**Microbial Rhizoremediation as A Strategy for Decontaminating Polluted Sites and Augmenting Plant Growth**

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**Abstract**

Soil pollution due to the persistent nature of recalcitrant and xenobiotic compounds is one of the major constraints of the twenty-first century that limits crop productivity. Several researchers are actively working in this area and devoting great attention to mitigating this challenge. Physiological and conventional strategies opted by scientists to date for remediation are generally uneconomical, and unsafe in long run. Hence, the target is to decontaminate such polluted soil using economical, eco-friendly, and sustainable means. Here the essence of rhizoremediation comes in the frontline. Rhizoremediation through plant-associated microbes (PAM) is one of the potent strategies for decontaminating polluted soil naturally. There are several microbial metabolites including enzymes, biosurfactants, exopolysaccharides, etc that can be used singly or in association with PAM as a novel approach for cleaning up contaminated sites and augmenting crop productivity. Thus, the main aim of this chapter is to highlight the promising role of microbes and their secondary metabolites in reclaiming degraded soils and augmenting plant growth. In the process, it also brings up certain recent advancements in the area of omics technology and nanotechnology that can further strengthen our knowledge in this expanse and help us to minimize the problem of soil contamination with high precision and less time.

**Keywords:**Biosurfactants, Exopolysaccharides, Metabolites, Omics, PAH, Rhizoremediation

**Introduction**

Soil, is a nutrient rich growth medium that harbours diverse microorganism including bacteria, fungi, protists, and animals within it. It is a living ecosystem, that is linked to food production, land usage, and human health. Hence, it serves as the principal foundation of a country’s agricultural resources, survival, global economy, and sustainability (Oh *et* *al*., 2013; Gomeiro, 2016; Pathak *etal*., 2020).

Soil contamination is a sought of soil pollution in which certain chemicals/elements are present at concentration higher than the permissible limits. Soil/land degradation can occur naturally due to environmental process including salinity, drought, discharge of contaminated surface water, oil and fuel dumping, leaching of wastes from landfills) (Ashraf *etal*., 2014; Liedekerke*etal*., 2018). Various human activities like smelting, mining and manufacturing of coal and oil; livestock, domestic and municipal wastes; immoderate application of fertilizers, herbicides, pesticides in agriculture; heavy metals and disintegration of petroleum products in the environment participates in contaminating the soil (Ashraf *etal*., 2014; Liedekerke*etal*., 2018). The inappropriate disposal of emerging biological pollutants (endocrine disruptors, pharmaceuticals, biological contaminants) as well as e-wastes (old electronics) are of great concern nowadays. Contaminants present in soil, interacts with the soil in different ways like complexion, sorption, and precipitation and ultimately alters the soil fertility by changing bioavailability of essential nutrients, pH, and ion exchange capacity (Mishra and Arora 2019).­­

Soil pollution, is a gradual process, that may be not visible at once but, the effects of soil pollution are clearly visible and may be seen in long term. It can affect food production, water regulation, nutrient recycling, climate change and the biodiversity of terrestrial ecosystems. Soil contaminants can cause deleterious changes in the structure, biology and productivity of soils and can also cause harm to environment and human health. The biomagnifications and non-biodegradable characteristics of soil contaminants degrade the soil fertility and food quality, which eventually effect food production and economy of the country (Yousuf *etal*., 2020).

Calculating full degree of soil pollution around the world is a daunting task, but an approximate estimation is reported in many studies. A study reported that nearly 75% of the land area on earth is degraded (Gibbs and Salmon 2015). As per the findings of United Nations environment programme published on December 2020, reported that around 40% people of world’s population are affected due the soil/land degradation. Research data obtained from China, shows that pollution ratio of C­­hinese farmlands is 22.10%, with 1.23% of severe pollution and 20.8% of soil cause carcinogenic risk in children (Zeng *etal*., 2019). Soil pollution has been recognised as the third most important threat to soil functions in Europe and Eurasia followed by North Africa, Asia, Northwest Pacific, North America, and sub-Saharan Africa and Latin America (FAO and ITPS, 2015). According to Superfund National Priorities List (NPL) of United States Environmental Protection Agency (USEPA) there are 40,000 federal superfunds sites in the country and in year 2021, 1322 are listed in NPL. NPL is US based list of sites contaminated with hazardous waste that are entitled for long-term remediation funded under federal Superfund program. Data collected through European Environment Agency reports that in Europe around 3,40,000 sites are contaminated with elements released from fertilizers (cadmium) and fungicides (copper) and require remediation (EEA, 2019b, Silva et.al 2019). The total number of polluted sites in Australia is believed to be around 80,000 (Rodríguez-Eugenio*etal*., 2018). According to reports from India, crop yield has reduced by 15–25% throughout the years as a result of deleterious effects of soil pollution (Mishra and Arora 2019). According to Central Pollution Control Board (CPCB) in India, total 112 sites are contaminated with different type of contaminants and require remediation. Odisha, Uttar Pradesh, and Delhi have maximum contaminated sites in India. Due to excess use of fertilizers which contain heavy metals such as mercury, lead, cadmium, etc. large numbers of farmers are suffering from cancer in Punjab and Haryana (Saha *etal*., 2017).

As per the data, analysed from the Indian as well as global scenario, it could be elucidated that high rate of soil contamination is the burgeoning problem. Hence, the remediation of such contaminated sites has become a priority, as it is affecting human as well as environment health and the global economy. Removing of contaminants from such sites involve the usage of various *in-situ* or *ex-situ* technologies. In the last few decades, new environmental criteria have been developed, as well as many measures are opted for the restoration and repair of polluted sites. Several chemical and physical treatments like chemical oxidation, thermal desorption, photochemical degradation, incineration, soil washing, solvent extraction and solidification have already been introduced for remediation of polluted sites (Sessitsch*et* *al*., 2013; Mishra and Arora 2019). These techniques are though used predominately but, holds certain disadvantages too such as, chemical oxidation dependent on highly acid pH, which may reduce the soil pH, harmless organic materials of soils also get oxidized, disturb normal microflora of soil (Rosas *etal*., 2014; Cheng *etal*., 2016, Baldissarelli*etal*., 2018). In soil washing process, the pollutants are transported to a washing solution for the complete destruction, but treating these solutions are challenging due to presence of highly diverse pollutants and their concentration (Santos *etal*., 2015). Solvent extraction is dependent on soil conditions and presence of contaminants; therefore, it is location limited. Most of these physical and chemical treatments require high economic cost for complete remediation and affect soil quality negatively (Oh *et* *al*., 2013). As a result, an eco-accommodating and highly effective strategy is urgently needed for the repair of soil damage. Although, soil has some capacity of adsorption and with favorable environmental conditions some contaminants may degraded naturally. In natural degradation process, relationship between soil microflora and plants plays an important role. Here, the essence of rhizoremediation comes in the frontline, in this, plants and microorganism show symbiotic relationship and degrade the toxic compounds present in the soil. In starting the degradation of herbicides and pesticides was the main focus of rhizoremediation but later, it was used for other contaminants too, like heavy metals, crude oil, organic compounds etc. Rhizoremediation is a cost-effective and environmental beneficial strategy. It offers numerous advantages over other traditional approaches, like it increases the level of bioavailable part of contaminants in soil, it conserves the fertility of soil, no waste is accumulated after the excavation of contaminants, there is no need of special equipment and maintenance, degradation of hazardous compounds into non-hazardous compounds and this technique is easy to implement (Mishra and Arora 2019). It has emerged as the most potent approach for remediating contaminated soil (Kamaludeen and Ramasamy 2008).

Thus, this chapter aims to present and discuss the potentials of rhizoremediation technology and its genetically modified or nanobiotechnological approaches to remediate soil, contaminated with various contaminants.

**CONCEPT OF RHIZOREMEDIATION**

Rhizosphere is the area which encircles the root system of the plant (1-2 mm) and the highly active zone of soil (Brink, 2016; Razavi*etal*., 2016). In comparison of bulk soil, it is 100 times richer and metabolically active (Erickson *et* *al*., 1995). Rhizobacteria are mostly rod shaped and Gram-negative, however, a minor proportion of Gram-positive rods, cocci are also present (Pathak *etal*., 2020). *Pseudomonas* is the dominating specie of gram-negative rod present in the rhizosphere (Kuiper *etal*., 2004).

Recently, the use of rhizospheric microbes such as *Bacillus*, *Pseudomonas*, *Paenibaciluus*, *Rhodococcus*, etc to clean contaminated soil has emerged as a suitable alternative to other techniques, since the inputs provided by these microbes are not toxic (Kuiper *etal*., 2004; Ullah *etal*., 2015; Oberai and Khanna 2018). There are several mechanisms by which rhizosphere microbes carry out the remediation process such as through acidification, chelation, precipitation, complexation and redox reactions (Mishra *etal*., 2017).

Rhizoremediation which is also known as microbe-assisted phytoremediation, rhizosphere degradation, or rhizodegradation is an emerging technique of phytoremediation. It involves interaction between rhizospheric microbes and plant to degrade different type of toxic xenobiotic compounds. Contribution of the rhizomicrobial population is referred to as rhizoremediation (Anderson *etal*., 1993; Schwab and Banks 1994). The plant exudates enhance the growth of microbes and in return microbes makes compounds bioavailable for plants and at the same time microbes make soil pollutants free. The microbes may not remove pollutants completely, but they have capabilities to reduce pollution significantly, Microbes degrade xenobiotic compounds like heavy metals, polyaromatic hydrocarbons (PAH), and some other contaminants present in the soil without producing any toxic by-products unlike traditional methods (Alkorta and Garbisu 2001; Morikawa and Erkin 2003; Barea *et* *al*., 2005; Truu*et* *al*., 2015). Important parameters for rhizoremediation are soil structure and hydrogeology, nature of the pollutants present in soil, microbe-plant interaction, nutritional state of soil, and the microbial composition of soil (Mishra and Arora 2019). The success of rhizoremediation depends on many factors such as meteorological conditions, soil conditions, suitable plant species, and related microbes (Mohy-Ud-Din *etal*., 2020).

Rhizoremediation is the promising approach used in the area of rhizosphere technology, where rhizospheric microbes break majority of soil pollutants by utilizing available plant root exudates. Microbial degradation halts, when soil microbes are nutrient deprived. Hence, in laboratory the researchers described an enrichment method for the isolating soil microbes, using combination of soil pollutant and root exudates as nutrient source (exudates such as alcohol, sugar, and organic acids etc.) resulting in degradation of soil contaminant, enhancing root colonization, increasing root adhering soil/root tissue ration, thereby raising plant growth significantly ( Shukla *etal*. 2013).

Rhizobacteria can remediate soil by volatilizing PAHs, by increasing the organic pollutants humification and releasing metal by chelating ligands, protons, and oxidoreductive systems that are present on cell surfaces and membranes (Salt et al., 1998; Singh, 2021). Various bacteria in the rhizosphere produce exopolysaccharides (EPS), enzymes, and biosurfactants, which also aid in the absorption of metal ions and form a coating to protect themselves and plant roots from metal toxicity (Mishra et al. 2017).

**FUNGI**

Fungi particularly used in rhizoremediation, belonging to *Basidiomycota* and *Ascomycota*. Large number of arbuscular mycorrhizal fungi have been found in places exposed to heavy metals because they have the ability to adhere plant roots and colonize large volumes of soil through the hyphae (Khan et al. 2000). Plants that have potential for rhizoremediation are mostly symbionts with arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (ECM). Mycorrhizal fungi play an important role in nutrient cycling and in functioning of ecosystem, hence; have an effect on composition of microbes and contaminants. It is found that, under natural condition 60% or more of the root system of poplar plant and 80% or more of the willow plant, is colonized with ECM. They are ubiquitous, show positive resistance on abiotic stress, and capable to degrade contaminants (Bucking 2011). In a study, conducted with total 58 fungi, including 22 ECM, it is found that the PAH degrading capability of ECM fungi is lower as compared to wood and straw degrading basidiomycetes. In the same study, it also found that the majority of ECM fungi was able to degrade PAHs to a certain degree (Add Reference). Among all of the examined ECM fungi*, Hebelomacrustuliniforme*,*Hebelomahiemale*,and *Lactariusdeliciosus* showed the highest removal and degradation as compared to wood and straw-degrading fungi (Gramss et al. 1999). Ectomycorrhiza and saprophytic basidiomycetes have shown remarkable activity in the decomposition of soil pollutants. AMF demonstrate a mechanism for reducing heavy metals toxicity in plants by keeping heavy metals in mycorrhizal structures such the fungal mycelium and vesicles, where large concentrations of heavy metals were concentrated, preventing their mobilization to aerial plant tissues (Dhalaria et.al, 2020). Some arbuscular mycorrhizal fungi such as *Rhizophagusintraradices*, *Glomus versiforme*and*Funneliformismosseae*have also been reported to complex with heavy metals such as Cd, Pb, and Cu through EPS and glomalin production (Gonzales Chavez et al. 2004). In ground, the high concentration of glomalin induced the formation of aggregated soil, carbon accumulation, and reduce soil erosion. Its deposition in soil contributes approx. for 5-10 % organic soil carbon and 5-13% nitrogen (Surtiningsih et al., 2017). The inoculation of *Cellulosimicrobiumcellulans* in contaminated soil reduces the toxic Cr (VI) to non-toxic Cr (III), thus reducing the absorption of Cr (VI) in the contaminated soil by 56% in the roots of the chili plant green and by 37% in shoots it is reduced in (Chatterjee et al. 2009).

**PLANTS ROLE**

In rhizoremediation, plant species plays a secondary role in the remediation process. There are many studies which proves that various plant species are suitable for the rhizoremediation process. *Alfalfa* is a leguminous plant, suitable for remediation because they can produce large biomass above and below ground, develop an extensive root system, establish a niche for rhizosphere microorganisms, can form symbiotic relationship with nitrogen-fixing microbes and can easily grow in a soil with high C/N ratios and can adapt different climate condition easily (Kuiper et al. 2004; Agnello 2015). Prairie grass can maintain high number of bacteria in the extensive root system, and can accumulate complex mixture of heavy metals within them and can reduce the formation, bioavailability, or mobility of heavy metals (Deka et al., 2009; Pandey et al., 2020). Trees such as *Populus* and *Salix* because have perennial growth, high biomass production and extensive roots system, hence, they are resistance to contaminants and high absorption surface areas (Guerra et al., 2011).

Successful utilisation of plant species from the genera *Populus* (poplar) and *Salix* (willow) for rhizoremediation of PHC contaminated soils is likely attributable to the oxygenation of deeper soil layers via specialised root channels called aerenchyma. In the rhizosphere, nutrients for bacteria are produced by the mucigel generated via root cells, lost root cap cells, starved root cells, or decaying entire roots of these plants (Bisht *etal*., 2015). Silver birch (*Betula pendula*) and red mulberry (*Morus rubra)*has the ability to colonize nutrient-depleted soils efficiently and produce high biomass (Rezek et al. 2008). All of these plant species can maintain a huge number of bacteria in their root systems (Qiu et al., 1994; Shann et al., 1994; Kuiper *etal*., 2001). Root exudates releases organic compounds which may serve as a nitrogen and carbon sources for the microbes that can degrade the organic contaminants present in soil (Anderson *etal*., 1993; Salt *etal*., 1998; Kupier*etal*., 2004; Bisht *etal*., 2014)

Rhizoremediation has a number of advantages, including the fact that microbial degradation usually results in complete mineralization of the pollutant, and it can be employed *insitu* on the contamination site without disturbing the soil matrix (Heitzer et al., 1993). The approach is also reported to boost soil organic matter, insoluble compound bioavailability, and nutrient cycling, all of which increase biomass output and make the soil more fertile and productive for agronomic purposes. Limitation of rhizoremediation is that, the pollutants must be bioavailable to rhizospheric microbes. The naturally residing microbial population compositions and their remediation strategy in rhizosphere change with the concentration and composition of contaminants, so only those microbes can survive and work on remediation of pollutant which are resistant to that particular environment.

**Precarious effects of contaminants on soil**

In the case of soil contamination, the total concentration of contaminants does not provide complete information on the potential risk. It is important to identify the existing and non-existing forms of the contaminants, in order to intentionally remediate the contaminated soil in order to prevent non-existent forms from arising. Biological tests can be used to determine the toxicity and bioavailability of metals and metalloids in the soil, but in this case, it is necessary to correct the standards or thresholds of soil quality and soil properties as texture, pH, and organic matter ( Romero-Freire et al., 2015; Martin et al., 2015).

**HEAVY METALS**

The 'Soil-Plant Barrier' idea, coined by the Chaney for the metals and metalloids over 40 years ago, asserts that some contaminants are highly dangerous, even in low levels for plants (Chaney 1980). Heavy metal pollutants are non-biodegradable, and their poisonous nature causes negative changes in soil biology, structure, production, and eventually find their way into human food via the interconnected food chain (Yousuf *etal*., 2020). As it enters in the tissues of living species, they create a serious threat to the environment and human health. Barium, aluminium, cadmium, arsenic, nickel, zinc, lithium, mercury, copper, chromium, manganese and cobalt are some common heavy metals that found in contaminated soil (Adriano *etal*., 2005; Sparks 2005; Karthik *et* *al*., 2017).

For the remediation of toxic heavy metals, various technologies such as membrane separation, ion exchange, electrochemical treatment, reverse osmosis, chelation, precipitation, ultrafiltration, and electrodialysis have been used. These treatments have some drawbacks, such as at low metal concentrations low productivity of execution and high establishment costs. In the substitute of these techniques, rhizoremediation is considered as the best approach, because it remediates without changing the physiochemical properties and is thus considered as eco-accommodating strategy for toxic heavy metal remediation (Yaashikaa et al., 2020).

Alder (*Alnus tenuifolia*), silver birch (*Betula pendula*), willow (*Salix*), conifer trees, and black locust (*Robiniapseudoacacia*) are the most popular trees that have a high capacity to accumulate heavy metals (Wislocka et al., 2006). Cadmium (Cd) is the most frequently studied heavy metal in the food chains (Grant et al., 1999). In flooded rice-based cropping systems, food chain contamination by cadmium and arsenic occurs predominantly. It is one of the serious concerns in many Southeast Asian countries, China, Bangladesh and India (Brammer and Ravenscroft 2009; Rahman and Hasegawa 2011; Bhattacharyya and Jha 2012).Generally, arsenic remediation by microbes involves two different types of arsenic resistant bacteria, one which reduce the bioavailability of arsenic in soil and protects crops and another which increase arsenic bioavailability to plants for better remediation (Cavalca et al., 2010; Ghosh et al., 2011; Wang et al., 2011; Yang et al., 2012; Pandey et al., 2013). In a study, two novel strains named as *Kocuria flava* and *Bacillus vietnamensis* were found that can accumulate arsenic intracellularly and can help in remediation . They are halophilic arsenic resistant bacteria and possess plant growth promoting (PGP) traits, like siderophores and IAA. These isolates produced EPS, which helped to form *invitro* biofilms and biofilm-like association with plant roots. These isolates were capable of effective adsorption and accumulation of arsenic under hypersaline condition. Inoculation of these isolates in rhizosphere significantly raised rice seedlings growth in arsenic-amended hypersaline soil and also it minimized arsenic uptake in plants. Isolates, *Kocuria flava* and *Bacillus vietnamensis*, could tolerate 35 mM and 20 mM of arsenite respectively (Mallick et al., 2018). The ongoing studies on these strains are essential to understand the complete mechanism of arsenic adsorption. The possible reasons of arsenic accumulation by these strains may be due to the adsorption of the negatively charged arsenic ions by positively charged amino groups in the bacterial cell, methylation followed by reduction and oxidation of arsenic ions, or sequestration by a range of cysteine-rich peptides walls (Bai and Abraham, 2001; Bai and Abraham, 2003; Thomas et al., 2007; Thomas et al., 2010; Dhankher et al., 2002).

**FLY ASH**

Fly ash is a ferro-alumino silicate mineral having the primary components Silicon, Calcium, Potassium, Iron, Sodium, Zinc, Lead, Nickel, Manganese, Molybdenum, Magnesium, Fluorine, Copper, Cobalt, Cadmium, Boron and Aluminium (Gupta et al., 2002). Fly-ash has play significant role in plant growth promotion in a dose-dependent manner. For example when the roots of *Beta vulgaris* were cultivated in fly-ash-amended soil, it was found that low doses of fly ash up to 2 % (kg/m2 plot) raised sugar production, while higher doses (up to 4 and 8 %) were inhibitory to it. It is stated that the excessive usage of fly ash, changes pH and raise soil salinity. The rhizosphere and plant roots are both harmed by the high alkaline pH and excess quantities of soluble elements produced from fly-ash. The high pH of fly-ash is precarious to the primary rhizospheric bacteria that perform nitrogen fixation (Gupta et al., 2002). In this case most herbaceous plants such as *Melilotus*, *Agropyronryens*, and *Festuca* have been found to grow better on fly ash (Gupta et al., 2002). Some studies indicate that as time and nutrients accumulate in fly ash, microbial diversity increases. The use of fly ash of approximately 40 t/ha with the phosphate solubilizer *Pseudomonas striata* increased bean yield by approximately 14% that means 35 g/pot (Gaind and Gaur 2002). Enterobacter *sp*. NBRI K28, is a metal tolerant plant growth promoting bacteria and its siderophore, if overproduces the NBRI K28 SD1 mutant, they can stimulate plant biomass and can increase the phytoextraction of metals (Cr, Ni and Zn) from fly ash by the *Brassica juncea* (Indian mustard) plant (Kumar et al. 2008). Siderophore producing microbes are *Brochothrixcampestris*, *Bacillus*, *Serratiamarcescens*, *Microbacteriumbarkeri*, *Enterococcuscasseliflavus* and *Pseudomonasaeruginosa* (Pandey and Singh 2010). Inoculation of fly ash tolerant *Rhizobium s*trains in *Cassia surattensis* gave the plant tolerance to grow under fly ash stress conditions (Vajpayee *etal*., 2000). Fly ash is also used in combination with cyanobacteria as green manure for the cultivation of *Brassica juncea* (Gupta *etal*., 2002). *Anabaenadoliolum*, is able to reduce heavy metals such as Zn, Cu, Ni, Fe and Mn in fly ash through bioaccumulation in its tissue (Rai *etal*., 2000).

**PAH**

PAHs are typical soil pollutants that cause a change in the grain size, porosity and water holding capacity of the soil and negatively affect the microbial population. It also leads to changes in permeability, volume, plasticity, etc. These are toxic and persistent. The degree of industrial development, the proximity of the contamination sites to the source of production and the type of PAH transport affects the concentration of PAH in the environment (Bisht et al., 2010). PAHs generally clog pores in the soil, which could reduce soil aeration and water infiltration. Soil contamination by PAH can affect the microbial population and microbial or enzymatic activity. One study reported that PAH contamination has a significant impact on the structure of the bacterial community in the soil (Khomarbaghi et al., 2019). Genus including *Agromyces*, *Janthinobacterium*, *Pseudomonas*, *Serratia*, *Streptomyces* and *Flavobacterium* inoculation showed a high potential for rhizodegradation of PAH (Kuffner et al., 2008). For *Sorgumbicolor*, the bacterial strains that promote PAH degradation are *Bacillussubtilis*, *Brevibacteriumhalotolerans*, *Brevibacteriumpumilis*, *Pseudomonaspseudoalcaligenes*, and *Pseudomonasmontellili* (Duponnois et al., 2006; Shanab et al., 2008).

**CRUDE OIL**

Crude oil contaminants have been identified as a key factor responsible for minimizing agri-food production. Various types of serious impact are made by these contaminants such as oxidative stress which arose due to accumulation of reactive oxygen species (ROS), accelerated senescence. Crude oil is composed of various hydrocarbons, that have small density, higher viscosity and low emulsifying ability (He et al, 1999; Wang 2009). Due to these traits, they get easily absorbed in the soil, thereby inhibiting the enzymatic activity of the microbes and sometimes there number too. Besides crude oil, the sewage in oil and gas fields also lead towards soil pollution. If they are untreated, they may show serious impact on soil and water pollution (Gu et al, 2007; Lu, 2009; Mariana et al, 2010). These kinds of waste are not only responsible for soil salinization but also can show various effects by destroying the soil environment.

A variety of physical and chemical approaches have been utilized for decades to remove oil spills from soil, including incineration and land filling but they neither cost effective nor eco-friendly. Incineration is a process in which spilled oil is simply burnt with a consequence of raising atmospheric CO2, NO2, and SO₂ levels resulting in global warming. Land filling is reported to produce hazardous leachates in the form of gases and liquids which potentially results in intoxicating the ground water. Therefore, the unpredictable hazards associated with the use of these approaches can be lethal and can limit implementationFrom the perspectives of rhizoremediation, microorganisms such as *Fusariumculmorum*, *Fusariumsolani*, *Fusariumoxysporum*, *Macrophominaphaseoli*, and *Bacillus* can colonise cotton root (Ghaffar and Parveen, 1969). Some bacteria, such as the *Rhodococcus* strain, can thrive at the oil-water interface and create a capsule containing mycolic acid, which aids in crude oil degradation (Wang et.al, 2010). Plant roots of Wheat (*Triticum*) shows interaction with *Azospirillumlipoferum* for mitigating crude oil from soil environment (McGuinness et al., 2009).

**TRICHLOROETHANE**

In addition to contaminants, Trichloroethane is extensively applied as a pesticide to the agricultural soil and its continuous use shows obvious possibility of destroying the fauna of soil. DDT is widely used organochlorine compound for its activity of a pesticide, but its residues are detected in agricultural soils around the world and resulting in disturbing the soil environment (Zhou et al., 2013). Despite being quite effective in destroying plants and animals that are harmful to the environment, this substance can also pose a great deal of danger to humans and the environment. 1,1,1-trichloroethane, also called dichlorobiphenyl trichloroethane it was first synthesized in 1874. These chlorinated compounds do not degrade easily in the soil and shows persistent behaviour.

In one of the experiments, the researchers took 101.6 kg of 5% DDT dust per acre and plot were laid out in a random block consisting of four replicates of each treatment and control. The size of each plot was 6 ft. square with 6 ft. guard rows between plots. The insecticides were applied to the surface of the plots as dusts and thoroughly mixed into the soil to a depth of 6 inch by double rotovator. All plots were kept fallow during this period of the trial by regular application of the weedicides. After the experiment, when sample of soil was extracted, it was observed that considerable changes in the fauna of soil occurred and changes in population of various groups of organisms have been seen (Wang et al., 2010). Wheat (*Triticum spp*.) mixed with a Trichloroethane degrading bacteria *Pseudomonas fluorescens* protected grass seed against Trichloroethane toxicity, and the expanding roots carried the Trichloroethane degrading bacteria into soil that would have been too deep without roots (McGuinness et al., 2009).

**Microbial metabolite assisted rhizoremediation**

The biodegradability of the microorganisms, and the expression of the necessary microbial genes in the rhizosphere are of great importance for rhizoremediation. Most organic pollutants are hydrophobic and cannot be dissolved in water. These organic pollutants form insoluble complexes with soil particles and are not biologically available to recovery organisms. Root exudates increase the bioavailability of contaminants by improving their solubility and making them more available for microbial attack. Microorganisms use different approaches to promote the bioavailability of hydrophobic contaminants. The degradation of toxic compounds take place by nutrients, enzymes, biosurfactants which are result of microbes-plants symbiotic relationship.

**Enzymes**

Biodegradable enzymes are also responsible for breaking down various pollutants in the soil such as trichloroethane, crude oil, heavy metals, PAHs, fly ash etc. They are responsible for the decomposition of hydrocarbon-based pollutants through oxygen enrichment of the terminal methyl group, different alkane-breaking microbes have various genes such as hydroxylases, as they are responsible for decomposing a large number of alkanes (Beilen et al., 2002; Parthipan et al., 2017b). Some enzymes for certain hydrocarbons are methane monooxygenase, alkane monooxygenase, alcohol dehydrogenase, and laccase (Parthipan et al. 2017b). A wide range of bacterial strains have been studied such as *Pseudomonas* species, *Stenotrophomonas nitritireducens*, *Pseudomonas aeruginosa*, etc. for its ability to produce these degrading enzymes during the biodegradation of hydrocarbons and trichloroethane (Mishra and Singh 2012). Enzymes are correlated with catabolic genes involved in the degradation of PAHs. The main enzymes used are oxygenase, dehydrogenases, phosphatases and lignolytic enzymes. These enzymes require an optimal temperature and most of these degrading enzymes are reported to operate at mesophilic temperatures and their activity decreases at very high and low temperatures Some extracellular enzymes, such as lignin, peroxidases, laccase, and manganese peroxidase, are fungal lignolyticenzymes . They catalyse the formation of radicals through oxidation to destabilize the bonds in a molecule. In fungi, the degradation of hydrocarbons is mainly an extracellular process that involves the release into the environment of oxidoreductases of wide specificity, such as laccases, manganese peroxidases and lignin peroxidases (Harms et al., 2011). Spent mushroom compost (SMC) increases the rate of degradation of PAH. SMC is the remaining compost waste which is generated by the mushroom industries. The most frequently reported enzymes in SMC are laccase and Mn-dependent peroxidase, however traces of lignin is also present. Lignolytic enzymes do not show substrate specificity like the rest of the enzyme. They act in a non-specific way with the help of cationic radicals on phenolic and non-phenolic compounds. Hydroxy radicals are produced by *Pleurotusostreatus*laccase, while a wide range of PAHs are directly decomposed by Mn-dependent peroxidase (from the fungus *Nematolomaforwadii*) into carbon dioxide and polar fission products (Haritash et al., 2009). Recent research from 2021 reported that PAH dioxygenase, produced by bacteria is the key enzyme for breaking down PAH. It mainly breaks the cyclic ring of PAHs into small intermediate molecules by oxidizing it to carbon dioxide and water (Min Wei et al., 2021).

Degradation is initiated by attacking the ring structure by oxygenation, resulting in the formation of 2,3 dihydrodiol DDT and after forming 2,3-dihydroxy DDT, meta cleavage occurred in successive steps results in the formation of 4-chlorobenzoic acid. During the process of oxygenation, two molecules of oxygen are incorporated in the presence of enzyme dioxygenase, in some cases it was observed that production of specific enzyme can be influenced by a secondary carbon source. *Alcaligenes*, effectively degraded over 65% of the DDT present in the soil and this degradation rate is significantly enhanced in the presence of some amount of glucose (Xie et al., 2011) although, in some cases it is seen that glucose inhibit DDT degradation such as degradation by *Serratia marcescens*.

Degradation of low molecular PAHs by aerobic bacteria is initiated by the dioxygenase enzyme. The dioxygenase enzyme catalyses the oxidation of aromatic compounds and then produce dihydrodiols. After this, the extradiol dioxygenase enzyme catalyses the dehydrogenation process of this dihydrodiols. The newly formed dehydroxylated intermediate than further undergo ortho or meta cleavage pathways to form protocatechuates or catechol. This catechol further undergoes either ortho or meta cleavage pathways and by the subsequent series of enzymatic actions they are converted into the tricarboxylic acid cycle intermediates. PAHs such as phenanthrene and pyrene are reported to degraded by many bacterial species such as *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Diaphorobacter*, *Enterobacter*, *Flavobacterium*, *Phanerochaetechrysosporium*, *Polysporus*, *Pseudomonas*, *Pseudoxanthomonas*, *Rhodococcuswratislaviensis*, *Sphingomonas* and *Stenotrophomonas* (Sivaram et al., 2020).

Some species of anaerobic bacteria that exhibit hydrocarbon degradation ability belongs to genus *Dechloromonas*,*Thauera*,*Desulfococcus*, and *Azoarcus* (Ahmad *etal*., 2020). Strains s22 and t15 of *Dechloromonas*degrade contaminants through key enzymes like glutathione S-transferase (GST), which plays a role in detoxification metabolism of contaminants. For controlling osmotic stress, they use the EnvZ-OmpR two component system and for quorum sensing they use QseC-QseB system. They have around 63 core genes that are responsible for their survival in polluted environment (Zhang et al., 2021).

*Enterobacter* sp. NBRI K28 isolated from fly ash contaminated soils exhibited 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity (Pandey and Singh 2010).

**Biosurfactants**

Surfactants are a group of amphiphilic chemicals, i.e., contain hydrophilic and hydrophobic components in their molecular structure. Biosurfactants are surface-active biomolecules that are produced by microbes. Biosurfactants form lamellar micelles, when the surfactant concentration exceeds a critical micellar concentration that is specific for each compound. Critical micellar concentration (CMC) is the concentration above which micelles formation occur. Hydrophobic impurities are solubilized in hydrophobic nuclei of micelles, increasing the transition of compounds from solid to aqueous phase. Impurities in aqueous phase are more easily accessible to bacteria.

The prominent role of biosurfactant is to induce antimicrobial activity and to make substrate readily available for uptake by cells in adverse environmental conditions. It also reduces the surface and interfacial tension (Fakruddi 2012). Surfactants shows diverse application in various fields such as in agriculture, food production, chemistry, pharmaceuticals and microbial-enhanced oil recovery. Biosurfactants have various advantages as compared to synthetic surfactants such as low toxicity, biodegradability, antimicrobial activity, tolerance to range of temperature and pH, ionic strength, and emulsifying and demulsifying ability (Chakrabarti, 2012).

Biosurfactants CMC is lower than the chemical surfactants, that is less surfactant is necessary for maximal decrease on surface tension. As they are produced by different microbes, they are capable to tolerate different range of temperature and pH. Biosurfactant are natural products, when compared to synthetic surfactants they can be easily degraded and that’s why they are suitable for the process like bioremediation or biosorption (Mulligan et al., 2001; Vijayakumar and Saravanan 2015). There are few literatures that report the role of biosurfacatant in biofilm formation (Vijayakumar and Saravanan 2015).

Biosurfactants are classified primarily according to their chemical structure and microbial origin. The main class of biosurfactants are glycolipids, phospholipids, polymeric biosurfactants, and lipopeptides. Several microorganisms including (add microbes name) have been identified and studied that have the ability to produce biosurfactants using various substrates such as oils, alkanes, sugars and agro-industrial waste, various biosurfactants.

Some contaminants or organic compounds are not directly degraded by microbes. Biosurfactants increase the microbial uptake of these contaminants or organic compounds by increasing their apparent solubility at concentration above CMC. Itoh and Suzuki (1972) show that the rhamnolipid producing strain of *P*. *aeruginosa* can degrade hydrocarbons.

Biosurfactants such as lipopeptides, glycolipids, phospholipids, etc., come from the genera *Bacilli*, *Candida*, *Pseudomonas* or*Thiobacillus*. Lipid-polysaccharide compounds are produced by *Acinetobacter* species, they have an important role in the degradation of crude oil (Van Dyke et al., 1991; Youssef et al., 2004). Biosurfactants often play an important role during proliferation in water-immiscible substrates (Youssef et al., 2004; Ibrahim et al., 2016). Metagenomic biosurfactant protein 1 is extracted from the protein purification and the surfactant is obtained from the bacterial cultures which shows emulsification activity towards different hydrocarbons.Amongest all the tested hydrocarbons, it was observed that emulsification indices was high in toluene and xylene by 56.7% and 51.9% respectively. One of the important groups of bacterial surfactants are glycolipids and of which rhamnolipids are the major representatives. Rhizosphere bacteria produce biosurfactants that, by complexing with heavy metals, reduce the activity of the metal solution and thus increase the desorption of heavy metals (Gupta and Kumar 2017). *P. aeruginosa* produces biosurfactant called rhamnolipid, which increases the solubility of heavy metals in the soil (Maeir and Chavez 2000; Rufino et al., 2014). Rhamnolipids and surfactins together detoxify the effects of heavy metals such as Lithium, Calcium, Zinc, and Barium (Nielsen and Sørensen 2003; Mulligan and Wang 2004). In corn, sundar grass and tomatoes, an increase in biomass production was observed after the inoculation of Rhizosphere *Bacillus* under metal-contaminated soil (Sheng et al., 2008). Rhamnolipids are reported to increase the biodegradation rate of pollutants. Kuiper and his colleagues in 2004 reported that they isolated a strain of *Pseudomonas putida* from plant roots at a site contaminated with PAH, that produce two lipopeptide biosurfactants. These lipopeptides (Putisolvins) increased the formation of protein emulsions with toluene (Kuiper et al., 2004a).

The bacterial biosurfactants are well explored, but there are few fungal species, that could also be exploited for biosurfactant production. *Candidabombicola* (Casas et al., 1997), *Candidalipolytica* (Sarubbo et al., 2007), *Trichosporonashii* (Chandran and Das, 2010) and *Aspergillusustus* (Cortes-Sanchez et al., 2011) are few fungi that can produce biosurfactants. Fungal biosurfactants can be a good area of research for improving bioremediation techniques as they are known to produce surfactant on low-cost raw materials. Generally, the biosurfactant produce by fungal species is sophorolipids (Vijayakumar and Saravanan 2015). EXPLAIN SOPHOROLIPIDS

The search for rhizobacteria that promote the bioavailability of pollutants is therefore of great interest in the field of rhizoremediation. This property is also of interest because several bio degenerative microbes show positive chemotaxis towards contaminants. Therefore, the combined effect of biosurfactants and chemotaxis can contribute to bacterial reproduction and microbial spread in contaminated soils, leading to the cleaning of larger areas (Parales, 2004).

**Biofilm and EPS**

Biofilms are the group of microorganisms of same or different species in which cells are often surrounded by a self-producing EPS (add reference).It is an association of the abiotic or biotic surface and microorganisms. The surface and microorganisms are strongly united by the production of an extracellular polymeric matrix. This surface is submerged in water or surrounded by a humid environment. The biofilm formation is a multistep process it begins with attachment of microbes onto a surface and then EPS production, cell-cell communication via signalling molecules occurs. In the end cells are dispersed and again attach to another surface (Characklis1990; Azeredo et al., 2017).

Biofilm development is highly ordered pathway which is regulated by some specific genes in each microorganism. In *Pseudomonasaeruginosa* biofilm formation is dependent on three two-component systems, for biofilm initiation, biofilm maturation and microcolony formation. These three two-component systems are BfSR, BfmSR, and MifSR (Petrova and Sauer 2009; Petrova et al., 2017).

The microbial cell communicates with each other by releasing some signalling molecules, this phenomenon is called as quorum sensing and the signal molecules are known as autoinducers (Waters and Bassler, 2005). In microbial species, quorum sensing is an important phenomenon for regulation gene expression, virulence, resistance, sporulation, formation of biofilm, production of EPS, biosurfactant production and bioremediation of contaminants from natural environment (Li and Tian 2012; Mangwani et al., 2016).

Biofilm(add microbe name) cells are robust and can tolerate the xenobiotic compounds (Halan et al., 2012). Biofilms can play a vital role in remediation due to high microbial biomass and their immobilizing ability, sticky nature and presence of charged molecules(add molecule name) (Singh et al., 2006; Balan et al., 2021). Biofilm(add microbe name) can trap variety of contaminants because of stickiness and charged molecules present in them (Balan et al., 2021). Autoinducers (add name) produced by different bacterial species can enhance the degradation of xenobiotic compounds in both natural and engineered environments (Feng, Wu, & Yu, 2013). Similarly, like autoinducers, EPS produced by biofilm can confine pollutants, such as heavy metals, PAH, pesticides present in the soil (Mangwani et al., 2016). (explain mechanism of biofilm and eps)

Biofilm-mediated rhizoremediation is an economical and environmentally friendly method of removing contaminants including spilled oil, heavy metals, pesticides, and xenobiotics. Its remediation has been particularly useful in the treatment of crude oil, hydrocarbons, trichloroethane, etc.

The use of bacterial biofilms in the rhizoremediation process has been elucidated by various workers (Tremaroli et al., 2010; Demeter et al., 2015). *Micrococcus* established biofilms significantly increases the breaking of the hydrocarbon chain present in crude oil compared to other bacterial strains. Furthermore, the culture of *Stenotrophomonas acidaminiphila* biofilms efficiently degraded 71% and 41% of phenanthrene and pyrene, respectively, in 7 days. PAHs, along with other carcinogenic wastewaters, are highly present in groundwater and soils (Kargi and Eker 2005). Mixed microbial biomass from activated sludge culture and *Pseudomonas putida* have been used for creating biofilm reactor, that has been used for removing almost 100% of 2,4-dichlorophenol from artificial wastewater (Gisi et al., 1997). Similarly, dinitrotoluene was degraded in a fluidized bed biofilm reactor using mixed microbial culture (name of culture and reference). Biofilms promote the production of useful plants by colonizing the soil, roots and shoots if they facilitate reproduction in the desired niche and increase soil fertility (Kour et al., 2021). *Acinetobacter* PDB4 species is a potential degrader that can be used with plants at a PAH-contaminated site for remediation purposes (Kotoky et al., 2017). In the degradation of crude oil and DDT, it was shown that a microbial consortium formed by *Bacillus subtilis* and *Acinetobacter* radioresistant with a surfactant-producing strain degrades better than microbial consortia composed solely of degraders (Mnif I, Mnif S, Sahnoun R, et al., 2015). Remediation of heavy metals like arsenic, lead, mercury or zinc via biofilm are explored recently (Nocelli et al., 2016; Meliani and Bensoltane 2016; Tay et al. 2017).

The EPS are the primary macromolecular components in microbial collections. EPS generally made up of polysaccharides, proteins, DNA, lipids, uronic acid, organic and inorganic compounds (Raj et al. 2018)**.** EPS plays a vital role in remediation of heavy metals. The negatively charged functional group present in EPS can trap heavy metals from their immediate vicinity (Geesey and Jang 1989; Pal and Paul 2008; Li and Yu 2014). In a study, it is reported that on coarse sand, biofilm, produced by a consortium of *Bacillussubtilis* and *Bacilluscereus* eliminated 98% of Cr (III) (Das et al. 2017). Consortium of sulfate-reducing bacteria such as *Pseudomonas*, *Proteus* can precipitate metal sulfides of copper, iron, nickel or zinc, and can remove 82% of iron, and 98% of copper, nickel or zinc (Jong and Parry 2003). The EPS produced by rhizobacteria forms a heavy metal complex of EPS that binds and trap precipitated metal oxides and sulfides, leading to remediation of heavy metals (Xu et al. 2012; Kaushal and Wani 2016). Various EPS bacterial genera such as *Arthrobacter*, *Pseudomonas*, *Rhizobium*,and *Azotobacter* are copious producers of EPS (Gupta and Diwan 2016). EPS produced by *Azotobacter*, immobilize 15.17 +/- 0.58 mg/g of Cd2+ and 21.9 +/- 0.08 mg/g of CrO42- (Joshi and Juwarkar 2009). Strains of rhizobacteria *Microbacterium*and*Curtobacterium*are considered to be strong candidates for the remediation of Pb (II), As (V), Zn (II) and Cu (II) in agroecosystems (Romano *etal*., 2017). These microbes can tolerate the effects of heavy metal toxicity and can increase the bioavailability and solubility of heavy metals. The *Sphaeranthus indicus* plant was selected in the Cu (II) exposed area of tannery effluent in one investigation, and the distributed *Pantoea* bacteria was isolated as a Cu (II) resistant bacterium (Yaashikaaa 2020). Gram-negative bacteria, such as *Mycobacterium*, were found to be able to consume polycyclic aromatic hydrocarbons (PAH) as a source of carbon and energy. *Acinetobacteria*, *Arthrobacteria*, *Bacillus*, *Enterobacteria*, *Flavobacteria*, *Polysporous*, *Pseudomonas* and other rhizosphere bacteria have been found to breakdown PAHs. Actinomycetes are the major members of the rhizosphere microbial population that actively participates in rhizoremediation (Bhattacharyya and Jha 2012; Pathak *etal*., 2020).

The bioremediation efficiency of some biofilm producing microbes can be improved by gene transfer from genetically engineered microbes, engineered enzymes, increasing the number of degradative gene in microbe, modified metabolic pathway (Singh et al., 2006; Balan et al., 2021).

**Chemical messengers**

Phytohormones are signalling molecules that are produced by plants and plays a role in plant growth, physiologic and metabolic process. Some microbes can also release phytohormones, where they are regarded as a secondary metabolite instead of hormones. Rhizobacteria such as *Acinetobacter*, *Agrobacterium*, *Azotobacter*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Clostridium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Rhizobium*, and *Xanthomonas* are known for releasing a phytohormone indole acetic acid (IAA) (Tewari and Arora 2013). Microbes released IAA, helps plants in tolerating the harmful effects of heavy metals by causing their roots to lengthen the accumulation of metal through the production of IAA and ACC-Deaminase (Ganesan 2008). In recent research, it is reported that the *Pseudomonasaeruginosa* and *Gordoniaamicalis* can degrade hydrocarbon present in soil as well as promote growth of *Azadirachtaindica* plant. Both of these bacteria can solubilize phosphate, produce siderophore and IAA even in the crude oil contaminated soil. *P.aeruginosa* and *G.amicalis* in combination with the *Azadirachtaindica* plant can remove 95.71% and 89.88% TPHs respectively (Bhuyan and Pandey 2022).

Some metal-binding peptides, i.e., phytochelatins and metallothioneins (MT) can remove free metal ions through sequestration, compartmentalization, or transport (Cai and Ma 2002; Solanki and Dhankar 2011). Phytochelatins shows high affinity towards a broad range of metal ions, example arsenic, cadmium, copper, lead, mercury, nickel, silver, zinc, therefore plays an essential role in remediation process (Chia, J. C. 2021).

In Rhizobacteria *Pseudomonas putida*, the expression of EC20, a metal-binding peptide, enhanced cell growth in Cd-contaminated soils (Wu et al., 2006). *Azotobactervinelandii* produces metalophores like azotocheline and protocheline, these organic ligands increases the bioavailability of some oxo anions and cations(add action/role) (Deicke et al., 2013). Many *Bacillus* species produce xenobiotic contaminants degrading, secondary metabolites such as fatty acids, isocoumarins, lipopeptides, macrolactones, polypeptides and polyketides (Qadir et al., 2022).

Heavy metal toxicity is also reduced by microbial methylation. Biomethylation of Hg to gaseous methylmercury is carried out by bacterial species of *Bacillus*, *Clostridium*, and *Pseudomonas* (Pongratz and Heumann 1999). The toxicity of heavy metals in their niche can also be reduced by some plant growth promoting bacteria that can convert metals such as selenium, lead, tellurium, and tin to the gaseous state by adding a methyl group which due to instability, methylated metals diffuse away from the cell wall (Etesami 2018). Organic acids such as oxalic acid, gluconic acid, and citric acid are secreted by microbes in the rhizosphere. They dissolve or mobilize the heavy metals present in the soil (Rajkumar et al., 2012; Ullah et al., 2015). The solubility of Zn compounds is improved by a derivative of gluconic acid 5 ketogluconic acid, which is produced by *Gluconacetobacterdiazotrophicus*(Saravanan et al., 2007; Mishra and Arora 2019). (add content)

**Recent research and emerging challenges**

**Biotechnological interventions**

Application of genetically engineered microorganisms (GEM) has increased the remediation efficiency in recent times. Microorganisms utilized in the process of rhiizoremediation can be genetically modified by introduction of catabolic genes, construction of hybrid pathways, promoter modification and by the construction of recombinant strains. Recombinant strains are the strains that possess two or more trait in combination such as degradation of the contaminant, production of biosurfactant, excellent colonization capability and PGP traits. There are certain restrictions on the release of recombinant microorganisms in many countries, and these legal restrictions, along with some ongoing scientific concerns, may limit the development of this area (Segura, et al., 2009).

Numerous studies have been done to examine how microorganisms react to various contaminants that are present everywhere in nature, even in more delicate conditions (Yergeau et al., 2012, 2015a, b). Modern meta transcriptomic studies in the rhizosphere have highlighted several key taxa that respond to the breakdown of hydrocarbons in crude oil. Various transcripts related to alpha-proteobacteria, beta-proteobacteria, gamma-proteobacteria, and acid bacteria are more common in the rhizosphere of contaminated soil than in control soils. Some functional genes associated with the breakdown of aromatic and aliphatic hydrocarbons were more abundant in the rhizosphere of contaminated soils (Yergeau et al., 2014). These genes have been seen in bacteria of various orders, *Actinomycetetales*, *Rhodospirillum*, *Burkholderiales*, *Alteromonadales*, *Soliurubrobacterales*, *Caulobacterales* and *Rhizobiales* (Page et al., 2015). Similarly, ryegrass stimulates the expression of bacterial PAH-ring dioxygenase hydroxylation genes such as nidA3, pdoA, nahAc, and phnAc (Guo et al., 2017a, b). Genes for CYP153 alkane hydroxylase were found in *Stenotrophomonas* and *Rhodococcus*, and these organisms can grow in n-hexadecane (the sole carbon source) (Pawlik*etal*., 2017).

Genetically modified microbes to be used as heavy metal bio sorbents were constructed with metal-binding peptides to improve selectivity and affinity for target metals. In *Staphylococcus xylosus*and *S. carnosus*, surface display systems express two different polyhistidyl peptides, i.e., His3GluHis3 and His6, to improve metal-binding functionality and surface accessibility (Samuelson et al., 2000). By immobilizing an intracellular phosphate-binding protein on their cell surface, *Pseudomonas putida* and *Escherichia coli* show increased phosphate biosorption (Li et al., 2009; Mosa et al., 2016). GEMs can also be used as an alternative to treat PAH-contaminated soils, where native microbial activity is inhibited and bioavailability is low. *Trichoderma* a fungus that degrades pyrene, benzopyrene, and phenanthrene, is an example of genetic manipulation. It was genetically modified with a hygromycin resistance gene and an organ phosphohydrolase gene. The wild-type and modified strain colonized natural substrates quickly and efficiently and remains persistent in the soil (Fernadezluquano et al., 2010). The strategies proposed to eliminate PAHs in the soil are the following: clone a complete dissipation pathway, create new metabolic dissipation pathways, improve the genetic stability of catalytic activities, improve the stability of enzymes, change the degradation kinetics of enzymes, avoiding gene transfer in autochthonous strains, reducing the proliferation of new strains, chemotaxis and production of biosurfactants (Paul et.al).

Endophytic bacteria were developed for remediation with toluene (Barac et al., 2004). They transferred the plasmid pTOM by conjugation of *B. cepaciaG4* to *B. cepacia*L.S.The plasmid pTOM encodes the toluene degradation genes. This was followed by a horizontal gene transfer of the toluene monooxygenase (TOM) operon to various members of the endogenous community. This revealed new avenues for introducing desirable properties into the community. Although PCB breakdown is ineffective due to multiple PCB breakdowns. In a study, Narasimhan and his colleagues (2003) used *P*. *putida* PML 2 to break down phenylpropanoid compounds to break down PCBs. 37% of the *Arabidopsis thaliana* root exudates were flavonoids and mainly phenylpropanoids. However, there is no practical information on where GEM can be used. Before GEMs can be used to remove contaminants from soil, a few questions need to be answered. When using these GEMs, some measures must be taken so that they do not displace native strains or that negative traits do not spread through the soil microbial population (Fernadezluquano et al, 2010).

**Omics based tools**

Studies on the degradation of various pollutants focused on isolated metabolic properties of different bacteria that helps in the breakdown of toxic substances in the soil. OMIC technologies are used to study the ability of microorganisms to degrade hydrocarbons and other pollutants. Modern next generation genomic sequencing technologies and genome editing techniques can be used to investigate the biosorption capacity of organisms (ElMetwally et al., 2014; Bao et al., 2016; Mosa et al., 2016). When remediation processes are explored in greater depth, an attempt is made to isolate and characterize the organisms responsible for remediation. The main disadvantage of culture-based approaches is that more than 99 percent of microorganisms found in various natural habitats are either uncultivable or extremely difficult to cultivate. The recovery of microbial isolates involved in rhizoremediation processes is extremely significant. As it allows researchers to examine their biodegradation reactions as well as physiological characteristics, that are thought to govern growth and other activities in polluted environments. A number of DNA-based molecular approaches have been developed to investigate the microorganisms, responsible for rhizoremediation. In order to overcome these limitations and shortcomings 16S rRNA sequences have been analyzed using denaturizing gradient gel electrophoresis (DGGE) to assess complex microbial diversity and deduce phylogenetic association among these microbial communities (Malla et al., 2018). The use of omics-tools to study the taxonomic and functional characteristics of microbial communities from contaminated locations has resulted in the discovery of several novel bacteria that would not have been discovered otherwise using traditional culture procedures.

One of the newest additions to the omics family is metabolomics, which is the study of a cell's metabolite profiles under specific conditions. This approach has great potential to study and understand the role of metabolites in response to carious pollutants. Recently, integrative studies of proteomics and metabolomics have been carried out in order to better understand the reactions of microorganisms to the biodegradation of environmental pollutants (Finley et al., 2009). When soil microbial populations were exposed to phenanthrene, the quantity of transcripts linked to dioxygenase, stress response, and detoxification increased. Similarly, the rhizosphere of willows growing in contaminated soils was highly enriched in transcripts associated to PAH degradation. Most of the genera related to them are *Actinomycetales*, *Rhodospirillales*, *Burkholderiales*, *Alteromonadales*, *Solirubrobacterales*, *Caulobacterales* and *Rhizobiales* members (Finley et al., 2009).

Models that can predict microbial activities under various remediation procedures have been developed using metabolome-based methodologies applied to environmental data. Metabolomics, enables us to gain a deeper understanding of microbial communities' dynamic operations and functional contributions to the environments in which they dwell. A lot of recent research have been conducted on the biodegradation of anthropogenic contaminants using metabolome analysis. The comparative metabolome analysis of *Sinorhizobium* during phenanthrene production is an example of this. The metabolite profiles (fatty acids, polyhydroxy alkanoates, and polar metabolites) were evaluated with an untargeted metabolome analysis, and the intracellular metabolomes were compared to those from carbon sources. These kinds of studies demonstrate the value of metabolomic data in remedial research (Bharagava et al., 2019).

In addition to that Proteomics is an OMIC technology useful for studying the complete set of proteins expressed in a given biological sample and helping to understand the pattern and function of proteins (Varga B, Somogyi V et al. 2019). Mass spectrometric-based proteomic approaches have been utilized to explore the translational level response of heavy metals stress, and the changes in protein expression brought on by the accumulation of high levels of toxic metals in cells. It is also used to identify siderophores-related proteins and their functional roles (Italiano et al., 2009; Chen et al., 2013).

Roots release a variety of chemicals, such as flavonoids and fatty acids, that boost microbial proliferation and pollutant degradation activity, so concomitant effective rhizodegradation may occur spontaneously (Booth SC et al. 2011). PAH-degrading bacteria, for example, were found to be significantly more abundant in the rhizosphere of Spartina plants than in unplanted sediments. Recent integrative omics-based methodologies, can help in exploring the genome, transcriptome, proteome, and metabolome of single organisms and even mixed groups. Thus, it assists in opening new avenues for deciphering molecular pathways of PAH breakdown in contaminated environments (Bell TH et al., 2015). Shotgun metagenomics involves the shearing and sequencing of all DNA, and functions and taxonomy are derived via database homology searches (Bell TH et al., 2015). Functional metagenomics involves inserting large DNA fragments into vectors and expressing them in hosts. These hosts are then tested for activity, and only clones that exhibit the desired activity are sequenced (George I., et al., 2010 ). Combining these methods can aid in determining which organisms are performing specific tasks *insitu* and how much of that function is being expressed. Effective degrader strains have been found to belong to a few numbers of genus groupings, such as *Sphingomonas*, *Burkholderia*, *Pseudomonas*, and *Mycobacterium*, based on cultivation-dependent approaches. The majority of these bacteria can utilize PAHs as their only source of carbon and energy. The biochemical catabolic routes of aromatic chemicals (in particular PAHs) have been explored and characterized using these isolated isolates ().

During environmental remediation, genome-enabled approaches provide a framework for plant-microbe interactions. Metagenomic investigations can help researchers to understand the microbial diversity associated with plants in polluted environments. This knowledge will serve as a foundation for better understanding of the indigenous microbial communities and will aid in the development of remediation techniques for damaged settings.

**Nanotechnological approach**

Nanotechnology is an area of ​​research and innovation that plays a role in designing, producing and usage of materials and devices by modifying atoms and molecules at the nanoscale. Nanotechnology can be used for both, accelerating as well as reducing the performance of material or any other process. In soil remediation, nanotechnology can play a role in sensing, detection, and pollution prevention (Carata et al., 2017). Nanotechnology used nanoparticles. Nanoparticles are engineered material that possess size between 1 nm to 100nm. Nanoparticles can be categorized into two types, organic (carbon nanoparticles) and inorganic (magnetic, noble metal, semiconductor nanoparticles) nanoparticles (Tripathi et al., 2018).

The use of nanoparticles to clean pollutants from contaminated soils is one of the latest technologies (Pan and Xing 2012). In remediation process, nanoparticles are used to degrade the heavy metals, herbicides, pesticides from the contaminated sites. Due to their small size and innovative surface coatings, these nanoparticles have highly desirable properties for application *in situ*. They allow for both chemical reduction and catalysis to reduce harmful contaminants. Using nanotechnology for the remediation purposes is more advantageous in comparison to other technologies including chemical oxidation, thermal desorption, photochemical degradation, Nano-remediation technology is ecofriendly and economical, it can reduce the overall cost of large-scale clean-up. Due to small size of nano-particles they can enter in those contaminated zones, where other entities cannot. In *in situ* application, it can reduce the contaminant level near zero (Tripathi et al., 2018). Most of the reported advantages of nano-remediation are laboratory tested as *ex situ* nano-remediation is still under research stage. Even potential risk associated with nano-remediation is still unclear.

For remediation purpose, the nanoparticles are selected on the basis of the contaminant nature. To remove heavy metal from the contaminated soil, a magnetic nanoparticle can be used for example, magnetite, a nano iron material. Carbon based nanoparticles also can be used to remove heavy metals but they are generally used to trap organic pollutants from the contaminated soil. Carbon tubes (carbon-based nanoparticle) and Arthrobacter can degrade PCBs. Pesticides such as chlorpyrifos and herbicides such as atrazine, molinate are examples of organic pollutants that can be eliminated using nZVI (zerovalent nano ions) (Tripathi et al., 2018). nZVI strains are highly reactive reducing agents that can efficiently break down organochlorine pesticides and chlorinated hydrocarbons (Singh et al., 2011; Zhanqiang, 2010). Carbon nanotubes have a high absorption capacity for radionuclides(check word) (Ren et al., 2011), organic compounds (Pan and Xing, 2008) and metal ions (Rao et al., 2007). Therefore, both nZVI and carbon nanotubes can be used as suitable remediation material. Huge nanoscale materials have been investigated for remediation, such as nanoscale zeolites, enzymes, various precious metals such as bimetallic nanoparticles. In a study it was found that *Geobactermetalireducens*, when bound to iron oxide, can reduce highly polluting organic compounds called 4-nitroacetophan (Braunschweig et al., 2003). According to a study, PAHs can be removed from contaminated locations using amphiphilic polyurethane nanoparticles produced from polyurethane acrylate anionomer or polyethylene glycol modified urethane acrylate precursor chains that are emulsified and cross-linked in water (Tungittiplakorn et al., 2004). The minimal toxicity of PVP-Ag-NPs (silver) to three bacteria, *B*. *amyloliquefaciens*, *S*. *meliloti*, and *P*. *putida* was reported in a study, implying that AgNPs-cell contact is significant in mitigating Ag-NPs toxicity. The impact of TiO2-NPs on soils was recently investigated, and it was discovered that culturing *T*. *aestivum*with*Paenibacilluspolymyxa*, *Alcaligenesfaecalis*, *Bacillusthuringiensis*, and a mutant strain of *P*. *polymyxa* alone or in different combinations improved the growth of *T*. *aestivum*. The effects of TiO2-NPs on wheat drought, salt, and disease responses were also investigated at the same time. It has been suggested that TiO2-NPs can boost the growth of PGPR when plants are co-inoculated with *P*. *polymyxa*, *B*. *thuringiensis*, or *A*. *faecalis*, based on the accumulation of shoot biomass in wheat. When plants were cultivated in the sand, however, no growth improvement was observed after exposure to TiO2-NPs (Ameen et al., 2021). Toxicity of nanoparticles can be evaluated by using *Saccharomycescerevisiae* as a model organism. This combination can further be used to investigate arsenite resistance in eukaryotes. *P*. *chrysosporium* is well known for its cellulolytic uses. As a result, it is widely used for rhizoremediation of lead-contaminated soil and the breakdown of other xenobiotic chemicals (Ameen et al., 2021).

There are some research gaps in nano-remediation process like *exsitu* application, large scale application, toxicity of nanoparticles on the environment. Once these gaps were disclosed, then nano-remediation can be used as promising remediation technology. On the basis of recent researches, in future, the combination of nanotechnology with bioremediation can be used as a sustainable, effective and efficient solution for decontaminating polluted sites.

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