Rhizosphere: An Innovative Approach for Remediation of Contaminants

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RHIZOSPHERE

The vast amount of organic carbon secreted by plant roots forms, sustains and drives the rhizosphereic zone, a variety of microbial populations colonizing niches from plant's interior and into the bulk soil, responding to the plant, interacting with each other and impacting upon their environment. Within this continuum, the rhizosphere forms a transition zone between the bulk soil and the plant root surface. Plant roots exert strong effects on the rhizosphere through 'rhizodeposition' (root exudation, production of mucilages and release of shoughed-off root cells) and by providing suitable ecological niches for microbial growth (Bais et al.2006). Bacteria residing in the rhizosphere most likely originate from the surrounding bulk soil and will thrive under conditions prevailing in the neighbourhood of plant roots. It must therefore be assumed that bacterial communities in the rhizosphere form a subset of the total bacterial community present in bulk soils. Important parameters, like the quantity and the quality of available carbon compounds originating from plants, as well as novel sites for microbial attachment discriminate rhizosphere from bulk soil (Curl and Truelove 1986). Other parameters, intrinsic to the plant's physiology, genetic make-up, life history and ecology, and the soil itself, certainly have major influences on the structure of rhizomicrobial communities, impacting on their spatial, temporal and functional components. It is a narrow zone of soil affected by the presence of plant roots which is defined as rhizosphere. The rhizosphere is known to be a hot spot of microbial activities. This is caused by an increased nutrient supply for microorganisms, since roots release a multitude of organic compounds (e.g., exudates and mucilage) derived from photosynthesis and other plant processes (Brimecombe et al. 2007).The root system of higher plants is associated with a vast community of metabolically active microorganisms alongwith that of soil environment composed of inorganic and organic matter. The living plants create a unique habitat around the roots and the microbial population on and around the roots is found to the considerably higher than that of root free soil environment. It is the zone/region of soil immediately surrounding the plant roots together with root surfaces, or it is the region where soil and plant roots make contact, or it is the soil region subjected to influence of plant roots and characterized by increased microbial. The rhizosphere region is a highly favourable habitat for the proliferation, activity and metabolism of numerous microorganisms.

Microorganisms present in the rhizosphere play important roles in the growth and in ecological fitness of their plant host. Important microbial processes that are expected to occur in the rhizosphere include pathogenesis and its counterpart, plant protection, as well as the production of antibiotics, geochemical cycling of minerals and plant colonization (Kent and Triplett 2002).

COMMONLY OBSERVED MICROBIAL POPULATION IN THE RHIZOSPHERIC REGION

1. Bacteria

Bacterial communities are not uniformly distributed along root axes, but differ between root zones. Distinct bacterial community compositions are obtained by molecular fingerprints in different root zones, like those of emerging roots and root tips, elongating roots, sites of emergence of lateral roots, and older roots (Yang and Crowley 2000). It has been proposed that populations residing in the rhizosphere oscillate along root axes in a wave-like fashion (Semenov et al. 1999). Accordingly, bacterial communities temporarily profit from the nutrients released younger roots in the root hair zones, and wave-like by death and lysis of bacterial cells upon starvation when nutrients become depleted, followed by cell divisions in surviving and thus viable populations as promoted by the release of nutrients from dead and decaying cells (Semenov et al. 1999). Bacterial communities in rhizosphere soils are thus not static, but will fluctuate over time in different root zones, and bacterial compositions will differ between different soil types, plant species, plant growth seasons and local climates. Changes induced in the soil by the growing root provide additional niches for soil microbes. Rhizosphere conditions sustain communities which differ from those found in bulk soil. Hence, these communities exhibit a "rhizosphere effect" (Curl and Truelove 1986; Lynch and Hobbie 1988). The greater number of bacterial species has been found to be commonly within and around the rhizospehric zone than that of fungi and actinomycetes varieties. Gram-negative, rod shaped, non-sporulating bacteria which respond to root exudates are predominant in the rhizosphere***(Pseudomonas, Agrobacterium),*** **w**hile gram-positive, rods, cocci and aerobic spore forming ***(Bacillus, Clostridium)*** are comparatively rare in the rhizosphere. The most common genera of bacteria are: ***Pseudomonas, Arthrobacter, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas, Micrococcus*** and others have been reported to be either abundant or sparse in the rhizosphere. The abundance of nitrogen fixing and phosphate solubilizing bacteria in the rhizosphere is found to have much importance. The aerobic bacteria are relatively less in the rhizosphere because of the reduced oxygen levels due to root respiration. The bacterial population in the rhizosphere

is enormous in the ranging from 10^8 to 10^9 per gram of rhizosphere soil. They cover about 4-10% of the total root area occurring profusely on the root hair region and rarely in the root tips. There is predominance of amino acids and growth factors required by bacteria, are readily provided by the root exudates in the region of rhizosphere.

2. FUNGI

The plant roots do not alter / enhance the total count of fungi in the rhizosphere as that of bacterial population. However, specific fungal genera ***(Fusarium, Verticillium, Aspergillus*** and ***Penicillium)*** are found to be stimulated**.** The mycelial forms of fungi are more dominant in the field. The zoospore / forming lower fungi such as ***Phytophthora, Pythium, Aphanomyces*** are strongly attracted to the roots in response to particular chemical compounds excreted by the roots and cause diseases under favourable conditions. Several fungi ***eg: Gibberella*** and ***Fujikurio*** produce ***phytohormones*** and influence the plant growth.

3. Actinomycetes, Protozoa and Algae

Stimulation of actinomycetes in the rhizosphere has not been studied in much detail so far. It is generally understood that the actinomycetes are less stimulated in the rhizosphere than bacteria. However, when antagonistic actinomycetes increase in number they suppress bacteria. Actinomycetes may also increase in number when antibacterial agents are sprayed on the crop. Among the actinomycete, the phosphate solublizers (eg.***Nocardia, Streptomyces)*** have a dominant role to play. Because of large bacterial community, an increase in the number or activity of protozoa is expected in the rhizosphere. Flagellates and amoebae are dominant and ciliates are rare in the region.

An important consequence of the high diversity is an intense microbial activity with feedback effects on root development and plant growth in general. In general, the microbes serve as intermediary between the plant, which requires soluble mineral nutrients, and the soil, which contains the necessary nutrients but often in low concentrations and/or complex and inaccessible forms. Thus rhizosphere microorganisms provide a critical link Between Plants And Soil (Lynch 1990).

ENDOPHYTIC BACTERIA

Endophytic bacteria can be defined as those bacteria that colonize the internal tissue of the plant showing no external sign of infection or negative effect on their host (Holliday, 1989; Schulz & Boyle, 2006), and of the nearly 300 000 plant species that exist on the earth, each individual plant is host to one or more endophytes (Strobelet al., 2004). Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents (Berg et al., 2005). Indeed, numerous reports have shown that endophytic microorganisms canhave the capacity to control plant pathogens (Sturz &Matheson, 1996; Duijff et al., 1997; Krishnamurthy & Gnanamanickam, 1997), insects (Azevedo et al., 2000) and nematodes (Hallmann et al., 1997, 1998).Along with the production of novel chemicals, many endophytes have shown a natural capacity for xenobioticdegradation or may act as vectors to introduce degradative traits. The ability of some endophytes to show resistance to heavy metals/antimicrobials and degrade organic compounds probably stems from their exposure to diverse compounds in the plant/soil niche (Table 1). This natural ability to degrade these xenobiotics is being investigated with regard to improving phytoremediation (Siciliano et al., 2001; Baracet al., 2004; Germaine et al., 2004, 2006; Porteous-Moore et al., 2006; Ryan et al., 2007a).

Table 1: A non-exhaustive list of pollutants that has been associated with bacterial endophyte phytoremediation strategies

Compound	Plant association	Organism	Reference
Mono- and dichlorinated benzoic acids	Wild rye (*Elymus dauricus*)	*Pseudomonas aeruginosa* strain R75 and *Pseudomonas savastanoi* strain CB35	Siciliano *et al.* (1998)
2,4-D	Poplar (*Populus*) and willow (*Salix*)	*P. putida* VM1450	Germaine *et al.* (2006)
Methane	Poplar tissues (*Populus deltoidesnigra* DN34)	*Methylobacterium populi* BJ001	Van Aken *et al.* (2004)
TNT, RDX, HMX	Poplar tissues (*Populus deltoidesnigra* DN34)	*Methylobacterium populi* BJ001	Van Aken *et al.* (2004)
MTBE, BTEX, TCE	*Populus* cv. *Hazendans* and cv. *Hoogvorst*	*Pseudomonas* sp	Germaine *et al.* (2004), Porteous-Moore *et al.* (2006)
Toluene	Poplar (*Populus*)	*B. cepacia* Bu61(pTOM-Bu61)	Taghavi *et al.* (2005)
TCP and PCB	Wheat	*Herbaspirillum* sp. K1	Mannisto *et al.* (2001)
Volatile organic compounds and toluene	Yellow lupine (*Lupinus luteus L.*)	*Burkholderia cepacia* G4	Barac *et al.* (2004)

TNT, 2,4,6-trinitrotoluene; 2,4-D, 2,4-dichlorophenoxyacetic acid; TNT, 2,4,6-trinitrotoluene; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazene; HMX, octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine; NDAB, aliphatic nitramine 4-nitro-2,4-diazabutanal; BTEX, benzene, toluene, ethylbenzene, and xylene; TCP, 2,3,4,6-tetrachlorophenol; PCB, polychlorinated biphenyl.

Microbial diversity using molecular studies in the rhizosphere as compared with bulk soil and that the rhizosphere selects for a specific subset of genotypes from the bulk soil, and this effect is plant species specific and highly reproducible (Marilley et al., 1998; Kowalchuk et al., 2002). The reasons for the reduction in diversity in the rhizosphere and the plant–microbe interactions that determine host specificity of microbial community composition are in general assumed to be that roots through rhizodeposition, are able to regulate the soil microbial community in their immediate surroundings (Walker et al., 2003). Rhizodeposits are chemically diverse, with components ranging from simple sugar and organic acid monomers to polymeric lignocellulose containing root debris (Uren, 2001;Walker et al., 2003). The diversity of rhizodeposition not only sustains multitrophic rhizosphere foodwebs (Phillips et al., 2003), but also mediates chemical communications which include signal traffic between roots of competing plants and between roots and beneficial or detrimental rhizosphere dwelling microorganisms. The existence of such diverse below-ground communications has been likened to an 'information superhighway' (Bais et al., 2004), and conversations between beneficial rhizo-bacteria and plant roots as a 'love parade beneath our feet' (Somers et al., 2004).

FLAVONOIDS

A good example is the molecular integration of legume flavonoid signals by compatible rhizobial population during the initiation of nitrogen fixing symbiosis. Flavonoids are a diverse class of natural compounds produced as a result of plant secondary metabolism. They are polyaromatic compounds with a 15-carbon skeleton and can be divided in to subclasses depending on their structure. A wide variety of flavonoids (chalcones, fla-

vanones, isoflavones, flavonols) have been shown to have nod gene inducing activity in different legume/ rhizobia interactions (Aoki et al., 2000). There is also evidence that flavonoids are involved in regulation of nodulation in actinorrhizal associations; where nitrogen- fixing nodules are formed as a result of colonization by an actinomycete, Frankia (Benoit and Berry, 1997; Hughes et al., 1999; Hocher et al., 2006). Increasingly, it is being appreciated that flavonoids also form part of a cluster of signals that are exchanged between arbuscular mycorrhizal fungi (AMF) and their host plants at all stages of the symbiosis: presymbiotic, colonization and symbiotic. Flavonoids represent, to those rhizosphere microorgan- isms in possession of appropriate catabolic enzymes, a carbon rich source. Several studies (summarized in Table 2) have quantified and characterized aerobic fla- vonoid biodegradation for a number of bacterial species. From examination of those studies which attempt to clarify the pathway, a common flavonoid biodegradative route can be identified (reviewed in detail by Cooper, 2004).

Table 2: Aerobic flavonoid biodegradation by bacteria

Bacterial species	Flavonoid	Findings	Reference
Acinetobacter calcaoceticus MTC 127	(+)-Catechin	Used as a sole carbon source and mineralized via protocatechuic acid (PCA) and phloroglucinol carboxylic acid (PGCA) intermediates	Arunachalam et al. (2003)
Bradyrhizobium japonicum	Catechin	Cleaved to produce PGCA and PCA. Mono-aromatics were further metabolized via ortho-ring cleavage, Degradative pathway was inducible.	Bradyrhizobium japonicum
Actinobacterial strain isolated from roots of *Cicer arietium*	Formononetin	The strain was also able to assimilate daidzein, quercetin, luteolin and various other isoflavonoids and flavonoids hydroxy-substituted at the 3-, 5-, 7-, 3′-or 4′-position	
Rhizobium loti NZP2042 and LC22 *Bradyrhizobium sp.* CC814s and CC829	Quercetin	Catabolized to produce PCA and phloroglucinol	Rao and colleagues (1991)
Bradyrhizobium japonicum USDA 110spc4, *Rhizobium fredii* HH103, *Rhizobium sp.* NGR234	Daidzein and genistein	Biodegradation proceeded via closed C ring modification followed by C ring fission and production of A (phloroglucinol, PGCA, resorcinol) andB(p-coumaric acid, p-hydroxybenzioc acid) ring products	Rao and Cooper (1995)
Pseudomonas putida DSM3226	Quercetin	Biodegradation via A ring cleavage	Rao and Cooper (1994)
Agrobacterium tumefaciens C6-6	Naringenin	Non-specific ring fission, no conserved A or B ring products	Rao and Cooper (1994)
Various rhizobial strains	Naringenin, quercetin, 7,4′-dihydroxyfla	Biodegradation proceeded via C ring	Rao and Cooper (1994)

	vone, luteolin, genistein, daidzein, apigenin	cleavage and production of A and B ring products	

Root Exudates

Plant secretes chemical compounds which acts as communication signal between the adjacent plant and microbial community present in the rhizosphere of the root. Root exudates correspond to an important source of nutrients for microorganisms in the rhizosphere and seem to participate in early colonization inducing chemotactic responses of rhizospheric bacteria (Bacilio et al., 2002). An wide range of compounds exuding from roots include sugars, amino acids, peptides, enzymes, vitamins, organic acids, nucleotides, fungal stimulators, inhibitors and attractants and attracting factors and miscellaneous compounds. Organic acids, sugars, amino acids, lipids, coumarins, flavonoids, proteins, enzymes, aliphatics and aromatics are examples of the primary substances found within rhizosphere of the root. Among them, the organic acids have received considerable attention due to their role in providing substrates for microbial metabolism and for serving as intermediates for biogeochemical reactions in soil (Philippe, 2006). Root exudation is an element of the rhizodeposition process, which is a major source of soil organic carbon released by plant roots (Hutsch et al., 2000; Nguyen, 2003). roots typically respond by secreting certain small molecules and proteins (Stintzi et al., 2000; Stotz,2000). Root secretions may play both positive and negative communication in the rhizosphere. The positive communication includes symbiotic associations with beneficial microbes, such as *mycorrhizae, rhizobia* and plant growth promoting *rhizobacteria* (PGPR). The rhizospheric bacteria are responsible for the elimination of the contaminants while the roots are responsible for providing nutrients (root exudates) used by the microorganisms to proliferate (Bais et al., 2008).

Selected microbes can degrade most environmental pollutants. The process stops when the microbe isstarved of food. In order to determine that such microbes can have access to the best food source available in soil, workers have described an enrichment method for the isolation of microbes, which combine the properties of:
(1) degradation of a selected pollutant and
(2) excellent root colonization.

Chemical Nature and Composition of Root Exudates

In general, the component of carbohydrates compounds extracted by the root is arabinose, glucose, galactose, fructose, sucrose, pentose, rhamnose, raffinose, ribose, xylose and mannitol. Sugars such as glucose dominate root exudates (Jonesand Darrah, 1995; Lugtenberg, 1999; Toal, 2000). All 20proteinogenic amino acids, l-hydroxyproline, homoserine ,mugineic acid, and aminobutyric acid are included in amino acids exudates. Acetic acid, succinic acid, lasparticacid, malic acid, l-glutamic acid, salicylic acid,shikimic acid, isocitric acid, chorismic acid, sinapic acid, caffeic acid, *p*-hydroxybenzoic acid, (Alexa et al., 2004)gallic acid, tartaric acid, ferulic acid, protocatacheuic acid,*p*-coumaric acid, mugineic acid, oxalic acid, citric acid,and piscidic acid are categorized in organic acids and phenolic compounds (Jeremy et al., 2004). Some of these compounds, especially the phenolics, influence the growth and development of surrounding plants and soil microorganisms. Naringenin, kaempferol, quercitin, myricetin, naringin, rutin, genistein, strigolactone and their substitutes with sugars are the flavonols (Antonio etal., 2009; Siegridet al., 2007). Catechol, benzoic acid, nicotinic acid, phloroglucinol, cinnamic acid, gallic acid, ferulic acid, syringic acid are derivatives of lignin (Dayakar et al., 2009), sinapoyl aldehyde, chlorogenic acid, coumaric acid, vanillin,sinapyl alcohol, quinic acid, pyroglutamic acid.Coumarins (Umbelliferone), aurones (benzyl auronessynapates, sinapoyl choline), glucosinolates (cyclobrassinone, desuphoguconapin, desulphoprogoitrin, desulphonapoleiferin, desulphoglucoalyssin), anthocyanins (cyanidin, delphinidin, pelargonidin and theirsubstitutes with sugar molecules), indole compounds(indole-3-acetic acid, brassitin, sinalexin, brassilexin, methyl indole carboxylate, camalexin glucoside), fattyacids (linoleic acid, oleic acid, palmitic acid, stearic acid),sterols (campestrol, sitosterol, stigmasterol), allomones (jugulone, sorgoleone, 5,7,4′-trihydroxy-3′, 5′-dimethoxyflavone,DIMBOA, DIBOA) (Bais et al., 2006) and proteins and enzymes (proteins, lectins, proteases, acidphosphatases, peroxidases, hydrolases, lipase) are the compounds found on root exudates, which is isolated from various plant roots (Narasimhan et al., 2003; Uren,2000).

Root-root communication

Root exudates play important role as phytotoxins in mediating chemical interference (allelopathy) and significant indirect roles in resource competition by altering soil chemistry, soil processes and microbial populations. Plants that produce and release potent phytotoxins can reduce the establishment, growth or survival of susceptible plant neighbors, thus, reducing competition and increasing resource availability. Plants release phytotoxins in decomposing leaf and root tissue, in leachates from live tissue, in green leafy volatiles, and in root exudates (Bertin

et al., 2003). Different phytotoxins in root exudates affect metabolite production, photosynthesis, respiration, membrane transport, germination, root growth, shoot growth and cell mortality in susceptible plants (Weir et al., 2004).Studies indicate, a variety of phytotoxic compounds are released as root exudates for example 7,8-benzoflavone [Acroptilon repenst (italics), Russianknapweed], (±)-catechin (*Centaurea maculosa*, spotted knapweed), DIMBOA and DIBOA (*Triticum aestivum,*wheat), juglone (*Juglans nigra*, black walnut), 8-hydroxyquinoline (*Centaurea diffusa*, diffuse knapweed),sorgoleone (*Sorghum* spp.), and 5, 7, 4′-trihydroxy-3′, 5′-dimethoxyflavone (*O. sativa*, rice). These compounds possess some structural components, such asaromaticity (with the exception of sorgoleone), hydroxyl and/or ketone groups. However, the structures of thecompounds also vary considerably, and include flavonoids [7, 8-benzoflavone, (±)-catechin, and 5, 7, 4′-tri-hydroxy-3′, 5′-dimethoxyflavone], quinones (juglone and sorgoleone), quinolines (8-hydroxyquinoline), and hydroxamic acids (DIMBOA- 2, 4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), DIBOA) (Stermitz et al., 2003; Baiset al., 2002; Wu et al., 2000; Jose and Gillespie, 1998;Vivanco et al., 2004; Nimbal et al., 1996; Kong et al.,2004).

Root-microbe communication

Microbial diversity in the soil has been linked to plant diversity, though it is unclear whether this is through increased habitat heterogeneity, the increased plant biomass commonly observed with highly diverse plant communities or increased diversity of carbon substrates and signaling compounds provided by the plants. The signal components largely responsible for these specific host-microbe relationships belong to a class of compounds termed flavonoids. Data on flavonoids assignaling compounds are available from several symbiotic and pathogenic plant-microbe interactions. More than 4000 different flavonoids have been identified in vascular plants, and a particular subset of them is involved in mediating host specificity in legumes (Perret et al., 2000). In several *Fusarium* plant interactions, an effect of flavonoids on micro- and macroconidia germination has been reported; Striglactones only recently have been identified as important signals in the AMF-plant interaction and thus, are “hot issues” in mycorrhizal research. Rhizobia-legume interactions are very specific, allowing specific rhizobial strains to nodulate with specific host legumes. *S. meliloti* effectively nodulates species of the *Medicago*, *Melilotus* and *Trigonella* genera, whereas *Rhizobium leguminosarum bv viciae* induces nodules inthe *Pisum*, *Vicia*, *Lens* and *Lathyrus* genera (Bais et al.,2006). Scientists demonstrated that roots selectively secrete L-MA (malic acid) and effectively signal beneficial rhizobacteria establishes a regulatory role of root metabolites in recruitment of beneficial microbes, as well as underscores the breadth and sophistication of plant microbial interactions (Thimmaraju et al., 2008).*Brassicaceae* have also been found to have a stimulatory effect on ectomycorrhizal fungi (Zeng et al.,2003). Few researchers demonstrates that two model plant species (*Arabidopsis thaliana* and *Medicago truncatula*) are able to maintain resident soil fungal populations, but unable to maintain nonresident soil fungal populations. This is mediated largely through root exudates: the effects of adding *in vitro*-generated root exudates to the soil fungal community were qualitatively and quantitatively similar to the results observed for plants grown in those same soils (Yanhong et al., 2009).

Mycorrhizal Association

Most plants have symbiotic relationships with soil microorganisms. For example, root nodule bacteria that have symbiotic relationships with legumes are involved in nitrogen fixation. Vesicular-arbuscular (VA) mycorrhizal fungi have symbiotic relationships with most (approximately 80%) plants and are involved in the uptake of nutrients, such as phosphorus. For mycorrhizal plants, mycorrhizal colonization is the rule rather than the exception in natural ecosystems (Smith and Read, 1997); the term mycorrhizosphere is more accurately used to describe the zone of soil influenced by both the colonized root and the extraradical hyphae of the mycorrhizal fungus (Johansson et al., 2004) (fig 1).

The term 'mycorrhizosphere' is generally used to identify a sphere of influence encompassing plant roots, the root symbiotic mycorrhizal fungi, and soil in the immediate vicinity of the mycorrhizal roots (fig. 2). Most plants living in nature are dependent on their mycorrhizal fungi. The activity of the microbes in the mycorrhizosphere has obvious effects on soil chemistry and podzol profile formation (Ghosh *et al*., 2005). The mycorrhizosphere of an individual plant comprise to a large extent of fungal biomass. The symbionts cooperate closely in the uptake of nutrients from the soil and in defense against metal stress. VAM induces changes in the host root exudation pattern, which alters the microbial equilibrium in the mycorrhizosphere by establishing the host root colonization (Korade and Fulekar, 2009).

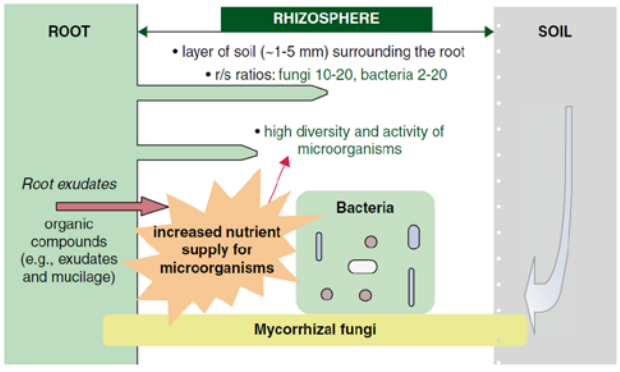

Fig 1:Rhizosphere microorganisms as a critical link between plants and soil. *(Adapted from Richardson et al. 2009)*

Arbuscular mycorrhizal fungi: A potential tool for successful bioremediation

The arbuscular mycorrhizal fungi (AMF) are universal and ubiquitous rhizosphere microflora forming symbiosis with plant roots and acting as biofertilizers, bioprotactants, and biodegraders (Xavier and Boyetchko, 2002). AMF belongs to Glomales, form symbiotic relationships with roots of 80%~90% land plants in natural and agricultural ecosystems (Brundrett, 2002), including halophytes, hydrophytes and xerophytes (Khan, 1974; Khan and Belik, 1995; Schussler *et al*., 2001; Khan, 2003; Khan, 2006), and are known to benefit plant nutrition, growth and survival, due to their greater exploitation of soil for nutrients (Smith and Read, 1997). Fungi play a central role in many microbiological and ecological processes, influencing soil fertility, decomposition, cycling of minerals and organic matter, as well as plant health and nutrition. Fungi are heterotrophs, requiring external sources of carbon for energy and cellular synthesis and they have adopted three different trophic strategies to obtain this carbon, occurring as saprotrophs, necrotrophs, and biotrophs (Pichardo et al. 2012). By acquiring phosphate, micronutrients and water and delivering a proportion to their hosts they enhance the nutritional state of their hosts (Clark and Zeto, 2000; Turnau and Haselwandter, 2002). Similarly, heavy metals are taken via the fungal hyphae and can be transported to the plants by active or passive import. Thus, mycorrhizosphere can show enhanced heavy metal uptake and root-shoot transport. The result of mycorrhizal colonization on clean-up of contaminated soils depends on the plant-fungus-HM combination and is influenced by soil conditions.

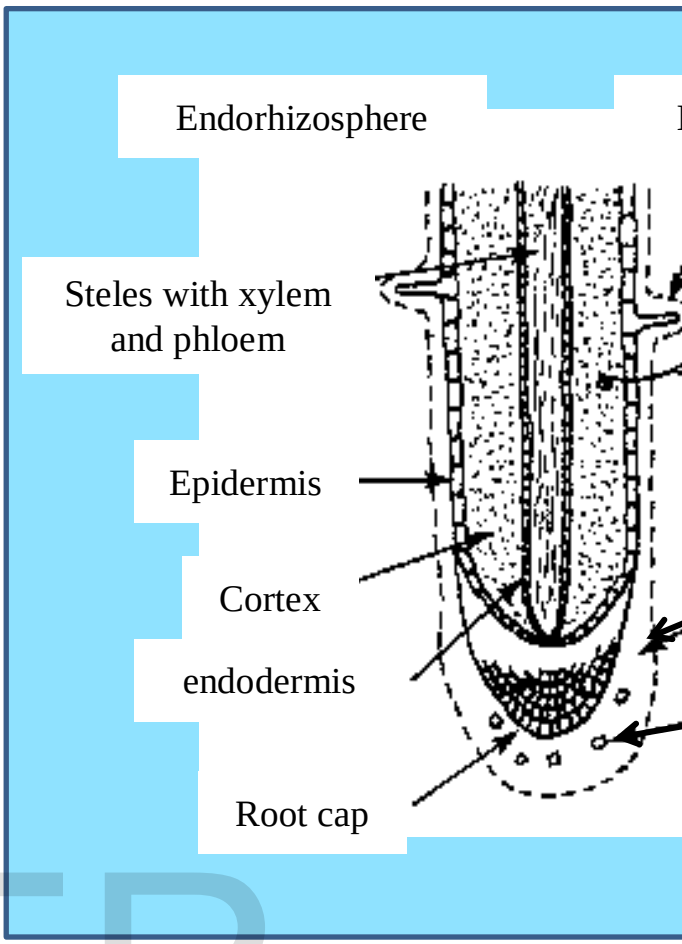

Fig. 2: Mycorrhizosphere

Rhizosphere is considered as an Ecological Remediation Unit to treat the contaminated soil. Microorganisms like bacteria, fungi and Actinomycetes form a symbiotic association along the root zone of the plant in the rhizosphere. The plant releases exudates such as short chain organic acids, phenolics, sugars, alcohols and small concentration of high molecular weight compounds (enzymes and proteins) in the rhizosphere. Research at the U.S. Environment Protection Agency (EPA) laboratory in Athens, Georgia has examined fine plant enzyme systems in sediments and soils, i.e. dehalogenase, nitroreductase, peroxidase, laccase and nitrilase.

- Dehalogenase enzymes are important in dechlorination reactions of chlorinated hydrocarbons
- Nitroreductase is needed in the first step for degradation of nitroaromatics
- Laccase enzyme serves to break aromatic ring structures in organic contaminants
- Peroxidase and nitrilase are important in oxidation reactions

These enzymes are active in rhizosphere soils in close proximity to the root (1 mm) for transformation of organic contaminants. The addition of plant root systems

creates ecology in mycorrhizal soil in the bioreactor which treats the contaminants. Rhizosphere increases the organic carbon bacteria and mycorrhizal fungi, all factors that encourage degradation of organic compounds in mycorrhizal soil (Fulekar, 2006). Experiments have shown that the number of beneficial bacteria increased in the root zone of hybrid poplar trees relative to unplanned reference sites. Also plants release exudates help to stimulate the degradation of organic chemicals by inducing enzyme systems of existing bacterial populations stimulating growth of new species that are able to degrade the wastes and/or increasing soluble substrate concentrations of the microorganisms (Fulekar, 2005). Microbial assemblages are abundant in the rhizosphere which has importance for biotreatment.

Plants and fungi in arbuscular mycorrhiza symbioses

AMF associations in plant roots in metal contaminated soil are widely recognized as a potential tool for successful bioremediation. Plants in symbiosis with AM fungi have the potential to take up heavy metal from an enlarged soil volume. Mycorrhizal associations also offer a biological means of assuring plant health and also increased growth and yield in such degraded ecosystems. So in heavy metal contaminated sites requiring clean up the beneficial dimensions of mycorrhiza should be utilized (Pal 2011). The basic principles of detoxification mechanisms include the extracellular heavy metal –chelation by root exudates and/ or binding of heavy metals to the rhizodermal cell walls uptake of metal avoiding. Active plant efflux systems control cytosolic concentrations of heavy metals. Intracellularly the plant cell produces chelating agents such as phytochelatins and metallothioneins, which have high-affinity heavy metal binding properties. The resulting complex can finally be exported from the cytoplasm across the tonoplast and sequestrated inside the vacuole (Hall, 2002). Heavy metal detoxification mechanisms of plants and fungi in arbuscular mycorrhiza symbioses is illustrated in Fig. 3.

Detoxification mechanism of plants and fungi in mycorrhizosphere includes:

- Chelating agents are secreted that bind metals in the soil, eg. histidine and organic acids from the plant, glomalin from the fungus.
- Binding of HM to cell wall components in plants and fungi.
- The plasma membrane as a living, selective barrier in plants and fungi.
- Specific and non specific metal transporters and pores in the plasma membrane of plants and fungi (active and passive import).
- Chelates in the cytosol, e.g., metallothioneins (plants and fungi), organic acids, amino acids and metal specific chaperons (shown for plants, assumed for AM fungi).
- Export via specific or non specific active or passive transport from plant or fungal cells.
- Sequestration of HM in the vacuole of plant and fungal cells.
- Transport of HM in the hyphae of the fungus.
- In arbuscules, metal export from the fungus and import into plant cells via active or passive transport.

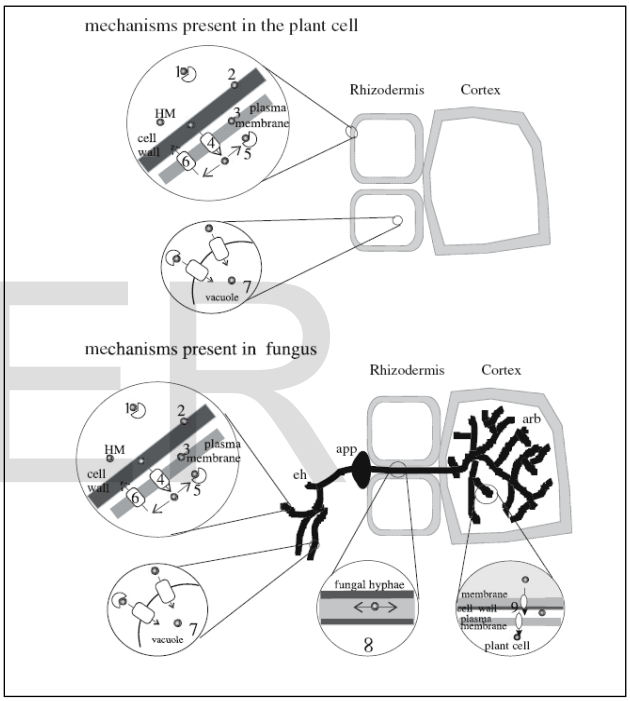

Fig 3: Heavy metal detoxification mechanisms of plants and fungi in arbuscular mycorrhiza symbioses. (Adapted from Göhre and Paszkowski, 2006).

Bioengineering of Rhizosphere for enhanced degradation/removal of organic compounds and heavy metals

Bioengineering of Rhizosphere by application of mycorrhizal inoculum (natural biofertilizer) for removal of heavy metals

The pot culture technique has been employed for development of mycorrhizal soil in the green house. Mycorrhizal soil is a symbiotic association of bacteria, fungi and Actinomycetes which provides the effective rhizosphere for the growth of plants. The enrichment of microbial enzymes and plants exudates in mycorrhizosphere influences phytoremediation. The heavy

metals (cadmium, lead and zinc) toxicity at varying concentrations, viz. 5, 10, 20, 50, 75 and 100ppm has been assessed for seed germination and growth of *Medicago sativa* plants both in mycorrhizal soil and non mycorrhizal soil. Mycorrhizosphere has found to provide suitable conditions for seed germination and growth of plants at concentrations ranging from 5-50ppm. The germination rate was found comparatively lower in NMS at the metal concentrations 5-50ppm. The seed germination *M. sativa* in cadmium amended mycorrhizal soil was found 87, 80, 70 and 55% at concentration of 5, 10, 20 and 50ppm, respectively; while for lead amended mycorrhizal soil, the percentage of seed germination was observed 80, 75, 70, and 60% at 5, 10, 20 and 50ppm, respectively. The seed germination percentage was 95, 90, 91 and 89% when zinc was amended with the concentration of 5, 10, 20 and 50ppm, respectively in mycorrhizal soil. Similarly the root/ shoot growth of *M. sativa* for each of this metal was propound in mycorrhizal soil. The higher metal concentrations i.e. 75 and 100ppm were found inhibitory for the seed germination and root/shoot growth. The enzymes studied; in particular acid & alkaline phosphatase and dehydrogenase in mycorrhizal soil have propounded the growth of plants in the mycorrhizosphere. The present research study has proved the effect of mycorrhizal soil for phytoremediation of heavy metals at concentrations ranging from 5-50 ppm using *M. sativa* as a potential candidate (Anamika and Fulekar, 2010).

Arbuscular mycorrhizal symbiosis is most often associated with improved plant growth. Plants associated with AM fungi are known to have enhanced inorganic nutrition (Cooper, 1984) greater rate of photosynthesis (Allen *et al*., 1981) proved resistance to drought, pathogens and heavy metals. Colonization of roots by arbuscular mycorrhizal fungi imparts several important changes on host plant physiology and soil ecology. AM colonization affects the autotrophic portion of C cycling by stimulating photosynthetic rate at both the whole plant and leaf level and increasing photosynthate demand belowground (Smith and Read, 1997). Langley *et al*., 2005 reported that colonization by AM fungi increased belowground respiration rates in sunflower rhizocosms. The increase in respiration appeared to be mediated through enhanced nutrition in mycorrhizal plants. The mycorrhizal stimulation of soil respiration was in close proportion to mycorrhizal increase of plant mass which suggests that AM colonization did not affect respiration independently of its effects on plant growth. Agely *et al*. (2005) hypothesized that AM fungi have a role in maintaining its productivity and may contribute significantly to plant arsenic uptake. Their data indicate that AM fungi have increased plant growth and arsenic accumulation by the hyperaccumulator Chinese brake fern (*Pteris vittata* L.).

In a greenhouse trials, the effects of arbuscular mycorrhizae fungi (AMF) on growth and uptake of N, P, K and Pb by *Eucalyptus rostrata* L., grown with or without *Phaseolus vulgaris*, in lead (Pb) contaminated soil were investigated by Bafeel (2008). Inoculation of the host plants with AMF, *Glomus deserticola* spores, significantly increased the dry weight, shoot length, total N, P and K as well as chlorophyll concentration in *Eucalyptus*. The inoculation with mycorrhizal fungi enhanced the amount of Pb absorbed and accumulated by *Eucalyptus*. The results showed that inoculation of the host plants with AMF protects them from the potential toxicity caused by increased uptake of Pb. At all cases, *E. rostrata* growth and other parameters performed better at the presence of *P. vulgaris* plants. The stusy has proved that arbuscular mycorrhizae have a potential in phytoremediation of the heavy metal contaminated soils.

The remediation of nutrients, trace metals, or organics is realized by roots of plants in the soil–root–shoot chain. Rhizosphere microorganisms have an important effect on plant nutrient uptake, on the morphology and on the development of roots, and on a number of physiological and developmental processes of plants. Arbuscular mycorrhizal fungi (AMF) are important root symbionts which live in strong associations with the 80–90% of higher plants, and they are regarded as an as an essential component of soil microflora in a phytoremediation system (Khade 2005). Arbuscular mycorrhizal fungi have been recovered from numerous metal-enriched habitats (Del Val *et al*. 1999; Hildebrandt *et al*. 1999; Pawlowska *et al*., 1996), and glomalean hyphae may contribute directly to the uptake and translocation of metals to host roots, including micronutrients such as Cu and Zn, as well as toxic element Cd (Bukert and Robson 1994; Joner and Leyval 1997; Li *et al*. 1991).

Janoušková *et al.* (2006) concluded from his investigation that mycorrhiza may decrease Cd toxicity to plants by enhancing Cd immobilisation in soil. The results therefore suggest a potential role of AM symbiosis in the phytostabilisation of contaminated soils, where high Cd availability inhibits plant growth. The contribution of arbuscular mycorrhiza (AM) to immobilisation of Cd in substrate was studied in two experiments. In the first experiment, substrates prepared by cultivating tobacco, either non-mycorrhizal or inoculated with the AM fungus *Glomus intraradices* were enriched with a range of Cd concentrations, and Cd toxicity in the substrates was assessed using root growth tests with lettuce as a test plant. The tests revealed lower Cd toxicity in the mycorrhizal than in the non-mycorrhizal substrate, and the difference increased with increasing total Cd concentration in the substrates. In the second experiment, extraradical mycelium (ERM) of *G. intraradices* exposed in vivo to Cd was collected and analysed on Cd concentration. The ERM accumulated 10–20 times more Cd per unit of biomass than tobacco roots. While Cd concentrations were lower in the biomass of

mycorrhizal plants than of non-mycorrhizal plants, Cd immobilisation by ERM did not affect the total Cd content in mycorrhizal tobacco.

The effect of arbuscular mycorrhiza (AM) on cadmium (Cd) uptake by tobacco (*Nicotiana tabacum* L.) was studied in a pot experiment. Three commercial varieties, Basma BEK, K326 and TN90, representing three distinct tobacco types, were each grown in a different soil with nutritional conditions matching as closely as possible their requirements for field production. Cd concentrations in these soils were within the background range. Each variety was either non-mycorrhizal or inoculated with one of five AM fungal isolates. Cd concentration in leaves was decreased by inoculation with selected isolates in the K326 and TN90 variety grown in acidic soils. In contrast, it was increased by inoculation with most isolates in the Basma BEK variety grown in a basic soil with low Cd availability. Besides, plants of all three varieties had significantly higher leaf concentrations of phosphorus and nitrogen in some inoculated treatments. The percentage of root colonisation was mostly low in the inoculated treatments. In the Basma BEK and TN90 variety, the tested AM fungal isolates differed in their ability to colonise roots, but no correlation was found between the root colonisation of an isolate and its effects on the Cd concentrations in tobacco leaves. One isolate influenced most pronouncedly Cd concentrations and improved mineral nutrition in all the three combinations of variety and soil despite its low colonisation levels (Janoušková *et al*., 2007).

Arbuscular mycorrhizal fungi (AMF) have repeatedly been demonstrated to alleviate heavy metal stress of plants. Hildebrandt *et al*., 2007, have summarized the colonization of plants by AMF in heavy metal soils, the depositions of heavy metals in plant and fungal structures and the potential to use AMF-plant combinations in phytoremediation, with emphasis on pennycresses (*Thlaspi* ssp.).

The phytoremedial potential of *Ipomoea aquatica* and role of arbuscular mycorrhizal fungi (AMF) during Cadmium uptake was studied under two different soils i.e., soil inoculated with and without AMF by Bhaduri and Fulekar, 2012. The plants were treated with different concentrations of Cd(NO)3 starting from 0, 5, 10, 25, 50, and 100 ppm in three replicate design in soil with and without AMF inoculation. Results showed that AMF enhanced accumulation of cadmium in plant tissues at all concentrations. Plants in AMF exhibited tolerance for Cd up to 100 mg/l and accumulated 88.07% in its tissues with no visual symptoms of toxicity, whereas those in non-AMF showed marked growth reduction at the same concentration with a metal accumulation of 73.2%. A significant variation of antioxidant enzymes under different environments evaluated the defense pathways of plants during uptake of Cd. Physiological changes and nutrient uptake showed that plants inoculated in AMF were more unwavering during stress conditions. The study established that phytoremedial potential of *I. aquatica* depends on rhizospheric conditions which enhanced Cd uptake. Finally, it was established that AMF was able to maintain an efficient symbiosis with *I. aquatica* in soil moderately contaminated by Cd, viable due to relation between fungus and plant.

Bioengineering of Rhizosphere by application of vermicompost for remediation of heavy metals

This plant based technique is essentially an agronomic approach and its success depends ultimately on agronomic practices applied at the site. Biological processes such as composting followed by vermin-composting to convert vegetable waste (as valuable nutrient source) in agriculturally useful organic fertilizer would be of great benefit. The composting followed by vermin-composting of vegetable waste with earthworm (*Eisenia foetida*) develops in to a natural fertilizer (Maharashtra Nature Park Bulletin, 2003). The vermicompost contain high nutrient value, increases fertility of soil and maintains soil health (Suthar et al., 2005). Application of compost and vermin-compost in contaminated soil improves soil fertility and physical properties as well as helps in successful approach to phytoremediation which has been demonstrated by Zheljazkov and Warman (2004). It also enhances quality of growing plants and increased biomass which could suggest that more metal can be taken up from the contaminated growth media and the tolerance to the metal toxicity is improved (Tang et al., 2003). The use of vermin-compost developed from vegetable waste by vermin-culture biotechnology with soil would provide natural environment for phytoremediation (Elcock and Martens, 1995). To investigate the effectiveness of soil- vermicompost media, greenhouse experiment was conducted to determine the phytotoxic effect of heavy metals such as Cd, Cu, Ni, Pb and Zn on the growth of Sunflower (*Helianthus annuus*): on the seed germination, root/shoot growth and uptake of metals in soil-vermicompost media (Jadia and Fulekar, 2008). The selected metals were dosed at various concentrations ranging from 0, 5, 10, 20, 40 and 50 ppm separately in soil - vermicompost media (3:1) in pot experiment. The seed germination, root and shoot growth were found significantly affected by these metals at higher concentration of 40 and 50 ppm. However, the lower concentration of heavy metals ranging from 5 to 20 ppm doses were observed to be stimulating the root and shoot length and increase biomass of the sunflower plant. Sunflower was able to germinate and grow efficiently at all Zn concentration evaluated in this study. The research study of the sunflower indicates the heavy metal uptake at the concentrations 5, 10, 20, 40 and 50 ppm. Sunflower is a

very fast-growing industrial oil crop with a high biomass producing plant to be used for phytoremediation (uptake) of toxic metals (Cu, Zn, Pb, Hg, As, Cd, Ni) from soil in heavily contaminated areas. Vermicompost can be used to remediate metals –contaminated sites because it binds metals and increase uptake by providing nutrients such as sodium, magnesium, iron, zinc, manganese and copper which can serve as a natural fertilizer giving high yield of biomass and microbial consortium helped the overall growth of the sunflower plant. The use of vermicompost amended soil would be effective to remediate the heavy metals from contaminated environment. The present technology will help to remediate the higher concentrations of metals by the application of vermin-compost as a natural fertilizer in soil. This technology will be directly applicable at the site to remediate the heavy metals.

Bioengineering of Rhizosphere by mycorrhizal inoculum (natural biofertilizer) for degradation of organic compounds

Deepali and Fulekar (2009) studied Rhizosphere bioremediation of chlorpyrifos in rye grass mycorrhizosphere. The results indicate that chlorpyrifos rapidly dissipated in rhizosphere soils within first 7 days and continue to degrade completely till the end of the experiment over the period of 28 days. The percentage dissipation of the chlorpyrifos in soil amended at varying concentrations (10–100 mg/kg) ranged from 58.73 to 100% at the first harvest period. Whereas at the end of the experiment, the lower concentrations (10–50 mg/kg) spiked in the soil were totally degraded and percentage degradation at higher concentrations (75 and 100 mg/kg) was found to be 94.25 and 91.6%, respectively. The research findings thus show that ryegrass is an able candidate which efficiently degrades chlorpyrifos in its root zone area. The grass varieties are reported to be the most suitable plant species for rhizosphere bioremediation of organic contaminants due to their ability to harbor large number of bacteria on their highly branched root system. In the rhizosphere, an increase in microbial density, diversity and metabolic activity is estimated to be effective due to release of plant root exudates, mucigel and root lysates (enzymes, amino acids, carbohydrates, low molecular mass carboxylic acids, flavonones and phenolics) [Kidd et al., 2008]. These rhizo-deposits also stimulate the survival and action of bacteria which subsequently results in efficient degradation of pollutants [Kuiper, et al. 2001]. In present research study, the chosen plant species—ryegrass proved competent for rhizosphere bioremediation of chlorpyrifos in the soil which may be credited to its advantageous features of having fibrous root system providing large specific surface area to interact with microorganism [Dzantor, et al. 2000] and capacity to release high amount of exudates in the rhizosphere [Meharg and Killham 1990].

The research study has been carried out to evaluate the potential use of two grass species *Cenchrus setigerus*, and *Pennisetum pedicellatum* as a monocropping and co-cropping system for the rhizospheric bioremediation of pesticides Chlorpyrifos, Cypermethrin and Fenvalerate .The effect of the three pesticides on the germination of grass seeds was investigated using pesticide spiked soil at the concentrations 10, 25, 50, 75 and 100 mg/kg, while unspiked soil has been taken as control. The heterotrophic microbial numbers were also enumerated in the developing rhizospheric zone and in the bulk soil in order to assess developing microbial associations for biodegeradation of pesticides in mycorrhizosphere. The research finding shows that Chlorpyrifos was more toxic than Cypermethrin and Fenvalerate at higher concentrations (75 and 100mg/kg) for the germination, survival and subsequent growth of Cenchrus setigerus, and *Pennisetum pedicellatum*. The heterotrophic microbial populations were found to be higher in the mycorrhizosphere soil of co-cropping system of Cenchrus setigerus and *Pennisetum pedicellatum* as compared to individual mycorrhizospheres of *Cenchrus setigerus* and *Pennisetum pedicellatum*, for all the three pesticides at each concentration ranging from 10 mg/kg to 100mg/kg. This study will help in selection of plants for further investigation of the rhizospheric bioremediation of Chlorpyrifos, Cypermethrin and Fenvalerate contaminated soil (Dubey and Fulekar 2011).

Rhizoremediation is a specific type of Phytoremediation involving both plants and their Rhizosphere associated microbes. In the present study *Pennisetum pedicellatum* and rhizosphere associated degrading strains were evaluated for chlorpyrifos remediation. Time-course pot experiments were conducted in greenhouse with *P. pedicellatum* grown in soil amended with chlorpyrifos at the concentrations of 10, 25, 50, 75 and 100 mg/kg for 60 days. The half life of chlorpyrifos varied from 19.25 to 13.02 days in planted treatments. Residual concentrations of chlorpyrifos were negatively correlated with abundance of degrading microorganisms in rhizosphere. The isolated species of *Bacillus*, *Rhodococcus* and *Stenotrophomonas* were evaluated for their degrading potential in mineral medium. A novel isolated strain of potential degrader *Stenotrophomonas maltophilia* named as MHF ENV20 showed better survival and degradation at high concentration of chlorpyrifos. Degradation of chlorpyrifos by strain MHF ENV20, 100, 50 and 33.3% degradation within the time period of 48 h (h), 72 and 120 h at 50,100 and 150 mg/kg concentrations, further the gene encoding the

organophosphorous hydrolase (mpd) was amplified using PCR amplification strategy and predesigned primers. Our findings indicate that rhizosphere remediation is effective

bioremediation technique to remove chlorpyrifos residues from soil. *P. pedicellatum* itself, in addition to the Rhizosphere bacterial consortium, seemed to play an important role in reducing chlorpyrifos level in soil. High chlorpyrifos tolerance and rhizospheric degradation capability of *P. pedicellatum*, makes this plant suitable for decontamination and remediation of contaminated sites. The ability to survive at higher concentration of chlorpyrifos and enhanced degrading potential due to presence of mpd gene make *S. maltophilia* MHF ENV20 an ideal candidate for its application in chlorpyrifos remediation (Dubey and Fulekar 2012).

Bioagumentation of rhizosphere for enhanced Bioremediation of organic compounds in mycorrhizosphere

Bioaugmentation has been utilized in agriculture for many years. The inoculation of legumes with symbiotic, nitrogen-fixing *Rhizobium* spp. dates back to the 1800s. Attempts have also been made to utilize bioaugmentation with free-living or plant-associated nitrogen-fixing bacteria such as *Azotobacter* or *Azospirillum* spp. to increase plant yields (Monib et al. 1979; Ramos et al. 2002). Now-a-days Rhizosphere Bioaugmentation is an emerging technology to remediate numerous environmental contaminants from the polluted soils (fig.4). The potential microbial inoculants have been applied to the soil as live microorganisms in either a liquid culture or attached to a carrier material. When applying the inoculant to a harsh environment such as soil, it may be desirable to use a carrier material since it can provide a protective niche and even temporary nutrition for the introduced microorganism. Numerous different carrier materials have been used including biosolids, charcoal-amended soil, clay, lignite, manure, and peat. Bioaugmentation with microorganisms has been shown to increase degradation of numerous compounds including chlorinated solvents, methyl *tert*-butyl ether, nitrophenols, oil, pentachlorophenol, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and several pesticides such as atrazine, dicamba, and carbofuran (Alexander 1999;Lendvay et al. 2003; Salanitro 2000; Schwartz and Scow 2001; Silva et al. 2004).

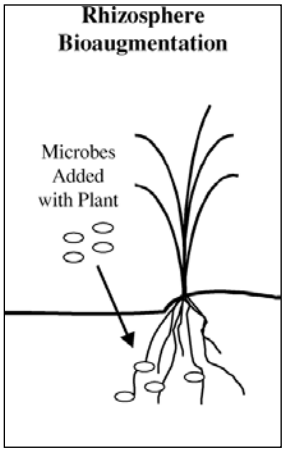

Fig. 4: Rhizosphere Bioagumentation: An Emerging Technology for decontamination of polluted soils

Deepali and Fulekar used this rhizosphere Bioagumentation strategy for degradation of chlorpyrifos in ryegrass mycorrhizosphere. The potential microorganisms existing in rhizospheric soil which have capacity to survive and multiply at higher concentration of chlorpyrifos were selected for bioaugmentation of this hazardous compound. The chlorpyrifos degrader capable of using chlorpyrifos as the only source of carbon in the minimal salt medium was then identified by 16s rDNA analysis. The analysis indicated that the bacterium was most similar to *Pseudomonas nitroreducens* PS-2 (accession no. FJ588866.1).This bacterium was cultured further into an inoculum for bioaugmentation of chlorpyrifos. Bioaugmentation of chlorpyrifos by inoculation of *P. nitroreducens* PS-2 into ryegrass rhizosphere soil was performed. Along the period of incubation, the degradation of chlorpyrifos was about 1.35 times higher for 25 mg/kg and ranged from 1.38 to 2.02 for 50 mg/kg in bioaugmented soil than the non-bioaugmented soil. Chlorpyrifos dissipation was greater in inoculated soil during the experimentation and ranged from 1.31 to 2.64 times when compared to the non-inoculated soils for 100 mg/kg spiked soil. The concentrations 25 and 50 mg/kg were examined to dissipate completely by 14th day and 21st day as compared to the 21st and 28th day as observed in non-inoculated rhizospheric soil. Chlorpyrifos was completely dissipated at the initial spiked concentration of 100 mg/kg for the inoculated soil in contrast to the non-inoculated soil (6.68 mg/kg) by the end of the bioaugmentation experiments. The differences in percentage dissipation of chlorpyrifos occurred in inoculated and the non-inoculated soil in all the concentrations are shown in Table 3. These results indicated that bioaugmentation by adding the isolate PS-2 into the Rhizosphere soil of ryegrass significantly improved the degradation of chlorpyrifos ($p < 0.05$) and can be used for enhanced degradation of chlorpyrifos from the contaminated soils. This study presented has research findings in accordance with Yu et al. (2003), where the

degradation of butachlor in wheat rhizosphere soil at the initial concentration of 10 mg/kg was five times improved by bioaugmentation of butachlor degrader.

Table 3: Percentage dissipation of chlorpyrifos in the soil inoculated and non-inoculated along the period of time.

Chlorpyrifos mg/kg	non inoculated rhizospheric soil	non inoculated rhizospheric soil
25	76.24% (14 days)	100% (14 days)
50	90.36% (21 days)	100% (21 days)
100	90.80% (28 days)	90.80% (28 days)

Soil sites contaminated with several different chemicals, such as metals, radionuclides and oraganics, pose special problems for bioaugmentation. In such cases, it may be necessary to use multiple microbial cultures or consortia for bioaugmentation (van der Gast et al. 2003). Roane et al. used a dual-bioaugmentation strategy to remediate soil contaminated with both Cd and 2,4-dichlorophenoxyacetic acid (2,4-D). The researchers inoculated the soil with the metal-resistant bacterium *Pseudomonas* strain H1 and/or the 2,4-D-degrading bacterium *Ralstonia eutropha* JMP134. Bioaugmentation with both *Pseudomonas* strain H1 and *R. eutropha* JMP134 increased 2,4-D degradation in the presence of Cd, as compared to microcosms not bioaugmented or bioaugmented with only one strain. Another interesting point from this paper is that the authors added the *Pseudomonas* strain H1 48 h before adding the *R. eutropha* JMP134. It was hypothesized that by staggering the bioaugmentation, the metal-resistant *Pseudomonas* strain H1 was able to partially detoxify the Cd (reportedly by intracellular sequestration) prior to introduction of the Cd-sensitive *R. eutropha* JMP134.

Arbuscular mycorrhizal fungi used for ecological restoration

A greenhouse experiment conducted by Chen *et al.*, (2007) have provided evidence for the potential use of local plant species in combination with AMF for ecological restoration of metalliferous mine tailings. They investigated the potential role of arbuscular mycorrhizal fungi (AMF) in encouraging revegetation of copper (Cu) mine tailings. Two native plant species, *Coreopsis drummondii* and *Pteris vittata*, together with a turf grass, *Lolium perenne* and a leguminous plant *Trifolium repens* associated with and without AMF Glomus mosseae were grown in Cu mine tailings to assess mycorrhizal effects on plant growth, mineral nutrition and metal uptake. Results indicated that symbiotic associations were successfully established between *G. mosseae* and all plants tested, and mycorrhizal colonization markedly increased plant dry matter yield except for *L. perenne*. The beneficial impacts of mycorrhizal colonization on plant growth could be largely explained by both improved P nutrition and decreased shoot Cu, As and Cd concentrations.

AM associations are important in natural and managed ecosystems due to their nutritional and non-nutritional benefits to their symbiotic partners. They can alter plant productivity, because AMF can act as biofertilizers, bioprotectants, or biodegraders (Xavier and Boyetchko, 2002). AMF are known to improve plant growth and health by improving mineral nutrition, or increasing resistance or tolerance to biotic and abiotic stresses (Clark and Zeto, 2000; Turnau and Haselwandter, 2002). Their potential role in phytoremediation of heavy metal contaminated soils and water is also becoming evident (Chaudhry *et al*., 1998; Khan *et al*., 2000; Khan, 2001; Jamal *et al*., 2002; Hayes *et al*., 2003).

Mycorrhizosphere microorganisms play a critical role in cycling of rhizo-deposit carbon and in interacting biogeochemical functions like denitrification (Cheneby et al., 2004; Mounier et al., 2004) and have important plant growth promotion functions (e.g. biocontrol, hormone production, bioremediation) (Dobbelaere et al., 2003; Kuiper et al., 2004). Thus, understanding the drivers of rhizosphere microbial communities to allow rational manipulation or engineering of the rhizosphere for beneficial function is an important biotechnological aim.

Conclusion

The above studies imply that the microbial community of soil rhizosphere play important role to solve the environmental problems, such as contamination of soil with metals and organics. Soil microorganisms, including plant root associated free-living as well as symbiotic rhizobacteria and mycorrhizal fungi in particular, are integral part of the rhizosphere biota. The overall result of plant–rhizosphere microbe interactions is a higher microbial density and their metabolic activity in the rhizosphere, even in metal/organic contaminated soils. Plant root exudates provide nutrition to rhizosphere microbes, thus increasing microbiological activity in the rhizosphere, which in turn stimulate plant growth and help in degradation/ removal of organics / metals from the contaminated environment. Further bioengineering of rhizosphere with mycorrhizal fungi and vermicompost as well as rhizosphere bioagumentation strategy offers a novel approach for enhanced degradation/removal of organics/metals from contaminated soils. This is especially important, since it is clear that the greatest application of rhizoremediation will be in developing countries, where this technology can

provide a low-cost means of controlling widespread environmental contamination.