CHAPTER 5

GENERAL DISCUSSION

5.1 Overview

The results obtained over the course of the experiment on screening and rate of degradation experiment included: changes of soil physico-chemical parameters, plant survival rate, growth, biomass production, amount of TPH degradation in soil, amount of TPH accumulated in plant roots and shoots, amount of heavy metal degradation in soil, amount of heavy metal accumulation in plant roots and shoots, MPN quantity in soil and influence of fertilizer amendments during phytoremediation. The physico-chemical characteristics used in the screening experiments changed after addition of crude oil and fertilizer. Screening experiments for 6 months in different concentrations of crude oil contaminated soil showed that *C. rotundus, C. brevifolius, C. odoratus, C. laevigatus*, and *M. pudica* can survive and tolerated up to 10% crude oil contaminated soil but with very low survival rate and tolerance. These plants showed good survival rate and tolerance up to 8% crude oil contaminated soil. These plants could not survive in 12% crude oil contaminated soil. Rate of degradation experiments in 8% crude oil contaminated soil showed that presence of plants had significant impact in the changes of soil physico-chemical characteristics during 720 days of study. Plant growth and biomass production reduced due to the toxicity of TPH. TPH degradation was significantly higher in planted treatments in comparison to unplanted treatments. Fertilized treatments in the presence of plants showed significantly higher degradation of TPH in soil TPH accumulation in roots and shoots, heavy metal degradation in soil, heavy metal accumulation in roots and
shoots and microbial numbers. However, discussions from these experiments are presented under following heads.

5.2 Screening of plant species for TPH and heavy metals degradation

The physico-chemical characteristics used in the screening experiments was suitable growth condition for plants after addition of fertilizer. All the physico-chemical characteristics were almost similar condition except the differences in concentration of crude oil in different treatments. The contaminated soil had significantly higher C:N ratio and organic carbon than control treatments. High values of C:N ratio and organic carbon in contaminated soil can be attributed to the presence of high concentration of carbon compounds added by hydrocarbon contamination. Screening experiments revealed that survival rate, height and biomass of *C. rotundus*, *C. brevifolius*, *C. odoratus*, *C. laevigatus*, and *M. pudica* decreased with increase in crude oil contaminated soil. Screening experiments for 6 months in different concentrations of crude oil contaminated soil showed that *C. rotundus*, *C. brevifolius*, *C. odoratus*, *C. laevigatus*, and *M. pudica* can survive upto 10% crude oil contaminated soil but with very low survival rate. These plants showed good survival rate upto 8% crude oil contaminated soil. However, plants grown in 2%, 4%, 6% and 8% crude oil contaminated soil showed higher survival rate and less mortality rate than the plants grown in and 10% crude oil contaminated soil. Plants in uncontaminated soil showed 100% survival with 0% mortality rate. These plants could not survive in 12% crude oil contaminated soil. The average values of height and biomass components of *C. rotundus*, *C. brevifolius*, *C. odoratus*, *C. laevigatus* and *M. pudica* were also found to be high in 2%, 4%, 6% and 8% crude oil contaminated soil. In 10% crude oil contaminated soil average height and biomass production were very low.
During the experimental period it was observed that the plants could show good tolerance up to 8% crude oil contaminated soil. The limit of tolerance of these plant species is 10% concentration with very low survival rate. Plant tolerance in crude oil contaminated soil can be attributed to high antioxidant defense that acts to protect the cells from possible oxidative damage. Thus, it appears that these plant species have high antioxidant defense that conferred them the capacity to survive in the soil with petroleum. Moller 2001 and Gratão et al. 2005 also found high antioxidant property in *S. macrophylla, H. campechianum*. Martí et al. (2009) found a correlation between the increase in antioxidant enzyme activity and plant tolerance to petroleum muds in *Medicago sativa*. For *Melilotus albus* developing in the presence of diesel, an over production of reactive oxygen species (ROS) was registered that stimulated the activity of antioxidant enzymes (Hernández-Ortega et al. 2011). On the other hand, microorganisms also play an important role in the reduction of oxidative damage caused by the presence of TPH. Debiane et al. (2009) found that the fungus *Glomus intraradices* notably reduced the oxidative damage in *Chichorium intybes* caused by the presence of anthracene and benzo (a) pyrene. On the other hand, Guerrero-Zúñiga and Rodríguez-Dorantes (2009) found that plants of *Cyperus hermaphroditus* responded to the toxicity of phenanthrene with a greater enzyme activity and expression of proteins in the rhizospheric zone, and that these exudates can transform or participate in the partial transformation of toxic products to less toxic substances which may be more available to the roots or the rhizospheric microorganisms (Gianfreda et al. 2005). In our study it was observed that there was less impact to the plant species, being able to survive in the presence of
petroleum in the soil which can be attributed to the reduction of oxidative damage in the presence of microorganisms.

Due to very low survival rate, height, biomass production and tolerance in 10% crude oil contaminated soil further rate of degradation experiments were designed in 80,000 mg kg\(^{-1}\) (8%) crude oil contaminated soil.

5.3 Rate of degradation of TPH and heavy metals with selected plant species

5.3.1 Changes of soil physico-chemical characteristics

The pH of vegetated soil was more than in unvegetated soil during the study period in all the treatments. Plants provide root exudates of carbon, energy, nutrients, enzymes and sometimes oxygen to microbial population in the rhizosphere (Campbell 1985; Cunningham et al. 1996b; Vance 1996). These exudates provide sufficient carbon and energy to support large number of microbes in the rhizosphere (Erickson et al. 1995). This plant induced enhancement of the microbial population is called rhizosphere effect (Atlas and Bartha 1998) and is believed to result in enhanced degradation of PHC and heavy metal contaminants in the rhizosphere. With sufficient oxygen, soil moisture, and an acclimated population of microorganisms the soil column acts as a natural biofilter within which PHC vapours are degraded at sufficient fast rates (USEPA 2011e). This dissipation of PHCs might have increased moisture content in soil. At lower soil pH, the bioavailability of cations generally increases due to replacement of cations on soil CEC sites by H\(^+\) ions (Taiz and Zeiger 2002). In the present study, increase in N level and bacterial utilization of PHCs might have declined the C:N ratio. The decrease of P and K could possibly be due to exhaustion of these nutrients from sandy loam soil as a result of their use by plants during their growth and development in the experimental period. The
decrease in total organic carbon in the vegetated pot might be due to utilization of hydrocarbon by plants and microbes. As an additional compartment, plant roots can interact with both microbes and organic pollutants (Bossert and Bartha 1994). Root proliferation of the plant can support a flourishing microbial consortium, thus accelerating biodegradation of PHCs (Cai et al. 2010). In turn, the healthier microbial consortium can benefit better growth of the plant, thus improving phytoremediation efficiency and characteristic of soil. However, due to the complexity of rhizosphere, more information about relationships between plant root–microbial interactions in respect of TPH degradation needs to be further extracted.

The physical conditions that affect metal bioavailability are temperature and moisture. In the present study, increase in moisture content increased bioavailability of TPH and heavy metals in planted treatments which enhanced the rate of degradation and hyperaccumulation. Higher temperatures accelerate physical, chemical and biological processes. Irrigation stimulates general plant growth and higher soil moisture increases migration of water-soluble pollutants. Proper watering and fertilization had positive effect in the increased soil moisture and migration of water-soluble pollutant during the course of study. Bioavailability of pollutant is also enhanced by chelators that are released in rhizosphere by plants and bacteria. Chelators such as siderophores, organic acids, and phenolics can release metal cations from soil particles (Taiz and Zeiger 2002) which make the metals more available for plant uptake. Furthermore, plants extrude H⁺ via ATPases, which replace cations at soil CEC sites, making metal cations more bioavailable (Taiz and Zeiger 2002).
5.3.2 Plant growth and biomass production

The effects of crude-oil on plants are attributed to phytotoxicity, which depends on several factors; concentration of oil in soil, oil type and its content of phytotoxic compounds (e.g., Polycyclic aromatic hydrocarbons, which includes most phytotoxic substances), environmental conditions and plant species (Baker 1970b). The reduction in growth and development by reduction of both height and biomass exhibited by all the plant species in contaminated soil in comparison to uncontaminated soil during the study period can be attributed to the phytotoxicity of crude oil. Several studies have shown that crude oil has an inhibiting effect on plant and root growth (Wiltse et al. 1998; Brandt et al. 2006). Brandt et al. (2006) observed 50% decrease in total biomass and 40% decrease in plant height in 5% crude oil contaminated soil compared to uncontaminated soil in 6 month old Vetiveria zizanioides (L.) Nash. In the study reported here, the same phenomenon was found for all the plant species studied. The average yields were significantly lower in contaminated soil than in uncontaminated soil ($p = 0.05$). In the crude oil contaminated soil, average reduction of plant height during the study of 720 days was 13.3 - 21.6% in case of C. rotundus, 28 - 31.8% in case of C. brevifolius, 25 - 33.8% in case of C. odoratus and C. laevigatus and 8 - 12% in case of M. pudica respectively compared to uncontaminated soil, regardless of the fertilizer in the contaminated soil compensating for the higher C/N ratio. However, reduction of height in presence of contaminants was less in M. pudica followed by C. rotundus, C. brevifolius, C. odoratus and C. laevigatus respectively. High rates of plant mortality and reduction in height and biomass are typical reactions caused by oil contamination (Lin and Mendelssohn 1998). These plants are herbaceous with extensive fibrous root system, can
multiply both vegetatively as well as sexually. Despite these characteristics, growth of plant was inhibited due to the presence of crude oil during the initial stage of growth. After 60 days the species showed adaptability to the toxic environment, as shown by the high rates of tillering and biomass production. The rate of tillering was more in *C. rotundus* (89 - 95%) followed by *C. brevifolius* (82 - 95%), *C. odoratus* (75 - 95%), *M. pudica* (87 - 92%) and *C. laevigatus* (73 - 92%) respectively. Regarding mortality rate, *C. rotundus* had less mortality rate (< 3%) followed by *C. brevifolius* (< 4%), *C. odoratus*, *C. laevigatus* and *M. pudica* (< 5 - < 8%). All the plants produced significantly (*p* < 0.05) more biomass in fertilized soils in comparison to unfertilized soil during the first 180 days of growth. The differences in biomass production diminished in the later periods. Comparison of average biomass reduction in contaminated soil in comparison to uncontaminated soil was less in *M. pudica* (8 - 12%) followed by *C. rotundus* (13.3 - 21.6%), *C. brevifolius* (28 - 31.8%), *C. odoratus* (25 - 33%) and *C. laevigatus* (26 - 34%) respectively.

Hydrocarbons create a hydrophobic layer around the root which limits the absorption of nutrients and the interchange of gases. Under these conditions, the plants suffer a metabolic imbalance generated by a condition of oxidative stress which disrupts cellular homeostasis (Mittler 2002, Gill and Tuteja 2010). Oxidative stress is the toxic effect caused by chemical substances that are highly reactive (ROS) produced during the reduction of molecular oxygen (Halliwell 2006). Upon entering into a stressed state the plants act to reduce the production of ROS by processes such as anatomical and physiological adaptions, mechanisms which re-organize the photosynthetic apparatus, or suppress photosynthesis (Gechev et al 2006). Another form of defense is the action of
antioxidant molecules to eliminate ROS. If the intercellular concentration of ROS is not controlled, a direct consequence is the damage of cell structure, as well as the interruption of metabolic pathways (Blokhina et al 2003, Moller et al 2007), conditions which can cause plant death. Possibly, it is by this phenomenon that *C. rotundus*, *C. brevifolius*, *C. odoratus*, *C. laevigatus* and *M. pudica* were seen to be affected in the production of biomass, height and especially, plant mortality. Upon harvesting the plants it was observed that their roots were completely covered in a layer of petroleum, being more notable in the soil with the highest concentration. Thus, it appears that these plant species possess good antioxidant defense that acts of protect the cells from possible oxidative damage. These conferred them the capacity to survive in the soil with petroleum, with only a little effect to height and biomass.

**5.3.3 TPH degradation in soil**

The concentration of oil dropped sharply in the vegetated soil than in unvegetated soil during the course of study in the experiment with *C. rotundus*, *C. brevifolius*, *C. odoratus*, *C. laevigatus* and *M. pudica*. During the first 120 days TPH degradation was faster, degradation slowed down subsequently during 180, 240, 300 and 360 days. During 420 days onwards there was marginal degradation of TPH that continued up to 720 days. Despite inhibition of plant growth and root development, the decrease of crude oil in soil was significantly more (*p* < 0.05) in vegetated soil than in unvegetated soil. Merkl et al. (2005a) and Merkl et al. (2005b) showed enhanced degradation of crude oil under the influence of a tropical grass after only a few months. Muratova et al. (2008) showed TPH reduction up to 52% during 3 years of rye cultivation. Diab (2008) recorded 30%, 16.8% and 13.8% reduction of TPH in rhizosphere soil of broad bean, corn and wheat
respectively. In addition, Peng et al. (2009) noted 41.61 - 63.2% removal of TPH by *Mirabilis jalapa*. In the present study, *C. rotundus* could decrease more TPH (90% in fertilized soil, 64.5% in unfertilized soil) followed by *C. brevifolius* (86.9% in fertilized soil, 57.3% in unfertilized soil), *C. odoratus* (82.2% in fertilized soil, 50.3% in unfertilized soil) and *C. laevigatus* (76.4% in fertilized soil, 44.5% in unfertilized soil). *M. pudica* showed less degradation of TPH (62.6% in fertilized soil, 42.6% in unfertilized soil) in comparison to other plants. The degradation however, is significantly more (*p* < 0.05) in fertilized soil in comparison to unfertilized soil in the experiment with all the plants. The fertilized soil could compensate the nutrient demands of plants during phytoremediation. Unfertilized soil seemed to be insufficient to meet the nutrient demands of plants. Hutchinson et al. (2001) also observed better degradation of TPH using grasses with N/P amendments than without inorganic amendments. In addition, Merkl et al. (2005a) found enhanced degradation of crude oil by using *B. brizantha* in NPK fertilized soil in comparison to control. In the present study, the degradation is due to capability of plant to tolerate and degrade crude oil. Lopez-Martinez et al. (2008) also found significant reduction of TPH by *Cyperus laxus* Lam. in 24-months when plants were cultivated on hydrocarbon-contaminated soil and spiked perlite. The slower and less degradation of TPH in unvegetated soil can be attributed to its lack of plants. Volatilization of lighter fractions of crude oil might have contributed to the rapid degradation. In addition, microbial degradation might have also contributed to reduction of crude oil in soil. Both these actions constitute part of the natural attenuation phenomenon (Margesin and Schinner 2001; Pichtel and Liskanen 2001; Bento et al. 2005; Chaîneau et al. 2005; Sarkar et al. 2005; Scow and Hicks 2005; Atagana 2010a).
The slow rate of removal after day 60 might be due to removal of volatile components and the remaining components of the crude oil required intervention to be removed from the soil. A comparison of TPH removal in the vegetated treatments and unvegetated treatments clearly showed that phytoremediation by experimental plants was responsible for increased removal of TPH. In the present study, removal of TPH in vegetated treatments is believed to be due to the interaction of rhizospheric microorganisms and plants which is similar to the findings of Liste and Prutz (2006b), Ho et al. (2007) and Muratova et al. (2008). As roots grow, they penetrate through the soil, exposing entrapped contaminants that have been previously inaccessible, increasing their availability to degradation which is similar to the findings of Fava et al. (2004) and Bogan and Sullivan (2003). Moreover, the root apparatus increased soil aeration, reducing soil moisture content and changing physicochemical and biological characteristics which is in agreement with the findings of Bowen et al. (1991) and USEPA (1999b).
5.3.4 TPH accumulation in plant roots and shoots

Concerning hydrocarbon accumulation, the plants in the presence of fertilizer showed significantly more accumulation of TPH than unfertilized one. It is believed that the inhibition in growth caused by crude oil contaminated soil in unfertilized treatments, which resulted in the slow removal of oil in the soil accounts for the phytotoxicity of oil in the roots and shoots of the plants grown in these experiments. It can be argued that the metal affected the physiological processes responsible for movement of solutions in the plants such as imbibitions and transpiration, resulting in slow movement of water and solutes in the plant. On the other hand the fertilized soil allowed for larger accumulations of oil in plant roots and shoots. Atagana (2010a) also found similar results during his study on bioremediation of co-contamination of crude oil and heavy metals in soil by phytoremediation using Chromolaena odorata (L) King and H.E.Robinson.

*C. rotundus*, *C. brevifolius*, *C. odoratus*, *C. laevigatus* and *M. pudica* possess good capacity to degrade and accumulate TPH. There was positive relationship between the TPH concentrations of the soils with the plants. The uptake of TPH by these plant species may be more dependent on the adaptive physiological characteristics of the plant and the mobility and other properties of the hydrocarbon itself than on the TPH concentrations. Adaptive strategies including rejection, metabolism, and excretion of the TPH would be adopted selectively by the plant to regulate the heavy metals when growing in polluted environments. Study of TPH accumulation in plants indicated that *C. rotundus*, *C. brevifolius*, *C. odoratus* and *C. laevigatus* showed better ability to accumulate TPH among the five investigated plant species under the present net house conditions. From the weighted mean, it was found that the accumulation abilities for TPH
are as follows, in decreasing order: *C. rotundus* > *C. brevifolius* > *C. odoratus* > *C. laevigatus* > *M. pudica*. However, comparison of the accumulation abilities amongst the plant species showed that these plants can accumulate TPH. This indicates that there are different mechanisms operating to provide TPH tolerance variations amongst the plant species. The tolerance mechanism is probably related to the ecological and physiological behavior. This mechanism remains to be further elucidated.

The accumulation potential of a plant is determined not only by its capacity to absorb high oil concentration, but also by its ability to translocate the hydrocarbons from roots to aerial parts and produce simultaneously a high biomass. In our study, hydrocarbon content of the plant species was relatively high during the initial stages of root growth; after 240 days of experimentation the degradation and accumulation were high and during 720 days highest degradation and accumulation were observed in fertilized soil relative to unfertilized soil which can be attributed to the addition of inorganic fertilizer.

### 5.3.5 Heavy metals degradation in soil

The high rate of metal removal from the soil in the planted treatments can be attributed to the presence of plants in the soil. This high rate of removal of metal ions have significant correlation with plant growth parameters, as it was noted that plants with higher biomass (fertilized treatments) showed higher metal removal. Correlation was observed with species of the plant and concentration of pollutants in soil. Higher removals of metals were observed in fertilized soil in comparison to unfertilized soil. The least degradation of heavy metals in unvegetated soil can be attributed to the absence of plants. Significant difference in the concentrations of metals between vegetated and
unvegetated pots for these species were rare, with unvegetated soil removing high proportions of metals. Read et al. (2008) also found similar result in their study of variation among plant species in pollutant removal from stormwater in biofiltration systems. Other studies have also shown that the soil medium in vegetated and non-vegetated biofilters removes high proportions (e.g. >90% on average) of influent metals, including Pb, Cu and Zn (Hatt et al. 2007a; Sun and Davis, 2007; Hatt et al. 2008b). Although some metals are required by plants in trace amounts, they are substantially less important than N and P, and concentrations are typically low in plant tissue, other than in specialists that hyperaccumulate some metals (Baker and Brooks, 1989c). The biofilter media therefore play the dominant role in metal uptake. This study shows how variation among plant species in their growth and morphology may influence effective pollutant removal in phytoremediation, and consequently, inform species selection for efficient phytoremediation design. However, there are still substantial gaps in our knowledge. The trends found in this study require investigation across a broader range of plant species, and under a range of environmental conditions.

5.3.6 Heavy metals accumulation in plant roots and shoots

The results obtained from this study suggest that soil properties significantly influence metal degradation and hyperaccumulation in *C. rotundus*, *C. brevifolius*, *C. odoratus*, *C. laevigatus* and *M. pudica*. Metal concentration and uptake in roots and shoots differed among the studied soils among different plant species and may be attributed, in part, to the experimental soil properties, such as organic carbon, soil pH, clay and free Fe contents. The highest concentration of uptake of metals occurring in the plant species may be related to the soils of highly acidic pH and organic carbon content,
free Fe content, and clay content. The combination of these properties suggest that a greater fraction of soluble metals were available for plant uptake in this soil which is similar to the findings of O’Neill (1990); Smith et al. (1998a) and McLaren et al. (2006). The higher accumulation of metals in the roots and shoots of the plant in the fertilized treatments, further explains the rapid metal removal in different treatments. It is well documented that free Fe oxides are the dominant soil constituents responsible for metal sorption (Fendorf et al. 1997), and soil organic matter can also adsorb metals, thus reducing its availability (Redman et al. 2002). Our results corroborate the findings of earlier studies that have indicated acidic soil pH and low clay content caused low sorption on inorganic pollutants (Xu et al. 1991; Smith et al. 1999b; McLaren et al. 2006).

*C. rotundus, C. brevifolius, C. odoratus, C. laevigatus* and *M. pudica* possess good capacity to degrade and accumulate some heavy metals. There was positive relationship between the heavy metal concentrations of the soils with the plants. The uptake of heavy metals by these plant species may be more dependent on the adaptive physiological characteristics of the plant and the mobility and other properties of the metal itself than on the metal concentrations. Cu, Fe, Mn, Zn, Cu, and Ni are essential micronutrients to the plants, while Cr, Pb, Cd, As, Se and Hg are non-essential and toxic metals. As an essential element for the growth, the plants might have accumulated the essential elements which were found in both contaminated and uncontaminated soil. Non-essential elements were found in roots and shoots of plants grown in contaminated soils only which might be due to the presence of these heavy metals in hydrocarbon contaminated soil and capability of plants to accumulate these metals. Adaptive strategies including rejection, metabolization, and excretion of the heavy metals would be adopted
selectively by the plants to regulate the heavy metals when growing in polluted environments. Deng et al. (2004) reported that the metal tolerance mechanism in plants may be related to the special root anatomy in plants, the alleviated metal toxicity by the reduced rooting conditions and the relatively high innate metal tolerance in some species. It also indicated that metal concentrations in plant parts were significantly different amongst plants (Otte and Wijte 1993). To compare plant accumulation abilities for heavy metals more accurately, the accumulation was studied separately in roots and shoots. The results indicated that *C. brevifolius*, *C. odoratus* and *C. rotundus* showed better ability to accumulate heavy metals among the five investigated plant species under the present net house conditions. From the weighted mean, it was found that the accumulation abilities for heavy metals in terms of multi-metal are as follows, in decreasing order: *C. odoratus* > *C. brevifolius* > *M. pudica* > *C. laevigatus* > *C. rotundus*. Whereas, the accumulation capabilities in terms of percentage of particular metal are as follows, in decreasing order: *C. rotundus* > *C. brevifolius* > *C. odoratus* > *C. laevigatus* > *M. pudica*. The plant species are tolerant to heavy metals; this tolerance may come from the plant’s good capability to transfer metals, with higher heavy metal concentration. Ye et al. (1997) investigated metal concentrations in shoots and in roots of seedlings of four different ecotype populations of *Phragmites australis* cultivated in the MPCS substrate and found the shoots containing 47~60 mgkg$^{-1}$ Zn and 2.5~4.0 mgkg$^{-1}$ Pb, while the roots containing 100~164 mgkg$^{-1}$ Zn and 8.4~13 mgkg$^{-1}$ Pb, which shows the accumulation ability of root is much higher than that of shoot, without any mention about accumulation by foliage. In our study, there was no significant difference of heavy metal accumulation in roots in comparison to shoots. Some metals may be retranslocated and selectively
stored in the vacuoles of older leaves (Windham et al. 2001). A comparison of the accumulation abilities amongst the plant species showed that these plants can accumulate Cu, Zn and Pb, either for essential elements or for non-essential elements, at a high level. This indicates that there are different mechanisms operating to provide heavy metal tolerance variations amongst the plant species. The tolerance mechanism is probably related to the ecological and physiological behavior. This mechanism remains to be further elucidated.

The phytoremediation potential of a plant is determined not only by its capacity to absorb high metal concentration, but also by its ability to translocate the metal from roots to aerial parts and produce simultaneously a high biomass. In our study, metal content of the plant species were relatively high during the initial stages of root growth; after 360 days of experimentation the degradation and accumulation was high and during 720 days. Highest degradation and accumulation were observed in fertilized soil relative to unfertilized soil which can be attributed to the addition of inorganic fertilizer. This is in agreement with the earlier reports on many plants, which have shown significant accumulation of metals in the roots from the externally supplied metal solution (Banks et al. 2006b; Diwan et al. 2010).

5.3.7 Microbial population in soil

During the initial stage of experiment the population of microorganism was very low in soil with all the experimental plant species. The slow and gradual adaptation of the microbial population to the crude oil was observed in the fertilized treatments, which is probably due to the beneficial effect of adding inorganic fertilizer, resulting in a higher number of MPN. In all the plant species studied the population of microorganism was
more than double in fertilized soil and less than double in unfertilized soil. In uncontaminated soil the increase in MPN was 4 - 6 times in comparison to initial count. In our work the plant promoted increase in MPN is believed to be due to the presence of root exudates of carbon, nitrogen and organic acids.

Microbial population in the planted treatments in the presence of fertilizer showed a significant increase in the population of MPN bacteria at the end of the experiment (720 days) in relation to the initial (time 0) population in the experiment with *C. rotundus, C. brevifolius, C. odoratus, C. laevigatus*, and *M. pudica*. The slow and gradual adaptation of the microbial population to the crude oil contaminated soil was observed in the fertilizer containing treatments, probably due to the beneficial effect of adding inorganic fertilizer, and, thus, resulting in the greatest reduction of TPH. The uncontaminated soil (TE) showed the highest MPN number, due to rapid increase in population in the absence of contaminants. In the unvegetated pots no significant increase in bacterial population was observed in this study. The increase in bacterial population in the vegetated pots might have had positive impacts on the degradation of TPH. The increased microbial numbers are primarily due to the presence of plant exudates and sloughed tissue, which serve as sources of energy, carbon, nitrogen, or growth factors (Lee and Banks 1993). Huang et al. (2004) reported the enhanced phytoremediation of organic contaminants using grass species inoculated with a mixture of beneficial bacteria such as *Pseudomonas putida, Azospirillum brasilense* and *Enterobacter cloaeae*. Microorganisms have a fundamental influence on the health conditions of plants. In our work, plant-promoted degradation of hydrocarbon may be due to the complexity of plant and microbe interactions.
Rhizosphere microbial populations may enhance a plant’s adaptation to petroleum hydrocarbons by detoxifying contaminated soils through direct mineralization of these organic contaminants (Siciliano and Germida 1998; Jeffries et al. 2003; Barea et al. 2005). In the present study, the bacterial population was found to be 2 - 6 times in vegetated pots during the study of 720 days. Sabaté et al. (2004) also found similar levels of soil bacteria in hydrocarbon contaminated soil. Ghazali et al. (2004) reported a ten-fold increase in soil microorganisms. Microorganisms have a strong influence on the health conditions of plants. In our work, a plant promoted degradation of hydrocarbon may be due to the complexity of plant-microorganism interactions which in addition brought about the changes of soil physico-chemical characteristics, hence, improved soil property.

5.3.8 Influence of fertilizer

Fertilizers increase plant growth in oil-polluted soils in the case of nutrient deficiency (Lin and Mendelssohn 1998; Hutchinson et al. 2001). However, over fertilization usually leads to yield depressions (Brandt et al. 2006). In our study, there was significant difference of plant growth between the fertilized and unfertilized soil during the first 60 days but the differences diminished towards the end of the experiment in the experiment with all the plant species. Concerning TPH degradation, accumulation, heavy metal degradation and accumulation, there was significant influence of fertilizer application during the experiment. However, the fertilized soil could show significant (\( p < 0.05 \)) amount of TPH degradation in soil, accumulation in plant roots and shoots, heavy metal degradation in soil and accumulation of metals in plant roots and shoots in comparison to unfertilized soil. For the soil used, the unfertilized soil seemed to be insufficient to meet the nutrient demands of plants. The fertilized soil could compensate
the nutrient demands of plant for phytoremediation. Hutchinson et al. (2001) also observed better degradation of TPH using grasses with N/P amendments than without inorganic amendments. In addition, Merkl et al. (2005a) found enhanced degradation of crude oil by using *B. brizantha* in NPK fertilized soil in comparison to control. However, in this study, application of basic doses of fertilizer had significant influence on plant growth, biomass production, TPH degradation in soil, accumulation of TPH in plant roots and shoots, heavy metal degradation in soil and accumulation of heavy metals in plant roots and shoots.