Int.J.Curr.Microbiol.App.Sci (2018) *7*(3): 1673-1697

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 03 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.703.200

Rhizobia and their Bio-Partners as Novel Drivers for Functional Remediation in Contaminated Soils

Sharanappa Kuri*, G. Prakasha, Vishwajith and S.S. Shree Harsha Kumar

Department of Agronomy, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

**Corresponding author*

Keywords

Rhizobia and their Bio-Partners, Contaminated soils

Article Info

Accepted:
12 February 2018
Available Online:
10 March 2018

A B S T R A C T

Soil contamination as part of land degradation is caused by the presence of xenobiotic chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals or improper disposal of waste. Rhizobiales, belonging to the alphaproteobacteria, are Gram- negative bacteria of agronomic importance because some species form nitrogen fixing symbiotic relationships with leguminous plants. Recently, rhizobia have been demonstrated to be available for the elimination of various types of organic pollutants from the environment, ranging from aromatic to linear hydrocarbons, chlorinated compounds, phenolic compounds, pesticides, and others. The genus Rhizobium was one of the most abundant members of the degrading microcosm in dibenzofuran contaminated soil. However, the bacterial catabolic enzymes and the pathways involved in the degradation of these compounds are only partially known. In addition to organic compounds, rhizobia have also been shown to have the potential to be a powerful tool for heavy metal bioremediation.

Introduction

Soil contamination or soil pollution as part of land degradation is caused by the presence of xenobiotic (human-made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals, or improper disposal of waste. The most common chemicals involved are petroleum hydrocarbons, poly-nuclear aromatic hydrocarbons (such as naphthalene and benzo (a) pyrene), solvents, pesticides, lead, and other heavy metals. Contamination is correlated with the degree of industrialization and intensity of chemical usage. The concern over soil contamination stems primarily from health risks, from direct contact with the contaminated soil, vapors from the

contaminants, and from secondary contamination of water supplies within and underlying the soil. There are physical, chemical and biological means to remediate pollution; among them, bioremediation has become increasingly popular.

Reasons for soil contamination

Increasing urbanization and industrialization.

Disposal of untreated wastes.

Indiscriminate use of agrochemicals.

Unscientific mining.

Dumping industrial wastes on land.

Accidental pollution or leakages.

Outdated technology, inadequate treatment and safety management of chemicals and waste.

Lack of designed engineered landfills.

These are the reasons for the contamination of agricultural soils leads low agriculture productivity and human and animal health risks.

Status of soil contamination in India

In India 175 million hectare are considered as degraded out of 329 million hectare.

There are more than 125 million ha major contaminated sites across the country.

More than 40 per cent of chemical fertilizers leached into soil leads to contaminated soil.

In India there is 14 States are affected by Fluoride contamination area leads lot of human health hazards.

Heavy metals beyond permissible limits affecting ground water of 40 districts from 13 states in India.

More than 65 per cent of Indian villages are exposed to residual pesticides risks like endoshulpan, DDT, *etc*., agrochemicals.

Types of contaminated soils

Organic contaminated or polluted soils

Heavy metal contaminated soils

Pesticide contaminated soils

Possible remediation methodologies

Transfer of contaminated solid wastes to common TSDFs or possible options for utilization of the waste removed.

Capping the waste in a secured landfill (SLF).

Confinement of the contaminated area by concrete / bentonite side walls and capping. Contaminated soil excavation, soil washing and refilling.

Physical and chemical remediation techniques like soil vapour extraction, pump & treat chemical precipitation *etc*.

Bioremediation of contaminated sites.

Among these remediation methods bioremediation is most appropriate, effective, eco-friendly and economical approach.

Bioremediation makes effective better approach

Bioremediation: Either by destroying or render them harmless using natural biological activity. Natural biological agents like microorganisms and plants.

Why microorganisms are so important in this process?. Because of they have extraordinary metabolic diversity.

Bioremediation through

Bioremediation through microorganisms (Rhizobia) for organic pollutants.

Phytoremediation especially suited for heavy metals remediation.

Rhizobia assisted phytoremediation (micro-plant cross interactions) become more synergetic effect than indusial.

Rhizobium as component of agriculture

Rhizobium, a root nodule bacterium, has the ability to fix atmospheric nitrogen in symbiotic association with host legumes.

It was estimated that a total of 175 million metric tons of N is fixed per year globally through biological nitrogen fixation involving legume - *Rhizobium* symbiosis.

Characteristics of *rhizobium*

Rhizobiales, belonging to the alpha proteo bacteria, are Gram- negative bacteria of agronomic importance because some species form nitrogen fixing symbiotic relationships with leguminous plants.

Some the characteristics, rhizobia invade legume roots through root hairs, form effective pink colored nodules in the roots and lives symbiotically inside the nodules and fix nitrogen. Then converts atmospheric nitrogen into plant accessible forms of nitrogen.

Most rhizobia are host specific in nature.

Hydrogen (H_2) is a by-product of the symbiotic nitrogen fixation process and has recently been revealed to be a common element with novel bioactive properties that enhances plant tolerance to abiotic factors (*i.e*., oxidative stress and heavy metal toxicity).

Functions of *Rhizobium* and their bio-partners

Rhizobia invade the roots of legumes (*i.e*., alfalfa) and form nodules. During the process of biological nitrogen fixation in nodules, dinitrogen (N_2) is reduced to two ammonia (NH_3) molecules by the rhizobial nitrogenise (Teng *et al.*, 2015). Hydrogen (H_2) is a by product of the symbiotic nitrogen fixation process (Fig. 1). This hydrogen (H_2) is responsible for degradation of organic contaminates in contaminated soils.

Rhizobiales, belonging to the alpha proteobacteria, are gram- negative bacteria of agronomic importance because some species form nitrogen fixing symbiotic relationships with leguminous plants.

Rhizobia and their bio-partners are producing plant growth promoting substances like IAA, siderophores, HCN, ammonia, exo-polysaccharides, cytokinin, heavy metal mobilization *etc*., these are responsible for the degradation of contaminates in soils (Subramaniam *et al.*, 2015) (Table 4).

Recently, rhizobia have been demonstrated to be available for the elimination of various types of organic pollutants from the environment, ranging from aromatic to linear hydrocarbons, chlorinated compounds, phenolic compounds, pesticides, and others.

Kaiya *et al.*, (2012) reported that genus *Rhizobium*was one of the most abundant members of the degrading microcosm in dibenzofuran contaminated soil. However, the bacterial catabolic enzymes and the pathways

involved in the degradation of these compounds are only partially known. In addition to organic compounds, rhizobia have also been shown to have the potential to be a powerful tool for heavy metal bioremediation.

Potential mechanisms involved

Some of the potential mechanisms involved in biodegradation of contaminates through rhizobia and their bio-partners.

Rhizobia and their bio-partners able to utilize organic pollutants as a source of carbon and nitrogen.

Adsorption and accumulation of heavy metals.

Microbial secretion of enzymes and bioactive metabolites (*i.e*., extracellular polymeric substance, siderophores, and organic acids) to lessen their toxicity by altering the redox state of metals and increasing the complexation and bioavailability of metals.

Microbial volatilization of heavy metals and their transformed products can also facilitate bioremediation, although this process has yet to be identified in rhizobia.

Heavy metal resistant microbes can enhance plant growth and decreases metal phytotoxicity.

Effectiveness of phyto- or microbial-remediation is dependent on

The symbiosis between Rhizobiuam and plants has been employed for the elimination of environmental contaminants to achieve high effectiveness and ecological sustainability. The effectiveness of phyto or microbial remediation is dependent on.

Soil physio-chemical properties- pH, nutrient/ OM content, soil surface properties, soil texture and BD, which influence plant–soil–water relationships and nutrient availability.

Toxicity or bioavailability of the targeted contaminants that reduce the productivity of the impacted soils, the biomass of plants and the degradative ability of microorganisms.

Plant species and traits.

The diversity and richness of the indigenous soil microbial communities or flora.

However, these limitations can be addressed through the exploitation of the chemical interactions between the plants and the related rhizospheric microbes or endophytes. For the phytoremediation of heavy metals, heavy metal- resistant microbes can enhance plant growth, decrease metal phytotoxicity, and affect metal translocation and accumulation in plants.

Rhizobia: Bioremediation for organic pollutants contaminated soil

Many free living rhizobial strains in the genera *Agrobacterium*, *Rhizobium*, *Sinorhizobium*, and *Bradyrhizobium*have a demonstrated capacity to thrive in or utilize PAHs, PCBs, aromatic heterocycles (*i.e.*, pyridine), or other toxic organic compounds first isolated 22 strains of *Rhizobium* capable of degrading phenolic compounds (*i.e*., catechol, protocatechuic acid, *p*-hydroxybenzoic acid, and salicylic acid). Among them, *Rhizobium* sp. and *R. phaseoli* 405 dissimilated *p*-hydroxybenzoate to salicylate and then to gentisic acid before oxidation.

Catechol and protocatechuic acid were also directly cleaved by these species, whereas *R. japonicum* converted catechin to protocatechuic acid (Muthukumar *et al.*, 1982).

Degradative mechanisms involved in organic pollutants

The presence of rhizobia can also exert direct or indirect impacts on microbial-degrader communities in the soil, thereby comprehensively facilitating restoration (Li *et al.,* 2013). The mechanisms involved in this process include: (i) improvement of environmental conditions (*i.e*., pH) and nutrient availability (*i.e*., nitrogen) and (ii) changes in the amounts and constituents of root exudates due to the enhancement of plant metabolic activities following inoculation with rhizobia (Johnson *et al.,* 2005).

Acenaphthylene and phenanthrene are ubiquitous PAHs in the environment. Acenaphthylene (600 mg/liter) can be totally degraded by *Rhizobium* sp. Strain CUA1 within three days through the naphthalene 1, 8-dicarboxylic acid metabolism pathway (Poonthrigpun *et al.,* 2006). *Sinorhizobium* sp. C4 can utilize phenanthreneasa sole carbon source, and 16 intermediate metabolites involved in this degradation pathway have been identified (Keum *et al.,* 2006).

Tu *et al.,* 2011, reported that, 2,4,4-TCB biodegradation by *S. meliloti* resting cells The degradation dynamics of 2,4,4-TCB by *S. meliloti* in liquid culture is presented in Figure 4. After 6 days of incubation, the concentration of 2,4,4-TCB decreased in both treatments. However, significant difference was observed between *S. meliloti* treatment (0.20 ± 0.02 mgL^{-1}) and the autoclaved control (0.91 ± 0.03 mgL^{-1}, $p < 0.05$). Calculated percent biodegradation of 2,4,4-TCB by *S. meliloti* was also described in Figure 2. The percentage of 2,4,4-TCB biodegradation received 34.6%, 52.4%, and 77.4% on Day 1, Day 3, and Day 6, respectively. Loss of 2,4,4-TCB in the control was possibly caused by non-biological processes such as volatilization and photo degradation during the extracting process due to the volatile physicochemical property of 2,4,4-TCB.

Tu *et al.,* 2011, reported that the total concentrations of 21 PCB-Congener-Mix in the soil microcosms are presented in Table 5. After 30 days of bioremediation, total PCB concentrations across all treatments ranged from 126.7 to 198.0 g kg^{-1} dry soil. Inoculation with *S. meliloti* significantly reduced soil PCB concentrations compared with the uninoculated control ($p < 0.05$). A remarkable enhancement in total PCB degradation was also observed between 20% and 10% inocula treatments, except for the data from Day 30.

Soil culturable bacteria, fungal and biphenyl-degrading bacterial counts from different treatments are presented in Table 6. Counts of culturable bacteria, fungi and biphenyl degrading bacteria in the soil were increased from all the inoculated treatments than from the uninoculated control. Moreover, the bacterial counts in 20% inocula were significantly higher than in corresponding 10% inocula treatment ($p < 0.05$) but there was no significant differences in counts of fungi or biphenyl degrading bacteria between the two inocula treatments (Tu*et al.,* 2011). Percent biodegradation of single congener from the 21 PCB mixtures was calculated for each treatment. Inoculation of *S. meliloti* significantly increased the percent biodegradation of all the 21 PCB congeners at the end of the experiment (Fig. 3). In the uninoculated control, the percent biodegradation of 21 PCB congeners varied from 7.8% to 100%, with only 5 of them got a percentage higher than 50%. While in the *S. meliloti* inocula treatments, 14 of the 21 PCB congeners received more than 50% degradation. Moreover, *S. meliloti* inoculation was proved to shorten the time for PCB 126 and PCB 200 depletion from 20 days to less than 10 days (Tu *et al.,* 2011).

In conclusion, *S. meliloti* plays an important role in the biodegradation of PCBs in liquid cultures and soil. The biotransformation product of 2,4,4-TCB by *S. meliloti* was 2-hydroxy-6-oxo-6- phenylhex-2,4-dienoic acid (HOPDA). Inoculation with *S. meliloti* may greatly increase the counts of soil culturable biphenyl degrading microbes. However, further studies are needed to investigate the metabolic pathway of PCB degradation by *S. meliloti* and the genes that encoding the key enzymes in the pathway. These results may provide evidence for the potential application of Rhizobia in bioremediation of PCB-contaminated soil (Tu *et al.*, 2011).

Johnson *et al.*, (2005), reported that the soil microbial biomass did not differ between treatments at the start of the experimental period (Fig. 6). However, by the end of the experimental period there was a significantly greater biomass in the inoculated planted treatments than in the unplanted treatments (P=<0.01). No significant differences were observed between planted treatments and planted inoculated treatments.

Treatments

Planted with host legume (Claver + Ryegrass), Rhizobia: *Rhizobium leguminosarum*bv. *Trifolii.*

Johnson *et al.*, (2005), reported that the most probable number of PAH degraders was influenced by planting regime (Fig. 4). Initially there were only small differences between the numbers of PAH degraders. However, after 180-days, numbers of microorganisms capable of degrading PAHs were greater in the planted treatments relative to the unplanted treatments. However, unlike total soil biomass, the planted treatment that had received a rhizobial inoculum had a greater number of PAH degraders than the planted treatments with no inoculum.

Treatments

Planted with host legume (Claver + Ryegrass), Rhizobia: *Rhizobium leguminosarum*bv. *Trifolii.*

Inoculated plots had an average of 1.5 clover nodules g^{-1} soil as opposed to 0.7 nodules g^{-1} soil in the planted treatment that had received no inoculum. The presence of 0.7 nodules g^{-1} confirms that indigenous rhizobia were present in the soil. However, both root and shoot growth were also greater in the inoculated plots (Table 7), confirming that the inoculum did have a positive impact upon plant vigour (Johnson *et al.*, 2005).

The influence of the inoculated rhizobia is reflected in a larger shoot and, most importantly, root biomass in the inoculated pots (Table 7). Microbial measurements reveal that the microbial biomass was significantly greater in planted treatments than in unplanted treatments (Fig. 6). However, there is no significant difference between the biomass of planted treatments that had received a rhizobial inoculum and those that had not. In contrast, the numbers of PAH degraders increased in the presence of a rhizobial inoculums (Fig. 7). This suggests that selective enhancement of PAH degraders within the rhizosphere leads to enhance PAH loss (Johnson *et al.*, 2005).

In conclusion, our results support the hypothesis that the enhanced dissipation of PAHs in the rhizosphere was due to the stimulation of the microbial community within the soil rhizosphere. However, this loss was only greater in soils that received a rhizobial inoculum. It is therefore likely that rhizobia play an important role in the rhizoremediation of high molecular weight PAHs. It would appear that microbes responsible for PAH degradation are selectively enhanced within the rhizosphere of soil that has received a

rhizobial inoculum. The exact mechanisms involved in this process are not revealed and further work is required to further elucidate the processes involved (Johnson *et al.*, 2005).

Teng *et al.*, (2016), reported that the biodegradation of 3,3,4,4-TCB (PCB77) by strain ZY1 in a liquid culture is presented in Figure 8. After 10 days of incubation, the concentration of PCB77 decreased in both treatments. A significant difference between the active and inactivated (control) ZY1 treatments ($P < 0.05$) was observed. PCB77 concentrations in treatment with active ZY1 were 14.58 mg L^{-1}, 6.39 mg L^{-1}, 6.07 mg L^{-1}, and 5.91 mg L^{-1} on days 0, 4, 7, and 10, respectively. A total of 62.7% of PCB77 was degraded after 10 days of incubation. The loss of PCB77 in the control was possibly caused by non-biological factors, such as volatilization during the extracting process due to the volatile physicochemical property of PCB77.

Treatments

Control: Inoculated with sterilized strain ZY1; Inoculated with strain ZY1.

The concentration was 9.41 mg kg^{-1} dry soil when active ZY1 was inoculated in the soil, but it decreased to 3.55 mg kg^{-1} dry soil after 28 days of bioremediation. The concentration of PCBs in the control inoculated with sterilized ZY1 was 9.93 mg kg^{-1} dry soil and 8.38 mg kg^{-1} dry soil, respectively. Inoculation with active ZY1 significantly degraded PCBs compared with control ($P < 0.05$). A remarkable enhancement in the total PCB degradation was also observed (57.6%) in the active inoculants treatment (Teng *et al.*, 2016).

Teng *et al.*, (2016), reported that the bacterial inoculation significantly enhanced the numbers of bacteria able to use PCBs as the sole carbon source in the soil compared with control (Fig. 10). The bacterial population in the active ZY1 inoculation treatment was 6.4 X 10^5 MPN g^{-1}, 4.0 X 10^6 MPN g^{-1} and 4.7 X 10^6 MPN g^{-1} dry soil at days 0, 7, and 14, respectively. These values are 7.1 = 8.9 times that of the native PCB-degrading bacteria of the control.

Control (C): Soil inoculated with sterilized bacteria ZY1;

Treatment (T): Soil inoculated with bacteria ZY1.

PCBs were extracted and detected at days 0, 4, 7, 14 and 28.

The mean percent composition of di-, tri- and tetra-PCBs in different treated soils during the cultivation is shown in Figure 11. The percentage of tetra-CB reduced in active ZY1 inoculated treatment, which was mainly caused by bacterial degradation. With the degradation of tetra-CB, the proportion of tri-CB increased. The percentage of di-CB increased to 36.7% in the first 7 days and then decreased to 14.7 % at the 28th day (Teng *et al.*, 2016).

Teng *et al.*, (2016), reported that the combined remediation effects of planting *A. sinicus* inoculated with *Mesorhizobium sp.* ZY1 are presented in Figure 12. After 100 days of cultivation, there were significant differences between all treatments and the control ($P < 0.05$). The final PCB concentrations of all treatments ranged from 111.5 = 189.1 mg kg^{-1} dry soil, while the unplanted and uninoculated control was 237.9 mg kg^{-1}. The results showed that the soil PCB concentrations of the single incubation of *Mesorhizobium sp.* ZY1 (R) and single planting *A. sinicus* (P) were decreased by 20.5% and 23.0%, respectively. Planting *A. sinicus* inoculated with *Mesorhizobium sp.* ZY1 decreased PCBs amount in soil by 53.1% compared with control. This indicated that

planting *A. sinicus* inoculated with ZY1 had a better effect on the transformation of PCBs than single planting or inoculation.

Treatments

Control (CK): soil with neither planting *Astragalussinicus L*. nor inoculated with bacteria ZY1

Rhizobia (R): soil inoculated with bacteria ZY1 only

Plant (P): soil planting *Astragalussinicus L*. (Chinese milk vetch) only

Plant + Rhizobia (PR): soil treated with *Astragalussinicus L*. and bacteria ZY1.

The combined remediation effects of planting *A. sinicus* inoculated with *Mesorhizobium sp*. ZY1 are presented in Figure 12. After 100 days of cultivation, there were significant differences between all treatments and the control ($P < 0.05$).

The final PCB concentrations of all treatments ranged from 111.5 = 189.1 mg kg^{-1} dry soil, while the unplanted and uninoculated control was 237.9 mg kg^{-1}. The results showed that the soil PCB concentrations of the single incubation of *Mesorhizobium sp.* ZY1 (R) and single planting *A. sinicus* (P) were decreased by 20.5% and 23.0%, respectively. Planting *A. sinicus* inoculated with *Mesorhizobium sp*. ZY1 decreased PCBs amount in soil by 53.1% compared with control. This indicated that planting *A. sinicus* inoculated with ZY1 had a better effect on the transformation of PCBs than single planting or inoculation (Teng*et al.*, 2016).

Regarding *A. sinicus* planting treatments, Figure 13 shows the fresh biomass of *A. sinicus* and the PCB concentration in *A. sinicus* after harvest. The biomass of *A. sinicus*inoculated with *Mesorhizobium sp*. ZY1 (PR) was significantly higher than that of single A. sinicus planting (P) ($P < 0.05$), and the PCB concentrations in *A. sinicus* exhibited the same trend (Teng*et al.*, 2016).

Johnson *et al.*, (2004), reported thatthe populations of rhizobia were monitored throughout the 180-day rhizo remediation trial. At the end of the experimental period, the treatments that had received inocula contained significantly larger viable populations of rhizobia than those that had received no inocula (Fig. 14).

There were six treatments in this trial:

Sterile soil with plants

Sterile soil with plants and rhizobial inoculum

Sterile soil without plants

Unsterilised soil with plants

Unsterilised soil with plants and rhizobial inoculum

Unsterilised soil without plants

Treatments: Host legume: White clover (*Trifoliumrepens*) +Ryegrass (*Loliumperenne* L.)

Rhizobia: *R. leguminosarumbv. trifolii*

This is consistent with the lack of toxicity shown by PAHs towards the indigenous rhizobia and demonstrates good survival of the introduced rhizobial strain throughout the 180-day experimental period.

The higher rhizobial populations in the inoculated pots were reflected in the clover nodulation. Inoculated pots had 2.2 clover nodules g^{-1} soil as opposed to 1.1 nodules g^{-1}

soil in the planted treatment that had received no inoculum. Similarly both root and shoot growths were greater in the inoculated pots (Table 8).

In sterile soil, planted treatments showed slightly higher extractable concentrations of chrysene throughout the 180-day experimental period than the comparative unplanted treatments (Fig. 15), although these differences were not statistically significant ($p>0.05$). However, in non-sterile soils, after 180 days the extractable concentrations of chrysene were significantly lower ($p \leq 0.01$) in planted treatments containing a rhizobial inoculum than planted treatments without an inoculum and unplanted treatments (Fig. 16). Surprisingly, chrysene concentrations were only significantly lower than the unplanted treatments in the planted treatments that had received a rhizobial inoculum. Planted treatments with no inoculum did not show a significant reduction in chrysene concentrations at the end of the 180-day trial relative to similar unplanted treatments (Johnson *et al.,* 2004).

In conclusion we can partially support the hypothesis that the dissipation of chrysene is enhanced in a soil planted with a mixed clover/ryegrass sward. However, this loss is only greater in soils that received a rhizobial inoculum. It is therefore likely that rhizobia play an important role in the rhizo remediation of high molecular weight PAHs. However, in this study the role appears to be by stimulating root growth and therefore soil microbial populations as opposed to direct degradation by the rhizobia themselves. To determine the exact mechanisms involved, the soil microbial populations must be measured during a similar trial, with particular emphasis placed on the response of total soil biomass, soil microbial community structure and, if possible, the response of known PAH degraders within the soil (Johnson *et al.,* 2004).

Heavy metallic compound

Heavy metal

Any metallic chemical element that has a relatively high density and is toxicor poisonous at low concentrations**.** Eg: Mercury (Hg), Cadmium (Cd), Arsenic(As), Chromium (Cr), Thallium (Tl), Lead (Pb) *etc*.,.

There has been increasing concern over heavy metal resistance (*i.e*., arsenic, cadmium, zinc, copper, and lead) in symbiotic rhizobia and its effects on their potential for bioremediation (Hao*et al.,* 2014). Heavy metal resistant strains are commonly isolated from nodules of the metallicolous legume (*i.e*., *Robiniapseudoacacia, Anthyllisvulneraria, and Glycine max)* from mining tailings or contaminated sites.

Remediation of metallic contaminants: Mechanisms

Such mechanisms includes

Changes in the metal efflux of microbial cell membranes;

Intracellular chelation due to the production of metallothione in proteins.

The transformation of heavy metals to their less toxic oxidated forms through microbial metabolism.

The pumping of metal ions exterior to the cell,

Accumulation and sequestration of the metal ions inside the cell,

Biotransformation - transformation of toxic metal to less toxic forms

Adsorption/ desorption of metals.

Based on studies in other bacteria, the metal resistance of rhizobia might be attributed to (Fig. 17): (i) changes in the metal efflux of microbial cell membranes; (ii) intracellular chelation due to the production of metal lothionein proteins (Nies, 1995); and (iii) the transformation of heavy metals to their less toxic oxidated forms through microbial metabolism (Nies, 2003). For example, the increased contents of reductive agents (*i.e.*, glutathione concentrations) in microbial cells might reduce the toxicity of cadmium, there by contributing to cadmium resistance (Bright and Bulgheresi, 2010). Moreover, the metabolism of rhizobia also increases metal bio availability in the soil through alterations in the soil pH, resulting in the release of chelators (*i.e*., siderophores) and organic acids capable of enhancing the complexation of metals and their mobility (Schalk *et al.*, 2011). Microbial volatilization is another preferred method of metal bio-removal (*i.e.*, selenium and mercury) in many rhizosphere bacteria (Souza *et al.*, 2001; Zhang *et al.*, 2012), although the mechanisms for the volatilization of metals in rhizobia have yet to be identified. Studies have suggested that engineering rhizobia for the volatilization of heavy metals could be a valuable avenue for tackling soil pollution. For example, Chen *et al.*, (2014) demonstrated that *Pseudomonas putida*KT2440 endowed with the *ars M*gene encoding the As (III) *S. adenosyl* methionine (SAM) methyl transferase from *Rhodopseudomonas palustris* could remove arsenic from contaminated soil through microbial arsenic methylation and volatilization.

The concentration of lead in polluted soils of mathura road and exhibition ground, aligarh and non-polluted soils of faculty of agricultural sciences, AMU, Aligarh, India was determined by atomic absorption spectrophotometer. The concentration of lead in polluted soils of Mathura road exhibition ground was 195 and 191 mg kg^{-1} soil, respectively.

Wani and Khan (2012) reported that the strain RL9 showed plant growth promoting activities like IAA, siderphore, HCN and ammonia under in vitro conditions. The lead tolerant strain RL9 demonstrated the production of substantial amounts of Indole Acetic Acid (IAA) during 24 hr growth in Luria Bertain broth supplemented with 20, 60 and 100 mg /ml of tryptophan. A maximum amount of IAA (33mg /ml) was observed in LB Broth having 100 mg/ml of tryptophan, which was followed by 15.2 and 6.4 mg/ml of IAA at 60 and 20 mg tryptophan/ ml respectively (Table 9).

Wani and Khan (2012) reported that the generally, length, total dry weight and nodulation at 90 DAS, decreased progressively with increase in the concentration of lead. Lead at 390 mg/kg soil had the greatest phytotoxic effect and significantly ($p \leq 0.05$) decreased the length of roots and shoots by 33 and 39 %, nodule numbers and nodule dry weights by 42 and 33% and total dry weight at 90 DAS by 22%, at 390 mg/kg of lead compared to control plants. In contrast, plant inoculated with strain RL9 increased the measured parameters, even in the presence of different concentrations of lead (Table 10). Rhizobial strain RL9 when used with 195 mg pb/kg had the highest stimulatory effect and increased the root length, shoots length, nodule numbers, nodule dry weight and total dry weight by 67, 87, 100 138 and 172 % at 90 DAS, compared to un-inoculated but amended with 195 mg pb/kg soil in the presence of bio inoculants.

Wani and Khan (2012) reported that the chlorophyll, leghaemoglobin and N content in roots and shoots at 90 DAS decreased consistently with increase in the concentration of nickel (Table 11) without the inoculation of

strain RL9. Lead at 390 mg pb/kg had the greatest effect on the photosynthetic pigments of lentil plants and decreased the chlorophyll, leghamoglobin and root N and shot N by 7, 44, 9 and 5 %, respectively compared to un-inoculated control (Table 11). On the other rhizobium Sp RL9 species with 195 mg pb/kg, increased the chlorophyll, leghaemoglobin and root, leghaemoglobin content in fresh nodules, N content in roots and N content in shoots by 221, 100, 11 and 7%, respectively compared to un-inoculated but having 195 mg pb /kg soil. Furether more, chlorophyll, leghaemoglobin content, N content in roots and shoots also increased even at 390 mg pb /kg soil in the presence of rhizobium speces RL9 (Table 11).

The uptake of lead by plant organs (roots and shoots) at 90 DAS was maximum at 390 mg pb/kg of lead (Fig. 18) both in the presence and absence of bio- inoculants. Moreover, the accumulation of lead in roots and shoots were less in the presence of bio- inoculants RL9 compared to the un-inoculated but lead amended plants. Generally, roots accumulated more concentrations of lead compared to those observed for shoots, under both inoculated and metal stressed condition (Wani and Khan, 2012).

The dry weight (DW) of soybean roots ($F = 3.17$, $df = 3$, $P = 0.047$) and shoots ($F = 21.28$, $df = 3$, $P<0.001$) decreased as the As concentration of the solution increased and was greater in the roots ($F = 4.87$, $df= 1$, $P =0.039$) and shoots ($F = 6.75$, $df = 1$, $P = 0.017$) of inoculated compared to non-inoculated plants (Fig. 19). The mean total DWs of inoculated plants were 0, 31, 32 and 38 % greater than non-inoculated plants in the 0, 1, 5 and 10 mMAs treatments respectively. However, the as in solution by inoculation interaction was not significant for the DW of roots ($F = 1.77$, $df = 3$, $P = 0.184$) or shoots ($F = 0.57$, $df = 3$, $P =0.640$) (Reichman, 2007).

Soil samples were collected in situ at four different zones with regard to the toxic spill. Zone 0 is the nearest zone to the pond where the dam collapsed, between 100 and 500 m from the pond. Two other zones (1 and 2) situated approximately 5 and 10 km down the Guadiamar River respectively and zone 3 was located 1 km upstream the spill. The content in all toxic elements was much higher in soils from Aznalco´llar, especially in zones 0 and 2 (Table 12). In the case of As, Pb and Cu the concentrations of these elements are from 20 to 100 fold the concentration found in the control soil. Furthermore, changes over the past 3 year showed an increase of the content in toxic elements at the most contaminated zone 2 in spite of mechanical work to clean the area (Jose *et al.*, 2005).

As shown in Table 14, the *S. meliloti* Alf 12 has the capability of bio-accumulating 3-fold more As and 20-fold more Pb than the non-resistant strain Ism6. The most part of As and Pb are adsorbed to cell surface of Alf12, although there is also a 20% of the total Pb accumulated inside the Alf12 cell. For these strain we observed a great amount of polysaccharide in pellets (not shown), thus it could be that the metal was adsorbed to the polysaccharide, as it has been widely reported (Valls and de Lorenzo, 2002). This might be an indication of a great potential of this strain in bioremediation experiments. On the other hand, the Cu resistant strain Med4D has a similar behavior, and it is able to bio-accumulate up to 3-fold more Cu than the control strain, being 75% of the accumulated Cu located in the cell surface (Jose *et al.*, 2005).

The symbiotic characteristics of 10 S. meliloti strains isolated from contaminated soils are shown in Table 15. We found that non-inoculated plants had very low mass and N content in shoots and showed severe symptoms of N deficiency (not shown).

Table.1 Type and source of the most relevant group of soil contaminants

Contaminants	Example of compounds	Sources of contamination
Heavy metals	Cu, Zn, Cd, Pb, Hg, Cr	Application of animal manure (D) Military facilities (P) Gasoline stations (P) Sawmills and wood preservation sites (P) Mining and metallurgical industry (P,D)
Oil hydrocarbons, chlorinated compoundes	Alkanes, alkenes, cycloalkanes, PCBs	Oil industry (P,D) Manufacture of pesticide and herbicide (D) Wood preservation site (P) Pulp and Paper production (P) Municipal waste incineration (P,D) Plastics, fire-retardants manufacture (P,D)
Monomeric aromatic hydrocarbons	Benzene, toluene, ethylbenzine, xylene	Oil industry (P.D) Gasoline station (P)
PAHs	Benzo(a)pyrene, chrysene, fluoranthene	Oil industry (P.D) Gasoline station (P) Manufactured gas plants (P, D) Wood preservation sites (P) Municipal waste incineration (P,D) Automobile exhaust (D)
Nitroaromatics	Nitrobenzene, nitrophenols, atrazine	Manufacture of aniline, dyes, drugs (P, D) Explosive industry, military facilities (P,D) Manufacture of pesticides and herbicides (D)

P-point contamination D-diffuse contamination (Valentin *et al*., 2013)

Table.2 Rhizobia and their bio-partners available species (Rivas *et al*., 2009)

Genus	No. of species	Major host plants
Rhizobium	33	*Pisum, Phaseolus, etc.,*
Sinorhizoum	12	*Acacia, Medicago, etc.,*
Mesorhizobium	19	*Cicer, Prosopis, etc.,*
Bradyrhizobium	08	*Glycine, Pachyrhizum, etc.,*
Azorhibium	02	*Sesbania*

Table.3 Contribution of *Rhizobium* in biological N fixation

Crop	*Rhizobium* species	Quantum of N fixed (kg N/ha/yr)
Soybean	*R. japonicum*	60-80
Alfalfa	*R. meliloti*	100-200
Clover	*R. trifolii*	100-200
Groundnut	*R. sp.*	50-60
Pea	*R. leguminosarum*	52-77
Cowpea	*R. sp.*	80-85
Green/ Black gram	*R. leguminosarum*	50-55
Bengal gram	*Bradyrhizobium sp.*	85-110

Table.4 Plant growth promoters by *Rhizobia* and their bio-partners

Rhizobia	Plant growth promoting substances	References
Rhizobium sp. (pea)	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2012)
Rhizobium sp.(lentil)	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2011)
Rhizobium phaseoli	IAA	Zahir*et al*. (2010)
Mesorhizobium sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2012)
Rhizobium leguminosarum	IAA, siderophores, HCN, ammonia, Cytokinin	Wani*et al*. (2007)
Bradyrhizobiumjaponicum	IAA, Siderophores	Shaharoona*et al*. (2006)
Bradyrhizobium sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2012)
Bradyrhizobium sp.	IAA, siderophores, HCN, ammonia	Wani*et al*. (2007)
Bradyrhizobium sp. 750,	Heavy metal mobilization	Dary*et al*. (2010)
Mesorhizobium sp.	IAA, siderophores, HCN, ammonia	Wani*et al*. (2008)
Mesorhizobiumciceri	IAA, siderophores	Wani*et al*. (2007)

Subramaniam *et al*. (2015)

Table.5 Total concentration of 21 PCB-Congener-Mix in soil microcosms under different treatments

Times (days)	Control		10 % inocula		20 % inocula	
	Concentration (mg/kg)	Biodegradation (%)	Concentration (mg/kg)	Biodegradation (%)	Concentration (mg/kg)	Biodegradation (%)
Initial	335.9 ± 9.1	0	335.9 ± 9.1	0	335.9 ± 9.1	0
0	328.1 ± 9.1	2.5±2.3b	317.8 ± 9.9	5.4±3.0b	296.4± 11.6	11.8±3.5a
5	293.9 ± 1.8	12.5±0.5c	270.0 ± 2.8	19.6±0.8b	246.5 ± 5.2	26.6±1.5a
10	238.2 ± 4.6	29.1±1.4c	182.5 ± 3.8	45.7±1.1b	156.0 ± 7.3	53.5±2.2a
20	206.9 ± 3.3	38.4±1.0c	178.4 ± 1.5	46.9±0.4b	146.0 ± 4.4	56.5±1.3a
30	198.0± 17.6	41.1±5.2b	135.1± 14.3	59.8±4.3a	126.7 ± 3.9	62.3±1.2a

Table.6 Soil cultural bacterial, fungal and biphenyl-degrading bacterial counts

Soil microbial counts	Control	10 % inocula	20 % inocula
Bacteria (10^7 cfu /g)	0.7 ± 0.6b	2.7 ± 0.6b	7.3 ± 3.1a
Fungi (10^6 cfu /g)	0.4 ± 0.1b	1.4 ± 0.3a	1.5 ± 0.3a
BD Bacteria (10^5 MPN/g)	0.4 ± 0.1b	1.8 ± 0.4a	2.5 ± 0.7a

Table.7 Influence of *Rhizobial* inoculum on dry mater of root biomass and shoot weight at end of the 180 day pot trail

Treatment	Root biomass mg /g soil	Shoot weight g/pot
Planted + inoculated	8.21 (0.92)	2.23 (0.18)
Planted	4.13 (0.56)	1.59 (0.36)

Table.8 Influence of *Rhizobial* inoculum on root and shoot growth at end of the 180 day experimental period

	Root density(mg/ g soil)	Shoot weight/1 kg pot
Planted + inoculated	10.40 (0.82)	5.26 (0.22)
Planted	6.24 (0.68)	3.79 (0.36)

Table.9 Plant growth promoting activities of *Rhizobium* species RL9

PGP activities	values	Where,
Sidarophores		T: Tryptophan,
CAS agar (mm)	12±2	+: Positive
SA (mg /l)	15±2	-: Negative for the strain,
2,3 DBA (mg /l)	18.3±3	CAS: Chrome Azurol S agar,
IAA (micro gram /ml)		SA: Salicylic acid,
20 T	6.4±0.9	DBA: Dihydroxy benzoic acid,
60 T	15.2±1.5	IAA: Indole acetic acid,
100 T	33±3.0	HCN: Hydrogen cyanide,
HCN	+	
Ammonia	+	

Table.10 Effect of inoculation with *Rhizobium* sp. RL9 on biological characteristics of lentil subjected to different soil lead concentrations

Treatment		Length /plant (cm)		Dry weight (mg / plant)		Nodulation		Total dry weight (mg /plant)
	Pb (mg/kg soil)	Root	shoot	Root	Shoot	No. /plant	dry weight (mg/plant)	
Uninoculated	Control	21c	18c	44c	123c	12e	13c	180d
	97.5	21c	18c	40b	118c	10c	8b	166c
	195	18b	15b	38b	110b	8b	8b	156b
	390	14a	11a	33a	102a	7a	6a	141a
Inoculated	Control	24d	21d	60d	125d	13f	15d	200e
	97.5	29e	26e	101d	205a	14g	16d	322f
	195	30e	28e	135e	270e	16h	19e	424g
	390	20c	20c	47c	128d	11d	13c	188d
LSD		1.4	2.0	3.7	6.6	0.7	1.1	8.4
F-value								
Inoculation (df=1)		233	486	1953	457	505	502	1909
Metals (df=3)		54.1	62.4	370	116	71.7	44	398.6
Interaction (df=3)		15.1	30.2	349	120	57.7	29.1	406.5

Table.11 Effect of inoculation with *Rhizobium* sp. RL9 on the biological and chemical characteristics of lentil plants subjected to different soil lead concentration

Treatment		Chlorophyll content (mg/g)	Leghamoglobin content (mmol/g FM)	N content (mg/g)	
	Pb (mg/kg soil)			Root	Shoot
Uninoculated	Control	0.28a	0.09b	13.7b	41.0a
	97.5	0.29a	0.08b	13.1a	40.1a
	195	0.28a	0.07a	12.9a	39.7a
	390	0.26a	0.05a	12.5a	38.8a
Inoculated	Control	0.30a	0.10b	14.2b	42.0b
	97.5	0.80b	0.13c	14.2b	42.1b
	195	0.90c	0.14c	14.3b	42.4b
	390	0.60b	0.10b	13.7b	39.4a
LSD		0.27	0.023	1.15	1.8
F-value					
Inoculation (df=1)		256.9	212.6	83.9	21.1
Metals (df=3)		33.2	21.0	9.3	9.9
Interaction (df=3)		31.7	16.6	3.1	1.9

Table.12 Distribution of rhizobium strains resistant to As, Cu and Pb isolated from Aznalcollar soils

Element	Host plant	Number of resistant strains	Nodulation test
As	Medicago sativa	9	+
	Trifoliumsubterraneum	1	+
	Lotus corniculatus	0	0
Cu	M. Sativa	3	+
	T. Subterraneum	1	+
	L. corniculatus	1	+
Pb	M. Sativa	18	+
	T. Subterraneum	7	+
	L. corniculatus	2	+

Table.13 Bioaccumulation and bioadsorption of toxic elements in resistant and non-resistant strains from *Sino rhizobium meliloti*

Concentration of toxic element	*Sinorhizobiummelilotis*train	Bioaccumulation + bioadsorption (mg /kg dry matter)	Bioaccumulation (mg/kg dry matter)
As (200 mg/l)	Alf 12	60.6/62.4	21.3/30.3
	Ism6	21.6/18.9	19.0/22.2
Pb (500 mg/l)	Alf 12	193.7/304.0	42.6/47.1
	Ism6	10.7/12.5	9.4/8.5
Cu (125 mg/l)	Med4D	912.0/804.2	253.3/216.3
	Ism6	304.3/335.3	85.9/70.7

Table.14 Symbiotic effectiveness of *Sino rhizobium meliloti* strains isolated on Alfalfa from contaminated Aznalcollar soils

Sinorhizobium meliloti strain	Number of nodules /plant	Nodules fresh weight (micro g/plant)	Shoot dry weight (mg/plant)	N content in shoots (%)
l-1	0.56	120	10.6	1.2
l-2	1.56	250	9.75	ND
l-4	1.00	140	6.94	ND
l-5(Alf2L4)	11.4	5100	22.5	2.07
l-7	0.34	120	6.5	ND
l-8	2.77	360	4.1	ND
l-9 (Alf 12)	17.4	12500	70.0	2.57
4 (Med4D)	8.6	2690	27.5	1.83
2-3	0.14	170	9.5	1.06
2-4	1.8	840	6.8	1.05
Ism6	12.1	4900	32.3	2.24
Non inoculated	0	0.0	8.5	1.1

Table.15 Atrazine removal from soil by different bio-augmentation treatments at different time

Treatments	Atrazine (500 mg/L) Time (days)			
	5	10	20	40
Trichoderma sp. (T)	114.9452b	168.5607a	442.3395d	447.1820d
Rhizobium sp. (R)	293.1485c	302.2429c	256.7403c	417.5677c
Rhizobium + *Trichoderma* (RT)	284.418c	198.7616a	219.523c	403.6424c
Negative Control (NC)	29.6909b	67.0079b	59.9570b	207.0662b
Positive Control (PC)	119.8873a	174.3655a	112.2000a	147.4810a
Standard error	±12.8536	±12.8536	±12.8536	±12.8536

Table.16 Atrazine removal from soil by phytoremediation and bio-augmentation in greenhouse

Treatments	Atrazine concentration (mg /L) and removal (%)			
	1000	%	2000	%
Bean + *Trichoderma* sp. (BT)	558.5515e	55.85	1532.603e	76.63
Bean + Rhizobium sp. (BR)	313.7177d	31.37	1224.677d	61.23
Bean + Rhizobium + *Trichoderma* (BRT)	434.9978c	43.49	1248.323d	62.41
Bean (B)	244.4174b	24.44	1040.574c	52.02
Negative Control (NC)	98.9121a	9.89	391.000b	19.55
Positive Control (PC)	130.8838a	13.08	885.453a	44.27
Standard error	±32.4016		±51.6385	

For each line, letters indicate significant difference (with a D 0.05, Tukey).

Conventional Methods Vs Bioremediation

Conventional Methods	Bioremediation technology
Costly	Cost-effective
Create new waste	Eliminate problem to a greater extant
Does not eliminate problems	Generate no or low waste
Low public acceptance	High Public acceptance

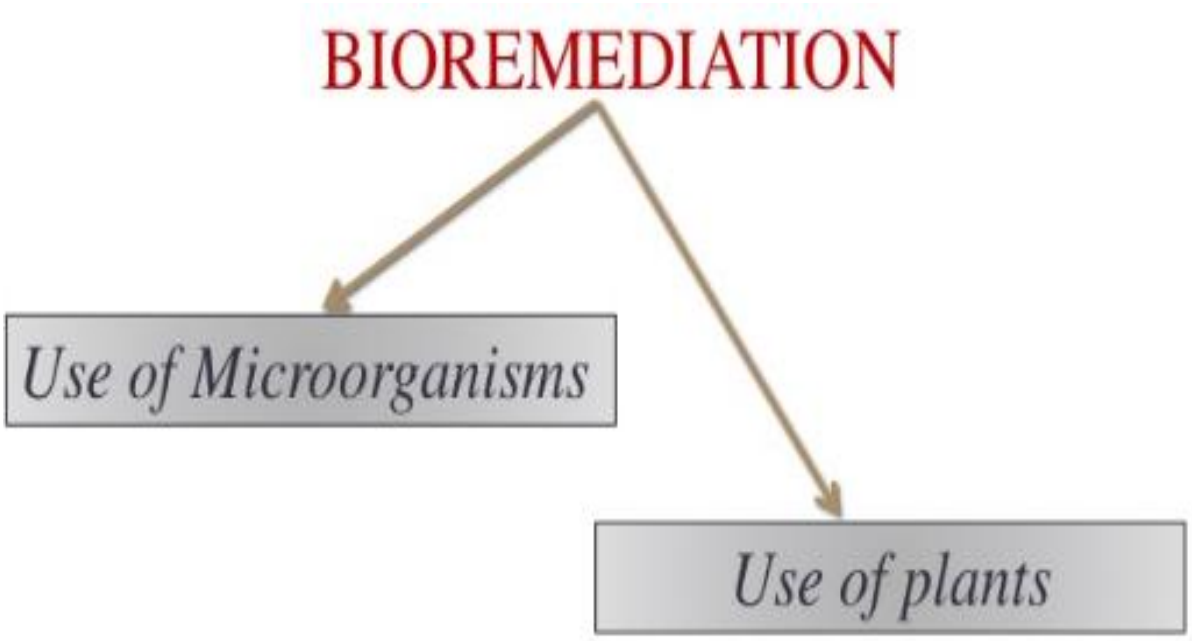


Fig.1 Schematic drawing representing the N-fixing process associated with rhizobia

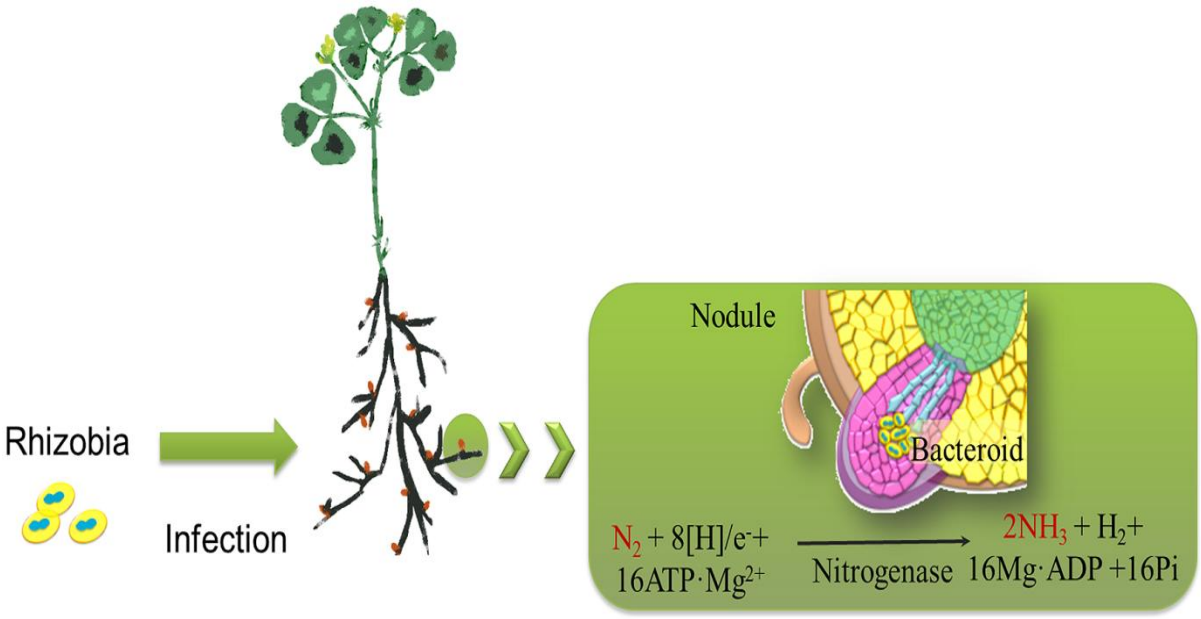


Fig.2 Time course of 2,4,4-TCB concentration and % biodegradation in *S. meliloti* 1021 resting cells

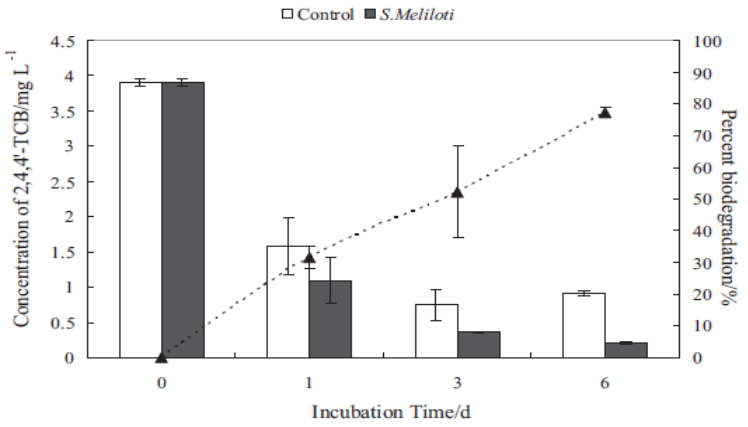


Fig.3 Mean percent biodegradation of PCB congeners in the soil under different treatments

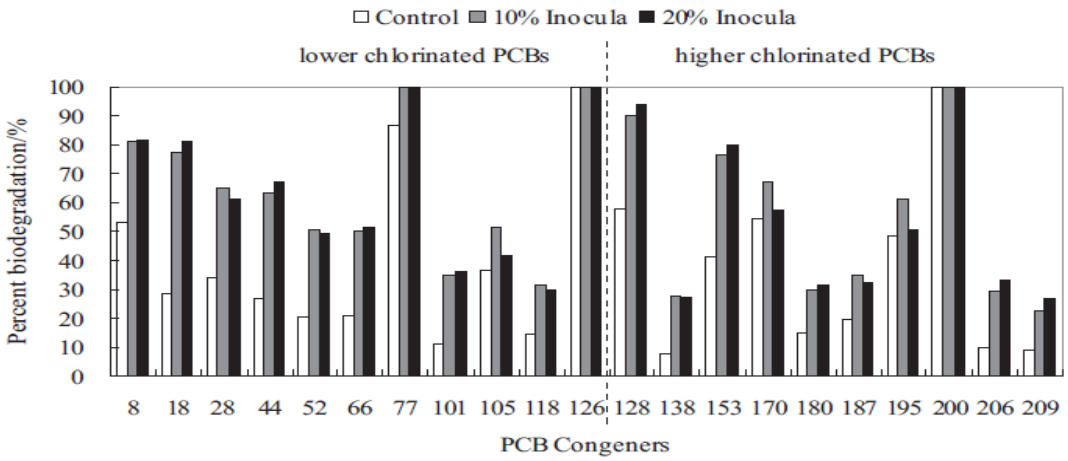


Fig.4 Most probable number of PAH degrading microbes in soil at the end of the 180 day experiment

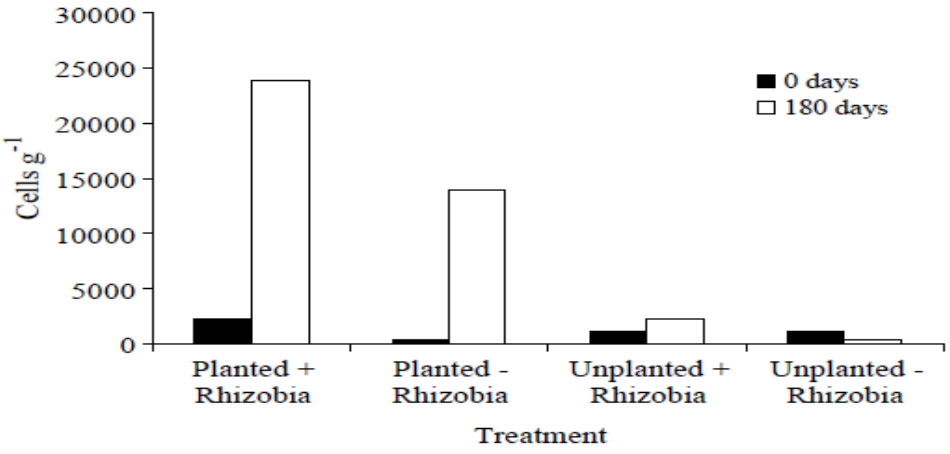


Fig.5 Degradation of PCB77 by strain ZY1 in basic salt medium. And Fig. 6: Degradation of PCBs in artificially contaminated soil by strain ZY1. And Fig. 9. Shows the content of PCBs in the artificially contaminated soil

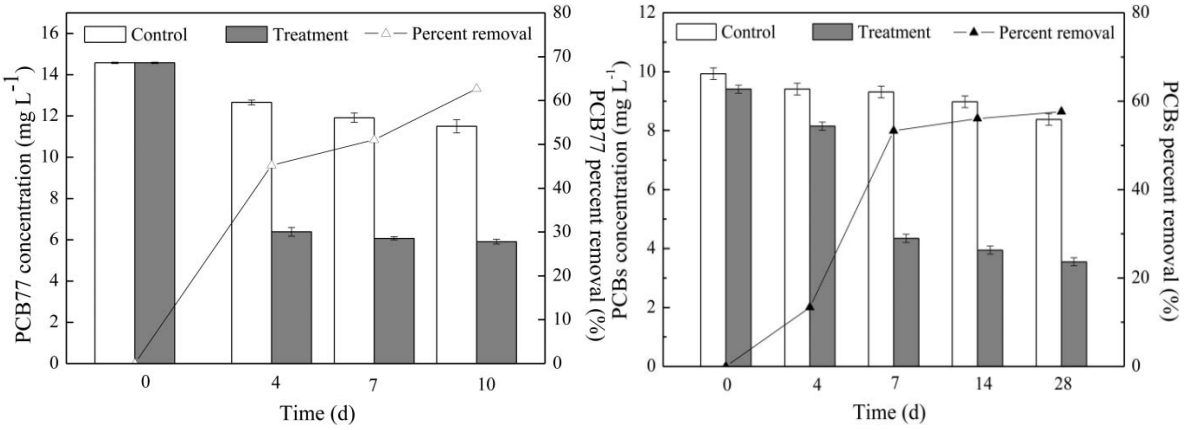


Fig.6 Changes in soil microbial biomass throughout the 180 day pot trail

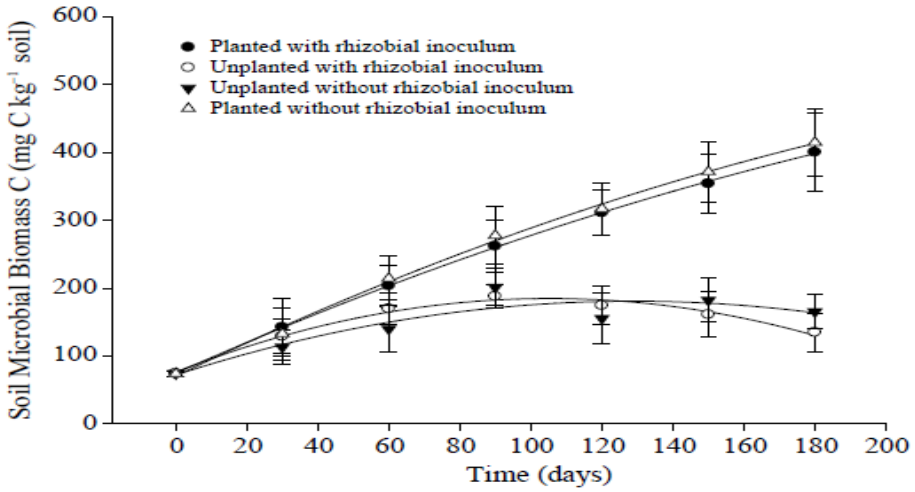


Fig.7 Concentration of PCBs in different treated soils. **Fig.8** Plant fresh biomass and PCB concentrations in plants

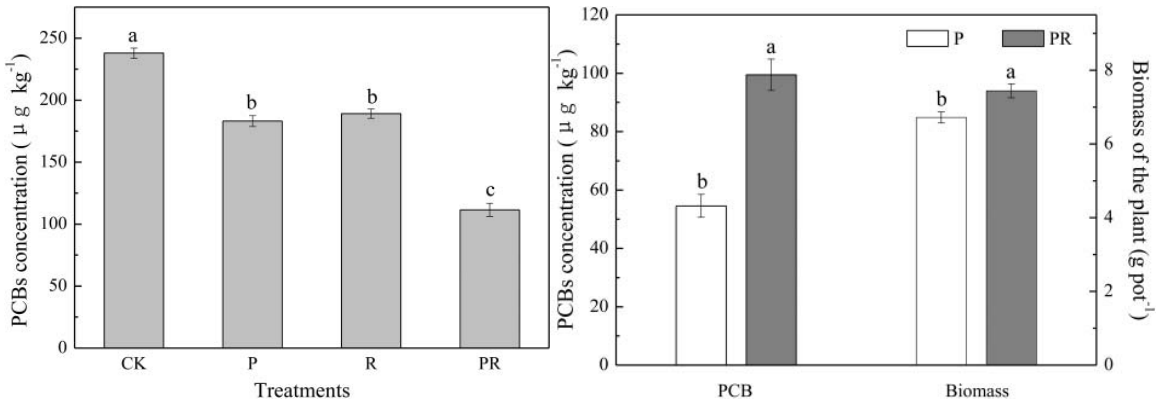


Fig.8 Populations of *rhizobia* in soils at the end of the 180 day experimental period

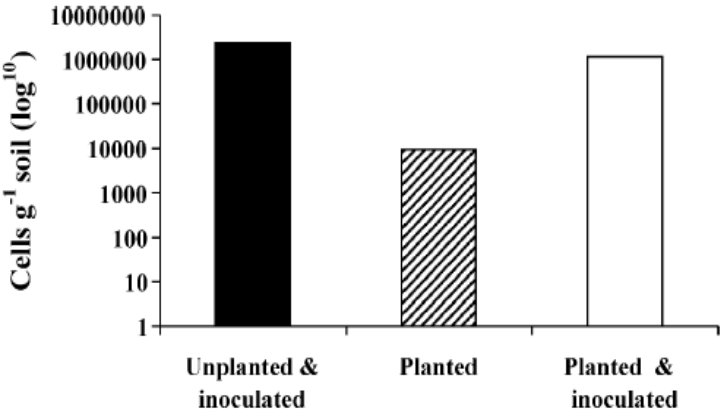


Fig.9 Numbers of soil biphenyl-degrading bacteria for different treatments. And **Fig.10** The mean per cent composition of PCB homologues in artificially contaminated soil

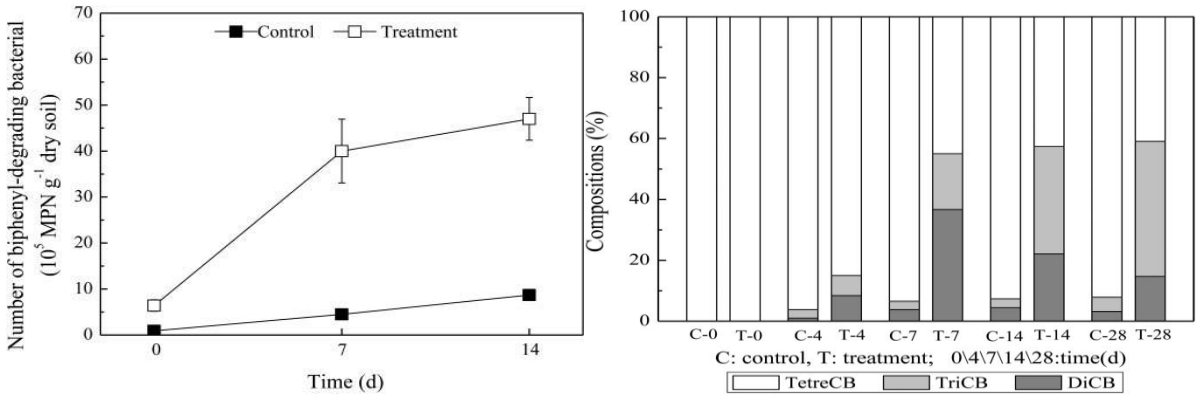


Fig.11 Total extractable chrysene concentrations throughout the 180 day experimental period in sterile soils. And **Fig.12** Total extractable chrysene concentrations throughout the 180 day experimental period in non-sterile soils

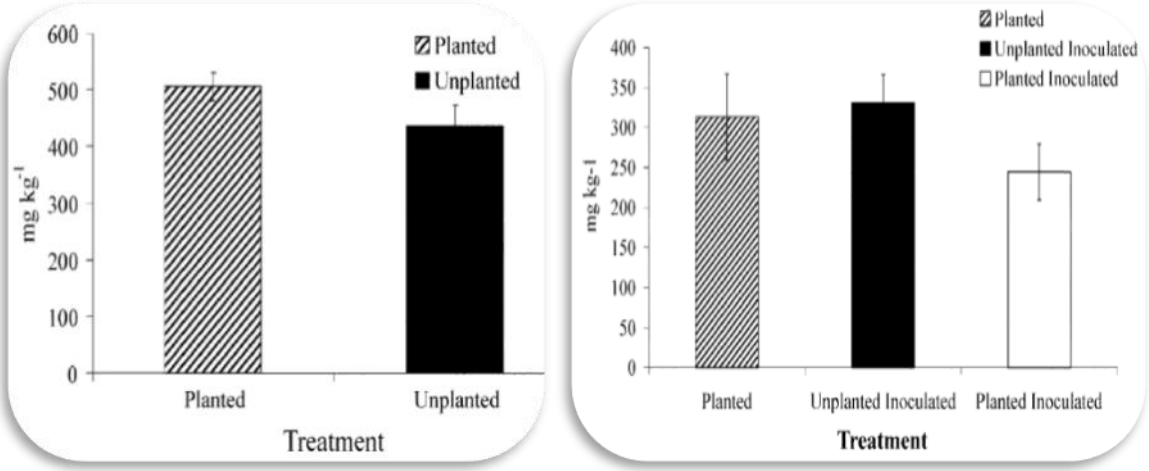


Fig.13 The biodegradation mechanisms involved in the legume- rhizobia symbiosis for the removal of heavy metals

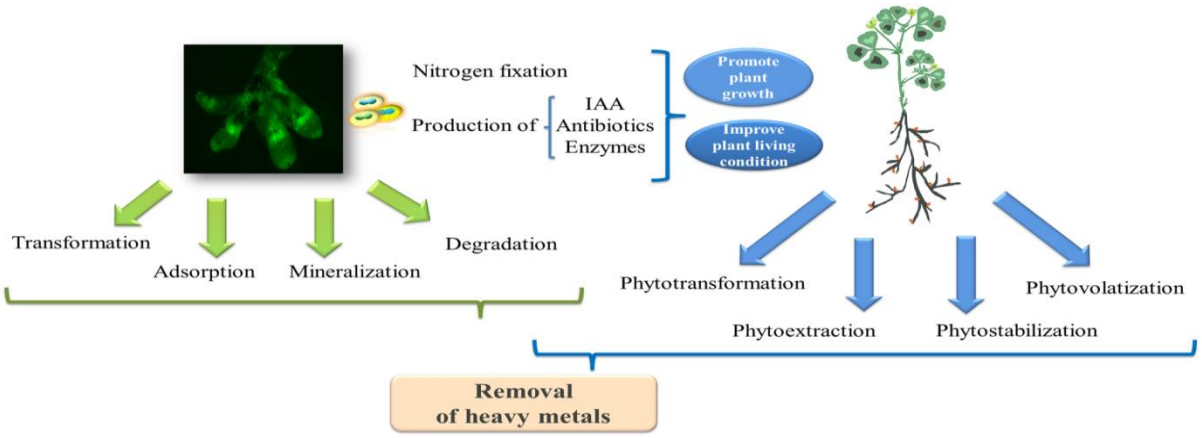


Fig.14 Lead accumulation in (a) roots and (b) shoots at 90 days after seeding lentil in the absence and presence of bioinoculant strain *Rhizobium* RL9 with 97.5, 195 and 390 mg Pb /kg soil.

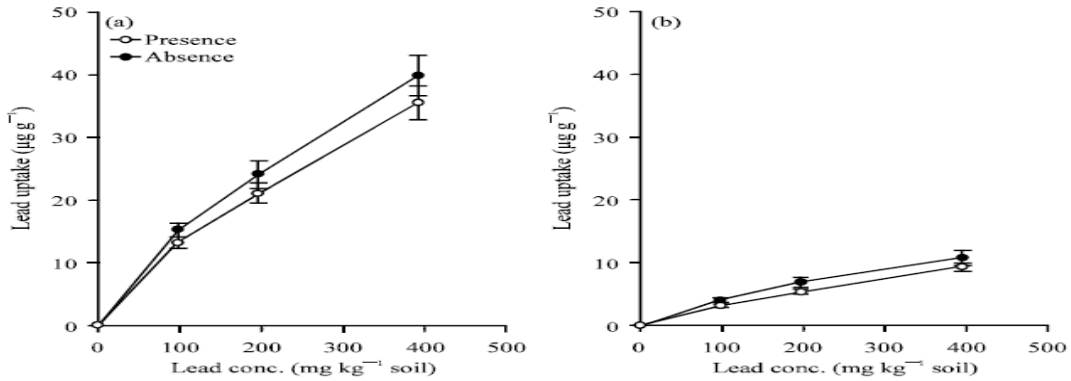
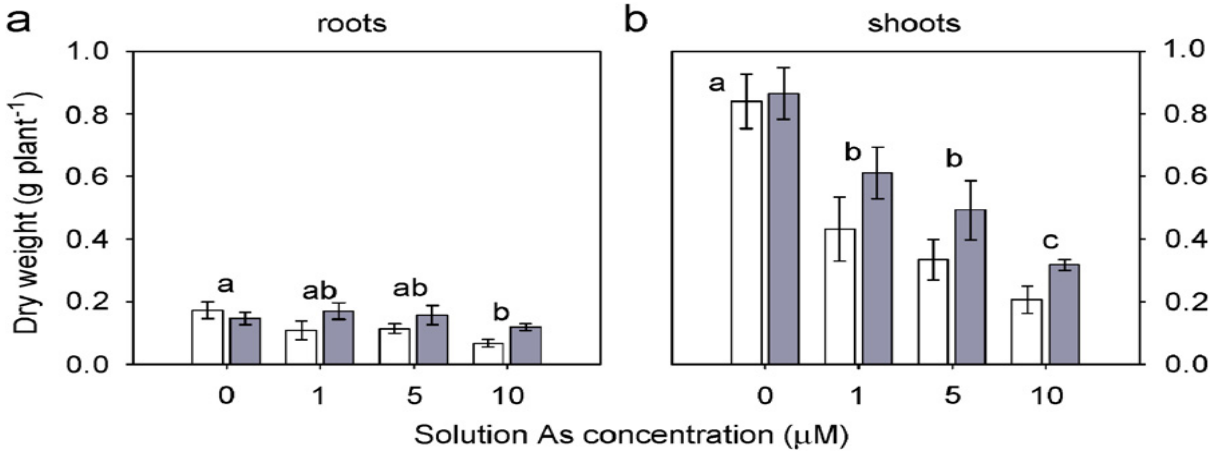


Fig.15 Effects of the concentration of As in the nutrient solution on the dry weight of (a) roots and (b) shoots of soybean grown without (white) or with (grey) the addition of *Bradyrhizobium japonicum* inoculum to the solution



On the other hand, plants inoculated with strains Alf12, Alf 2L4 or Med4D looked healthy and the N content in shoots increased significantly to a similar level produced by the effective laboratory strain Ism6 (Jose *et al.,* 2005).

Jose *et al.,* 2005, reported that the Alf12 was one of the most interesting Rhizobium meliloti strains, since it was resistant to the highest concentrations of both As and Pb. Specific nodulation and N2 fixation analyses were performed in non-contaminated and contaminated soils. The number of nodules in plants grown in contaminated soils was about one-third of the nodules found in plants grown in non-contaminated soils. However, the determination of nitrogenase activity in nodules of plants grown in contaminated or control soils did not show significant variations. In fact, nodules size was similar and seemed perfectly functional.

It is necessary to isolate and study the native rhizobial strains from heavy metals contaminated soils, to identify the potential of rhizobium–legume symbiosis of particular strain for the remediation of the affected area. Such studies with their contribution are presented in Table 15. Rhizobia, such as *R. fredii, R. meliloti, R. etli, R. leguminosarumbv. viceae, R. leguminosarumbv. trifolii, Bradyrhizobium sp*. and *B. japonicum* had been evaluated for heavy metal resistance and of which *R. fredii* and *R. meliloti* alone were found to exhibit higher metal tolerance against Tellurium (Te) and Selenium (Se) (Kinkle *et al.,* 1994). Nonnoi *et al.,* (2012) demonstrated differences in the heavy metal resistance spectrum of *S. medicae* and *R. leguminosarumbv. trifolii* strains isolated from mercury-contaminated soils. Heavy metals are reported to cause harm not only to benefiting microbes, but also to host plants. Paudyal *et al.,* (2007) reported the negative effect of heavy metals such as Al, Fe and Mo on two Rhizobium strains and their symbiotic efficiency on host plants. Chaudri *et al.,* (2000) observed greatly reduced symbiosis of *R. leguminosarumbv. viciae* with pea and *R. leguminosarumbv. trifolii* with white clover under Zn toxicity as a consequence of reduced numbers of free living rhizobia in the soil indirectly affecting N fixation and Zn phytotoxicity. Severe yellowing of plants, small leaves, lack of nodules and reduced rhizobial counts has also been observed as the symptoms of heavy metal toxicity in these toxicity affected plants.

Besides nitrogen fixation and heavy metal resistance, some rhizobia exhibit PGP traits under contaminated conditions as reported in soybean cv. Curringa and its rhizobialsymbiont *B. japonicum* at higher arsenic (As) concentrations (Reichman 2007). Guo and Chi (2014) reported cadmium (Cd) tolerant *Bradyrhizobium sp.*

To exhibit several PGP traits including synthesis of IAA, ACC deaminase, siderophores, increased shoot dry weights and high level accumulation of Cd in roots of *Lolium multiflorum* than in un-inoculated control. They also reported that the strain enhanced the extractable Cd concentrations in the rhizosphere, whereas it decreased the Cd accumulation in root and shoot of G. max by increasing Fe availability.

Alfredo *et al.,* (2017) reported that the treatments of 10 mg of atrazine 50 g^{-1} soil under laboratory conditions showed significant differences ($\alpha < 0.05$) between treatments from the fifth day and until day 20. At the end of the experiment (40 days), the amount of atrazine eliminated by *Trichoderma sp*. (T) was significantly decreased compared to both the negative control (NC) and the positive control (PC) at the same time (Table 17).

As for *Rhizobium sp.*, the greatest rate of degradation occurred on day 10, decreasing at day 20 and peaked at 8.35 mg 50 g^{-1} soil (417.5677 mg L^{-1}) at day 40.

This result places the bacteria second in terms of bio-augmentation treatments, and the mixed culture of *Rhizobium* + *Trichoderma* ranked third overall (Alfredo *et al.,* 2017).

Alfredo *et al.,* (2017) reported that the bioremediation results from the greenhouse trials of two atrazine concentrations in soil are presented in Table 18.

The results can be summarized as follows: (i) significant differences were found among the four treatments (Bean +*Trichoderma* [BT], Bean + Rhizobium [BR], Bean + Rhizobium +*Trichoderma* [BRT] and Bean [B]) compared to the controls; (ii) the treatment of that included only the bean plant (B), eliminated 24.44% and 52.02% of the atrazine in the soil, which corresponds to 16.6 and 33.3 mg of atrazine 50 g^{-1} soil, respectively, and (iii) a combination of phytoremediation (bean plant) + bio-augmentation with *Trichoderma sp*. (BT) was the best treatment for herbicide removal.

The analysis of variance and post hoc tests reported that the removal herbicide increased by 25% when combining bio-augmentation and phytoremediation (Table 19). This leads us to the conclusion that common dry bean (*Phaseolus vulgaris* L.) can be viable as a model plant for phytoremediation of atrazine contaminated soils. Additionally, bio-augmentation with rhizospheric microorganisms plays an important role in the removal of compounds like atrazine, not only as promoters of plant growth by increasing nutrient availability, but because they increase the bioavailability and metabolism of the contaminants, due to its organizational structure (Alfredo *et al.,* 2017).

In the second greenhouse experiment, the combination treatments using bio-augmentation and phytoremediation + BT (bean *Trichoderma sp*.) removed 25.51 mg of atrazine 50 g^{-1} soil in 40 days. The BR (Bean Rhizobium) and BRT (bean- *Rhizobium* - *Trichoderma*) treatments were able to remove an average of 20 mg of atrazine 50 g^{-1} soil each, between 35 and 40 experimental days. The group consisting of only the bean plant (B) eliminated 17.32 mg of atrazine 50 g^{-1} soil in 35 days. Finally, the negative control (NC) eliminated 6.5 mg and positive control (PC) eliminated 14.74 mg of atrazine 50 g^{-1} soil, after 40 days (Table 19).

Table 19 summarizes the results of the atrazine contaminated soil remediation at three experimental concentrations, using the same initial inoculum of 1 X 10^9 CFU mL^{-1} of *Rhizobium sp.*, and 1 X 10^5 conidia mL^{-1} of *Trichoderma sp*. For the firstexperiment (laboratory level), an initial concentration of 10 mgof atrazine 50 g^{-1} soil was used and the bio-augmentation treatment using *Trichoderma sp.* (T) eliminated 8.94 mg 50 g $^{-1}$ soilin 40 days at 25°C and a pH of 7.7 (Alfredo *et al.*, 2017).

Other strategies to address contaminated soil

Optimization of pollutant-degrading microbial consortia

The augmentation of the diversity and richness of degrading microbial consortia in contaminated sites has been regarded as one of the key reasons that rhizobia enhance the biodegradation of organic pollutants by manipulating sterile and non-sterile soils.

Rhizobia have the potential to directly modify rhizosphere microflora by improving environmental conditions and nutrient availability. Nitrogen is a major limiting factor in bioremediation and is often added to contaminated soils to stimulate the existing microbial communities.

Synergistic interactions with other microbes

The collaboration between multiple beneficial microbes has been exploited for more comprehensive and sustainable rehabilitation. Simultaneous inoculation of multiple beneficial

microbes often provides complementary and additive benefits to plants, revealing the compatibility, and synergy between distinct mutualisms.

Transgenic rhizobia in bioremediation

Recent advances in 'omics' technologies have provided opportunities to exploit genomic, transcriptomic, proteomic, and metabolomic means to modify the traits of 'biological designers' in order to maximize their phytoremediation efficiency.

Rhizobia possess the biochemical and ecological capacity to degrade environmental organic chemicals and to decrease the risk associated with metals in contaminated soils. Rhizobia assisted phytoremediation provides environmental and economic benefits for bioremediation.

The wide adoption of these biological adaptation strategies result in the development of environmentally friendly management techniques.

Future line of work

Need to investigate the metabolic pathway of contaminate in soil for rhizoremediation and rhizobial- assisted phytoremidiation.

Need to conduct field experiments on rhizobial bioremediation.

References

Alfredo, M. N., Blanca, R. R. P., José, R. V., Otilio, A. A. S., Gregory, P. and Margarita, I. P. 2017, Bioremediation model for atrazine contaminated agricultural soils using phytoremediation (using *Phaseolus vulgaris* L.) and a locally adapted microbial consortium. *Journal of Environmental Science and Health.* PART B. 0 (0):1-9.

Hao, X., Taghavi, S., Xie, P., Orbach, M. J., Alwathnani, H. A., and Rensing, C., (2014). Phytoremediation of heavy and transition metals aided by legume-rhizobia symbiosis.*Int. J. Phytoremediat.* 16, 179–202.

Jabeen, H., Iqbal, S., and Anwar, S. (2014). Biodegradation of chlorpyrifos and 3, 5, 6-trichloro -2- pyridinol by a novel rhizobial strain *Mesorhizobium*sp.HN_3. *Water Environ. J.* doi: 10.1111/ wej.12081.

Johnson, D. L., Anderson, D. R. and McGrath, S. P., 2005, Soil microbial response during the phytoremediation of a PAH contaminated soil. *Soil Biology and Biochemistry*.37: 2334-2336.

Johnson, D. L., Maguire, K. L., Anderson, D. R., and Mcgrath, S. P. (2004). Enhanced dissipation of chrysene in planted soil: the impact of a rhizobial inoculum. *Soil Biol. Biochem.* 36, 33–38.doi:10.1016/j.soilbio.2003.07.004.

Johnson, D., Anderson, D., and Mcgrath, S. (2005). Soil microbial response during the phytoremediation of a PAH contaminated soil. *Soil Biol.Biochem.*37, 2334– 2336. doi:10.1016/j.soilbio.2005.04.001.

Jose, A. C., Patricia A., Eloisa, P., Araceli B., Miguel A. C., Rafael, L., Manuel, A. Chambera, and Antonio J. Palomaresc, 2005, Isolation and characterisation of symbiotically effective Rhizobium resistant to arsenic and heavy metals after the toxic spill at Aznalcollar pyrite mine. *Soil Biology & Biochemistry* 37: 1131–1140.

Jose, A. C., Patricia, A., Pajuelo, E., Burgos, A., Miguel, A. C., Rafael, L., Manuel A. C., and Antonio, J. P., 2005, Isolation and characterization of symbiotically effective *Rhizobium* resistant to arsenic and heavy metals after the toxic spill at the Aznalcollar pyrite mine. *Soil Biology and Biochemistry*.37: 1131-1140.

Kaiya, S., Utsunomiya, S., Suzuki, S., Yoshida, N., Futamata, H., Yamada, T., 2012, Isolation and functional gene analyses of aromatic- hydrocarbon- degrading

bacteria from a polychlorinated- dioxin-dechlorinating process. *Microbes Environ.* 27: 127–135.

Keum, Y. S., Seo, J. S., Hu, Y. T., and Li, Q. X. (2006). Degradation pathways of phenanthrene by *Sinorhizobium* sp. *C*4. *Appl. Microbiol. Biotechnol*. 71, 935–941.doi: 10.1007/s00253-005-0219-z.

Li, Y., Liang, F., Zhu, Y. F., and Wang, F. P. (2013). Phytoremediation of a PCB-contaminated soil by alfalfaa and tall fescue single and mixed plants cultivation.*J. Soil. Sediment.*13, 925–931.doi:10.1007/s11368-012-0618-6.

Muthukumar, G., Arunakumari, A., and Mahadevan, A. (1982). Degradation of aromatic compounds by *Rhizobium* spp. *Plant Soil.*69, 163–169. doi: 10.1007/BF02374511.

Nies, D. H. (1995). The cobalt, zinc, and cadmium efflux system CzcABC from *Alcaligenes eutrophus* functions as a cation–proton antiporterin *Escherichia coli*. *J. Bacteriol.* 177, 2707–2712.

Poonthrigpun, S., Pattaragulwanit, K., Paengthai, S., Kriangkripipat, T., Juntongjin, K., and Thaniyavarn, S. (2006). Novel intermediates of acenaphthylene degradation by *Rhizobium* sp. Strain CU-A1: evidence for naphthalene-1,8- dicarboxylic acid metabolism. *Appl.Environ.Microbiol*. 72, 6034–6039.doi: 10.1128/AEM. 00897-06.

Reichman, S. M. (2007), the potential use of the legume–rhizobium symbiosis for the remediation of arsenic contaminated sites. *Soil Biology & Biochemistry* 39: 2587–2593.

Stringfellow, J. M., Cairns, S. S., Cornish, A., and Cooper, R. A. (1997). Haloalkanoated ehalogenase I1 (DehE) of a *Rhizobium* sp. Molecular analysis of the gene and formation of carbon monoxide from trihaloacetate by the enzyme. *Eur.J.Biochem.*250, 789–793.doi:10.1111/j.1432-1033.1997.0 0789.x.

Subramaniam, G., Arumugam, S., Rajendran, V., Rajeev, K., Laxmipathigowda, C. L. and Lakshmanan, K., 2015, Plant growth promoting rhizobia: challenges and opportunities. *Biotech*., 5:355–377.

Teng, Y., LI, X., Chena, T., Zhang M., Wang, X., Li, Z. and Luo, Y., 2016, Isolation of the PCB-degrading bacteria *Mesorhizobium* sp. ZY1 and its combined remediation with *Astragalussinicus* L. for contaminated soil. *International Journal of Phytoremediation.*18 (2): 141-149.

Teng, Y., Wang, X., Li, L., Li, Z. and Luo, Y., 2015, Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. *Frontiers in plant science*.32 (6).

Tu, C., Teng, Y., Luo, Y., Li, X., Sun, X., Li, Z., Liua, W. and Peter, C., 2011, Potential for biodegradation of polychlorinated biphenyls (PCBs) by *Sinorhizobiummeliloti*. *Journal of Hazardous Materials*.186: 1438-1444.

Valentín, L., Nousiainen, A., and Mikkonen, A. (2013). "Introduction to organic contaminants in soil: concepts and risks," in *Emerging Organic Contaminantsin Sludges: The Handbook of Environmental Chemistry*, eds A.G. Kostianoy and D. Barceló (Berlin, Heidelberg: Springer-Verlag), 1–29.

Wani, P. A. and Khan, M. S., 2012, Bioremediation of lead by a plant growth promoting *Rhizobium* Species RL9. *Bacteriology Journal*.2 (4):66-78.

How to cite this article:

Sharanappa Kuri, G. Prakasha, Vishwajith and Shree Harsha Kumar, S.S. 2018. Rhizobia and their Bio-Partners as Novel Drivers for Functional Remediation in Contaminated Soils. *Int.J.Curr.Microbiol.App.Sci.* 7(03): 1673-1697. doi: https://doi.org/10.20546/ijcmas.2018.703.200