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Chapter 11

Plant Growth Promoting Rhizobacteria's (PGPRS) Enzyme Dynamics in Soil Remediation

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65267

Abstract

Soil is the basis of agriculture and consists of organic matters, minerals, water, and several gasses. All plants require soil both as an anchor to attach and as water and nutrient source. Unfortunately, lifestyles of humans, industrial progress, chemicals used in agriculture contaminate soil and cause soil pollution. A pollutant may be natural or human-made in origin such as petroleum hydrocarbons, pesticides, heavy metals, and solvents. Since the quality of the soil affects the growth and product yield of plants, soil pollution is a crucial problem needs to be addressed urgently. Plant growth promoting rhizobacteria (PGPR) are microorganisms living in soil, on the plants roots, or inside the plant. PGPRs synthesize chemicals to stimulate plant growth and promote nutrient uptake, help degrading soil pollutants and fending off pathogens. While some pollutants can be degraded by enzymes produced by bacteria and fungi, degradation of heavy metals requires alternative methods. In this chapter, three enzymes produced by PGPRs are reviewed briefly. Aminocyclopropane-1-carboxylate (ACC) deaminase is responsible of lowering the ethylene levels of plants during stress conditions, whereas nitrogenase is responsible for N2 reduction to NH3. Moreover, phytase enables the degradation of phytate which is a main storage form of phosphate in plants.

Keywords: PGPR, enzyme, soil remediation, plant growth, organic farming

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1. Introduction

Soil, typically formed from decomposed rock and organic matter, is a mixture of minerals, several gases and liquids, and many organisms that supports life on Earth. Soil is the basis of agriculture on which all crops for human food and animal feed depend. Its properties vary from one place to another, due to bedrock composition, climate, and other factors. Soil and its properties are typically affected by several factors including current and past land use and distance to pollution sources. In certain location or climate conditions, some soil elements may reach toxic levels for humans, animals, or plants [1]. Soil pollution is majorly caused by the large quantities of either natural or man-made waste products.

Pollution is an undesirable change in the physical, chemical, and biological characteristics of air, water, and soil, which in turn, affects lives of humans, plants, and animals, as well as albeit more indirectly, industrial progress, socioeconomical welfare, and cultural assets. Accordingly, a pollutant can be anything that adversely interferes with health, comfort, property, or environment of living matter; ranging from certain chemical elements naturally occurring in soil as mineral components, to anything that may be produced through human activities. Last point also covers the use of pesticides, fertilizers, and other amendments to soil, as well as accidental spills and leaks of chemicals used for commercial or industrial purposes. Even some contaminants are transported via the air and deposited on plants as dust or by precipitation. Lastly, exploitation of natural resources while contributing to the socioeconomic growth of countries also causes environmental problems, in particular potentially contaminated soil. Additionally, storage, transportation, and distribution of hazardous substances and oil-derived liquid fuels; oil activity in the refining phase; and agricultural and forestry activities can be source of pollutants. Controlling the soil pollution is an important problem, needing urgent solution to preserve the soil fertility while increasing the productivity [2].

1.1. Major soil pollutants

More formally, soil pollution is the accumulation of persistent toxic compounds, chemicals, salts, radioactive materials, or pathogens in soil, with undesired effects on plants, animals, and human health [3]. Soil becomes a significant source of contamination release when combined with the action of air and water. Similarly, several factors affect the mobility and final destination of soil compounds, such as the existence, depth, and runoff direction of the groundwater; porosity; temperature; absorption capacity and ionic interchange of soil particles; air and water content; and the soil microbiota. For humans, the risk will mainly depend on their exposure to pollutant sources. These can be through direct inhalation, contact, and consumption of water, meat, or vegetables affected by pollutants [4].

A significant concept in soil pollution is the bioavailable portion, defined as the chemical amount that directly affects plants, animals, or humans as it can be taken up. This depends on several soil and land characteristics, e.g., how the contaminant is kept by soil and the contaminant's solubility: greater solubility typically implies more bioavailability, but in turn, the pollutant may leach out of the soil. Typically, only a portion of a soil contaminant is biologically

http://dx.doi.org/10.5772/65267

available and interestingly, certain chemicals exhibit an "aging effect" and a decreased bioavailability over time.

Bioavailability of a contaminant can be affected by fluctuations in soil conditions, e.g., soil pH, texture, clay type or organic matter content. Unfortunately, quick determination of bioavailable portion is lacking. Soil tests that are commonly available quantify a considerable part of the total amount of a specific pollutant in the sample, and not just the bioavailable portion, which in turn can be a small fraction. Most direct way of estimation for bioavailability, however, albeit being slow, expensive, or generally not available, are by using bioassay tests whereby uptake of pollutants by plants or microorganisms is quantified. Therefore, only the total or chemically extractable amounts of a particular pollutant are usually quantified.

Several substances contribute to the pollution of soil, major ones accounted as: petroleum hydrocarbons, pesticides, heavy metals, and solvents.

Additional to the potential adverse health effects on humans, elevated levels of soil contaminants negatively affect all living matter, including the plant vigor, microbial processes via enzymatic processes, and animal health. In particular, the effect of contaminants to biochemical reactions can affect all metabolic processes and decrease yield for crops. These can be effective at even relatively low concentrations of contaminants as these can alter soil chemistry and impact organisms that depend on the soil or plants for their nutrition and habitat. The exact effects of contaminants on living matter and soil within a given system will depend on the properties of the soil, the levels of contamination, and the sensitivity of a particular organism to existing contamination. For example, zinc contamination affects nitrogen fixation process in Rhizobium bacteria, which is specifically sensitive to zinc. This in turn affects the nitrogen availability to plants and cause reduced yield for legume plants and crops (including beans, peas, peanuts, and lentils) since these plants fix nitrogen via symbiotic relation with the aforementioned Rhizobium bacteria in their root nodules.

Contaminants mobilize in soil in several forms and this phenomenon depends on many factors. Chemical changes or degradation into less toxic material are observed for organic (carbon-based) contaminants. In contrast, metals do not degrade further, but these may undergo chemical changes in such a way to be taken up by living matter. Furthermore, soil pollutants have different *preference* in their final destination: some are transported to water or either present in soil or to groundwater, some others vaporize; or stay bound to the soil. A major factor in the fate of the contaminants is the characteristics of the soil, which in turn is affected by land use and site management and readily available mechanism of uptake of these by plants or animals. Some important soil features that potentially affect the fate of pollutants contain soil texture in the form of its mineralogy and clay content, the pH, temperature, amount of organic matter, moisture level, and the presence (or absence) of other chemicals.

As for the living matter, people are generally exposed to soil contaminants via either ingestion (eating and/or drinking), inhalation (breathing), or dermal exposure (skin contact). Expectedly, human contact to contaminant of soil depends on the pollutant and on the condition and (past) activities at a specific location. Children ingest, typically unintendedly, small amounts of soil (younger children do more than older ones and adults) during playing, gardening, or per-

forming other yard work, or even during indoor activities if soil is transported in via, e.g., shoes, clothing, or pets. Many pesticides enter the body by passing through the skin, i.e., being touched. Contaminants bound to soil particles or vaporized directly from soil, therefore becoming airborne, e.g., windblown dust, may also be inhaled. Not seldom animals raised for nutrition take in contaminants from soil, and pass these to people via animal foodstuffs such as eggs, milk, and even meat. Lastly, in case contaminants are directly dumped into a water source or reach surface water via overflow, drinking water may also contain contaminants.

1.1.1. Soil contamination by heavy metals

Heavy metals are mostly found at specific absorption sites, and these typically are strongly retained by organic or inorganic colloids. These are present also in all uncontaminated soils resulting from residues from the parent materials. A list of basal heavy metal concentrations in soils and plants is given in **Table 1**. Heavy metal accumulation is toxic to all living matter. Exposure to heavy metals is typically chronic, i.e., occurs over a long time period, due to food chain transfer. Some chronic problems associated with long-term heavy metal exposures, e.g., are: lead—mental lapse; cadmium—affects kidney, liver, and GI tract; and arsenic—skin poisoning, affects kidneys, and central nervous system. Immediate poisoning is comparatively rare and typically occurs via ingestion or (dermal) contact.

Heavy metal	Lithosphere	Soil range	Plants
Cadmium (Cd)	0.2	0.01–0.7	0.2–0.8
Cobalt (Co)	40	1–40	0.05–0.5
Chromium (Cr)	200	5–3000	0.2–1.0
Copper (Cu)	70	2–100	4–15
Iron (Fe)	50,000	7000–550,000	140
Mercury (Hg)	0.5	0.01–0.3	0.015
Manganese (Mn)	1000	100–4000	15–100
Molybdenum (Mo)	2.3	0.2–5	1–10
Nickel (Ni)	100	10–1000	1
Lead (Pb)	16	2–200	0.1–10
Tin (Sn)	40	2–100	0.3
Zinc (Zn)	80	10–300	8–100

Table 1. Heavy metal basal concentrations in the lithosphere, soils and plants (μg/g dry matter) [2].

From there, these are spread in the environment and to all living matter, e.g., plants and animal tissues as well as in soil. Interestingly, some of the heavy metals are essential for microbes, animals, and plants, but at very low levels. Their deficiency (essential ones) reduces growth and induces physiological abnormalities in plants [5]. The pollution thereof is mostly seen at urban and industrial aerosols from burning off leaded fuels, mining wastes, and chemical residues in both agricultural and farming practices. Heavy metal contamination of urban and

http://dx.doi.org/10.5772/65267

agricultural soil depends on many factors, e.g., fertilizers, mining, tailings, and waste sludge, also the use of synthetic products (e.g., pesticides, insecticides containing arsenic as active ingredients), paints, batteries containing heavy metals, industrial waste, and industrial or domestic sludge applied on land and industrial areas where chemicals may have been buried or in areas downwind to these. It should be noted that heavy metals do also occur naturally, but seldom at levels to be considered as toxic [6].

The risk associated to the pollution is when these spread into the food chain, simply because this is closely related to (increased) bioavailability, in particular, phyto-availability, i.e., availability to plants, which in turn, are the first stage of terrestrial food chain as essential components of natural ecosystems and agroecosystems. Despite its importance in the food chain, plants would be a threat to animals and human, if these are grown on contaminated soils, due to the accumulation of heavy metals up to toxic levels in the tissues. A common example is the Itai-Itai disease (caused by Cd metal) affecting farmers working with heavy-metal contaminated rice on long term.

Fertilizer	Co	Cr	Cu	Mn	Mo	Ni	Pb	Zn
Nitrochalk	–	–	22	24	–	2	–	15
Calcium	0.1	Traces	Traces	Traces	–	–	–	1
Nitrate	–	–	To 10	To 5	–	–	–	–
Ammonium sulfate	<5	<5	0.800	0.80	<0.05 to 0.2	<5	Traces to 200	0.800
Super phosphate	0.02-13	0–1000	Traces to 1000	Traces to 2842	Traces to 35	Traces to 32	Traces to 92	70-3000
Potassium chloride	001	–	0–10	Traces-8	<0.05	<1	<1	0–3
Potassium sulfate	<5	<5	0–300 to 80	Traces to .33	0.09	<5	<50	<50

Table 2. Heavy metal content of fertilizers (μg/g) [2].

Heavy metals do not only cause diseases on plants, animals, and humans, but also sharply reduce the yield of the crops, causing economic damage to farmers, in particular on sites located near smelters or mine spills.

In contrast to naturally present levels of heavy metals in soils, these are typically significantly higher in agricultural soils. This is because of the applications and accumulation of heavy metals thereof of several chemicals, pesticides, increased doses of fertilizers, farm slurries, other agricultural chemicals, sewage sludge, etc. A short list pointing to the heavy metal content of some fertilizers is given in **Table 2**. In particular, some phosphate fertilizers do contain small amounts of cadmium, which in turn accumulates in the soils whereby these fertilizers are applied.

Along the same line, the heavy metal content of sludges is listed in **Table 3**.

Heavy metal	Range (μg/g)
Cadmium	<60–1500
Cobalt	2–260
Chromium	40–8800
Copper	200–8000
Iron	6000–62,000
Manganese	150–2500
Molybdenum	2–30
Nickel	20–5300
Lead	120–3000
Zinc	700–49,000

Table 3. Heavy metal contents in sludges (μg/g) [2].

Physical, microbial, or biological processes will determine the fate of the heavy metal pollutants in soil. As a result of being transported via natural routes (via water, nitrogen cycle, etc.) and their level at the destination, these may as well be retained in soluble or insoluble form, which in turn affects their bioavailability. It is reported that the soil organic matter has large affinity to heavy metals, which in turn reduced the nutrient content simply because heavy metals form stable complexes with organic matter in plant [7, 8].

The management of polluted soils requires great deal of knowledge on plant pathways in which biochemical reactions use these heavy metals in one way or another. Therefore, all biochemical processes including intracellular transport, adsorption, exchange with environment, complex formation with organic and inorganic ligands, subcellular precipitation-dissolution upon, e.g., intracellular pH change, and redox reactions need to be investigated [9, 10]. Like all biochemical reactions, the extent of these reactions is a function of mineral content of the soil (e.g., for ionic strength) in the form of available silicate layers, carbonates, affecting in turn soil pH, and/or available organic matter (e.g., humic and fulvic acids, polysaccharides, and organic acids), and temperature and humidity.

An important point is the heavy metal bioavailability, which depends on a wide range of soil properties, including uptake and secretion rates, pH, clay and organic matter content, temperature, and coexistence of other (trace) metals in soil, which itself correlates with the soil redox potential and pH [11, 12]. Trace metal bioavailability is reduced as a result of reduced redox potential. Heavy metals' availability depends also on the soil type: these are typically higher in sandy soils when compared to soils with high clay content. The metals typically form complexes on clay surfaces, the localization (outer layer, inner layer) has been described for SiOH and AlOH groups [13] and for amorphous hydroxides and oxides, gibbsite, and allophane clay [14]. Significant differences in Cd uptake, in soils with high Fe and

Mn oxides and low organic matter versus soils with low oxides and high organic matter were found [14].

Organic matter in soil contains negatively charged sites, e.g., humic compounds, suitable for (heavy) metal complex formation [15]. Metals can therefore be either adsorbed on the surface of precipitated organic matter, or in certain cases can dissolve as soluble organic complex with, e.g., organic acids. Expectedly, plant uptake decreases as the amount of insoluble organic matter increases. An important concept investigating the availability of trace elements is the cation exchange capacity (CEC), which itself is a function of organic matter and clay content of soil. Therefore, the metal uptake in plants decrease as CEC increases [16]. Focusing on individual metals, the Cd adsorption is reported to be controlled by calcium, following a competition for available absorption sites at the root surface [17]. Typically, mercury, copper, lead, cadmium, nickel, copper, zinc, and chromium are found as positively charged metal ions. On the other hand, arsenic, selenium, and molybdenum are present in their neutral forms. Both neutral and positively charged heavy metals are found in soil via sewage, industrial waste, or mine washings (USDNCRS 2000). Additionally, radioactive materials such as thorium, uranium, and strontium also constitute as source of dangerous soil pollution as concentrated in sediments [18]. Decontamination procedures include the use of chelate amendments.

This negative correlation between the plant uptake and metal availability have been investigated for the negative impact of macronutrients on trace element uptake [19]. In that work, phosphate ions are reported to reduce Cd and Zn uptake in plants, and reduce the toxic effects of arsenic, typically observed on soils treated with arsenic pesticides [20]. This is especially important when considering the substantial amounts of trace metals in fertilizers. The long-term use of these fertilizers is expected to increase the levels of trace elements in soils and in long-term accumulation in plants [21]. Similar antagonistic effect among micronutrients is also common. An example is leaf chlorosis resulting from Fe deficiency, which can result from a surplus of other metals such as Zn, Ni, and Cu, which in turn decrease the Fe uptake by plant roots. This is important since Fe in turn affects the toxic metal Cd absorption. Another antagonistic metal couple reported by Smilde et al. is the well-known Cd/Zn antagonism. These two metals are chemically similar in their electronic configuration and reactivity with organic ligands: Zn lowers Cd uptake [17], while at low concentrations the interaction is reported to be synergistic [22].

Some plants, known as "hyper-accumulators" adapt quite well to stressful environmental conditions, holding (heavy) metals in their tissues higher than 1% of the metal and up to 25% on a dry matter basis. As a rule-of-thumb, fast-growing plants (lettuce, spinach, carrots) take up more metals than grasses. Similarly, leafy vegetables accumulate trace metals more than root vegetables which, in turn, accumulate metals more than grain crops [10].

1.1.2. Soil contamination by inorganic toxic compounds

An important class of contaminants is the inorganic residues from industrial waste causing severe problems in their disposal. These typically form complexes with (heavy) metals and therefore have very high toxicity potential. Examples are the arsenic fluorides and sulfur

dioxides from industrial wastes, reported in Ref. [23]. These fluorides typically emerge from superphosphate, phosphoric acid, aluminium, steel, and ceramic industries. Along this line, emitted SO_2 makes the soil highly acidic, promoting again metal complex formation, causing further leaf injury and hampered vegetation. In addition to the above-mentioned contamination, some of the fungicides containing copper and mercury, as well as exhaust gases from automobiles running in leaded fuel gets adsorbed by soil particles, therefore adding to soil pollution and is toxic for the plants.

1.1.3. Soil contamination by organic wastes

Various types of organic wastes, e.g. improperly disposed domestic garbage, sewage, industrial waste, agricultural effluents from animal farms, and drainage of water sources, cause soil pollution and adversely affect human health as well as vegetative growth of plants [24–27]. These typically contain large amounts of borates, detergents, and phosphates. For soil, the main contaminants are coal and phenols, combustible materials, aerosols, H_2S, and carbon mono-/dioxides.

A typical source of organic waste contamination is irrigation with sewage water, which typically causes both physical changes such as leaching, changes in porosity, and humus content, as well as chemical changes such as salinity, changes in nitrogen, and phosphate content. An important effect of sewage sludge is the heavy metal pollution. This further leads into the phytotoxicity of plants. Alekseev reported that solubility and availability of heavy metals increase as a result of decrease in soil pH, which results from the release of soluble organic carbon following sludge decomposition [28].

1.1.4. Soil contamination by organic pesticides

Pesticides are often used to control pests and may cause harm to microorganisms and to plants and humans accordingly. Generally, pesticides, particularly aromatic compounds, decompose over much longer time and are known as persistent organic pollutants (POPs). They are the main cause of accumulation, which in turn are highly toxic. Chief examples are aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, hexachlorobenzene, toxaphene, chlordecone, lindane, and endosulfan. Being undecomposed for long periods of time (ranging from months for diuron to tens of years for DDT), these pesticides move into water streams and into food and the food chain thereof. With their high degree of persistence, they can also be easily transported to far away distances from their sources.

These pesticides typically contain heavy metals such as cadmium, mercury, and arsenic, and these are the major problems in pesticide pollution. Currently, several organochlorine compound containing pesticides, including DDT has been banned from USA, Europe, and other countries [29, 30].

The harmful organochlorines have currently been substituted by alternative pesticides containing organophosphate, more toxic, yet little to no residue is left and therefore do not pollute the soil. Common practice for controlling the pesticidal pollution is to increase the organic matter level of the soil and choose the nonpersistent pesticides.

http://dx.doi.org/10.5772/65267

1.2. Causes of soil pollution

Soil gets polluted via either man-made matter or due to natural causes. The natural causes include rupture of underground storage links, water reservoir, while man-made causes cover application of pesticides, oil and fuel dumping, direct discharge of industrial wastes, or leaching of wastes from landfills. The more industrialized the area, the more polluted the soil gets, which naturally decreases soil quality.

A significant cause of pollution is uncontrolled use of fertilizers to supply soil deficiencies. These are known to contaminate with impurities, such as ammonium nitrate, phosphorus as P_2O_5, and potassium as K_2O. Important pollutants from fertilizers are the heavy metals, such as, As, Pb, and Cd present in traces in rock phosphate mineral being transferred to super phosphate fertilizers. Being not degradable, heavy metals accumulate in soil above toxic levels for crops. The uncontrolled use of NPK fertilizers therefore reduce the overall yield as well as protein content of vegetables and crops grown on that soil [31].

Another cause of pollution is the rampant use of insecticides and herbicides, which are used majorly to protect plants from insects, fungi, bacteria, viruses, rodents, and other animals. Large-scale use of insecticides dates back to the 1950s and do include DDT and gammaxene. Over time, insects became resistant to DDT and farmers had to use increasing amounts of DDT to be effective against pests. Add to that the fact that DDT does not readily decompose, quickly created significant contamination. Being soluble in fat, DDT biomagnified in the food chain [32].

Solid wastes, including domestic trash, of discarded commercial operations typically contain recyclable material, e.g., paper, cardboards, plastics, glass, old construction material, packaging material, and toxic or otherwise hazardous substances. However, albeit small, hazardous wastes, e.g., battery metals, organic solvents, and oils are significant soil pollutants [33].

Another point to consider is the pollution of surface soils materials (e.g., vegetables, rotten and decomposed leaves, wooden pieces, animal wastes and carcasses, and papers) and many nonbiodegradable materials (such as plastic bags, bottles and other wastes, cloths, glass pieces, bottles) [34, 35]. In case the pollution is left uncollected and decomposed, they are a cause of several problems such as clogging of drains, including the burst/leakage of drainage lines; barrier to natural waterways, causing damage to nature but also man-made constructions; foul smell; and elevated microbial activity in particular along with decomposition of organic material. Specifically, if the source is from hospitals, the microbiota would include several pathogens. Lastly, underground soil may be polluted in particular where industrial activities exist, cities by chemicals released and sanitary wastes. Heavy metals in particular are likely to be accumulated.

1.3. Effects of soil pollution

Although some of them are obvious and have been enumerated above, it is worth noting that soil pollution affects many aspects of life, majorly food chain but not limited to this. To start with, polluted soil causes reduced crop yield and reduced soil fertility. Polluted soil fixes less nitrogen and has increased erodability. Due to the latter, soil loses more nutrients and soil fauna

and flora becomes more imbalanced in its nutrients (becomes extremely salty, acidic, alkali, etc.). In particular, as a result of industrial activities, water gets polluted and drinking water becomes more inaccessible to humans. Again with industrial activities, greenhouse and other pollutant gases release to the atmosphere, which decreases the quality of the air, causing an increase in public health and waste management problems.

The rest of this article focuses on enzymes used for soil remediation as a special case of bioremediation via so-called plant growth promoting rhizobacteria (PGPRs). As such, it represents one of the alternative tools for soil remediation, such as thermal soil remediation, air sparging, encapsulation, chemical oxidation, stabilization, and soil washing.

2. Using enzymes and PGPRs for soil remediation

Plant growth promoting rhizobacteria naturally exist at plant roots or they are used as inocula that are applied to the roots of plants to stimulate growth by changing the soil environment. PGPRs generally produce important substances for plants, facilitate the uptake of nutrients, and have a role in soil remediation. Soil remediation is an important process for plant health, in which soul pollutants, contaminants, or plant pathogens are reduced or eliminated.

Due to industrialization, soil is polluted together with water and air. The most encountered pollutions can be from organic substances such as total petroleum hydrocarbons (TPHs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated terphenyls (PCTs), perchloroethylene (PCE), trichloroethylene (TCE), and pesticides like atrazine and bentazon or from inorganic substances, which are mostly heavy metals, cadmium, chromium, copper, lead, mercury, zinc, and others.

In a study, different concentrations of TPH contamination was successfully reduced with the help of *Enterobactor cloacae* UW4 and *E. cloacae* CAL2 strains which are PGPRs. Aminocyclopropane-1-carboxylate (ACC) deaminase helped the process by lowering the ethylene levels of TPH. PAHs are remediated by dehydrogenases (e.g., 1,2-dihydroxy-l,2-dihydronaphthalene dehydrogenase), dioxygenases (e.g., 1,2-dihydroxynaphthalene dioxygenase), and aldolases (e.g., cis-2′-hydroxybenzalpyruvate aldolase) produced by *Pseudomonas paucimobilis* Q1. PAHs include napthalene, accenaphtene, phenanthrene, fluoranthene, pyrene, benzo[a]pyrene, and others. PCBs are remediated with biphenyl dioxygenases. TCE is remediated with toluene o-monooxygenase produced by recombinant *Pseudomonas fluorescens*. Biopolymers such as kraft and lignin or trinitrotoluene (TNT) are remediated with Mn-dependent peroxidase (MnP) and lignin peroxidase (LiP).

There are a variety of insoluble substances, whether natural or synthetic, in origin and can be hydrolyzed by specific enzymes. Cellulose, chitin, keratin, Kraft pulp, and sewage sludge are examples of natural insoluble substances. Cellulose can be degraded by cellulase while chitin by chitinase, keratin by keratinase, Kraft pulp by both xylanase and β-xylosidase, and sewage sludge by protease and phosphatase. For synthetic insoluble substances, nylon can be hydrolyzed by MnP, poly-L-lactic acid by depolymerase and alkaline protease, polyacrylate by cellobiose dehydrogenase, and polyurethane by esterase.

http://dx.doi.org/10.5772/65267

Fungi are the most common known yet not the sole producers for such enzymes. Many bacteria which are used as PGPRs can also produce them.

Chemical degradation of heavy metals is not possible, and other alternative methods should be used to relieve the soil from heavy metal accumulation. Alternative methods for remediation of soil include immobilization, separation, extraction, and isolation of metals, as well as reduction of toxicity and mobility.

This section of the chapter focuses on selected enzymes such as ACC deaminase, phytase, and nitrogenase, which can be used in bioremediation of soil.

2.1. 1-Aminocyclopropane-1-carboxylate (ACC) deaminase

PGPRs help plant growth and development directly and indirectly. In case of direct stimulation, it fixes the nitrogen present in the air, produces the phytohormones necessary for plants and enables uptake of some metals including iron and soluble phosphate. The indirect stimulation covers biocontrol actions, i.e., mediating fight with plant pathogens. Both direct and indirect mechanisms operate via specific enzymes. An important enzyme is 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) that plays a well-described role in plant hormone and ethylene regulation (an important stress inducer in plants).

It has been extensively reported that ACC deaminase is found in numerous microbial species of Gram-negative and Gram-positive bacteria, rhizobia, endophytes, and fungi. Also the biochemical and physical aspects of ACC deaminase have been investigated broadly by numerous researchers. **Table 4** summarizes both the plant growth promoting microorganisms and results of the relevant studies.

Microorganism	***Pseudomonas* sp. strain ACP**	***Hansenula saturnus***	***P. putida* GR 12-2**	***Penicillium citrinum***	***P. putida* UW4**
Molecular mass (Da)	104–12,000	69,000	105,000	68,000	a
Subunit mol. mass (Da)	36,500	40,000	35,000	41,000	41,800
Estimated nm. of subunits	3	2	3	2	a
Optimum pH	8.0–8.5	8.5	8.5	8.5	8.0
Optimum temperature (°C)	a	a	30	35	a
K_m for ACC (mM)	1.5–9.2	2.6	a	4.6	3.4
K_{cat} (min^{-1})	290	a	a	a	146

Table 4. Biochemical characterization of 1-aminocyclopropane-1-carboxylate (ACC) deaminase from selected microorganisms [65].

There are several mechanisms in which the ACC deaminase concurrently catalyzes the reaction where ACC breaks down to α-ketobutyrate and ammonia along with the regulation of ethylene production which under stress conditions inhibits the plant growth [36]. When plants were treated with bacteria producing ACC deaminase, relatively extensive root growth

was observed due to presence less ethylene [37, 38] and improved resistance to various stresses was reported [37, 39]. Therefore, using PGPRs which are showing ACC deaminase activity and genetic manipulation of other microorganism to express ACC deaminase genes to stimulate plant grown and development, under either normal or stress conditions, is now a hot topic in biotechnology [39, 40].

2.1.1. Mode of action of bacterial ACC deaminase

The model which explains the mode of action of PGPR containing ACC deaminase is given in detail in [41]. They extensively investigated the competition between ACC deaminase with a low affinity for ACC and ACC oxidase. ACC oxidase is the plant enzyme that has a high affinity for ACC, and it decreases plant's endogenous ethylene concentration. They suggested that there is a relation between ACC deaminase and ACC oxidase in the system and the ACC deaminase level must be at least 100- to 1000-fold greater than the ACC oxidase level for the biological activity of PGPR to be able to decrease plant ethylene levels.

Brassica campestris	*Methylobacterium fujisawaense*	Bacterium promoted root elongation in canola.
Brassica campestris	*Bacillus circulans DUC1, Bacillus firmus DUC2, Bacillus globisporus DUC3*	Bacterial inoculation enhanced root and shoot elongation.
Brassica napus	*Alcaligenes* sp. *Bacillus pumilus* *Pseudomonas* sp. *Variovorax paradoxus*	Inoculated plant demonstrated more vigorous growth than the control (uninoculated).
Brassica napus	*Enterobacter cloacae*	A significant increase in the root and shoot lengths was observed.
Dianthus caryophyllus	L.*Azospirillum brasilense* Cd1843	Inoculated cuttings produced longest roots.
Glycine max	*Pseudomonas cepacia*	Rhizobacterium caused an early soybean growth.
Pisum sativum L.	*Rhizobium leguminosarum bv. viciae* 128C53K	Bacterium enhanced nodulation in plants.
Vigna radiata L.	*Pseudomonas* sp. *Bradyrhizobium* sp.	Bacterium promoted nodulation in mung bean.
Vigna radiata L.	*Pseudomonas putida*	The ethylene production was inhibited in inoculated cuttings.
Zea mays L.	*Enterobacter sakazakii* 8MR5 *Pseudomonas sp.* 4MKS8 *Klebsiella oxytoca* 10MKR7	Inoculation increased agronomic parameters of maize.
Zea mays L.	*Pseudomonas* sp.	Bacterium caused root elongation in maize.
Brassica campestris	*Methylobacterium fujisawaense*	Bacterium promoted root elongation in canola.

Table 5. Inoculation with plant growth promoting rhizobacteria, containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase and subsequent physiological changes in plants [66].

Indole-3-acetic acid (IAA), which is synthesized from tryptophan and other small molecules and secreted by PGPR, gets absorbed on the seed or root surface of the plants [42, 43]. Plants take up a part of the newly synthesized IAA; with the endogenous plant association IAA can stimulate plant cell proliferation and elongation. By the way, IAA induces the activity of the ACC synthetase enzyme to turn S-adenosylmethionine (SAM) into ACC [44]. It seems from the model outlined in [41] that a considerable amount of ACC might be leaked from plant roots and received by the microbes in soil or hydrolyzed by the microbial enzyme ACC deaminase to provide ammonia and α-ketobutyrate. Soil microorganisms containing ACC deaminase enzyme encourage plants to synthesize more ACC than the plant would otherwise need. The excess ACC would leak into the rhizosphere. Uptake and afterwards hydrolysis processes of ACC by the microorganisms reduces the level of ACC outside the plant [41]. In order to keep the balance of ACC between the internal and external ACC levels, more ACC flows into the rhizosphere. This cycle provides the microorganisms with a perfect source of nitrogen (ACC), and hereby, ACC deaminase containing microorganisms grow rapidly around the plant roots when compared to the other soil microorganism. With this action, while ACC level is decreasing in the plant, biosynthesis of the stress hormone ethylene is also inhibited [41]. Therefore, when a plant is inoculated with ACC deaminase containing microorganisms more root growth would be observed. ACC deaminase containing bacteria and the physiological effects of the latter have been described in **Table 5**.

2.2. Nitrogenase

Proteins, nucleic acids, and most of the other biomolecules contain reduced nitrogen as the complementary component. Therefore, obtaining the metabolically consumable form of nitrogen is necessary for all organisms to grow and survive. Earth's atmosphere is rich in elemental dinitrogen, N_2, but it is actually inert at room temperature in the absence of an appropriate catalyst. The reduction of N_2 into ammonia is a good example for this situation. However, the activation energy which is necessary for reduction of N_2 into ammonia is very high even though thermodynamically advantageous. This has been evidently demonstrated in the industrial fixation of nitrogen by the Haber-Bosch process. This process allows formation of NH_3 from N_2 only if temperature is between 300 and 500°C and pressure is higher than 300 atm with Fe-based catalysts in the environment.

Despite the abundance of N_2, obstacle of chemically using this source reveals a problem but nature has already figured it out via the process called biological nitrogen fixation (BNF), for example, the reduction of N_2 to the metabolically consumable form of ammonia. While 60% of the fixed nitrogen is provided by BNF, unfortunately, in the nature, only a few numbers of microorganisms called diazotrophs are able to carry out this process [45]. Hence, the presence of diazotrophs is a major necessity for organisms to generate their own nitrogenous monomers which are used for the synthesis of nucleic acids, proteins, etc. via different biochemical pathways.

Diazotrophs are spread across a wide range of habitats. While they can be found in free forms, they also can be associated with various plants. Despite this difference, they all use the same

fundamental mechanism for N_2 fixation which is carried out by the nitrogenase enzyme system.

Nitrogenase contains two metalloprotein components: (i) the homodimeric Fe-protein: acting as a reductase which has a high reducing power and is responsible for the providing of electrons and (ii) the heterotetrameric MoFe-protein: a nitrogenase which utilizes the electrons supplied to reduce N_2 to NH_3.

The rate-determining step in the overall nitrogenase enzyme kinetics is built on the complexation of Fe-protein and MoFe-protein [46]. Although the definitive structural properties of the nitrogenase complex are unknown, some possible properties can be determined by the characteristics of these individual metalloproteins.

2.2.1. ATP hydrolysis and electron transfer in the nitrogenase system

In the overall reaction which explains the electron flow during the nitrogenase activity, electrons are introduced by Fe-protein and leave the system as reduced products. Although the intermediate steps have not been experimentally validated, there is a "consensus" model which suggests the order of compounds that electrons follow. The suggested occurrence can be found below:

Fe-protein ➔ P-cluster pair ➔ MoFe-cofactor ➔ substrate

Degradation of substrate by nitrogenase is done via three elementary electron transfer reactions. In the first basic reaction Fe-proteins are reduced by electron carries (i.e., flavodoxin, ferrodoxin, or dithionite). Second reaction is a MgATP-dependent process where a single electron moves from Fe-protein to MoFe-protein. Third, the substrate, bound to the active site of the MoFe-protein, is reduced by an electron transfer.

When optimum requirements are provided, the overall stoichiometry for the reaction where nitrogenase reduces the N_2 to NH_3 can be summarized as [47]:

$$N_2 + 8H+ + 8e^- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

with an overall negative enthalpy of reaction which is $\Delta H0 = -45.2$ kJ mol^{-1} NH_3 and a very high activation energy which is EA = 230–420 kJ mol^{-1}.

Mainly nitrogenase is responsible for N_2 reduction to NH_3 while simultaneously catalyzing the reduction reactions of protons and other small unsaturated molecules (i.e., acetylene, cyanide) [48]. With this property, nitrogenase can be considered as a hydrogenase with an ATP-dependent evolution activity. Uptake hydrogenase can play an important role in energy saving via recycling H_2 released by nitrogenase. Furthermore, uptake hydrogenase allows some organisms such as *A. lipoferum*, *Derxia gummosa*, and *P. diazotrophicus* to grow chemolithoautotrophically even under N_2-fixing conditions. Electron donor limitation can improve expression of the uptake hydrogenase. Like nitrogenase, hydrogenase activity is sensitive to oxygen.

http://dx.doi.org/10.5772/65267

2.2.2. N_2 fixing bacteria

Several bacteria fix nitrogen, a short list is given in **Table 6**. Rhizobium bacteria, listed as the first kind as "symbiotic bacteria," is typically linked to leguminous plants, frankia, or cyanobacteria with nonlegume plants. The "nonsymbiotic" second kind (also referred as "free-living" bacteria), exist either in water or soil. Examples of the nonsymbiotic N_2 fixing bacteria are cyanobacteria (blue-green algae, *Anabaena*, and *Nostoc)* and genera such as *Azotobacter*, *Beijerinckia*, and *Clostridium*. The third kind typically is found around roots of the plant rhizosphere and stream the fixed nitrogen to the plant. This group is typically referred as "associative nitrogen fixation" bacteria and includes *Azospirillum*, *Klebsiella sp.*, *Azotobacter paspali*, and *Alcaligenes*. The fourth kind is "endophytic nitrogen fixation" linked with cereal grasses such as sugarcane and includes *Azoarcus sp.* and *Burkholderia sp.*

PGPR	Relationship to host	Host crops
Azospirillum sp.	Rhizospheric	Maize, rice, wheat
Azoarcus sp.	Endophytic	Kallar grass, Sorghum, rice
Azotobacter sp.	Rhizospheric	Maize, wheat
Bacillus polymyxa	Rhizospheric	Wheat
Burkholderia sp.	Endophytic	Rice
Cyanobacteria*	Rhizospheric	Rice, wheat
Gluconacetobacter diazotrophicus	Endophytic	Sorghum, sugarcane
Herbaspirillum sp.	Endophytic	Rice, Sorghum, sugarcane

*Numerous species; predominantly of the genera Anabaena and Nostoc, E.C. number: 1.18.6.1.

Table 6. Plant growth promoting rhizobacteria (PGPR) and their relationship to hosts [67].

The abundantly available PGPRs are diazotrophs and can fix N_2 via the biological nitrogen fixation, this characteristic is not the main mechanism with which they promote growth to their host plant. The plant growth stimulations primarily occur due to bacteria's enzymatic activities such as nitrogenase.

2.3. Phytase

Phosphorus (P) is an essential element for plants to grow and develop. Although P is found in soil both as insoluble inorganic and organic forms, it is unavailable for plants [49]. In soil, there are phosphate-solubilizing bacteria (PSB) which can turn the insoluble inorganic phosphates in organic acids, into an available form. Therefore, these microorganisms have been generally studied to improve the growth properties and yield of crops. Despite being the most abundant form of phosphates in soil (10–50% of total P) [50, 51], phytates should be

hydrolyzed by phytases (myo-inositol-hexakisphosphate-phosphohydrolases) to be consumed by the plants [52, 53].

Phytic acid (myo-inositol hexa-phosphate, IP6) has six phosphate groups. It is present mainly in plant-based nutrients, particularly in cereals and legumes. Phytic acid is thought to be a major stock component for plant germination and growth [54]. IP6 forms a vigorous structure called "chelating agent" by its six P groups and this structure plays a role in binding minerals such as Ca^{2+}, Mg^{2+}, Fe^{3+}, and Zn^{2+}. Presence of phytates may also have a negative effect on digestion of protein [55, 56], starch [57], and lipids [58]. Endogenous phytases in most seeds of higher plants may degrade the IP6 partly to produce penta-, tetra-, or tri-phosphate compounds through food processing and digestion [59].

Phytases are the enzymes which catalyze the degradation reaction of phytate which is the primary reserve form of P in plants. Phytases are a different type of phosphatases and they can hydrolyze phytate to a set of lower phosphate esters of myo-inositol and phosphate. Phytates are present in wide range of living things including plants and microorganisms. In the last decade, the number of researches, which focuses on how to lower the phytate levels found in animal feed by improving the enzymatic reaction of phytases, has been increased [60–62].

A great deal of phytases assumes broad specificity to substrates and can therefore hydrolyze different phosphorylated compounds, irrespective of their similarity to phytic acid, including phosphorylated sugars (e.g., G6P). In contrast, few phytases, e.g., the one from Bacillus sp. and few other bacteria and fungi, e.g., *Aspergillus* sp., are characterized to be highly specific to phytic acid and/or to the class of protein tyrosine phosphatase-PTP-like pyhtases.

2.3.1. Pathways of phytic acid dephosphorylation

Pythase degrade phytic acid at various rates and order. The mechanism of hydrolysis is reported to be step-wise, the product of each step is the substrate of the subsequent one. Depending on the mechanism, this enzyme is recognized having three subclasses: 3-phytase (EC 3.1.3.8), 4-phytase (EC 3.1.3.26), and 5-phytase (EC3.1.3.72), each class depending on the position of the first phosphate hydrolyzed. Note that, phytases are mostly able to hydrolyze five out of six available phosphates.

2.3.2. Phytase and plant growth promotion

There are several microorganisms in rhizosphere which interact with plant roots and affect plant nutrition in different ways. Direct effects of these microorganisms are altering the uptake and availability of plant nutrition. Indirect effects include promoting plant growth. For instance, in a study phytate was used as the unique source of phosphate to grow *Trifolium subterraneum*, as a result secretion of phytase in a very low grade from plant roots was observed. Following *A. niger* phytase was added to the medium and liberation of sufficient phosphates was observed. This step enables *T. subterraneum* seedlings to grow and plants supplied with inorganic phosphorus.

http://dx.doi.org/10.5772/65267

Phytase source	Host plant
Burkholderia sp.	Lotus
Discosia sp.	Maize, pea, chickpea
Bacillus sp., *Pseudomonas fluorescens, Serratia marcescens*	Arabidopsis
Rhizobacteria	Tomato, Pigeon pea
Serratia marcescens Pseudomonas sp., *Bacillus circulans*	Pearl millet
Emericella rugulosa	Pearl millet
Chaetomium globosum	Wheat, pearl millet
A. rugulosus	Wheat, chick pea
Sporotrichum thermophile	Wheat
A. niger	Sub clover
Bacillus subtilis	Tobacco, Arabidopsis
A. niger	Arabidopsis
Medicago truncatula	Arabidopsis
Bacillus mucilaginosus	Tobacco

Table 7. Microorganisms expressing (extracellularly) phytase and their affectees of the resulting enzyme [68].

Since fungi hydrolyze several organic phosphorus compounds efficiently, they are considered as sufficient utilizers of organic phosphorous which can beneficial to the plant growth. Therefore, fungi which produce phytase and phosphatases were applied to seeds as inoculant, for effective use of phytate phosphorus in soil [63]. For instance, *Chaetomium globosum* is a fungus which produces phosphatase and phytase was used as the inoculation agent for wheat and pearl millet crops [64]. As a result, a remarkable progress in plant biomass, root length, plant phosphate concentration, seed and straw yield, and seed P content was obtained after inoculation with the fungus. A brief summary of phytase sources and their host plants can be found on **Table 7**.

3. Conclusion

Soil pollution is an important problem affecting millions of individuals, and surely this effect is not restricted to humans. Therefore, sustainable methods that are suitable for large-scale methods, to remediate soil, become increasingly interesting for both fundamental and applied research. In particular, using biological systems (microbes and enzymes produced by these plants) has shown considerable progress. This needs to be applied in different agro climatic zones of the world.

A key element in these remediation methods is the fundamental (underlying principles) and executive (application principles) understanding of the microbe-plant interaction, that may be physical, chemical, and biological. This will further draw attention to generating engineered agro-lands, as mass production of these organisms and enzymes also economically interesting.

Despite important progress made in, particularly for PGPRs, growth conditions, enzyme portfolio vis-a-vis to soil remediation, and other (symbiotic) interaction with plants, the research is still at its infancy, especially about the interaction with plant roots and other bacteria.

A still unexplored aspect is the molecular engineering of these microbes and/or plants that would enhance the efficiency of these organisms for soil remediation. This has a large potential, as some PGPR can increase plant tolerance to degraded soil and other extreme conditions such as heavy metal contamination and increased salinity.

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