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Bioremediation: A Sustainable Tool for Environmental Management – A Review

Sonal Bhatnagar^{1*} and Reeta Kumari²

¹*Department of Botany, Deshbandhu College, University of Delhi, Kalkaji, New Delhi - 110019, India.*

²*Department of Botany, Sri Venkateswara College, University of Delhi, Dhaula Kuan, New Delhi - 110021, India.*

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Review Article

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ABSTRACT

Bioremediation is considered as one of the safer, cleaner, cost effective and environmental friendly technology for decontaminating sites which are contaminated with wide range of pollutants. Various industrial and anthropogenic activities resulted in increased contaminated sites due to unawareness regarding production, use and disposal of hazardous substances. The process of bioremediation uses various agents such as bacteria, yeast, fungi, algae and higher plants as major tools in treating oil spills and heavy metals present in the environment. A continuous search for the new biological forms is required to regulate increasing pollution and environmental problems faced by man residing in an area. As microorganism shows wide range of mechanisms, there are still few mechanisms which are not known, therefore bioremediation is still considered as a developing technology. Thus, there is an urgent need to for us to review and modify the available options for environmental clean up. The objective of this paper is to conduct a comprehensive review on various sources of bioremediation agents and their limitations in treating pollutants present in the environment.

Keywords: *Biological agents; conservation; natural resources; mycoremediation; phycoremediation; phytoremediation; pollutants.*

*Corresponding author: Email: sbhatnagar.du@gmail.com;

1. INTRODUCTION

The planet 'Earth' is endowed with rich wealth of natural resources such as forests, wildlife, land, soil, air, water, wind, plants and animals. The race begins when humans started living a stable life rather than a nomadic life. Since the advent of civilization the use and overuse, and now the misuse has led to depletion of various natural resources to an extent that today half of our natural wealth are either depleted or at the edge of depletion [1]. In early times, it was believed that our land and its resources are in abundance and will remain available for decades. However, today existing state of our resources shows carelessness and negligence in using them. There are various examples which indicate the exploitation of natural resources by the use of chemical fertilizers in agriculture, release of industrial waste, anthropogenic activities and burning of fossil fuels etc.

The use of chemical fertilizers, pesticides and herbicides in agriculture has improved the crop yield and productivity but led to the addition of detrimental amount of nitrogen and phosphorus in soil and terrestrial ecosystem. Similarly, the release of toxic contaminants from various man made sources resulted in contamination of natural resources leading to scarcity of clean water and loss of soil fertility [2,3]. The industrial and anthropogenic activities had also led to the contamination of agricultural lands resulting in loss of biodiversity. The biodiversity of plant and animal species play important role in the development of healthy and productive ecosystem and, thus contribute to large number of economic benefits to man and environment. Unfortunately, rapidly growing population and increased human activity has threatened many of these species. The natural processes such as crude oil formation, soil formation, waste disposal, nitrogen fixation, biological pest control, pharmaceutical production, dispersal of fruits and pollination are all accomplished by the enormous biodiversity available worldwide [4]. A study by Buchman and Nabhan [5] also showed that one third of world's food production relies either directly or indirectly on insect pollination which in turn depends on rich and diverse vegetation. Therefore, these problems are of major concern, as the estimated number of contaminated sites is increasing significantly and is becoming a major challenge worldwide.

According to the Environmental Protection Agency (EPA) report, the United States has more than 40,000 contaminated sites till May 2004. In Western Europe, some industrialized countries possess even more contaminated sites in a comparatively smaller area [6]. Major incidents in the past few decades such as the Exxon Valdez oil spill, Minimata disease in Japan, the Union-Carbide (Dow) Bhopal disaster, large-scale contamination of Rhine river, release of radioactive material in Chernobyl accident and progressive deterioration of aquatic habitats and conifer forests in the Northeastern US, Canada and parts of Europe has revealed the necessity to prevent the escape of effluents into the environment [7]. There are various methods by which contaminated sites can be clean up and one such method is conventional technique. This technique removes the contaminated soil to a landfill or covers the contaminated sites. However, this may create significant risks in the excavation, handling, and transport of hazardous material. In addition, it is expensive and very difficult method to find new landfill sites for the final disposal of material. Some other technologies have also been used such as high-temperature incineration and chemical use for decomposition (e.g., base-catalyzed dechlorination and UV oxidation). These techniques can be effective in reducing level of wide range of contaminants (namely chlorinated solvents, petroleum, polynuclear aromatic hydrocarbons, ketones, PAHs, TNT, RDX, HMX, BTEX, inorganic nitrogen (NO_3 , NH_4), explosives, pesticides, herbicides and heavy metals) however, this shows several demerits such as technical complexity, lack of public acceptance, increased contaminants exposure to site workers and nearby residents [8].

Various other physico-chemical treatments such as coagulation with alum or lime followed by adsorption on powdered activated carbon (PAC) is reported to yield high removal efficiency for phenolics and COD. These processes generate large volume of hazardous sludge and do not lead to ultimate destruction of the pollutants [9].

To overcome these drawbacks, a much better perspective is to completely destroy the pollutants, or to transform them into some biodegradable substances. This approach can be achieved by using a technique known as bioremediation. This acts as an option to clean environment and its resources by destroying various contaminants using natural biological activity. It is considered as safer, cleaner, cost effective and environment friendly technology which generally have a high public acceptance and can often be carried out at any site. According to van Dillewijn et al. [10] "Bioremediation" is defined as the process by means of various biological agents, primarily microorganisms to degrade the environmental contaminants into less toxic forms. The first patent for a biological remediation agent was registered in 1974, using a strain of *Pseudomonas putida* [11] to degrade petroleum. In 1991, about 70 microbial genera were reported to degrade petroleum compounds [12] and almost an equal number has been added to the list in the successive two decades [7]. U.S. EPA has defined bioremediation agents as microbiological cultures, enzyme and nutrient additives that significantly increase the rate of biodegradation to mitigate the effect of various pollutants. The main advantages of bioremediation over conventional treatment includes: low cost, high efficiency, minimization of chemical and biological sludge, selectivity to specific metals, no additional nutrient requirement, regeneration of biosorbent and the possibility of metal recovery [13]. Bioremediation can occur on its own in nature (natural attenuation or intrinsic bioremediation) or can be spurred via addition of fertilizers for the enhancement of bioavailability within the medium (biostimulation). Bioventing, bioleaching, bioreactor, bioaugmentation, composting, biostimulation, land farming, phytoremediation and rhizofiltration are all examples of bioremediation technologies [14].

On the basis of removal and transportation of wastes, bioremediation technology can be classified as *in situ* and *ex situ*. *In situ* bioremediation involves treatment of contaminated material at the same site, while *ex situ* involves complete removal of contaminated material from one site and its transfer to another site, where it has been treated using biological agents. When both the methods have been compared, it was found that the rate of biodegradation and consistency of process outcome differs between *in situ* and *ex situ* methods. With the need for excavation of contaminated samples for treatment, the cost of *ex situ* bioremediation is relatively high as compared to *in situ*. *In situ* and *ex situ*, both the bioremediation methods depends essentially on microbial metabolism, however, so far *in situ* methods are preferred over *ex situ* for ecological restoration of contaminated soil, water and environment [8].

The major advantage of using biological sources is its ability to multiply and magnify in terms of initial inoculum as compared to physical and chemical means of treatment. The process of bioremediation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant, which is further dependent on accessibility and bioavailability [15]. The process of remediation can be enhanced by the addition of various microorganisms (called seeding or inoculation) to a polluted environment to promote increased rate of biodegradation. The inoculums may be a blend of nonindigenous microbes from various polluted environments (specially selected and cultivated for their various pollutant degrading characteristics) or it may be a mixture of microbes selected from the site to be remediated or mass-cultured in the laboratory. Addition of nutrients along with seeding process shows enhanced results for bioremediation [16].

This review paper deals with the significance of bioremediation, as it plays important role in the restoration of degraded land which is an important conservation effort for sustainable development and environmental management. This review also summarizes the available information on various attributes of microbial utilization and plant derived biomass for bioremediation. The major aim of present review is to focus on the role of various biological agents used in bioremediation and their wide term application and acceptance. The review will be useful for human value to better understand the feasibility of bioremediation and as an aid in selecting its products. The collected information can be useful in treating contaminated environment at regional, national and global level.

1.1 Agents of Bioremediation

The process of bioremediation involves the use of various microorganisms or plants to treat environment contaminated with organic molecules which can be living or non-living, natural or genetically engineered. These toxic heavy metals molecules are otherwise very difficult to break or mitigate by transforming them into innocuous products [14]. Currently, a wide range of microorganisms (bacteria, archaeobacteria, yeasts, fungi and algae) and plants are being studied for use in bioremediation processes (Table 1-3). Some of these microorganisms have already been employed as biosorbents of various pollutants [17]. In the case of oil spills, the process exploits catabolic ability of microorganism feeding on oil. Several workers [7,17-21] have also described the application of bioremediation using microorganism in oil spills contaminated sites where the process exploits catabolic ability of microorganism feeding on oil.

1.1.1 Microorganisms

Microbes have been widely used in the process of environmental clean-up and are known as bioremediators. The process of bioremediation involve the use of microorganisms which are native to the contaminated sites by providing them sufficient nutrients and a few chemicals essential for their growth and development. This enables them to destroy the pollutants present in the contaminated sites [18,22]. Amongst bacteria, *Bacillus* [23], *Pseudomonas* [24], and *Streptomyces* [25] acts as a potent metal biosorbents. The potential of *Streptomyces* strain to retain trace elements from polluted waters has recently been confirmed by many workers. Some other common microorganisms used in the process of remediation (Table 1) are species of: *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Cinetobacter*, *Corynebacterium*, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Pseudomonas*, *Vibrio*, *Rhodococcus* and *Sphingomonas* [22-24,26-30]. These microorganisms are used for the treatment of contaminated sites containing a wide variety of pollutants.

Table 1. Examples of bacteria, archaeobacteria and yeast widely used and studied in bioremediation

Organisms	Genus/species	Toxic Chemicals/Elements	Reference	
Bacteria	<i>Arthrobacter</i> sp.	p-nitrophenol	[28,30]	
	<i>Bacillus</i> sp.	Cu, Zn, Cd, Pb, Fe, Ni, Ag, Th, Ra and U	[22,23,26]	
	<i>Citrobacter</i> sp.	U	[31]	
	<i>Cupriavidus metallidurans</i>	Zn and Cu	[32]	
	<i>Escherichia coli</i>	Zn and V	[32]	
	<i>Escherichia hermannii</i>	V and Zn	[33]	
	<i>Enterobacter cloacae</i>	Pb, Cu, V and Cr	[33]	
	<i>Exiguobacterium aurantiacum</i>	phenolics, heterocyclics and (PAHs)	[34]	
	<i>Geobacter metallireducens</i>	Fe	[35]	
	<i>Micrococcus</i> sp.	Th and U	[29]	
	<i>Pseudomonas aeruginosa</i>	Cd, Pb, Fe, Cu, U, Ra, Ni, Ag, Zn, Th and Atrazine	[24,27]	
	<i>Ralstonia eutropha</i>	2,4-Dichlorophenoxyacetic acid	[37]	
	<i>Streptomyces</i> sp.	Cu, Zn, Cd, Pb, Fe, Ni, Ag, Th, Ra and U	[25]	
	Archeobacteria	<i>Zoogloea ramigera</i>	Pb, Cu and Cr	[38]
		<i>Filo crenarchaeota</i>	Cd, Cu, Ni, and Zn	[39]
	Yeast	<i>Candida utilis</i>	Cd	[40]
		<i>Hansenula anomala</i>	Cd	[41]
<i>Rhodotorula mucilaginosa</i>		Zn and Cd	[42]	
<i>Rhodotorula rubra</i>		Hg	[43]	
<i>Streptomyces</i> sp.		Pb	[25]	
<i>Saccharomyces cerevisiae</i>		Cu, Zn, Cd, Pb, Fe, Ni, Ag, Th, Ra, U and Hg	[17,43]	

A study by Asku [38] demonstrated that *Chiarella vulgaris* and *Zoogloea ramigera* showed biosorption of copper through adsorption and formation of bonds between metals and amino or carboxyl groups of cell wall (polysaccharides). A study by Doyle et al. [26] also indicates that heavy metal cations showed adsorption to the cells walls of Gram-positive bacteria. Many bacteria, such as *Actinomycetes*, *Azotobacter* and *Pseudomonas*, synthesizes different substances to capture Fe^{2+} which they require for their metabolic activity and biosorption [27]. A study by Jayashree et al. [24] proved that the *Pseudomonas* acts as fuel-eating bacteria which can degrade the hydrocarbons. *Pseudomonas syringae* also showed the formation of bond which play important role in the accumulation of calcium, magnesium, cadmium, zinc, copper and mercury *Geobacter metallireducens* is a Fe (III) reducing organisms that can oxidize a variety of aromatic contaminants such as benzene and naphthalene and removes uranium (a radioactive waste) from drainage water in mining operations and from contaminated groundwater [35]. A study by Jeswani and Mukherji [34] showed that pure culture of *Exiguobacterium aurantiacum* has the capability of phenol degradation and PAHs in batch culture when provided with pure compounds as a source of carbon and energy. This activated sludge consortia and batch culture form a good biofilm on

the rotating discs in a Rotating Biological Contractor (RBC) resulting in removal of COD and TOC [44].

The removal of heavy metals cations from industrial waste water or recovery of metals from their solutions can be accomplished by methods that use microorganisms as cation sorbents [45]. The mechanisms of metal binding to microbial biomass is divided into three types (i) intracellular accumulation (this process requires live cells), (ii) sorption or complex formation on cell surface (it takes place on both live and dead cells) and (iii) extracellular accumulation or precipitation (the process may require viable cells) [13,46]. The uptake of metal ion by living and dead cells consists of two modes. In the first mode metal ions binds to the surface of cell wall and extra cellular material whereas, the second mode involves the metal uptake into the cell across cell membrane and is referred as intracellular uptake, active uptake or bioaccumulation. Both living and dead cells shows first mode however, the second mode which is metabolism dependent occurs only in living cells. Metal uptake is also facilitated by the production of metal-binding proteins in living cells. Therefore, both living and dead cells are capable of metal adsorption. The use of dead biomass is preferred over living due to the absence of toxicity limitations, absence of growth media and nutrients requirements, and high capacity of binding metals [47]. In solutions the process of metal removal takes place by the formation of complex between the pollutants (metal) and the active groups on cell surface after interaction.

Yeasts are readily available source of biomass which shows the ability to resist under unfavorable environment. Many metals and metalloids can be accumulated by yeasts and some of them are essential for structural and catalytic functions, whereas others are of no metabolic importance [48]. Yeasts are also known for dye decolorization of food industry effluents mainly by three mechanisms such as biosorption, bioaccumulation and biodegradation. The removing mechanisms of dyes color from industrial effluents, by yeast cells are either through absorption or adsorption at the cell surface [49]. The yeast species such as *Candida*, *Clavispora*, *Debaryomyces*, *Leucosporidium*, *Pichia*, *Rhodospodium*, *Rhodotorula*, *Sporidiobolus*, *Sporobolomyces*, *Stephanoascus*, *Trichosporon* and *Yarrowia* are used in bioremediation process and shows biodegrading properties [50]. Microbial biomass derived from yeasts, particularly *Candida utilis* has the ability to accumulate metal ions and radionuclides from the environment. A study by Kujan et al. [40] also showed that *C. utilis* biomass can conveniently be used for cadmium biosorption from aqueous solutions. There are some yeast like *Rhodotorula mucilaginosa* which is efficient in bioadsorption of lead and are also known to accumulate free and complexed silver ions by metabolism dependent and independent processes [51]. A comparative study was made by Ksheminska et al. [52] on the sensitivity of yeast *Pichia guilliermondii* to Cr (III) and Cr (VI) as well as on the uptake potential of Cr. The results indicated accumulation of Cr (III) and Cr (IV) by *Pichia* sp. and also showed increase in Cr tolerance by the addition of riboflavin.

Besides used for metal removal, microbes are also finding their application in removing various hydrocarbons and hazardous chemicals. These chemicals are produced by the burning of fossil fuels have been successfully bioremediated at contaminated sites using bioremediation procedures. Gasoline and the other components such as benzene, toluene, ethylbenzene and xylene (BTEX) in particular, has been the focus of substantial biodegradation and bioremediation research. Several studies have indicated that extent of oil and total petroleum hydrocarbon (TPH) biodegradation is linked to oil type and its molecular composition [53-56]. According to Lal and Khanna [57] 58% degradation in 15 day period was recorded in Indian crude oil samples by *Acinetobacter calcoaceticus* and *Alcaligenes odorans* in combination. A study by Ijah [58] reported that both bacteria and yeast isolates

from tropical soils are capable of degrading 52% and 69% of crude oil in 16 days, respectively. Endosulfan, a pesticide residual (used extensively to control insect pests) is extremely toxic to fish and aquatic invertebrates and has been implicated increasingly in mammalian gonadal toxicity, genotoxicity, and neurotoxicity [59]. It can bind to soil particles and persist for a relatively long period with half-life of 60-800 days [60]. The bacteria such as *Pseudomonas* sp. and *Arthrobacter* sp. can degrade upto 57-90% of α -endosulfan and 74-94% of β -endosulfan in a period of 7 days. It is believed that many species of microorganisms such as bacteria, yeasts and fungi obtain both energy and tissue building material from petroleum [61].

1.1.2 Algae and fungi

Algae and fungi plays important role in returning the environment to its original state altered by various contaminants (Table 2). The process of phycoremediation is defined as the use of algae to remove pollutants from the environment or to convert them into harmless form. Phycoremediation in a much broader sense is the use of micro or macroalgae for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO₂ from air. Algae are highly adaptive and can grow autotrophically, heterotrophically or mixotrophically in any environment. In natural environments, algae play a major role in controlling metal concentration of lakes and oceans. It possess the ability to degrade or accumulate toxic heavy metals and organic pollutants such as phenolics, hydrocarbons, pesticides and biphenyls from the environment, resulting in higher concentrations within themselves as compared to surrounding water [62]. Pollutant-degrading mixotrophic algae are excellent agents for remediation and carbon sequestration [63]. An alga fixes CO₂ and produce O₂ by the process of photosynthesis and increases BOD level in contaminated water along with the efficient removal of excess of nutrients [64]. Uptake of metals by living microalgae occurs in two steps. The first step is independent of cell metabolism and involves "adsorption" onto the cell surface (physical adsorption) afterwards these ions are transported slowly into the cytoplasm known as chemisorption [65]. The second step is dependent on cell metabolism and involves absorption or intracellular uptake of heavy metals. Many studies have showed that various metals such as Pb, Cu, Cd, Co, Hg, Zn, Mg, Ni and Ti are sequestered in polyphosphate bodies of algae and perform two functions i.e. storage and detoxification of metals [66]. Due to its role in sequestration of heavy metals by algal cell wall, these are considered as an ideal source of multifunctional polymers [67]. Algae are also known for effective removal of nitrogen from soil or water through the process of absorption and store it as biomass. As the time passes, the biomass decomposes and releases the nitrogen back into the soil (ammonia or urea) or atmosphere (N₂O), where it may be recycled or lost [68].

Algae plays important role in pH correction, sludge removal and TDs reduction, whereas in conventional treatment, separate methods or stages are required. Amongst algae, blue green algae or cyanobacteria are susceptible to various physical and chemical alterations of light, salinity, temperature and nutrient composition. Recently, there has been increasing worldwide interest in using cyanobacteria as an economic and low-maintenance remediation technology for contaminated and polluted sites. Cyanobacteria have been used efficiently for remediating dairy waste water by converting the dissolved nutrients into biomass. However, the beneficial application of cyanobacteria in bioremediation of contaminated waters, either natural aquatic environments or industrial effluents, still needs further research. The other algal species such as *Aphanocapsa* sp., *Oscillatoria salina*, *Plectonema terebrans* and *Synechococcus* sp. develops mats in aquatic environments and have been successfully used in bioremediation of oil spills in different parts of the world [20,21,29]. Various species

of algae such as *Anabaena inaequalis*, *Chlorella sp.*, *Stigeoclonium tenue*, *Synechococcus sp.* and *Westiellopsis prolifica* are resistant to heavy metals and thus used for the removal of heavy metals [20,69,70].

Table 2. Examples of algae and fungi widely used and studied in bioremediation

Organisms	Genus/species	Toxic chemicals/elements	Reference	
Algae	<i>Ascophyllum nodosum</i>	Pb, Cu and Cr	[23]	
	<i>Anabaena inaequalis</i>	Cr	[70]	
	<i>Chlorella vulgaris</i>	Cd, Ag, Cu, Th, Zn, Pb, Ni, Ra, Fe and U	[38,69,71]	
	<i>Cladophora glomerata</i>	Cu, Pb, Cd, Cr, Ni, Fe, Zn, Mn, Sr and Cs	[65]	
	<i>Cyanobacteria</i>	Pb, Hg and Cd	[20]	
	<i>Nostoc sp.</i>	Hg, Pb, Cd and Gamma-hexachlorocyclohexane	[20,72]	
	<i>Oedogonium rivulare</i>	Cr, Ni, Zn, Fe, Mn Cu, Pb, Cd and Co	[48]	
	<i>Oscillatoria spp.</i>	Cu, Pb, Cd and Co	[21]	
	<i>Sargassum spp.</i>	Pb, U, Cd, Ni, Zn, Cu and Cr	[73]	
	<i>Scenedesmus obliquus</i>	Cd and Zn	[71]	
	<i>Spirogyra spp.</i>	Ni, Cr, Fe and Mn	[21,23]	
	<i>Spirulina spp.</i>	Pb and Cd	[74,75]	
	Fungi	<i>Aspergillus tereus</i>	Cr	[76,77]
		<i>Aspergillus niger</i>	Pb, Zn, Cd, Cr, Cu, Ni and Chlorpyrifos	[78,79,80]
<i>Funalia trogii</i>		Hg, Cd and Zn	[81]	
<i>Ganoderma lucidumk</i>		Cr and Cu	[78]	
<i>Penicillium chrysogenum</i>		Pb, Fe, Ni, Ra, Th, U, Cu, Zn, Ag, and Cd	[25]	
<i>Phanerochaete chrysosporium</i>		2,4-dicholorophenol	[82]	
<i>Pleurotus ostreatus</i>		PAHs and Orange 3, 4-(4-nitrophenylazo) aniline	[83,84]	
<i>Rhizopus sp.</i>		Cr	[85]	

Accumulation of Cd and Zn was recorded with alga *Scenedesmus obliquus*, it also showed enhanced absorption with increased concentration of phosphorus in the media, where Se accumulation was found to be inhibited. Metals such as Cu, Pb, Cd and Co are also accumulated by *Cladophora glomerata* and *Oedogonium rivulare*. *Spirogyra hatillensis* a fresh water filamentous alga showed continuous uptake of Ni, Cr, Fe and Mn from aqueous solution [66]. The algae are significantly efficient in treating more than one problem at a time, which is not possible by conventional process of chemical treatment. The phycoremediation shows advantage over other chemical methods as the removal of algal mass from the treated effluents is easy and economic.

Another type of bioremediation is mycoremediation which uses fungal mycelium to decontaminate or filter the toxic waste from contaminated area. The fungal mycelia secrete various extracellular enzymes and acids that break down the lignin and cellulose. The key to mycoremediation is to determine the right fungal species to target a specific pollutant. Fungi (*Ligninolytic fungi*) such as the white rot fungus *Phanaerochaete chrysosporium* and

Polyporus sp. are promising candidates for bioremediation, as it shows the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), explosives, polychlorinated biphenyls (PCBs), and organochlorine pesticides [82,86]. It has also been reported to degrade a wide variety of organopollutants because of lignin-degrading or wood-rotting enzymes. The extracellular enzymes secreted by white-rot fungi during lignin decay have been proposed as promising agents for oxidizing pollutants. *Cyathus bulleri*, a bird's nest ligninolytic fungus that colonizes with dead herbaceous stems, wood chips, dung, sticks and other woody debris are found ecologically suitable for lignin degradation. It produces a single laccase the internal peptides which bears similarity to enzyme laccases of several white rot fungi, the only difference lies in proportion of some basic and hydrophobic amino acids. Laccases are copper containing enzymes which catalyze the oxidation of a broad range of phenolic compounds and aromatic amines by using molecular oxygen as the electron acceptor [87]. Many researchers have showed that the extracellular enzymes secreted by these fungi oxidizes woody plant material i.e. lignin which shows structural similarity with PAHs [88]. This process of degradation is called mineralization, and the end product is carbon dioxide [89]. The mechanisms of degradation are not yet conclusively mapped but it is likely that intracellular and extracellular enzymes are responsible for oxidizing different pollutants under different conditions.

The biosorption of uranium, cadmium, zinc, copper and cobalt by dead biomass of microorganisms takes place through electrostatic interactions between the metal ions in solutions and cell walls [73,85]. The fungi *Ganoderma lucidum* and *Aspergillus niger* are used for chromium biosorption through ion exchange mechanism [78,79]. *Pleurotus ostreatus* (tasty oyster mushroom) has a wide range of application in bioremediation. The potential use was proved when a plot of soil contaminated with diesel oil was inoculated with mycelia of oyster mushrooms. After four weeks, when the soil was analyzed more than 95% of PAH (polycyclic aromatic hydrocarbons) was reduced to non-toxic components in the mycelial-inoculated plots [83]. Wood-degrading fungi are particularly effective in breaking down aromatic pollutants (toxic components of petroleum), as well as chlorinated compounds [81]. It appears that natural microbial community participates with the fungi to break down contaminants, eventually into carbon dioxide and water.

1.1.3 Plants

Phytoremediation is a fast remediation technique to clean contaminated sites. It has been accepted and utilized widely as an effective and environmental friendly green technology for permanent removal of pollutants [18]. Phytoremediation has been defined as the use of green plants and their biomass to degrade or render harmless environmental contaminants. A large number of plant species have found to be efficient in phytoremediation of organic pollutants as mentioned in Table 3. Phytoremediation, when compared to physical and chemical remediation, shows several advantages as it helps: (1) in preserving the natural properties of soil, (2) acquiring energy mainly from sunlight and, (3) maintaining high levels of microbial biomass in the rhizosphere [90]. Although with these advantages, some plants show very low tolerance to soil contaminants, which limits the degradation efficiency to an insufficient level for soil remediation. There are approximately 400 plant species from 45 different families which act as a hyperaccumulator plants. The major families include *Brassicaceae*, *Fabaceae*, *Euphorbiaceae*, *Asteraceae*, *Lamiaceae* and *Scrophulariaceae*. There are various crops such as *Astragalus racemosus*, *Haumaniastrum robertii*, *Ipomea alpina*, *Thlaspi caerulescens* and *Sebertia acuminata* showed the potential of accumulating Se, Co, Cu, Cd/Zn and Ni respectively [91,92].

A very few studies have been carried out on uptake, mobilization and transport of hazardous heavy metals (Pb, Cd, Cu, Zn, U, Sr and Cs) mechanisms. There are two approaches for phytoremediation of organic polluted soils based on the difference in remediative mechanism. Firstly, organic pollutants can be taken up directly by plants, resulting in the degradation of pollutants inside plants known as phytoextraction. Secondly, organic pollutants can be degraded by plant secreted enzymes or plant modified microbial community in rhizosphere called plant-assisted rhizoremediation [93,94]. It may be possible that some organic compounds are able to enter into plant cells by penetrating cell membrane. Certain plants are able to extract hazardous substances such as arsenic, lead and uranium from soil and water. A study by McCutcheon and Schnoor [95] indicates alpine pennycress (*Thlaspi caerulescense*), acts as hyperaccumulator of metals and are known for accumulating high levels of Cd and Zn from the environment. According to Kramer et al. [96] it has been reported that *Alyssum lesbiacum* acts as a Ni hyperaccumulator and uses histidine, as an excellent Ni chelator, to acquire and transport Ni. The hyperaccumulator plants possess genes which regulate the amount of metal uptake from the soil by roots and deposit the metals at other locations within the plant. These genes govern various processes which increase the solubility of metals in the soil and help in the transport of proteins that translocate metals into root cells vacuoles thereby protects cell metabolism from metal toxicity. Thereafter, the metals enter the plant's vascular system and transported further to other parts of the plant and get deposited in leaf cells. Non-hyperaccumulator plants require addition of some chelated agents in soil to promote the uptake of heavy metals. The heavy metal tolerance mechanism ranges from exclusion, inclusion and accumulation by plants depending on species [97]. Willow (*Salix viminalis*) has a significant potential as a phytoextractor of Cd, Zn and Cu, as it shows high transport capacity of heavy metals from root to shoot [98]. Another example of phytoremediation is *Pteridium esculentum* which extracts arsenic from soil at a much faster rate than other plants. Arsenic is stored in the fern's leaves at a much higher level than present in the soil, thus enabling effective environmental clean-up [99]. *Brassica juncea* was reported as a valuable plant for the removal of Se from soil. The volatilization of Se in the form of methyl selenate is a major mechanism of Se removal by plants [100]. Some plants can also remove Se from soil by accumulating nonvolatile methyl selenate derivatives in the foliage. Plants such as *Medicago sativa*, *Zea mays*, *Tagetes patula* and *Helianthus* sp. are potential candidates for the phytoremediation of soils contaminated with PAEs, PAHs and uranium respectively [92,101-103].

Plant roots and shoots can be used to absorb and concentrate hazardous compounds, particularly heavy metals from aqueous solutions known as rhizofiltration. Hydroponically cultivated plants rapidly remove heavy metals from water and concentrate them in the roots and shoots. The process of rhizofiltration is effective in wetlands where contaminated water is allowed to come in contact with roots. The plant root exudates namely organic acids, sugars and phenolics are commonly used as source of carbon and energy by soil microbes which show the ability to degrade various organic pollutants [104]. Plant roots not only secrete enzyme (laccase, nitrilase, dehalogenase and nitroreductase) degrading organic pollutants but also improve the degrading ability of microorganisms present in rhizosphere [94,105].

A large proportion of heavy metals remains sorbed to soil particles and to acquire these soil-bound metals. phytoextracting plants play important role in the mobilization of metals into soil solution. The process of mobilization can be carried by various methods such as metal-chelating molecules that can be secreted into the rhizosphere to chelate and solubilize 'soil-

bound' metal [106]. Secondly the 'soil-bound' metal ions can be reduced by roots with the help of specific plasma membrane bound metal reductase, which increases metal availability [107]. The plant roots can solubilize soil-bound toxic metals by acidifying their soil environment with protons extruded from roots. A similar mechanism has been observed for Fe mobilization in some Fe-deficient dicotyledonous plants [108]. The iron-chelating compounds, termed phytosiderophores, have been studied well in plants for e.g. Mugineic and deoxymugeneic acids from barley and corn and avenic acid from oats. These phytosiderophores are released due to iron deficiency and help in mobilization of Cu, Zn and Mn from soil. Lastly, the roots can also employ rhizospheric organisms (mycorrhizal fungi or root-colonizing bacteria) in increasing the bioavailability of metals [106,109].

Though many microorganisms are capable of degrading organic compounds, microbial bioremediation technology suffers a number of limitations for their widespread application as compared to phytoremediation [110]. Harvested plants containing heavy metals can be disposed off or can be treated to recycle the metal. Scientists have identified various plants demonstrating high biomass production and metal removal capacity for a wide variety of metals. Rhizofiltration has many advantages over other phytoextraction techniques, including low cost and minimal environmental disruption. A continuous flow system circulates the contaminated water through specially designed plant containment units. According to Zhuang et al. [111], the addition of PGPR increased the organic pollutant polycyclic aromatic hydrocarbon and creosote removal by enhancing plant germination and their survival in soil.

Table 3. Examples of plants widely used and studied in bioremediation

Organisms	Genus/species	Toxic Chemicals/Elements	Reference
Plants	<i>Ambrosia artemisifolia</i>	Pb	[112]
	<i>Apocynum cannabinum</i>	Pb	[112]
	<i>Brassica juncea</i>	Se, Pb and Cu	[100,112, 113]
	<i>Helianthus annuus</i>	As and Ur	[101,112]
	<i>Medicago sativa</i>	Benzopyrene, PAEs and PAHs	[101-103]
	<i>Melastoma malabathricum</i>	Al	[113]
	<i>Nephrolepis exaltata</i>	Hg	[18]
	<i>Pteridium esculentum</i>	As	[99]
	<i>Pteris vitata</i>	As, Hg, Cs and Sr	[18,112]
	<i>Salix viminalis</i>	Cd, Zn and Cu	[98]
	<i>Raphanus sativus</i>	Cu	[114]
	<i>Silene vulgaris</i>	Zn and Cd	[99]
	<i>Thlaspi caerulescens</i>	Cd and Zn	[95]

1.1.4 Role of biotechnology in bioremediation

Biological agents have proved their capacity for remediation however; their long term and large scale use needs the application of genetic tools. Breeding programs and genetic engineering are powerful methods for enhancing the natural tendencies of plant which can be more suitable for environmental conditions. Thus, researchers are now diverting their focus towards ways to augment contaminated sites with various other non-native microbes especially genetically modified microorganisms (GMM). Genetically modified microorganisms have shown potential for bioremediation applications in soil, groundwater, and activated sludge environments, exhibiting enhanced degradative capabilities encompassing a wide range of chemical contaminants. The recombinant DNA and other

molecular biological techniques have (i) enabled amplification, disruption and modification of the targeted genes that encodes the enzymes of metabolic pathways, (ii) minimized bottlenecks pathway (iii) enhanced redox and energy generation, and (iv) played important role in recruiting heterologous genes to give new characteristics [115]. It is possible that this process, known as bioaugmentation, will open a new range of possibilities for future process of bioremediation.

There are several examples where the use of biotechnology has increased the natural capacity of biological forms. *Bacillus thuringensis* has been used for the removal of oil spills, *Deinococcus radiodurans* (radioactive resistant bacteria) has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste. There is an example where bacterial cell wall is equipped with metal ion-binding polypeptides by the fusion of protein IgA protease of *Neisseria gonorrhoeae*, metallothionein (MT) from rats and lpp-ompA to act as anchor [116,117]. Besides this, transgenics of plants has also become a powerful tool for enhancing the efficiency of phytoremediation of organic-polluted soil [118]. The genetically modified strategies are supposed to achieve the goals of enhancing the degrading rates of pollutants *in planta* or enhancing the release of degrading enzymes from roots leading to accelerated degradation of pollutants *ex planta* [119]. The genes coding for CYP and GSTs are the usually modified targets for stimulating the degradation of organic pollutants in plants [120]. A recent study suggested that transgenic *Medicago sativa* plants co-expressing GST and human CYP2E1 showed great potential for phytoremediation of organic pollutants [121].

Various organisations, R & Ds and Universities are working towards increasing the natural tendency of these microorganisms artificially. One such organization in India is TERI (The Energy and Research Institute). TERI developed an indigenous bacterial consortium, named Oilzapper by assembling five different bacterial strains isolated from various oil contaminated sites in India. These bacteria are immobilized and are mixed with a carrier material (powdered corncob) which degrades total petroleum hydrocarbon (TPH) present in oily waste. Bioremediation by oilzapper technology is an ongoing investigation whose results is highly encouraging and costs 30% less than the conventional physico-chemical treatments [54,55]. TERI has treated more than 1,50,000 metric tonnes of oil contamination at various oil installations in India and abroad and ~ 60,000 tonnes of oil contamination is under the process of treatment. This technology has helped various oil industries in ecorestoration and management of hazardous oily wastes from contaminated sites. The end product of bioremediation is CO₂, water and dead biomass which is environment friendly. Successful fish culturing was also carried out in one of the oil contaminated lake after bioremediation. The bioremediated soil contains TPH content to the extent of <1% which is not toxic and natural vegetation can be grown on the same.

In the recent past a need was felt to address the anticipated risks due to uncontrolled survival or dispersal of GMMs or recombinant plasmids into the environment. It is essential to perform field experiments to acquire the requisite information for determining overall effectiveness and risks associated with GEM introduction in natural ecosystem [122,123]. Some attempts have been made and need to be explored further for the safe disposal and acceptance of these GMM in the natural environment.

1.1.5 Limitations of bioremediation

As bioremediation is limited to only those compounds that are biodegradable and not all compounds are susceptible to rapid and complete degradation. There are some concerns

that the products of biodegradation may be more persistent or toxic than the parent compound. The biological processes are highly specific with culture requirements and at time are difficult to extrapolate the results from lab to field. It also often takes longer time than other treatment such as excavation and removal of soil. There are various factors affecting the process of bioremediation such as depletion of preferential substrates, lack of nutrients, toxicity and solubility of contaminants, oxidation or reduction potential and microbial interaction. The outcome of each degradation process depends on microbes (biomass concentration, population diversity and enzyme activities), substrate (physico-chemical characteristics, molecular structure and concentration), and a range of environmental factors (pH, temperature, moisture content, Eh, availability of electron acceptors, and carbon and energy sources).

These parameters affect the acclimation period of microbes to the substrate. The molecular structure and contaminant concentration have been shown to strongly affect the feasibility of bioremediation. The type of microbial transformation depends on whether the compound serves as a primary, secondary or co-metabolic substrate [16]. The limitations of phytoremediation lie in the fact that the process is limited to the surface plants and the area occupied by the roots. Moreover, the system is not efficient enough to put a complete check on the process of heavy metals leaching. There is always a danger of bioaccumulation and biomagnification of the contaminants into the plants and then to higher levels through food chains. The biggest hurdles lie in the fact that few plants are bigger in size and cannot be moved from one place to other to be used for the process of bioremediation.

All the contaminants are not easily treated, accumulated or degraded by bioremediation using microorganisms and the effects of microorganisms on the metal leaching associated with phytoremediation have yet not received proper attention [124]. The past studies have suggested that the plant-aided remediation may not always accelerate contaminant degradation but can have a negligible or even negative effect [125]. Thus, there is a need to search new techniques such as genetically modified microorganisms or to combine plants, fungi and bacteria for providing interesting opportunities in bioremediation process. A continuous search for the new biological form is required for proper management of increasing pollution and contamination. Therefore, bioremediation is still considered as a developing technology to regulate the day to day environmental problems faced by man residing in an area.

2. CONCLUSION

Bioremediation is considered to be very safe and helpful technology as it relies on microbes that occur naturally in the soil and pose no threat to environment and the people living in that area. The process of bioremediation can be easily carried out on site without causing a major disruption of normal activities and threats to human and environment during transportation. Bioremediation is less expensive than other technologies that are used for clean-up of hazardous waste. Even though various sources of bioremediation such as bacteria, archaeobacteria, yeasts, fungi, algae and plants are available but, the biological treatment alone is not sufficient enough to treat the pollutants or contaminated sites. Every biological forms has a different growth requirements (temperature, pH and nutrients) so we need to isolate those forms, which can cultured easily in the lab, with minimal requirement and can be useful in treating variety of pollutants. Use of genetically engineered microorganisms is probably not needed in most cases because of wide availability of naturally occurring microbes. Besides using these natural or genetically engineered microbes, there is an urgent need for us to educate and aware local people about the

various life forms, their potential applications and tendencies to absorb/adsorb the contaminants whose existence can harm our environment. A detailed study of area wise and pollutant type data base is much needed to finalize the priority area and the need for the effective removal of the pollutant from the contaminated sites.

As natural resources are major assets to humans their contamination resulted in long term effects of pollution (noise and radiation), global warming, ozone depletion and greenhouse gases. The decontamination of these natural resources is essential for the conservation of nature and environment using bioremediation process. Thus, there is an urgent need to study the effect of various microorganisms in combination against various pollutants for the conservation of natural resources and environment management.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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