Chapter 3
Cell Wall Extract of the Endophytic fungus *Piriformospora indica* Promotes Growth of *Oryza sativa* under Heavy Metal Stress

3.1 Introduction

Contamination of soils with heavy metals, either by natural causes or due to pollution, often has pronounced effects on the vegetation, environment and human health. Due to the continual influx of heavy metal contaminants and pollutants into the biosphere from both natural and anthropogenic sources, these metals and metalloids accumulates in soil and have noxious effect on both plants and animals, which are strongly deleterious to metal-sensitive enzymes and result in growth inhibition and death of the organism (Zhang *et al.* 2001). Some of these metals are micronutrients which are necessary for plant growth, such as Zinc (Zn), Copper (Cu), Manganese (Mn), Nickel (Ni) and Cobalt (Co), while others have no known biological function, such as Cadmium (Cd), Lead (Pb) and Mercury (Hg) but are extremely toxic to the plants. The metal pollution is of major importance and relevant to the present scenario due to the increasing levels of pollution and its evident impact on human health through the food chain.

Despite a worldwide intensification of agriculture and tremendous progress toward increasing yields in major crops over the last decades, the goal to reduce the problems associated with heavy metal toxicity in plants is far from being achieved. The metal concentrations in soil range from less than 1 mg/kg (ppm) to high as 100,000 mg/kg (Blaylock and Huang 2000). The high metal concentrations in contaminated soils result in decreased soil microbial activity, soil fertility, and yield losses (McGrath *et al.* 1995). Several technologies and methods have been developed to remove them from polluted soil. These methods such as excavation and land fill, thermal treatment, acid leaching and electroreclamation which are being used for heavy metal remediation are not suitable in practical applications due to their high cost, low efficiency, large destruction of soil structure and fertility and high dependence on the contaminants of concern, soil properties, site conditions, and so on (Jing *et al.* 2007). The knowledge of metal-plant interactions is
not only important for the safety of the environment, but also for reducing the risks associated with the introduction of trace metals into the food chain.

Zn is an essential element that is employed in a wide range of biochemical and biophysical roles, though excess of it has toxic effects. Zn toxicity in plants is characterised by chlorosis in young leaves, probably via competition with Fe and Mg resulting in growth reduction (Marschner 1995). Depending on the degree of toxicity chlorosis can progress to reddening due to anthocyanin production in younger leaves (Harmens et al. 1993; Fontes and Cox 1995; Lee et al. 1996). Plants exhibiting Zn toxicity have smaller leaves than control plants (Ren et al. 1993). In severe cases plants may exhibit necrotic lesions on leaves and eventually entire leaf death (Harmens et al. 1993). In roots, Zn toxicity is apparent as a reduction in the growth of the main root, fewer and shorter lateral roots and a yellowing of roots (Ren et al. 1993). Cd and Hg are common hazardous constituents of industrial effluents. These are the non-essential elements that negatively affect plant growth and development and are toxic to living organisms even at low concentrations. Cd can be easily taken up and accumulated by plants and crops through their root systems and is present in all food (Alam et al. 2003). Research undertaken over the past 40 years has identified the irrefutable relationship between long term consumption of Cd-contaminated rice and human Cd diseases such as itai-itai and proximal tubular renal dysfunction (Simmons et al. 2005). Due to the potential toxicity and high persistence of metals, soils polluted with heavy metals are a critical environmental issue, which requires an effective and affordable solution.

Plants have evolved potential mechanisms involved in the detoxification and tolerance to heavy metal stress. 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. The mutualistic interactions between microbes and agroforestrical, horticultural and medicinal plants have been attractive for many years, since mutualists can improve the growth, biomass and seed production on poor soil with little input of chemical fertilizers and pesticides. Mycorrhizal associations are among the most prevailing, intimate and important symbioses in terrestrial ecosystems. Mycorrhiza is the highly evolved, mutualistic symbiotic association (non-pathogenic) of a specific group
of soil-borne fungi with the roots of higher plants generally characterized by bi-directional exchange of plant-produced metabolites to fungus and fungal-acquired nutrients to the plant. Mycorrhizae, as a major interface or connection between soil and plants, are a keystone in integrated system. The mycorrhizal fungi classified in two groups as endo or ecto-mycorrhizae based on the position of hyphae in relation to the root epidermis of the host plant. Mycorrhizae have been reported in plants growing on heavy metal contaminated sites (Chaudhry et al. 1998) indicating that these fungi have evolved a heavy metal tolerance and that they may play a role in the phytoremediation of the site. Mycorrhiza fungi can process heavy metals might provide efficient and ecologically sound approaches to sequestration and removal. The accumulation of heavy metals in the plants depends on many factors, including transport of metals from the soil to the plant. Thus, the development of strategy to overcome the problem of heavy metals in contaminated soil with isolated biotic factor from Arbuscular mycorrhizal fungi (AMF) can unfold this problem. AMF have the ability to improve phosphorus nutrition, to withstand water stress and offers a natural potential for biological control of root pathogen and are also reported to be present on the roots of plants growing on metal-contaminated soils and play an important role in metal tolerance and accumulation. The AM fungi can play significant roles in the growth of plants in metal contaminated soils and in salt marshes and considered to be the primary determinant of plant health and soil fertility in terrestrial ecosystems (Hildebrandt et al. 2007, Jeffries et al. 2003). AM fungi are ubiquitous, important for terrestrial ecosystems and have a potential application. AM fungi found under all climates in all ecosystems, regardless of the type of soil, vegetation and growing conditions, can affect the water balance of both amply watered and droughty host plants (Auge 2001). Joner and Leyval (1997) found that Cd-tolerant Glomus mosseae isolates were responsible for uptake, transport and immobilization of Cd. Cu was absorbed and accumulated in the extra-radical 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 (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; Hrishikesh Upadhyaya1 et al. 2010), mycorrhizal inoculation with suitable fungi has been proposed as a promising tool for improving phytoremediation of metal contaminated soil. Therefore, heavy metal-stress
tolerance can be evoked in crops by the exploitation of worldwide abundant endophytic AMF, which live in reciprocally beneficial relationships with land plants.

The endophytic root-colonizing AM fungus *Piriformospora indica* (*P. indica*) was isolated from the roots of plants grown in the Indian desert Thar (Verma et al. 1998). The axenically culturable fungi *P. indica* has been found to stimulate nutrient uptake, plant growth promotion and confers resistance to various biotic and abiotic stresses (Varma et al. 1999; Peskan-Berghoefer et al. 2004; Oelmüller et al. 2004). Waller et al. (2005) had shown that *P. indica* re-programmes barley to salt stress tolerance, resistance to diseases and higher yield. It mediates the Phosphorus and Nitrogen uptake from the soil and translocates to the host in an energy dependent process. *P. indica* produces significant amount of acid phosphatases for the mobilization of broad range of insoluble forms of phosphate, enabling the accessibility of the host plant to adequate phosphorus from immobilized reserves in the soil (Varma et al. 2000). The fungus associates with promotion of nutrient uptake, plants survival under water, temperature and salt stress, conferring (systemic) resistance to toxins, heavy metal ions and pathogenic organisms and stimulating growth and seed production (Verma et al. 1998; Varma et al. 1999, 2001; Sahay and Varma 1999; Oelmüller et al. 2004, 2005; Pham et al. 2004a,b; Peskan-Berghoefer et al. 2004; Kaldorf et al. 2005; Shahollari et al. 2005, 2007a, Sherameti et al. 2005, 2008a,b; Vadassery et al. 2008, 2009a,b; Waller et al. 2005, 2008). Since it can colonise the roots of many plant species including trees, agricultural, horticultural and medicinal plants, monocots and dicots and even mosses the interaction between the symbiotic partners should be based on general recognition and signalling processes.

The isolated fraction i.e. cell wall extract (CWE) of *P. indica*, can mimic the presence of the fungus in the initial stages and induces cytosolic calcium level and growth promotional effect in *Arabidopsis thaliana* (Vadassery et al. 2009). Considering the role of *P. indica* in stimulating the resistance to the heavy metal stress in plants, the CWE was supplied to the plant to study the response of the plant to the isolated fraction under heavy metal stress. This is the first time report that the CWE of *P. indica* can help plant to detoxicify the heavy metal.
3.2 Material and Methods

3.2.1 Plant and Fungus Material

In order to analyze the effect of CWE of *P. indica* on *Oryza sativa* (*O. sativa*), the plants were grown under high concentrations of heavy metals Zn, Cd and Hg. The two days old germinated seeds of *O. sativa* plants were grown hydroponically in half Hoagland’s solution in a growth chamber at 22-25°C temperature and a photoperiod of 12 hours day/night. The plants were then exposed to different concentrations of the following heavy metals: ZnSO₄ (2µM, 50µM, 100µM), CdSO₄ (0.5µM, 1µM) and HgCl₂ (0.02µM, 0.04µM, 0.1µM). The cell wall component of *P. indica* was isolated and to the plants growing with these heavy metal concentrations in half Hoagland’s solution. To minimize variation in the bioavailability of these heavy metals, we used a hydroponic rather than soil-based culturing system. The plants established to grow in half Hoagland’s solution only i.e. without heavy metals were kept as control for all other plants growing with heavy metals concentrations. Forty plants of each concentration of Zn, Cd and Hg were grown and compared with forty plants of each concentration of Zn, Cd and Hg supplied with CWE of *P. indica*. To analyze the effect of CWE on *O. sativa* growing with excess amount of heavy metals, the root length of the plants were measured and a comparison was done with the control plants.

The CWE was prepared using the protocol of Vadassery et al. (1975). Mycelia from 14-day-old liquid cultures were homogenized using mortar and pestle in 5 ml water g⁻¹ mycelia. The homogenate was filtered using a coarse sintered glass funnel. The residue was washed three times with water, once with chloroform/methanol (1:1) and finally in acetone. This preparation was air dried for 2 hour and the mycelial CW material was immersed in 100 ml water and autoclaved for 20 min at 121°C temperature. Autoclaving releases the active fraction recovered. The CWE fractions were prepared from mycelial CWs by suspending 1 g of CW. The suspension was filtered using a Whatmann filter paper no. 1 and was further used for studying its effect on plant growth.

3.2.2 Analysis of Root and Shoot Micronutrient Content

The micronutrient content of root and shoot tissues were analyzed using Energy Dispersive X-Ray Fluorescence (EDXRF).
3.3 Results

3.3.1 Effect of Cd on *O. sativa* and Detoxification with CWE

The *O. sativa* plants were grown for four weeks under Cd concentrations (0.5 μM Cd, 1 μM Cd). The plants were chlorotic and shorter compared with control plants grown for the same time under half Hoagland’s solution without Cd (Fig. 1A, 1B, 1C). Cd in the nutrient solution significantly reduced plant height, root length, plant biomass, and chlorophyll content. Young leaves of the plants were chlorotic and some wilted and died (Fig. 1B, 1C). Leaf veins and sheaths were reddish and the roots were shortened with some necrotic areas. The reduction in plant growth became more pronounced as Cd in the solution increased. Increasing concentrations of bioavailable Cd in the nutrient solution resulted in shorter plants and leaf chlorosis, showing clear symptoms of Cd toxicity as described earlier by Arduini *et al.* 1996. Visual symptoms of Cd-damage as noted here are reported in certain other plant species also e.g. radish (Khan and Frankland, 1983), pea (Hernandez and Cooke, 1997) and of leaf chlorosis in oilseed rape (Baryla *et al.* 2001; Carrier *et al.* 2003) grown in Cd-contaminated soils. The root growth of the plants was severely affected (Fig. 1B, 1C) due high Cd. The average root length of the plants was reduced by ~1.5 and ~2 fold on exposure to Cd concentrations of 0.5 μM Cd and 1 μM Cd respectively (Fig. 2A).

To minimize the effect of Cd stress on the plant, the CWE of *P. indica* was added to the plants and the phenotypic response of the plant was studied. The significant increase in average root length of the plants was observed when plants were supplemented with CWE (Fig. 3D and 3F) as compared with the plant only with Cd (Fig. 3C and 3E). The root length of the plants were measured on 15th day, which showed fold increase of ~1.39, and ~1.92 for 0.5 μM Cd and 1 μM Cd respectively (Fig. 2B). The height of the CWE treated plants was also increased and leaves were less chlorotic as compared to Cd stressed plant. There was no significant effect of CWE observed on root length of control plants, however the leaves of these plants were more green (Fig. 3A, 3B). There was improvement in the overall plant health as seen in (Fig. 3A-3I). Sigma Plot Version 12.0 was used to perform one-way analyses of variance (ANOVA) to statistically analyze the data.
3.3.2 Micronutrient Enhancement in Roots of CWE Treated Plants

The micronutrient mineral content of CWE treated plants was analyzed by Energy Dispersive X-Ray Fluorescence (EDXRF). To determine the mineral content, the plants were grown hydroponically as described above. The root of four weeks old plants were dried and crushed with mortar and pestle. This dried powder was used for further mineral assay in EDXRF. Fifteen plants were pooled together for this mineral assay. The roots of the plants treated with CWE found to have high Zn and Mn content in them as compared to the control plants. The Zn content of the plants treated with CWE was enhanced by ~1.8, ~2 and ~1.8 fold for control, 0.5µM Cd and 1µM Cd as compared to non-treated plants respectively. Also, the Mn content of the plants treated with CWE was enhanced by ~1.2, ~4.5 and ~5.2 fold than non-treated plants respectively (Data shown in Table 1).

Table 1. Mineral content of *O. sativa* roots treated with CWE

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Control + CWE</th>
<th>0.5µM Cd + CWE</th>
<th>0.5µM Cd + CWE</th>
<th>1µM Cd + CWE</th>
<th>1µM Cd + CWE</th>
</tr>
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<tr>
<td>Zn</td>
<td>834.652</td>
<td>1461.165</td>
<td>851.541</td>
<td>1632.430</td>
<td>566.616</td>
<td>1032.232</td>
</tr>
<tr>
<td>Mn</td>
<td>50.054</td>
<td>59.340</td>
<td>87.825</td>
<td>395.344</td>
<td>140.774</td>
<td>740.199</td>
</tr>
</tbody>
</table>

[Figure 1: Phenotypic effect of *O. sativa* grown in half Hoagland’s solution under Cd concentrations. (A) Control (B) 0.5 µM Cd; (C) 1 µM Cd]
Figure 2: Effect of Cd supplies (0.5 and 1 µM) in half Hoagland’s solution on root length of *O. sativa* plants. (A) Root length on 15th day; (B) Effect of Cd supplies in combination with CWE on root length on 15th day
Figure 3: Phenotypic effect of CWE of *P. indica* on *O. sativa* grown in half Hoagland’s solution under different Cd concentrations. (A) Control; (B) Control + CWE; (C) 0.5 µM Cd; (D) 0.5 µM Cd + CWE; (E) 1 µM Cd; (F) 1 µM Cd + CWE; Comparison of root length of single plant: (G) Control and Control + CWE plant; (H) 0.5 µM Cd and 0.5 µM Cd + CWE; (I) 1 µM Cd and 1 µM Cd + CWE

### 3.3.3 Response of *O. sativa* Plants to Zn and CWE

The response of *O. sativa* to different Zn supplies was studied in a similar way as for Cd. As, Zn is an essential microelement for the plants, Zn concentrations to which the plants were exposed were increased while studying the effect of excess concentrations of Zn on the plant. The plants were grown for four weeks under high Zn concentrations (50 µM Zn,
100 μM Zn). The higher concentrations of Zn resulted in toxic effect on plants. The plants were chlorotic and shorter compared to control plants with 2μM Zn (Fig. 4A, 4B, 4C). The average root length of the plants was reduced by 1.2 fold for the plants with 50 μM Zn, but there was no significant effect found on root length of the plants with 100 μM Zn on 15th day (Fig. 5A). However the shoot height was reduced and the leaves were chlorotic for the plant with 50 μM and 100 μM Zn showing symptoms of Zn toxicity.

To study the effect of CWE of *P. indica* on the plant affected with high Zn, the CWE was added to the plants growing solution with different Zn concentrations. Significant increase in average root length of the plant was observed for CWE treated 50 μM Zn plants as compared to non-treated plants (Fig. 5B, Fig. 6C and 6D). The root length of the plants was measured on 15th day, which showed a significant increase of 1.2 fold for plant with 50 μM Zn supplemented with CWE (Fig. 5B, 6H)). The height of the plant with 50 μM Zn supplemented with CWE also increased (Fig. 6D). There was no significant effect observed on the plant height and leaf chlorosis for CWE treated control as well as 100 μM Zn plants (Fig. 6A-6B, 6E-6F, 6G and 6I). The plant height was still shorter and leaves were chlorotic for treated as well as non-treated plants. Sigma Plot version 12.0 was used to perform One-way analyses of variance (ANOVA) to statistically analyzes the data.

![Figure 4: Phenotypic effect of O. sativa grown in half Hoagland’s solution under different Zn concentrations. (A) Control; (B) 50 μM Zn; (C) 100 μM Zn](image-url)
Figure 5: Effect of different Zn supplies i.e. 2 µM (control), 50 µM and 100 µM Zn in half Hoagland’s solution on root length of *O. sativa* plants at 15th day. (A) Root length; (B) Effect of different Zn supplies in combination with CWE on root length
Figure 6: Phenotypic effect of CWE of *P. indica* on *O. sativa* grown in half Hoagland’s solution under different Zn concentrations. (A) Control; (B) Control + CWE; (C) 50 µM Zn; (D) 50 µM Zn + CWE; (E) 100 µM Zn; (F) 100 µM Zn + CWE; Comparison of root length of single plant: (G) Control and Control + CWE; (H) 50 µM Zn and 50 µM Zn + CWE; (I) 100 µM Zn and 100 µM Cd + CWE

3.3.4 Response of *O. sativa* Plants to Hg and CWE

Similarly, we have also studied the effect of Hg on *O. sativa* plants. The *O. sativa* plants were exposed to 0.02 µM, 0.04 µM and 0.1 µM concentrations of Hg. There was no visible phenotypic effect of Hg observed on the plant (Fig. 7A-7C). The shoot, root and leaves of all plants were healthy as the control plants. It can be possible that this low concentration of Hg was not toxic to the plants.
The plants were also treated with CWE of *P. indica* and there was a significant increase in the root length of the CWE treated 0.02 μM Hg plant observed (Fig. 8B). No significant effect of CWE was observed on the root length control, 0.04 μM and 0.1 μM Hg plants (Fig. 8B, 9A-9B, 9E-H). However the leaves of the CWE treated 0.02 μM Hg plants were more chlorotic as compared to non-treated plants only with 0.02 μM Hg (Fig. 8B; Fig. 9C-9D).

**Figure 7:** Phenotypic effect of *O. sativa* grown in half Hoagland’s solution under different Hg concentrations. (A) Control; (B) 0.02 μM Hg; (C) 0.04 μM Hg; (D) 0.1 μM Hg.

**Figure 8:** Effect of different Hg supplies (Control, 0.02 μM, 0.04 μM and 0.1 μM Hg) in half Hoagland’s solution on root length of *O. sativa* plant. (A) Root length after 15th day; (B) Effect of different Hg supplies in combination with CWE on root length on 15th day.
Figure 9: Phenotypic effect of CWE of *P. indica* on *O. sativa* plants grown under different Hg concentrations. (A) Control; (B) Control + CWE; (C) 0.02 μM Hg; (D) 0.02 μM Hg + CWE; (E) 0.04 μM Hg; (F) 0.04 μM Hg + CWE; (G) 0.1 μM Hg; (H) 0.1 μM Hg + CWE; Comparison of root length of single plant: (I) Control and Control + CWE; (J) 0.02 μM Hg and 0.02 μM Hg + CWE; (K) 0.04 μM Hg and 0.04 μM Hg + CWE; (L) 0.1 μM Hg and 0.1 μM Hg + CWE
3.4 Discussion
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 and Cd. 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. Zn, being an essential trace element for normal growth and development of all organisms plays an important role in several plant metabolic processes such as enzyme activation, protein synthesis and metabolism of carbohydrate, lipid and nucleic acid (Cakmak 2000). In addition to having their most significant role in maintaining the structural and functional integrity of cell membranes in higher plants (Welch and Norvell 1993), Zn ions are integral parts of transcription factors which control the cell proliferation and differentiation (Vallee and Falchuk 1993). The requirement of Zn in stabilization of plant cell membranes is due to its ability to control the level of oxidizing O₂ species by an NADPH oxidase (Pinton et al. 1994) as well as being an integral metal component of the O²⁻ detoxifying superoxide dismutase (Cakmak and Marschner 1988). Zn at higher concentration have deleterious effect on plant growth and development by interfering in different metabolic processes (Ebbs and Kochian 1997; Prasad et al. 1999; Bonnet et al. 2000). So, the reduction in plant root length and height can be due to its toxicity as it is a non-redox metal and its toxicity can result in oxidative damage as well as induction of antioxidative defence mechanisms against it. Together with this, it has been shown that exposure of cells to Zn can enhance the intracellular level of reactive oxygen species (ROS) (Kim et al. 1999).

Similarly, Cd and Hg which are non-essential elements and have negative effect on the growth and development of the plant are extremely significant pollutant due to its high toxicity and large solubility in water. Duxbury (1985) classified Cd as an element of intermediate toxicity. The plants grown in high concentration of Cd were chlorotic and with reduced height and shorter root length. The reduced height and the root length of the rice plants with high Cd (0.5 µM and 1 µM) may be due to the alteration in the uptake of minerals as Cd can alter the uptake by plants through its effect on the availability of minerals in the environment. The chlorosis in the Cd affected plants may be due to Fe-deficiency, Phosphorus deficiency or reduced Mn-transport as the inhibition of root Fe(III)
reductase induced by Cd led to Fe(II) deficiency. Cd affects the stomatal opening and transpiration due to which the chlorosis occurs in plants when it is present in nutrient solution. Despite the different mobility of metal ions in plant, metal content is generally greater in root then in other ground tissue of the plant. In most environmental conditions, the Cd first enters the root tissues by penetrating root through the cortical tissue and gets translocated to the above ground tissue. Cd ions mainly retained in the root, only small amount of Cd transported to the shoot, thus causing severe damage to the root tissue. the effect of Hg on rice have also been studied. Hg deposition in environment is also big threat to the ecosystem. Though, Hg is also non-essential element for the plants and causes toxicity to the plant. In our study, we have exposed rice plants to 0.02 µM, 0.04 µM and 0.1µM concentrations of Hg, but these concentrations of Hg have not shown any visible phenotypic effect on the plant.

The toxic effect of these heavy metals in rice is of great concern as rice is the staple food for half of the global population. In addition to being an important crop, it is also a powerful model system for cereals. To cope up with this problem of metal toxicity, the strategy should be developed for the improvement of the plant health. Mycorrhizas are the extracellular strategy which can be helpful in avoiding the metal toxicity. 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 non-mycorrhizal microbes such as Phialocephala fortinii, Cryptosporiopsis spp, dark septate endophyte (DSE), P. indica, Fusarium spp. and Cladorrhinum foecundissimum have been shown to improve the growth of their hosts after root colonization (Schulz 2006). P. indica is a root interacting fungus that is able to associate with the roots of various plant species in a manner similar to AMF and promotes the plant growth. In contrast to AMF, this fungus can easily be cultivated in axenic culture where it produces spores (Pham et al. 2004b). The axenically culturable fungi P. indica is able to confer beneficial effects to its hosts; this phenomenon distinguishes P. indica from ectomycorrhizal and AM fungi. 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.

The active component of the cell wall of *P. indica* i.e. CWE was isolated and this CWE was found to having good effect on the root length as well as overall health of the plant. The CWE of *P. indica* was added to the plants growing solution with Cd concentrations and significant increase in average root of the plant when solution was supplemented with CWE was observed (Fig. 3A-3L). Similarly, there was an increase in the root length of the plants when the CWE was added to the nutrient solution of the plant having high Zn (50 µM). But no significant effect found with 100 µM Zn. The 0.02 µM, 0.04 µM and 0.1 µM concentrations of Hg were not showing any toxic effect on the plant. When CWE added to these plants, there was a significant increase in the root length of the plant with 0.02 µM Hg. Thus, when the CWE was added to the plants with high heavy metal concentrations, there is an increase in the root length of the plant and there is an improvement in the plant health. Thus, it may be concluded here that CWE of the endophytic fungi *P. indica* can help to overcome the problem of metal toxicity in plants.

Plants were analysed for their mineral assay after four weeks of the CWE treatment. The Dried root and shoot of the plants were analysed and it was found that there was a significant increase in Zn and Mn in the root of the plants when treated with CWE. Similar results observed for the plants under high Cd concentrations (0.5 µM and 1µM) when treated with CWE.