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IN SITU AND EX SITU BIOREMEDIATION OF HEAVY METALS: THE PRESENT SCENARIO

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Highlights

- Microbial associated bioremediation techniques.
- Use of ANN and computational approach.
- Use of omics approach.

Abstract. Enhanced population growth, rapid industrialization, urbanization and hazardous industrial practices have resulted in the development of environmental pollution in the past few decades. Heavy metals are one of those pollutants that are related to environmental and public health concerns based on their toxicity. Effective bioremediation may be accomplished through "ex situ" and "in situ" processes, based on the type and concentration of pollutants, characteristics of the site but is not limited to cost. The recent developments in artificial neural network and microbial gene editing help to improve "in situ" bioremediation of heavy metals from the polluted sites. Multi-omics approaches are adopted for the effective removal of heavy metals by various indigenous microbes. This overview introspects two major bioremediation techniques, their principles, limitations and advantages, and the new aspects of nanobiotechnology, computational biology and DNA technology to improve the scenario.

Keywords: bioremediation, "in situ" and "ex situ" bioremediation, bioattenuation, heavy metals.

Introduction

Environmental pollution is increasing rapidly due to urbanization and industrialization. It leads to the rise in toxicity and threat to human life and the environment and becomes a major global concern (Manisalidis et al., 2020). The increase in loads of heavy metals like cadmium (Cd), arsenic (As), chromium (Cr VI), mercury (Hg) and lead (Pb), in aerial, aquatic and terrestrial system results in deterioration of the environment. Enhancement in the concentration of heavy metals within the body causes serious health hazards. Heavy metal contaminations into the environment appear due to both natural and man-made sources. Anthropogenic sources include industrial wastes, metal mining, agricultural practices, automobile emissions and atmospheric deposition (Pazirandeh et al., 1998; Rajendran et al., 2003). Natural resources are affected by contamination due to anthropogenic sources which lead to contamination of agricultural and food products. Various physical and chemical methods exist for the removal of pollutants (Vargas-García et al., 2012). Conventional methods such as excavation, solidification/stabilization are used to remediate heavy metals contaminated site but these technologies permanently do not remove heavy metals. Biological methods are easy to operate and it encourages the establishment of plants on polluted soils. Heavy metals have high density and are toxic at low concentrations (Shazia et al., 2013). Bioremediation is a natural process that involves microorganisms to reduce pollutant concentration. Microorganisms possess a potent role in for maintaining the sustainability of the ecosystem as they adjust themselves to the rapid change of the environment. They possess the ability to evolve life long and they are omnipresent as they bring about its effect to the entire biosphere (Seigle-Murandi et al., 1996). Microbial cells are responsible for various processes that include nitrogen fixation, carbon fixation, sulphur metabolism

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and methane metabolism which on contrary regulated the biogeochemical cycles (Dash & Das, 2012). The metabolic enzymes being produced by these organisms help in the safe removal of the various types of contaminants by the mechanism of converting complex toxic substances to lesser toxic materials (Dash & Das, 2012).

The process of bioremediation can be classified based on aerobic and anaerobic conditions, but remediating processes in aerobic conditions are faster compared to anaerobic conditions (Jeyasingh & Philip, 2005). Type of environment, pollution nature, depth of pollution, location and cost are some of the criteria which are taken into consideration while choosing any bioremediation technique (Frutos et al., 2012; Smith et al., 2015). Apart from these criteria, temperature, pH, nutrient and oxygen concentration determines the success of bioremediation.

Heavy metal contaminated soil or aquatic system can be remediated with the help of "ex situ" and "in situ" techniques. "In situ" bioremediation involves treating soil without excavation. It is a simple process to apply and reduces the risk of contaminants spreading through transport. "In situ" techniques involve minimum technologies for the addition of air, nutrients and microorganisms. "Ex situ" bioremediation involves removing contaminants from the original site, which involves landfill, acid leaching and thermal treatment. A wide spectrum of technologies exists for "ex situ" techniques which are based on different degrees of complexity having a common feature, treatment of excavated soil. "Ex situ" techniques are complex and have a higher cost compared to "in situ" techniques.

This mini-review would focus on various mechanisms of "in situ" and "ex situ" bioremediations of heavy metals by microbial systems. It will focus on the latest systems on machine learning, deep learning and artificial neural networks (ANN) for understanding the mechanisms of bioremediations.

1. Principle of bioremediation

Bioremediation is defined as the process where the contaminants present in soil, sediments and groundwater can be biologically degraded to innocuous substances. It requires three essential components like micro-organisms food and nutrients that are together known to be a bioremediation triangle. Microorganisms play an effective role as it metabolizes the chemicals to produce water, carbon dioxide or methane and biomass. Bioremediation depends on many parameters like the nature of pollutants, type of microorganisms present in the soil, pH, moisture content, soil properties, temperature and reduction-oxidation (redox) potential. The main aim of bioremediation is to create the optimum conditions for biologically degrading the contaminants through bio-stimulation (addition of nutrients, aeration, organic substrates etc.) or bioaugmentation (addition of microorganisms) Mostly bioremediation systems operate under aerobic conditions, but when operated under anaerobic conditions it allows microbial organisms to degrade. The techniques involved in bioremediation are more favorable than the traditional methods as it can be implemented on-site, reducing risks for personnel. The technology is more focused on natural processes so the public considers it more acceptable and greener compared to others. The technique is considered cost-effective and it provides a permanent solution that is less expensive compared to other physicochemical methods and has become more prevalent in treating soils contaminated by heavy metals.

Organisms serve as important agents for bioremediation. *Pseudomonas putida* was firstly patented in the year 1974 as an important agent for the biological remediation of petroleum (Latha & Reddy, 2013).

2. Heavy metals toxicity

Heavy metal toxicity means the potentiality of the metal to bring about detrimental effects on microorganisms that are dependent on the absorbed dose and the bioavailability of the metals (Rasmussen et al., 2000). Heavy metals toxicity includes various mechanisms such as destruction of ion regulation, degradation of enzymatic activity, production of reactive oxygen species (ROS) and degradation of DNA as well as the associated proteins (Patrick & Violaine, 2014; Hildebrandt et al., 2007). Due to the presence of heavy metals, the biochemical and physiological properties of microorganisms can be altered. Chromium (Cr) and Cadmium (Cd) can bring about oxidative denaturation and damage of microorganisms resulting in weakening the bioremediation capacity of the microbial cells.

Cr(III) can change both the structure and activity of enzymes by reacting with their thiol and carboxyl groups (Cervantes et al., 2001). Heavy metals like copper, Cu(I) and Cu(II) catalyze the production of ROS by Fenton and Haber-Weis reactions, which will itself act as soluble electron carriers and causes serious injury to DNA, lipids, cytoplasmic molecules and other proteins.

Cadmium (Cd) and Lead (Pb) can damage cell membranes, destroys the structure of DNA, and have a dangerous impact on microbes. They displace the metals from their native binding sites or ligand interactions (Olaniran et al., 2013).

Aluminum (Al) is responsible for the damage of DNA and can stabilize superoxide radicals. Heavy metal changes the configuration of enzymes by stopping their vital enzymatic functions with substrates by competitive or non-competitive interactions (Patrick & Violaine, 2014).

2.1. Effect of heavy metal on environment

Heavy metals bring about negative impact on all the biotic components of the ecosystem including human being and its desired species. Various heavy metals like copper, cadmium, nickel, lead and methyl mercury produce serious adverse effects upon the environment above a threshold concentration (Hopkins et al., 2000; LeFauve & Connaughton, 2017). These metals generate negative impacts on the germination of seeds, metabolism of plants, resistance mechanisms and growth (Aydinalp & Marinova, 2009). It has also been observed that accumulation of heavy metals results in oxidative stresses on algae leading to the reduction in their chlorophyll contents (Pinto et al., 2003). Metals like arsenic and barium can cause nausea, diarrhea, abdominal pain, decreased blood pressure alongwith lower counts in blood cells. Exposure to zinc is known to have erosive effects on skin (Ismail et al., 2013). Heavy metals like silver and selenium are reported to have abdominal, respiratory and neurological abnormalities causing arygria and selenosis respectively (Martin & Griswold, 2009).

2.2. Heavy metal toxicity in human

Heavy metals bring about several negative impacts upon human health mainly on kidney, liver, respiratory system and nervous system (Godwill et al., 2019). Some of the heavy metals are highly carcinogenic (Godwill et al., 2019), delays growth within human, brings about disruption in the bioregulatory systems resulting in the development of neurodegenerative diseases, chronic fatigue and Alzheimer's diseases (Poey & Philibert, 2000). It has been observed that lead and hexavalent chromium can bring about severe hemotoxic effect (Ray, 2015, 2016). Lead and mercury are also responsible for the development of autoimmunity phenomenon like rheumatoid arthritis, nervous problem, kidney and circulatory system (Lauwerys et al., 2007).

3. Microbial bioremediation of heavy metals

Microbial bioremediation of heavy metals is an effective and ecofriendly way to achieve a pollution free environment by reducing industrial exploitations of chemical methods (Rajendran et al., 2003). Metals that play an important role in various metabolic and redox functions include chromium (Cr), calcium (Ca), copper (Cu), manganese (Mn), magnesium (Mg), sodium (Na), nickel (Ni) and zinc (Zn). Metals such as cadmium (Cd), gold (Au), aluminum (Al), mercury (Hg) and silver (Ag) have no biological role and are toxic to soil microbes. In the process of adsorption of heavy metals, temperature plays an important role. As temperature increases, the rate of adsorbate diffusion across the external boundary layer also resulting in the increased solubility of heavy metals increases which improves the bioavailability of heavy metals (Bandowe et al., 2014). Chromium exists in the environment in two forms: Cr^{6+} in highly toxic form and Cr^{3+} in less soluble toxic form. Cr^{3+} which exists as a trivalent form of chromium is an essential trace element that acts as a cofactor for enzymes in various biological systems e.g. insulin receptor tyrosine kinase gets activated (Davis & Vincent, 1997). Cr^{6+} which is toxic and mutagenic can be reduced to a less toxic trivalent form by several bacteria (Garbisu et al., 1998). Chromate (Cr^{6+}) can be reduced to non-toxic Cr^{3+} and chromate efflux. Chromate efflux is regulated by the sulphate uptake system as accumulation interferes with sulphate metabolism (Peitzsch et al., 1998). Soil and marine sediments which contain several anaerobic and facultative bacteria are capable of reducing Cr^{6+} to Cr^{3+} (Francis, 1990). Anaerobic bacteria which have the ability to reduce iron and sulphate can indirectly reduce Cr^{6+} via Fe(II) and hydrogen sulphide (HS-) respectively (Davis & Vincent, 1997; Pettine et al., 1998).

4. Factors affecting bioremediation of heavy metals by microorganisms

The heavy metals to be acting as stimulator or inhibitor to microorganisms is determined by the total available concentration of heavy metals, chemical form in which the metals exist and the various redox potential. Various factors of environment like pH, temperature, low molecular weight organic acids and humic acids regulate the mechanisms of transportation, transformation, the bioavailability of heavy metals and valence state of the heavy metals towards the microbial cells. Enhancement in the concentration of hydrogen ions results in the development of a positive charge upon the adsorbent surface resulting in the reduction of attraction between the adsorbent and metal cations causing the development of metal toxicity. The adsorption of heavy metals is considerably dependent upon the temperature thus enhancement in temperature

Table 1. Factors affecting bioremediation by the microorganisms (Boopathy, 2000)

Factors	Activities
Microbial cells	Induction of enzymes, production of toxic metabolites, mutations, horizontal gene transfer, help in the proliferation of bacterial cells
Substrates	The structure and chemical composition of the contaminants, variations in the concentrations of the contaminants, amount of toxicity of the contaminants, solubility of the contaminants
Environmental	Depletion of the preferential substrates, inhibitory environmental conditions, lack of nutrients
Limitations of Mass Transfer	Oxygen diffusion and solubility, variation is solubility and miscibility in water and amount of nutrient diffusion
Co-Metabolism Vs Growth substrates	Interactions among the microbial cells, variation in the concentration and alternate carbon sources available
Aerobic and Anaerobic Biological processes	Site comprising of various microbial populations, redox potential, availability of various types of electron acceptors

increases the rate of diffusion across the external boundary layer. The increase in temperature enhances the solubility of the metals thus enhances the bioavailability of these metals (Bandowe & Meusel, 2017). Thus, the increase in the temperature increases the activity of the microorganisms thus increases the metabolism of the microbial systems accelerating the rate of bioremediations (Table 1).

5. Types of bioremediation [“ex situ” and “in situ” bioremediation]

Both “in situ” and “ex situ” bioremediation approaches depend on microbial metabolism. The methods of “in situ” bioremediation are preferred for restoring contaminated soil and water environments (Jørgensen et al., 2007) as they involve the mechanisms of removing target pollutants from the natural environment with the help of the metabolic potential of the microbial systems without the process of excavation of contaminated samples (Fruchter & Demian, 2002). On the other hand, the “ex situ” bioremediation is the process of intervention that brings about degradation of chemical pollutants, present within the excavated samples (Carberry & Wik, 2001). The techniques of “ex situ” remediation appear to be more expensive in comparison to that of “in situ” techniques. These mechanisms of bioremediation show significant differences in their experimental controls and the consistency of the process outcome. The process of “ex situ” bioremediation can be manipulated with the help of various physicochemical treatments as it is carried out in a non-natural environment (Kim et al., 2005). The mechanism of “in situ” remediation is mainly targeted to the restoration of the normalcy of the contaminated environment by the usage of various treatment technologies.

5.1. “Ex situ” bioremediation techniques

This involves excavation or removal of contaminated soil and subsequently transporting them to another site for treatment. The geographical location of the polluted site, cost of treatment, type of pollutant, depth of pollution and degree of pollution are the main criteria for “ex situ” bioremediation techniques. “Ex situ” bioremediation techniques are easier to control and are used to treat a wider range of toxins and soils. Here the material is typically mixed, well-aerated and plenty of nutrients are available so the breakdown of contaminants is must faster compared to “in situ” techniques.

5.1.1. Biopile

Biopile mediated bioremediation involves heaping contaminated soils into piles, followed by nutrient amendment and aeration to augment bioremediation by increasing microbial activities. Different terms of biopiles such as bioheaps, biocells, biomounds are used for remediating petroleum contaminants present in soil and sediments. Nutrient, temperature, moisture, pH are adequately controlled to enhance biodegradation and for this constructive features use of this particular “ex situ” technique is increasing (Whelan et al., 2015). Biopile is considered as a better pollutant removal strategy as it has higher efficiency compared to land farming and composting based on mass transfer of water, nutrients and air. This technique is used to remove heavy metals from soil (Tampouris et al., 2001). It can be applied to remediate extremely polluted environments such as very cold regions. It can also treat large volumes of polluted soil in limited space. This process can be easily scaled up to a pilot system for achieving the same performance which was obtained during laboratory studies (Chemlal et al., 2013). Sieving and aeration of contaminated soil are important to maintain the efficiency of biopile (Delille et al., 2008).

5.1.2. Windrow

To enhance bioremediation, windrows focus on periodically turning the piled polluted soil by enhancing the rate of degradation of indigenous or transient hydrocarbonoclastic bacteria observed within polluted soil. The periodic turning of polluted soil increases the rate of bioremediation by increasing aeration with the addition of water, uniform distribution of nutrients, pollutants and microbial degrading activities which can be performed by assimilation, mineralization and biotransformation In windrows, the release of greenhouse gas, methane (CH_4) was due to the development of anaerobic zones within polluted soil (Hobson et al., 2005). Windrow treatment is not a suitable option for remediating soil polluted with toxic volatiles as it involves periodic turning. Humic substances were found to be effective for the removal of heavy metals like Cd, Ni. (Zhang et al., 2019). The fractionation of metals like Cu, Zn, Fe and Mn, Cd are accomplished by this method (Achiba et al., 2009).

5.1.3. Land-farming

Landfarming requires less equipment for operation and low-cost characteristics. The polluted soils are either tilled or excavated, but the site of treatment depends on the type of bioremediation. The production of leachate compounds must be taken into account while applying land farming as a leachate collection and treatment system is required depending on the nature of the site, to prevent groundwater contamination. Landfarming creates air pollution problems and health risks for the workers due to the emission of volatile organic compounds (VOCs), which can be reduced by covering the area with a greenhouse structure for minimizing the dust. Bioremediation proceeds without excavation, when a pollutant lies <1 m below the ground surface, whereas the pollutant, is transported to the ground surface for bioremediation when it lies >1.7 m (Nikolopoulou et al., 2013). Tillage enhances bioremediation by stimulating the activity of autochthonous microorganisms during landfarming. The major operations of tillage are the addition of nutrients (nitrogen, phosphorus and potassium), irrigation and aeration. Both tillage and irrigation having appropriate biological activity enhance the rate of bioremediation by increasing the heterotrophic bacterial counts. Microbial dehydrogenase activity can be used as a biological parameter in landfarming as it was observed to be a good indicator of biostimulation treatment (Silva-Castro et al., 2015).

Though landfarming is the simplest bioremediation technique, it has some limitations. It requires a large operating space, additional cost due to excavation, microbial activity reduction due to unfavorable environmental conditions and reduced efficiency in inorganic removal of pollutants (Khan et al., 2004; Maila & Cloete, 2004). These limitations make the technique time-consuming and less efficient compared to other *ex situ* remediation techniques.

5.1.4. Bioreactor

Bioreactor, as the name suggests, is a vessel designed for the removal of pollutants from wastewater or pumped groundwater using microbes. It is an "ex situ" biological processing system used for the treatment of contaminated soils in solid and liquid (slurry) phases. The different operating modes of the bioreactor are batch, fed-batch, sequencing batch, multistage and continuous. The operating mode of the bioreactor is based on the total economy of the market and capital expenditure. For providing optimum growth conditions, bioreactor supports natural processes of cells by maintaining their environment. Temperature, pH, moisture, inoculum concentration, aeration rate, agitation rate and substrate concentration are the parameters important for bioreactor. The parameters in a bioreactor can be controlled and manipulated which implies that the biological reactions present within can be augmented to effectively reduce bioremediation time. The limiting factors of bioremediation process in a bioreactor are addition of nutrients, controlled bioaugmentation, increased bioavailability of pollutants and mass transfer. Heavy metal removal in an Anaerobic Upflow (ANFLOW) bioreactor is similar to an ion exchange process, where a saturation transient begins at the bottom of the column and moves upward (Rivera, 1983). About 80% for Cu(II), 98% for Pb(II), 50% for Ni(II) and 77% for Zn(II) bioremediation was found to be achieved in a Membrane bioreactor (MBR) system (Katsou et al., 2011). It was found that the fixed bed bioreactors was able to increase the efficiency towards the removal of metals (Cu^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, and Fe^{3+}) in polluted environmental samples by a strain of *Pseudomonas aeruginosa* to control many harmful health hazard substances (Ibrahim et al., 2019). On the other hand, removal of heavy metals including nickel, arsenic, cadmium, antimony and lead by membrane bioreactor was investigated by Komesli (2014). Aftab et al. (2017) effectively removed Chromium (Cr) and Lead (Pb) metals, from wastewater by osmotic membrane bioreactor (OMBR). About 90% removal of heavy metals (nickel and chromium) was done through biosorption in fixed packed-bed bioreactor at alkaline pH for 20 days (Barros et al., 2006).

Due to some reasons, bioreactor- based bioremediation is not a particular practice for heavy metal removal. Firstly, it requires more manpower, capital and safety measures to treat the large volume of polluted soils or other substances and transporting pollutants to the site of treatment, which make this technique cost ineffective (Philp & Atlas, 2005). Secondly, having so many bioprocess variables of a bioreactor, if any variable is not properly controlled it becomes a limiting factor which results in the reduction of microbial activities making the technique less effective. Thirdly, pollutants respond to different bioreactors in different way so the availability of the most suitable design is of utmost importance.

5.2. "In situ" techniques of bioremediation

"In situ" bioremediation techniques involve treating the contaminants at the site of pollution where they can be biologically degraded under natural conditions. This technique does not require any excavation, so it involves much less effort of transportation and physical displacement than required for "ex situ" bioremediation techniques. Apart from removal of heavy metals, "in situ" bioremediation is used for the treatment of dyes, chlorinated solvents and hydrocarbon polluted sites (Kim et al., 2014; Frascari et al., 2015; Folch et al., 2013; Roy et al., 2015). But, "in situ" treatment generally requires longer time and there is less certainty about the uniformity of treatment because of the variations in soil characteristics and efficacy of the process is very difficult to verify. For a successful "in situ" bioremediation, moisture content, temperature, pH, oxygen supply and nutrient supply are the major environmental conditions that need to be suitable, of which, the availability of molecular oxygen is the major problem.

5.2.1. Intrinsic methodology of bioremediation

The mechanism of natural attenuation or intrinsic bioremediation is an inherent technique of "in situ" remediation process which deals with both microbial aerobic and anaerobic processes to remediate the pollutants or contaminants without using any external input. This process is the first choice for bio treatment as it does not require any intervention. The method allows the ecosystem to revert to its original condition, avoids damaging the habitat and enables detoxification of toxic compounds. The term, monitored natural attenuation (MNA) represents a more comprehensive approach to intrinsic bioremediation. Bioavailability is a crucial step in case of intrinsic bioremediation, as in this case biological materials are used to investigate the presence of heavy metals. The major disadvantage of intrinsic bioremediation is the prolonged time it needs to reach the target level.

5.2.2. Bioattenuation

The technique relies on transforming pollutants to less harmful forms or immobilized state (Smets & Pritchard, 2003). On the basis of physical and chemical principles, the costs are 80–90% lower than other clean-up technologies (Davis et al., 1994; Mulligan & Yong, 2004). Fauziah et al. (2017) *reported about the successful removal of* metals like As, Ni and Al by proteobacteria mediated bioattenuation, whereas Acinetobacteria were found to take part in bioattenuation process for natural removal of metals like U, Al, Cd, Co, Cu, Mn, Ni (Schmidt et al., 2005).

5.2.3. Bioaugmentation

Bioaugmentation, the process where enriched consortia or strains that are added as the required microbial populations do not exist in sufficient number for degrading the target compounds (Robles-González et al., 2008). Hence for achieving success, mixed cultures having variety of microorganisms are used in practice (Di Toro et al., 2006). Various biotic and abiotic factors responsible for the purpose of effective bioaugmentation (Bento et al., 2005); which includes cadmium enriched soil by a newly isolated strain of *Bacillus* sp. and a *Streptomyces* sp. (Lebeau & Jézéquel, 2008) and removal of heavy metals (Ni, Pb, and Zn) by a consortium of filamentous fungi using bioaugmentation procedure are reported.

Although various adverse biotic and abiotic factors affect autochthonous microorganisms and they may alter their surroundings by releasing organic compounds, the success of bioaugmentation can be improved by selecting the proper operating strategies, aimed at improving the survival and long-term efficiency of the inoculated microbial species.

5.2.4. Biostimulation

Biostimulation for heavy metals depends on the supply of nutrients (carbon, nitrogen, phosphorus), temperature, oxygen, pH, redox potential and concentration and type of organic pollutant for accelerating the microbial turnover of chemical pollutants (Carberry & Wik, 2001; Bundy et al., 2002; Atagana, 2008; Al-Sulaimani et al., 2010).

Addition of biodegradable compounds which act as primary substrates is another strategy of biostimulation where the pollutant is degraded as secondary substrate but at acceptable rates.

Temperature increases biomass activity which plays a positive role in biostimulation, and temperature control can be achieved only in engineered systems such as slurry bioreactors and contained vessel composting. Fulekar et al. (2012), carried out a biostimulation study for removing Fe, Cu, and Cd using aerobic bacteria cultured from isolated heavy metals, Kanmani et al. (2012) also performed biostimulation for Cr removal by using heterogeneous groups of bacteria isolated from contaminated sites.

5.2.5. Bioslurping

Bioslurping involves the simultaneous application of vacuum enhanced recovery, soil vapor extraction and bioventing to achieve soil and groundwater remediation by indirectly incorporating oxygen and stimulating biodegradation of contaminants (Gidarakos & Aivalioti, 2007). It is designed for free product recovery such as light non aqueous phase liquids (LNAPLs) without extracting vast quantities of ground water. The system uses a "slurp" tube that extends into the free product layer that draws up liquid (including soil gas and free product) from the layer in a way similar to a straw draws liquid from any vessel. Bioslurping of cadmium ions from soils was done using *Arabidopsis helleri (*However, the technique is not suitable for remediating soil with low permeability and moisture content as it dries out the soil and curbs the effectiveness of bioremediation). The technique also can not treat residual contamination in saturated soils and is applicable at sites with water table greater than 30 feet. The major concern of this particular "in situ" technique is the establishment of vacuum on fluctuating water table and deep high permeable site which creates saturated soil lenses that are difficult to aerate (Figure 1).

5.2.6. Bioventing

The ultimate goal in bioventing is to achieve microbial transformation (Table 2) of pollutants to a harmless state by addition of nutrients and moisture to enhance bioremediation (Philp & Atlas, 2005). The two basic criteria for successful bioventing are to maintain aerobic conditions and to obtain reasonable biodegradation rates, natural hydrocarbon degrading microorganisms must be present in enough concentrations and hence air injection rate is one of the main parameters for pollutant dispersal. "In situ" treatment of heavy metals generally involves pumping oxygen/nutrients (bioventing/biostimulation) into the soil (Kapahi & Sachdeva, 2019).

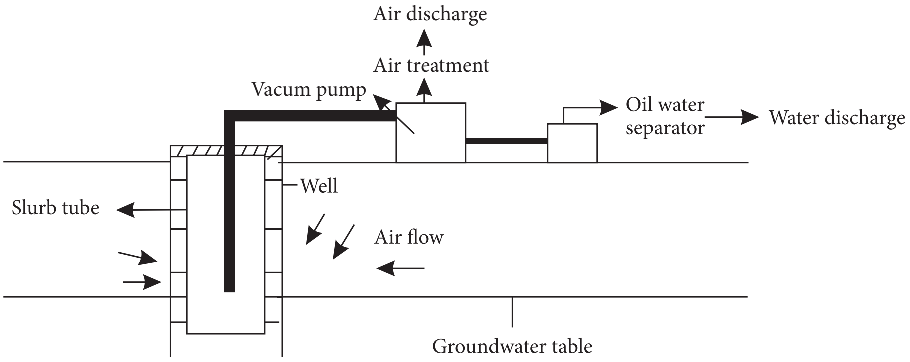

Figure 1. "In situ" bioremediation by the technique of Bioslurping

Table 2. Potential mechanisms of microbial transformation of metals

Types of Metabolite Reaction	Microorganisms Involved	Potential of the Microorganisms	Reference
Redox Reaction			
Oxidation and Reduction Reaction	*Leptospirillum ferrooxidans, Acidithiobacillus thiooxidans, Rhodococcus* sp., *Streptomyces lividans* sp.	Enhances the mobility of As, Cd, Cu, Hg and Zn	Yang et al. (2012)
Polymeric Substances			
Glomalin	*Glomus mosseae*	Immobilizes Pd, Cd and Cu	González-Chávez et al. (2004)
Extracellular Polymeric Substances	*Azotobacter* sp.	Immobilized Cr and Cd	Joshi and Juwarkar (2009)
Biosurfactant			
Organic Acid			
5-ketoglutaric acid, Gluconic acid	*Pseudomonas aeruginosa, Gluconacetobacter diazotrophicus*	Solubilized ZnO, $Zn_3(PO_4)_2$, $ZnCO_3$	Saravanan et al. (2007)
Siderophores			
Azotobactin, Azoto chelin	*Azotobacter vinelandii*	Helps in the acquisition of Mo	Wichard et al. (2009)
Coelichelin and Desferrioxamine	*Streptomyces tendae*	Enhanced uptake rate of Fe and Cd by plants	Dimkpa et al. (2009)

At the vadose zone, bioventing enhances microbial degradation process by moderately injecting air (Figure 2) whereas soil vapour extraction (SVE) relies on maximization of volatile organic compound volatilization (Magalhães et al., 2009). In soil-vapour extraction, the flow rate of air is higher compared to that of bioventing (Baker & Moore, 2000).

5.2.7. Biosparging

This technique involves injection of air into soil subsurface to enhance the rate of biological degradation of contaminants by naturally occurring bacteria. The effectiveness of biosparging depends on two factors: pollutant biodegradability and soil permeability (Philp & Atlas, 2005). The bacteria, in stressful condition produce metal-adsorbing materials, which chemically interact with pollutants causing their precipitation. These contaminants get degraded during biosparging as oxygen input creates aerobic condition suitable for the degradative action of indigenous microbes (Adams & Reddy, 2003). This results in the raise in concentration of dissolved oxygen, redox potentials, sulphate, nitrate and reduction in methane, sulphide and iron (Table 2).

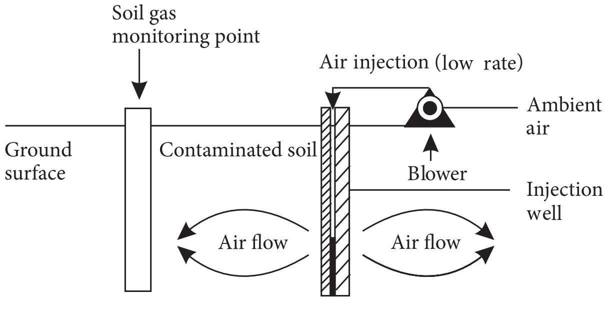

Figure 2. "In situ" bioremediation by the technique of bioventing

5.2.8. Permeable reactive barrier (PRB)

This technique also referred to as permeable reactive treatment zone (PRTZ), is used for remediating contaminated groundwater. The wall is "permeable" which means that groundwater can flow through it. This process involves in remediating the polluted ground water that usually comprise of chlorinated compounds and heavy metals. In this technique, the reactive barrier (medium) used is made of zero valent iron which is submerged in the trajectory of the polluted groundwater (García et al., 2014; Zhou et al., 2014). When the polluted water flows through the barrier, pollutants get trapped and undergo various reactions resulting in the flow of clean water (Thiruvenkatachari et al., 2008; Obiri-Nyarko et al., 2014). The barriers are quite reactive enough to trap the pollutants and allow the flow of water but not pollutants (De Pourcq et al., 2015). For removal of contaminants from groundwater, there are four types of reactive processes used in PRB: abiotic reduction, biotic reduction-oxidation, chemical precipitation and sorption of ion exchange.

Researchers are more focused on coupling PRB with electrokinetics method for treating various kinds of pollutants (García et al., 2014; Mena et al., 2015). Maximum removals of Zn, Pb, Cu and Cd were achieved under different experimental conditions. The voltage gradient and processing time were shown to have significant effects on the removal of Cu and Cd, whereas the addition of the oxalic acid had a more significant influence on the removal of Pb (Huang et al., 2015). The effectiveness of the technique depends on the biogeochemical and hydrogeological conditions, contaminant distribution and cost. The effectiveness of the technique can be increased by improving PRB designs and advanced site characterization methods (Gibert et al., 2013). The use of iron sulphide (FeS) barrier will help to overcome the problems faced due to the use of zero valent iron (ZVI) (Henderson & Demond, 2013).

5.3. Phytoremediation

Phytoremediation or plant-based bioremediation is a very cost-effective, ecofriendly and prominent technique which has been used for years (Azubuike et al., 2016). This technique involves the use of plants and associated microorganisms to completely remediate the selected contaminants from soil, sediments, sludge, wastewater and groundwater. In phytoremediation, based on the type of pollutants (organic or elemental) there are several mechanisms available such as filtration, degradation, accumulation or extraction, stabilization and volatilization. Organic pollutants such as hydrocarbons and chlorinated compounds can be removed by degradation, stabilization, rhizoremediation, volatilization and mineralization (Meagher, 2000). Elemental pollutants such as radionuclides and toxic heavy metals can be removed by transformation, extraction and sequestration.

All plants have the ability to accumulate essential metals (such as Ca, Cu, Co, Mg, Mn, Na, K, Zn, Se) as well as non-essential metals (As, Au, Cd, Cr, Pb, Hg, Sb, Pd, Pt) from the soil. Non-essential metals have no known biological function. While choosing a plant as a phytoremediator, some factors to be considered are root system (tap or fibrous) depending on the depth of the existence of pollutant, plant survival based on the prevailing environmental conditions, growth rate of the plant and time required to accomplish the desired level of cleanliness. Plants should be resistant to pests and diseases (Lee, 2013). The process of removal of contaminants by plants involves: uptake by passive process, translocation from roots to shoots which is carried out by flow of xylem and accumulation in shoot (San Miguel et al., 2013). Both translocation and accumulation depend on transpiration. Plants which grow in any polluted site are good phytoremediators. Hence, optimizing the remediation potential of plants growing in any polluted sites by either bio stimulation or by bioaugmentation with exogenous or endogenous plant rhizobacteria is of immense importance. Plant growth promoting rhizobacteria (PGPR) which play an important role in phytoremediation, enhance biomass production and help the plant to tolerate the stress of heavy metals and other unfavorable soil conditions (Yancheshmeh et al., 2011; de-Bashan et al., 2012). It has been reported that *Zea mays* and *Brachiaria mutica* act as potential phytoremediators of heavy metal contaminated soils (Ijaz et al., 2015; Tiecher et al., 2016).

Rhizoremediation which is an elemental component of phytoremediation can occur naturally or can be triggered by using plant growth promoting microorganisms. The presence of contaminants in soil which is deeper than the root zone of plants requires excavation or selection of trees with deeper roots. Another type of phytoremediation involving trees is Dendroremediation, which is useful in attenuating certain pollutants such as trichloroethylene and 2,4,6-trinitrotoluene from groundwater and soil. Various secondary plant metabolites (SPMEs) produced by plants includes phytohormones/phytoalexins, phytosiderophores, root exudates and phytoanticipins.

Phytoremediation has various advantages, like it restores habitat, reduce remedial costs, low installation and maintenance cost, cleanup the contaminants in place rather than transporting the problem to another site. In phytoremediation, soil fertility might be improved due to the input of organic matter (Mench et al., 2009). The microbes associated with the rhizosphere of the plants play an important role in the mechanism of phytoremediation by changing the bioavailability through alterations in pH, redox potentials and release of some chemicals like chelators such as organic acids, siderophores, biosurfactants etc. (Miransari, 2013).

5.4. Mycoremediation

Fungi play an important role in the mechanism of remediating contaminants within the environment and help in the restoration of normalcy from the weakened condition. The process of mycofiltration shows similarity to the filtration of toxic substances by the use of the fungal mycelia. The various types of fungi including endophytic, saprophytic and mycorrhizal help in the recovering of the soil-water ecosystem thus help in balancing the living population. Various types of extracellular enzymes are produced by the myecelium which helps in the breakdown of the cellulose, lignin and other types of the building blocks of the plant fibres. The mechanism of mycoremediation utilizes the right type of fungal species in remediating a specific type of pollutant (Stamets, 2005). Various studies showed that fungi like *Penicillium* sp., *Ganoderma lucidum, Cladosporium resinae, Aureobasidium pullulans*, *Aspergillus niger, Rhizopus arrhizus and Trametes versicolor* can be effectively used in the process of remediating the heavy metals (Loukidou et al., 2003; Say et al., 2003; Taştan et al., 2010). Studies also showed that *Aspergillus versicolor* help in remediating chromium from waste water effluents.

5.5. Cyanoremediation

The influx of heavy metals like Cd, Ni and V into the atmosphere occurs at much faster rate in comparison to the degradation by natural processes. This results in the accumulation of toxic metals within various ecosystems. Various organisms like bacteria, green algae (Deng et al., 2007), microalgae (Norström et al., 2004) and blue-green algae (Tripathi et al., 2008; Yin et al., 2012) has played an effective role in treating the contaminated wastes being present within the aquatic eco-system. The mechanism of cyanoremediation of heavy metals played an effective role in controlling pollution (Yin et al., 2012).

The degradation of heavy metals was achieved by the use of varieties of microbes (Table 3).

5.6. Nanoparticle associated bioremediation

An emerging "in *situ*" technology is *nanoremediation*, to reduce the source and/or to manage the contaminant plume along the pathway. Nanoparticles derived from plants, fungi and bacteria play an important role in remediating environmental toxic wastes. This mechanism is also known as Nanobioremediation (NBR), which is an emerging field of remediating toxic wastes being present within the nature. Various roles are played by different types of nanomaterials (NM) or nanoparticles (NPs) or nanostructure materials, nanocomposites or nanoclusters in the mechanism of bioremediation (Anjum et al., 2016; Kardam et al., 2012). The nanoparticles prove to be effective agents in cleaning up of the environmental pollutants although possessing zero-valency since they can easily penetrate up to the region of contamination where other types of microparticles do not possess the ability to reach. They possess higher reactivity to the contaminants in comparison to the other types of the microsized particles being used for clearing of the contaminants.

6. Artificial neural network (ANN) mediated bioremediation

ANN is a type of computational simulation based on mathematical models which works on the basis of the neurological functions of the brain (Manahan, 2010). The Application of ANN involves mathematical modelling that can be used for the purpose of quantification of Cr concentration with the dwarf bean and the amount of phytoremediation being performed by the plant (Hattab et al., 2013).

Table 3. Heavy metal bioremediation by microbes

Type of Microbes	Name of the Species	Heavy Metal Remediated	Reference
Bacteria	Bacillus cereus	Cr	Dong et al. (2013)
	Escherichia coli, Pseudomonas sp., *Micrococcus, Bacillus, Streptococcus, Salmonella*	Fe, Cu, Cd	Fulekar et al. (2012)
	Bacillus cereus	Cr	Kanmani et al. (2012)
	Bacillus cereus	Cd, Zn	Hrynkiewicz et al. (2012)
	Pseudomonas aeruginosa	U	Choudhary and Sar (2011)
	Kocuria flava	Cu	Achal et al. (2011)
	Bacillus sp.	Cd, Cu, Pb	Guo et al. (2010)
	Pseudomonas and Bacillus	U	Kumari and Singh (2011)
	Rhizobacteria sp, Bradyrhizobium sp.	Cd, Pb, Cu	Dary et al. (2010)
	Burkholderia sp.	Cd, Pb	Jiang et al. (2008)
	Pseudomonas veronii	Cd, Zn, Cu	Vullo et al. (2008)
Fungi	Rhizopus, Penicillium, Aspergillus, Mucor	Fe, Cu, Cd	Fulekar et al. (2012)
	Cladonia rangiformis	Pb	Mani and Kumar (2013)
	Aspergillus fumigatus	Pb	Mani and Kumar (2013)
	Penicillium sp., *Ganoderma lucidum*	Ar	Mani and Kumar (2013)
	Penicillium canescens	Cr	Mani and Kumar (2013)
	Aspergillus versicolor	Ni, Cr, Cu	Mani and Kumar (2013)
Algae	*Spirulina* sp. and *Spirogyra* sp.	Zn, Cu, Fe, Mn, Cr, Se	Mani and Kumar (2013)
	Rhizoclonium sp., *Oedogonium* sp. and *Hydrodictylon* sp.	As, V	Mani and Kumar (2013)
	Spirullina sp. and *Spirogyra* sp.	Cu, Fe, Cr, Mn, Zn	Mani and Kumar (2013)
	Cladophora sp. *and Spirogyra* sp.	Cu, Pb	Mani and Kumar (2013)
	Cladophora fascicularis	Pb	Mani and Kumar (2013)
	Chlorella pyrendoidosa	U	Mani and Kumar (2013)
	Ascophyllum nodosum, Chlorella fusca, Aspergillus niger, Bacillus firmus	Ni, Cd, Pb, Zn, Cu, Cr,	Mani and Kumar (2013)

7. System biology and gene editing tool bioremediation studies

7.1. Gene editing for "ex situ" bioremediation

The mechanism of gene editing involves the process of manipulation of DNA with the help of engineered nucleases. The use of nucleases has proven to have immense application in vast areas of researches including animals, plants and microbes (Butt et al., 2018). Studies has shown that the gene editing tools (Figure 3) possess the ability to enhance the rate of bioremediation by the mechanism of conversion of toxic compounds to lesser toxic substances, elimination of the xenobiotics, remediation of heavy metals and ability to degrade complex forms of the pesticides (Basu et al., 2018). At present condition the major gene editing tools include ZFN, TALEN and CRISPR-Cas has proven to enhance the mechanism of bioremediation. The mechanism of CRISPR-Cas is one of the most effective mechanisms for the purpose of gene editing comprise of three types and various subtypes (Zhu et al., 2018; Behler et al., 2018). TALEN or Transcription Activator Like Effector Nuclease which also acts as an efficient tool for the purpose of gene editing and helps in enhancing the rate of bioremediation. Another commonly used mechanism of gene editing is Zinc Finger Nucleases or Zinc Finger Proteins (ZFPs) which helps in accurate target gene editing.

7.2. Biodegradation network for ex situ bioremediation

Bioinformatics and computational tools are alternate approaches towards the mechanism of bioremediation (Malla et al., 2018; Vanacek et al., 2018). The online database provides a platform (Table 4) for the purpose of retrieving

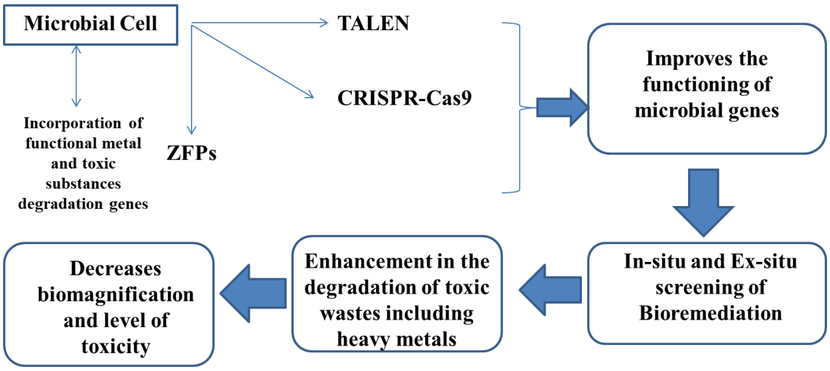

Figure 3. Various types of gene editing tools for bioremediation (TALEN: Transcription Activator Like Effector Nuclease, ZFPs- Zinc Finger Proteins)

Table 4. Computation oriented biodegradation studies

Databases for Understanding Bioremediations	Links for the Servers	Utility in the Process of Bioremediation	Reference
The Environmental Contaminant Biotransformation Pathway (EAWAGBBD/PPS)	https://envipath.org/	Provides the multi-omics information	Malla et al. (2018)
Microbial Genome Database (MBGD)	http://mbgd.genome/	Provides information about comparative aspect on microbial genome	Fory et al. (2014)
Metarouter	http://pdg.cnb.uam.es/MetaRouter	It helps in maintaining diverse information related to biodegradation or bioremediation	Paliwal et al. (2012)
Metabolic Pathway Database (MetaCyc)	https://metacyc.org/	It helps in assessing the catabolic pathways and helps in predicting various metabolic pathways	Millacura et al. (2017)
Pathway/Genome Databases (BioCyc)	https://biocyc.org/	It provides information regarding the genetics and biochemistry of toxic waste degradation by microorganisms	Arora and Bae (2014)
Oxygenase Database (OxDBase)	http://crdd.osdd.net/raghava/oxdbase/	It provides information regarding various types of oxygenase activities	Arora and Bae (2014)
Biodegradation Network-Molecular Biology Database (Bionemo)	http://bionemo.bioinfo.cnio.es/	It provides information regarding the regulation of degradation by metabolic pathways and the transcriptional factors involved in the mechanism of degradation	Arora and Bae (2014)
University of Minnesota Biocatalysis/Biodegradation Database (UMBBD)	https://www.msi.umn.edu/content/university-minnesota-biocatalysis-and-biodegradation-database	It provides information pertaining to the mechanisms involved in degradation of toxic substances and the various genes, enzymes and various types of microbial enzymes involved in the processes	Arora and Bae (2014)

information on the mechanism of biodegradation by microorganisms and the pathways that are involved in the mechanism of degrading the toxic chemicals (Nolte et al., 2018). Various databases are involved that provide the information and mechanism of bioremediation of toxic chemicals.

7.3. Computational tool for assessing "ex situ" bioremediation

The interactions between the microbes and various chemical compounds can be analyzed with the help of various scientific technologies and system biology approaches. The process of improving the soil health can be analyzed by the integration of various computational methods (De Sousa et al., 2018). Molecular interactions of the microbial enzymes with targeted toxic compounds or heavy metals can be analyzed with the help of in silico approaches (Malla et al., 2018). Computational Biology help in understanding the in silico approaches of proteins and genes by considering a cellular system (Purohit et al., 2018). The in silico technique provides the concept of various metabolic pathways that are involved in the mechanism of bioremediation of toxic metals (Liu et al., 2018). This technique also helps in the mechanism of data mining by understanding the knowledge on various mechanisms involved in the metabolic network for the enhancement of cellular processes involving the mechanism of bioremediation (Ostrem Loss & Yu, 2018). The stoichiometric analysis of metabolic network can be analyzed by the determination of metabolic flux analysis (MFA), flux balance analysis (FBA) and metabolic pathway analysis (MPA) (Zhang & Xiu, 2009).

7.4. Multi-omics approach

The utility of computational application in the field of biological sciences help in understanding the interactions of various proteins encoded by genes with a cellular model or the model developed by organisms (Figure 4). Hence provides a suitable platform in studying the metabolic processes involved in biodegradation or bioremediations. The field of Genomics helps in studying various molecular level genetics approaches (Jaiswal et al., 2019).

Conclusions and future prospects

This review categorically depicted the scientific progress and various applications of the biotechnological tools for environmental management and its implication in the process of bioremediation to protect the environment and mitigate hazardous heavy metals. Various environmental problems are to be seriously analyzed and properly addressed to prevent environmental degradation. The mechanism also involved the multi-faceted use of systems biology and computational techniques for determining the mode of degradation and various types of interactions that are involved. There are various types of approaches like the use of microbial induced calcite precipitation by the involvement of the urease hydrolyzing bacteria, development of nanoparticles and the use of biosensors in the detection of remediation of toxic metals from the environment. Another important mechanism addressed is genetic modification for developing microbes possessing greater efficacies in degradation of various heavy metals being present within the environment. The future prospects of the remediation techniques include the adoption of better strategies by the policymakers and implementation of highly advanced techniques by the bioremediation practitioners under the surveillance of the environmentalists to clean up the toxic heavy metals from the environment to make it more habitable for our future generations.

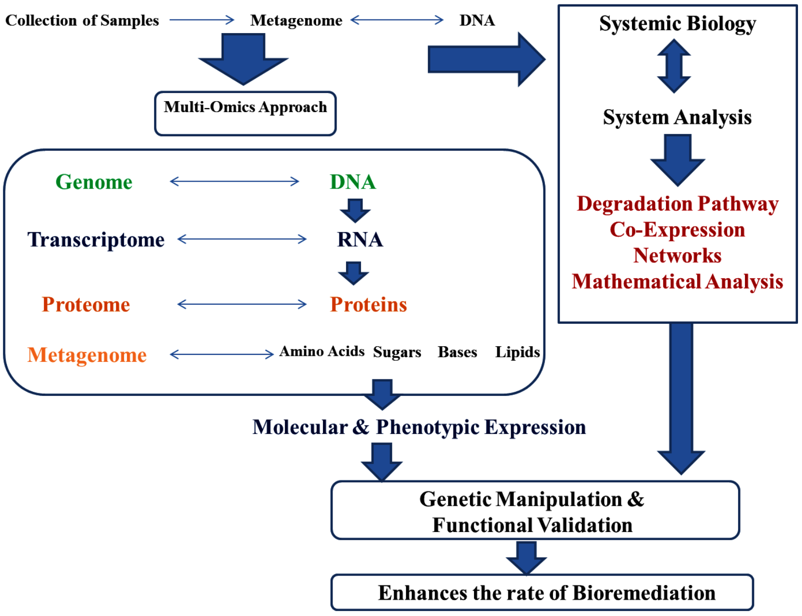

Figure 4. Multi-omics approach for improving bioremediation

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Authors' contributions

All the authors contributed equally.

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