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# The Future Insight on Bioremediation of Contaminated Soils - Review

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## Abstract

Biodegradation is very fruitful and attractive option to remediating, cleaning, managing and recovering technique for solving polluted environment through microbial activity. The speed of unwanted waste substances degradation is determined in competition with in biological agents, inadequate supply with essential nutrient, uncomfortable external abiotic conditions (aeration, moisture, pH, temperature), and low bioavailability of the pollutant. Mainly, the advantages are greater than that of disadvantages which is evident by the number of sites that choose to use this technology and its increasing popularity through time. The difficult part of evaluating bioremediation techniques is that there are no standard criteria for evaluating among methods. The applicability of bioremediation techniques requires particularly close evaluation of each site. Soil conditions such as porosity, pH, moisture content, and presence terminal electron acceptors all affect remediation technique can be used and technique selection depends on the pollutant that is targeted. By now, the ability of certain microbes to engage in the degradation of environmental pollutants has been well established. Different factors ultimately ensure the applicability of the ex or in situ bioremediation technique to be implemented. The future awaits the discovery of expeditious technologies that would rise above the current challenges and lead the world toward a cleaner and greener environment.

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## Introduction

Bioremediation is the use of microbes to clean up contaminated soil and groundwater. It stimulates the growth of certain microbes that use contaminants as a source of food and energy. Contaminants treated using bioremediation include oil and other petroleum products, solvents, and pesticides. Bioremediation is the use of microorganisms for the degradation of hazardous chemicals in soil, sediments, water, or other contaminated materials. The intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds to the environment. The accumulation of harmful waste in excess has resulted in scarcity of clean water and disturbances of soil thereby limiting crop production (Kamaludeen *et al.*, 2003). The environmental contaminants accumulation like petroleum products, pharmaceutical compounds, chloro and nitrophenols and some other polycyclic aromatic hydrocarbons, organic dyes, pesticides and heavy metals is a serious problem (Mohamed *et al.*, 2016; Rodgers-Vieira *et al.*, 2015). Microorganisms can grow in various environmental conditions as a result they are widely distributed and found in the biosphere having an impressive metabolic activity. The decomposition of pollutants can be carried out exploiting the nutritional versatility of microorganisms. The process in which certain microorganisms convert, modify and utilize toxic contaminants to obtaining energy and biomass production is termed as bioremediation (Tang *et al.*, 2014). It is continued through based on the ability of certain microorganisms to convert, modify and utilize toxic pollutants in order to obtaining energy and biomass production in the process (Tang *et al.*, 2007)

Microorganisms are the most important tools for removal pollutants in soil, water, and sediments. Contamination of the soil with pollutants, toxic chemicals or any contaminant in such a quantity that reduces soil quality and makes it inhabitable to organisms such as insects and other microbes could be termed as soil pollution. It is the addition of chemicals to the soil in quantities that are toxic to the environment and its residents. This anthropogenic activity such as mining, modern practices in agriculture, deforestation, indiscriminate dumping of human generated trash and unregulated disposal of untreated wastes of various industries Pollutants mostly found in soil are categorized as organic and inorganic pollutants. Organic pollutants from industries are built up in soil. Anthropogenic activities contribute immensely to the contamination of soil (Vidali, 2001).

Inorganic pollutants are not biologically degradable substances disposed from industrial chemicals or waste, such as from refineries, pharmaceuticals factories, and sometimes agrochemicals; fertilizers, pesticides, and herbicides (Alori and Fawole, 2017). Other inorganic pollutants heavy metals and some trace elements, sulphates, mineral acids, metals, metal compounds, and inorganic salts (Alori *et al.*, 2017). The inorganic pollutants can persist for longer time in the surrounding environment causing health hazards.

## Cause/sources of soil pollution

The pollution of soil arises from a wide range of sources and the causes could be ample. These might be discrete point sources, or diffuse sources, and the pollution process itself may be deliberate, as in fertilization processes or following an accident, as in the case of radio nuclear accidents or oil spills.

## Industrial and Mining Activities

Large numbers of Industries coming up since the dawn of industrial era without having information in proper waste management systems; and are the biggest contributor to soil pollution. Different industrial and mining by products are contaminants and they are not discharged in a manner that can be considered safe to the environment.

## Modern Agricultural Practices

To enhance the yield from small area of agricultural land, and to meet the increasing demand of food for ever increasing population, synthetic chemical pesticides and fertilizers are being used rampantly in last few decades leading to pollution of the soil. They seep into the ground after they mix with water and slowly reduce the fertility of the soil. Other chemicals damage the composition of the soil and make it easier to erode by water and air. Plants absorb many of these pesticides and when they decompose, they cause soil pollution since they become a part of the land.

## Lack of proper Waste Disposal

Modern lifestyle, urban as well as rural, produces ample wastes and lack of waste management procedures adds to the problem of soil pollution. Urban wastes comprise of both commercial and domestic wastes consisting of dried sludge and sewage, garbage and rubbish materials; plastics, glasses, metallic cans, fibres, paper, rubbers, street sweepings, fuel residues, leaves, containers, abandoned vehicles and other discarded manufactured products. Plastic and other non- biodegradable wastes are the major cause of concern.

## Radioactive Pollutants

Radioactive substances resulting from explosions of nuclear testing laboratories, radioactive fallout and industries giving rise to nuclear dust and radioactive wastes penetrate the soil and accumulate giving rise to soil pollution.

## Biological Agents

Soil gets a large amount of human, animal and bird excreta which constitute a major source of land pollution by biological agents. For instance, heavy application of manures and digested sludge can cause serious damage to plants within a few years.

## Accidental Oil Spills

Oil leaks can happen during storage and transport of chemicals (mostly at fuel stations). The chemical present in the fuel deteriorates the quality of soil and make them unsuitable for cultivation. These chemicals can enter into the groundwater through soil and make the water undrinkable.

## Acid Rain

Acid rain causes pollution in the air mixes up with the rain and fall back on the ground. The polluted water could dissolve away some of the important nutrients found in soil and change the properties of soil.

## Urbanization

Man is blamed for most of the land degradation; productive area is fast reducing because of developmental activities such as human settlement, industries, roads, railways, airports (Nawrot *et al.,* 2006). Pollution of surface soils by materials like vegetables, animal wastes, papers, wooden pieces, carcasses, plant twigs, leaves, cloth wastes as well as sweepings and many non- biodegradable materials such as plastic bags, plastic bottles, plastic wastes, glass bottles, glass pieces, stone cement pieces.

## Mining

The main source of metal pollutants in soils is mining, smelting activities, some garage area by products, and mining can contaminate soils over a large area. Agricultural activities near a mining project may be particularly affected from the released contaminants (Manceau *et al.,* 2000). Mining operations routinely modify the surrounding landscape by exposing previously undisturbed earthen materials, erosion of exposed soils, extracted mineral ores, tailings, and fine material in waste rock piles can result in substantial sediment loading to surface waters and drainage ways. In addition, spills and leaks of some toxic materials and the deposition of contaminated windblown dust lead to soil contamination (Manceau *et al.,* 2000). Fugitive dust poses environmental problems at some mines. The inherent toxicity of the dust depends upon the proximity of environmental receptors and type of ones being mined. Soils contaminated from chemical spills and residues at mine sites may pose a direct contact risk when materials are misused.

## Land degradation

Soil Erosion occurs when the weathered soil particles are dislodged and carried away by wind or water. Deforestation, agricultural development, temperature extremes, precipitation including acid rain, and human activities contribute to the soil pollution through soil erosion. Anthropogenic activities speed up this process by construction, mining, cutting of timber, over cropping and overgrazing and this result in floods and cause soil erosion (Leon, 2008). Forests and grasslands are an excellent binding material and support many habitats and ecosystems, which provide innumerable feeding pathways or food chains to all species. Scientists believe that a wealth of medicinal substances including a cure for cancer and aids, lie in the forests. The precious rain forest habitats of South America, tropical Asia and Africa are coming under pressure of population growth and development especially timber, construction and agriculture. Removal of forest is slowly destroying the most productive flora and fauna of World, which form vast tracts of a very valuable sink for CO2 (Leon, 2008).

## The soil environment

Soil is the core and facilitator of terrestrial ecosystem and its particulate and colloidal composition, either in organic matter, minerals, or microorganisms form, which are not unconnected beings.

They are, interacting with one another in affecting the transformation and fate of inorganic and organic pollutants (Chen *et al.,* 2019). Turgut *et al.,* (2004), considered soil as natural bodies that cover the earth’s surface that is known to support plants’ growth and that have properties due to integration of the effects related to climate and organisms acting on parent material as conditioned by relief over a span of time. Soil pollution, generally means the presence of a chemical substance or compound foreign and/or at higher concentration in the soil that poses adverse threats to un-targeted organisms (FAO and ITPS, 2015). Naturally occurring compounds in soils; metals and petroleum seeps, may also be very problematic.

Contaminants released at the surface can be transported vertically and laterally into surface and ground waters and eventually ingested by water users, thereby, representing fatal human health risks, among others.

They can also be taken up by plants, accumulated in animal tissues, and be eventually found in the foods ingested by the human kinds. Literally, there exist thousands of chemicals used in industry and agriculture for human benefit. As such, there are diverse types of compounds that become lethal soil contaminants.

## Hydrocarbon Contaminations and Their Sources

Hydrocarbons are naturally occurring compounds principally formed from plant and animal fossils due to natural or anthropogenic actions. They are made up entirely of carbon and hydrogen atoms and serve as the foundation for crude oil, natural gas, and coal, providing a significant proportion of the world's energy (Ehis-Eriakha *et al.,* 2020). Hydrocarbons and their derivatives intentionally or accidentally released into the soil and oceans; and principally existed in solid, liquid, or gaseous states (Liu *et al.,* 2019). Garages, gas station services, mining, wastes from chemical and petrochemical industries, agriculture wastes, incomplete combustion of organic matter, sludge wastes, gasification, automobile exhaust and processing, production, transportation, run-off asphalt pavements, volcanic eruption, the distillation of wood, waste disposals, vehicular emission, and combustion of fossil fuel can be contaminant hydrocarbon sources (Singh and Haritash, 2019). The difficulty of cleaning up of environment contaminated with hydrocarbons is the ability to identify potential sources either point or nonpoint source. As some studies indicated, hydrocarbons have a significant adverse impact on ecosystem (Ben Ayed *et al.,* 2015). Introduction of hydrocarbons into the soil restricts the supply of water, nutrients, oxygen, light, and other parameters for the biological processes. It can affect soil fertility (plant growth and seed germination) and consequently agricultural productivity (Varjani and Upasani, 2019). Hydrocarbon contaminants cause immediate or latent effects like genetic mutations, immunotoxicity, teratogenicity, carcinogenesis, high bioaccumulation potential, and deterioration of the ecosystem functioning and treating of animal and plant life (Pattabhiramaiah, 2018) also causes cancers of the skin, lung, bladder, liver, and gastrointestinal.

## Bioremediation

Bioremediation is a process of converting harmful substances to environmentally safe substances by the action of the invisible workforce. Ecologically, bioremediation refers to the interaction between three factors; contaminant, invisible workforce, and environment. Their interaction in turn ensures the mobility of contaminant in the environment, the presence of suitable conditions to degrade the contaminant, and degradation of the contaminants by converting it into an environmentally friendly substance. Mobility or bioavailability of any contaminant is about the ease with which the contaminant is available for biodegradation to microorganisms (Tiedje, 1993). The microorganisms need suitable conditions to function like availability of electron acceptors, pH, and availability of nutrients to run well and convert the environmentally harmful substances to harmless benign substances. The biodegradability of the contaminant depends upon the presence of suitable microorganisms to eradicate that contaminant under the required conditions.

Eradication of contaminants depends on the its nature of the contaminants; pesticides, herbicides, heavy metals, hydrocarbons, sewage, plastics, degree of contamination, environmental factors, contaminated sites, cheap policies for conserving environment are important selection study criteria that are considered while choosing any bioremediation technique (Smith *et al.,* 2015). Other factors involve the aerobic and anaerobic nature of the area under study, pH, and moisture content are equally important to be considered.

Bioremediation strategies make it possible to increase the efficacy of the contaminant removal process. Mostly, the bioremediation techniques work for the removal of hydrocarbon contaminating types from soil or water, cost-effective techniques are efficiently applied to the contamination sites for the eradication of hydrocarbons (Kim *et al.,* 2014).

## Factors affecting microbial bioremediation

Microorganisms are involved through enzymatic pathways act as biocatalysts and facilitate the progress of biochemical reactions in degrading the desired pollutant. Microorganisms act against the pollutants when they have access to different materials to help them generate energy and nutrients to build more cells. The efficiency of bioremediation depends on the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their availability to microorganisms (El Fantroussi and Agathos, 2005). The ability of the microbes to reduce organic materials to serve as sources of energy is among major variables that affect microbial activity. Oxidation of carbon in a contaminant serves as an effective source of energy for an aerobic heterotrophic organism. The result of biodegradation practice hinges on microbial; population diversity, enzyme activities, and biomass concentration, physical, chemical characteristics, molecular concentration, and structure of substrate, and pH, moisture content, temperature, EC, availability of electron acceptors and carbon, and energy sources an array of environmental factors. The contaminants concentration and molecular structure considerably affects bioremediation practicability and the type of microbial transfiguration that occurs, and whether or not the compound will serve as a primary, secondary, or co-metabolic substrates. (Salilh and Tarekegn, 2020).

### Biological factors

Competition between microorganisms for limited carbon sources, antagonistic interactions among microorganisms or the predation of microorganisms by protozoa and bacteriophages are biotic factors affecting the degradation of organic compounds. Amount of catalyst represents the number of organisms able to metabolize the contaminant as well as the amount of enzyme produced by each cell (Madhavi and Mohini, 2012).

### Environmental factors

The interaction between soil microorganisms and contaminants depends on the environmental situations of the site of the interaction. The activity and growth of microorganism are affected by pH, temperature, moisture, soil structure, solubility in water, nutrients, site characteristics, redox potential and oxygen content, lack of trained human resources in this field and Physico-chemical bioavailability of pollutants (contaminant concentration, type, solubility, chemical structure and toxicity) (Adams *et al.*, 2015).

### Nutrient availability

To survive and continue their normal activities microbes needs nutrients such as carbon, nitrogen, and phosphorous. Biodegradation in aquatic environment is limited by the availability of nutrients and oxygen (Thavasi *et al.*, 2011). Presence of essential nutrients needed for the growth and working of microbes is necessary for the proper outcomes of bioremediation. Nitrates, phosphates, and electron transport sources are required to be present in the soil and water environment for bioremediation to be carried out accordingly.

### Nature and concentration of contaminants

Concentration and nature of the contaminant are among the limiting and important factors for the bioremediation process to be considered. The presence of heavy metal and some impurities inhibits the growth of microbes and it tends to inhibit the bioremediation process to be carried out in the removal of this contaminants. Similarly, increased concentrations of contaminant in the particular bioremediation site affect the microbial colonies and distribution both in terms of growth and enzymatic functioning of the microbes.

### Oxygen availability and moisture content

The availability of oxygen is a very important as it ensures the oxidative and educing environment in both soil and water environment. Soil rich in sand and gravel content helps to retain moisture as well as aerate the soil well. More heavy the clay form or soil rich in organic content reduce the availability of oxygen and thereby inhibiting the functioning of microbes.

### Temperature

Temperature is the most important in determining the survival of microorganisms and composition of the hydrocarbons. Biological enzymes are participated in the degradation pathway have an optimum temperature and will not have the same metabolic turnover for every temperature.

Temperature either speeds up or slows down bioremediation process and it strongly affects microbial physiological properties (Macaulay, 2014). It is among the important factors that determine the moisture content and chemical composition of the site. Generally, the temperature between 20 and 40°c is considered good for the efficient performance of microbes.

### pH

The pH measurement in soil could indicate the potential for microbial growth. Higher or lower pH values showed inferior results; metabolic processes are highly susceptible to even slight changes in pH (Asira and EnimEnim, 2013). The pH of the soil or water determines the nature of the species present in it, as it can cause a change in the chemical composition due to the basicity or acidity nature of the site. The pH ranges from 5 to 9 is taken as optimum for the working of different microbial colonies. As biological reactions are all pH-sensitive so existence and functioning of enzymes are highly pH-sensitive.

### Soil Contaminants

Soil contamination is part of soil degradation caused by anthropogenic interferences or alteration of the natural soil environment. The soil is considered contaminated when the concentration of chemicals, nutrients, or other elements in the ground is greater than the standard concentration.

Soil is considered a vital resource, and considered as nonrenewable. Soil pollution is a worldwide problem that draws its origins from anthropologic and natural sources. Bioremediation techniques applied on the basis of strategies to resolve this problem can be classified in situ and ex situ.

## Organic Soil Contaminants and Their Sources

Organic contaminants have enormously large list, including hydrocarbons, different solvents, PAHs (polycyclic aromatic hydrocarbons), petroleum products, pesticides, herbicides, PCBs (polychlorinated biphenyls), phthalate esters, phenols, and their derivatives (Oleszczuk, 2006; Moore, 2006). These pollutants affect the soil properties, with the presence of little amount by altering the microorganisms distribution and their metabolic activities as well.

Unluckily, the classical technologies for elimination of contaminants from the soil system are costly, which discourage industries to take care of their effluent discharge (Alkorta *et al.*, 2001). To remove organic pollutants from the contaminated soil, bioremediation serves as a very good option as it is eco-friendly and cost-effective (Glazer *et al.*, 1995). Rhizoremediation is a process in which plants are used to remediate pollutants from soil with the help of microbes present in their rhizospheric region (Oberai *et al.*, 2018). Here plants serve homely environment to microbes by providing them carbon source in the form of root exudates, and the microorganisms do the degradation work and the residues can be taken by the plants with the water transport (Cannon *et al.*, 2005). Soil acts as bed not only for seeds and microbes but also for the environmental pollutants. There are many organic pollutants of the soil which are categorized in three classes by UNEP in 2001; these are industrial chemicals pesticides, and by-products (UNEP, 2011).

## Fate of Organic Pollutants in Soil

Once an organic pollutant enters into the ecosystem, its movement within the ecosystem depend on its chemical nature; broadly, if it is biodegradable, it will be mineralized in its components with time and if it is non-biodegradable then it will persist in the environment and may impose a series of events on the surrounding environment. The concentration of pollutants deposited during a long course of time can be known by measuring the concentration of the same in sediments, which is a well-accepted compartment for accumulation of these pollutants (Guzzella *et al.*, 2005). Sediments are made up of several components; biological, organic, and inorganic originated from different sources and containing various features.

## Bioremediation of organic and inorganic compounds

Bioremediation methods with low operational costs for the removal or minimization of inorganic and organic pollutants (hydrocarbons, halogenated organic compounds, halogenated organic solvent, non-chlorinated pesticides and herbicides, nitrogen compounds, and heavy metals (lead, mercury, chromium, radionuclide) at least to the level that they cannot cause a serious effect to ecological functions (Yap *et al.,* 2021). Various applications of both macro- and microorganisms are used, but bacteria are selectively useful to degrade a variety of hydrocarbon pollutants and are ubiquitous (McFarlin *et al.,* 2014).

## Organic pollutants Bioremediation in the Soil

Organic compounds (OCs) such as biocides and flame retardants have been widely used and are now considered as a threat to forms of life on the planet. Most OCs; polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBEs), and polycyclic aromatic hydrocarbons (PAHs), are biodegradable by microbes and possible to eradicate from environment. Biodegradation is the process by which microbes break down organic compounds into less toxic or entirely non-toxic residues (Yaashikaa *et al.,* 2022). To obtain organic carbons and energy, the microbes consume the organic substrate. Isolated from other microbes, an individual microbial species usually does not degrade any organic substrate and does well in a community. As a result of community microbe interactions, resistance, chemical-degrading ability, and tolerance are all conferred by the exchange of genetic information among microbial species (Bhatt *et al.*, 2021). A successfully bioengineered microbe requires the identification of the relevant species and strains for each substrate. A viable alternative to the recombinant degradation of resistant organic compounds is biodegradation by microbes using readily-available organic carbon and energy sources in the surrounding environment. Microbes use the fluctuation in chemical gradients in their environment to determine the most favorable conditions for growth (Mbé *et al.,* 2021).

## Bioremediation for Inorganic Pollutants

The disposal of toxic metals as a pollution source to soils and waters on or below the surface causes unacceptable health risks and as a result many environmentalists took as a concern (Joutey *et al.*, 2013). Several microorganisms have been reported for the bioremediation of organic and inorganic pollutants. Microbes create chelation as agents and acids that can change the physicochemical properties of redox potential in their environment can cause significant changes in the environment by increasing the bioavailability of metal ions (Masindi and Muedi, 2018).

Some lists of steps in the interaction between metals and microbial cells are physical adsorption, biosorption, and ion complexation. Microbes have a capability of adapting to heavy metals, such as iron, zinc, chrome, magnesium, mercury, and barium in textile waste, was demonstrated in the multidrug resistant *Pseudomonas aeruginosa* T-3 isolate from tannery effluent (Nkrumah *et al.*, 2018). Such type of ability of microbes implies that they have an ability to adapt to the changing environment

## Principles of Bioremediation

Microbes possess enzymes that allow them to destruct contaminants that are environmental contaminants as a food. All metabolic reactions are mediated by enzymes. These enzyme groups are oxidoreductases, hydrolases, lyases, transferases, isomerases and ligases.

Enzymes have a remarkably wide degradation capacity as they are either nonspecific or specific affinity to the substrate. Bioremediation is effective where environmental conditions permit microbial growth and activity and its application encompasses the manipulation of environmental parameters to permit growth of microbes and degradation to proceed at a faster rate (Kumar *et al.*, 2011). Technology involving bioremediation is principally dependent of biodegradation. Considerable mechanisms and pathways have been elucidated for the biodegradation of a wide variety of organic compounds; for instance, it is completed in the presence and absence oxygen (Pankaj Kumar Jain and Vivek Bajpai, 2012).

## Bioremediation: A Conventional Approach

Rhizoremediation is a method where soil contaminants are degraded by the rhizospheric microorganisms. In another words it is also termed as rhizodegradation, microbe-assisted phytoremediation technology, and rhizosphere bioremediation. The rhizosphere is the very active region around roots affected by plant activity (Liu *et al.*, 2014).

## Bioremediation process and classification

Various microbial processes are involved in bioremediation-based cleaning; many were developed and utilized since the advent of this field fundamental principle of all the methods is to stimulate the microbe to yield optimum microbial enzymatic activity. The utilization of microbial communities employs the enzymatic metabolic pathways that have evolved over a long period of time.

Under specific conditions, microbes degrade organic contaminants and gain energy for growth and reproduction through the consumption of the contaminants as substrate. To breakdown of chemical bonds energy is involved and the free electrons released are transferred to an electron acceptor such as oxygen. Basically, this process involves redox reaction in which the organic matter is oxidized through loss of electrons while the compound that accepts the electron is said to be reduced.

## Current status of bioremediation

Bioremediation is applied for the removal of a wide range of pollutant and laboratory trials extended to a large contaminated site. In every case, some modifications are carried out into the basic technologies for the optimization of parameters for the successful applicability of the bioremediation. These parameters depend on the place of contamination, nature of the contaminant, and the source of contamination.

## Techniques of Bioremediation

Superficially, bioremediation techniques can be carried out ex-situ and in-situ site of application. Pollutant nature, concentration, depth and amount of pollution, type of environment, location, cost, and environmental policies are the selection standards to be considered for selection of this technique.

Performance based on some factors and environmental conditions like oxygen and nutrient concentrations, temperature, pH, and other abiotic factors that determine the success of bioremediation processes.

## Ex Situ Remediation Techniques

Ex situ remediation includes techniques such as land farming, biopiling, land farming, composting and processing by bioreactors along with thermal, chemical, and physical processes. (Koning *et al.,* 2000) Ex situ thermal processes involve the transfer of pollutants from the soil to a gas phase. The pollutants are released by vaporization and the burned at high temperatures. Ex situ thermal remediation is completed in 3 steps: soil conditioning, thermal treatment, and exhaust gas purification. (Van Deuren *et al.,* 2002). The ex situ remediation process known as soil scrubbing uses mechanical energy to separate the pollutants from the soil. Composting consists of excavating the soil and then mixing organics such as wood, hay, manure, and vegetative waste with the contaminated soil (Van Deuren *et al.,* 2002) It involves the excavation of contaminants and transporting them to the treatment sites above the earth's surface. Indigenous microbes that have been living there in the soil act as the remediating agents provided other environmental factors are kept monitored. This method is tailored by altering the degradation environment and maintaining the optimum conditions required for microorganisms to work efficiently as well as accordingly. Sometimes, the amendments are added to the soil. On the basis of phases of contaminant material, ex situ method can be for a solid phase or slurry phase in which solid agricultural waste or domestic, sewage sludge, industrial waste, and municipal solid waste are treated to get compost, employed for the conditioning of soil. Before compost formation different treatments has done to enhance the biological treatment potential. Depending on the availability of O2 and suitability of the working pH, the enzymes secreted by microorganisms detoxify the surrounding area. Ex situ treatment to as it e that has some compositional limitations or nutrient deficiency for microbial activity needs tailoring of the site by adding site-specific compost. Adjustment of pH and water availability in the bioremediation process site assures the efficiency of microbial colonies at ex situ operating sites. In this method supply of air, maintenance of proper pH, temperature, micronutrients are needed for the growth of microbial colonies (http://www.online). Ex situ refers to the destruction or treatment of the contaminant from the site by using different and appropriate methods that fits the condition.

## Land filling

Land farming is the simplest way and an outstanding bioremediation technique because of its low cost, less equipment for operation and it is an aerobic bioremediation process carried out for a long time. Polluted soils are transported to a land farming area, incorporated into the soil surface over large areas, and periodically tilled to aerate the mixture. In land farming, polluted soils are regularly excavated and tilled and site of treatment speciously regulates the type of bioremediation. When excavated polluted soil is treated on-site, it is ex-situ as it has more in common than other ex-situ bioremediation techniques. Excavated polluted soils are carefully applied on a fixed layer support above the ground surface to allow aerobic biodegradation of pollutant by autochthonous microorganisms (Silva *et al.,* 2012). Over all, land farming bioremediation technique is very simple to design and implement, and can be used to treat large volume of polluted soil with minimal environmental impact and energy requirement (Maila and Colete, 2004).

## Composting

It is a method involving the stacking of polluted soil along with organic substances. These are added to complement the measure of nutrients and organic matter readily degradable in the topsoil, stimulating bacterial growth by adding nutrients results in efficient biodegradation within a relatively brief timeframe (Sharma, 2019). Compositing technique provides oleophilic microbial population and higher temperatures making it more promising than land farming which is based exclusively on native soil biota. Likewise, compost manure, which is useful for agricultural purposes, is generated as an end product (Sharma, 2019). The benefits of composting include the enrichment of soil quality and characteristics as well as its ecofriendly nature to the contaminated site. Some limitations of composting are continuous site monitoring, time consuming as it may take a month and labor intensive. In addition to the above listed drawbacks, toxic and odorous (green house) gas may be released into the environment as well.

## Biopiles

Biopiling also called as bioheaps, biocells, or biomounds, is mostly used to address a wide variety of petrochemical pollutants in soils and sediments. This entails building piles of contaminated soils or dried sediments and encouraging aerobic microbial populations to degrade the material by fostering ideal or nearly ideal growth conditions inside the pile (Sharma *et al.,* 2019). Some techniques used are aeration, adjustment of pH and humidity levels, and addition of nitrogen, phosphorus, and introduction of heat. Because of this optimal condition for growth, the increase in microbes' activity disintegrates bioavailable pollutants. Biopiles is a way in which excavated soil polluted with aerobically remediable hydrocarbons, can be treated in "biopiles". In this process, air is supplied to the biopile system during a system of piping and pumps that either forces air into the pile under positive pressure or draws air through the pile under negative pressure. The microbial activity is enhanced through microbial respiration then the result in degradation of adsorbed petroleum pollutant became high (Emami *et al.,* 2012). Biopiles are in a way similar to landfarms due to the fact that this technology also uses oxygen as a way to stimulate bacterial growth.

## Bioreactors

The conversion of polluted media via a series of biological processes in a vessel to a specific product is an effective bioremediation technique. Bioreactors are designed to provide optimum conditions for microbial growth and biodegradation were designed for use in bioremediation strategies to improve the various anticipated objectives (Robles-González *et al.,* 2018). Bioreactors can be in batch, continuous, fed batch and multistage mode and are aimed at optimizing microbial processes concerning polluted media as well as the kind of pollutant. Slurry bioreactors offer an ex-situ eco-sustainable way to remediate most soils and sediments from petroleum hydrocarbons and explosives when formed into slurry (Samer, 2015). The most fundamental biofilter bioreactor consists of a sizeable media bed which allows microbes to pass through the pollutants for degradation. Biofilters are among the earliest known bioremediation techniques used in the environment (Samer, 2015). Microorganisms grow on the surface of the packaging material in biofilm forms and are responsible for the degradation of effluent pollutants. Membrane bioreactors use a membrane to create a biological filtration system (Samer, 2015). The membrane offers a barrier that excludes the solid from the liquid component while ensuring good effluent quality.

## In-Situ Remediation Techniques

In situ bioremediation is the subsurface treatment of contaminants by the biological system of that area. It is a sustainable method as it does not require any excavation and transportation of contaminants. Some in situ bioremediation techniques like biosparging, phytoremediation, and bioventing, have been enhanced to get good outcomes for onsite decontamination while some other techniques like natural attenuation or intrinsic bioremediation proceed without any enhancement (Frascari *et al.,* 2014). In situ remediation includes techniques such as bioventing, biosparging, bioslurping and phytoremediation along with physical, chemical, and thermal processes. In situ remediation is less costly due to the lack of excavation and transportation costs, but these remediation techniques are less controllable and less effective. (Koning *et al.,* 2000). In situ thermal processes are still in the developmental phase. The process involves injecting a steam-air mixture at 60-100oC into the soil. This method is successfully used to treat chemically contaminated sites like; industrial effluents dumping sites containing dyes, chlorinated solvents, and hydrocarbons polluted sites, and heavy metals (Folch *et al.,* 2013). Intrinsic bioremediation involves the conversion of contaminant to nontoxic form by the microbial communities naturally present in soil and water. Annual water flow through the area understudy determines the presence of various minerals and pH of that soil which in turn tells about the working of microbes under such conditions. The presence of heavy metals hindered the growth of microorganisms present in the soil and water. The time of exposure of microorganisms to the contaminant is also an important parameter that should be studied at a pilot scale before conducting the bioremediation on a wide surface area. In situ bioremediation is laborious as compared to other methods (http://www.online). In situ bioremediation refers to treating contaminants at the site with minimal disturbance using methods such as bioventing, biostimulation, bioaugmentation, and/or natural attenuation.

## Bioventing

Bioventing is involved in venting of oxygen through soil to stimulate growth of natural or introduced bacteria and fungus in the soil by providing oxygen to existing soil microorganisms; indeed, it is functional in aerobically degradable compounds. Bioventing uses low air flow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil by means of wells. Adsorbed fuel residuals are biodegraded, and volatile compounds also are biodegraded as vapors move slowly through biologically active soil (Agarry and Latinwo, 2015). This technique uses low pressure air and focuses more on the deeper unsaturated soil zone.

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### Bio simulation

Bio-stimulation is linked through the injection of nutrients at the site (soil/ground water) to stimulate the activity of indigenous microorganisms. It is the stimulation of indigenous or naturally existing bacteria and fungus community. Firstly, by supplying fertilizers, growth supplements and traces minerals. Secondly, by providing other environmental requirements like pH, temperature and oxygen to speed up their metabolism rate and pathway (Adams *et al.,* 2015 and Kumar *et al.,* 2011). The Presence of small amount of pollutant can also act as stimulant by turning on the operons for bioremediation enzymes. This type of strategic path is most of the time continued in the addition of nutrients and oxygen to help indigenous microorganisms (Madhavi and Mohini, 2012). It is the addition of growth-limiting factors such as substrates, vitamins, oxygen, and modifying the environmental conditions (temperature, moisture, pH, redox potential, terminal electron acceptors) in the hydrocarbon-contaminated environments (Dindar *et al.,* 2016). When hydrocarbon contaminants are greatly available, they provide high carbon and energy but nitrogen or phosphorus for bacterial growth (Galitskaya *et al.,* 2016). Certain limitations for effective biostimulation application are rapid depletion of inorganic nutrients (NP), the nature of the soil, and the scarcity of indigenous hydrocarbon-degrading bacteria. Supplementing the biosimulation by nutrients in the form of fertilizers; KNO3, NaNO3, NH3NO3, K2HPO4, and MgNH4PO4 when they are not fast enough to degrade the pollutant hydrocarbons is important (Maletic *et al.,* 2019).

### Bioaugmentation

It refers to the introduction of potential microbial strain or consortia isolated from other contaminated sites or the genetically modified microbes to support the remediation (Galitskaya, 2016). This strategy is applied if natural attenuation is ineffective because of different factors like low indigenous hydrocarbon-degrading population, sluggish decontamination activities, and high-stress situations to start the bioremediation process (Bidja Abena *et al.,* 2019). For the bioremediation approaches, microbial cell bioaugmentation is a commonly used technique for the removal of contaminants. In addition, there are several bioaugmentation techniques: phytoaugmentation, rhizosphere bioaugmentation, gene bioaugmentation that have been exercised for cleaning up contaminated sites. In order to rapidly increasing the natural microorganism population growth and enhance degradation that preferentially feed on the contaminants site. Microbes are collected from the remediation site, separately cultured, genetically modified and returned to the site. For convince, all essential microorganisms are found in sites where soil and groundwater are contaminated with chlorinated ethenes, such as in tetrachloroethylene and trichloroethylene. It is used to ensure that the in situ microorganisms can totally remove and alter these contaminants to ethylene and chloride, which are non-toxic (Niu *et al.,* 2009). Bioaugmentation is the process of adding engineered microbes in a system which act as a bioremediators in order to quickly and totally eliminate complex pollutants. Genetically modified microorganisms showing and proving that can increase the degradative efficiency of a wide range of environmental pollutant. Natural species are not fast enough to break down certain compounds so to facilitate must be genetically modified through DNA manipulation; genetically engineered microbes act as break down pollutants much faster than the natural species and highly compete with the indigenous species, predators and also various abiotic factors (Malik and Ahmed, 2012; Alwan, 2013).

### Natural attenuation/ intrinsic bioremediation

Natural attenuation refers to the use of indigenous microbial populations to eliminate or detoxify hazardous hydrocarbon pollutants into less or nontoxic forms (Maletic *et al.,* 2019). Time required for natural attenuation depends on contamination, site conditions, and applicability of potential degrading bacteria and it implies that the use of natural attenuation is cost-effective and efficient if there is no need for a complex remediation process (Maletic *et al.,* 2019). During this process, the indigenous microbes utilize hydrocarbon contaminants as the sole carbon and energy sources based on their natural metabolic pathways. When the soil is contaminated with hydrocarbon contaminants, the number of indigenous hydrocarbon-degrading microorganisms increases rapidly and start to adapt to and metabolize (degrade) the pollutant (Kostka *et al.*, 2014). Natural attenuation depends on indigenous hydrocarbon degraders and its effectiveness is contingent on nutrient availability, type and concentration of contaminants, physical parameters, the fate of contaminants, and potential microbial communities with necessary catabolic genes for complete hydrocarbon degradation (Varjani and Upasani, 2019). Bioattenuation or natural attenuation is the eradication of pollutant concentrations from surrounding. It is carried out with in biological processes it maybe include (aerobic and anaerobic biodegradation, plant and animal uptake), physical phenomena (advection, dispersion, dilution, diffusion, volatilization, sorption/desorption), and chemical reactions (ion exchange, complexation, abiotic transformation). Terms such as intrinsic remediation or biotransformation are included within the more general natural attenuation definition (Mulligana and Yong, 2004).

## Bioslurping

The technique of bioslurping combines many of processes, such as vacuum enhanced pumping, bioventing, and soil vapor extraction, for the removal of soil and groundwater pollutants using an indirect supply of oxygen and stimulating microbial biodegradation. The limitation of this technique is the low permeability of soil which reduces the oxygen transfer rate and further decreases the microbial activity. These techniques generally are used for the removal of volatile and semivolatile organic contaminants from soil and liquid (Vidali, 2001).

## Biosparging/ air sparging

In this technique, microbial activities in polluted sites are improved by injection of air into the soil subsurface (Sharme, 2020). In bioventing high air-flow is required for volatilization of pollutant, whereas injected air in biosparging stimulates biodegradation. Soil permeability and pollutant biodegradability are two major factors that determine the success of biosparging technique (Sharme, 2020).

## Application of Bioremediation

Bioremediation is helpful for decomposition, eradication, immobilization, or detoxification of variable chemical wastes and physical hazardous materials from the surrounding through the all- inclusive and action of microorganisms. Bioremediation can occur naturally or stimulated, e.g. by the application of fertilizers (biostimulation), by the addition of similar microbe strains, the effectiveness of the resident microbe population to degrade contaminants may be increased.

The biological community exploited for bioremediation generally consists of the native soil microflora. Higher plants can also be manipulated to enhance toxicant removal hytoremediation, for remediation of metal contaminated soils.

## Phytoremediation

Phytoremediation is an in situ technique that uses plants to remediate contaminated soils. Phytoremediation is most suited for sites where other remediation options are not costs effective, low-level contaminated sites, or in conjunction with other remediation techniques (Schnoor, 2000). The direct use of green plants and their associated microorganisms to stabilize or reduce contamination in soils, sludges, sediments, surface water, or ground water is defined as Phytoremediation. This technique depends on the use of plant interactions (physical, biochemical, biological, chemical and microbiological) to contaminated sites to mitigate the toxic effects of pollutants. It is an alternative technology that can be used along with or in place of mechanical conventional clean-up technologies that often require high capital inputs and are energy intensive. Depending on pollutant type (elemental or organic), there are several mechanisms (accumulation or extraction, degradation, filtration, stabilization and volatilization) involved in phytoremediation. Elemental pollutants (toxic heavy metals and radionuclides) are mostly removed by extraction, transformation and sequestration.

## Mycoremediation

It is an important form of bioremediation by the use of fungi. Fungi are an excellent source to remove toxic pollutants from the environment and easily colonize both biotic and abiotic surfaces (Bharath *et al.,* 2019). The most suitable fungi to be used in soil remediation are basidiomycetes and the ecological groups of saprotrophic and biotrophic fungi. Various steps are involved in mycoremediation (Treu and Falandysz, 2017).

✓ Fungi freely present in the soil, or in symbiotic association with plant roots (ectomycorrhizal and endomycorrhiza)
✓ They are decomposers, decompose dead organic matters.
✓ Fungi are saprotrophs, feed on dead organic matters.
✓ Fungal hyphae produce and secrete special acids and enzymes that decompose, lignin (White-rot fungi) and cellulose (brown-rot fungi)
✓ Fungal mycelium by microfiltration removes toxic substances
✓ Fungi are useful in the degradation of oils, petroleum compounds, hydrocarbons, aromatic compounds, and pollutants in soil and water
✓ Mushrooms Agaricus, Amanita, Cortinarius, Boletus, Leccinum, Suillus, and Phellinus are used for mobilization/complexation of different heavy metals in soil.

### Phytovolatilization

Plants absorb contaminants from the soil and release them into the gaseous atmosphere in an unstable form through the process of transpiration.

### Rhizodegradation

It is the symbiotic relationship between plants and microbes. It is the breakdown of the contaminants due to the presence of protein and enzymes by plants or soil organisms in the rhizosphere.

### Phytoextraction

Plants take up the contaminants from water and pass them from the roots to the plant's upperparts.

### Phytostabilization

Certain plant species are used to bring contaminants from water and soil

### Phytoextraction

Algae are used to extract pollutants from soils, sediments, or water into harvestable plant biomass (hyperaccumulators, organisms that take larger than normal amounts of contaminants from the soil). Phytoextraction is more effective for extracting heavy metals than for organic contaminants. The plants translocate contaminants through their root systems to stems and leaves. Different plants absorb different elements and accumulate them into different organs of the plant. Sunflower (*Helianthus annuus*), Chinese Brake fern (*Pteris vittata*) are effectively used for the removal of Arsenic. Chinese Brake fern, act as a hyperaccumulator, and accumulates arsenicinits leaves. Willow, a common plant, has significant potential as a phytoextractor of cadmium (Cd), zinc (Zn), and copper (Cu).

This plant has some unique characteristics like a high transport capacity for heavy metals from root to shoot and large biomass production. Willow can also be used to produce energy in a biomass fueled power plant. Alpine pennycress (*Thlaspi caerulescens*), a hyperaccumulator, is effective for the removal of metals Cadmium and Zinc, although its growth appears to be inhibited by copper.

### Phycoremediation

It is an excellent form of remediation in aquatic ecosystems. Microalgae, "wonder organisms," are capable of accomplishing bioremediation efficiently by bioassimilation and biosorption. They have the capability to grow in polluted water as "algal blooms" and assimilate various pollutants. Metal pollutants can easily enter the food chain if heavy metal-contaminated soils are used for the production of food crops.

### Microorganisms

Microorganisms are the beneficial source for removing pollutants from soil and water. Bioaugmentation is the addition of microorganisms to the soil, where biostimulation is the modification, addition, reduction, or genetic engineering of the microbial colonies to degrade pollutants

### Nematodes

Nematode parasites are a sensitive indicator of heavy metals in the aquatic ecosystem showing sharing of more burden of environmental pollution of the sea and also act as bioremediator of heavy metals in fish. In rhizosphere, they are involved in cleaning, nutrient mobilization, nitrification, enzyme activation.

**Fig.1** Source of soil pollution

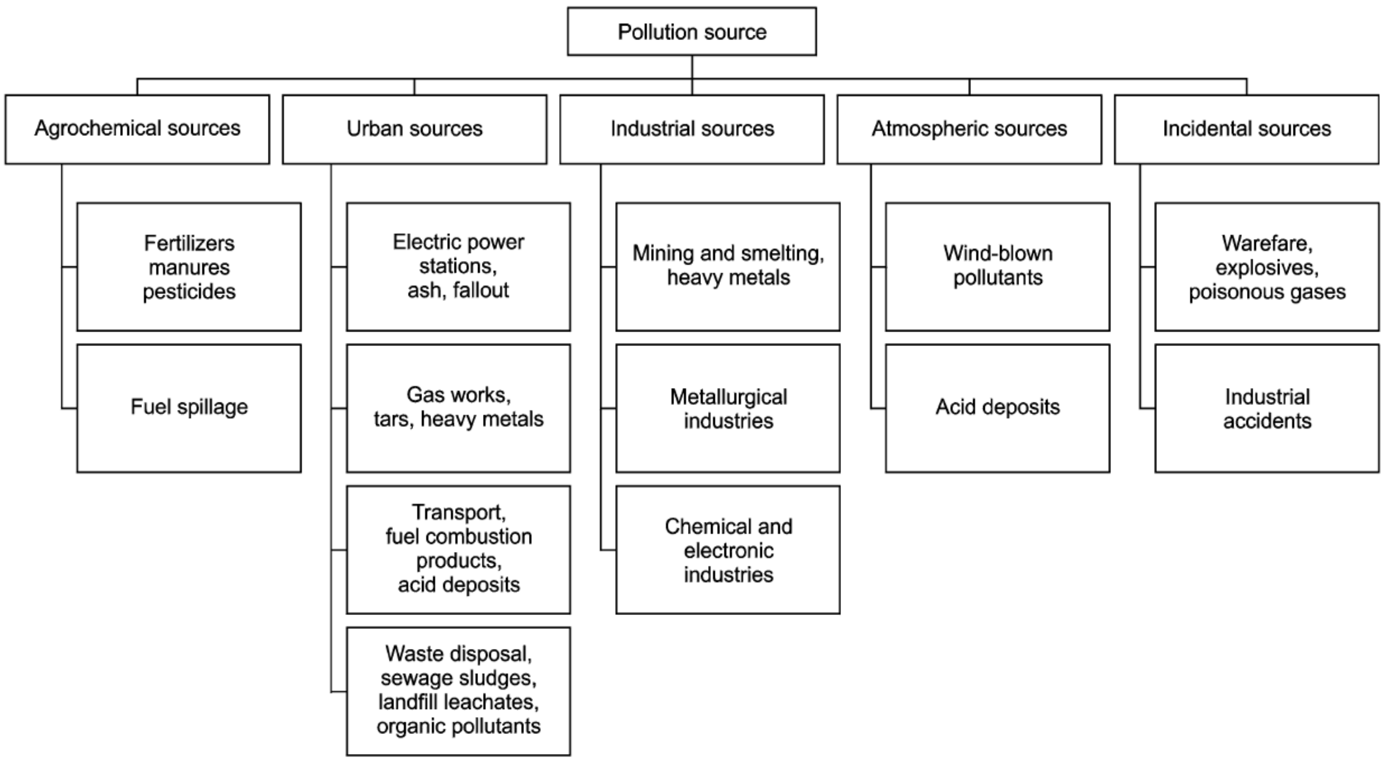



**Fig.2** Ecological Interpretation of factors governing Bioremediation

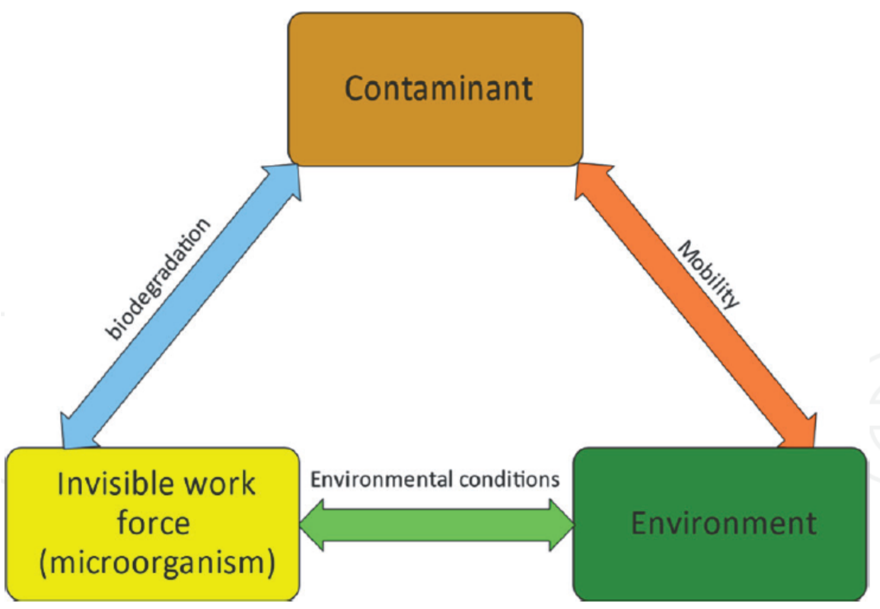


**Fig.3** Major sources of pollutants in soil

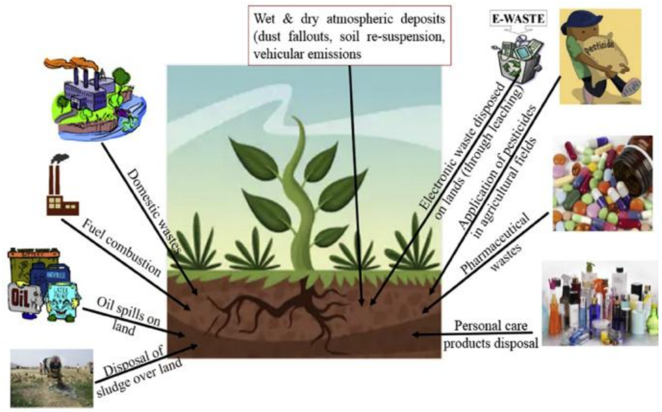



**Fig.4** Plant-microbe interactions during biodegradation of organic contaminants

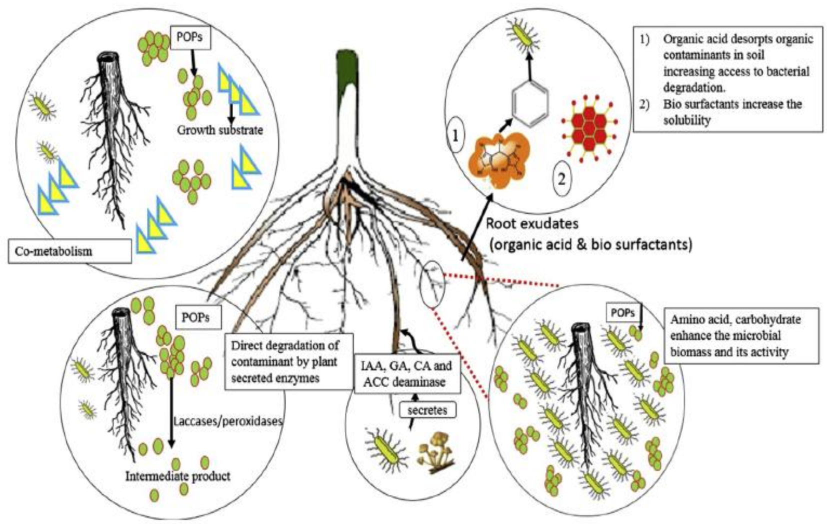



**Fig.5** Novel bioremediation approaches to facilitate removal of pollutants

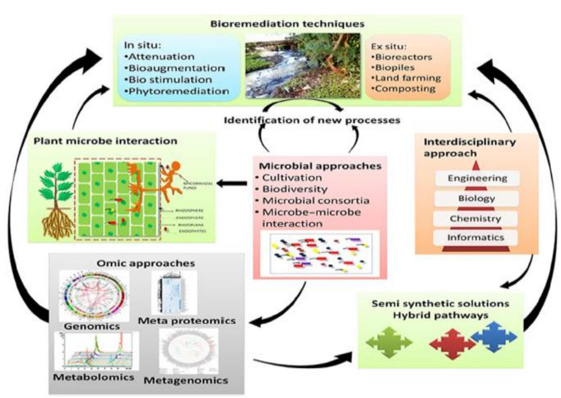


**Fig.6** Components of phytoremediation

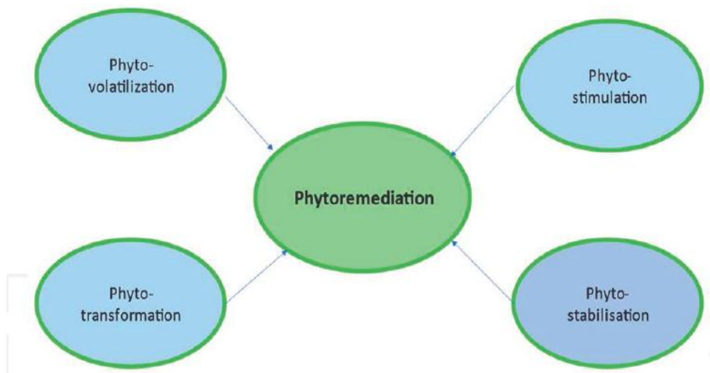
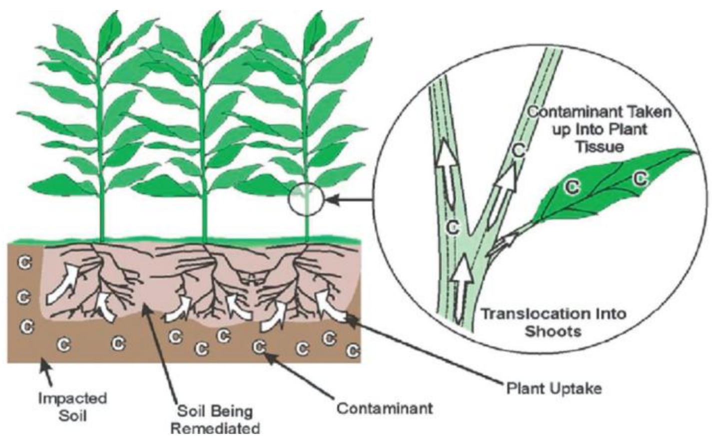



**Fig.7** Phytoextraction for environmental remediation




Bioremediation, an appropriate method, can be applied to different states of matter in the environment. * Soils, sediment, and sludge as solids *Groundwater, surface water, and industrial wastewater as liquids *Industrial air emissions as gases *Saturated and vadose zones as sub-surface environments

## Bioremediation Organisms

Microorganisms that carry out biodegradation in many different environments are identified asactive members of microbial consortiums. These microorganisms include: Acinethobacter, Actinobacter, Acaligenes, Arthrobacter, Bacillins, Berijerinckia, Flavobacterium, Methylosinus, Mycrobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctomia, Serratio, Trametes and Xanthofacter. Microorganisms individually cannot mineralize most hazardous compounds. Complete mineralization results in a sequential degradation by a consortium of microorganisms and involves synergism and co metabolism actions. Natural communities of microorganisms in various habitats have an astonishing physiological versatility, are able to metabolize and mineralize an enormous number of organic molecules. Certain communities of bacteria and fungi metabolize a multitude molecules that can be degraded is not known but many are known to be destroyed as a result of microbial activity in one environment or another. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions (Colberg and Young, 1995) may permit microbial organisms to degrade otherwise recalcitrant molecules.

## Role of Microorganisms in Heavy Metal Bioremediation

Heavy metals are environmental contaminants globally and at certain concentrations can have long term toxic effects within ecosystems and have a clear negative influence on biologically mediated soil processes. They have polluted agricultural soils and caused detrimental effects on our ecosystem (Levin *et al.*, 2004). Heavy metal concentrations above the threshold limit also causes disturbances in microbial activity and soil health (Hu *et al.,* 2013). The non-biodegradable nature of metals enhances their availability and longevity in soils. The longer persistence of metals in soils causes carcinogenic and mutagenic effects and becomes part of our food chain. Neurological disorders, Parkinson, Alzheimer, depression, schizophrenia, cancer, poor nutrition, lack of hormones balance, obesity, abortion, respiratory and cardiovascular disease, damage in organs (liver, kidneys and brain), anorexia, arthritis, hair loss, osteoporosis and death (in severe cases) are adverse effects of heavy metals in the human body are researched by many scholars (USEPA, 2004). Many mechanisms are adopted by plants to combat heavy metals. Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, extra and intracellular precipitation and active uptake (Gadd, 1993).

## Advantages of bioremediation

It is a natural waste treatment process. The treatment products are commonly harmless including cell biomass, water, and carbon dioxide. It needs a very less laborious and commonly carries out on-site, regularly without disturbing normal microbial activities. It eradicates the transport amount of waste off-site and the possible threats to human health and the environment. It is a cost-effective process in comparison to other conventional methods that are used for clean-up of toxic hazardous waste for the treatment of oil-contaminated sites. It supports in complete degradation of the pollutants; many of the toxic hazardous compounds can be transformed into less harmful products and disposal of contaminated material. It is chemically benign. Enzymes of microorganisms decontaminate the environment without the addition of toxicants in the environment. This way of remediating the environment is an ecofriendly and economically sustainable approach.

## Disadvantages of bioremediation

Various limitations are associated with bioremediation despite the research and development going on in this field. The process of bioremediation is only applicable to those materials that are biodegradable and cannot be applied as the generalized treatment method for all types of wastes. Research is going on to find out more about the persistent and toxic nature of the products of bioremediation. As it is a biological process involving the microbial communities so more site specific environmental specifications are needed for the microorganism to work. In order to maintain that environment, the cost-effectiveness remains there no more. Often it becomes difficult to conduct the pilot-scale bioremediation study in a field study. Genetic engineering of the microbes is needed in order to enhance the efficacy of the bioremediation process.

## Bioremediation Future Prospects

The future use of genetically engineered microorganisms (GEM) in enhancing bioremediation ability will be a favorable approach. This is because engineering a designer biocatalyst target pollutant, including recalcitrant compounds by combining a novel and efficient metabolic pathways, will widen the substrate range of existing pathways and will increase the stability of catabolic activity (Fodelianakis *et al.,* 2015). More also, using parallel gene transfer and multiplication of GEM will be an encouraging approach. In addition, using derivative pathway of GEMs genetically engineered microorganisms with a target polluted compound will increase bioremediation efficiency. There is need for further understanding of the relevant genomic and proteomic sequences of bioremediators that exist in nature. The understanding of their diversity and evolutionary relationships existing between the bioremediator will help to develop a more efficient bioremediation system in the future (Patel *et al.,* 2022). Hydrocarbon contaminants are considered a serious issue among environmental pollutants due to their high toxicity to human and environmental health. Several studies have shown that microorganisms can break down hydrocarbons utilizing a wide range of enzyme. Rhizoremediation is the most accepted, cost-effective bioremediation techniques focusing on the application of rhizospheric microorganisms in combination with plants for the remediation of organic and inorganic contaminants from the sites. The contamination of organic substances is posing threat to the human health and harming our environment, so there is an immediate need to resolve this problem with efficient solution (Kuiper *et al.,* 2004). rhizoremediation process, a rhizospheric microbial strain is inoculated with the plant seeds by coating them with microbes, so that microbes can grow in association with the roots of that plant easily and increase the speed of remediation of pollutant. Such type of inoculation of bacteria help the microbes to grow along the underground part and increase the chances of spreading in soil have screened out a plant microbe relation, which showed the ability to degrade naphthalene and the capacity to provide tolerance to plant seed against high concentration of this pollutant in soil (Kuiper *et al.*, 2001). The rhizoremediation has proven that a successful strategy for the removal of organic pollutants from the soil. There is a great emphasis toward sustainable and green rhizoremediation technologies and the use of plant-associated bacteria to degrade toxic synthetic organic compounds from environmental soil. If the efforts are taken seriously, then this technology may provide an efficient, economic, and sustainable green remediation technology for the abatement of organic pollutants from the soil

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