General Discussion and Conclusion
Micronutrients play an important role in various pathways in plants. Some micronutrients or heavy metals such as Zn and Cu are essential for normal growth and development of plants since they are constituents of many enzymes and proteins. The elevated concentrations of both essential or non-essential micronutrients in soil leads to the toxicity symptoms and inhibition of growth of most plants and causes detrimental effects on ecosystems and are a risk to human health as they can enter the food chain via agricultural products or contaminated drinking water. Heavy metals like Cu, Zn, Cd, Cr, Pb, Hg, As and Fe under toxic concentration inactivates enzymic antioxidant defense system in plants resulting into increased ROS signalling generally leading to death of a plant. At high concentrations, heavy metal interferes with essential enzymatic activities by modifying protein structure or by replacing a vital element resulting in deficiency symptoms (Halliwell 1987; Mittler 2002; Tiwari et al. 2008). Removal of these heavy metals from the environment has become an issue of major concern for the human welfare.

Depending on the extension, depth and kind of the contamination, different remediation approaches have been proposed (Mulligan et al. 2001). In general, three strategies are possible: the containment of the contaminants, their removal from the environment or their *in situ* stabilisation. Physical containment is the least expensive approach but this leaves the contaminant in place without treatment. As *ex situ* techniques are expensive, environmentally invasive and labor intensive, *in situ* approaches are generally preferred. One of these *in situ* techniques, phytoremediation, uses plants to remove pollutants from the environment or to render them harmless (Salt et al. 1995; Flathman & Lanza, 1998). Although there is much literature on the behaviour of heavy metals in soils and on the use of soil micro-organisms for phytoremediation or heavy metal extraction, but, the goal to reduce the problems associated with heavy metal toxicity in plants is far from being achieved. As mycorrhiza are the key-players of nutrient turnovers, they certainly play an important role in restoring the biological quality of perturbed ecosystems undergoing long-term remediation. This study tried to elucidate the consequences of (i) Heavy metal stress on the rice plant; (ii)
Use of cell wall component of AM fungi in the partial relief of this stress; (iii) Use of wsCNT in the partial relief of this heavy metal stress; (iv) Use of wsCNT in enhancing growth of AM fungi *P. indica*. Rice which is the primary food for over two billion people in Asia, Africa, and Latin America and has been used as a model plant in studies of Cd toxicity and tolerance.

First, we have investigated the effect of heavy metals Cd, Zn and Hg on the rice plant which were grown in half Hoagland’s solution with different concentrations of these heavy metals CdSO₄ (0.5µM, 1µM), ZnSO₄ (2µM, 50µM, 100µM) and HgCl₂ (0.02µM, 0.04µM, 0.1µM). (Chapter 2). The 0.5µM and 1µM concentrations of heavy metal showed toxic effect on the root and shoot length of the plant. The leaves were chlorotic and shorter as compared to the control plants. The chlorosis may appear due to the Fe deficiency (Haghiri 1973), phosphorus deficiency or reduced Mn transport (Godbold and Hutterman 1985). The inhibition of the root Fe (III) reductase induced by Cd led to Fe (II) deficiency which severely affect photosynthesis. Cd interfere with uptake, transport and use of several essential elements (Ca, Mg, P, K) and water by plants. The activity of Nitrate reductase also inhibited by Cd thus reducing the absorption of nitrate and its transport from root to shoot. The higher concentration of Cd severely affect the root growth. It causes the root inhibition in the plant. It may be because in most environmental conditions, Cd first enters into the roots, so the damage first appears to the roots. Cd by penetrating the root through the cortical tissue can get translocated to the shoot and affect the shoot growth.

Zn, which is an essential micronutrient element for plants, plays a critical role in plants as an essential component of key enzymes such as Cu-Zn superoxide dismutase, alcohol dehydrogenase, RNA polymerase, and DNA-binding proteins (Marschner, 1995; Guerinot and Eide, 1999). The present study showed that toxic effect of high Zn on rice plants. Growth retardation is the most general symptom of Zn toxicity in plants. The influence of Zn on plant cell division has been investigated earlier (Powell *et al.* 1986; Francis *et al.* 1995). We have studied the effect of high concentrations on rice plants. Plants were exposed to 2 µM, 50 µM and 100 µM Zn. In general, plants showed all the characters of reduced plant growth and
development by interfering in different metabolic processes (Ebbs and Kochian 1997; Prasad et al. 1999; Bonnet et al. 2000), chlorosis of the younger leaves (Harmens et al. 1993; Ren et al. 1993; Fontes and Cox, 1995) and reduction in the growth of the root. The 50 µM and 100 µM Zn concentrations showed the toxic effect on the plant in comparison to the control plants with 2 µM Zn, which is normally required by the plant for its normal growth and development. The leaves of the plant exposed to 50 µM and 100 µM Zn were chlorotic and small. The high Zn in the cell damages the normal cellular functionality, which is caused via binding of the cation at inappropriate sites in proteins (Eide, 2003). When the Zn concentration in the plant cell is too high, the cell cannot detoxify the high Zn concentration, the tissues are observed to be affected and chlorotic as observed in our study. Hg in low concentration did not show visible phenotypic effect on the plant growth in our study.

Plants have a range of potential mechanisms that might be involved in the detoxification and thus tolerance to heavy metal stress. Some plant species, however, have evolved tolerant races that can survive and thrive on such metalliferous soils, presumably by adapting mechanisms that may also be involved in general homeostasis of, and constitutive tolerance to, essential metal ions as found in all plants. A great interest has developed recently in the use of terrestrial plants as a green technology for the remediation of contaminated soils with toxic heavy metals, which may help to reduce or even reverse these pollution problems (Pence et al. 2000). This developed a new field of environmental biotechnology, termed phytoremediation, which uses plants to extract heavy metals from the soil and to concentrate them in the harvestable shoot tissue (Salt et al. 1995). Although natural hyperaccumulators can tolerate and accumulate high concentration of toxic metals, they usually produce little biomass, they grow slowly and cannot be easily cultivated. However, as phytoremediation is a slow process, improvement of efficiency and thus increased stabilization or removal of heavy metal from soils is an important goal. According to Kapoor et al. 2008, the plant performance and its yield can be improved under stress conditions by the intervention of the microorganism particularly beneficial fungi and bacteria. Therefore, AM fungi can provide an attractive system to advance plant-based environmental clean-up.
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 has also become evident (Chaudhry et al. 1998; Khan et al. 2000; Khan, 2001; Jamal et al. 2002; Hayes et al. 2003). Mycorrhizae have been reported in plants growing on heavy metal-contaminated sites indicating that these fungi have evolved a heavy metal-tolerance and that they may play a role in the phytoremediation of the site. Joner and Leyval (1997) found that cadmium-tolerant Glomus mosseae isolates were responsible for uptake, transport and immobilization of cadmium. Cu was absorbed and accumulated in the extraradical mycelium of three AMF isolates, as observed in a study with Glomus spp. Mycorrhizae were found to ameliorate the toxicity of trace metals in polluted soils growing in soybean and lentil plants by Jamal et al. 2002. As mycorrhizae may enhance the ability of the plant to cope with water stress situations associated to nutrient deficiency and drought (Schreiner et al. 1997), mycorrhizal inoculation with suitable fungi has been proposed as a promising tool for improving phytoremediation of metal contaminated soil

Traditionally, fungi have been regarded as pathogens by agronomists. However, in recent years, symbiotic fungi providing benefits to crop plants have become an additional focus of research. In addition to the AMF that constitute a distinct fungal phylum, the Glomeromycota (Schüßler et al. 2001), endophytes mainly belonging to the Ascomycota or Basidiomycota have been shown to improve the vigor of their hosts (Ernst et al. 2003; Hashiba and Narisawa 2005; Schardl et al. 2004; Varma et al. 1999). The novel root endophyte P. indica fungus was screened from the desert soil of western Rajasthan by Varma and co-workers from School of Life-Sciences, Jawaharlal Nehru University, New Delhi. This mycorrhizal fungus mimics the capabilities of typical AM. However, the unique feature of this fungus is that it is axenically
cultivable. This is a golden lining in AM history. Based on it’s characteristically typical pear shaped chlamydospores, the fungus was named as *Piriformospora indica* (Verma *et al.* 1998). The mutualistic symbiosis of crop plants and Sebacinales has a great potential for sustainable agriculture (Deshmukh *et al.* 2006). Its presence causes beneficial activities such as an increase in vegetative biomass and grain yield, local and systemic disease resistance, and tolerance to abiotic stresses (Waller *et al.* 2005; Aschhiem *et al.* 2005). Its application in horticulture or agriculture as a potent biofertilizer and biocontrol agent is economically and practically feasible through the facilitated propagation of fungal inoculum using liquid or axenic cultures. In this study the cell wall component of the novel endophytic root colonizing fungus *P. indica* was isolated. The present study showed that the CWE of *P. indica* have significant effect on the rice plant growing under heavy metal stress caused due to presence of higher level of Zn, Cd and Hg. As discussed above, when the rice plants were supplied with higher concentrations of these heavy metals, there was significant reduction in the root length of the plant and the plants were chlorotic and shorter compared with plants grown for the same time under half Hoagland’s solution. CWE was found to have increase in the root length of the plant.

Heavy metals, such as, Cd, Pb, Ni and Zn can be removed from water using sorbents. The rate and extent of removal may be enhanced by choice of appropriate sorbents. Among the several materials and methods used for the removal of heavy metals, CNT can play a strategic role due to their ability of adsorbing the elements. Zn, Pb, Ni, Cd, Cr and Hg (Li *et al.* 2002, 2003; Lia *et al.* 2003; Lu and Chiu, 2006; Lu *et al.* 2008; Rao *et al.* 2007; Yang *et al.* 2009). The present study showed that the wsCNT have significant effect on the rice plant growing under heavy metal stress caused due to presence of higher level of Zn, Cd and Hg and improves the plant health.