SUMMARY

Accumulation of metals in the soils affects the microbial communities bringing about the loss of soil fertility and reduced crop yields. Metals tend to accumulate in the plant parts making health problem for human and animals. Agricultural sites irrigated for long term with water polluted by industrial effluents provide natural ecosystems to study the effect of pollution on microbial communities. Besides these sites may harbor certain plant beneficial bacteria with metal resistance and multiple plant growth promoting traits which could when reapplied to the soil serve as effective biofertilisers for enhancing crop yields in polluted soils. The work presented in the thesis dealt with the influence of Copper (Cu) on microbial populations in the plant rhizosphere. Rhizobacterial community dynamics is inherently complex to understand as this population may be influenced independently as well as cooperatively by soil edaphic factors as well as plant species and plant health. Cu was chosen for studies since it is an important pollutant of agricultural fields and ranks high terms of its toxicity. Cu is considered as a double edged sword, being an essential metal and showing deficiency symptoms as well as toxic above threshold concentrations. It gets accumulated in agricultural fields due to wide application of fungicides, pesticides, sewage sludge, and irrigation water polluted by industrial effluents. Numerous studies of influence of Cu on plants and soil communities have been carried out yet these studies have to be extended to newer geographic and climatic areas for better understanding of how microorganisms respond to metals and how this information can be exploited for improvement of crop growth in affected soils.
The work presented in the thesis is broadly categorized into three sections (Chapters 2-4) which deal with the following aspects.

1. Impact of Cu on rhizobacterial communities in naturally polluted and artificially constructed Cu contaminated microcosms.
2. Screening of potential Cu resistant plant growth promoting bacteria from the sites under study.
3. Understanding the effect of Cu on some of the early determinants of symbiotic association between rhizobia and legumes using Sinorhizobium-Medicago as the model.

The effect of Cu on rhizobacterial communities was studied by a sensitive and powerful technique of denaturing gradient gel electrophoresis which allows fingerprinting of microbial communities either as total eubacterial population or specific subgroups (phyla) within the eubacterial domain. The study included culture dependent as well as culture independent approaches. The samples used were from the rhizospheres of plants grown in field soils from agricultural fields situated around the industrial belt of Gujarat, where practice of using water contaminated with the treated industrial effluent is prevalent. In addition to naturally affected soils, microcosms with unpolluted soil that was artificially polluted with Cu in the laboratory were developed. The microcosms were of two distinct types, one in which the Cu treated soils were sown with plants (Vigna radiata, mung bean) and their rhizospheres allowed to develop under laboratory conditions in the presence of Cu. In another study, the rhizospheric soil was collected from the same plants that were field grown (in unpolluted soil) and the already developed rhizosphere community was treated with different levels of Cu and the direct effect seen without the presence of the plants.

Bacterial communities from plants grown in Cu contaminated agricultural fields along the industrial zone of Gujarat, India varied in the magnitude of their Cu tolerance index indicating differences in long term pollution effects. A strong correlation between Cu concentrations in the soil samples with the Cu tolerance index of microbial communities was observed. Culture dependent denaturing gradient gel electrophoresis (CD-DGGE) of bacterial communities revealed the diverse
composition at the sampling sites and a reduced total diversity due to Cu toxicity. Mung bean plants were grown in agricultural soil amended with CuSO$_4$ under lab conditions. In plants, Cu exhibited toxic effects by reduction in growth with root system being affected prominently and showing elevated Cu accumulation. Culture-independent (CI) and CD DGGE fingerprinting techniques were employed to monitor rhizobacterial community shifts upon Cu amendment. In group specific PCR-DGGE, a strong negative impact was seen on $\alpha$-Proteobacteria followed by $\beta$-Proteobacteria with concomitant dose dependent decrease in diversity indices values. No significant changes were observed in Firmicutes and Actinomycetes populations. In CD DGGE rhizobacterial community shift was observed at moderate Cu concentrations, however certain bands were predominantly present in all treatments. This study emphasizes that amongst the rhizobacterial communities $\alpha$-Proteobacteria are adversely affected even at low Cu treatments and hence can be considered as sensitive bio-indicator for Cu toxicity. The effect of CuSO$_4$.5H$_2$O on culturable microbial communities tolerance and structure of mung bean rhizosphere soil in a microcosm study over a period of 10 days was also undertaken. A gradual increase in Cu induced community tolerance (Cu ICT) of rhizobacterial communities was observed during initial days due to a pronounced effect at lower concentrations that later randomized at high concentrations inferring an immediate effect. Microbial community shifts were pronounced with respect to both time and Cu concentrations from (CD DGGE) profiles affecting the diversity index. After 10 d, on 16S rDNA sequencing of isolates from control treatment found to be distributed among the phylum Proteobacteria, Firmicutes and Actinobacteria, while from high Cu treatment members belonging to Firmicutes and Actinobacteria were predominant. Hence the use of culturable approach for assessment of microbial activity measurements was demonstrated to complement culture independent diversity analysis in microbial community shift analysis. Proteobacteria group is of particular significance since this group includes majority of plant growth promoting rhizobacteria in particular nitrogen fixers. Therefore future remediation of Cu contaminated agricultural fields shall address plant beneficial bacteria belonging to Proteobacteria, the most sensitive group of microbial community members.

Analysis of 16S rRNA gene diversity of Cu tolerant rhizobacteria isolated from these sites indicated the presence of Enterobacter spp. and Pseudomonas spp. being
predominant. Cu tolerant isolates able to promote growth of mung bean plants *in vitro* at phytotoxic Cu concentration were isolated from these soil samples. Cu tolerant rhizobacterium, isolate P36, identified as *Enterobacter* sp., exhibited multiple plant growth promoting traits and significantly alleviated Cu toxicity to mung bean plants in metal amended soils.

*Sinorhizobium meliloti* 1021 belongs to α-Proteobactreia and nodulates host legumes *Medicago sativa* and *Medicago truncatula*. Cu is an essential micro nutrient required during nitrogen fixation but becomes toxic if present in excess. The effect of Cu stress in early symbiotic factors of *S. meliloti* with its host *M. tranculata* was investigated. On exposure to Cu, *S. meliloti* 1021 growth was inhibited to 50% at 120 µM, while above 200 µM Cu growth was completely ceased. Proteomic analysis *S.meliloti* total proteins under Cu stress antioxidative proteins, GroEL, WrbA, were highly expressed. Interestingly upon Cu supplementation, *S.meliloti* exhibited pleomorphism with undifferentiated cells showing slight elongation and branches showing similarity in shape with bacteroids. *S. meliloti* exopolysaccharide production, lipopolysaccharides (LPS) integrity, particularly smooth LPS was significantly affected under Cu stress as a result biofilm formation was reuduced. In hydroponic, *M. tranculata* roots showed 50% inhibition at 4 µM Cu. Histo-chemical staining of *Medicago* roots exposed to Cu revealed loss of plasma membrane integrity and damage to root membrane. In hydroponics, *S.meliloti* was unable to show efficient attachment on *Medicago* roots above 3 µM Cu possibly due to root surface modifications and root hair inhibition, implying macrosymbiont being more sensitive than microsymbiont. Under stress conditions root signals and root architecture are important for initiating the symbiotic interactions by microsymbiont.