteration is the multiplex combination of bio-waste that includes metals, organic chemicals, and organisms in the effluvium from infected network. The bio-availability of heavy metals is affected by physio-chemical and biological factors. Adsorption, sequestration, temperature, phase association, complexation kinetics, thermodynamic equilibrium, lipid solubility, trophic interactions, and adaptation play a principal role in the soil accumulation of heavy metals (Okodua 2018; Tchounwou et al. 2012; Kapahi and Sachdeva 2019).

Anthropogenic activity responsible for heavy metal and metalloid generation in soil are the use of pesticides, and industrial effluents deposited. They make soil unfertile after a period resulting in economic loss (Kaur et al. 2017; Liu et al. 2020; Yang et al. 2017; Bi et al. 2018; Bidar et al. 2020; Ortiz-Hernandez et al. 2014; Song et al. 2020; Abdo et al. 2020; Fu et al. 2020). Mercury, arsenic and lead are active ingredient of these pesticides (Wallace 2015). Pulp and paper industries wastewater, sludge, municipal solid wastes painting pigments are the main source of Fe, Cd, Pb, Ni, Cr, Cu, Zn, Co, As, Hg, and Cu (Sharma et al. 2021).

Toxicity of heavy metals

The metals are appended and differ considerably above the soil surface. The crucial heavy metals found in the soil are manganese (Mn), arsenic (As), aluminum (Al), zinc (Zn), nickel (Ni), vanadium (V), cadmium (Cd), iron (Fe), cobalt (Co), gold (Au), mercury (Hg), and chromium (Cr). Metals cannot be degraded or destroyed, and after ingestion via food, drinking water, and air, they get accumulated in living cells and lead to toxicity (Wuana and Okieimen 2011). Like amino acids, heavy metals are also grouped into essential

and non-essential metals. The essential heavy metals act as a micronutrient and take part in several biochemical and physiological functions in the living system. It includes copper (Cu), nickel (Ni), iron (Fe), molybdenum (Mo) and zinc (Zn), etc. Insufficient supply of these micronutrients leads to a variety of deficiency diseases (WHO 1996). Essential heavy metals act as coenzymes and play an important role in the oxidation–reduction reaction of several biomolecules (Tchounwou et al. 2012; Garrett 2000). Ions of heavy metals bind to sulfur-containing amino acids strongly and bio-accumulate within cells, allowing them to reach high localized concentrations (Jia et al. 2012). Metal ions have been reported to interact with the cell membrane, DNA, and nuclear proteins inducing DNA damage, and various conformational changes that may cause cell cycle alteration, carcinogenesis, or apoptosis (Gjorgieva et al. 2013). Heavy metals such as silver, lead, aluminum, cadmium, gold, and mercury that have no biotic character and are hazardous to living organisms are known as non-essential heavy metals (Rahman and Singh 2019; Kumar and Sharma 2019). Some of the potential heavy metals contaminants levels in drinking water are discussed below.

Human exposure to toxic heavy metals denotes serious health issues in the body and can be responsible for death in accidental exposure (Rahman and Singh 2019). Arsenic toxicity is a global health problem affecting many millions of people. It is a persistent bioaccumulative carcinogen existing as arsenite or arsenate, disrupting enzymatic functions of cells, by interfering with phosphate uptake and utilization. The major pathway of As metabolism is oxidative methylation and glutathione conjugation (Sattar et al. 2016). As can also be actively sequestered in plant and animal tissues (Kaur et al. 2011; Nurchi et al. 2020). The current



recommended dose of arsenic in drinking water and soil is 10 µg/L and < 50 µg/L, respectively (<https://www.who.int/news-room/fact-sheets/detail/arsenic>; Ravenscroft et al. 2005). Rhizospheric bacteria generally play a defensive role against As, toxicity in the rhizosphere. They affect As level and burden through detoxification or mobilization into crop plants (Mohd et al. 2019). This phenomenon is of environmental significance, for the survival of the healthy living organism in an As-contaminated rhizospheric environment (Mohd et al. 2019). In the process of remediation, microorganisms are involved in the cycling of toxic heavy metals and the remediation of metal-contaminated sites. Microbes show resistance to As exposure, through the expression of arsenic resistance ars operon (Kaur et al. 2011).

Mercury (Hg) exposure is the second-most common cause of toxic metal poisoning (Patrick 2002). The maximum contaminant level of Hg is 2 µg/L set by the US Environmental Protection Agency for drinking water (Ware 1989). Hg is present in the environment in various forms like methylmercury in aquatic species, mercury vapors in dental amalgams, or ethylmercury in various immunogenic vaccines. Human exposure to Hg is mostly chronic and results from fish consumption or dental amalgam (Bernhoft 2012). There is a need to develop more sensitive and refined tools to monitor the effects and susceptibility to adverse metal-mediated health risks.

Cadmium (Cd) exposure mostly damages the microbial life in soil (Xiao 2020). It is a non-essential environmental toxicant that shows toxicity at a low concentration. The USEPA has established a maximum contaminant level (MCL) of 0.005 mg per liter (mg/L) for cadmium in drinking water (Cadmium 2015). Studies reported that environmental and occupational cadmium exposure is responsible for various types of cancer like kidney, breast, pancreas, lung, nasopharynx, and prostate (Genchi et al. 2020b).

Antimony (Sb) is commonly found in sulfide mineral forms such as stibnite (Sb_2S_3) (Park et al. 2020). Due to its potential to cause carcinogenicity in humans, it is listed in the pollutant category by USEPA and European Union (Bolan et al. 2022). The guideline value set by USEPA and WHO for antimony in drinking water is 6 ppb and 20 ppb, respectively (Nishad et al. 2017). Exposure and direct ingestion of the metal can lead to respiratory irritation, osteoporosis, pneumoconiosis, skin spots, and symptoms of gastrointestinal problems (Romero-Freire et al 2017; Liu et al. 2018; Zhong et al. 2020).

Chromium (Cr) is a toxic environmental pollutant responsible for degrading rhizobia, legumes, and their symbiosis. Cr occurs in different oxidation states of which trivalent Cr(III) and hexavalent Cr(VI) are the most stable. Cr(III) is an essential oligo-element, and Cr(VI) is mutagenic and carcinogenic, has DNA-damaging effects, and is involved in several allergic reactions (Joutey et al. 2015). The EPA has

established a drinking water standard of 100 parts per billion for all forms of chromium. The toxic effect of Cr (VI) is due to inhibition of pigment synthesis, increased production of ROS, and modification of various cellular components (Stambulska et al. 2018). It enters the human body through the food chain and affects human health (Tang et al. 2021).

Lead (Pb) is a highly toxic metal in aquatic environments and gets accumulated in fish tissues causing oxidative stress and inducing synaptic damage, neurotransmitter malfunctioning leading to neurotoxicity in fish (Lee et al. 2019). The Guideline for EPA's maximum acceptable concentration for lead is 0.01 mg/L (EPA-hse-drinking-water-consumer-advice-note-lead.pdf). Occupational exposure is the main cause of lead poisoning (Fouad et al. 2020). Lead speciation is influenced by soil organic matter, colloids, pH, clay minerals, and iron oxides (Kushwaha et al. 2018).

Nickel (Ni) is a transition element. Its role as a trace element in humans and animals has not yet been fully discovered. It can occur in mono-, bi-, trivalent forms in living organisms. The highest acceptable nickel concentration is 0.1 mg/L, which puts it in the group of the most toxic metals. Various forms of nickel are introduced into the environment through sulfidic and lateritic nickel ores. Industrial, municipal effluents, solid, and liquid fuels wastes contain various concentrations of Ni (Pruett et al. 2020; Genchi et al. 2020a). In healthy human tissue, the concentrations of Ni range from 0.04 to 2.8 mg/g on a dry basis (Solomons et al. 1982).

Zinc (Zn) is an important trace element that has the potential to regulate cellular metabolism, particularly in the immune system and other physiological functions such as absorption, excretion, and homeostasis (Roohani et al. 2013). In many cases, it retards the biochemical reactions leading to oxidative stress (Rasmussen et al. 2010). In comparison with other metal ions, zinc is less toxic (Plum et al. 2010). In humans, the total zinc content in the body has been estimated to be 30 mmol (Hambidge 1987). However, a high concentration of zinc in humans can lead to anemia and reduce enzyme activities and absorption of copper and iron (WHO 553 2009). Zinc-contaminated agricultural land has been recorded to have reduced bacterial diversity (Genova et al. 2022).

Copper (Cu) is an essential trace element and plays an important role in iron consumption, production of RBC, metabolism of cholesterol, and glucose in humans (Attar 2020). In the environment, it is released from wastewater of industries that contaminates agricultural products and aquatic bodies.

Selenium (Se) is yet another element that is beneficial for plants and the normal functioning of the body. But due to the increase in activities of mining, emission of harmful wastes, and agricultural practices, its accumulation is increasing in crops threatening human health indirectly (Fu et al. 2021).

The guideline value of selenium in drinking water suggested by WHO is 40 µg/l. (WHO 2011). Acute and chronic selenosis may occur depending upon the dose and time of selenium consumption. Genotoxicity, embryotoxicity, cytotoxicity, immunotoxicity, and reproductive toxicity may be induced due to its excessive concentration (Lv et al. 2021).

Aluminum (Al) is a potentially toxic metal when exposed or ingested beyond its acceptable limits. It can cause bone disease, microcytic anemia, and neurological problems (Oleti et al. 2014; Klotz et al. 2017) In 2011, the WHO declared the guideline limit of 900 µg/L of health-based drinking water. But this guideline was not applied because the expected concentration of aluminum was exceeded in drinking water even after treatment with aluminum-based coagulants (WHO 2011).

Remediation of metals and metalloids

Previous studies reported that eradication of metal and metalloids is not fully possible, but they can be effectively converted or neutralized into a low toxic form that reduces their detrimental effect on the environment (Pratish et al. 2018). Enzyme machinery inside the microbial system plays a significant role in transforming heavy metals. Biodegradation of other environmental pollutants is inhibited by heavy metals by inhibiting the function of metabolizing enzymes (Schroder et al. 2009; Wuana and Okieimen 2011). The approach for heavy metal remediation from the soil includes physical, chemical, and biological.

Physio-chemical approach

Physical remediation includes soil flushing, soil washing with various acids, and mixing contaminated and uncontaminated soil (Lim et al. 2014). Soil washing with varying concentrations of chemicals such as nitric acid, sulfuric acid, hydrogen bromide, and phosphoric acid is an effective way of metal removal from the soil (Mahimairaja et al. 2005). Because of the high cost of chemicals, soil washing can be used for only small-scale processes. Mixing leads to an acceptable level of metal in the contaminated soil (Mahimairaja et al. 2005). The chemical remediation operations involve immobilization, adsorption process with the use of specific media, modified coagulation along with filtration, precipitations, and complexation reactions (Fan et al. 2016; Guerra et al. 2018; Fu et al. 2021). Chemical remediation is famous because of its great success rate but is found to be expensive when used for the remediation of a large area (Mahimairaja et al. 2005). The combination of physio-chemical remediation techniques includes soil washing, stabilization, solidification, and thermal treatment to control soil contamination (Xu et al. 2015). Thermal desorption, electrokinetic, soil flushing, and washing treatments mobilize

and capture insoluble metal species in the contaminated regions. Solidification, stabilization, and vitrification processes immobilize various metals by converting them into less soluble forms (He et al. 2015). Physicochemical remediation is applicable for moderate-to-high/severe contaminated soil in both in situ and ex-situ conditions. The requirement of human power for the physicochemical method may vary depending upon the type of contaminants and the choice of the method adopted for their removal. A study on the efficacy of soil washing for the removal of heavy metals and soil quality revealed it as a two-sided coin where washing that removed Pb and Cd in soil, also reduced soil quality. Neutralization of soil has alleviated the adverse effects induced by soil washing (Wang et al. 2020).

Biological remediation

There is an imperative need for green approaches to explore efficient and cost-effective methods in the eradication of heavy metals from the polluted site. One such eco-friendly, cheaper, solar-driven, novel and effective alternative to abolishing heavy metals is biological remediation or bioremediation (Mahimairaja et al. 2005; Rudakiya and Patel 2021). It is a technique that includes the recruitment of biological organisms like microorganisms, green plants, biochar, genetic engineered microbes, or enzymes for the eradication of heavy metals, organic and inorganic contaminants present in soil or water (Azubuike et al. 2016; Verma and Shrama 2017; Kalogerakis et al. 2017; Ye et al. 2017). The recruited biological organisms can be unicellular or multicellular. Over the years, bioremediation of hazardous metals has gained much more interest. Based on site, its bioremediation can be divided into intrinsic or engineered bioremediation (Mahimairaja et al. 2005). Intrinsic bioremediation involves the degradation of heavy metals by naturally occurring microorganisms at the native site with no interference from humans. This process is appropriate for the remediation of soil having less concentration of toxic compounds (Mahimairaja et al. 2005). In engineered bioremediation techniques, human intervention is required for optimizing the environmental conditions to accelerate the proliferation and activity of microorganisms in a particular area. In the highly contaminated region, engineered bioremediation method is more suitable (Mahimairaja et al. 2005). Although bioremediation has many limitations, these approaches have gained interest due to their cost-effectiveness in the removal of heavy metals and metalloids. Some important characteristics of physicochemical and biological remediation are compared in Table 1.

The association of biological action of invertebrates, plants, and microorganisms in the soil has a great potential of creating new and low toxic metabolites. It depends on the use of pollutants as derivatives of energy and food and their



Table 1 Comparison of characteristics of physicochemical and bioremediation techniques

S.No	Characteristics	Physicochemical remediation	Bioremediation
1	Nature	Physical and Chemical Based	Natural Process
2	Site of Treatment	May be <i>in situ</i> or ex-situ	Mostly in situ
3	Sensitivity toward Toxicity	Not sensitive	Highly sensitive
4	Removal of Toxicant	Particular toxicant will be removed	Complete destruction of contaminant
5	Cost	Highly Costly	Cost effective
6	Instrument requirement	Tedious instruments and chemicals are required	Instruments not required
7	Time required	Less time taking	Tedious/Time taking
8	Technological need	Highly advanced technique required	Techniques involved in construction of Genetically Engineered Microorganism
9	Specificity	Specific	Highly Specific
10	Regulation Requirement	Required	Not Required except GEM
11	Action Against Compounds	Selected compounds will be degraded	All biodegradable compounds can be degraded
12	Scale up	Can be easily scaled up	Tough to scale up remediation process
13	Effect of soil environment (pH, temperature, water, oxygen, nutrients, microbial population)	Not affected	Highly affected
14	Control	Highly controlled environment	Less control over the process

transformation into water and carbon dioxide without the generation of secondary metabolites in the ecosystem. The mechanisms involved in heavy metal degradation include immobilization, photocatalytic degradation, Fenton-like oxidation, reduction reaction, and collective processes (Qian et al. 2020b). Tang et al. 2019 have summarized the role of microbiological indices to monitor soil pollution caused by heavy metals. They have used microbiological indicators such as microbe's abundance, diversity, structure, and functional activity to observe the microbial reaction to stress induced by heavy metals in soil. They have found an increased number of heavy metals in the soil, which promotes the growth of plant growth-promoting bacteria

(PGPB) in the gut of earthworms that may alleviate the phytoremediation of heavy metals (Liu et al. 2020a). Based on the biological organism involved in the remediation of heavy metals from the land, it can be of the following types. The processes involved in the bioremediation of various metals and metalloids from contaminated sites are summarized in Table 2.

Phytoremediation

Phytoremediation is an eco-friendly alternative technique that requires low capital cost in removal of metal and metalloids (Haq et al. 2020). It is an environmental clean-up

Table 2 Bioremediation processes used for removal of various soil contaminants

Soil Pollutants	Sources	Bioremediation processes	Principle of degradation	References	
Inorganic Pollutants	Heavy metals	Natural and anthropogenic	Biosorption, Biostimulation, Biotransformation, Bioaccumulation, Bioprecipitation, Biofilm, Bioleaching	Enzymatic	Alloway 2013; Igiri et al. 2018; Kapahi and Sachdeva 2019; Dhaliwal et al. 2020; Qin et al. 2021. Choudhury and Chatterjee 2022
	Metalloids	Mostly anthropogenic	Biodegradation, phytoremediation, bioventing, bioleaching	Enzymatic, solubilization, stabilization, precipitation, etc.	Raffa et al. 2021; Nguyen et al. 2021
	Radionuclids	Natural and anthropogenic	Biosorption, phytoremediation	Solubilization, precipitation, stabilization, etc.	Yan et al. 2021



process for pollutant remediation using plants and associated microorganisms (Nedjimi 2021). Plants can spread their roots more than 100 million miles per acre, which enhances their potential for the removal of toxic compounds in natural environments. It has the potential versatility to eradicate a wide variety of hazardous pollutants. It is a non-invasive, sustainable, environment-friendly eco-technology that can be easily implemented to soil pollutants for large-scale clean-up and eco-restoration of polluted sites. The plants that bring out phytoremediation of metals are referred to as metallophytes. Metallophytes are of three types; metal indicators, metal excluders, and metal hyperaccumulators (Awa et al. 2020). Metal indicators are plants that accumulate high concentrations of heavy metals in plant parts such as shoots and leaves from soils. The plant later dies due to metal phytotoxicity (Ahmad et al. 2019; Awa et al. 2020). Plants that collect the absorbed heavy metals in their root and terminate the translocation to their aboveground shoots and leaves are known as metal excluders (Masarovicova et al. 2010). Metal hyperaccumulators are plants that accumulate heavy metals in high concentrations, inside their tissues without being affected or showing signs of metal phytotoxicity (Shrivastava et al. 2019). Based on the mechanisms for removal of contaminants, phytoremediation techniques can be categorized into phytoaccumulation, phytostabilization, phytoextraction, phytodegradation, phytofiltration, phytovolatilization, and hydraulic control (Muthusaravanan et al. 2018; Yadav et al. 2018). Phytostabilization and phytoextraction are frequently used remediation techniques that can be differentiated in the accumulation of metals or heavy metals in the root portion of the plant or above the ground, respectively (Ghosh and Singh 2005). High contamination affected sites inhibit the growth of plants, including roots through oxidative stress, and limit the speed of phytoremediation in *situ* conditions (Jagat et al. 2020). Microbe assisted-phytoremediation enhances phytoremediation efficacy by producing growth hormones, siderophores, secondary metabolites, and developing an effective antioxidant system (Sharma 2021).

The literature works suggest that phytoremediation causes the successful eradication of toxic heavy metals from the soil with time (Ayan et al. 2020). Heavy metal accumulating plant, *Eleocharis acicularis*, has the potential to phytoremediate copper-contaminated soil sediments naturally (Sakakibara et al. 2011). A wetland plant *Cyperus alternifolius* showed high metal accumulation ability for the uptake of Pb, Zn, Cd, from the rhizospheric region and root in flooded conditions of metal-contaminated wetlands (Yang et al. 2017). It has been found that three types of grass namely Elephant grass, Rhodes, and Vetiver showed metal uptake efficiency of Mn, Cu, and Zn under the hydroponic condition in municipal solid waste generated leachate (Hassan et al. 2020). Water hyacinth, *Eichhornia crassipes*, effectively lowers the potential environmental risk by phytoremediation

of untreated wastewater polluted with cadmium, arsenic, and mercury (Nazir et al. 2020). Phytovolatilization of arsenic has been studied in plant *Arundo donax* (Guarino et al. 2020). The energy crop of *Miscanthus giganteus* indicates good phytostabilization efficiency for Hg and Cd (Zgorelec et al. 2020). The root and shoot of *Ricinus communis* have potential of phytoextraction of metals iron, copper, zinc, manganese, nickel, and lead which indicates that it has high hyperaccumulation and translocation efficiencies of metals and heavy metals (Kumar et al. 2021). Phytofiltration of heavy metals such as iron, copper, nickel, zinc, and manganese from wastewater has been shown by *Monosoleum tenerum* (Sut-Lohmann et al. 2020).

The need for a longer duration time in remediation because of the slow growth of plants is the main demerit of the phytoremediation strategy. It is also limited by the change in climate and characteristics of the soil. The contaminants may enter the environment again by litter effects and exudates of roots increasing the solubility of contaminants to enhance their rate of distribution in soil (Jacoby et al. 2017).

Phycoremediation

The algae are widely used for clean-up strategies of wastewater, resulting in treated waters as well as the formation of useful algal biomass which serves as feedstock for a range of valuable products, such as food, fodder, biofertilizer, pharmaceuticals, and biofuel (Phang et al. 2015). Phycoremediation involves the participation of various algae and cyanobacteria effective for heavy metal remediation by either removal or deterioration of contaminants (Chabukdhara et al. 2017). Metal-binding sites are present on algae that are composed of carboxyl, hydroxyl, phosphate, and amide chemical moieties. Algae are autotrophic in nature, need low nutrients, and produce a large amount of biomass in comparison with other microbial biosorbents. These algal biosorbents have been used in the treatment of heavy-metal-contaminated effluent through adsorption or by integration into cells (Abbas et al. 2014). Microalgae growth determines the duration of the phycoremediation process. Phycoremediation by *Spirulina* sp. removed 88 to 98% of Ca^{2+} and Cd^{2+} from municipal wastewater and aqueous solution (Anastopoulos and Kyzas 2015; Al-Homaidan et al. 2015). Goher et al. 2016 used *Chlorella vulgaris* dead cells to eliminate Cd^{2+} , Cu^{2+} , and Pb^{2+} ions in an aqueous solution under conditions of pH, biosorbent dosage, and contact time. Cultivation of freshwater algae (*Cladophora glomerata*, *Oedogonium westii*, *Vaucheria debaryana* and *Zygnema insigne*) in industrial wastewater lowers the biological oxygen demand, chemical oxygen demand, electrical conductivity, total dissolved solids, and nitrate level. *C. glomerata* showed a high accumulation of Cd about 80.3%, and *O. westii* showed high



removal capability of Ni about 66.3% (Khan et al. 2017). Studies on heavy metal biosorption from the biomass of seaweeds (*Chaetomorpha* sp., *Polysiphonia* sp., *Ulva* sp., and *Cystoseira* sp.) have shown the effective removal of Zn in aqueous solution (Deniz and Karabulut 2017). Genetic modification leads to overexpression of metal tolerance protein performed in *Chlamydomonas reinhardtii*, which showed a two-to-threefold increase in tolerance of Cd²⁺ and uptake, respectively, in comparison with its wild type (Ibuot et al. 2017). Studies by Apandi et al. (2019) showed more than 90% phycoremediation potential of *Scenedesmus* sp. to exclude Cd, Cr, Fe, and Zn from wet market wastewater. Microalgae *S. obliquus* has phycoremediation potential and high tolerance against lead (Pb²⁺). The cells undergo oxidative stress in presence of Pb, which is defended by the antioxidant system through enzymatic and non-enzymatic mechanisms (Danouche et al. 2020). The mechanisms utilized in the removal of heavy metal ions by microalgae are biosorption and bioaccumulation (Salama et al. 2019). Green algae, *Botryococcus brurauni*, significantly reduced the level of Pb, Cd, and Cu from wastewater effluent (Uddin and Lall 2019). Biosorption is a physio-chemical process in which contaminants or hazardous elements are removed by the cellular structures or surfaces through passive adsorption (Mohita et al. 2020). *Chlorella* and *Scenedesmus* sp. are found to decrease the level of heavy metals such as copper, sulfate, nickel, lead, and zinc in pharmaceutical effluent (Pratibha et al. 2020). Algae *Cladophora fracta* has shown effective phycoremediation of silver (Ag) and gold (Au) from mine gallery water (Murat et al. 2020). The transgenic strain of

Chlorella sp. DT. modified with mercuric reductase (MerA) obtained from *Bacillus megaterium* B1 showed improved two-fold Hg²⁺ removal ability, in comparison with control and low level of oxidative stress (Leong and Chang 2020). Sangeetha et al. 2020 studied the phycoremediation efficacy of *Spirogyra* sp. using sugar plant cushion and showed that it can exclude metals such as Ni, Cr, Fe, and Mn. *Nostoc commune*, *Oscillatoria limosa* and *Chlorella vulgaris* were studied for phycoremediation potential, demonstrating up to 90% removal of lead in contaminated industrial wastewater (Atoku et al. 2021). Diatoms *Pseudostaurosira brevistriata* and *Staurosira construens* were found effective in phycoremediation of silver (Ag). The rate of photosynthesis of these diatoms affects the adsorption of silver (Asiandu and Wahyudi 2021). Development, screening, and identification of macro- and microalgae that have special attributes can be efficient for nutrient removal algal systems. Phycoremediation of heavy metals /metalloids are summarized in Table 3.

Rhizoremediation

Rhizoremediation is an *in situ* phyto-restoration system that involves plant and related rhizospheric microbes in the breakdown of pollutants. Rhizoremediation strategy requires the microorganisms which are capable to divide and proliferate in the root system (necessary for enhancing the catalytic potential) and operative catabolic pathways responsible for remediation of the pollutants (Segura et al. 2009). The bacteria residing in the rhizosphere may convert some metals or metalloids (Hg, Te, Tn, Se, As, Pb, Sb) into volatile form,

Table 3 Algae used in heavy metal /metalloids remediation at contaminated site

S.No	Algae	Remediation of heavy metals/metalloids	References
1	<i>Spirulina</i> sp.	Cu ²⁺ and Cd ²⁺	Anastopoulos and Kyzas 2015; Al-Homaidan et al. 2015
2	<i>Chlorella vulgaris</i> dead cells	Cd ²⁺ , Cu ²⁺ , and Pb ²⁺ ions	Goher et al. 2016
3	<i>Cladophora glomerata</i>	Bioaccumulation of Cd (80.3%)	Khan et al. 2017
4	<i>Oedogonium westii</i>	Ni removal capacity about 66.3%	Khan et al. 2017
5	<i>Chaetomorpha</i> sp., <i>Polysiphonia</i> sp., <i>Ulva</i> sp. and <i>Cystoseira</i> sp.	Biosorption of Zn	Deniz and Karabulut 2017
6	<i>Scenedesmus</i> sp.	Ability to exclude Cd, Cr, Fe, and Zn	Apandi et al. 2019
7	<i>Botryococcus brurauni</i> ,	Pb, Cd and Cu	Uddin and Lall 2019
8	<i>S. obliquus</i>	Pb ²⁺	Danouche et al. 2020
9	<i>Chlorella</i> and <i>Scenedesmus</i> sp	Cu, SO ₄ ²⁻ , Ni, Zn and Pb	Pratibha et al. 2020
10	<i>Cladophora fracta</i>	Ag and Au	Murat et al. 2020
11	<i>Nostoc commune</i> , <i>Oscillatoria limosa</i> and <i>Chlorella vulgaris</i>	90% removal of Pb	Atoku et al. 2021
12	<i>Geotrichum</i> sp.	Cu, zn, Ni	He et al. 2022
13	<i>Spirogyra</i> sp. <i>Cladophoras</i> p.	Cd(II), Cu(II)	Mane and Bhosle 2012
14	<i>Spirulina</i> sp.	Cr, Cu, Fe, Mn, Zn	Mane and Bhosle 2012
15	<i>Hydrodictyon</i> , <i>Oedogonium</i> , <i>Rhizoclonium</i>	As	Kaur et al. 2019; Christobel and Lipton 2015
16	<i>Oedogonium</i> spp., <i>Chlorella</i> spp., <i>Scenedesmus</i>	Hg	Quiroga-Flores et al. 2021



through a detoxification mechanism. The bioavailability and toxicity of many metalloids are influenced through microbial oxidation, reduction, and methylation process. Some microbes have the ability to antimonate Sb (V) as a terminal electron acceptor and convert it into antimonate Sb (III) during anaerobic respiration (Alloway 2013). During this process, the root of plants secretes the exudates which either enhance or stimulate the growth and activity of microorganisms in the rhizosphere, resulting in effective pollutant degradation (Saravanan et al. 2019). The interaction is mutually beneficial for plants and microorganisms; plants provide necessary nutrients to the microbe, and in turn, microbes prevent the pathogenic attack (Glick 2014). The action of microorganisms on heavy metals is accomplished through chelation, precipitation, transformation (oxidation–reduction, methylation), biosorption, and accumulation (Verma and Rawat 2021). The rhizoremediation process is influenced by various physicochemical and biological factors such as temperature, pH, soil conditions, microbial communities, aeration, the content of organic matter, exudation rate, age of the plant, and nutritional requirements, and contaminants contents (Oberai and Khanna 2018). The significant advantage of rhizoremediation over phytoremediation is the higher rate of contaminant degradation through assistance received by microbes (Sathishkumar et al. 2008). A rhizobacterium, *Pseudomonas* sp. CPSB21, has also been found to be involved in rhizoremediation of *Helianthus annuus* in chromium (Cr)-contaminated soil (Gupta et al. 2018). *Methylobacterium oryzae* strain CBMB20 and *Burkholderia* sp. strain CBMB40 have been reported to decrease the toxicant level of nickel and cadmium in tomato by decreasing the uptake and transportation of heavy metals from root to stem and fostering plant growth in metal-contaminated soil (Madhaiyan et al. 2007). A study done found that copper-resistant *Pantoea dispersa* strains increase the bioaccumulation of copper ions in *Sphaeranthus indicus* with increasing concentration of copper through the rhizoremediation process (Yaashikaa et al. 2020). Filamentous fungal strain *Purpureocillium* sp., found in wheat farmland soil, showed Cd-resistant nature. Rhizo-inoculation of the fungus showed a decrease in Cd stress to wheat, by a significant reduction in Cd concentration of wheat seedlings (Zheng et al. 2021).

Mycoremediation

Mycoremediation is a profitable, environment-friendly, and reliable method for remediation of environmental pollutants with the use of fungi or its derivatives. It is an advantageous method to other conventional bioremediation methods. The soil fungi show adaptation to various adverse environments making them play an effective role in the degradation of various pollutants such as PAHs, pharmaceutical wastes, and agricultural and heavy metals. Fungal accumulation

capacity for various heavy metals can be potentially utilized for their removal from contaminated sites. The importance of fungi in environmental remediation is due to their ability to biotransform xenobiotics and accumulate heavy metals. The mycoremediation process is controlled by soil type, pH, temperature, organic matter content, concentration and nature of heavy metals, dose, and characteristics of fungal biomass. The composition, concentration of impurities, and age of mycelium affect the remediation process. The content of mushroom fruiting bodies is correlated with the emission of pollutants (Muszynska et al. 2017). Metallotolerant fungi have been utilized for bioleaching of heavy metals from industrially contaminated soil (Khan et al. 2019).

The biomechanisms involved in the removal of heavy metals by fungi include bioaccumulation, biosorption, biosynthesis, biomineralization, bio-reduction, bio-oxidation, extracellular and intracellular precipitation, surface sorption, etc. (Javanbakht et al. 2014; Xu et al. 2020). C-S stretching, C-N stretching, C=O, C=N, C-H, and N-H bending are the important functional groups entangled in the mycoremediation of metals. The bioaccumulation of heavy metals is influenced by the species, ecological types, morphological traits, parts, the lifetime of mycelium and fruiting bodies, the intervals between fructifications, the ecological environments, and bioaccumulation characteristics and genetic potentials of macrofungi (An and Zhou 2007). The mushroom, *Galerina vittiformis*, has the potential to uptake heavy metals, from soil artificially polluted with Cd (II), Cu (II), Cr (VI), Pb (II), and Zn (II). It has been observed that within 30 days, *G. vittiformis* effectively removes added metals from soil (Damodaran et al. 2014). It has also been possible that a single fungus can effectively remediate multiple heavy metals. An entomopathogenic fungi *Beauveria bassiana* has the capacity to remove various heavy metals individually or in multiple (Gola et al. 2016). Indigenous metallotolerant fungal strains of *Aspergillus* from the Pb and Hg contaminated soil samples can be utilized in *in situ* and *ex-situ* remediation of soils polluted with Pb and Hg. The semi-solid culture system can provide valuable information for the remediation of heavy metal-polluted soil by fungi. Another strain *A. fumigatus* has the potential to eradicate some Cd from the contaminated soil in the semi-solid culture system. (Chen et al. 2017).

In psycho-remediated soil, treatment of *H. annuus* with wood rot fungi *Trichoderma* shows an effective increase in the soil's extracellular enzyme activities. The activities of biochemical enzymes like invertase, cellulase, amylase, dehydrogenase, urease, and phosphatase significantly increased in arsenic and lead-affected soil. *Trichoderma* sp. can be an effective candidate for the remediation of arsenic and leads in polluted soil (Govarthanan et al. 2018). Another study on fungi-assisted phytoextraction of Pb explored the potential of five non-pathogenic fungal strains namely



Trichoderma harzianum, *Penicillium simplicissimum*, *Aspergillus flavus*, *Aspergillus niger*, and *Mucor* sp., to promote phytoextraction of lead by enhancing Pb phytoavailability in soil. It also improves plant biomass production (Manzoor et al. 2019). In another experimental study, it is observed that *A. niger* is the most successful strain in removing heavy metals from the soil with the highest bioaccumulation efficiency for cadmium and chromium. It indicates that new strategies could be developed for the remediation of soil polluted with heavy metals (Cd and Cr) through *in situ* or *ex-situ* mycoremediation (Khan et al. 2019). A study by Lin et al. (2020) revealed that some fungi have a strong capability to decrease the toxicity of heavy metals by biodegradation and create a proper soil environment to help to grow food crops. Fungal cellular sulfur and phosphorus compounds are also involved in Ag(I) binding (El-Sayed 2020). The secretion of organic acids from filamentous fungi is responsible for the dissolving of metals and is able to tolerate a high number of heavy metals (Dusengemungu et al. 2021).

Vermiremediation

In addition to improving soil fertility and crop production, earthworms can resist high concentrations of soil pollutants and play a vital role in their removal effectively (Dada et al. 2021). Vermiremediation is the use of earthworms to remove contaminants from soil or help to degrade non-recyclable chemicals. It is proved to be an alternative, very effective, low-cost technology for treating heavy-metal-contaminated sites (Zeb et al. 2020). The eco-biotechnology employs earthworms as natural bioreactors for decomposing organic matter that may be used for remediation of pollutants present in various organic matter sources. The valorization potential of earthworms and their ability to detoxify heavy metals in industrial wastes is because of their strong metabolic system and involvement of earthworm gut microbes and chloragocyte cells (Bhat et al. 2018). The addition of earthworms in sewage sludge composting enhances the degradation of polycyclic aromatic hydrocarbons and heavy metals (Rorat et al. 2016). The study has shown that earthworm *Eudrilus eugeniae* could be applied as possible bioremediation of heavy metals and petroleum hydrocarbons in diesel contaminated soil (Ekperusi and Aigbodion 2015). It is demonstrated that the addition of another earthworms *Eisenia fetida*, in various sludge wastes generated from industries, increases the degradation rate of heavy metals and hydrocarbons found in it (Suthar et al. 2014; Martinkosky et al. 2016; Kavehei et al. 2018). It decreases the bioavailability of Cd in soil and simultaneously improves soil fertility (Cheng et al. 2021). *Metaphire posthuma* was used in the vermiremediation of toxic jute mill waste (Das et al. 2015). A combination of earthworms, with biochar or *Bacillus megatherium*, has

been found as an alternative method for Cd-contaminated soil remediation (Xiao et al. 2021).

Nanobioremediation

It is very fragile to develop a feasible remediation method that does not disturb the ecosystem and ensures a safe and healthy environment. Microbes have various mechanisms of metal sequestration that hold greater metal biosorption capacities (Ojuederie and Babalola 2017). Nanotechnology includes the use of engineered nanomaterial to create innovative approaches for soil remediation. The microbial biosorbents are eco-friendly and cost-effective, providing an efficient alternative remediation pathway for the removal of heavy metals and metalloids from contaminated environments. The goal of microbial biosorption is to eliminate metals and metalloids from the contaminated site, using living or dead biomass or their components. It is a new safe and sustainable technique for persistent organic compounds and heavy metals remediation (Ganie et al. 2021). It is possible because nanomaterials have a high surface area to volume ratio, and unique features of high reactivity, selectivity, and versatility (Guerra et al. 2018). It assures quick and efficient performance with minimized adverse effects on the ecological system (Esposito et al. 2021). The combination of nanotechnology with phytoremediation and micro-remediation becomes very popular and effective in remediation (Xue et al. 2018). The conjugate nanoparticles have a bio-interface strategy, hierarchical architecture, water-dispersibility, long-term colloidal stability in environmental media, and non-specific toxicity (Basak et al. 2020). Rhizospheric fungal isolates along with silver nanoparticles (AgNPs) show degradation of crude oil (Al-Zaban et al. 2020). White rot fungi (WRF) along with metal nanoparticles are used in the bioremediation of contaminants. Metallic nanoparticles act as supports or synergistic agents that enhance the stability and bioremediation performance of WRF in wastewater treatment and the biosynthesis process (He et al. 2017). Zero-valent iron has been reported as a successful remediation agent for environmental issues, due to its high specific surface area. These are extensively used in soil and groundwater remediation for removal of pollutants from contaminated sites (Corsi et al. 2018; Galdames et al. 2020; Qian et al. 2020a). Ligand-coated dense nanoparticles have been used for the remediation of heavy metals from the contaminated site of soils (Huang and Keller 2020).

Bacterioremediation

Decontamination of metal and metalloid polluted sites can be done through microbial/enzymatic metal immobilization. Microbial remediation is an innovative technique for the removal of polluted toxic heavy metals into less toxic

forms by microbes or their enzymes (Banerjee et al. 2018). Some of these metals are incorporated into enzymes and cofactors (Cu, Fe, Mn, Co, Zn, and Ni), whereas some heavy metals exert toxic effects on microbial cells (i.e., mercury, lead, cadmium, arsenic, and silver). In various bacteria, metal resistance has been found due to genes present on plasmids, whereas bacterial chromosomes contain genes for resistance to many of the same heavy metals' cations and oxyanions as do plasmids (Igiri et al. 2018). Bacteria have evolved various processes including biosorption, entrapment, precipitation, efflux, and reduction in heavy metal ions to a less toxic state inside the cell (Nies 1999; 2003).

Xanthomonas citri subsp. *citri* (Xcc) has developed resistance to copper as a consequence of using copper bactericides (Behlau et al. 2012). Copper resistance genes have taken place from strains of *X. alfalfae* subsp. *citrumelonis* and *Xanthomonas citri* subsp. *citri* (Behlau et al. 2013). *Sulfolobus metallicus*, *Frankia* strains may precipitate the Cu^{+2} -phosphate complex to the hyphae. *Acidithiobacillus ferrooxidans* have the potential to detoxify Cu^{+1} metal by formatting phosphate granules through stimulation of polyphosphate hydrolysis and formation of metal-phosphate complexes (Alvarez and Jerez 2004). The *Staphylococcus aureus* and *Citrobacter*

freundii accumulated Pb as an intracellular lead-phosphate (Levinson and Mahler 1998). CadA ATPase of *Staphylococcus aureus* and the ZntA ATPase of *Escherichia coli* has been reported as the efflux of Pb(II) (Rensing et al. 1998). *Cupriavidus metallidurans* show resistance to zinc and cadmium (Hynninen 2010). *S. aureus*, *Citrobacter freundii* (Levinson et al. 1996), and *Vibrio harveyi* (Mire et al. 2004) precipitate lead as a phosphate salt. In *Streptococcus thermophilus* Strain 4134, two genes (cadCSt and cadASt) were confirmed to constitute in cadmium/zinc resistance (Prabhakaran et al. 2018). Selenite reduction may be catalyzed by *Thauera selenatis*, *Enterobacter cloacae*, *Thiosphaera pantotropha*, *Thauera selenatis*, and *Clostridium pasteurianum*. Recent studies have indicated that NADPH-/NADH-dependent selenate reductase enzymes bring about the reduction in selenium (selenite/selenate) oxyanions. Selenite can be reduced to inert elemental selenium, which occurs in the selenite-resistant *Frankia* strains CN3, EuI1c, EUN1f, and DC12 (Richards et al. 2020).

Microorganisms (algae, fungi, and bacteria) used in the remediation of various heavy metals and metalloids at the contaminated site are summarized in Table 4.

Table 4 Microorganisms used in heavy metal remediation of contaminated site

Microorganisms	Name of Microorganism	Remediation of Heavy Metals/metalloids	References
Bacteria	<i>Kokuria flava</i> , <i>Desulfovibrio desulfuricans</i> (immobilize on zeolite)	Cu, Ni	Kim et al. 2015; Kumar et al. 2018
	<i>Flavobacterium</i> sp., <i>Bacillus firmus</i> , <i>Micrococcus</i> sp.		
	<i>Actinobacter</i> sp., <i>Bacillus cereus</i> , <i>B. subtilis</i> , <i>Sporosarcina saromensis</i> (M52), <i>Pseudomonas aerogenosa</i>	Cr(II)	Kim et al. 2015; Zhao et al. 2016
	<i>Sporosarcina ginsengisoli</i>	As(III)	Chen et al. 2017; Sher and Rehman 2019
	<i>Pseudomonas veronii</i> , <i>Bacillus firmus</i>	Cd, Zn, Cu	Peng et al. 2018
	<i>Pseudomonas putida</i> , <i>Enterobacter cloacae</i> B2-DHA, <i>Bacillus subtilis</i>	Cr(VI)	Rahman et al. 2015; Basu et al. 2015
	<i>Pseudomonas</i> and <i>Burkholderia</i> sp.	Ni, Zn	Meng et al. 2021
	<i>Fusibacter</i>	Pb, Zn, Cu, Co, Ni, Cd, and Ag	Meng et al. 2021
	<i>Syntrophorhabdus</i>		Meng et al. 2021
	Fungi	<i>Aspergillus versicolor</i>	Ni, Cu
<i>Aspergillus niger</i>		Cd, Cr	Khan et al. 2019
<i>Aspergillus fumigatus</i>		Pb	Khatoun et al. 2021
<i>Gloeophyllum sepiarium</i>		Cr(VI)	Ojuederie and Babalola 2017
<i>Cladosporium cladosporioides</i>		Cr(VI)	Garcia-Hernandez et al. 2017
<i>Candida parapsilosis</i>		Hg	Muneer et al. 2013
<i>Pleurotus</i> species		Cd, Cr, Pb, Cu, Ni, Zn, Hg	Kapahi and Sachdeva 2017; Rhaman et al. 2021
Yeast	<i>Saccharomyces cerevisiae</i>	Pb, Cd, Cr	Fathima et al. 2010; Ozturk et al. 2021
	<i>Yarrowia lipolytica</i>	Pb, Cr, Ni, Zn, Cu, As	Mamaev and Zvyagilskaya 2021

Genetically engineered microorganism (GMO) as bioremediator

Pollutants directly harm soil microbial sustainability and the assistance provided by the afflicted organisms. Nowadays, several genetically modified microorganisms (GEMs) are used for heavy metal bioremediation of soil and water (Paliwal et al. 2012; Sharma et al. 2021). GEMs are competent microorganisms that bioremediate various ranges of contaminants (Ojuederie and Babalola 2017). To improve the capability of bacteria for bioremediation, several strategies have been developed. Desirable properties are added to the microorganism through the transfer of genes, for modifying metabolic pathways (Urgun-Demirtas et al. 2006). Microorganisms can be genetically modified with the use of genetic engineering techniques. A wide variety of substances such as heavy metals, pesticides, dyes, oils, radioactive wastes, organic pollutants have been treated with GEMs to elevate degradative potential (Pant et al. 2021). Removal of pesticides can be done through the expressing plasmid gene and chromosome gene in the bacteria. Manipulation of gene sequences is based on the basis of genetic interactions between toxic compounds and microbes (Jaiswal and Shukla 2020). *Deinococcus radiodurans*, a radioresistant bacterium, was genetically engineered to metabolize ionic mercury and toluene found in radioactive nuclear wastes (Gogada et al. 2015).

Rhizospheric or endophytic bacterial species obtained from the harsh environment can be genetically modified to produce specific enzymes and enhance their degradative ability against toxic substances. The construction of GEMs for bioremediation involves several steps. 1) Identification and cloning of efficient degradation genes. 2) Enhancement in the expression of enzymes involved in effective degradation. 3) Construction of super-engineered microorganisms degrading various pollutants. 4) Incorporation of genes from both parents through protoplast fusion for bioremediation of pollutants (Joutey et al. 2013). The advantage of using these modified microorganisms having a high degradation capacity within a small amount of cell mass and speeding up in recovery of polluted wasteland sites (Abatenh et al. 2017).

Metal regulatory genes possessed by microbes encode proteins that convert heavy metals into their low toxic forms (Singh et al. 2011). Genetically engineered *Escherichia coli* strain M109 and *Pseudomonas putida* consisting of the *merA* gene have been found to be effective in eradicating mercury from contaminated sediments and soil (Verma et al. 2020). Chromium-containing industrial wastewater has been remediated through GEMs containing heavy metal degradation genes. Cadmium at naturally polluted sites can be remediated through genetically modified *Bacillus subtilis* BR151 [pT0024] (Ivask et al. 2011). *Rhodospseudomonas palustris*, a recombinant photosynthetic bacterium, has been constructed

for mercury degradation in wastewater (Verma and Kulia 2019). Yang et al. 2010 have reported conversion to a non-toxic form of arsenic through TTHB128 and TTHB127 genes that encode enzyme arsenite oxidase produced by *Thermus thermophilus* HB8. For effective phytoremediation, microbial genes are introduced and expressed in plants for improving heavy metal tolerance (Kumar et al. 2018). *E. coli* strain overexpressing ELP153AR (metalloregulatory protein ArsR) has been reported to be remediated arsenic in contaminated drinking groundwater (Kostal et al. 2004). The major risk of using GMOs is horizontal gene transfer which interferes with native microbial genetic structure (Bhayani et al. 2020). “Suicidal genetically engineered microorganisms” (S-GEMS) have been introduced to reduce the risk on native microbes and ensure a safer effective degradation system (Paul et al. 2005). Releasing genetically modified microbes into the environment can impose potential risks related to safety concerns, legislative issues, and public perceptions. These risks can be minimized by regulatory guidelines provided by environmental protection agencies (EPA) and other regulating bodies. A set of advisory guidelines have been proposed to control GEMs by International Technical Guidelines for Safety in Biotechnology for microorganisms (Hussain et al. 2018). Some genetically engineered microorganisms (GEMs) used in the bioremediation of metals and metalloids are summarized in Table 5.

Limitations of physicochemical remediation and bioremediation

The physicochemical approaches have numerous environmental hazards. Physicochemical remediation of heavy metal is technically challenging and costly, depending on the heavy metal involved in contamination. The physicochemical remediation process is influenced by soil type, and chemical and metal content (Khalid et al. 2016). Application of physicochemical methods for the removal of metal and metalloid causes extreme soil disturbances (mechanical), loss of soil minerals, color, and diffusion of refractory chemicals or surfactants in soil. This process requires fine soil with low permeability for the effective removal of contaminants. With its moderate efficacy in performance, toxicant removal, much human power is needed in the application of the physicochemical method. Although they are rapid, effective, and conventionally used, they are uneconomical and time-consuming. These processes are laborious but can be applied at the highly contaminated site. They deteriorate soil properties and disturb soil's native flora and fauna (Ali et al. 2013). The existing physicochemical technologies also generated hazardous waste that necessitates their proper disposal in a regulatory manner.

Bioremediation is cost-effective, effective, eco-friendly, solar-driven, novel, and an efficient alternative to



Table 5 Some genetically engineered microorganisms (GEMs) used in bioremediation

Heavy metal	Year of study	Microorganism	Bioaccumulative capacity**(DW, dry weight)	References
Nickel and Cobalt	2013	<i>Escherichia coli</i> JM109	0.88 mgNi gDW – 1	Krishnaswamy and Wilson 2000
	2014	<i>Escherichia coli</i> K-12 MG1655	4.8 mgCo gDW – 1, 6 mgNi gDW – 1	Duprey 2014
	2015	<i>Deinococcus radiodurans</i> R1	0.012 mgCo gDW – 1	Gogada 2015
Arsenic Sp	2013	<i>Escherichia coli</i> BL21 (DE3)	5.24 mgMMA gDW – 1 3.92 mgDNA gDW – 1	Yang et al. 2013
	2014	<i>Corynebacterium glutamicum</i> 13,032	2.16 mgAs4 + gDW – 1	Villadangos et al. 2014
Cadmium	2013	<i>Escherichia coli</i> BL21, MG1655	7.5 mgCd gDW – 1	Chang and Shu 2013
	2015	<i>Escherichia coli</i> BL21	~6 mgCd gDW – 1	Gong et al. 2015
Copper	2017	<i>Saccharomyces cerevisiae</i> BY4743	103.3 mgCu gDW – 1	Geva et al. 2016
Mercurial Species	2011	<i>Rhodospseudomonas palustris</i> GIM1.167	77.58 mgHg gDW – 1	Deng and Jia, 2011
	2018	<i>Escherichia coli</i>	4.012 mgHg gDW – 1	Shahpiri and Mohammadzadeh 2018
Uranium	2013	<i>Deinococcus radiodurans</i> R1	10,700 mgU gDW – 1	Kulkarni et al. 2013
Multi Metal	2012	<i>Escherichia coli</i> TB1, BL21(DE3), LF20012	1.51 mgCd gDW – 1 0.4 9 mgAs3 + gDW – 1 0.31 mgCu gDW – 1 0.94 mgHg gDW – 1 1.79 mgPb gDW – 1	Sauge-Merle et al. 2012
	2014	<i>Escherichia coli</i> BL21 (DE3)	0.13 mgCd gDW – 1 0.057 mgCu gDW – 1	He et al. 2014
	2015	<i>Escherichia coli</i> Rosetta (DE3)	2.24 mgCd gDW – 1 12.39 mgCu gDW – 1 0.82 mgHg gDW – 1	Li et al. 2015

abolishing heavy metals. Bioremediation consumes much time compared to other treatment options, such as excavation and removal of soil from contaminated site. Bioremediation process is largely specific and limited up to the biodegradable compounds only. The process of bioremediation takes place in the presence of metabolically capable microbial populations, suitable environmental growth conditions, and adequate levels of nutrients and contaminants at the contaminated sites. Too much effort is required to scale up of bioremediation from bench- and pilot-scale studies to full-scale field operations. More research is required to develop and advance bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly distributed in the environment, i.e., it may be present as solids, liquids, and gases. Regulatory uncertainty about completion of remediation is major drawback of bioremediation because we are unable to 100% sure about completion of metal and metalloids remediation at contaminated site, as there is no known definition of clean. Along with that performance evaluation of bioremediation is very complex, and there is no fixed endpoint for bioremediation treatments. Merits and demerits of different bioremediation techniques are discussed in Table 6.

Future prospective

Cleaning up of soil is a challenging venture due to the complexities of heavy metal/metalloids presence along with financial and technical implications. Based on the references cited, we are able to say that each bioremediation technique has its own merits and demerits. Phytoremediation will not only provide a link between researchers and farmers but also improves the economy of developing countries. The choice of bioremediation will be based on the level of metals and metalloids at the site of soil pollution. Rhizoremediation metabolizes the metals and metalloids present in the rhizospheric region and acts as a plant growth-promoting process. Metals and metalloid removal through edible mushrooms enables waste accumulation and production of proteinaceous food. Vermiremediation is an economically and environment-friendly technology that can accelerate the process of micro- and phytoremediation in combination. Nanobioremediation is an effective approach to designing novel catalysts and adsorbents for the metabolism of heavy metals and metalloids. Cited references advocate the rapid removal of metals and metalloids at the contaminated site by the application of genetically modified organisms by genetic engineering technique. Many genes responsible for heavy



Table 6 Merits and demerits of different bioremediation techniques

Bioremediation techniques	Merits	Demerits
Phytoremediation	Low capital cost Sustainable Effective bioremediation of organic contaminants Effective bioremediation of heavy metals such as Cd, As, Zn, Pb, Hg, Cu, Mn Effective accumulation of heavy metals	High contamination limits the speed of remediation Increases phytotoxicity Depends upon climate and characteristic of soil Contaminants may enter environment by litter effects
Phycoremediation	Algae require low nutrient for survival Produce large amount of biomass Cost reliable technique for wastewater treatment Effective in bioremediation of Cd, Cu, Pb, Cr, Fe, Ni	Expensive to harvest algal biomass Limited for large-scale clean-up
Rhizomediation	Higher rate of contaminant degradation than phytoremediation Bioremediation of Hg, Te, Tn, Se, Pb, Sb, As	Influenced by various physicochemical and biological factors such as temperature, pH, soil conditions, microbial communities, aeration, content of organic matter, exudation rate, age of the plant, nutritional requirements, and contaminants contents
Mycoremediation	Fungus adapts to various adverse environments Degradation of various pollutants such as PAHs, pharmaceutical wastes, agricultural and heavy metals Improves plant biomass production Secretion of organic acids helps to tolerate high number of heavy metals	Process is controlled by soil type, pH, temperature, organic matter content, concentration and nature of heavy metals
Vermiremediation	Earthworms can resist high concentrations of soil pollutants Degrade non-recyclable chemicals Low-cost technology Improves soil fertility	Proper care and attention for worms Requires maintenance of moisture level for worms
Nanobioremediation	New safer and sustainable technique for persistent organic compounds and heavy metals remediation Quick and efficient performance with minimized adverse effects	Synthesis of nanoparticles by biological enzymes may render the desired characteristic of it
Bacterioremediation	Thermophilic, acidophilic and presence of gene resistance to heavy metal/metalloids make them perfect choice for genetic engineering	Environmental variability may hinder the remediation process
Genetically modified organisms (GMOs)	Competent microorganisms that bioremediate various range of contaminants High degradation capacity within a small amount of cell mass Speed up in recovery of polluted waste land sites	Costly compared to other bioremediation process Risk on native microbes Potential risk related to safety concerns, legislative issues, and public perceptions

metals/metalloids resistance involved in enhanced expression of the selected protein can be incorporated into many more plant or microbial cells to resist heavy metal contamination. Legal and environment-safe application of chelating agents along with genetically engineered plant/microbial systems can be done through an effective transgenic approach. The invention of the enzyme-based biosensor is needed for rapid detection and detoxification of metal/metalloids from polluted soil. It remains interesting to explore the role of various microorganisms in the methylations, reduction, and oxidation of various metal compounds. This ensures a high potential application in the future. Along with it, awareness of various metabolic pathways used by biological organisms in remediation is required for the enhancement of bioremediation processes.

Conclusions

Contamination of soil by heavy metals and metalloids is a global concern. Reliable and economical remediation techniques are needed to protect soil quality and fertility. For effective clean-up of heavy metal polluted sites, various physical, chemical, and biological methods have been developed. In this review, we have explained briefly the various biological remediation techniques. The heavy metal remediation by various strains of bacteria, fungi, plants, and earthworms has been explained in detail. The new branch of science, i.e., nanotechnology has also been proved an effective approach for the remediation of various metals. The release of secondary contaminants is the main limitation of physicochemical remediation techniques. Biological remediation techniques enable us to remove heavy metals from

the large contaminated area without disturbing soil quality. It is a less destructive, eco-friendly, safe, and cheap method. Further basic and field-scale research-based technology is required to develop fast, effective, eco-friendly bioremediation techniques.

Declarations

Conflict of interest No potential conflict of interest was reported by the authors.

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