Impact of Biofertilisers on Crop Production Under Contaminated Soils

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1 Introduction

1.1 Biofertiliser: Significance in Sustaining Crop Productivity and Soil Health

Soil provides a vital habitat for various organisms including microbes such as bacteria, actinomycetes, fungi, etc. Soil microbes play a crucial role in regulating various soil reactions, organic matter decomposition, nutrient recycling and soil health improvement, thereby influencing crop quality and productivity. The soil microorganisms convert organic residues into biomass or mineralise them to CO_2, H_2O, inorganic nitrogen,

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phosphorus and many other nutrients including trace elements. These beneficial microorganisms also benefit soil–plant system in several ways through production of various biomolecules like enzymes, vitamins, antibiotics, hormones, organic acids, etc., that have the ability to bind soil particles leading to aggregate formation and improved soil structure (Harrier and Watson 2003; Kumar et al. 2015a, b, 2017; Suri et al. 2013).

In the present context, there is a growing concern about environmental hazards and threats to sustainable agriculture. The studies involving biofertilisers revealed that the long-term use of biofertilisers is economical, eco-friendly, more efficient, productive and accessible especially to marginal and small farmers in comparison with chemical fertilisers. Several researchers have evaluated that utilisation of soil microbes as biofertiliser in crop production not only improves crop quality and production (Kumar et al. 2017) but also exhibits significant influences on soil physical, chemical and biological properties (Bhardwaj et al. 2014; Bai et al. 2016a, b). Soil microbes such as *Rhizobium*, *Azotobacter*, etc., associated with legume roots or free living in soil supplements N supply to plants through biological N_2-fixation, while phosphate-solubilising bacteria, mycorrhizal fungi, etc., enhance the P availability and other nutrients especially the immobile ones from the soil (Suri et al. 2011; Bhat et al. 2015; Bai et al. 2017; Yadav et al. 2015a, b; Kumar et al. 2016a, b, c). The AM fungi also play an important role in P transformation (Kumar et al. 2014). Similar to the aforementioned microorganisms, there are several groups of microbes in soil that benefit from soil–plant system in different ways either directly or indirectly.

Primarily, soil microbes are responsible for organic matter decomposition. Several groups of soil microbes work on organic matter decomposition to humus formation, which is very fine material having very high surface area, possesses the ability to hold positively charged nutrients and retains soil moisture. Soil microbes such as phosphate-solubilising microorganisms play a crucial role in conversion of organic forms of nutrients in inorganic ones (mineralisation) by secreting various types of organic acids and enzymes (Rodriguez and Fraga 1999; Puente et al. 2004; Sharma et al. 2013). These inorganic or mineral forms of nutrients are then easily absorbed by growing plants. Certain groups of soil microorganisms such as AM fungi secrete polysaccharides and glycoproteins that have the ability to bind soil particles and form aggregates, thereby improving soil structure and overall physical properties of the soil (Wright and Upadhyaya 1998; Wright et al. 1998; Harrier and Watson 2003). Moreover, hyphae of AM fungi develop an extensive extra-radical hyphal network that grows into the soil matrix and holds primary soil particles together via physical entanglement. This hyphal network plays a crucial role in soil texture improvement and, in turn, water relations (Hamblin 1985; Tisdall 1991; Staddon et al. 2003; Rillig 2004). Nitrogen fixation is an important process carried out by soil microbes, that is, bacteria, especially by *Rhizobium* (symbiont) and *Azotobacter* (free living). The above bacteria has the ability to convert atmospheric nitrogen to ammoniacal form, thereby enriching soil with plant available nitrogen (Kass et al. 1971; Mila and Shamsuddin 2010). Certain soil fungi (*Trichoderma*) serve as biocontrol agents against fungal root diseases of plants (Harman 2006).

Moreover, some genera of fungi are utilised to control insect pests (Sahoo et al. 2013). Thus, the use of soil beneficial microorganisms as biofertiliser not only enhances nutrient and water use efficiencies of the crops but also improves overall soil health, crop quality and productivity in the long term.

2 Mechanism of Action of Various Biofertilisers

2.1 Arbuscular Mycorrhizal Fungi

2.1.1 Mechanism of Nutrient Absorption by AM Fungi

The arbuscular mycorrhizal fungi (AM fungi) expand the surface area of plant root system through ramification of hyphae and thus extend the exploratory area of plant roots for harnessing nutrients and water (Marschner and Dell 1994). The researchers have explored that in mycorrhizal plants, numbers of extension hyphae are usually far more in number as compared to root hairs of plant; the area of surface where AM fungi, plant and soil interacted increased greatly (Fig. 1), resulting into more nutrient and water absorption (Suri and Choudhary 2013a; Bai et al. 2016b; Kumar et al. 2016b). The AM fungi release low molecular weight organic acids such as oxalic, malic acids, etc., that have the ability to solubilise inorganic forms of phosphates; thus, P is released into soil solution and absorbed by the plants (Zou et al. 1995; Choudhary et al. 2013). Moreover, AM fungi attack complex organic compounds through secretion of various enzymes (*chitinase, peroxidase, cellulase, protease, phosphatase,* etc.) and converting them into simple ones, which can be taken up and utilised by fungi/host plants to fulfil their energy requirements for growth and reproduction (Chen et al. 2007).

Fig. 1 Association showing interactions between AM fungi, plant and soil (Brundrett et al. 1996)

2.1.2 Mechanism of Water Absorption by AM Fungi

The AM fungi-inoculated plants explore larger volume of soil profile through extension of root system by developing higher order laterals by ramification of fungal hyphae associated with it (Song 2005; Suri and Choudhary 2013b), thus absorbing water from larger area of soil profile as well as from deeper soil layers. The main absorption apparatus of mycorrhizal fungi is extension hyphae having a diameter of 2–5 μm, which penetrate soil pores inaccessible to root hairs (10–20 μm) and hence absorb water from these pores which otherwise is not available to non-mycorrhizal plants (Gong et al. 2000). In addition, colonisation of plant roots with AM fungi might change the root architecture and enhance the interaction area of root and soil (Atkinson et al. 1994). Studies undertaken by Hamblin (1985), Tisdall (1991), Staddon et al. (2003) and Rillig (2004) revealed that AM fungi inoculation improves soil structure by binding of soil aggregates with their hyphal network and enhances moisture retention capacity of the soil. The fungal hyphae have a unique capacity of producing glomalin (a glycoprotein) that has ability to bind soil particles and form aggregate. The aggregation improves soil structure and moisture retention capacity (Wright and Upadhyaya 1998; Wright et al. 1998). As per reports in the literature, AM symbiosis also enhances resistance of plants towards various biotic and abiotic stresses (Harrier and Watson 2003).

2.2 Phosphate-Solubilising Microorganisms (PSMs)

2.2.1 Mechanisms of Inorganic Phosphate Solubilisation by PSMs

Several theories elaborated the mechanism of inorganic phosphate solubilisation by PSMs; however, most of theories primarily put emphasis on mechanism involving production of siderophores, organic acids, hydroxyl ions, protons, etc., that dissolve mineral compounds and make them available for plant use (Rodriguez and Fraga 1999; Sharma et al. 2013). As per the concept of Zhao et al. (2014), organic acids are produced in the periplasmic space by direct oxidation pathway. Organic acids produced along with their carboxyl and hydroxyl ions reduce the pH or cause chelation of cations to release P in the soil solution (Seshachala and Tallapragada 2012). As per Goldstein (2000), gluconic acid is one of the most frequent agents of mineral phosphate solubilisation amongst different organic acids produced and released by PSMs; it actually chelates the cations bound to phosphate and in turn makes phosphate available for plant use.

The researchers also explained the mechanisms, where PSMs solubilise mineral phosphate by producing inorganic acids, namely, carbonic, sulphuric, nitric acids, etc., and certain chelating substances. With time, however, the researchers found that the organic acids released by PSMs are more effective in releasing phosphorus in soil as compared to inorganic acids and chelating substances produced by PSMs. Therefore, Kim et al. (1997) suggested that organic acid production by PSMs for

P solubilisation is not only the cause for increased phosphorus concentration in culture medium; rather, liberation of enzymes or enzymolysis by PSMs also plays a critical role in phosphate solubilisation (Zhu et al. 2011).

2.2.2 Mechanisms of Organic Phosphorus Mineralisation by PSMs

Halvorson et al. (1990) proposed sink theory of solubilisation of organic P, where they highlighted that the continuous removal of P results in dissolution of Ca-P compounds. As per the concept proposed by Dighton and Boddy (1989), phosphorus decomposition in organic substrates is directly related with P content in the biomass of phosphate-solubilising microorganisms. The studies carried out by researchers elaborated that several groups of enzymes are associated with biological process of organic phosphorus mineralisation by PSMs. One group of enzymes has dephosphorylate, phosphor-ester or phosphoanhydride bond of organic compounds, which are nonspecific acid phosphatases (NSAPs). Amongst various NSAP enzymes released by phosphate-solubilising microorganisms, phosphomonoesterases are mostly studied, which are also called as phosphatases (Nannipieri et al. 2011). The aforementioned NSAP enzymes can either be acid or alkaline phosphomonoesterases (Jorquera et al. 2011). Another enzyme produced by PSMs in organic-P mineralisation process is phytase, which is responsible for the release of P from organic material stored in the form of phytate and makes it available for plant use (Richardson and Simpson 2011).

2.2.3 Mechanism of N-Fixation by Rhizobium

As we know, legume crops such as pea, lentil, berseem, pulses, clovers, etc., form a symbiotic relationship with soil-dwelling bacteria that takes gaseous nitrogen from the air present in soil pores and feeds it to the legume crop plants, and in turn the plant provides carbohydrates to the bacteria for its growth and reproduction; due to this reason, legume crops are said to ‘fix’ atmospheric nitrogen (*N-fixation*). Likewise other beneficial soil microbes, *Rhizobacteria*, are also present naturally in the soil, but due to their low populations, they did not maximise nitrogen fixation. Hence, inoculation of seed with *Rhizobium* biofertiliser culture is usually recommended to attain a maximum potential of N-fixation by legumes.

The actual process of N-fixation starts with nodule formation in the root of legume plant. *Rhizobia* (bacteria) invade legume root and multiply within cortex cells. The plant supplies all the necessary nutrients and energy for growth and multiplication of the bacteria. Within 6–7 days after infection, small nodules appear and are visible with naked eyes. Depending on legume species and germination conditions, small nodules can be seen within 2–3 weeks after sowing. Initially at younger stage, nodules are usually white or grey inside (*yet not started fixing N*), but as nodules grow in size, they gradually turn pink or reddish in colour (*N-fixation started*). Leghemoglobin (*controls oxygen flow to the bacteria*) imparts pink or red colour to the nodules. The root nodules that are no longer able to fix nitrogen turn green and may usually be discarded by the plant. In general, pink nodules must predominate on the roots of legume plant during mid of growing season, as pink nodules are considered to be most efficient and active in N-fixation. However, the predominance of white, grey or green nodules in the roots of legume indicated inefficient *Rhizobia* strain that led to poor N-fixation and in turn resulted in poor plant nutrition, pod filling and susceptibility of plant to various stresses.

The legume–*Rhizobium* symbiosis is a mutual association, and several researchers have widely exploited symbiotic N-fixation as a medium for increasing crop yields (Boholool 1990; Sharma et al. 1993). There are several genera of *Rhizobia* that belong to the *Rhizobiales*. They are characterised by their unique ability to infect root hairs of legume plant and bring out effective N_2-fixing nodules (Mila and Shamsuddin 2010). In soil, leguminous plants usually secrete dicarboxylic acid exudates that attract *Rhizobium* bacteria. As evident from studies, flavonoids play a crucial role in attracting the bacteria as they are easily absorbed through the membrane of organisms (Maj et al. 2010). Once the bacteria detect these chemicals, they actively move towards legume root and attach to it. Besides attracting bacteria, exudates and flavonoids also play a crucial role in activating genes involved in producing 'Nod factors' (Maj et al. 2010).

For the preparation of symbiotic relationship, *Rhizobium* attraction towards legume roots is usually followed by transcription of 'Nod genes'. Nod factors in turn stimulate the branching of root hair, hydrolysis and deformation of cell wall. In addition to attraction of *Rhizobium*, exudates and flavonoids also change the plant roots making it easier for the *Rhizobium* to enter the cells of the root hair for symbiosis. When the *Rhizobium* bacteria come in contact with root hair, they invade plasma membrane of the cells. As the bacterium penetrates the cell, the plant produces new cell wall material at the site that covers the bacteria as well as allows them to enter deeper into the root hairs (Gage 2017). Similar explanations have also been provided by Matiru and Dakora (2004), Dakora (1995) and Lhuissier et al. (2001), where they highlighted that different species such as *Rhizobium*, *Mesorhizobium, Bradyrhizobium*, *Azorhizobium, Allorhizobium, Sinorhizobium*, etc., respond chemotactically to flavonoid molecules released as signals by legume plant (host) and form intimate symbiotic relationships with them. Above plant compounds induce the expression of nodulation (nod) genes in *Rhizobia* and produce lipo-chitooligosaccharide signals that trigger mitotic cell division in roots and lead to nodule formation.

2.2.4 Mechanism of N-Fixation in Anabaena azollae

Azolla is a freshwater floating fern (a pteridophyte) and lives in symbiotic relationship with a diazotrophic cyanobacterium. All the species of this genus harbour a filamentous nitrogen-fixing cyanobacterium in their fronds that is usually referred to as *Anabaena azollae* (*Nostocaceae*) (Papaefthimiou et al. 2008). The *Azolla* occurs naturally on the surface of the lakes, slow-moving rivers, canals, ponds, etc., and in warm temperate to tropical climates. The *Azolla* has the ability to fix atmospheric nitrogen; hence *Anabaena–Azolla* association holds the potential to substitute application of nitrogenous fertilisers, if used as biofertiliser. Besides the above benefit, *Azolla* is also used as 'green manure' in several countries to fertilise paddy field and play a significant role in enhancing yield (Van Hove and Lejeune 2002) as it has the potential to fix more nitrogen as compared to plants. As per reports in literature, *Anabaena–Azolla* has the capacity to fix nearly about 1 kg N ha^{-1} day^{-1} in paddy field, thereby providing sufficient nitrogen for sustainable rice cultivation. Moreover, owing to its faster multiplication rate, *Azolla* covers the surface of water bodies very rapidly, thus helping to reduce the volatilisation of water and ammonia in rice fields.

The *Azolla* sporophyte bears a multibranched rhizome originating, on ventral surface, adventitious roots hanging down into the water in order to absorb nutrients directly. Further, rhizome has small leaves (about 1 mm in length) consisting of an aerial chlorophyllous dorsal lobe and a partially submerged colourless ventral lobe, which is cup-shaped to provide buoyancy. Dorsal lobe contains a specialised cavity, where cyanobiont is permanently housed. The interior surfaces of the mature cavities which are ellipsoid in shape are covered with mucilaginous layer, where usually 2000–5000 cyanobacterial cells are embedded and immobilised. There are several trichomes (hairs) that extend from the cavity surface into the mucilage layer and establish an intimate contact between the symbiotic partners, thus helping in the exchange of metabolites. Hence, leaf cavity is one type of natural microcosm having a self-organisation and an ecological well-defined structure. This behaves as both physiological and dynamic interface units of symbiotic relationship, where main metabolic and energetic flows occur (Peters and Perkins 1993; Rai 2000).

The *Anabaena* filaments lack heterocyst in the younger leaves of the water fern, whereas in mature leaf cavity, these gradually increase in frequency to 30–40%, relative to photosynthetic cells, reaching the cyanobacterial cells in *Anabaena* population of mature leaf cavities. The 50–90% of fixed nitrogen in the form of ammonia is delivered to the fern by *Anabaena*. Carbohydrate is synthesised in vegetative cells probably in the form of glucose and moves into heterocysts. In this way, nitrogen fixed in heterocysts moves to the vegetative cells in the form of amino acids (Herrero and Flores 2008).

2.3 Soil Contamination and Agriculture

Soil contamination is becoming a major confront that we need to overcome for establishing a healthy environment (Okrent 1999). A large part of bacterial biodiversity, other microscopic and macroscopic living organisms occur in the soil. In general, soil contamination is a major problem at several stages. The groundwater which interacts with and goes underneath the soil could also become contaminated due to soil contamination. Further, the contaminant (*heavy metals and pesticides*) passes to animals feeding on vegetation grown in contaminated soil, and similar is the case with humans (Kirpichtchikova et al. 2006).

2.4 Sources of Soil Contamination

Several sources are responsible for the contamination of soils. Past land use that has used substances may have probably entered the soil as contaminant (Raymond and Okieimen 2011). A gas station or mechanics garage is a perfect example for this, where different fuels and lubricants may have entered the soil inadvertently through poor storage practices or spillage onto the ground leading to contamination of the soil. There are several other good examples highlighting different sources of contamination that affect soils directly or indirectly such as microplastics, oil spills, intensive farming systems, agrochemicals (pesticides, herbicides, fertilisers, etc.), petrochemicals, industrial accidents, waste disposal, etc. The different techniques to overcome soil pollution are as follows:

2.5 Physical Soil Remediation Techniques

Physical soil remediation techniques in general involve soil washing, vitrification and encapsulation of contaminated soils/areas by impermeable vertical and horizontal layers, electrokinesis and permeable barrier systems (Audrone and Vasarevicius 2005). Encapsulation of contaminated areas is commonly used for remediation by pollution prevention or by containment. Most of above techniques have been adapted for the use in the field of environmental engineering from the watertight encapsulation of construction pits. There is extensive literature available on most of these techniques, available for further reading.

2.6 Biological Soil Remediation Techniques

The biological remediation techniques are performed in situ and include microbial remediation, phytoremediation, fungal remediation and composting techniques.

2.6.1 Microbial Remediation

In microbial remediation, microbes degrade the contaminants into a less toxic form. The microbial remediation technique proved to be very effective in the treatment of hydrocarbons and pesticides. The cost of this technique is relatively low and less time consuming as compared to other techniques of soil remediation; however, there is possibility of increased toxicity of certain metals.

2.6.2 Phytoremediation

The process of using plants to extract contaminants or to degrade them in the soil is known as phytoremediation. Effectiveness in bringing soil up to agricultural standard varies because for one type of contaminant only one plant species is generally used, potentially leaving a range of contaminants behind. Moreover, contaminated plants used for extraction must be disposed of.

2.6.3 Fungal Remediation

The use of certain species of fungus to degrade contaminants is known as fungal remediation. Remediation of contaminants following different species of fungus is still in the development phase and is not commercially available till now.

2.6.4 Compost Remediation

This remediation technique involves the addition of compost to the soil. This is a cheaper and quick method of remediation of contaminated soils. However, this technique is not considered a true remediation technique because the contaminants usually remained intact in the soil. The addition of compost in soil, however, could be used to create a raised bed, where plant roots cannot reach the contaminated soil. The bioremediation techniques in general are conditionally effective in bringing soil up to agricultural standard. Phytoremediation may take longer time to show effects, and the plants used must be disposed of after the completion of the project. However, these techniques are inexpensive and easy to implement and are environmentally friendly (Azubuike et al. 2016).

The following mechanisms are involved in soil contamination:

- Deposition of solid waste
- Accumulation of non-biodegradable materials
- Toxification of chemicals into poisons
- Alteration in soil chemical composition, that is imbalance of chemical equilibrium

Some of Agriculture Measures to Control Soil Contamination

(i) Reduction in the usage of pesticides
(ii) Judicious use of chemical fertilisers along with organic ones
(iii) Improved crop production techniques to ensure less weed growth
(iv) Dumping of wastes in garbage pit to prevent soil pollution
(v) Controlled grazing of animals and ensuring best forest management

(vi) Reduction in wind erosion through plantation of wind breaks and wind shield
(vii) Afforestation and reforestation

3 Role of Biofertiliser in Bioremediation

Pesticides are regarded as one of the indispensible means of agricultural production. Soil-applied as well as foliar-applied pesticides contaminate soil directly and after wash off crop stands. There are several microorganisms which have been used to improve the supply of nutrients to crop plants for their vigorous growth as well as to restrict the activity of plant pathogens. They also play an important role to improve the physical health of the soils in numerous ways. Other more recent objectives for the introduction of microorganisms into soil are the mineralisation of organic pollutants (bioremediation of polluted soils, Van Veen et al. 1997).

As a detrimental consequence of environmental stresses, productivity of crops is declining at an unprecedented rate. Our too much dependence on chemical fertilisers and pesticides has encouraged the industries to produce life-threatening chemicals as a form of pesticides or fertilisers. To tackle this adverse condition, biofertiliser can put aside the agriculture from the severity of various environmental stresses (Mahanty et al. 2016).

Although PGPR are mainly considered for promoting the plant growth and disease control, much attention has recently been focused on xenobiotic bioremediation using PGPR (Bishnoi 2015). As bio-inoculants, PGPR are widely used to support survival of plants under stressed conditions, such as pesticide contamination of soil.

4 Case Studies on Bioremediation Using Biofertilisers

The isolated new bacterium (*P. rhizophila* S211), from an agricultural contaminated soil, displayed both pesticide solubilising and plant-growth-promoting activities and genes involved in xenobiotic biodegradation (Hassen et al. 2018).

The three *Pseudomonas* strains (K03, Y04 and N05) isolated from tobacco seeds that could produce siderophores, indole-3-acetic acid and 1-aminocyclopropane-1-carboxylate deaminase fix nitrogen, dissolve phosphorus and potassium and tolerate heavy metals. The Pb stabilisation in soil and reduction of Pb in tobacco content might be due to the rational application of the above species (Li et al. 2019).

The Indian mustard in conjunction with rhizospheric bacteria can be used for enhancing plant Se accumulation, and volatilisation can be used for the removal of heavy metals such as Se from contaminated soils in the San Joaquin Valley and other places where Se contamination is a problem (Mark et al. 1999).

The overall 11 cadmium-tolerant bacterial strains were isolated from the root zone of Indian mustard (*Brassica juncea L. Czern.*) seedlings grown in

Cd-supplemented soils as well as sewage sludge and mining waste highly contaminated with Cd. The ability of these bacteria to protect plants against the inhibitory effects of high concentrations of heavy metals is related to the bacteria providing the plants with adequate iron (Belimov et al. 2005).

A pot experiment was conducted with bioremediation strategies: natural attenuation, phytoremediation with alfalfa (*Medicago sativa* L.), bioaugmentation with *Pseudomonas aeruginosa* and bioaugmentation-assisted phytoremediation, for the treatment of a co-contaminated soil presenting moderate levels of heavy metals and petroleum hydrocarbons. The conclusion focused on the combined use of plant and bacteria was the most advantageous option for the treatment of the co-contaminated soil, as compared to natural attenuation, bioaugmentation or phytoremediation applied alone (Agnello et al. 2016).

B. alba can be considered as a Cr hyperaccumulator plant, based on Cr concentration recorded in its shoots which exceeds the standard values of hyperaccumulator plants (1000 mg kg^{-1}). In particular, both compost and *B. licheniformis* MBBL1 strains are able to induce a significant metal accumulation in shoots and/or roots of tested Brassicaceae. Due to the low bioconcentration factors of tested species (less than 1), these cannot be considered the appropriate choice for metal phytoextraction from the polluted soils examined (Brunetti et al. 2012).

The General Organization of Agriculture Fund, Ministry of Agriculture, Egypt, suggest that bioremediate may act as potential candidates for soil inoculation (phosphoren, microbien, cerealin and azospirillum) to bioremediate pesticide (organophosphate, carbamate and chlorinated organic compounds)-contaminated soil (El-Kabbany 1999).

The plant-growth-promoting bacteria supports in improving agricultural yields, maintaining the soil health by improving physical and chemical properties of soil. Several microorganisms such as bacteria and fungi play a key role in providing conducive environment to the plants to flourish in a healthy way as well as diminishing the pollution possibilities (Fig. 2).

5 Future Perspectives and Way Forward

The biofertilisers have shown the impact and need throughout the world keeping in view the economic and environmental factors. In developing countries such as India, we always put forward to save the economy of the nation (Al-Masri 2001; Santra et al. 2015). Plant-growth-promoting rhizobacteria, having multiple activities directed towards plant growth promotion vis-à-vis exhibiting bioremediating potentials by detoxifying pollutants like heavy metals and pesticides and controlling a range of phytopathogens as biopesticides, have shown spectacular results in different crop studies. The productive efficiency of a specific PGPR may be further enhanced with the optimisation and acclimatisation according to the prevailing soil conditions. Further research and understanding of mechanisms of PGPR-mediated phytostimulation would pave the way to find out more competent rhizobacterial

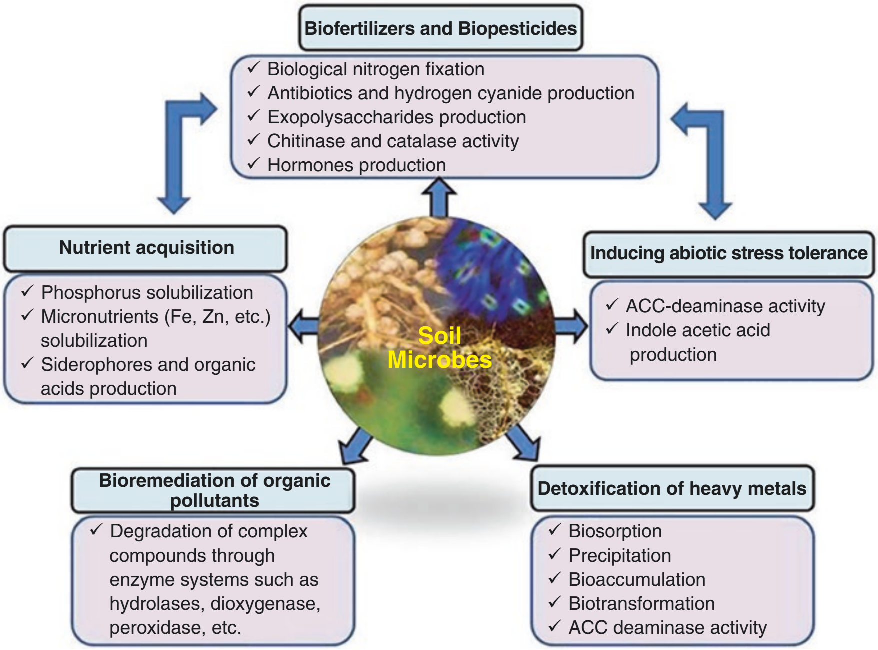

Fig. 2 Biofertilizers and Biopesticides. (Source adapted from: Ahmad et al. 2018)

strains which may work under diverse agro-ecological conditions (Ahemad and Kibret 2014). There is a need to tackle the contaminated soils with the biofertilisers as key member and bioremediation as key process.

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