New Bioremediation Technologies to Remove Heavy Metals and Radionuclides

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Abstract

Environment of this planet is facing hazards from various pollutants, among which heavy metal and radionuclide pollution is of great importance. This pollution is a resultant of both geological and anthropogenic activities. Various industrial and municipal solid wastes have been a major source of heavy metal contamination in soil, water and also as atmospheric aerosols in air. Similarly, radioactive wastes from nuclear plants and places where radioactive materials are used (e.g., medical centers) are contributing to radionuclide pollution of the environment. These contaminants are harmful for living beings and cause various health hazards to them. Proper management of wastes from these sources is required along with environment-friendly remedial techniques. Phytoremediation has been used in this regard for many years. However, nowadays, novel biotechnological tools are used for achieving paths in bioremediation through microorganisms. Microbes possess the ability to biotransform, biosorb, and biomineralize these metals and radionuclides. Techniques are now being availed to identify the microorganisms and study their biological functions in order to use them in remediating these hazardous pollutants from the environment.

Keywords

Heavy metal · Radionuclide · Phytoremediation · Microbes · Biosorption

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1 Introduction

Environmental pollution is a global concern of this century. One of the major areas of environmental pollution is the pollution caused by elements, which are now categorized by researchers as "emerging contaminants" (Yu et al. 2014). This pollution is mainly due to the presence of elements whose elimination results in harm and destruction of the natural environment. Elemental pollution is primarily due to anthropogenic activities involving rapid industrialization, advanced agricultural practices, and improper waste disposal methods. These practices have led to the increase in elemental concentration in the environment which can create immense toxicological and adverse effects on living organisms (Wijnhoven et al. 2007). Among the elemental pollution, heavy metals have emerged as a pollutant posing grave concern for the planet. These heavy metals have the capacity to enter the environment through high level single exposure and can also pose cumulative effect through low and high level exposures. However, when present in the environment, it can persist in its toxic form for a prolonged time period, causing ecological, nutritional, and environmental problems (Das and Osborne 2018). Heavy metal pollution comes from either natural or anthropogenic sources (Nagajyoti et al. 2010). But anthropogenic activities have resulted in heavy metal contamination in large parts of the world. In India, several researchers have conducted detailed studies on heavy metal contamination (Rajaganapathy et al. 2011; Paul 2017). Khan et al. (2005) reported heavy metal contamination in the surroundings of Neyveli Lignite Mines and associated industrial complex in Tamil Nadu. Similarly, Hejabi et al. (2011) reported pollution due to heavy metals discharged from industrial effluents in Kabini River of Karnataka. Similar to heavy metal pollution, another emerging pollutant of this world is radionuclide contamination (Pravalie 2014; Szarlowicz et al. 2019). Radionuclides are present in the environment from natural sources and in today's world majorly from anthropogenic sources. The advent of industrial revolution in the early nineteenth century and discoveries regarding nuclear weapons during World War II led to massive weapon production, experiments, and nuclear power generations. These things contributed to the increase of radionuclides in the environment (Hu et al. 2010). Both heavy metals and radionuclides pose detrimental threats to the living organisms (Das and Osborne 2018). Thus, heavy metal and radionuclide pollution is a worldwide phenomenon whose mitigation is of outmost importance.

Conventional methods employed in industries have been used for these purposes. Techniques like precipitation, oxidation, filtration, and adsorption have been used for removal of heavy metals and radionuclides from the environment. However, novel remedial technologies using organisms are being used. From conventional phytoremediation to the new age genetically modified microorganisms, various new approaches have been studied by the researchers in recent times. This chapter deals with such emerging technologies employed in remediation of heavy metals and radionuclides.

2 Heavy Metals and Its Sources

The term heavy metal is referred to as a metal element having atomic density more than 4 g/cm^3 imparting toxicity at low concentrations. These heavy metals (e.g., lead, cobalt, cadmium, iron, zinc, nickel, arsenic, manganese, chromium) naturally occur mostly in dispersed state in rocks. Industrialization and increasing urbanization of humans have resulted in their presence in the biosphere. Heavy metals are found dispersed mainly in soils and aquatic bodies and in lesser proportions as vapors or particulates in the atmosphere (Nagajyoti et al. 2010). Heavy metals are toxic to plants depending on parameters like metal concentration, pH, etc. but also are required by plants as essential elements. For example, metals like copper and zinc play the role of cofactors and activators of enzyme reactions. They also exert catalytic properties (e.g., prosthetic groups of metalloproteins). Besides these functions, they also play active role in redox reactions, nucleic acid metabolism, and electron transfer. However, metals like Cd, Hg, and As can also inhibit growth and death of organisms by targeting the metal-sensitive enzymes in organism's body (Nagajyoti et al. 2010).

In general, heavy metals are categorized as trace elements which are nonessential or class B and are highly toxic (e.g., nickel, mercury, lead). They accumulate and are not easily metabolized or eliminated from organism's body. These metals become a part of the food chain and remain in the ecosystem (Nagajyoti et al. 2010).

As mentioned earlier, there are different sources which contribute to heavy metal pollution in the environment. These can be broadly classified into:

(a) Natural sources.
(b) Anthropogenic sources.

2.1 Heavy Metals from Natural Sources

Heavy metals have their natural origin in the Earth's crust and are present naturally in the soil due to weathering. Thus, the geological parent rock is the primary source of heavy metals whose composition and concentration vary throughout the planet depending on the rock type and environmental conditions. In the soils, sedimentary rocks act as a lesser source of heavy metals in comparison to igneous rocks (Nagajyoti et al. 2010). Volcanoes, however, have considerable higher levels of heavy metals (Pb, Mn, Ni, Al, Zn, etc.). According to a very early research reported by Pacyna (1986), global emissions of various heavy metals are mainly from various sources which include windblown dust, volcanic particles, forest wild fires, vegetation, and sea salt.

2.2 Heavy Metals from Anthropogenic Sources

Various sources as a resultant of different anthropogenic activities contribute to the increase of heavy metals in the environment which are accessible to living organism at a level above their permissible limits. The various anthropogenic sources are illustrated in Fig. 1.

Agricultural sources contribute immensely to heavy metal pollution. Fertilizers both organic and inorganic serve as sources for heavy metal contamination. Fungicide contains heavy metals like Cd, Cr, Ni, Pb, and Zn in variable proportions (Nagajyoti et al. 2010). Pesticides like lead arsenate were used in Canada for six decades in the orchards which resulted in soil contamination with heavy metals. Again, Paul (2017) reported that the uncontrolled use of heavy metal-containing pesticides and fertilizers in agricultural fields have resulted in groundwater and surface water heavy metal contamination in India. It has even led to their presence in drinking water. Animal manure and sewage sludge used in agriculture add heavy metals like Mn, Zn, Cu, Cr, Pb, Ni, Cd, and Co in the soil. Liming process also contributes to this contamination (Nagajyoti et al. 2010). Various pedogenic and anthropogenic processes also lead to contamination of forest soils (Wuana and Okieimen 2011).

Industrial sources of heavy metal contamination occur due to processes like smelting, metal finishing and recycling, and transportation of ores. Mining processes also release metals in the environment. Coal mines act as sources of Cd, As, and Fe. Hg use in gold mines also results in Hg contamination (Lacerda 1997; Nagajyoti et al. 2010; Rajaganapathy et al. 2011). Heavy metal contamination of soil and aquatic bodies also occurs due to mine waste erosion, transportation of crude metals and metal leaching in water bodies. Thermal power plants and coal mining also contribute to these contamination processes.

Fig. 1 Various anthropogenic sources of heavy metal contamination (Nagajyoti et al. 2010; Wuana and Okieimen 2011; Das and Osborne 2018)

Domestic effluents, solid wastes, and untreated wastewater from industries also contribute to this list. These liquid wastes primarily pollute the water bodies and also contaminate soils.

Again, the application of various biosolids like municipal sewage sludge, manures, and composts in soils contributes to heavy metal contamination. Airborne sources of metal contamination are also prevalent. In industries, metals such as As, Cd, and Pb volatilize during processing at very high temperature. These metals are emitted as aerosols in the air from the stacks (Wuana and Okieimen 2011).

3 Heavy Metal Toxicity

Heavy metals are often required by plants and animals as trace elements (ppb range to less than 10 ppm) depending upon multiple parameters. Some often play their role in biochemical and physiological functioning. Being constituents of enzymes, they take part in oxidation-reduction processes in the body. For example, copper takes part as a cofactor in enzymes like peroxidases, cytochrome c oxidase, etc. It is also present in enzymes required for hemoglobin formation. Paradoxically, its cycling between C (II) and C (I) state makes it toxic to living organisms leading to diseases like Wilson's disease in human beings. Metals have often been found to interact with cell components leading to the disruption of normal cellular activity. They can cause DNA damage, changes in cellular confirmation, and even carcinogenicity (Tchounwou et al. 2012). In Table 1, the toxicity imparted by heavy metals in living organisms specially in human beings is given.

Table 1 Heavy metals, its anthropogenic sources, and toxicity in humans (Tchounwou et al. 2012; Paul 2017; Sengupta et al. 2017)

Metals	Sources (anthropogenic)	Toxicity
Zinc	Refineries, metal plating	Anemia, skin problems
Nickel	Batteries, electroplating	Respiratory disorder, kidney problems, gastrointestinal distress, dermatitis, cancer
Mercury	Pesticide, mining, paper industry	Respiratory disorder, neurological disorder, kidney problems
Lead	Paint, pesticide, thermal power plants, mining	Anemia, neurological disorder, renal damage
Copper	Pesticide, mining, electroplating	Gastric and neurological disorders
Chromium	Mining, tannery, textile	Respiratory disorder, cancer
Arsenic	Pesticide, fungicide, smelting	Neurological disorder, cardiovascular disease, hematologic disorders, cancer
Cadmium	Pesticides, fertilizer, nuclear power plants, batteries	Oxidative stress, cancer
Manganese	Fuels, welding	Respiratory disease, neurological disorder, Parkinson's disease

4 Methods of Heavy Metal Removal

Several methodologies have been adopted by researchers to successfully mitigate heavy metals from soil and water. Among the conventional methods used in heavy metal remediation, chemical precipitation is a widely used method in industries. In this method, the chemicals (hydroxides and sulfides) formed precipitates, and the metal ions were subsequently removed from the system. Furthermore, chemical precipitation was combined with other methods (e.g., sulfide precipitation with nanofiltration) for this purpose. Another approach that was used was heavy metal chelation. Chelating agent like 1,3-benzenediamidoethanethiol ($BDET^2$) dianion was used to chelate out heavy metals. Then method like ion exchange was also used having high treatment capacity and efficiency. Synthetic resins were used for this purpose. Recent advancements led to technologies like membrane filtration. The different types of this method include ultrafiltration, reverse osmosis, nanofiltration, and electrodialysis. Again, adsorption of metals onto various adsorbents like activated charcoal, clay, graphene oxide, carbon nanotube, rice husk, cellulose, etc. is used effectively by researchers. However, using biological organisms for this purpose has emerged as a green and sustainable route for this purpose. Plants, animals like earthworms and microorganisms like bacteria, fungi, and algae are being used for remediation purposes. Biomass of microorganisms, living or dead, is being effectively used. Genetic engineering of these organisms is also being carried out (Sengupta et al. 2017; Das and Osborne 2018). Various approaches adopted for bioremediation of heavy metals are given in Fig. 2.

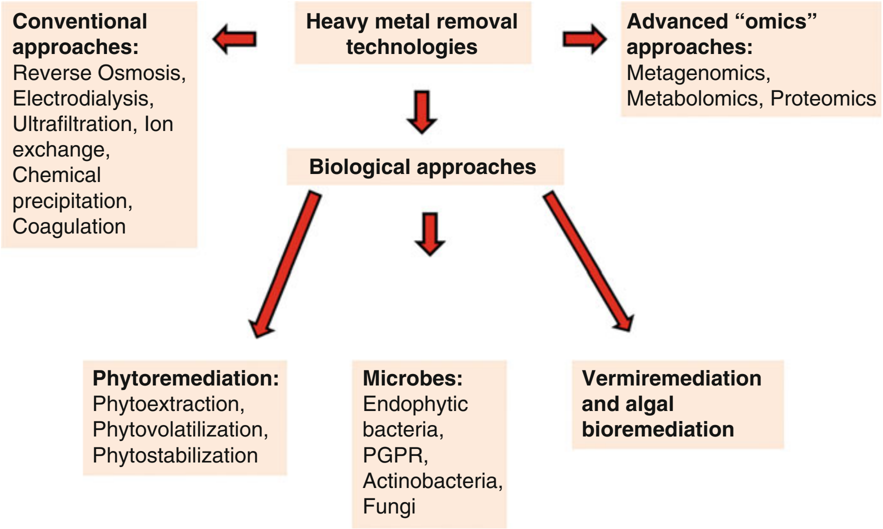

Fig. 2 Various heavy metal remediation approaches (Su et al. 2014; Das and Osborne 2018)

5 Bioremediation of Heavy Metals

Bioremediation of heavy metals is a widely sustainable, green, and sought-after method in today's age. This technique employs organisms to breakdown hazardous substances through biosorption, bioaccumulation, and biomineralization. The uses of biomasses are studied to be more effective than living organisms in case of heavy metal removal. Phytoremediation and use of earthworms are also some of the techniques which could be deployed individually or through integrated approach (Das and Osborne 2018).

5.1 Phytoremediation

Phytoremediation is one of the conventional bioremediation procedures, whereby plants and their physiological processes are used for metal remediation purposes. In nature, plants play the role of both accumulator and excluder of heavy metals. They possess the ability to biodegrade or biotransform the heavy metals in their tissues in inert forms. Whereas when plants exclude the heavy metals, they block the containment of the heavy metals in their tissues. The ion uptake mechanism through proton pumps (i.e., ATPases) plays an interesting role in the uptake of heavy metals also. This technique uses three processes, namely, phytoextraction, phytostabilization, and phytovolatilization (Tangahu et al. 2011).

(a) Phytoextraction is the uptake of heavy metals by plant roots and their storage in their biomass, e.g., *Brassica juncea* and Napier grass.
(b) Phytostabilization is another approach adopted by plants, whereby they immobilize metals in their rhizosphere, e.g., giant reed and silver grass.
(c) Phytovolatilization employs volatilization of heavy metals like Hg and Se, e.g., canola and Indian mustard (Das and Osborne 2018; Tangahu et al. 2011).

There are various factors which affect the phytoremediation process in plants like species of plant used, chemicals added as chelating agents in the soil, properties of the medium (pH, fertilizer added), bioavailability of the concerned heavy metal, root zone properties, and environmental conditions (Ginneken et al. 2007;Tangahu et al. 2011).

However, phytoremediation technologies are in general cheap, aesthetic, and eco-friendly methods for heavy metal containment, but as a method, it is time-consuming and dependent on various external and environmental parameters. Thus, researchers have been on the lookout of these remediation facilities by the use of microorganisms as they are more effective and employ less hassle-free technology.

5.2 Bioremediation Using Microorganisms

Bioremediation technologies using microorganisms like bacteria, fungi, and algae have been rapidly developed in recent times (Sengupta et al. 2017). In a recent review, Yin et al. (2019) highlighted various strategies adopted by microorganism for the effective remediation of heavy metals in the environment. They identified the anthropogenic and natural processes/activities as the primary origin of heavy metals in the environment emphasizing on the utility of bioremediation process to tackle the challenges related to heavy metal deposition. In that respect, both living and dead organisms have been used to get rid of the heavy metals. The straightforward and inexpensive protocols along with high adsorption capacity and abundant choice of the organisms have prompted the research to flourish at a faster rate than the other methods.

Among the living choices, bacteria, fungi, and algae are the primary candidates which are primarily considered for this remediation strategies. In case of bacteria, primarily two types of mechanisms for heavy metal adsorption have been identified: (1) through the functional groups (carboxyl, amino, phosphate, sulfate, etc.) on the polysaccharide slime layers of bacteria (Yin et al. 2016; Yue et al. 2015) and (2) via extracellular polymeric substances comprised of proteins, lipids, carbohydrates, nucleic acids, etc. (Fang et al. 2010). Though, the first case is more prevalent in the literature as exemplified by the recent reports of bioadsorption of Hg, Cr(VI)/Zn (II) by *Pseudomonas aeruginosa* (Yin et al. 2016) *and Staphylococcus epidermidis* (Quiton et al. 2018)*.,* \respectively. The second type of adsorption have also started to emerge (Wang et al. 2014). After the bioadsorption, subsequent transport of the heavy metal into bacterial cells and through the corresponding enzyme-dependent metabolism pathways, the oxidation state of the metal is changed to a less toxic oxidation state. Commonly, the uptake capacity of bacteria for the heavy metals falls between 1 mg/g and 500 mg/g, depending on the types and concentration of the metal as well as the types of bacteria used for the experiment.

In case of fungi, due to its ability to survive in heavy metal concentrations, they have also been used in various cases for the successful bioadsorption of heavy metal. Both functional groups (amine, carboxyl, etc.) and the ionizable sites (glucuronic acid, chitin-chitosan complex, etc.) affect the adsorption capacity as well as the affinity toward a particular heavy metal. For example, *Termitomyces clypeatus* effectively absorbs Cr (VI) with the help of various functional groups like hydroxyl, imidazole, carboxyl, etc. on the surface (L. Ramrakhiani et al. 2011). *Saccharomyces cerevisiae*, the most common fungus, has been employed to effectively remove copper, zinc, and cadmium at high-salt environment (Li et al. 2013:46–52). Algae on the other hand adsorb heavy metal ions on different peptide which in turn protect algae from heavy metal toxicity. For example, the green microalga *Desmodesmus* sp. (Rungini et al. 2018) and *Fucus vesiculosus* (Demey et al. 2018) have been shown to effectively remove Cu (II)/Ni (II) and Pb (II), respectively, with high capacity.

The major part of nonliving microorganisms, which are routinely used for the removal of heavy metal, primarily originates from biomass produced by debris of microorganisms. Though sounds promising due to the high abundance of biomass, in order to achieve satisfactory remediation capability, they are often modified using different techniques. While acid treatment allows to incorporate additional adsorption sites (Mao et al. 2013), the base treatment increases the adsorption capability by increasing the surface negative charge for the electrostatic attraction between the surface and the positively charged heavy metals (Yan and Viraraghavan 2000). Apart from chemical treatments, thermal treatment (Mane et al. 2011) including heating in the presence of oxygen/air/argon/nitrogen, autoclave methods, etc. is also routinely used to alter the nature and the amount of surface functional groups which allow better interaction between the surface and the metal leading to higher adsorption efficiency.

Based on the available reports where the bioadsorption mechanisms and the strategies are clearly explained, it is well-understood that for detoxification, active bioorganisms are preferred because of their potential to convert the toxic metal ion to their corresponding nontoxic counterparts. However, in case the requirement is aimed toward removal of heavy metal, biomass-based systems with modified surface properties are more conducive. However, the protocols that are to be adopted for different heavy metal adsorptions are essentially purpose-based, and any generalization on this may lead to oversimplification of the complexity.

While uptake of heavy metal into microorganisms emerges as effective remedial method mainly due to the active participation in various metabolic and enzymatic processes, the overdose of the metal ion concentration can be detrimental for the microorganisms leading to enzyme inhibition, damage of DNA and cell membranes, etc. Also, in some cases, metals such as Hg and Pb can inherently be toxic for those microorganisms. The related studies of such toxicological effects of such heavy metals on the microorganisms have impacted significantly in microbial ecology where adoptability of microbial stains in the presence of various heavy metal is investigated (Huertas et al. 2014). In case of exposure, the microorganisms have their own detoxification strategies which are mainly enzyme detoxification, transportation of heavy metals, and sequestration of intacellular and extracellular enzymes, by which they try to circumvent heavy metal overdose (Fig. 3). In fact, thorough knowledge of the resistance mechanism of microorganisms in the presence of heavy metal can potentially lead to their evolutionary background and their future fate.

These informations and strategies shows the path for development of techniques which uses these microorganisms for remedial measures and are truly the emerging technologies of our times.

5.3 Emerging Microbial Bioremediation Technologies

In recent researches, heavy metal adsorption by microbial biosorbents is used. This uptake is mainly done through the electrostatic interactions of the cell wall components with the heavy metals. However, it is a non-metabolic process. Whereas, accumulation of the metals occurs by the functional groups of the

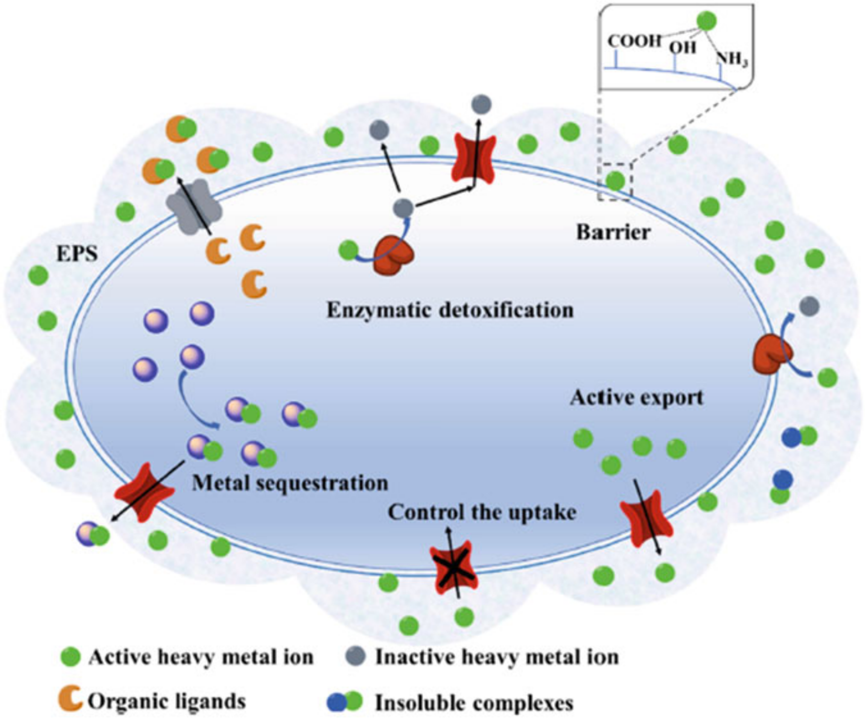

Fig. 3 Proposed detoxification pathways of microorganisms toward heavy metal ions. (Reproduced with permission from Yin et al. 2019)

polysaccharides/chitin/cellulose present in the cell walls (Yin et al. 2019). Thus, it is mainly an exchange process of the functional groups which makes these microorganisms effective absorbents. In this regard, genetic engineering plays an important role to improve and redesign the microorganisms having heavy metal uptake and sequestering properties. Genetic engineering can engineer the selectivity and also enhance the accumulative properties. Cell surface adsorption can be genetically engineered by embedding functional group proteins on the anchoring protein molecules on the cell surface. This cell surface engineering in bacterial cells has been achieved successfully. Again, cysteine-rich peptides like glutathione, phytochelatins, and metallothioneins have the ability to sequester metal ions. Genetically modified bacterial cells with overexpression of these proteins are able to absorb more metals. All these techniques can be used for better remediation of metal contamination (Sengupta et al. 2017).

With the advent of modern bioinformatics tools, information of microbial organisms and processes could be used more effectively. These information could be gathered through different approaches like metagenomics. By definition, metagenomics refers to the study of genetic material sampled directly from environmental sources. In the current context, metagenomics has been proven to be utilized to assess the correlation between the genetic materials for the microbial community and the potential of their remedial activity. This emergence of this field is majorly attributed to the fact that it provides direct information about the microbial communities irrespective of their culturability. Till date, two major classes of metagenomics techniques have been adopted for better understanding of interaction between the microorganisms and the foreign bodies/environment: (1) library-based targeted metagenomics and (2) direct sequencing. In the first case, isolated DNA from the environment is cloned inside a host (commonly *Escherichia coli*), followed by the selection and isolation of clone of interest depending on either their functions or their sequence homology to generate a metagenomic library. Despite the prevalence of *Escherichia coli* as a host, several other new strategies such as multiple hosts, etc. are also being adopted to overcome the difficulties related to gene expressions, toxicity, etc. In case of direct sequencing, the cloning step is omitted, and the genetic components of microorganisms are directly studied for the structural and functional information of the microbial communities as well their interaction with environment. For example, 16S rRNA genes and marker genes have been utilized for this approach. Recently, after the introduction of next-generation sequencing (NGS), the growth in the field of metagenomic has been quite sharp due to the possibility of parallel sequencing of genetic materials. All these approaches coupled with newer technological inventions are expected to help to identify the missing links of the complete picture of bioremediation process. This work has been carried out on various microbial species (e.g., *Lysinibacillus* sp. and *Rhodococcus* sp. for removal of Pb, Mn, and Cu). Similarly, metabolomics and proteomics approach designed to identify various chemical compounds, proteins, and metabolic pathways in remediation studies helps in investigating remedial studies. This information could be stored and used effectively for bioremediations of heavy metals in the future (Das and Osborne 2018).

6 Radioactive Elements and Its Sources

Radioactivity is a natural phenomenon present in the planet Earth. Radionuclides are found in the environment being present in air, water, soil, and living organisms. The natural radionuclides may be classified into three categories, based on their source:

(a) Primordial radioisotopes have presented since the Earth was originated, nearly 4.6×10^9 years ago.
(b) The second classes are produced from nuclear reactions, and its constituents are present in air or on the upper layer of this Earth.
(c) Third category includes the radioactive elements coming from different radioactive decay series, i.e., uranium-radium series (containing ^{226}Ra, ^{222}Rn, ^{218}Po, ^{214}Pb, ^{214}Bi, ^{214}Po, ^{210}Pb), uranium-actinium series (including ^{231}Th, ^{227}Ac, ^{223}Ra, ^{215}Po, ^{211}Bi, ^{207}Pb), and thorium series (such as ^{228}Ra, ^{228}Ac, ^{228}Th, ^{220}Rn, ^{212}Pb, 212Bi, ^{208}Tl) (Isaksson and Raaf 2016).

However, over the recent years, various anthropogenic activities have contributed largely to the increase of the amount of radionuclides in the environment and their exposure to living organisms. Different industrial processes like metal mining, mineral sands, activity associated with coal industry, etc. can give rise to drastically increased radiation exposures especially to naturally occurring radioactive material (NORM) (Szarlowicz et al. 2019). Another reason for exposure to radioactive elements is due to the commencement of the so-called atomic age which was a result of the experiments of mankind with nuclear weapons.

Various studies have been conducted regarding the presence of radioactive elements in the environment. There are reports on efforts being carried out to study the spatial boundary of highly radioactive sites of our planet and to note its exposure on human beings. (Pravalie 2014). Researchers have reported studies regarding the effects of radionuclides on living organisms especially on humans where the source of radioactivity is anthropogenic. Likewise, analyses have been carried out to correlate the incidence rate of the enhancement of thyroid cancer and the rate of the presence of radioactive isotope (^{131}I) at heavily contaminated nuclear testing sites of Nevada, USA. Radionuclide pollution was reported due to hydrogen bomb testing in Marshall Islands in the United States on the Bikini Atoll in 1954 (the Castle Bravo test) and also due to the radioactive experiments conducted in the Novaya Zemlya archipelago in the United States of Soviet Russia in 1961 (the Tsar test) (Goodby 2005). It was reported that most of the airborne fission products had been released through hydrogen bomb, vented and leaked over a great time span. Therefore, highly volatile fission outcomes like ^{129}Te, ^{131}I, ^{136}Cs, etc. were released into the atmosphere. These radioisotopes were also moved together with different air particles, and its subsequent wet and dry accumulation caused their clustering on the surface of the Earth. Radionuclides (iodine, caesium, and strontium), which yielded huge fission products, delivered a greater risk for inner radiation exposure through intake of contaminated farming products (Kinoshitaa et al. 2011).

Naturally occurring radionuclides are present either in their cosmogenic or terrestrial form in our surroundings. The major radioisotopes generated through the reaction of different gases with cosmic rays are basically ^{3}H, ^{7,10}Be, ^{14}C, ^{26}Al, and ^{39}Ar. The rocks, minerals, and also soil carry NORM which has been characterized by their greater half-life time spans (ATSDR 1999). The most potential terrestrial radionuclides are ^{238}U and ^{232}Th decline series, with ^{40}K. ^{226}Ra, ^{232}Th, and ^{40}K are mainly responsible for soil activity (UNSCEAR 2000). The term radioactive contamination defines the presence of unwanted or undesirable radioactive materials on the upper layer of soil or within solid particles, liquid substances, and different gaseous materials and also in several biotas (IAEA 2007). The source of any kind of NORM is associated with the generation of this planet. In other context, different anthropogenic activities regarding the designing of nuclear energy and its several usages have become significant source of radioactive pollution (Smičiklas and Šljivić-Ivanović 2016). Since the last century, radioactive isotopes have seemed through the discharge of anthropogenic radionuclides causing ionizing radiation one of the crucial environmental factors (Aleksakhin 2009). Each and every organism on the Earth is frequently exposed to natural ionizing radiation called background radiation. Origins of this type of radiation include cosmic rays coming from the Sun and stars, NORM found in rocks as well as in soil, radionuclides into animal tissues, and the products of radon, which are basically inhaled. Human beings are also exposed to background radiation from different anthropogenic activities, mainly through medical processes such as X-ray diagnostics. Therapy associated with radiation is normally focused only to the damaged tissues (Hazra 2018). Concern for radioactive contamination is enhanced by the invention of artificial radioactivity, deigning of nuclear weapons, and development of different nuclear reactors for producing electricity. Radioactive pollution coming from both common nuclear approaches and defense-associated nuclear activities poses an alarming problem for protecting and sustaining our environment for both present and future generations (Hazra 2018).

The radioactivity of soils relies not only on man-made activities. Soils and other naturally found objects carry NORM, and their presence is known as natural radioactivity. The natural radionuclides can be of different origins; its major sources are global fallouts of technogenic radionuclides due to greater radiation experiments and nuclear accidents with discharge of technogenic radionuclides into the environment. Special focus is imparted to the removal of technogenic radionuclides and their intake through soil-plant cover from the enterprises with nuclear fuel recycling plants. The soil-plant cover adjacent to nuclear contaminant disposal sites is subjected to radioactive pollution (Aleksakhin 2009). Some of the nuclear accidents resulted in the radioactive pollution of considerable areas (Aleksakhin et al. 2001). In 1986, after the most dangerous nuclear accident in Chernobyl, total area impacted by the radioactive contamination (with the degree of ^{137}Cs contamination above 1 Ci/km^2) was nearly 195,000 km^2 (Izrael et al. 1990, 1994). The Kyshtym accident in 1957 ensued in the generation of the East-Ural radioactive footprint (the level of the radioactivity with ^{90}Sr above 2 Ci/km^2) on the domain of approximately 23,000 km^2. Different mineral fertilizers and some agrochemical modifiers can become sources of several radioactive elements of the soil. Lower radioactive pollution can also happen when different phosphoric fertilizers and phosphogypsum containing ^{238}U, ^{232}Th, and their fallouts are used (Aleksakhin 2009).

7 Toxicological Effects of Radionuclide Pollution

Most of the radioactive elements did not exist naturally. Radioactive contamination has become a critical issue since nuclear bombs and reactors have been developed (Walker and Don 2013). Radionuclides have also been found in different types of seafood in India, variety of foods in the Balkans, and food as well as drinking water in Switzerland (Khan and Wesley 2011; Carvalho and Oliveira 2010; Brennwald and Dorp 2009). Risk assessments are carried out to ascertain that degree remains within permissible levels (Thompson and Darwish 2019). Moreover, several experimental approaches are undertaken to measure safety in ingestion pathways analyzing different food uptakes (Prohl et al. 2005). Radionuclide substances have many negative impacts on individual living creatures as well as whole ecosystems.

Radionuclide and their compounds contain double toxicity—chemical toxicity and toxicity induced by radioactive elements (known as radiotoxicity). Although the occurrence of radioactive pollution is relatively rare, it requires more attention because of utmost degrading effects of radioactive isotopes on different living cells (Smičiklas and Šljivić-Ivanović 2016). The degree of the detrimental effects of radioactive contamination depends on absorbed nuclear energy, permeable capability of radioactive ions, time period of exposure, as well as reproducibility of cells (ATSDR 1999). Exposure to radionuclides or ionizing radiation induces severe health effects including nausea, vomiting, headaches, etc. With more exposure, the victim may also suffer different physical abnormalities such as diarrhea, dizziness, disorientation, fatigue, fever, hair loss, weakness, low blood pressure, and eventually death. Fetuses are basically susceptible to the effects of radioactivity at the cellular level, which can ensue smaller brain formation, poorly developed eyes, retard growth, and mental problems (Bogutskaya et al. 2011; Al-Zoughool and Krewski 2009). Several contemporary literatures reported that long-term exposure to radioisotopes results in an elevated risk of leucopenia, leukemia, and genetic distortion which can cause lethal health issues that can be transferred into the next generation (Mohner et al. 2006). Radioactive isotopes present in aquatic system are components of uranium, thorium, and actinium radioactive series and also include radium and radon. These radioactive isotopes may cause many biological alterations (Bonavigo et al. 2009).

Uranium is an alpha-emitting, radioactive element that appears naturally in almost all rocks and soils. Uranium has its own carcinogenic property, and it also has the negative toxicological impact on kidneys. The main target organ of this radionuclide is the kidney. Most of the kidney damage reports have been found after inhaling or insertion of uranium compounds into the living body. On the other hand, kidney problems have not been consistently seen in militaries having radioactive metal fragments in their bodies for many years. Insertion of dissolved uranium compounds will mostly participate in kidney damage than following exposure to insoluble uranium. Persons who inhaled uranium hexafluoride have suffered with respiratory irritation as well as accumulation of fluid in the lungs. Adverse effects of uranium on the functioning of kidneys were previously reported including alterations in renal metabolism of xenobiotics (Souidi et al. 2005), homeostasis effect on vitamin D (Tissandié et al. 2007) and iron homeostasis (Berradi et al. 2008). More iron aggregation, apoptosis in the tubulointerstitial region, and oxidative stress influenced by uranium were also documented (Taulan et al. 2004). Renal tissue disruption was also reported mostly in the cortical part (Canu et al. 2011). Uranium increases blocking of osteoblastic activity, yielding decreased bone volume, and healing interference. In vitro studies revealed that uranium influences instability in genomes and neoplastic conversion in osteoblasts (Canu et al. 2011). This radioisotope alters oxidative metabolism and decreases bone structures (Tasat et al. 2007). According to Prat et al. (2010), uranium modifies the expression of the gene for a biomarker of bone resorption, osteopontin.

Ra stored in bones can cause different types of bone abnormalities and even cancers. Many contemporary researches investigated that radium has many detrimental effects on bone tissues (Canu et al. 2011). Both radioisotopes ^{226}Ra and ^{228}Ra rapidly enhance modifications in bone formation and hematopoiesis. Almost in all species bone sarcomas and leukemias were also reported after ^{224}Ra ingestion.

8 Conventional Methods for Removal of Radionuclide

Various conventional remedial technologies are available for removal of radioactive wastes (Fig. 4). Chemical-reducing agents can be introduced into radioactive contaminated soil or unconfined aquifers to form a subsurface blocker to trap or completely ruin target contaminants. Then water having associated by-products and other remaining reagents is pumped back out. The treatment barrier is a region of suitable redox potential. The aim is to efficiently convert dissolved metals and radionuclides to less soluble structures and to advance the demolition of organic compounds like chlorinated hydrocarbons. This advanced technology permits in situ treatment of groundwater pollutants and avoids disposal expenditure as radionuclides are immobilized in place. The techniques available for treating aqueous radioactive contaminants are generally chemical precipitation, evaporation, ion exchange, reverse osmosis, sorption, ultrafiltration, etc. Sedimentation, decantation, filtration, centrifugation, etc. are techniques applied mainly to remove the effluent wastes, insoluble particles, and other miscellaneous debris (Abdel et al. 2011; IAEA 2001).

Chemical precipitation are mainly applied for eliminating radionuclides from aqueous radioactive contaminants at fuel recycling facilities, different research centers, and power plants. Precipitation techniques are highly versatile and inexpensive, and it can cover a broad range of concentrations of liquid effluents. However, in order to improve the precipitation process, pretreatment steps like oxidation of organic contaminants, degradation of different composites, and alteration of pH and valence state should be carried out prior to the precipitation. Radioactive wastes can also be dispatched using coprecipitation or adsorption (Valdovinos et al. 2014; IAEA 2001).

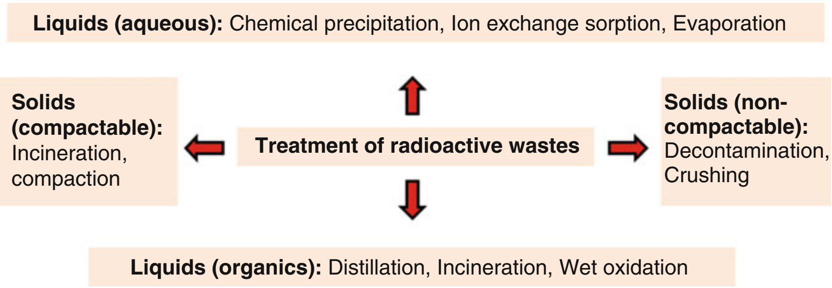

Fig. 4 Classification of different technologies applied to radioactive contaminants (Valdovinos et al. 2014)

Ion exchange process has broad use in order to dislodge soluble radioactive compounds fromliquid solutions generated from nuclear operations, radionuclide formation, and other research activities. It can significantly convert large proportions of radioactive substances into a trace amount of solid substance. Ion exchange technique exchanges positively or negatively charged ions in between solid matrices having ionizable groups and liquid system. Thus, ion exchangers may be reproduced and recovered with its high activity. If exchangers were completely utilized, they are mostly removed and treated as radioactive wastes (Valdovinos et al. 2014).

Varieties of substances are available for the ion exchange technology—(1) ion exchanges such as cellulose, collagen, clays, etc. and (2) synthetic materials like zeolites and oxides containing ionic groups—which has applicability in both continuous and batch systems. When high concentration of radionuclides are present, the water is pretreated before ion exchange process (IAEA 2001).

Evaporation is a potential approach for hazardous materials like radionuclide removal from different liquid wastes. This process is utilized for treatment of different levels of waste effluents. It may be conducted by the application of commercially available evaporation instrument (IAEA 2001).

Again, incineration is also used for removal of radioactive wastes (solid and liquid). This process burns contaminated substances at very high temperatures, producing gases like carbon dioxide, water vapor, sulfur dioxide, nitric oxide, and hydrogen chloride gases as end products of incineration procedure (Valdovinos et al. 2014).

Again, wet oxidation methods require insertion of chemicals which act as oxidizing sources to damage the contaminated compounds containing the radioactive wastes. In comparison to incineration, this method is economical as energy requirement is less (Chang 2001).

Distillation technology can reduce radionuclide volume of pretreating liquid solution and different solvent wastes in conventional equipment. This procedure is easy, well-known, and inexpensive as the valuable solvent is reused. The remaining part could be trapped and then destroyed by incineration (IAEA 2001).

Compaction is a process by which the volume of radioactive waste materials can be reduced and condensed and acts as a processing technique before further remedial measures can be used (Valdovinos et al. 2014; IAEA 2001).

9 Bioremediation Techniques for Removal of Radionuclide

Microbial bioremediation may be a lucrative alternative to excavate different types of contaminated soil. Biological strategies take facility of natural organisms like bacteria, algae, fungi, and plants to demolish or eliminate pollutants or to mineralize metallic pollutants and its associated radioactive elements, thus trapping them in a specific boundary. Microbes like *Rhodanobacter* sp., *Desulfuromusa ferrireducens*, etc. were reported to be capable to eliminate these contaminants (Green et al. 2012; Amachi et al. 2010). The interaction of bacteria induces solubility of modified radionuclides with addition or elimination of electrons, thus raising the motility of the radioactive pollutants and allowing it to be quickly irrigated from our surroundings (Amachi et al. 2010). Thus, microorganism-assisted biotransformation delivers the chances for bioremediation of radionuclides in this Earth, either to trap them in a particular place or to induce their removal. The bioremedial approaches for radioactive materials rely on the active metabolizing efficiencies of different types of microbial cells. Radionuclides may be solubilized by enzymatic oxidation-reduction reaction, alteration in pH and electronic activity, sorption by biomass, degradation of radionuclides using microbes, etc. (Hegazy and Emam 2011; Law et al. 2010; Holker et al. 2002). Activity of radionuclide-degrading microorganisms is highly induced by electron transfer mechanism, several nutrients, and different environmental factors.

Microbial degradation is basically employed to eliminate radioactive contamination from soil surface and also from groundwater where the other organic compounds are operating as chelating agents which influence contaminant movement (Gerber and Fayer 1994; CBCEC 1994). Demolition of the organic compounds assists to decrease rate of transportation which gives the time for radioactive contaminants to decay to harmless levels. Basically native or inoculated microbial species degrade organic pollutants in soil as well as groundwater under optimized either aerobic or anaerobic parameters. The in situ bioremediation of soil generally includes the percolation or insertion of uncontaminated water combined with nutrients and filled with dissolved oxygen. In different cases, microbial species and a source of oxygen are also injected. Many national initiative programs have begun for many years in order to study the bioremediation by microorganisms through noninvasive techniques to remove radioactive contamination. Bioremediation strategies require comparatively inexpensive, low-technology methods and produce very few or no residual waste, which normally have greater level of commercial acceptance (Lloyd and Renshaw 2005). Compared with different other more invading conventional approaches (IAEA 2004), biodegradation may offer a low-cost route for removing radionuclide from polluted soil and water. The conventional remediation approaches are expensive and cause ecological disturbances along with having other limitations. This has resulted in bioremediation becoming the most sought-after approach for radioactive waste removal. Microorganisms including *Deinococcus*, *Geobacter*, *Serratia*, *Kineococcus radiotolerans*, *Hymenobacter metalli* etc. are effective in removal of radioactive waste containing higher level of radionuclides (Roh et al. 2015).

The efficient bioremediation of radioactive contaminants depends on a complex interaction of biological, chemical, and physical procedures. Different types of mechanisms including oxidation, reduction, precipitation, sorption, etc. can influence the toxicity and transfer of radioisotopes in biogeochemical systems (IAEA 2004). Uranium (VI) and technetium (VII) have been reported to be prone to various microbial enzyme actions. The oxidized form of U and Tc is soluble in water, while the reduced ones are not. Thus, enzymatic reduction as mentioned in Fig. 5 is a possible approach for detection and removal of these from groundwater.

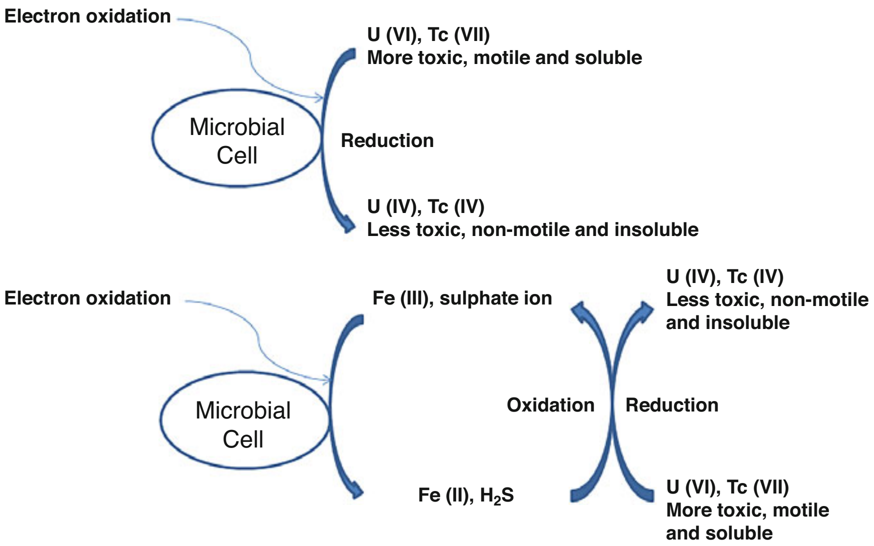

Fig. 5 Microbial approaches of dissolved radioactive (more toxic) metals into insoluble and less hazardous species (Roh et al. 2015)

Moreover, “indirect” methods of reduction can be essential in the immobilization of Tc (VII) as deposits, while the microbially reduced Fe (II) is able to transmit the electrons expeditiously to Tc (VII). Lovely and co-workers first reported that Fe (III)-reducing bacterial species *Geobacter metallireducens* and *Shewanella oneidensis* can be capable of acquiring energy for anaerobic growth by this reduction process. Other microorganisms such as *Clostridium* sp., *Desulfovibrio desulfuricans*, and *Desulfovibrio vulgaris* are also able to do this reduction process but cannot utilize this energy for growth purposes. The first reported microbial species in which enzyme system is accountable for U (VI) reduction was *Desulfovibrio vulgaris*. Payne and co-workers (2001) utilized cytochrome c3 mutant of the close relative *Desulfovibrio desulfuricans* G20 to affirm the function for this electron transfer protein in H_2-dependent reduction process of U (VI), but the electron donation pathway bypassed the use of cytochrome. It was suggested that c-type cytochromes in outer surface of *Geobacter sulfurreducens* might be responsible in U (VI) reduction, but it has now been reported efficient U (VI) reduction can take place at surfaces of electricity conducting pili or microbial nanowires. Orellana described that wild-type species did not precipitate uranium along pili as discussed in earlier studies but that U(IV) was precipitated at the outer layer of bacterial cells. These outcomes are consistent with earlier findings that have reported that *Geobacter sulfurreducens* does not need pili for reducing the extracellular electron acceptors but surface c-type cytochromes for this purpose. *Shewanella oneidensis* MR-1 has 42 putative c-type cytochromes which are necessary for reduction of metal (Roh et al. 2015).

10 Conclusions

Heavy metals and radionuclides are the emerging contaminants of today's world having highly toxic effect on living organisms including human beings. Though natural sources contribute to this contamination, exposure to heavy metals and radionuclides has increased considerably due to anthropogenic activities. We urgently need to curb these activities in order to save mankind from these harmful effects and also work toward removal of the existing contaminants as heavy metal and radionuclides are generally cannot be removed from the environment easily. They can be converted to a lesser toxic form, or contained in remedial organisms like plants and microbes or utilized by microorganisms in their metabolic activities. Modern biotechnological tools of genetic engineering are used nowadays to enhance the remedial properties of the microorganisms. Studies on various microbes and their pathways on one hand will lead to better research on better removal strategies, and also using bioinformatics tools will assist in storing of this information and using them accurately. The use of bioremedial measures over the previously used conventional ones will surely be the path forward in achieving a green, sustainable route for removal/containment of heavy metal and radionuclides from the environment and saving the living organisms from their exposure.

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