Bioremediation: Developments, Current Practices and Perspectives

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Abstract

Environmental contamination due to anthropogenic and natural sources is increasing day by day because of increase in population, industrialization and urbanization. The enigma for the public, scientists, academicians and politicians is how to tackle the contaminants that jeopardize the environment. Advances in science and technology, since industrial revolution has also increasingly enabled humans to exploit natural resources and cause damage to the environment. The ideal solution for pollution abatement is Bioremediation, the most effective innovative technology to come along that uses biological systems for treatment of contaminants. Although, this novel and recent technology is a multidisciplinary approach, its central thrust depends on microbiology. This technology includes biostimulation (stimulating viable native microbial population), bioaugmentation (artificial introduction of viable population), bioaccumulation (live cells), biosorption (dead microbial biomass), phytoremediation (plants) and rhizoremediation (plant and microbe interaction). Rhizoremediation, which is the most evolved process of bioremediation, involves the removal of specific contaminants from waste product of contaminated sites by mutual interaction of plant roots and suitable microbial flora. This paper represents an exhaustive evaluation with respect to developments, current practices and perspectives of a variety of approaches of bioremediation.

Keywords: Bioremediation; Phytoremediation; Polyaromatic hydrocarbons; Rhizoremediation.

Introduction

Vast number of pollutants and waste materials containing heavy metals are disposed into the environment per annum. Approximately $6 \times 10^6$ chemical compounds have been synthesized, with 1,000 new chemicals being synthesized annually. Almost 60,000 to 95,000 chemicals are in commercial use. According to Third World Network reports, more than one billion pounds (450 million kilograms) of toxins are released globally in air and water. The contaminants causing ecological problems leading to imbalance in nature is of global concern. The environmentalists around the world are trying to overcome it by several means. However, they are raising their voices at international platforms regarding the depletion of natural resources; little attention is given to their words and continues to use them without caring the adverse consequences.

Usually the contaminated sites are treated with traditional methods like physical, chemical and thermal processes resembling excavation and transportation. By this method, the cost of removal of 1 m$^3$ soil from a 1-acre contaminated site is estimated as US $0.6–2.5 million [1]. Billions of dollars are expected to be used to clean up all sites polluted with polycyclic aromatic hydrocarbon (PAHs) in coming decades [2]. Metal contamination in India is mainly due to industrial activities and it is estimated that about $3 billion are needed to remediate the metal contaminated sites alone in USA [3]. In the US alone, reinstatement of all contaminated sites will cost approximately $ 1.7 trillion [4]. The bioremediation technology is cost effective, eco-friendly and alternative to conventional treatments, which rely on incinerations, volatilization or immobilization of the pollutants. The conventional treatment technologies simply transfer the pollutants, creating a new waste such as incineration residues and not eliminate the problem.

Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site [5]. Compared to other methods, bioremediation is a more promising approach.

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and less expensive way for cleaning up contaminated soil and water [6]. Bioremediation uses biological agents, mainly microorganisms, e.g. yeast, fungi or bacteria to clean up contaminated soil and water [7]. Bioremediation, i.e. the use of living organisms to control or remediate polluted soils, is an emerging technology. It is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes. Some tests make an exhaustive examination of the literature of bioremediation of organic and inorganic pollutants [8, 9], and another test takes a look at pertinent field application case histories [10]. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules. Most important parameters for bioremediation are i) the nature of pollutants, ii) the soil structure, pH, Moisture contents and hydrogeology, iii) the nutritional state, microbial diversity of the site and iv) Temperature and oxidation-reduction (redox- Potential) [11]. In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources [12]. Bioremediation activity through microbe is stimulated by supplementing nutrients (nitrogen and phosphorus), electron acceptors (oxygen), and substrates (methane, phenol, and toluene), or by introducing microorganisms with desired catalytic capabilities [13, 14]. Plant and soil microbes develop a rhizospheric zone (highly complex symbiotic and synergistic relationships) which is also used as a tool for accelerating the rate of degradation or to remove contaminants.

Groundwater is one of the most vital sources of drinking water on earth. However, in the past few decades, it has been contaminated with petroleum hydrocarbons, which leaked from underground storage tanks. These organic compounds have caused serious public concern because benzene, toluene, ethylbenzene, and xylene (BTEX) are ubiquitous pollutants hazardous to human health [15]. In situ bioremediation technology is a widely used technology that can clean up BTEX-contaminated sites, indigenous microorganisms to enhance biodegradation of organic constituents in the subsurface. Bacteria have huge catabolic possibility for remediating wastes; however, the interactions between bacteria and pollutants are complex and suitable remediation does not always take place. Hence, molecular approaches are being applied to enhance bioremediation. The recent developments are taking place in bioremediation by utilizing rhizoremediation, protein engineering, metabolic engineering, whole-transcriptome profiling, and proteomics for the degradation of recalcitrant pollutants such as chlorinated aliphatic and polychlorinated biphenyl as well as for binding heavy metals [16]. Cell surface expression of specific proteins allows the engineered microorganisms to transport, bio-accumulate and/or detoxify heavy metals as well as to degrade xenobiotics [17].

Objectives of this Review

- Explore the current concepts of bioremediation.
- Provide an insight into the role of various developed processes like rhizoremediation and major controls that may be used for their management in degradation of inorganic and organic soil pollutants.
- Highlight the limitations and challenges associated with the various developed processes of bioremediation.

Development of Bioremediation

Bioremediation techniques are divided into three categories; in situ, ex situ solid and ex situ slurry. With in situ techniques, the soil and associated ground water is treated in place without excavation, while it is excavated prior to treatment with ex situ applications. Selection of appropriate technology among the wide range of bioremediation strategies developed to treat contaminants depends on three basic principles i.e., the amenability of the pollutant to biological transformation (Biochemistry), the accessibility of the contaminant to microorganisms (Bioavailability) and the opportunity for optimization of biological activity (Bioactivity) [18].

Simple hydrocarbons and petroleum fuels degradability decreases as molecular weight and degree of branching increase. Aromatic hydrocarbons one or two ring compounds degrade readily, higher molecular weight compounds less readily. Alcohols, esters, nitrobenzenes and ethers degrade slowly, chlorinated hydrocarbons decreasing degradability

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within increasing chlorine substitution - highly chlorinated compounds like PCBs and chlorinated solvents do not appreciably degrade aerobically, Pesticides are not readily degraded. Few environmental conditions are required for the soil remediation (Table 1).

Table 1: Environmental factors and optimum condition for microbial activity for soil bioremediation [5].

<table>
<thead>
<tr>
<th>Environmental Factor</th>
<th>Optimum conditions</th>
<th>Condition required for microbial activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available soil moisture</td>
<td>25-85% water holding capacity</td>
<td>25-28% of water holding capacity</td>
</tr>
<tr>
<td>Oxygen</td>
<td>&gt;0.2 mg/L DO, &gt;10% air-filled pore space for aerobic degradation</td>
<td>Aerobic, minimum air-filled pore space of 10%</td>
</tr>
<tr>
<td>Redox potential</td>
<td>Eh &gt; 50 mill volts</td>
<td></td>
</tr>
<tr>
<td>Nutrients</td>
<td>C:N:P = 120:10:1 molar ratio</td>
<td>N and P for microbial growth</td>
</tr>
<tr>
<td>pH</td>
<td>6.5-8.0</td>
<td>5.5 to 8.5</td>
</tr>
<tr>
<td>Temperature</td>
<td>20-30 °C</td>
<td>15-45°C</td>
</tr>
<tr>
<td>Contaminants</td>
<td>Hydrocarbon 5-10% of dry weight of soil</td>
<td>Not too toxic</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>700ppm</td>
<td>Total content 2000ppm</td>
</tr>
<tr>
<td>Type of soil</td>
<td>-</td>
<td>Low clay or silt content</td>
</tr>
</tbody>
</table>

**In-Situ Bioremediation**

**Bioventing**

Bioventing encourages the in-situ biodegradation of POLs (petroleum-oil-lubricants) by providing oxygen to microorganisms in the soil. The system supplies oxygen by injecting air directly into the residual contamination. In contrast to soil vapor vacuum extraction (SVE), bioventing uses low airflow rates to provide only enough oxygen to keep up microbial activity. Optimal flow rates maximize biodegradation as vapors move slowly through biologically active soil while minimizing volatilization of contaminants. A basic bioventing system includes a well and a blower, which pumps air through the well and into the soil [19].

**Biopiling**

Biopile treatment is a full-scale technology in which excavated soils are mixed with soil amendments, placed on a treatment area, and bioremediated using forced aeration. The contaminants are reduced to carbon dioxide and water. The basic biopile system includes a treatment bed, an aeration system, an irrigation/nutrient system and a leachate collection system. Moisture, heat, nutrients, oxygen, and pH are controlled to enhance biodegradation. The irrigation/nutrient system is buried under the soil to pass air and nutrients either by vacuum or positive pressure. Soil piles can be up to 20 feet high and may be covered with plastic to control runoff, evaporation and volatilization, and to promote solar heating. If volatile organic compounds (VOCs) in the soil volatilize into the air stream, the air leaving the soil may be treated to remove or destroy the VOCs before they are discharged into the atmosphere. Treatment time is typically 3 to 6 months [20].

**Ex-Situ Bioremediation**

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Composting

Composting is a process by which organic wastes are degraded by microorganisms, typically at elevated temperatures. Typical compost temperatures are in the range of 55° to 65° C. The increased temperatures result from heat produced by microorganisms during the degradation of the organic material in the waste. Windrow composting has been demonstrated using the following basic steps. First, contaminated soils are excavated and screened to remove large rocks and debris [21, 22].

The soil is transported to a composting pad with a temporary structure to provide containment and protection from weather extremes. Amendments (straw, alfalfa, manure, agricultural wastes and wood chips) are used for bulking agents and as a supplemental carbon source. Soil and amendments are layered into long piles, known as windrows. The windrow is thoroughly mixed by turning with a commercially available windrow turning machine. Moisture, pH, temperature, and explosives concentration are monitored. At the completion of the composting period, the windrows would be disassembled and the compost is taken to the final disposal area.

Table 2: Developmental methods applied in bioremediation [5].

<table>
<thead>
<tr>
<th>Technology</th>
<th>Examples</th>
<th>Benefits</th>
<th>Limitations</th>
<th>Applications</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>In situ</td>
<td>Biosparging</td>
<td>Most cost efficient</td>
<td>Environmental constraints, Extended treatment time, Monitoring difficulties</td>
<td>Biodegradative abilities of indigenous microorganisms, Presence of metals and other inorganic Environmental parameters, Biodegradability of pollutants, Chemical solubility, Geographical factors, Distribution of pollutants</td>
<td>[23, 24, 25]</td>
</tr>
<tr>
<td></td>
<td>Bioventing</td>
<td>Non-invasive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bioaugmentation</td>
<td>Relatively passive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Natural attenuation processes, Treats soil and water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex situ</td>
<td>Land farming (Solid-phase treatment system)</td>
<td>Cost efficient, Simple procedure, Inexpensive, self-heating</td>
<td>Space requirements, Slow degradation rates, Long incubation periods, Extended treatment time, Requires nitrogen supplementation, incubation periods, months to years, Need to control abiotic loss, Mass transfer problem, Bioavailability limitation</td>
<td>Surface application, aerobic process, application of organic materials to natural soils followed by irrigation and tilling, To make plants healthier, good alternative to land filling or incinerating, practical and convenient, Surface application, agricultural to municipal waste</td>
<td>[21, 22]</td>
</tr>
<tr>
<td></td>
<td>Composting (Anaerobic, convert’s solid organic wastes into humus-like material)</td>
<td>Low cost, Rapid reaction rate, Inexpensive, self-heating</td>
<td>Can be done on site</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biopiles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioreactors</td>
<td>Slurry reactors</td>
<td>Rapid degradation kinetic, Optimized environmental parameters</td>
<td>Soil requires excavation, Relatively high cost capital, Relatively high operating cost</td>
<td>Bioaugmentation, Toxicity of amendments, Toxic concentrations of contaminants</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Aqueous reactors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precipitation or Flocculation</td>
<td>Non-directed physico-chemical complex, -tion reaction between dissolved contaminants and charged cellular components (dead biomass)</td>
<td>Cost-effective</td>
<td>Yet to be exploited commercially</td>
<td>Removal of heavy Metals</td>
<td>[27]</td>
</tr>
<tr>
<td>Microfiltration</td>
<td>Microfiltration membranes are used at a constant pressure</td>
<td>Remove dissolved solids rapidly</td>
<td>Yet to be exploited Commercially</td>
<td>Waste water treatment; recovery and reuse of more than 90% of original waste water</td>
<td></td>
</tr>
<tr>
<td>Electrolysis</td>
<td>Uses cation and anion exchange membrane pairs</td>
<td>Withstand high temperature and can be reused</td>
<td>Yet to be exploited commercially</td>
<td>Removal of dissolved solids efficiently</td>
<td></td>
</tr>
</tbody>
</table>

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The first patent for a biological remediation agent was registered in 1974, being a strain of Pseudomonas putida [28] that was able to degrade petroleum. In 1991, about 70 microbial genera were reported to degrade petroleum compounds and almost an equal number has been added to the list in the successive two decades. These organisms belong to at least 11 different prokaryotic divisions [29].

**Heavy metal toxicity mechanism to microbes**

At high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities or organisms can develop resistance or tolerance to the elevated levels of metals. Unlike many other pollutants, metals can undergo biodegradation and produce less toxic, less mobile and/or less bio-available products, heavy metals are difficult to be removed from contaminated environment. These metals cannot be degraded biologically, and are ultimately everlasting, though the speciation and bioavailability of metals may change with variation in the environmental factors. Some metals such as, zinc, copper, nickel and chromium are essential or beneficial micronutrients for plants, animals and microorganisms [30] while others (e.g., cadmium, mercury and lead) have no known biological and/or physiological functions [31]. However, the higher concentration of these metals has great effects on the microbial communities in soils in several ways: (1) it may lead to a reduction of total microbial biomass [32] (2) it decreases numbers of specific populations or (3) it may change microbial community structure [33]. Thus, at high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities like protein denaturation, inhibition of cell division, cell membrane disruption etc or organisms can develop resistance or tolerance to the elevated levels of metals.

For endurance under metal-stressed environment, plant growth promoting rhizobacteria have evolved several mechanisms by which they can immobilize, mobilize or transform metals rendering them inactive to tolerate the uptake of heavy metal ions. These mechanisms include (1) exclusion—the metal ions are kept away from the target sites (2) extrusion—the metals are pushed out of the cell through chromosomal/plasmid mediated events (3) accommodation metals form complex with the metal binding proteins (e.g., metallothienins, a low molecular weight proteins) [34, 35] or other cell components (4) bio-transformation—toxic metal is reduced to less toxic forms and (5) methylation and demethylation.

The removal of different kinds of heavy metals including Cu, Zn, Ni and Cr by free and immobilized microalgae has been well demonstrated by previous workers [36]. An adsorption–desorption cycle was developed for repeated uses of the algal beads (micro-algal beads) for the removal and recovery of heavy metals. Workers reported that alginate-immobilized C. vulgaris beads effectively removed Cu (more than 95% removal) from industrial wastewater, the adsorbed Cu was completely eluted with 0.1 M HCl and the binding sites of algal beads were released for further treatment.

**Developments of Phytoremediation**

Green plants were proposed for in situ soil phytoremediation [37, 38], which has become an attractive topic of research and development. Plant-assisted bioremediation, or phytoremediation, is commonly defined as the use of green or higher terrestrial plants for treating chemically or radioactively polluted soils. Some workers quantified and compared the responses of soil microbial communities during the phytoremediation of polycyclic aromatic hydrocarbons (PAHs) in a laboratory trial [39, 40]. Scientists were showed that bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase regulates ethylene levels in plants by metabolizing its precursor ACC into α-ketobutyric acid and ammonia [41]. A recent publication of some workers describes the development of transgenic poplars (Populus) over expressing a mammalian cytochrome P450, a family of enzymes commonly involved in the metabolism of toxic compounds. The engineered plants showed enhanced performance about the metabolism of trichloroethylene and the removal of a range of other toxic volatile organic pollutants, including vinyl chloride, carbon tetrachloride, chloroform and benzene. Some workers suggested that transgenic plants might be able to contribute to the wider and safer application of phytoremediation [42]. Herbicides are economically important, but the non-point pollution that they cause may disrupt the surrounding environment. Phytoremediation of herbicides has been well studied using conventional plants. Transgenic plants...
produced for metabolizing herbicides and long-persisting pollutants can be used for phytoremediation of foreign chemicals in contaminated soil and water [43].

The major advantages of phytoremediation are as follows:

i) The cost of the phytoremediation is lower than that of traditional processes both in-situ and ex-situ.

ii) The plants can be easily monitored.

iii) The possibility of the recovery and re-use of valuable products.

iv) It uses naturally occurring organisms and preserves the natural state of the environment.

v) The low cost of phytoremediation (up to 1000 times cheaper than excavation and reburial) is the main advantage of phytoremediation.

**Phytoextraction**

Phytoextraction (or phytoaccumulation) uses plants or algae to remove contaminants from soils, sediments or water into harvestable plant biomass. Phytoextraction has been growing rapidly in popularity worldwide for the last twenty years or so [44]. Generally, this process has been tried more often for extracting heavy metals than for organics. At the time of disposal, contaminants are typically concentrated in the much smaller volume of the plant matter than in the initially contaminated soil or sediment. 'Mining with plants', or phytomining, is also being experimented with. The plants absorb contaminants through the root system and store them in the root biomass and/or transport them up into the stems and/or leaves. A living plant may continue to absorb contaminants until it is harvested. After harvest, a lower level of the contaminant will remain in the soil, so the growth/harvest cycle must usually be repeated through several crops to achieve a significant cleanup. After the process, the cleaned soil can support other vegetation. The fungal treated plants grown in Cd–Ni combination contaminated soils showed higher phytoextraction efficiency than those in Cd or Ni contaminated soils. Thus, it is suggested that the fungus *T. atroviride* able with organic-degrading capabilities could be exploited for fungi-assisted phytoremediation of mixed organic-metal contaminated soils [45].

The main advantage of phytoextraction is environmental friendly. The traditional methods those are used for cleaning up the heavy metal contaminated soil are responsible for disruption of soil structure and reduce soil productivity, whereas phytoextraction can clean up the soil without causing any kind of harm to the soil quality. Another benefit of phytoextraction is less expensive than any other clean up process. As this process is controlled by plant, so it takes more time than any traditional soil cleanup process.

**Phytotransformation**

In the case of organic pollutants, such as pesticides, explosives, solvents, industrial chemicals, and other xenobiotic substances, certain plants, such as *canas*, render these substances non-toxic by their metabolism. In other cases, microorganisms living in association with plant roots may metabolize these substances in soil or water. These complex and recalcitrant compounds cannot be broken down to basic molecules (water, carbon dioxide etc) by plant molecules, and hence the term phytotransformation represents a change in chemical structure without complete breakdown of the compound. The term "Green Liver Model" is used to describe phytotransformation, as plants behave analogously to the human liver when dealing with these xenobiotic compounds (foreign compound/pollutant). After uptake of the xenobiotics, plant enzymes increase the polarity of the xenobiotics by adding functional groups such as hydroxyl groups (-OH). This is known as Phase I metabolism, similar to the way that the human liver increases the polarity of drugs and foreign compounds. Whilst in the human liver, enzymes such as Cytochrome P450s are responsible for the initial reactions [46]. In plants, enzymes such as nitroreductases carry out the same role. In the second stage of phytotransformation, known as Phase II metabolism, plant biomolecules such as glucose and amino acids are added to

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the polarized xenobiotic to further increase the polarity (known as conjugation). This is again similar to the processes occurring in the human pancreas where glucuronidation (addition of glucose molecules by the UGT (e.g. UGT1A1) class of enzymes) [47] and glutathione addition reactions occur on reactive centers of the xenobiotic. Hence, the plants reduce toxicity (with exceptions) and sequester the xenobiotics in phytotransformation. Trinitrotoluene phytotransformation has been extensively researched and a transformation pathway has been proposed [48].

Rhizofiltration

Rhizofiltration is similar in concept to Phytoextraction but is concerned with the remediation of contaminated groundwater rather than the remediation of polluted soils. The contaminants are either adsorbed onto the root surface or are absorbed by the plant roots. Plants used for rhizofiltration are not planted directly in situ but are acclimated to the pollutant first. Plants are hydroponically grown in clean water rather than soil, until a large root system has developed. Once a large root system is in place, the water supply is substituted for a polluted water supply to acclimatize the plant. After the plants become acclimatized they are planted in the polluted area where the roots uptake the polluted water and the contaminants along with it. As the roots become saturated, they are harvested and disposed of safely. Repeated treatments of the site can reduce pollution to suitable levels as was exemplified in sunflowers were grown in radioactively contaminated pools [49].

Phytostabilisation

Phytostabilisation is the use of certain plants to immobilize soil and water contaminants. Contaminant are absorbed and accumulated by roots, adsorbed onto the roots, or precipitated in the rhizosphere. This reduces or even prevents the mobility of the contaminants preventing migration into the groundwater or air, and reduces the bioavailability of the contaminant thus preventing spread through the food chain. This technique can also be used to re-establish a plant community on sites that have been denuded due to the high levels of metal contamination. Once a community of tolerant species has been established, the potential for wind erosion (and thus spread of the pollutant) is reduced and leaching of the soil contaminants is reduced [50].

Phytodegradation (Phytotransformation)

Phytodegradation is the degradation or breakdown of organic contaminants by internal and external metabolic processes driven by the plant. Ex-planta metabolic processes hydrolyse organic compounds into smaller units that can be absorbed by the plant. Some contaminants can be absorbed by the plant and are then broken down by plant enzymes. These smaller pollutant molecules may then be used as metabolites by the plant as it grows, thus becoming incorporated into the plant tissues. Plant enzymes have been identified that breakdown ammunition wastes, chlorinated solvents such as TCE (Trichloroethane), and others that degrade organic herbicides [51].

Rhizodegradation

Rhizodegradation (also called enhanced rhizosphere biodegradation, phytostimulation, and plant assisted bioremediation) is the breakdown of organic contaminants in the soil by soil dwelling microbes which is enhanced by the rhizosphere’s presence. Certain soil dwelling microbes digest organic pollutants such as fuels and solvents, producing harmless products through a process known as Bioremediation. Plant root exudates such as sugars, alcohols, and organic acids act as carbohydrate sources for the soil microflora and enhance microbial growth and activity. Some of these compounds may also act as chemotactic signals for microbes. The plant roots also loosen the soil and transport water to the rhizosphere thus additionally enhancing microbial activity [52].
Phytovolatilization

Phytovolatilization is the process where plants uptake contaminants that are water-soluble and release them into the atmosphere as they transpire the water. The contaminant may become modified along the way, as the water travels along the plant’s vascular system from the roots to the leaves, whereby the contaminants evaporate or volatilize into the air surrounding the plant. There are varying degrees of success with plants as phytovolatilizers with one study showing poplar trees to volatilize up to 90% of the TCE they absorb [53].

Riparian corridors

Riparian corridors and buffer strips are the applications of many aspects of phytoremediation along the banks of a river or the edges of groundwater plumes. Phytodegradation, phytovolatilization, and rhizodegradation are used to control the spread of contaminants and to remediate polluted sites. Riparian strips refer to these uses along the banks of rivers and streams, whereas buffer strips are the use of such applications along the perimeter of landfills.

Vegetative cover

Vegetative cover is the name given to the use of plants as a cover or cap growing over landfill sites. The standard caps for such sites are usually plastic or clay. Plants used in this manner are not only more aesthetically pleasing they may also help to control erosion, leaching of contaminants, and may help to degrade the underlying landfill.

Limitations of Phytoremediation

Phytoremediation is limited to the surface area, depth occupied by the roots; slow growth and low biomass require a long-term commitment. With plant-based systems of remediation, it is not possible to completely prevent the leaching of contaminants into the groundwater (without the complete removal of the contaminated ground, which in itself does not resolve the problem of contamination). The survival of the plants is affected by the toxicity of the contaminated land and the general condition of the soil and possible bioaccumulation of contaminants, which then pass into the food chain, from primary level consumers upwards. These are the restrictions of the phytoremediation that is why researcher developed the novel concept of bioremediation parallel to rhizoremediation.

Table 3: Developmental process of phytoremediation with suitable examples of pollutants with their potential plants.

<table>
<thead>
<tr>
<th>Process</th>
<th>Function</th>
<th>Pollutant</th>
<th>Medium</th>
<th>Plants</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoextraction</td>
<td>Remove metals pollutants that accumulate in plants. Remove organics from soil by concentrating them in plant parts</td>
<td>Cd, Pb, Zn, As, Petroleum, Hydrocarbons and Radionuclides</td>
<td>Soil &amp; Groundwater</td>
<td>Viola baoshanensis, Sedum alfredii, Rumex crispus, Helianthus annus, Alfafla, poplar, juniper, fescue, Indian mustard, cabbage</td>
<td>[54, 55]</td>
</tr>
<tr>
<td>Phytotransformation</td>
<td>Plant uptake and degradation of organic Compounds</td>
<td>xenobiotic substances</td>
<td>Soil</td>
<td>Cannas</td>
<td>[56]</td>
</tr>
<tr>
<td>Phytodegradation</td>
<td>Plants and associated microorganisms degrade organic pollutants</td>
<td>DDT, Explosives, waste and Nitrates</td>
<td>Groundwater</td>
<td>Elodea Canadensis, Pueraria thunbergiana, Duckweed parrofeather, Hybrid poplar</td>
<td>[57, 58]</td>
</tr>
<tr>
<td>Rhizofiltration</td>
<td>Roots absorb and</td>
<td>Zn, Pb, Cd, As</td>
<td>Groundwater</td>
<td>Brassica juncea,</td>
<td>[59, 60]</td>
</tr>
</tbody>
</table>
adsorb pollutants, mainly metals, from water and aqueous waste streams | and Radionuclei | Helianthus annus (Sunflowers)

| Phytostabilization (Immobilization) | Use of plants to reduce the bioavailability of pollutants in the environment | Cu, Cd, Cr, Ni, Pb, Zn | Soil | Anthyllis vulneraria, Festuca arvenensis, Koeleria vallesiana Armeria arenaria, Lupinus albus | Hybrid poplar, grasses | [61] |

| Phytovolatilization (rhizovolatilization) | Use of plants to volatilize pollutants | Se, CC14, EDB, TCE | | Stanleya pinnata, Zea mays Brassica sp. | [62] |

| Vegetative cap or Evaporational landfill cover | Rainwater is evaporotranspirated by plants to prevent leaching contaminants from disposal sites, | - | - | - | - | [63] |

Development of Rhizoremediation

Selected microbes can degrade most environmental pollutants. The process stops when the microbe is starved of food. In order to ascertain that such microbes can have access to the best food source available in soil, namely root exudates. Workers have described an enrichment method for the isolation of microbes [64] which combine the properties of: (i) degradation of a selected pollutant, and (ii) efficient root colonization. They have termed this process ‘rhizoremediation’ instead of phytoremediation to emphasize the roles of the root exudates and the rhizosphere competent microbe. The high concentration of metals in soils and their uptake by plants harmfully influence the growth, symbiosis and consequently the yields of crops [65] by disintegrating cell organelles, and disrupting the membranes, acting as genotoxic substance [66] disrupting the physiological process, such as, photosynthesis or by inactivating the respiration, protein synthesis and carbohydrate metabolism. Pseudomonas putida KT2440 is a root colonizer of potential interest for the rhizoremediation of pollutants and the biological control of pests [67], some hypothesized that, when a suitable rhizosphere strain is inoculated together with a suitable plant (e.g., coating bacteria on plant seed), these well-equipped bacteria might settle on the root together with the indigenous population, thereby enhancing the bioremediation process. The rhizoremediation of heavy metal-contaminated soils consequently turn out to be significant, as these soils typically cover huge areas that are provided inappropriate for sustainable agricultural. Phytoremediation of organic pollutants, such as explosives, is often a slow and incomplete process, potentially leading to the accumulation of toxic metabolites that can be further introduced into the food chain. During the past decade, plants have been genetically modified to overcome the inherent limitations of plant detoxification capabilities, following a strategy similar to the development of transgenic crop. Bacterial genes encoding enzymes involved in the breakdown of explosives, such as nitroreductase and cytochrome P450, have been introduced in higher plants, resulting in significant enhancement of plant tolerance, uptake, and detoxification performances. Transgenic plants exhibiting biodegradation capabilities of microorganisms bring the promise of an efficient and environmental-friendly technology for cleaning up polluted soils, [68, 69] squeeze the process of rhizoremediation by worked on the introduction of two different genetically modified microorganisms Pseudomonas fluorescens into Salix sp. plants designed for rhizoremediation induces changes on native bacteria in the rhizosphere but not in the surrounding soil were analyzed via a polymerase chain reaction–thermal gradient gel electrophoresis (TGGE) approach.

To escape the metal strain, microorganisms of agronomic importance have evolved a number of mechanisms, which they use to tolerate the uptake of heavy metal ions. Such mechanisms include

i) Pumping of metal ions exterior to the cell.

ii) Accumulation and sequestration of the metal ions inside the cell.

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iii) Transformation of toxic metal to less toxic forms [70] and adsorption/desorption of metals.

Current status to organize genetically engineered plant–microbial systems to enhance rhizoremediation consists of gene cloning of plants containing bacterial gene for the degradation of organic pollutants such as PCBs [71] and of recombinant, root-colonizing bacteria (e.g. *Pseudomonas fluorescens*) expressing degradative enzymes (e.g. ortho-monooxygenase for toluene degradation. Related effort was offered to researchers [72] for the degradation of dioxine like compounds by a recombinant *Rhizobium tropici* strain expressing 1,9a-dioxygenase. Some worker prepares a transgenic plant to improve rhizoremediation of polychlorinated biphenyls (PCBs) [73]. *Sphingobium chlorophenolicum* is well known as some workers [74] did a pentachlorophenol (PCP) degrader; the potential of ryegrass for rhizosphere bioremediation of chlorpyrifos in mycorrhizal soil was investigated by the green house pot culture experiments by the researchers [75].

### Table 4: Detailed aspects of Rhizoremediation with respect to heavy metals.

<table>
<thead>
<tr>
<th>Examples of Pollutant</th>
<th>Microbe/Microbial communities</th>
<th>Characteristics properties of microbe</th>
<th>Plant</th>
<th>Soil nature</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td><em>Pseudomonas aeruginosa</em> <em>Citrobacter</em> spp.</td>
<td>-</td>
<td><em>Sunflower</em>, <em>Phragmites sp.</em></td>
<td>-</td>
<td>[80, 81, 82]</td>
</tr>
<tr>
<td>Co</td>
<td><em>Zooglea</em> spp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[83]</td>
</tr>
<tr>
<td>Zn</td>
<td><em>Bacillus</em> spp.</td>
<td>Bacterially mediated dissolution of Zn from non labile phase</td>
<td><em>Salix caprea, Alnus crispa, Thlaspi caerulescens</em></td>
<td>Pot Experiments</td>
<td>[84, 85, 86, 87]</td>
</tr>
<tr>
<td>As</td>
<td><em>Arthrobacter</em>, <em>Ochrobactrum</em>, <em>Bacillus</em>, <em>Serratia</em> spp <em>Pseudomonads</em></td>
<td>-</td>
<td><em>Helianthus annuus, Agrostis tenuis, Chinese Brake fern Pteris vittata</em> (in its leaves)</td>
<td>As contaminated cattle dip sites</td>
<td>[88, 89, 90, 91, 92, 93]</td>
</tr>
<tr>
<td>Cd</td>
<td><em>Bacillus subtilis</em> <em>Citrobacter</em> spp pseudomonad strains (MKRh1, MKRh3, and MKRh4)</td>
<td>Coinoculation of <em>Brevibacillus</em> sp. and AM Fungus, Cadmium resistant bacterial strains inoculated to plants (Indole acetic acid as auxin produced by the isolates for tolerance)</td>
<td><em>Trifolium repens, Brassica napus Salix viminalis, Thlaspi caerulescens</em></td>
<td>-</td>
<td>[94, 95, 96, 97, 98]</td>
</tr>
<tr>
<td>Cu</td>
<td><em>Bacillus</em> spp</td>
<td>Dissolution of Cu by addition of rhizobacterial strain MS12 &amp; <em>ampicillin 0.1mg/g</em>, Cu tolerant, exopolymer producing bacterial communities, predominantly, <em>Bacillus</em></td>
<td><em>Elsiolzia splendens</em> Willow (<em>Salix viminalis</em>)</td>
<td>Cu- contaminated soil (Near Cu mines)</td>
<td>[99]</td>
</tr>
<tr>
<td>Pb</td>
<td><em>Cupriavidus taiwanensis</em> <em>T268</em>, <em>Bacillus megaterium</em> <em>HKP-1</em></td>
<td>-</td>
<td><em>Mimosa pudica</em>, *Indian mustard (Brassica juncea), Ragweed (Ambrosia artemisiifolia), Hemp Dogbane (Apocynum cannabinum), or Poplar trees, which sequester lead in its biomass.</td>
<td>Experiments in green house</td>
<td>[100, 101]</td>
</tr>
<tr>
<td>Hg</td>
<td><em>Pseudomas fluorescens</em></td>
<td>-</td>
<td>Soybean</td>
<td>In green- house</td>
<td>[102]</td>
</tr>
<tr>
<td>Se</td>
<td>-</td>
<td>Bacteria volatilizes Se into nontoxic forms, such as dimethylselenide</td>
<td><em>Brassica juncea L.</em></td>
<td>-</td>
<td>[103]</td>
</tr>
<tr>
<td>Au</td>
<td><em>Chlorella vulgaris</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[83]</td>
</tr>
</tbody>
</table>
### Table 5: Rhizoremediation of BTEX compounds.

<table>
<thead>
<tr>
<th>Specific Examples of Pollutant</th>
<th>Probable Sources</th>
<th>Microbe/Microbial communities</th>
<th>Characteristics properties</th>
<th>Plant</th>
<th>Soil nature</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Benzene</td>
<td>Oil production and storage</td>
<td>Arthrobacter spp, Bacillus spp, Fusarium solani</td>
<td>Mesophilic, Thermotolerant</td>
<td>Paspalum vaginatum et Zosya tenutifolia</td>
<td>Rhizospheric soil</td>
<td>[104]</td>
</tr>
<tr>
<td>Toluene</td>
<td>Gas work sites</td>
<td>Pseudomonas boreopolis, Rhodococcus erythropolis, Pseudomonas migulae, P. oryzihabitans</td>
<td>Mesophilic, Thermotolerant</td>
<td>Galega orientalis</td>
<td>Rhizospheric soil</td>
<td>[105]</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>Airports</td>
<td>Pseudomonas boreopolis Fusarium solani</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[105]</td>
</tr>
<tr>
<td>Xylene</td>
<td>Paint manufacture</td>
<td>Pseudomonas boreopolis, Achromobacter xylonoxidans and Alcaligenesfaealis</td>
<td>Gram-negative bacteria</td>
<td>Galega orientalis</td>
<td>Rhizospheric soil</td>
<td>[105]</td>
</tr>
</tbody>
</table>

### Table 6: Rhizoremediation of Polycyclic aromatic hydrocarbons (PAH).

<table>
<thead>
<tr>
<th>Specific Examples of Pollutant</th>
<th>Probable Sources</th>
<th>Microbe/Microbial communities</th>
<th>Plant</th>
<th>Soil nature</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>Oil production and storage</td>
<td>Rhodococcus spp, Parvibaculum lavatiorans</td>
<td>Grasses, Betula pendula</td>
<td>Green house</td>
<td>[106]</td>
</tr>
<tr>
<td>Anthracene</td>
<td>Gas work sites</td>
<td>Pseudomonas spp, Rhizobium galegae, R. officinalis, R. orientalis, Pseudomonas migulae, Pseudomonas oryzihabitans</td>
<td>Grasses, Alfalfa, Galega orientalis</td>
<td>-</td>
<td>[107, 108]</td>
</tr>
<tr>
<td>fluoranthene</td>
<td>Coke plants</td>
<td>Soil microflora</td>
<td>Lolium perenne</td>
<td>Rhizospheric soil</td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>Engine works</td>
<td>Soil microflora</td>
<td>Lolium perenne, Brassica campestris, alfalfa (Medicago sativa)</td>
<td>Rdca, lupin, rape, dill, pepper</td>
<td>Rhizospheric soil</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>Landfills</td>
<td>Sphingomonas yanoikuyae</td>
<td>Salix alba matsudana,</td>
<td>Root extract and Rhizospheric soil</td>
<td>[110]</td>
</tr>
</tbody>
</table>

### Table 7: Rhizoremediation of pesticides, chlorinated phenol, chlorinated solvents, and polychlorinated hydrocarbons.

<table>
<thead>
<tr>
<th>Class of Pollutant</th>
<th>Specific Examples of Pollutant</th>
<th>Probable Sources</th>
<th>Microbe/Microbial communities</th>
<th>Plant</th>
<th>Soil nature</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticides</td>
<td>Atrazine, Carbarly, Carbofuran, Coumaphos, D slashing, Glyoxophosphate, Parathion, Propham</td>
<td>Agriculture, Timber treatment plants, Pesticide manufacture, Recreational areas, Landfills</td>
<td>Rhizobium sp</td>
<td>Corticula fluminea, Rice (cv. varuna)</td>
<td>Rhizospheric soil</td>
<td>[111]</td>
</tr>
<tr>
<td>Chlorinated Phenol</td>
<td>Pentachlorophenol</td>
<td>Timber treatment, Landfills</td>
<td>Rhizobium sp</td>
<td>Astragalus chrysanthem</td>
<td>mining taling region</td>
<td>[112]</td>
</tr>
<tr>
<td>Chlorinated solvents</td>
<td>Trichloroethylene</td>
<td>Drycleaners</td>
<td>Pseudomonas fluorescens</td>
<td>Mixture of grasses, legumes, herb and pine, Wheat</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polychlorinated biphenyl</td>
<td>4-Chlorobiphenyl</td>
<td>Electrical manufacturing</td>
<td>Pseudomonas fluorescens</td>
<td>Sugar beet (cv. Rea)</td>
<td>-</td>
<td>[115, 116]</td>
</tr>
<tr>
<td>2,4-Dichlorobiphenyl</td>
<td>Power station, Railway yards</td>
<td>Marine microalga Tetraselmis marina, Burkholderia cepacia</td>
<td>Rarely (Hordeum vulgare)</td>
<td>-</td>
<td>-</td>
<td>[115, 116]</td>
</tr>
</tbody>
</table>

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Conclusions

The main aim of this paper is to provide the scientific understanding needed to harness natural processes and to develop methods to accelerate these processes for the bioremediation of contaminated environments. Except few limiting factors, this technology has the ability to rejuvenate the contaminated environments effectively. However, rapid advances in the last few years has helped us in the understanding of process of bioremediation. The use of culture-independent molecular techniques has definitely helped us to understand the microbial community dynamics, structure and assisted in providing the insight in to details of bioremediation which has surely facilitated to make the technology safer and reliable. In this context, bioremediation in relation to process optimization, validation and its impact on the ecosystem can be performed and by judicious use of the models that can predict the activity of microorganisms that are involved in bioremediation with existing geochemical and hydrological models, transformation of bioremediation from a mere practice into a science is now a reality. With the exciting new development in this field and focus on interdisciplinary research and using it on gaining the fundamental knowledge necessary to overcome the obstacles facing current technologies and also with respect to ethical, legal, and social issues involved this technology will go a long way in cleaning the environment in near future.

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