BIOREMEDIATION: An emerging way for natural and stable environment

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ABSTRACT

Urbanisation, anthropogenic activities, increase in demand for the available resources, excessive agricultural and mining practices have led to increase in the concentration of heavy metals and xenobiotic compounds in the environment. Presence of these toxic compounds ultimately finds its way into the food chain and can cause serious damage to living beings. Several health issues related to accumulation of toxic metals in human body have been well documented in literature. Attention in this regard is majorly on an eco-friendly solution where bioremediation is one of the possible solutions. Bioremediation is a natural and environmentally friendly approach to treat contaminated land and restore using living organisms like bacteria, fungi, algae and plants. This method of treating contaminated land has received attention in the scientific community and with the newer methods like improved molecular biology techniques, genetic engineering and nanotechnology, improved efficiency is predicted in near future. This topic focuses on the origin, the treatment methods used originally and the futuristic approaches that might be employed for treatment of contaminated land.

Keywords--Bioremediation, Phytoremediation, Mycoremediation, Phycoremediation, futuristic trends

I. INTRODUCTION

Global industrialisation, migration and population explosion has resulted in the increase in demand for the available natural resources which subsequently resulted in pollution of the environment, waterways, and topsoil. Water quality has deteriorated because of human activities such as mining and the eventual purging of hazardous-metal effluents from steel plants, battery firms, and energy generating industries, raising serious environmental issues. Pollution caused by xenobiotics and other refractory substances has lately been recognised as a severe threat to both public wellness and ecology. A variety of pollutants, such as toxic metals, polychlorinated biphenyls, polymers, and other agrochemicals, are prevalent in the environment that are hazardous to the environment and are non-biodegradable. Over exploitation of natural resources and population explosion has led to indiscriminate use of available elements from our planet earth. The last few decades have witnessed an increase in pollution due to anthropogenic and natural sources. For instance, the concentration of toxic heavy metals and poly-aromatic hydrocarbons (PAHs), the use of pesticides in agriculture has increased sharply that has led to damage to the ecosystem. Newer methods for the treatment of these pollutants in an eco-friendly manner is in huge demand, even though there are conventional methods like physical and chemical treatments available with their own advantages and disadvantages. Alternative solution like the use of living organisms to remediate the polluted environment in environmentally favourable way has sparked interest over the last few decades. Bioremediation is a rapidly growing cleaning technology for eliminating harmful waste from damaged areas. Bioremediation employs a variety of microorganisms, including aerobes and anaerobes, plants and algae to remediate polluted locations. The history of modern bioremediation eras dates back to 1960 when George M Robinson (petroleum engineer) used microbes to remediate pollutants in a glass jar[1]. Romans have also used this technology of bioremediation to treat waste water in 600 BC[1]. However much attention to bioremediation was received only after the Exxon Valdez oil spill in Prince William Sound, Alaska incident that occurred on 24th March 1989 where an estimated 11 million gallons of crude oil was spilled in this region killing almost a variety of marine species especially seabirds. [2]

II. ENVIRONMENTAL POLLUTION

Rise in industries and unplanned urbanization has led to increase in the release of toxic pollutants in the environment. These pollutants could prove extremely hazardous depending on the nature of pollutant. Pollutants like antimony, chromium are released form dye manufacturing industries, mining activities release lead, arsenic, cadmium etc, use of herbicides and pesticides during agriculture can lead to release of aluminium, copper, zinc, nickel etc . [3] These heavy metals persist for a long time in the environment and may find its way into the food chain. A number of health hazards due to exposure or unintentional release of these toxic pollutants into the food and directly to the human body is well documented [4]–[6]. These heavy metals can cause a plethora of conditions in the human body damaging lungs, kidneys, CNS, variety of cancers as well as affecting the reproductive and immune system[7]. It is vital to shed light on the removal of these toxins and convert it into nontoxic product. Additionally heavy metal contamination can lead to deterioration of soil, loss of fertility, nutrient deprivation, loss or imbalance of microbiome, reduction in crop productivity etc [8]. Especially nitrogen dioxide and sulphuric acid can reduce plant yield and causes acidification of water respectively[8]. However, plants and microbes have developed metabolic, physiologic, and molecular traits to cope up with this situation, and these can be used for remediating polluted soil. A plethora of physical, chemical, and biological techniques have been suggested to rejuvenate contaminated land. [8] (Figure 1)

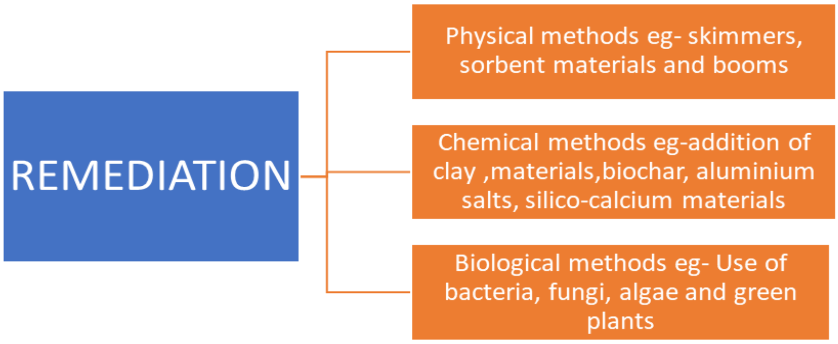

FIGURE 1: Different methods of remediation [3]

III. NEED FOR BIOREMEDIATION

Due to rapid industrialization, globalisation and various anthropogenic activities, the world is witnessing a decrease in natural resources and the increase in carbon emissions and pollution in the environment. Environmental pollution is known to cause a variety of side effects in human beings. The toxicity of certain elements and xenobiotic compounds is well documented in literature. For instance, there is increased risk of premature death when exposed to particulate matter in the environment [9]. These particulate pollutants cause irritation of the respiratory system, aggravate asthmatic condition and cause shortness of breath[10]. Carcinogenic volatile organic compounds like benzene is also linked to cause leukaemia on exposure[11]. Industrialization has caused release of contaminants which has serious health issues on long term exposure. This has led to intentional and accidental release of toxic and xenobiotic compounds in the environment.[12]. The heavy metals and toxic compounds accumulated over an extended period can be modified with the use of biosolids, compost, municipal solid waste has been found improve the texture and potency of solids as well as make the nutrients bioavailable to microbes[13]. Fewer metals that are required in less quantities in our body become toxic beyond a permissible limit[14].If the specific density of heavy metals exceeds 5 grams per cubic meter, it leads to unfavourable effects on the environment and human health[15]. Also, constant long exposure of some heavy metals can cause cancer[15]. The emerging field of Biotechnology encompasses the exploitation of engineering and scientific principles to the creation of materials using biological organisms to supply goods and services to human and environment [16]. The branch of biotechnology which includes microbes for remediating contaminated soil is called “Bioremediation”. “Phytoremediation” exploits plants for remediating the contaminated soil.

Bioremediation implies using living organisms to remediate polluted lands converting contaminants into harmless products. This technique ideally uses bacteria, archaebacteria, fungi, algae, and plants, working well for the eco-friendly mitigation of pollutants. The method adopted in bioremediation to treat the environment is greatly dependent on several factors like, the nature of the pollutant, the resident microbes, the microbes' capability to degrade the contaminant, the bioavailability of the contaminant, the concentration, and the chemical structure of the pollutant, along with the physicochemical properties of the environment.

IV. TECHNIQUES IN BIOREMEDIATION

Two approaches are practiced remediating contaminated land: In one approach, the main goal is to complete remove the contaminant completely from the polluted soil and the second approach is to convert the contaminant into less harmful forms using combination of different remediation techniques or advanced engineering technologies[17]. Bioremediation of contaminated soil can be carried out either by *In-situ* or *Ex-situ* approaches[17].

A. *In-situ* bioremediation:

This approach encompasses the use of a biological treatment for hazardous compound treatment and has been successfully used to degrade pollutants in saturated soils and groundwater as given in Figure 2[18]. For the elimination and detoxification of pollutants present in an area, resident microbial activity plays a significant role. In contrast, the ability of microorganisms to transform harmful pollutants into less harmful or non-harmful forms completely depends on the availability of nutrients, electron acceptors, and donors. As long as the need for groundwater pumping, treatment, and discharge to recipients are eliminated, bioremediation using an in situ technique is sustainable [18]. Furthermore, it has several benefits like using natural, harmless microbial species and treating huge amounts of contaminated soil or water without releasing hazardous chemicals.

This technique includes methods like biosparging, bioventing, bioaccumulation, biostabilization, biostimulation and biopiling.

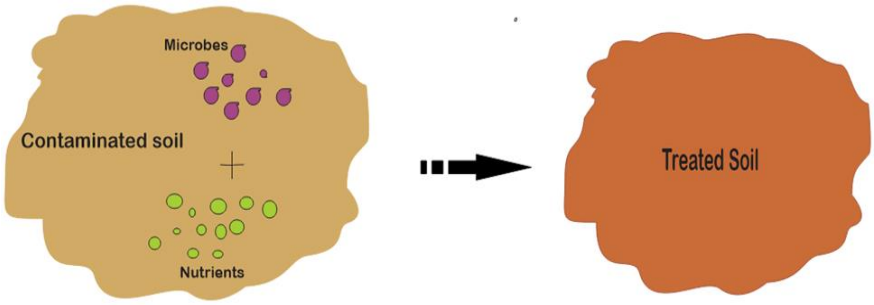

FIGURE 2 : *In-situ* Bioremediation

B. *Ex-situ* bioremediation:

This method entails successfully removal of pollutants from contaminated areas to another place for treatment as illustrated in Figure 3. A number of factors need to be considered for this technique, For instance, the extent of pollution, the nature and kind of pollutant, the microbes involved, accessibility of the pollutant to the microbe, form of the pollutant and the cost of treatment of the contaminated site[18]. Furthermore, the principle of the treatment is further classified into two phases depending on the state of pollutant; namely solid-phase and slurry-phase systems.

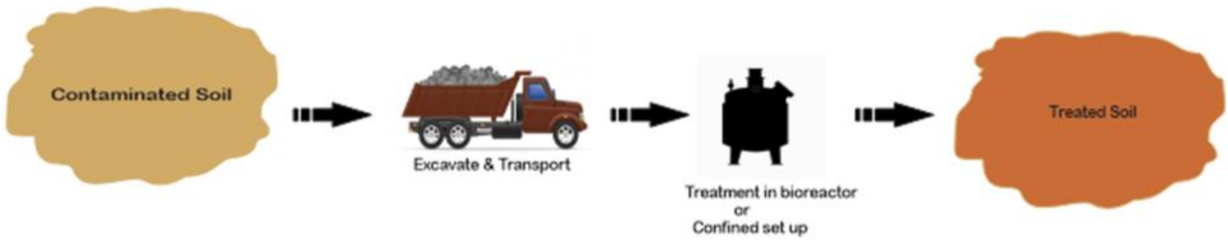

FIGURE 3 : *Ex-situ* Bioremediation

V. BIOLOGICAL ORGANISM IN BIOREMEDIATION PROCESS:

Although a variety of physical and chemical process are available for removal of contaminants from the environment, biological process is significant in that they are eco-friendly, can convert toxic to harmless products and safe to the environment [19].

Organisms documented in literature for biodegradation includes a variety of bacteria; namely *Bacillus licheniformis, Pseudomonas aeruginosa, Bacillus sonorensis, Aeromonas* etc. Amongst algae the major species involved are *Chlamydomonas reinhardtii, Microcystis aeruginosa, Chlorella sp., Isochrysis galbana,* *Scenedesmus*[4]. Amongst fungi, *Phanerochaete chrysosporium, Saccharomyces cerevisiae, Aspergillus sp.*, etc [4]. Improvised methods and newer molecular biology techniques has allowed scientists to explore the possibility of modifying proteins, genetically engineering organisms, systems like CRISPR-Cas and nanomaterials has been researched for their use in furture.

A. USE OF BACTERIA:

Heavy metals enter soil through a number of anthropogenic activities, industrialization, unplanned urbanization thus potentially threatening human health [20]. Detoxification of metals can be done by microbes either by valence transformation, extracellular precipitation and volatilization[20]. Bacteria have adapted different mechanisms to degrade pollutants especially heavy metals like biosorption, bioaccumulation, biotransformation, bioleaching, biomineralization etc.[21]. Also, microbes have the capability to mobilize heavy metals by chelation, redox transformation , leaching and methylation mechanisms.[21]

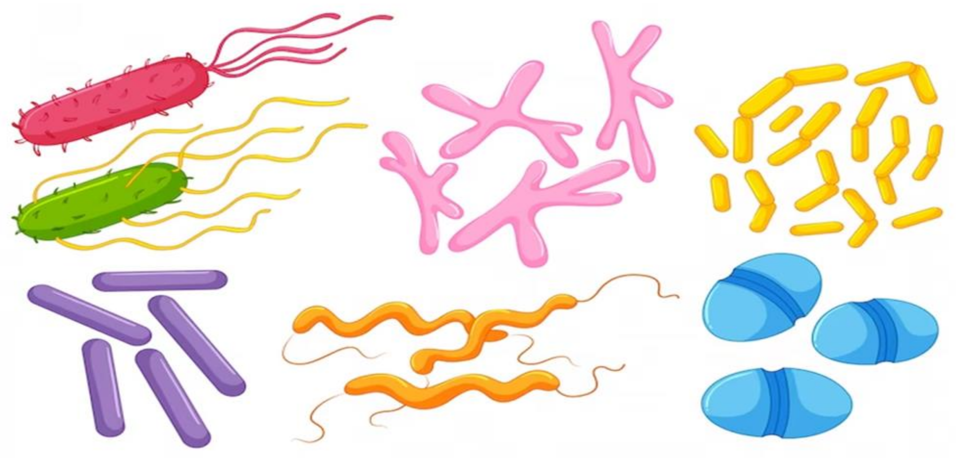

FIGURE 4: Different types of bacteria

B. MECHANISMS USED BY BACTERIA:

Microbes can use different mechanisms for removing pollutants namely, mobilization and immobilization.[22]. Mobilization encompasses process like bioleaching, bioaugmentation, enzymatic oxidation, enzymatic reduction.[4]. Immobilization includes process like bioaccumulation, biosorption, precipitation and complexation.[23]. Mineralization is a process where microbes transform pollutants and convert it into harmless end products like carbon dioxide and water, and immobilization is the process of converting the pollutants to a form where it becomes unavailable in the environment.[4].

B.1 Bioaugmentation: The word 'augment' means to add to, here microbes are added externally to augment those who are already present in the contaminated site. Additionally, the resident microbes may be isolated and genetically modified to degrade the pollutant (Figure 5). However, these organisms need to compete with the available resident microbes, as well as adapt to the new environment.[5]

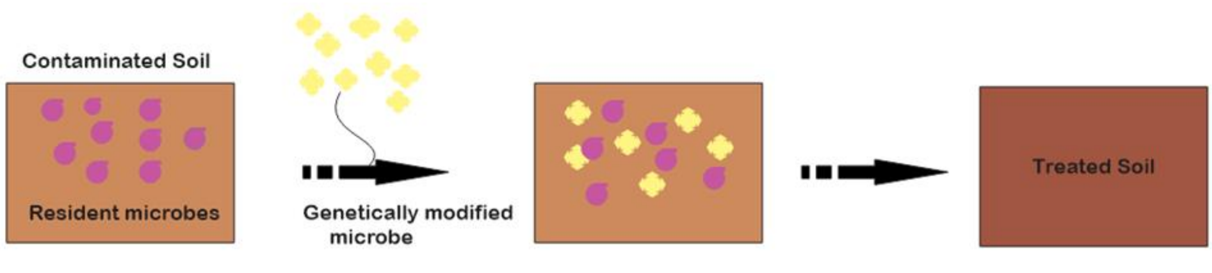

FIGURE 5: Bioaugmentation

B. 2 Biostimulation: It is important to stimulate the activity of microbes by addition of nutrients in the form of additives, enzymes, electron donors, metabolites, which may otherwise interfere with the organisms ability to utilize the pollutants (Figure 6) [6], [24].This method is comparatively economical and efficient than bioaugmentation[25]. Also , it has been recorded in literature that indigenous organisms are more competitive than added ones[26].

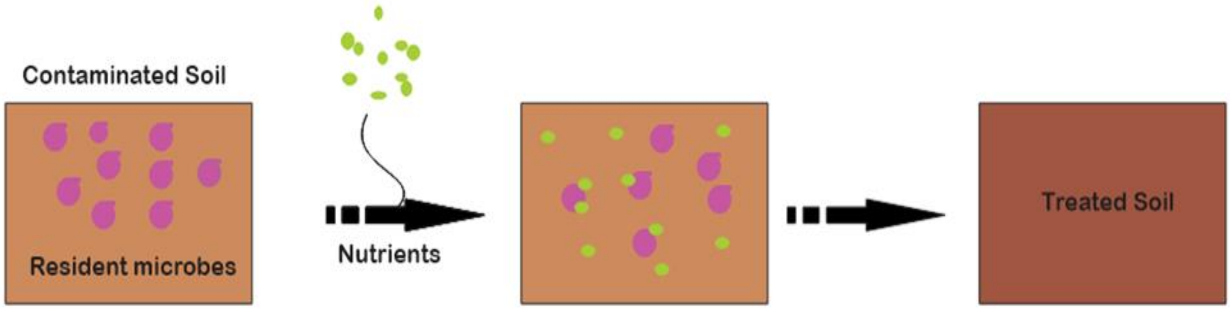

FIGURE 6: Biostimulation

B.3 Bioleaching: Acidophilic bacteria are used to mobilize metals from insoluble mineral ores by biological process like oxidation, acidification etc for extraction of metals is called bioleaching (Figure 7). This process is also called as biomining and has been successfully employed for the extraction of copper, cobalt, zinc, nickel etc. [27]

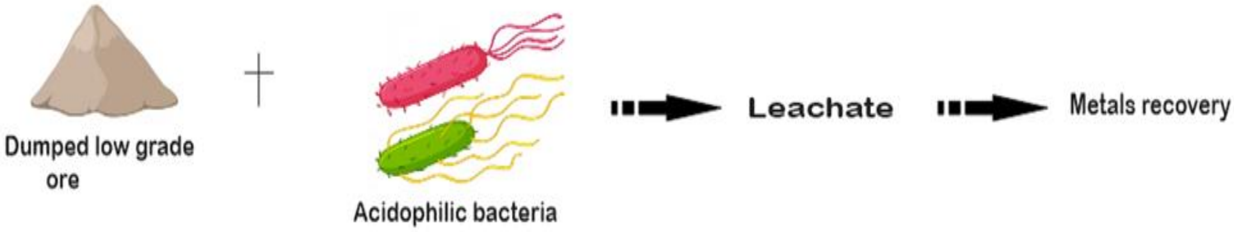

FIGURE 7: Bioleaching

B.4 Biosorption : It is the process where toxic metals are adsorbed on the surface of organisms by means of complexation, metal chelation or physical interaction is called biosorption (Figure 8) [28]. Organisms involved in biosorption are *Rhodococcus erythropolis*, *Streptomyces sp. K11*, and *Bacillus anthraci*.[29]

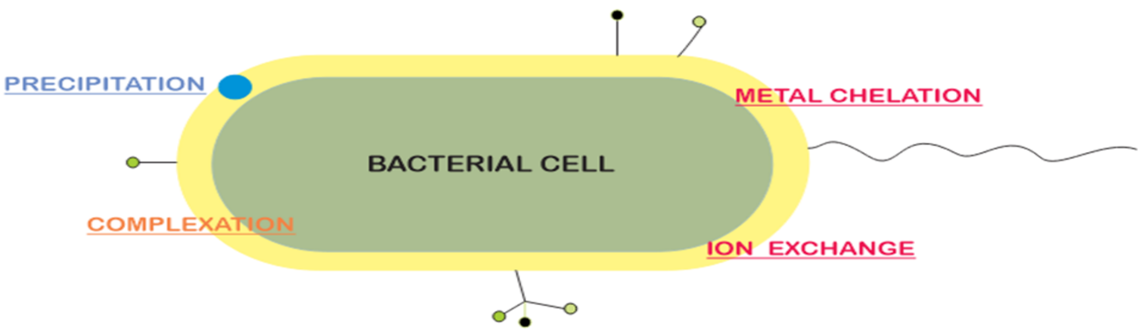

FIGURE 8: Biosorption

B.5 Bioaccumulation: It is a process where heavy metals are accumulated by microbes inside their cells. This leads to build up of heavy metals in organisms (Figure 9). Bioaccumulation occurs at both the surface as well as intracellular spaces. Through membrane associated metal transporters and a variety of transport mechanisms, bacteria can uptake metals from extracellular environment.[30]. Examples of bacteria involved in bioaccumulation are *Staphylococcus, Corynebacterium, Escherichia, Bacillus, Aeromonas, Klebsiella, Pseudomonas, Thiobacillus* and *Enterobacter* [31].

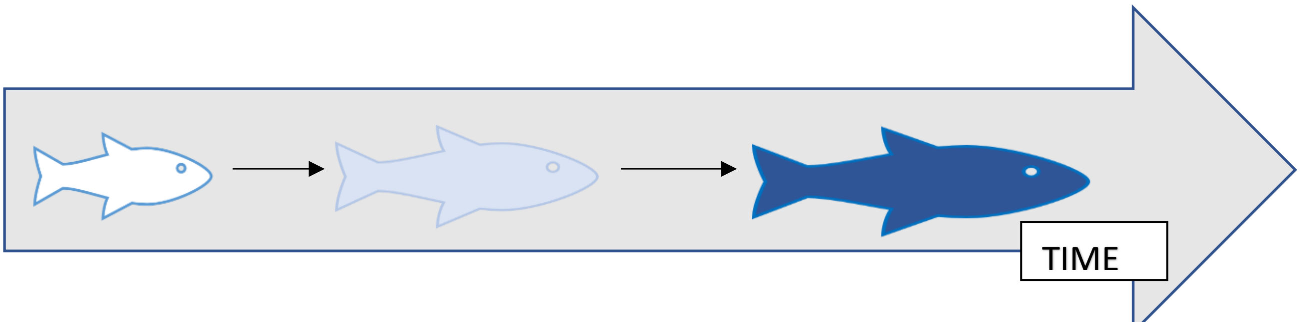

FIGURE 9 : The process of bioaccumulation

The above are few important mechanisms by which bacteria are exploited to degrade or to transform the available heavy metals.

C. BIOREMEDIATION WITH FUNGI:

Bioremediation mediated by fungi is called ‘Mycoremediation’. Diverse group of fungi, especially basidiomycetes, are capable of utilizing harmful pollutants from the soil (Figure 10) [32]. Fungi are also capable of complex long chain molecules naturally present in the environment [33]. Both filamentous and macro fungi can be employed in the degradation and removal of toxic compounds[32]. One saprotrophic fungus, the white rot fungi, has been widely studied for biodegradation. White-rot fungi (WRF) are recognized as key players in biodegradation, owing to their ability to efficiently degrade both lignin and cellulose biopolymers until complete mineralization. This fungi produces an extracellular enzymatic complex which encompasses lignin peroxidases (LiPs), manganese-dependent peroxidases (MnPs), versatile peroxidases (VPs), laccases, H_2O_2-generating oxidases and dehydrogenases, which are produced under nitrogen depletion [32]. Another fungi *Phanerochaete chrysosporium* has been the most extensively studied among these fungi for its exceptional ability to convert toxic or insoluble compounds into CO_2 and H_2O comparatively than other fungi. As early as 1985, Bumpus et al. first proposed using *Phanerochaete chrysosporium* in bioremediation studies, and the fungus soon became a model system in the field of mycoremediation [32].

Literature studies revealed that a variety of hydrocarbons like mineral oils, mono aromatic and polycyclic aromatic hydrocarbons (PAHs), , chlorinated hydrocarbons (CHCs), and phenols were demonstrated to be degraded by multiple fungal species[34]. Additionally, the potential to reduce the risk associated with heavy metals, metalloids, and radionuclides in soil was described[35].

FIGURE 10 : Fungi

In the current approaches, it is suggested to have a microcosm studies at lab scale to evaluate the potential degradative capacity among fungi. The biodegradation capacity of a variety of fungi has been well documented under microcosm studies [32].

Among fungi, mushrooms are researched for their biodegradable capability of toxic compounds. Several studies have reported the bioremediation of soil contaminated with engine oil by *Lentinus squarrosulus*, and the decontamination of soils polluted with cement and battery wastes using *Pleurotus pulmonarius*. Additionally, numerous works on the edible mushroom *P. ostreatus* have been published, including the biodegradation of the carcinogenic secondary metabolite aflatoxin B produced by *Aspergillus flavus* on rice straw and maize. Furthermore, the mycoremediation of heavy metal-contaminated soils by different *Pleurotus* species was reviewed and it was found that *Pleurotus spp*. are able to accumulate high levels of heavy metals, with each species having different sensitivities towards a variety of metals and at different concentrations [32]

D. BIOREMEDIATION BY ALGAE:

Treatment of contaminant or destruction of contaminant with the help of algae is called 'Phycoremediation'(Figure 11) . Algae has started gaining more importance in remediation, especially the marine algae is able to accumulate toxic contaminants [36]. Although surface adsorption is the primary mechanism for metal uptake in algae, both surface adsorption and internal diffusion also play a role in the accumulation of metals within the cells[36] .Microalgae by the process of biosorption can bind to heavy metals and metalloids without depending on cellular metabolism [37]. By biosorption in the cell wall or through the extracellular polymeric substances (EPS) that the living microalgae create in response to stress, heavy metals and metalloids (HMMs) are absorbed by living microalgae. HMMs are bioabsorbed into the EPS by a process that is dependent on metabolism [37]. In response to metallic stress, microalgae may control EPS synthesis, and they can also modify the properties of these biopolymers as necessary[37]–[39].

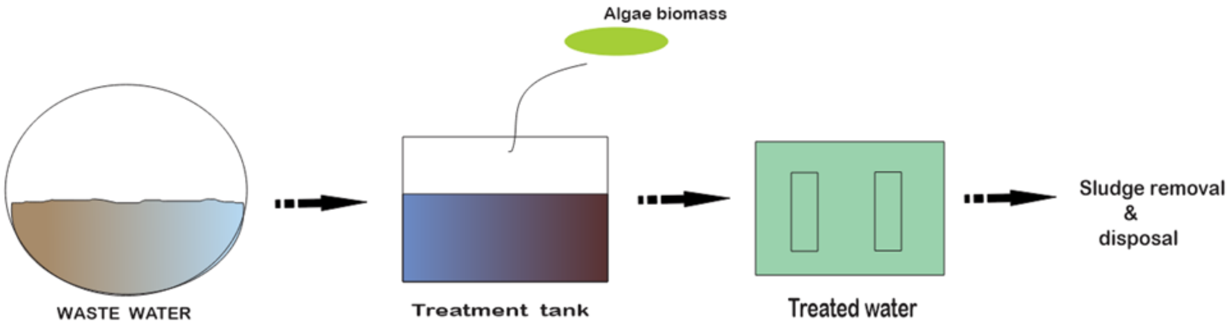

FIGURE 11 : Phycoremediation

E. BIOREMEDIATION WITH PLANTS:

The process of remediating the contaminated soil with the help of green plants is called "Phytoremediation" and the technique dates back to 1980's (Figure 12) [40]. One example of plant used for bioremediation is poplar trees that had been successfully demonstrated to remediate contaminated water or runoffs [40].

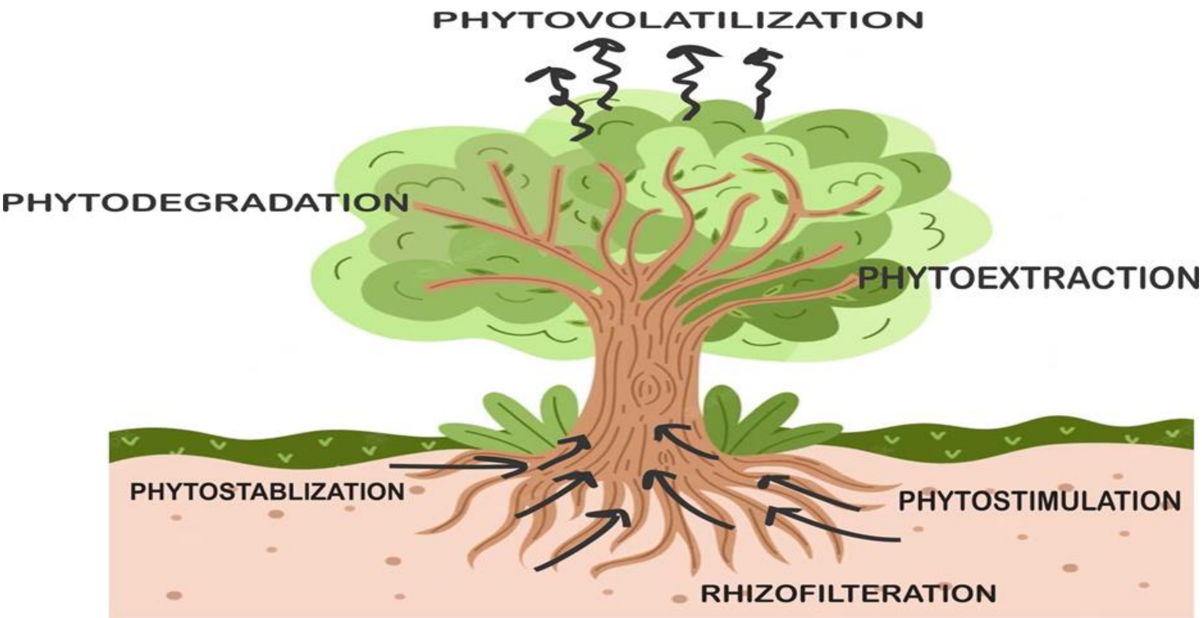

FIGURE 12: Phytoremediation

The process by which plants absorb pollutants from contaminated locations together with essential nutrients and water necessary for their growth is known as phytoaccumulation. The ingested pollutants are not eliminated, but rather accumulate in the shoots, leaves, and other plant parts as seen in the figure 12. For the purpose of restoring contaminated primary sources, such as soil and water, phytoremediation uses plants that accumulate metals[41].

Such plants are called hyperaccumulators that can accumulate heavy metals 10-100 times the normal plants. Furthermore, aquatic macrophytes have the capacity to accumulate heavy metals up to 1,000 times more than the amount of the related water, according to research. For instance, aquatic macrophytes species like *Eichhornia crassipes* and *Centella asiatica* have been observed for their capacity to absorb copper at various amounts from contaminated locations[42]. The phylum Chlorophyta, specifically the genera *Chlorella* and *Scenedesmus*, has recently produced the most often used microalgae strains in phycoremediation [31].

Even under comparable operating circumstances, the sensitivity and efficacy of microalgae biosorption differ by genus and species [32]. Due to the differing compositions and architectures of their cell walls, *C. sorokiniana* and *S. obliquus*, for instance, thrived in medium contaminated with copper (II), cadmium (II), lead (II), and chromium (VI) [33].

E. 1 MECHANISM OF PHYTOREMEDIATION:

Plants absorb pollutants from contaminated locations together with other nutrients and water needed for their growth through a process known as **phytoaccumulation** [41]. In another process known as **phytostabilization**, specific plant species are used to immobilize contaminants at contaminated locations by accumulating them in their roots through root hairs, adhering to their surfaces, or precipitating them in their rhizosphere[43]. In **phytovolatilization** process, pollutants are taken in by plants, grown, and then released into the atmosphere in a less dangerous form.[41]

Plants take up pollutants and convert them to less hazardous, simpler forms in a process known as **phytodegradation**. Two processes lead to breakdown of the contaminant using this process; namely through a plant's internal metabolic mechanism and using the plant's own enzymes

Many plants with special capabilities to absorb metals and other toxic components are being researched and studied for their tolerance levels. Though this process is eco-friendly and aesthetic solution to pollution problem, there are few disadvantages. For instance, plants show stunted growth due to heavy metal accumulation; problem of biomass degradation and the time taken by plants to adapt and grow in a contaminant laden environment.

VI. FUTURISTIC TRENDS IN BIOREMEDIATION

Essentially all living organisms depends on soil for their biosafety and ecological diversity[44]. However due to rapid industrialization, unplanned urbanisation, excessive use of pesticides and advances technologies has led to increase in the exposure of heavy metals, poly aromatic hydrocarbon (PAHs) to the environment in such a way that its harmful to living organisms and severely affecting the ecosystem. Even though, many remedial methods are under consideration, biological processes to remediate the environment in an eco-friendly way is the need of the hour. Using various living organisms like bacteria, archaebacteria, fungi, algae and plants, scientist are finding newer approaches in to solve the environmental hazards. These approaches have certain limitations like:

- Nature of the pollutant
- Resident microbe present in the contaminated area
- Capability of the microbe to degrade the contaminant
- Bioavailability of the contaminant
- Need to supply biosurfactant
- Concentration and the chemical structure of the pollutant
- The physicochemical properties of the environment

Thus, there is an urgent need for robust methods to transform the pollutants in the eco-friendly way. Few are enlisted below;

Using microbes, the bioavailability of pollutant is increased as microbial glycoconjugates help to lower surface tension. This improves the elimination of contaminants from the environment[4].Recently, the bioremediation of organic contaminants has also utilised microbial biofilms made of polysaccharides, extracellular DNAs, and proteins [45]. They are especially utilized in the removal of stubborn contaminants. Additionally, quorum sensing, environmental parameters, and other technologies are currently being improved to make the technique better.

Another cutting-edge technology called a bio electrochemical system combines the use of biological and electrochemical approaches to manage pollution [46]. This technology aids in the removal of petroleum hydrocarbon pollutants to a large extent, and the effectiveness of this technology is largely dependent on the syntrophic and cooperative relationships between the members of the microbial communities involved[46]

With the advent of genetic engineering and synthetic biology, it is now possible to modify and create new microbes with modified functions for a variety of bioremediation applications[44]. Genetically modified microbes (GEMs) are the ones manipulated to have an altered code through genetic engineering techniques. Numerous examples of bacteria having their code altered for heavy metal resistance by this technique includes *Ralstonia metallidurans*, *Pseudomonas putida*, *Staphylococcus aureus*, and *Bacillus thuringiensis*. The mechanisms in these bacteria includes energy-dependent heavy metal efflux, metal-binding proteins, enzymatic changes (such as oxidation, reduction, methylation, and demethylation), and regulatory proteins[12].

Gene editing**,** which involves changing the genomes in a targeted and precise manner, is a popular technique in contemporary biological research. It is an example of directed genetic improvement, which has enormous potential applications in genetic improvement breeding, genomics research, and the treatment of genetic diseases. Advanced genome editing technologies like zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) endonuclease system, has enabled scientists to alter DNA in a site-specific manner. This can sometimes be used to introduce mutations in organisms to increase their tolerance to pollutants[47]. The science of genome editing is being revolutionized by significant techniques in the CRISPR and CRISPR/Cas systems [48]. The discipline of bioremediation is now proposing new insights into CRISPR toolkits and creating guide RNA (gRNA) for expression of function-specific genes related to remediation in non-model organisms[44].Currently, certain research has a particular interest in the recovery of heavy metals and the reuse of contaminants. By genetically modifying the *E. coli* cells used as the host, a synthetic type VI secretory system (T6SS) cluster and a de novo synthetic heavy metal-capturing gene (encoding a protein called SynHMB) were introduced. This gave the synthetic cells (SynEc2) a high capacity for displaying the heavy metal-capturing SynHMB on the cell surface. Then, the magnetic nanoparticles (MNPs) and synthetic bacterial cells (SBCs) came together to form biotic/abiotic complexes with a self-developing feature that had a high removal effectiveness for heavy metals (HMs) (>90% even at 50 mg/L of Cd (II) and 50 mg/L of Pb (II)) and organic carbon pollutants (>80%)[49].

In the process of phytoremediation by plants, transgenic plants have been created that can accumulate or detoxify arsenic from contaminated soils or streams. PvACR3, an arsenite antiporter from the As-hyperaccumulating plant *Pteris vittate*, was expressed in *A. thaliana* as part of a transgenic approach to arsenic hyperaccumulation. In contrast to the control, the transgenic plants in which PvACR3 was produced through the CAMV promoter demonstrated tolerance to 80 mM and 1200 mM arsenite, which is known to be a deadly dose for wild-type *A. thaliana*. Comparing these genetically altered plants to their wild equivalents, as uptake increased by 7.5 times[50].

Not only the use advanced molecular biology comes to the rescue in bioremediation, but nanotechnology has also been used for bioremediation. For instance, the bioadsorbent quality of nanocellulose, which is functionalized through chemical and structural modification, contributes more to the applications and mechanisms of bioremediation. Because of its exceptional adsorption capacity, improved mechanical strength, hydrophilic qualities, renewable nature, and biodegradability, nanocellulose is chosen as a suitable bio-adsorbent for the removal of pollution. The kind of surface modification that nanocellulose has greatly affects the adsorption process's mode of action between the contaminant and the material. Nanocellulose with a negative charge (-ve charge) makes it easier to adsorb and remove pollutants that have a positive charge, such as heavy metals and dyes. In the event that the contaminant is oil, which has both hydrophobic and oleophilic properties, the nanocellulose is changed by adding hydrophobic groups, which have a lower surface energy and aid in adsorption to the nanocellulose[51].

Another emerging field in the area of bioremediation is the use of synthetic biology. Here, the possibility of changing the chemical make-up of biological molecules is provided by synthetic biology[52]. On the other hand, machine learning has great benefits for dealing with enormous amounts of data. Data-assisted synthetic biology is not used for bioremediation. On the other hand, by using data-aided assisted enzyme engineering, a natural enzyme's bioremediation characteristics or routes could be improved in future[52].

In conclusion, while efforts are being made in bioremediation research to improve the environment in the best natural way, challenges still persist. Comprehensive understanding of microbial metabolism and environmental factors are significant to improve biodegradation efficiency. It is essential to continue research and adopt sustainable, eco-friendly remediation methods and technologies to tackle the existing environmental pollution effectively.

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