



Bioremediation of Agricultural Soil

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DOI: 10.31080/ASAG.2023.07.1308

Received: August 10, 2023

Published: September 20, 2023

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Abstract

With the increase in the food demands of ever-expanding the population of our country, the tired use of fertilizers and pesticides in agricultural soil is quite common, which results in the contamination of cultivable soils. As our world is facing extreme challenges, using microbes for remediation is a pleasant moral alternative to remediate this problem and maintain the fertility and cleanliness of our soil, bioremediation is one of the best approaches where living microorganisms are used for the degradation of contaminants without causing any destructible effect on our ecosystem. The present review aims to describe the process of bioremediation strategies along with the challenges associated with evaluating the removal of contaminants from the soil environment.

Keywords: Bioremediation; Contaminants; Biodegradation; Phytoremediation

Introduction

Earth bestows us with the wealth of natural resources such as forests, wildlife, land, soil, air, water, wind, plants and animals, but now the misuse of them lead to the decline of natural resources to the extent that today half of our natural wealth is either depleted or at the verge of depletion. Over-exploitation of natural resources now has become common practice in current development initiatives. There are many anthropogenic actions that contribute towards the degradation of natural resources like the use of chemical fertilizers in agriculture, the release of industrial waste and burning of fossil fuels [45].

Agriculture is not only the backbone of our economy but the only way to feed the hungry mouths of the boundless expanding population. Increasing population emphasis on input-intensive agricultural practices with high yield appears to be a critical problem, as these require large-scale use of chemical fertilizers, pesticides, and irrigation water. According to the data by ICAR- National Institute of Agricultural Economics and Policy Research in the year 2019-20, there is 0.29 kg/ha of pesticides and 47.3 kg/ha fertilizer consumption all over India. Constraints to bringing in more area under cultivation and deficiency of various macro and micro-

nutrients in soils have forced Indian farmers to use more chemical fertilizers to increase yield [33]. Hence, the application of various chemical fertilizers and pesticides is a demanded need to maximize the yield. But the pesticides in the soil environment, in respect of pest control efficacy, have become a matter of environmental concern because of the hazardous effects of pesticidal chemicals on soil microorganisms that ultimately affect soil fertility. As the use of pesticides becomes more vigorous and continuous, significant quantities of pesticides and their degraded products may accumulate in the soil ecosystem. The potential of pesticides causing harmful effects is so hazardous that it may cause an accumulation of toxic material in the organisms. Moreover, hence influence and control health [24].

Pesticide that disrupts the activities of the soil microorganisms could be expected to affect the nutritional quality of soils and would, therefore, have ecological severe consequence [9]. Thus, the use of pesticides not only degrades the soil quality but also reaches to water table and hence enters the aquatic environment also, so it can be inferred that the fate of pesticides is often uncertain, thus decontamination of pesticide-polluted areas is a very complex process [42]. Positive effects of chemical fertilizers on production and

yield motivated the farmers further towards greater use of fertilizer inputs. The consequence of such excessive use of chemicals beyond the limit of consumption of the plants has been absorption of the same by the soil causing secondary effects on the soil itself, on plant produce and ground water too [32].

The harmful effects of fertilizer are likely to be

- Application of chemical fertilizers in an imbalanced ratio consume an indispensable part of the nutrients in the soil, reducing the number of minerals and vitamins in the food items [11].
- Acidification of soil can take place due to the decrease of organic matter in the soil by excessive use of chemical fertilizers causing threats to the survival of plants [44].
- Carbon dioxide and nitrous oxide, greenhouse gases, can be released into the atmosphere over and repetitive application of nitrogenous fertilizer beyond the crop's assimilation capacity contributing to global warming and erratic climatic conditions [13].
- Waterways and nearby water bodies can be affected by the application of excessive chemical fertilizers from chemical runoff through rainwater. As a result, the amount of oxygen is reduced in the water leading to hypertrophic action of the aquatic system. The living organisms are existing in the water use up oxygen. Such depletion of oxygen can cause the death of the majority of aquatic organisms, including fish [14].

Similarly, if untreated sludge water is directly dumped into the agricultural soil, it will lead to the accumulation of heavy metals, which is quite toxic for the soil environment, as well as soil fertility and productivity.

There are numerous causes of soil pollution that occur every day. Among the most common causes of soil contamination caused by human activity such as overuse of pesticides, the soil will lose its fertility. Also, the presence of excess chemicals will increase the alkalinity and acidity of the soil thus degrading the soil quality.

Moreover, microbes and plants are among the most critical biological agents that remove and degrade waste materials to enable their recycling in the environment. Soil microflora, mainly bacteria, algae, fungi and protozoa, make a valuable contribution to making the soil fertile through their primary catabolic role in the degradation of plants and animal residues in the cycling of the organic, inorganic nutrients content of the soil. Hence, to remediate the above concerned problem, bioremediation is a sustainable

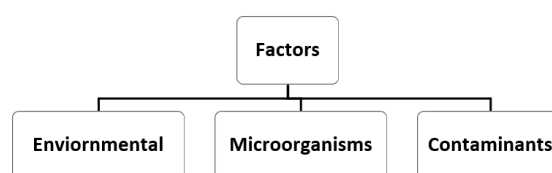
approach for ameliorating contaminated soil, which causes an uncertain effect on the soil and human health. Bioremediation is a biological process for recycling wastes into a form that can be used and reused by organisms. It is the process of cleaning up environmental sites contaminated with chemical pollutants by using living organisms to transform organic compounds into less/non-toxic substances or even essentially remove it from soil or water, or air. It is a cost-effective and eco-friendly technique that can destroy or render harmless contaminants using natural biological activity. It is proved to be an efficient tool for decontaminating polluted sites in the prevailing environment [29].

Principle of bioremediation

In the process of bioremediation, organic wastes are biologically degraded to the level below concentration limits established by the regulatory authorities. Bioremediation is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. It uses naturally occurring bacteria, rotifers or plants to degrade or detoxify substances hazardous to human health and the environment.

Factors affecting bioremediation in soil

Soil is the habitat of various type of microorganisms that receives and produce different kind of chemicals in diverse form and then act as a scavenger of hazardous substances. Factors like soil type, moisture, pH, organic matter, and temperature affect the binding of individual and mixtures of a contaminant in soil. This, in turn, influences the effectiveness of bioremediation strategies [45]. Therefore, these factors are kept under the three categories.



Environmental factors

- **Nutrients availability-** Microorganisms are present in contaminated soil, though they are not in the desired numbers required for bioremediation of the site; therefore, their growth and activity must be stimulated. Biostimulation usually involves the addition of nutrients to help indigenous microorganisms to grow their population. As microorganisms do require these nutrients as the basic building blocks of life and also to secrete the necessary enzymes for the breakdown of the contaminants [45].

- For instance, addition of readily metabolizable carbon source such as glucose maintain the cell viability, increases cell growth and degradation rates. Many xenobiotic compounds can be transformed by co-metabolism, in which the transformation does not serve as the energy source for the synthesis of cell biomass therefore it requires a separate carbon source for the building structure of cell [10]. Also, if we treat contaminated soil with nitrogen, it will increase the cell growth rate and decrease microbial lag phase. By the addition of NPK fertilizers biodegradation of oxyfluorfen (herbicide) in soil show an enhancement in the rate of the process [25]. Therefore, addition of nutrients shows enhanced results for bioremediation.
- **Moisture content:** Microorganisms require adequate water to meet their reproduction and proper life cycle. During the biodegradation process, water is the prime factor for the growth and diffusion of nutrients through the cell wall. There should be a sufficient amount of moisture content for growth. The optimum moisture condition for soil bioremediation is 25-85% of water holding capacity [8]. Low moisture conditions can restrict the movement of bacteria, and excess moisture may fill the smaller pores between particles and limit oxygen transport.
- **pH:** Soil pH shows greater significance because it affects nutrient availability to microorganisms. The growth of micro-

organisms can raise or lower the pH subsequently producing end products that correspondently affect pH or remove the parent organic compounds. The pH measurement in soil could indicate the potential for microbial growth [3]. The microbe species work under a specific pH range, i.e., 6.5-7.0. Experiments revealed the critical role of pH in the adsorption of compounds with acidic functional groups on activated carbon and soil because neutral and ionic forms display very different adsorption properties.

- **Temperature:** The survival of microorganisms and the stability of hydrocarbons are highly dependent on the temperature [11]. It is because the biological enzymes have the optimum range to tolerate the temperature during the degradation process. Hence, they cannot show ample metabolic activity at random temperatures. Generally, the speed of enzymatic reactions in the cell approximately gets doubled for each 10°C rise in temperature. Temperature influences the rate of the bioremediation process as it is highly affecting the microbial physiological properties [28]. The microbial process increases with an increase in temperature and reaches a maximum at optimum temperature after that, the process will fall down and influences the rate of enzymatic reactions within microorganisms.

Parameters	Condition required for microbial activity	Optimum value for degradation
Soil moisture	25-28% of water holding capacity	30-90%
Oxygen content	Aerobic, minimum air-filled pore space of 10%	10-40%
Nutrient content	N and p for microbial growth	C: N:P = 100:10:1
Temperature (°C)	15-45	20-30
Contaminants	Not too toxic	Hydrocarbon 5-10% dry weight of soil
Heavy metals	Total content 2000 ppm	700 ppm
Type of soil	Low clay or silt content	

Table 1: Optimum conditions required for bioremediation by microorganisms.

Microorganisms

Only those microorganisms that are metabolically capable, indigenous or genetically engineered species that can degrade the pollution are applied during the bioremediation process. Microorganisms are involved through their enzymatic pathways, act as biocatalysts and facilitate the progress of biochemical reactions that degrade the desired pollutant¹⁰. For the different kinds of contaminants, the diversified microbial species are used based on their metabolic and enzymatic reactions.

Types of contaminants

Contaminants are the provided surface at which enzymatic reactions take place. They absorb soil particles over which microorganisms start their metabolic process and release the compounds that are essential for the degradation of the contaminants. Also, some amount of carbon from the contaminant is used by microorganisms as their energy source. The contaminants can be the heavy metals accumulated through improper sewage treatment, the hydrocarbons released from the industries, and the toxic compounds of

various herbicides or pesticides. The toxicity, concentration, availability, solubility, and sorption of these contaminants directly influence the rate of absorption of contaminants by microorganisms. The toxic nature of the contaminant can create toxicity to microorganisms and slow down the degradation process. Some organic and inorganic compounds are toxic to targeted life forms [27].

Strategies for bioremediation

Microorganisms must possess the property to degrade the contaminants at a reasonable rate to a particular specific level. During the process of bioremediation, the thing which needs to be prioritized that the toxic by-products must not be produced, inhibitory chemicals must be absent; contaminants must be bioavailable and conditions must be optimized and maintained to support microbial growth and activity [2]. Various kinds of techniques are being underused depending upon the degree of saturation and aeration of an area.

In-situ

- That are applied at the site with minimal disturbance.

Ex-situ

- That are applied after being removed from the site.

In-situ technique for bioremediation

In-situ bioremediation is when the contaminated site is cleaned up exactly where it occurred; thus, it prevents the excavation and transport of contaminants. During this oxygen and nutrients are supplied through some external source of solutions in the contaminated site to stimulate naturally occurring microorganisms to degrade the organic contaminants. These are less expensive and generally the most desirable options due to the slightest disturbance caused on the site of application [36]. One of the essential techniques, i.e., chemotaxis, shows broad importance for the process of bioremediation. This is because the microorganisms with chemotactic abilities can move into an area containing contaminants; thus, the effectiveness of *in-situ* bioremediation is increased by improving microorganisms' chemotactic behaviour [37]. The commonly used *in situ* approaches included microbial remediation and phytoremediation.

Microbial remediation involves

- **Bioventing or biostimulation:** It involves the addition of air and nutrients to the contaminated soil through wells to stimulate the growth of indigenous microorganisms. It is a technique in which oxygen and nutrients are injected into

the soil so as to maintain bioremediation³⁸. Commonly the nutrients like Nitrogen and Phosphorous are added for that will stimulate the growth further³⁵. During the process, air with a low flow rate provides only the necessary amount of oxygen needed for bioremediation while minimizing the volatilization losses. This method includes both aerobic as well as anaerobic techniques. In the aerobic case, by using the supplies of oxygen, microbes utilize contaminants for the carbon source, while in the anaerobic, instead of air, nitrogen is injected, which displaces the soil oxygen, and the reductive dehalogenation is done by anaerobic bacteria. It works for simple hydrocarbons can be used where the contamination is deep under the surface [45].

- **Biosparging:** It is the process that involved injection of the air below the water table to increase the concentration of groundwater oxygen and thus enhance the rate of degradation of contaminants by naturally occurring microorganisms. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The process is not suitable for compounds that may volatilize too quickly. Sparging enhances both aerobic biodegradation and volatilization and is commonly applied to residual hydrocarbon source zone remediation [19].
- **Bioaugmentation:** It is the way to enhance the biodegradative capacity of the contaminated sites by inoculation of microbes with desired catalytic capabilities. The basic principle of this intervention is to enhance genetic diversity leading to a vast repertoire of biodegradative reactions. Bioaugmentation is the remediation process involving wild-type or genetically modified microorganisms for the treatment of sites contaminated with hazardous organic chemicals. Introducing specific microorganisms to decontaminate the soils when indigenous microbes are not efficient is considered an acceptable approach to remediate the contaminated soils. However, the strains for bioaugmentation must have a superior ability to degrade the target contaminants, easy to cultivate, fast growth, tolerance to the high concentration of contaminants and have the ability to survive in a wide range of environmental conditions/stress.

Two factors limit the use of added microbial cultures in a land treatment unit

- Non-indigenous cultures rarely compete well enough with an indigenous population to develop and sustain beneficial population levels.

- Most soils with long-term exposure to biodegradable waste have indigenous microorganisms that effectively degrade if the land treatment unit is well managed [22].

Bioaugmentation has been proven to be successful for a wide range of pollutants including pesticides such as DDT, lindane, endosulfan, pentachlorophenol (PCP), polyaromatic hydrocarbons (PAHs) and total petroleum hydrocarbons [1,17,18].

Phytoremediation

It is using green plants and the associated microorganisms, along with proper soil amendments and agronomic techniques, to either contain, remove or render toxic environmental contaminants harmless. It is an inexpensive, environmentally friendly, and effective method that can be applied *in situ* [40]. Here the plant furnishes a favourable microenvironment around the roots that facilitate the degradation of the contaminants. Both rhizospheric bacteria and endophytic bacteria are involved in the degradation of toxic contaminants in the soil environment.

Phytoremediation involves following practices

- **Phytoextraction:** Phytoextraction or phytoaccumulation is the process of using plants or algae to remove contaminants from soils, sediments, or water into harvestable plant biomass. The plants hold up the contaminants through the root system and store them in the root biomass or transport them up into the stems or leaves. After harvest, a lower level of the contaminant will remain in the soil, so the growth and harvest cycle must usually be repeated through several crops to achieve sustainable remediation [43]. It represents a green and environment-friendly tool for cleaning metal-polluted soil and water at a low cost as compared to conventional chemical and physical remediation technologies that are generally too costly and often harmful to soil characteristics (i.e., texture and organic matter), phytoremediation is rapidly ensuring the availability of metals by releasing compounds that can desorb metals from the soil matrix to form water-soluble metal complexes into the soil solution for plant uptake [7].
- **Rhizofiltration:** During this phenomenon, the contaminants are either adsorbed onto the root surface or absorbed by the plant roots. Plants used for hemofiltration are not planted directly *in situ* but are acclimated to the pollutant first. Plants are hydroponically grown in clean water rather than soil until an extensive root system has developed. Once a large root system is in place, the water supply is substituted for a polluted one to acclimate the plant. After the plants become acclimatized, they are planted in the polluted area where the roots uptake the polluted water and the contaminants along with it. As the roots become saturated, they are harvested and disposed of safely [8]. An experiment showed that the continuous clean-up system's heavy radioactive metal removal efficiency for hemofiltration using sunflower was over 99%, and the radioactive removal capability of sunflower roots used in the clean-up system exceeded 500 mg/kg of plant [20].
- **Phytodegradation:** This is the degradation of contaminants into smaller units by the plant enzymes or through the release of exudates that help to degrade pollutants via co-metabolism in the rhizosphere. Phytodegradation is likely most important in arid and semi-arid regions with open canopies, large amounts of standing dead material, and high radiative loads where microbial activity is inhibited by water limitation and possibly by exposure to high UV irradiance [39]. An experiment shows that phytodegradation of the water-soluble components of the soil is a source of biologically available substrates for bacteria isolated from the same soil⁶. These bacteria release some enzymes which help in the degradation of contaminants. Some of the enzymes produced by plants capable of degrading pesticides were identified [19]. These were aromatic dehalogenase for DDT, nitrilase for herbicides, O-demethylase for alachlor, as well as metal anchor and phosphatase for organophosphates, respectively.
- **Enhanced rhizospheric biodegradation:** In this method, naturally occurring rhizospheric microbes or selected microbes which have been isolated by enrichment method are used for the degradation of selected pollutants. Actually, the rhizosphere has excellent potential for remediation [12]. The reason is that the microbial population stimulate due to root exudates and the availability of improved soil moisture, oxygen, and nutrient conditions. Biodegradation is a "microbially driven chemical transformation of organic compounds," whereas microbial uptake is the direct removal of contaminants by adsorbing the compounds onto the membrane surface or absorbing compounds through the cell membrane [16]. Rhizospheric microbes play an essential role in the recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests³⁴. On the other hand, plant root exudates provide nutrition to rhizospheric microbes, thus increasing microbiological activity in the rhizosphere, which in turn stimulates plant growth and reduces metal toxicity in plants.
- **Phytovolatilization:** In this volatilization of contaminants from the plant, either from the leaf stomata or from plant

stems befalls [31] and the radial diffusion occurs through the stem tissues. Sometimes, breakdown products derived from the photodegradation or phytodegradation of the parent contaminant may also be phytovolatilized. Phytovolatilization is most applicable to those contaminants having high volatility, such as ethylene dibromide (EDB), trichloroethylene (TCE), methyl-tert-butyl ether (MTBE), and carbon tetrachloride (CTC). When water travels through the plant system i.e., in the xylem through transportation from roots to stem, some modification in the contaminants occurs along the way. Generally, the concentration of volatile compounds in the xylem decreases with increasing distance from the roots [23]. Once released into the atmosphere, compounds with double bonds, such as TCE, could be rapidly oxidized in the atmosphere by hydroxyl radicals. This technology can only be applied when we quantitatively study the transport and conversion processes taking into account the interaction of microorganisms in the soil and the plant. The analysis of the kinetics of phytovolatilization seems to be a viable means for studying the complex transport processes in the undisturbed plant-soil system [21].

Ex-situ technique for bioremediation

In this technique, there is the complete removal of contaminated material from one site and its transfer to another site, where it has been treated using biological agents. These methods are faster than the in-situ method. They are applicable to a broader range of contaminants and are more expensive. This technique involves two approaches, i.e., slurry phase treatment and solid phase treatment. The slurry treatment includes the bioreactors, while the solid phase treatment includes land farming, composting, and soil piles.

Slurry phase treatment

i. Bioreactors- In this technology, the polluted soil is excavated and transported to unique facilitates, where it is mixed with water in bioreactors. Oxygen and nutrients are added later, so the mixture is thoroughly mixed to form a thin slur. Temperature, oxygen, and nutrient concentration are controlled so that the organism may have the best condition to sustain its bioactivities leading to the degradation of the pollutants. The bioreactor may have been established on-site or elsewhere in a dedicated treatment area. The bioreactors are usually of the horizontal-drum and airlift type and may be batch or continuous, but are usually batch-mode. An acclimatized microbial population from a previously treated soil batch is usu-

ally introduced to each new batch to enhance the degradation rate. After treatment, the material is passed through a water-separation system, and the water was recycled. Contaminated groundwater/effluent may also be treated in bioreactors that are either the fixed-film or the stirred-tank type [46].

Solid phase treatments

- **Land farming:** Land farming is a technique in which contaminated soil is excavated and spread over a prepared bed. The polluted soil is then placed in layers over clean soil and allowing natural processes to detoxify, degrade and immobilize contaminants [41]. Oxygen is added by mixing via ploughing, harrowing or milling, along with nutrients and moisture are also added to favour the process of bioremediation. The main aim is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. It is a full-scale bioremediation technology in which the size and location of the spreading operation are decided based on the application rate of pesticides to avoid concentrations that would be unsafe in soil, groundwater, or crops [30]. Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative.
- **Composting:** It is a process by which microorganisms degrade organic wastes into smaller molecules at very high temperatures, usually in the range of 55° to 65°C. During this process, the heat released increases the temperature, consequently increasing contaminant solubility and higher metabolic activity in composts. The substrate level in composts is also high, which can lead to the co-metabolism of organic contaminants. The microbiological population can be more numerous and diverse in composts than in soils. While through the process, firstly, the contaminated soils are excavated and screened to remove large rocks and debris [5]. The soil is then transported to a composting pad with a temporary structure where the contaminant is added and thus also gets protection from extreme weather. The amendments (straw, alfalfa, manure, agricultural wastes, and wood chips) are used for bulking agents and as a supplemental carbon source. Soil and amendments are layered into long piles known as windrows. The windrow is thoroughly mixed by turning with a commercially available windrow turning machine. After the composting period, the windrows are disassembled, and the compost is finally disposed of. An experiment performed⁴ in the soil contaminated by the organic hydrocarbons released from the sewage sludge and the industries, the results show that under controlled con-

ditions, inoculation of compost could effectively accelerate the removal of such contaminants from the soil matrix.

- **Soil biopiles:** They are a hybrid of landfarming and composting. Biopiles provide a favourable environment for indigenous aerobic and anaerobic microorganisms. The basic pile system includes a treatment bed with a mound of contaminated soil, an aeration system, an irrigation/nutrient system and a leachate collection system. Moisture, heat, nutrients, oxygen, and pH are the factors that control and enhance the rate of biodegradation. The irrigation and nutrient system is buried under the soil to pass air and nutrients through the vacuum or by positive pressure. Soil piles can be up to 20 feet high and may be covered with plastic to control runoff, evaporation, and volatilization and to promote solar heating [8]. Suppose volatile organic compounds in the soil volatilize into the air stream. In that case, the air leaving the soil may be treated to remove or destroy the volatile organic compounds before these are discharged into the atmosphere.

Limitations of Bioremediation

Bioremediation is only limited to those compounds which are biodegradable in nature and though all compounds are not showing this nature therefore it is suitable for all the contaminants in the soil. It is considered to be problematic situation since the compound volatile sometimes show the very toxic effect. Also, in some cases the product of biodegradation is more persistent and toxic in nature than the parent compound. Biological processes are often highly specific. Important site factors required include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants. Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment.

Conclusion

Bioremediation is the sustainable method for the removal of contaminants may be naturally occurring compounds from the soil environment or occurring in unnaturally high concentrations. It is a promising technology that utilizes the ability of plants and microorganisms to remove harmful contaminants from the soil environment in an eco-friendly way. Various sources of bioremediation, such as bacteria, archaeobacteria, yeasts, algae, plants and rotifers are available. However, every biological forms have different growth requirements, so we need to isolate those which can be cultured easily in the lab, with minimal requirement and helpful in treating a variety of pollutants. Highly contaminated soils using chemical oxidants in safe concentrations to soil biota, followed

by bioremediation, appear as promising technology for intractable pollutants. It is low input and efficient technology to convert organic wastes into value-added products for sustainable land practice. Therefore, from the future perspective, bioremediation is one of the pleasing approaches to maintaining the rhythm of our ecosystem.

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