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Chapter 1

Microbial Inoculants-Assisted Phytoremediation for Sustainable Soil Management

Elizabeth Temitope Alori and Oluyemisi Bolajoko Fawole

Abstract Agricultural soil pollution refers to its accumulation of heavy metals and related compounds which could be from natural or anthropogenic sources. This threatens food quality, food security, and environmental health. The traditional physico-chemical technologies soil washing used for soil remediation render the land useless as a medium for plant growth, as they remove all biological activities. Others are labor-intensive and have high maintenance cost. Phytoremediation, sustainable and cheaper in situ remediation techniques was therefore considered. However, plants do not have the capability to degrade many soil pollutants especially the organic pollutant. It is therefore imperative to take advantage of the degrading ability of soil microorganisms. This chapter therefore focuses on phytoremediation techniques augmented by microbial inoculants.

Keywords Inoculants • Microbes • Phytodegradation • Phytoremediation • Soil pollution • Soil management • Sustainable

1.1 Introduction

Pollution of agricultural soils refers to its accumulation of heavy metals and related compounds which could be from natural or anthropogenic sources. This threatens food quality, food security, and environmental health [1]. Soil pollution produces change in the diversity and abundance of biological soil populations [2]. This is critical because of the role of soil organisms in plant establishment and survival. Such elimination of soil organisms can lead to problems with plant establishment and survival. Crops raised on polluted soil may contain harmful levels of pollutants that can be passed on to the animals and human that eat them [3]. Inhaling dust

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blown from polluted soil can be injurious to one that inhales it. More also, polluted soil cannot be used for commercial development, parks or recreation [4]. Soil pollutants alter plant physiology. It can cause cell membrane disruption, damage to photosynthetic apparatus, and can also alter the physical and chemical properties of the soil where plants are growing [5].

Cleaning of polluted soil may be very difficult because both soil pollutants and soil minerals carry small electric charges that cause each to bond with each other. It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be immobilized [6]. Traditionally, remediation of heavy metal-contaminated soils is either on-site management or excavation, and subsequent disposal to a landfill site [7]. However, this method of disposal merely shifts the contamination problem elsewhere. Soil washing for removing contaminated soil is an alternative to excavation and disposal to landfill. This method is however costly and produces a residue rich in heavy metals, which will require further treatment or burial. Moreover, these physico-chemical technologies used for soil remediation render the land useless as a medium for plant growth, as they remove all biological activities. Other technologies such as vitrification, leaching, electrokinetics soil vapor extraction, thermal desorption, chemical processing, etc., are labor-intensive and have high maintenance cost [8, 9]. It is therefore imperative to develop a sustainable on-site technique for remediation of heavy metal contaminated sites.

For better soil management, an increase in use of biological potential is important. Phytoremediation is one of the sustainable and cheaper in situ remediation techniques to be considered. Phytoremediation is a novel green technology that uses specialized plants and associated soil microbes to remove, destroy, sequester, or reduce the concentrations or toxic effects of contaminant in polluted soil and water [4]. The plant root-colonizing microbes or the plants themselves absorb, accumulate,translocate, sequester, and detoxify toxic compounds to non-toxic metabolites.

Five important approaches can be considered in the use of plants to clean up polluted soil. (1) Phytostabilization, a process in which pollutants are immobilized by plant activity resulting in attenuation of the wind and soil erosion and runoff processes into the ground water or air. (2) Hydraulic control, plants act like a pump, draws the groundwater up through their roots to

keep it from moving. This reduces the movement of contaminated groundwater toward clean areas off-site. (3) Phytovolatization involves use of plants to take up certain contaminants and then converts them into gaseous forms that vaporize into the atmosphere. (4) Phytofiltration refers torhizofiltration where contaminants such as metals are precipitated within the rhizosphere. (5) Phytoextraction (Phytoaccumulation) which involves metal hyperaccumulating plants which can contain more than 1% of metals in harvestable tissues [10, 11] (Fig. 1.1).

However, plants do not have the capability to degrade many soil pollutants. It is therefore imperative to take advantage of the degrading ability of soil organisms. Organic toxins containing carbon such as the hydrocarbons found in gasoline and other fuels can only be broken down by microbial processes [12]. Symbiotic root

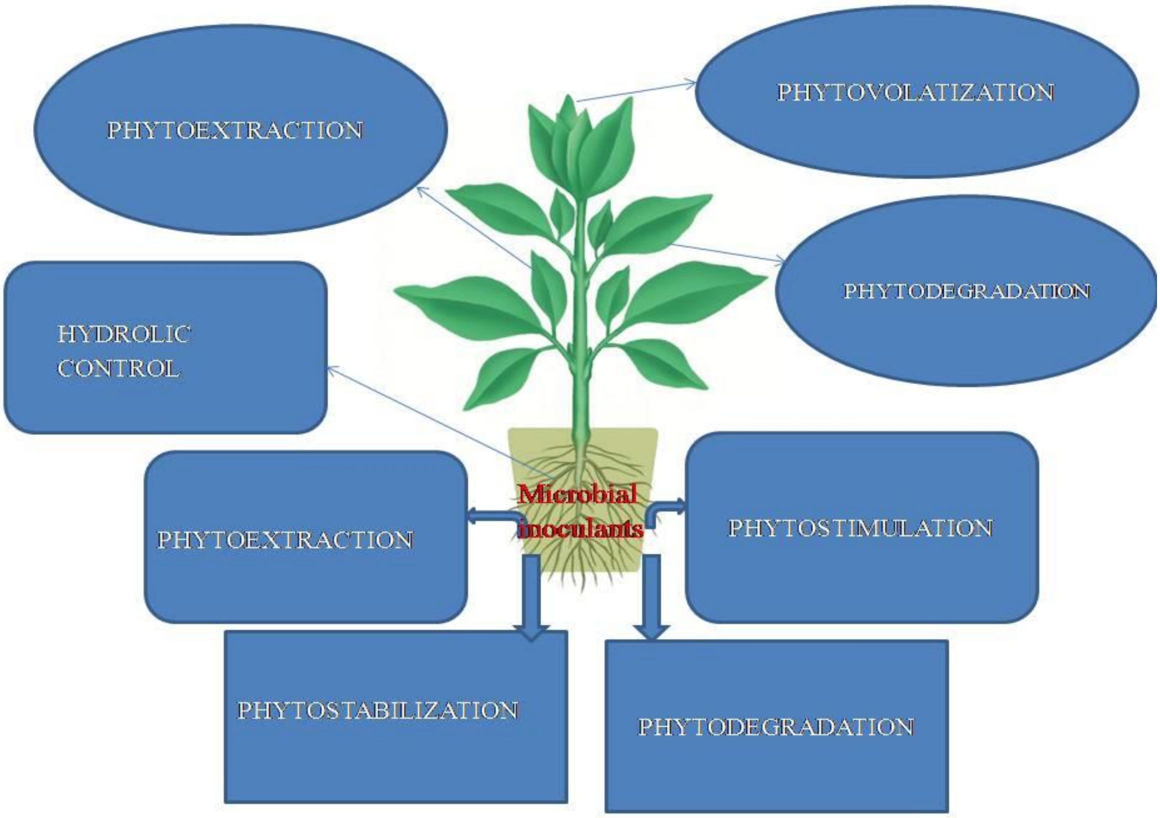

Fig. 1.1 Mechanisms of microbial-assisted phytoremediation

colonizing microorganism through metal sequestration increases metal tolerance in plants. The remediation by plant using the degrading ability of soil organisms is called phytodegradation. This helps us to understand integrated activity patterns between plants and microbes [13]. Some soil microbes such as the arbuscular mycorrhizal fungi (AMF) secret glycoprotein called glomalin. This can form complexes with metals. Microbial organisms within the rhizoplane can take part in phytoremediation by protecting the plants from the toxic effect of the contaminants while the plants in return provide the microbial processes the boost they need to remove organic pollution from the soil more quickly. Plants excrete organic materials that serve as food for microbes thus playing a key role in determining the size and health of soil microbial population. Bioaugmentation enables an increase of biodegradation of contaminated sites by the introduction of single strains or assemblages of microorganisms with the desired catalytic capabilities [14]. Microbial assemblages are found to be efficient since each partner can accomplish different parts of the catabolic degradation [15]. In this chapter, our focus is mainly on phytoremediation augmented by microbial inoculants. We begin with the contribution of plants and microbial inoculants in phytoremediation process. Then the methods of inoculating plants with microbial inoculants, the various mechanisms used by the microbial inoculants to assist plant in remediation, and the limitations of microbial inoculants-assisted phytoremediation are summarized and discussed.

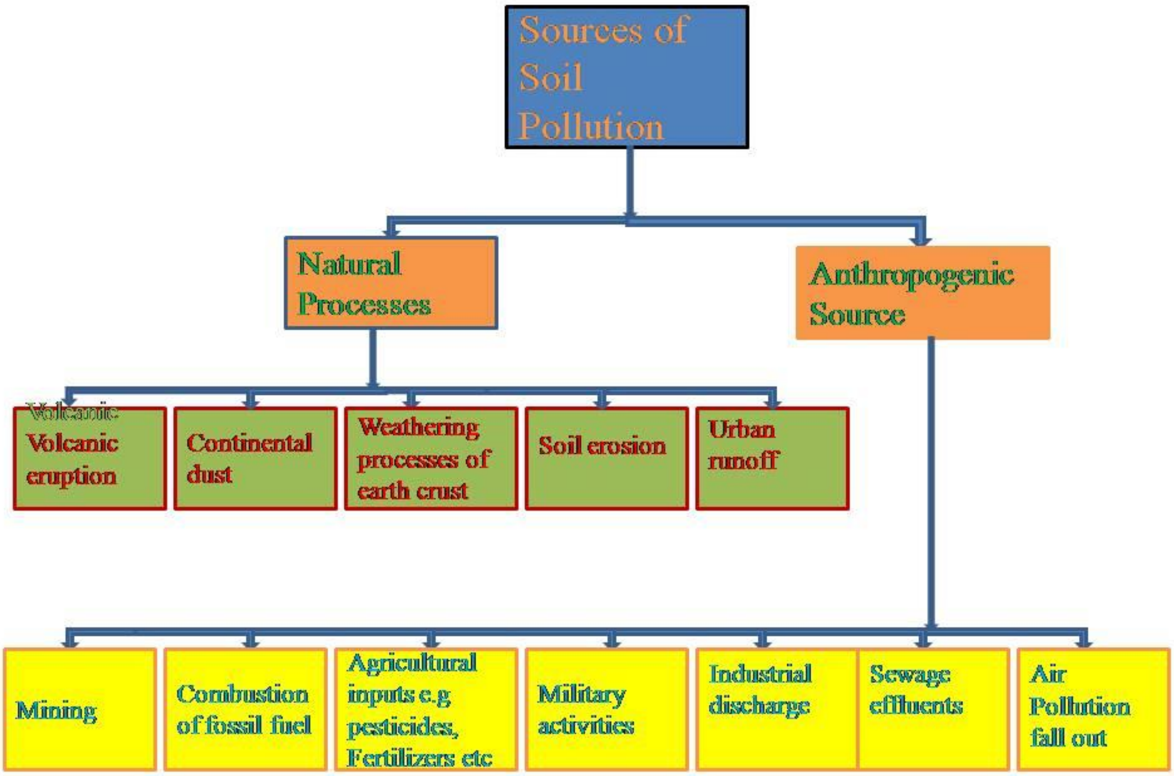

Fig. 1.2 Sources of Soil Pollutants

1.2 Sources of Soil Pollution

Soil pollutants get introduced to the soil from various sources ranging from natural (Lithogenic) to anthropogenic activities (Fig. 1.2). Heavy metals commonly get introduced via human activities that are related to energy and mineral consumption [5], while petroleum hydrocarbons usually come from accidental spills of petroleum-based products commonly used. Various industrial processes and anthropogenic activity es in urban areas induce the release of metals and metalloids (MM) (toxic and genotoxic compounds) in natural environments. Agricultural inputs such as chemical fertilizers, herbicides, and pesticides leaves the soil polluted with heavy metals [16]. According to Pietrzak and Uren [17], excessive use of fungicides and herbicides that are rich in heavy metal results in soil pollution. Copper for instance is used as a broad-spectrum bacterial and fungicidal agricultural pesticide and as fertilizer component because of its antimicrobial properties, but Cu is a common soil pollutant that persists in the soil providing a chronic, long-term stress on the soil microbial community [18]. Industrial activities such as chemical works, service stations, metal fabrication shops, paper mills, tanneries, textile plants, waste disposal sites, and intensive agriculture equally brings about the appearance of serious environmental problems such as soil pollution [19]. Indiscriminate waste disposal practices have led to significant build upon a wide range of metal(loid)s, such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), selenium (Se), and zinc (Zn) in soils [20]. Kierczak
et al. [21] found that soils in the areas around historic smelters are highly polluted

with metal(loids)s (up to 4000 mg/kg Cu, 1500 mg/kg Zn, 300 mg/kg As, and 200 mg/kg Pb). Fossil fuel combustion is another source of soil pollution reported by Krgović et al. [22]. Vehicle emissions, industrial processes, or waste incineration plants were revealed to introduce some pollutant such as heavy to what should have been valuable soil [23]. Soil pollutants could originate from the mining and smelting of metal ores [24], runoff of urban soils, fertilizer application, or effluents discharged [25].

1.3 Contributions of Plants and Microbial Inoculants in Phytoremediation

Microbial-assisted phytoextraction optimizes the synergistic effect of plants and microorganisms and has been used for the cleaning-up of soils contaminated by metals [2]. Plant translocates and sequesters pollutions such as heavy metals while microbes degrade organic contaminants. Plants can store many contaminants in biomass that

can later be harvested, while microbial assemblages can also convert contaminants such as heavy metals to stable and/or less toxic form. They can facilitate the uptake of pollutants such as heavy metals by plant roots. Microorganisms that reside on or within aerial plants tissue can help to stabilize and/or transform contaminants that have been translated which may limit the extent of volatization [13]. Plant root exudates such as enzymes, amino acids, aromatics, simple sugars, and aliphatics stimulate the growth of root-associated microorganisms; on the other hand, microbes can reduce the phytotoxicity of the contaminants in the soil or augments the capacity of the plant to degrade contaminant [3]. Ability of plant root to extend deeper into soil, allowing access to water and air and therefore changing the concentration of carbon dioxide, the pH, osmotic potential, redox potential, oxygen concentration, and moisture content of the soil, could lead to an environment that will better able to support high micro-biomass [26]. This enhanced trace element uptake by plants can be ascribed to an increase in root absorption ability and/or to an enhancement of trace metal bioavailability in the rhizosphere, mediated by microorganisms.

Plants can increase biodegradation through the transfer of oxygen to the rhizosphere and the release of soluble exudates that provide nutrient sources for micro-organisms [27]. Thus, plants enhance microbial growth and hence the associated contaminant-degradation processes. Microorganism contribution in immobilizing elements or facilitating plant absorption plants may significantly contribute to removal through uptake in biomass [28]. Microbial assemblages improve plant health and growth, suppress disease-causing microbes, and increase nutrient availabilityand assimilation [29].

Methods of Inoculating Plants with Microbial Inoculants

Plants to be used as phytoremediator to clean polluted soils could be inoculated with microbial assemblages via quite a number of techniques. These methods could include: (1) Seedinoculation, (2) Soaking plant roots with microbial suspension, when the root of ryegrass was soaked with a suspension of an endophytic *Massilia* sp. (Pn2) the same was found to have been translocated to the plant shoots [30]. (3) Painting plant leaves with microbial suspension [31–33]. Afzal et al. [34] discovered the cells of *Burkholderiaphytofirmans*PsJN in the internal tissue of the shoot and root when the plant was inoculated via leaf painting. Root colonization strategy was found to be the optimal colonization method for circumventing the risk of plant organic contamination [32].

1.5 Types of Soil Pollutants

Soil pollutant could be organic or inorganic present in the hydrosoluble fraction (complexed, adsorbed onto particles or dissolved). The most common inorganic contaminants are heavy metals and mineral oils such as Cd, Cr, Pb, Cu, Hg, NiSe, As, and Zn [35]. Industrial effluents release organic pollutants like hydrocarbons, polycyclic aromatic hydrocarbons, and anionic detergent. Other soil pollutants include plant organic materials, petroleum hydrocarbons, and organochlorines [36]. Table 1.1 reveals some examples of soil pollutants that could be removed from soil via a microbial-assisted phytoremediation technique.

1.6 Mechanisms of Microbial Inoculants in Phytoremediation of Polluted Soil

Microbial inoculants can improve pollutant removal through various mechanisms. Some has the potential to produce metal chelating siderophores, which could improve metal bioavailability [37]. Moreover, they produce biosurfactants (rhamnolipids) that can enhance the solubility of poor water-soluble organic compounds and the mobility of heavy metals [38]. Formation of biofilm is another mechanism by which microbial inoculants assist plants in remediation of polluted soils [39]. In addition, these microbes can transform metals into bioavailable and soluble forms through the action of organic acids, biomethylation, and redox processes [39].

Diverse soil microbes have the ability to secrete plant hormones such as indole-3-acetic acid (IAA), cytokinins, gibberellins (GAs), and certain volatiles which promote plant growth by altering root architecture [16]. The microbial plant growth stimulatory actions result from the manipulation of the complex and balanced network of plant hormones that directly are responsible for growth and root formation. For example, IAA produced by soil microbes has been demonstrated to enhance

Table 1.1 Some examples of soil pollutants that could be removed from soil via microbial-assisted phytoremediation technique

Plant	Microorganism	Pollutants	References
Helianthus annus	*Micrococcus* sp. MU1and *Klebsiella* sp. BAM1	Cd	Prapagdee et al. [50]
Polygonumpubescens	*Enterobacter* sp. JYX7and *Klebsiella*sp. JYX10	Cd	Jing et al. [51]
Zea mays L	*Azotobactorchroococum and Rhizobium leguminosarum*	Pb	Hadi and Bano [52]
Solanum melongena	*Pseudomonas* sp.	NaCl	Fu et al. [53]
Vignaunguiculata	*Scutelospore reticulate, Glomus phaseous*	Al, Mn	Alori and Fawole [2]
Solanum nigrum	*Pseudomonas* sp. LK9	Cd	Chen et al. [54]
Brassica napus	*Pantoeaagglomerans*Jp3-3, and *Pseudomonas thivervalensis* Y1-3-9	Cu	Zhang et al. [55]
Brassica juncea	*Paenibacillusmacerans NBRFT5, Bacillus endophyticus NBRFT4, B. pumilus NBRFT9*	Cu	Tiwari et al. [56]
Loliummultiflorum Lam	*Staphylococcus* sp. strain BJ06	Pyrene	Sun et al. [57]
Brassica oxyrrhina	*Pseudomonas sp. SRI2, Psychrobacter sp.* SRS8and *Bacillus sp. SN9*	Ni	Ma et al. [58]
Brassica napus	*Acinetobacter sp.* Q2BJ2 and *Bacillus* sp. Q2BG1	Pb	Zhang et al. [55]
Cytisusstriatus	*Rhodococcuserythropolis* ET54b *Sphingomonas* Sp. D4	hexachlorocyclohexane (HCH)-	Becerra-Castro et al. [59]
Cichoriumintybus	*Rhizophagusirregularis*	Diesel	Driai et al. [60]
Medicago sativa	*Pseudomonas aeruginosa*	(Cu, Pb and Zn and petroleumhydrocarbons	Agnello et al. [35]

Orychophragmus violaceus	*Bacillus subtilis, B. cereus, B. megaterium,* and *Pseudomonas aeruginosa*	Cd	Liang et al. [61]
Cytisusstriatus(Hill) Rothm	*Rhodococcuserythropolis*E T 54b and *Sphingomonas*sp D4		Becerra-Castro et al. [62]
Arabidopsis thaliana	*Achromobacterxylosoxidans*	phenolic	Ho et al. [63]
Solanum lycopersicum	*Penicilliumjanthinellum* LK5	Al	Khan et al. [64]
Brassica napus	*Rahnella* sp. JN6	Cd	He et al. [65]
Triticumaestivum	*Pseudomonas putida KT2440*	Cd, Hg, Ag	Yong et al. [66]
Brassica juncea	*Bacillus subtilis SJ-101*	Ni	Zaidi et al. [67]
Sedum plumbizincicola	*Bacillus pumilus* E2S2 and *Bacillus* sp. E1S2	Cd	Ma et al. [68]
Brassica napus	*Pseudomonas fluorescens G10and Microbacterium sp. G16*	Pb	Sheng et al. [69]
Trifoliumrepens	Arbuscular mycorrhizal fungi and *Bacillus cereus*	Heavy metals	Azcón et al. [70]
Iris pseudacorus	Arbuscular mycorrhiza fungi	Pb, Fe, Zn, and Cd	Wężowicz et al. [71]
Brassica juncea	*Rhizobium leguminozarum*	Zn	Adediran et al. [72]
Rahnella sp.	*Amaranthushypochondriacus, A.Mangostanus and S. nigrum*	Cd	Yuan et al. [73]
Brassica juncea	*Staphylococcus arlettae* NBRIEAG-6	As	Srivastava et al. [74]
Orycoprhagmus violaceus	*Bacilus subtilis, B. cereus, Flavobacterium sp.* and *Pseudo(Zhang et al. [55])monas aeroginosa*	Zn	He et al. [75]
Lupinusluteus	*Burkholderiacepacia*VM1468	Ni and trichloroethylene (TCE)	Weyens et al. [76]
Alnus firma	*Bacillus thuringiensis* GDB-1	As	Babu et al. [77]

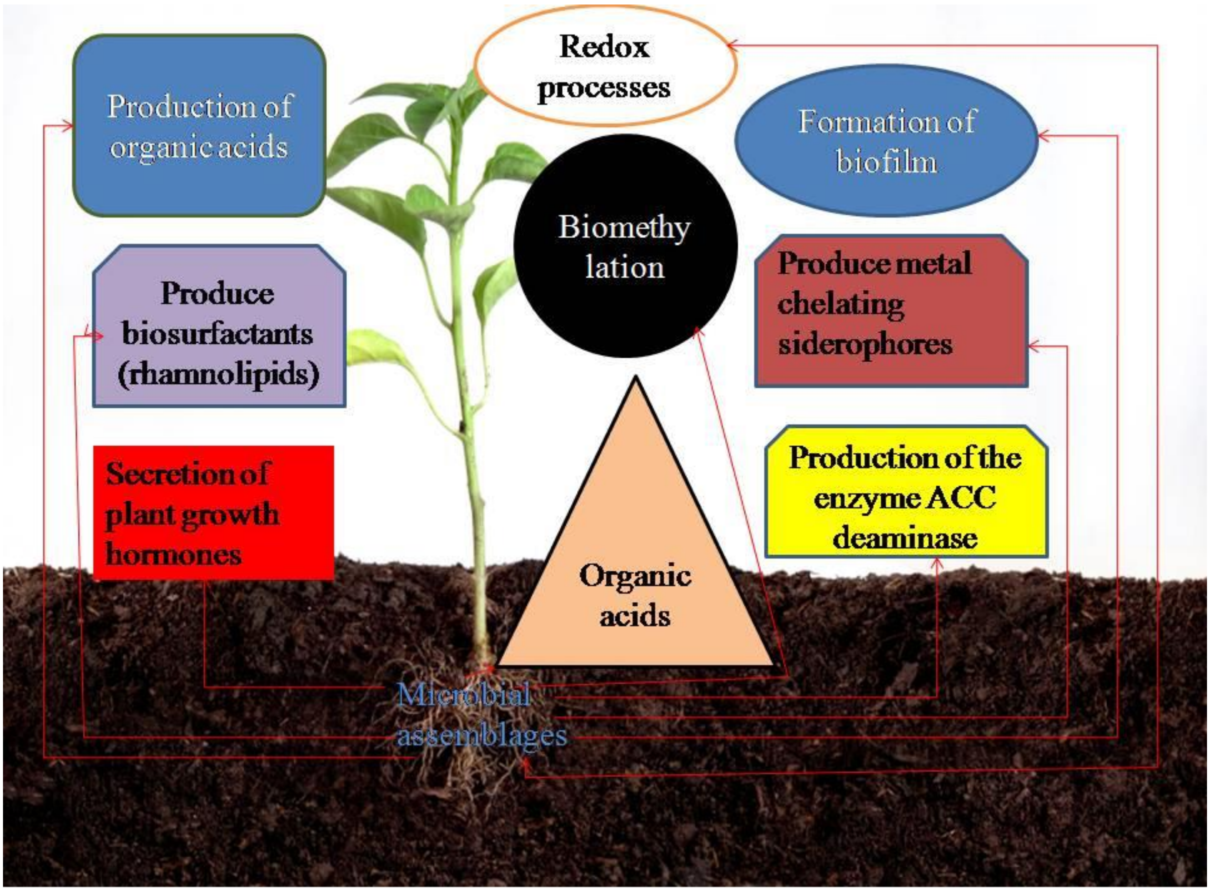

Fig. 1.3 Strategies of phytoremediation through microbial assemblages

root proliferation [40]. In addition, soil microbes possess growth- promoting traits, including phosphorus solubilization, nitrogen fixation, iron sequestration, and phytohormone, which improve plant growth and increase plant biomass [39].In addition to degrading soil pollutants microbial assemblages, also partake in phytoremediation by producing hormones, fixing atmospheric nitrogen, or solubilizing P [41]. One of the most important mechanisms by which microbial assemblages respond to stress condition such as from soil pollutant is by increasing ethylene levels that result to an increase in cell and plant damage [42]. Many microbes that augment phytoremediation destroy a precursor of the ethylene (1-aminocyclopropane-1-carboxylate (ACC)) that by producing the enzyme ACC deaminase, that in turn facilitates plant growth and development by decreasing plant ethylene levels [39]. Figure 1.3 depicts strategies of phytoremediation through microbial assemblages.

1.7 Challenges of Microbial Inoculants-Assisted Phytoremediation

The success of microbial inoculation-assisted phytoremediation encounters some set back due to the following reasons: (1) The number of degrading microbes available regarding the pollutant to be degraded may be low or non-detectable, (2).

The physical and chemical properties of pollutants. The various types of soil pollutants vary in their mobility, solubility, degradability, and bioavailability. These properties play very important role in the removal of the pollutants from the soil. Pollutant or mixtures of pollutants sometimes require several metabolic pathways operates simultaneously with sometimes metabolic intermediates whose toxicity toward indigenous microbes may be high, and (3) Some polluted areas requiring long microbial adaptation period of time justifying soil bioaugmentation [14, 43]. Other abiotic factors that also affect the success of microbial inoculation-assisted phytoremediation include; temperature, aeration, soil pH, cation exchange capacity (CEC), soil organic matter content, sorptive capacity of soil, and redox potential. According to Diels and Lookman [44], microbial inoculation-assisted phytoremediation is influenced by temperature in the range 5–30 °C. It therefore means that the success of microbial inoculation-assisted phytoremediation will depend largely on season as this will be ineffective during winter in temperate countries. Grundmann et al. [45] reported that the efficiency of microbial inoculation-assisted phytoremediation depends on pH in the range 5–8. Many metal cations like Cd, Cu, Hg, Pb, and Zn are reported to be more soluble and available in the soil solution at low pH (below 5.5) [46]. However, Phytoremediation of atrazine by two microbial consortia was seriously affected by pH and soil organic matter content. At pH 6.1 only one consortium degraded atrazine but at pH >7 atrazine was effectively degraded by the consortia, the microbial inoculants were ineffective at pH 5.7 because of their interaction with organic matter [47]. pH for the degradation of phenol and TCE was observed to vary from 6.7 to 10 depending on whether the microbial inoculant cells are free or immobilized [48]. As revealed by Bhargava et al. [46] higher CEC of soil permits greater sorption and immobilization of the metals. Depending on contaminant characteristics, different microbial-assisted phytoremediation mechanisms require different final electron acceptors. For example because of the highly reduced state of petroleum hydrocarbons, the preferred and most thermodynamically relevant terminal electron acceptor for microbial process is O2 while the degradation of chlorinated solvents, depending on the degree of halogenation, is different from that of petroleum hydrocarbons and other oxidized chemicals, and the preferred redox condition is anaerobiosis [44].

1.8 Characteristics to Consider in the Choice of a Plant for Microbial-Assisted Phytoremediation

A key aspect in biological remediation methods is the selection of appropriate plant–bacteria partnerships for the remediation of polluted soils [3]. Some of plant properties to be considered include: exceptional contaminant tolerance, ability to quickly grow on degraded land, and rapid biomass production. For instance alfalfa (*Medicago sativa* L.) that is often used in phytoremediation of contaminated soil is a fast growing species. Another critical characteristic to be considered is the

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composition of plant-recruited microbial communities. Plants that develop extensive tap root system favor the establishment of rhizosphere microorganisms. Plants ideal for phytoremediation should possess the ability to grow outside their area of collection, to produce high biomass, easy harvesting and accumulation of a range of heavy metals in their harvestable parts [49]. Poplar and willow possess deep root systems, produce great biomass, can be grown in a wide range of climatic conditions and these explain why they are effective phytoremediator of polluted soil [46].

1.9 Conclusions

Soil pollutant could be organic or inorganic present in the hydrosoluble fraction adsorbed onto particles or dissolved. Microbial-assisted phytoremediation remove, destroy, sequester, or reduce the concentrations or toxic effects of contaminant in polluted soils. Production of siderophores, biosurfactants, formation of biofilms,

organic acids production, biomethylation, and redox processes and plant growth hormones stimulation are mechanisms employed by microbial inoculants in phytoremediation. The number of available degrading microbes and the physical and chemical properties of pollutants determine the success of microbial inoculants-assisted phytoremediation. Exceptional contaminant tolerance, ability to quickly grow on degraded land, ability to grow outside their area of collection, and rapid biomass production are important plant characteristics to be considered in the choice of plant for phytoremediation.

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