NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 30, 2013
Print ISSN 1222-4227; Online ISSN 2067-5720

INOCULATION WITH ACC-DEAMINASE CONTAINING BACTERIA TO IMPROVE PLANT GROWTH IN PETROLEUM CONTAMINATED SOIL

Hafiz Naeem Asghar*, Muhammad Ayyaz Zafar, Muhammad Yahya Khan and Zahir Ahmad Zahir

Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Faisalabad-38040, Pakistan
*Corresponding author. Email: naeemasghar@yahoo.com

ABSTRACT

Plants growing in petroleum hydrocarbons contaminated soils are often subjected to the nutritional and chemical stress. that results in the production of excess ethylene which ultimately leads to plant growth inhibition. One strategy to eliminate this stress is inoculation of contaminated soils with bacteria having ACC-deaminase enzyme. When plant growth-promoting bacteria with this enzyme are bound to the seed coat of a developing seedling, they may act as a sink for ACC and thus the ethylene level is not elevated to the point where root growth is impaired. To evaluate this, bacteria were isolated from petroleum contaminated soils and tested for ACC-deaminase activity both qualitative and quantitative. A pot experiment was conducted in growth room to study the effect of inoculation with ACC-deaminase positive bacteria on the growth of canola seedlings in petroleum contaminated soil. Pots were contaminated with three different levels (1, 2 and 3%) of mixture of diesel and kerosene. Petroleum stress caused significant damage to plant growth but inoculation significantly recovered the negative effect of stress. It was revealed that inoculation with ACC-deaminase containing plant growth promoting bacteria was more effective at lower levels of contamination but at highest level it had comparatively less effect.

Key words: TPH, canola, enzyme.

INTRODUCTION

Soil contamination with organic and inorganic pollutants is gaining considerable attention due to their toxic effects on natural vegetation, wildlife and human health (Rahbar et al., 2012). Among the organic pollutants, petroleum hydrocarbons are potent soil contaminants threatening the life on earth (Alkorta and Garbisu, 2001; Villalobos et al., 2008). Mainly petroleum hydrocarbons are complex mixture of saturated hydrocarbons, aromatic compounds, asphaltenes and resin (Huang et al., 2005; Tang et al., 2012). Petroleum contamination could be due to road traffic, unchecked industrial and agricultural activities (Alkorta and Garbisu, 2001; Vasudevan and Rajaram, 2001; Peng et al., 2009). The use of petroleum as the principle source of energy is continuously causing pollution of petroleum hydrocarbons in the environment (Plohl et al., 2002; Kathi and Khan, 2011). Petroleum contamination also results from leakage of above ground and underground storage tanks, spillage during transport of petroleum products, abandoned manufactured gasoline sites, other unplanned releases and current industrial processes (Mishra et al., 2001; Sarkar et al., 2005; Atlas and Philp, 2005; Okoh, 2006; Akpor et al., 2007). Petroleum hydrocarbons are going to be sequestered in soil and sediments (Denys et al., 2006) due to relatively high hydrophobicity which may results in partitioning of these compounds into soil organic matter and/or diffusion into nanopores (Karthikeyan and Bhandari, 2001; Tang et al., 2012) ultimately decrease in their bioavailability for natural biological degradation (Parrish et al., 2005; Luepromchai et al., 2007). This sequestration of petroleum hydrocarbons in soil is affecting plant growth and development (Ferro et al., 1999; Joner et al., 2004; Das and Mukherjee, 2007). Toxicity of petroleum hydrocarbons on plants could be chronic or acute (Bona et al.,

Received 12 November 2012; accepted 22 March 2013. First online: 10 April 2013. DII 2067-5720 RAR-254

2011). Petroleum hydrocarbons interfere in hydric relations of plants, affects the seed germination, decrease photosynthetic pigments and reduce nutrient assimilation by creating hydrophobic conditions accompanied by anaerobic environment in soil (Racine, 1994; Odjegba and Sadio, 2002). Petroleum hydrocarbons cause oxidative stress, decrease growth, leaf deformation and tissue necrosis in plants (Al-Hawas et al., 2012). Root growth is also severely affected by petroleum hydrocarbons contamination (Alkio et al., 2005; Njoku et al., 2012; Rahbar et al., 2012). This root inhibition in contaminated soil might be due to generation of stress induced ethylene (Rodecap and Tingey, 1981; Zahir et al., 2005). Ethylene biosynthesis under contaminant stress is considered to be accelerated (Glazebrook, 2005; Weisman et al., 2010; Zahir et al., 2012) which could be key factor for inhibition of root growth (Hall et al., 1996). Extensive root system is primarily important for better plant growth and development as plant vigor is directly related to the better root system (Doty et al., 2007). Moreover, prolific root growth is also prerequisite for rhizo-degradation of petroleum hydrocarbons (Wenzel, 2009; Maqbool et al., 2012). For effective phytoremediation and better plant growth in contaminated soil, elimination of root inhibiting factor like production of ethylene is a major challenge (Arshsd et al., 2007). Nature has gifted the some microbes which stay in stress environment (Asghar et al., 2012) with a novel enzyme known as 1-aminocyclopropane-1-carboxylase (ACC) deaminase (Glick, 1995; Shaharoona et al., 2006; Wang et al., 2012). This enzyme regulates the biosynthesis of ethylene by metabolizing its immediate precursor ACC into α-ketobutyrate and ammonia (Glick, 2005; Arshad et al., 2007). Plants inoculated with bacteria containing ACC-deaminase enzyme produced more plant biomass with longer roots and better root density (Glick et al., 1998; Zahir et al., 2009). Proliferation of roots by the assistance of ACC-deaminase containing bacteria could enhance rhizo-degradation of petroleum hydrocarbons contaminations (Huang et al., 2005). Keeping in view the possible role of bacteria having ACC-deaminase enzyme in plant growth promotion by improving root system in petroleum hydrocarbons contaminations, the present study was carried out to evaluate the growth promotion potential of ACC-deaminase containing bacteria in petroleum contaminated soil.

MATERIAL AND METHODS

Isolation, purification and preservation of bacteria

For isolation of bacteria, soil samples were collected from previously petroleum exposed areas of Karachi and Faisalabad, Pakistan. Bacteria were isolated by dilution plate technique using glucose peptone agar medium. Isolated bacterial colonies were further purified by repeated streaking on glucose peptone agar medium. Purified bacterial colonies were preserved in Eppendorf by using glycerol at -40ºC temperature.

Characterization of bacteria for ACC-deaminase activity

Selected bacterial isolates were characterized qualitatively for ACC-deaminase activity by following the method described by Jacobson et al. (1994). ACC-deaminase containing bacteria were also quantitatively assayed for ACC-deaminase activity by following the method described by Saleh and Glick (2001) which measured the amount of α-ketobutyrate produced when this enzyme cleaved ACC.

Pot experiment

Sandy clay loam soil with pHs 8.2, saturation percentage 38%, EC_e 2.12 dS m^{-1}, CEC 4.42 $cmol_c$ kg^{-1} soil, organic matter 0.68%, extractable potassium 136 mg kg^{-1}, available phosphorus 6.48 mg $kg^{-1}$and total nitrogen 0.08%, was used to fill the pots. Soil was passed through 2 mm sieve and autoclaved for sterilization to kill indigenous microbes. The autoclaved soil was spiked with equal quantity of diesel and kerosene (1:1) to maintain three levels of contamination (1, 2 and 3% of total soil mass in each pot).

Control was kept with no added oil. The soil was thoroughly homogenized to get uniform contamination from top to bottom. These pots were then left for 15 days in the same temperature and light set for experiment, for natural weathering and to avoid toxicity to seedlings due to evaporation. The inoculum for pot trial was prepared by growing the selected isolates in glucose peptone broth medium. Flasks containing glucose peptone broth were inoculated with selected isolates and incubated at 28±1°C for 3 days. Bacterial cells were harvested by centrifugation at 4500 rev min^{-1} for 20 minutes. Then cells were washed and suspended in sterilized phosphate buffer saline (pbs) and uniform cell density (10^7-10^8 CFU mL^{-1}) was achieved by maintaining optical density of (OD=0.45) at 535 nm. The inoculum of each isolate was injected into sterile peat (100 ml kg^{-1}) and was incubated for 24 hrs at 28±1°C before using it for seed coating. For seed inoculation, seed dressing was carried out with inoculated peat mixed with clay and 10% sugar solution. In case of the un-inoculated control, the seeds were coated with the same but autoclaved inoculum suspension. Inoculated canola (*Brassica napus* L.) seeds were sown in contaminated pots keeping one treatment inoculated but without contamination and three treatments contaminated but un-inoculated to sort out the detrimental effect of petroleum stress on plants and recovery efficiency by ACC-deaminase containing bacteria. The experiment was conducted in the growth room under controlled conditions. The data recorded about different growth parameters were subjected to statistical analysis by using Statistix-9 computer software.

RESULTS

To evaluate the role of ACC-deaminase containing bacteria on growth of canola many bacteria were isolated, 10 isolates were found positive both qualitatively and quantitatively with ACC-deaminase activity. The strain with maximum (631α-ketobutyrate nmol g^{-1} biomass h^{-1}) ability to hydrolyze ACC into NH_3 and α-ketobutyrate was finally selected to inoculate the canola seeds grown at different levels of petroleum oil contamination.

Results of pot experiment indicated that petroleum hydrocarbons contamination significantly suppressed the plant growth and development as compared to plants grown on normal soil and this effect was more severe when contamination increased from 1% to 3%. However, the inoculation of bacterial isolates with ACC-deaminase enzyme enhanced the plant growth significantly at all three levels of contamination as compared to respective un-inoculated plants grown in contaminated soil but at variable rates. No doubt, the bacterial inoculation enhanced the plant growth in all contamination levels but the stress recovery was always less than 100%. Results showed (Table 1) that the decrease in the total fresh weight was up to 52, 69 and 87% at contamination levels of 1, 2 and 3%, respectively, as compared to normal soil. However, bacterial inoculation decreased the inhibitory effect of contamination up to 24, 51 and 66% at 1, 2 and 3% contamination levels, respectively. Data (Table 1) regarding shoot fresh weight indicated that in contaminated soil there were 48, 64 and 83% decrease in shoot fresh weight as compared to plants grown in normal soil. But, inoculation improved the shoot fresh weight up to 58, 58 and 190% by decreasing the negative effect of contamination up to 18, 44 and 60% at 1, 2 and 3% levels of contaminations, respectively. The reduction in root fresh weight (Table 1) was up to 69, 91 and 93% at 1, 2 and 3% contamination levels respectively as compared to normal soil, the root fresh weight reduction effect was statistically non-significant at 2 and 3% contamination level. Nevertheless, the bacterial inoculation increased the root fresh weight up to 41, 105 and 40% by decreasing the root fresh weight reduction effect up to 56, 82 and 90% at contamination levels of 1, 2 and 3%, respectively. Number of leaves was reduced (Table 1) up to 39, 49 and 57% at 1, 2 and 3% contamination levels respectively as compared to plants grown in normal soil. Though, bacterial inoculation increased the number of leaves up to 30, 36 and 9% at 1, 2 and 3% contamination levels, respectively.

*Table 1.*Effect of ACC-deaminase enzyme containing bacteria on total fresh weight, shoot fresh weight, root fresh weight and number of leaves per plant of canola in petroleum hydrocarbons contaminated soil

Treatments	Total Fresh Weight (g)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	No. of Leaves Plant^{-1}
Control	0.91 b	0.73 b	0.200 b	5.44 b
1 % Oil	0.44 e	0.38 e	0.062 d	3.33 d
2 % Oil	0.28 g	0.26 g	0.018 f	2.78 e
3 % Oil	0.12 h	0.10 h	0.015 f	2.33 e
Inoculation	1.99 a	1.75 a	0.240 a	7.00 a
1 % Oil + Inoculation	0.69 c	0.60 c	0.088 c	4.33 c
2 % Oil + Inoculation	0.45 d	0.41 d	0.037 e	3.77 d
3 % Oil + Inoculation	0.31 f	0.29 f	0.021 f	2.55 e

Means sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$).

Results showed that contamination levels of 1, 2 and 3% decreased the shoot length up to 21, 30 and 34% as compared to plants grown in normal soil (Table 2). Nonetheless, bacterial inoculation recovered the reduction in shoot length by increasing 21, 22 and 30% shoot length at 1, 2 and 3% contamination levels, respectively. Root length was decreased (Table 2) up to 34, 64 and 74% at 1, 2 and 3% contamination levels, respectively, as compared to normal soil. Bacterial inoculation improved the root length by decreasing this inhibitory effect of contamination up to 13, 26 and 37% by increasing root length up to 31, 102 and 142% at 1, 2 and 3% contamination levels, respectively. Contamination levels 1, 2 and 3% also significantly reduced the shoot dry weight up to 42, 53 and 89%, respectively, as compared to plants grown in normal soil (Table 2). Bacterial inoculation also enhanced the shoot dry weight up to 41, 38 and 350% at 1, 2 and 3% contamination levels, respectively, by reducing the inhibitory effect of contamination up to 18, 35 and 51% respectively. Results regarding root dry weight showed that 1, 2 and 3% contamination levels decreased the root dry weight up to 64, 71 and 79% as compared to plants grown in normal soil (Table 2). However, the increase in root dry weight with bacterial inoculation was up to 120 and 50% at 1 and 2% contamination levels respectively but the effect of bacterial inoculation on root dry weight at 3% contamination level was statistically non-significant. Results showed that inoculation with ACC-deaminase containing bacteria also improved plant growth in normal soil (non-contaminated) by increasing total fresh weight (118%) shoot fresh weight (139%), root fresh weight (20%), shoot length (6%), root length (37%), number of leaves plant^{-1} (29%), shoot dry weight (127%) and root dry weight (57%) as compared to un-inoculated plants grown in normal soil.

Table 2. Effect of ACC-deaminase enzyme containing bacteria on shoot length, root length, shoot dry weight and root dry weight of canola in petroleum hydrocarbons contaminated soil

Treatments	Shoot Length (cm)	Root Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)
Control	8.52 b	3.78 b	0.055 b	0.014 b
1 % Oil	6.73 e	2.49 e	0.032 de	0.005 cd
2 % Oil	5.97 f	1.37 f	0.026 f	0.004 d
3 % Oil	5.60 g	0.99 g	0.006 g	0.003 d
Inoculation	9.02 a	5.16 a	0.125 a	0.022 a
1 % Oil + Inoculation	8.18 c	3.27 c	0.045 c	0.011 bc
2 % Oil + Inoculation	7.30 d	2.78 d	0.036 d	0.006 cd
3 % Oil + Inoculation	7.27 d	2.40 e	0.027 ef	0.003 d

Means sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$).

DISCUSSION

Petroleum hydrocarbons are among the most persistent organic pollutants, which are causing environmental degradation and health problems. Soil contamination with petroleum hydrocarbons also severely affects the plant growth and development. Petroleum hydrocarbons tolerant plant growth promoting bacteria (PGPB) with ACC-deaminase enzyme have potential to enhance plant growth under petroleum hydrocarbons contamination. Plant growth promoting bacteria used in this experiment were much effective to improve plant growth in non-contaminated soil and their effect in contaminated soil was also significant as compared to un-inoculated and contaminated soil. In present study, the effect of petroleum hydrocarbons tolerant bacteria with ACC-deaminase activity on growth of canola at 1, 2 and 3% contamination levels revealed that contamination significantly decreased the plant growth, but inoculation with the ACC-deaminase containing bacteria enhanced the growth of plants in contaminated soil. The growth decrease was more prominent by increasing contamination levels as compared to non-contaminated soil. This hindrance in plant growth and development might be attributed to hydrophobicity of petroleum hydrocarbons which may result in drought conditions (Racine, 1994; Al-Moaikal et al., 2012) and might be due to impaired water and nutrient uptake from soil (Jones et al., 2004; Wenzel, 2009). This growth retardation could be also due to disturbance in normal physiological processes i.e. generation of reactive oxygen species (ROS), leading to cellular death (Weisman et al., 2010), degradation of chlorophyll (Alkio et al., 2005), decreased photosynthesis and respiration (Baker, 1970) and might be due to reduced starch assimilation due to inhibition of amylase and starch phosphorylase (Achuba, 2006). The reduction in plant growth may also be attributed to the biosynthesis of stress induced ethylene in roots of canola plant under petroleum hydrocarbons stress (Glick et al., 2007). Root growth is often inhibited by pollutant-induced stress. However, the plants inoculated with ACC-deaminase containing bacteria showed better growth as compared to plants growth without inoculation in contaminated, as well as non-contaminated soil. Numerous studies have reported that bacteria with ACC-deaminase enzyme enhanced the plant growth under stress conditions (Glick, 2003; Nadeem et al., 2006; Zahir et al., 2012; Chookietwattana and Maneewan, 2012; Khan et al., 2013) and also in normal conditions (Zafar-ul-Hye et al., 2007; Shahzad et al., 2010; Bhattacharyya and Jha, 2012). The inoculated plants grow well in petroleum hydrocarbons contaminated soil, with more root biomass probably alleviating ethylene production in stressed plant by bacterial consumption of 1-aminocyclopropane-1-carboxylate (ACC), an immediate precursor of ethylene (Glick et al., 1995; Ghosh et al., 2003; Shaharoona et al., 2006). This improvement in plant growth and development of canola under petroleum hydrocarbons stress might be due to production plant growth regulators or biologically active substances (Zahir et al., 2003; Asghar et al., 2004; Humphry et al., 2007), reducing or inhibiting harmful effects of contaminants in the rhizosphere (Tang et al., 2010; Divya and Kumar, 2011) and/or helping in availability and uptake of certain nutrients from the rhizosphere i.e. phosphorus solubilization and siderophore production (Gupta et al., 2002; Pena and Reyes, 2007). Growth improvement in petroleum hydrocarbons due to bacterial inoculation may also be attributable to rhizo-degradation of contaminants by these bacteria (Maqbool et al., 2012), which may result in reduction of contaminant stress. As prolific root growth can exploit rates of rhizo-degradation of organic pollutants which may be attributed by ACC-deaminase containing bacteria due to improvement in root growth (Huang et al., 2005). Petroleum hydrocarbons tolerant bacteria with ACC-deaminase activity may have the characteristics of utilizing petroleum hydrocarbons as well (Glick et al., 1998; Belimov et al., 2001), because it is evident from previous studies that some specific

enzymes are induced in microorganisms that can face contamination stress (Alexander, 1999). About 40% photosynthates in the form of sugars, organic acids, and larger organic compounds are released by plants in the rhizosphere through roots (Kumar et al., 2006) which are commonly used by soil microbes for energy source (Siciliano et al., 2003). These situations may result in interactions of roots, root exudates and soil microbes for enhanced rhizo-degradation and plant growth improvement in petroleum hydrocarbons contamination (Hontzeas et al., 2004). The improvement in total fresh weight, shoot fresh weight and root fresh weight by bacterial inoculation might also be due to improvement in water and nutrient uptake of plants by reason of decreased hydrophobic compound in rhizosphere due to rhizo-degradation of petroleum hydrocarbons (Chaineau et al., 2005; Gerhardt et al., 2009). Higher shoot and root dry weight of canola in petroleum hydrocarbons contamination by ACC-deaminase containing bacteria might be due to increase in chlorophyll pigments (a, b and carotenoids) of canola (Glick et al., 1997; Han and Lee, 2005). This may result in increased photosynthetic leaf area which ultimately improves photosynthesis and more biomass production due to bacterial inoculation under stress (Marcelis and Van Hooijdonk, 1999). These results were compatible with Huang et al. (2005) as they had described that the shoot biomass accumulation of plants improved in petroleum hydrocarbons contamination by ACC-deaminase containing bacteria. Reed and Glick (2005) also reported that bacterial inoculation improved the canola growth in petroleum hydrocarbons contamination. The improvement in plant growth under normal soil by inoculation might also be due to one or more above discussed mechanisms, but mainly it might be due to production of phytohormones (Humphry et al., 2007) and enhanced nutrient availability (Pena and Reyes, 2007) by bacteria. The results of our study regarding plant growth promotion under normal conditions are in line with work of Zafar-ul-Hye et al. (2007) and Shahzad et al. (2010), where they reported that bacteria with ACC-deaminase activity improves the plant growth under normal conditions.

CONCLUSIONS

On the basis of our results we concluded that biomass accumulation, particularly in root systems of plants is critical for better plant growth in stress conditions. ACC-deaminase containing bacteria might be lowering the endogenous ethylene levels by hydrolyzing its immediate precursor ACC. However, bacteria may also directly stimulate plant growth by providing the plants with phytohormones, phosphorus and sufficient iron through the action of bacterial siderophores. Exact mechanisms that improve plant growth in petroleum contaminated soils need to be further explored.

Ackmowledgements

This study was conducted in the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad and funds were provided by Higher Education Commission (HEC), Pakistan. Their contribution is highly acknowledged.

REFERENCES

Achuba, F.I., 2006. *The effect of sublethal concentrations of crude oil on the growth and metabolism of cowpea (Vigna unguiculata) seedlings*. Environmentalist, 26: 17-20

Akpor, O.B., Igbinosa, O.E., Igbinosa, O.O., 2007. *Studies on the effect of petroleum hydrocarbon on the microbial and physico-chemicals characteristics of soil.* African Journal of Biotechnology, 6: 1939-1943.

Alexander, M., 1999. *Biodegradation and bioremediation,* Academic Press Inc, San Diego, USA.

Al-Hawas, G.H.S., Shukry, W.M., Azzoz, M.M., Al-Moaik, R.M.S., 2012. *The effect of sublethal concentrations of crude oil on the metabolism of jojoba (Simmodsia chinensis) seedlings*. International Research Journal of Plant Science, 3: 54-62.

Alkio, M., Tabuchi, T.M., Wang, X., Colón-Carmona, A., 2005. *Stress responses to polycyclic aromatic hydrocarbons in Arabidopsis include growth inhibition and hypersensitive response-like symptoms.* Journal of Experimental Botany, 56: 2983-2994.

Alkorta, I., Garbisu, C., 2001. *Phytoremediation of organic contaminants in soils.* Bioresource Technology, 79: 273-276.

Al-Moaikal, R.M.S., Shukry, W.M., Azzoz, M.M., Al-Hawas, G.H.S., 2012. *Effect of crude oil on germination, growth and seed protein profile of jojoba (Simmodsia chinensis).* Plant Science Journal, 1: 20-35.

Arshad, M., Saleem, M., Hussain, S., 2007. *Perspectives of bacterial ACC deaminase in phytoremediation.* Trends in Biotechnology, 25: 8-12.

Asghar, H.N., Setia, R., Marschner, P., 2012. *Community composition and activity of microbes from saline soils and non-saline soils respond similarly to changes in salinity.* Soil Biology and Biochemistry, 47:175-178.

Asghar, H.N., Zahir, Z.A., Arshad, M., 2004. *Screening rhizobacteria for improving the growth, yield, and oil content of canola (Brassica napus L.).* Australian Journal of Agricultural Research, 55: 187-194.

Atlas, R.M., Philp, J., 2005. *Bioremediation: applied microbial solutions for real-world environmental cleanup.* ASM Press, Washington, D.C.: 1-292.

Baker, J.M., 1970. *The effects of oils on plants*. Environmental Pollution, 1: 27-44.

Belimov, A.A., Safronova, V.I., Sergeyeva, T.A., Egorova, T.N., Matveyeva, V.A., Tsyganov, V.E., Borisov, A.Y., Tikhonovich, I.A., Kluge, C., Preisfeld, A., Dietz, K.J., Stepanok, V.V., 2001. *Characterisation of plant growth-promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase.* Canadian Journal of Microbiology, 47: 642-652.

Bhattacharyya, P.N., Jha, D.K., 2012. *Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture.* World Journal of Microbiology and Biotechnology, 28:1327-1350.

Bona, C., Silva, M.Y.B., Rezende, I.M., Santos, G.O., Souza, L.A., Inckot, R.C., 2011. *Effect of diesel contaminated soil on root and leaf of Sebastiania commersoniana (Euphorbiaceae) and Schinus terebinthifolius (Anacardiaceae).* Acta Botanica Brasilica, 25: 277-285.

Chaîneau, C.H., Rougeux, G., Yéprémian, C., Oudot, J., 2005. *Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil.* Soil Biology and Biochemistry, 37: 1490-1497.

Chookietwattana, K., Maneewan, K., 2012. *Selection of efficient salt-tolerant bacteria containing ACC-deaminase for promotion of tomato growth under salinity stress.* Soil and Environment, 31: 30-36.

Das, K., Mukherjee, A.K., 2007. *Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India*. Bioresource Technology, 98: 1339-1345

Denys, S., Rollin, C., Guillot, F., Baroudi, H., 2006. *In-situ phytoremediation of PAHs contaminated soils following a bioremediation treatment*. Water, Air and Soil Pollution, 6: 299-315.

Divya, B., Kumar, M.D., 2011. *Plant–microbe interaction with enhanced bioremediation.* Research Journal of Biotechnology, 4: 72-79.

Doty, S.L., James, C.A., Moore, A.L., Vajzovic, A., Singleton, G.L., Ma, C., Khan, Z., Xin, G., Kang, J. W., Park, J.Y., Meilan, R., Strauss, S.H., Wilkerson, J., Farin, F., Strand, S.E., 2007. *Enhanced phytoremediation of volatile environmental pollutants with transgenic trees.* Proceedings of the National Academy of Sciences, 104: 16816-16821.

Ferro, A.M., Rock, S.A., Kennedy, J., Herrick, J.J., Turner, D.L., 1999. *Phytoremediation of soils contaminated with wood preservatives: greenhouse and field evaluations*. International Journal of Phytoremediation, 1: 289-306.

Gerhardt, K.E., Huang, X.D., Glick B.R., Greenberg, B.M., 2009. *Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges*. Plant Science, 176: 20-30.

Ghosh, S., Penterman, J.N., Little, R.D., Chavez, R., Glick, B.R., 2003. *Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, Brassica campestris.* Plant Physiology and Biochemistry, 41: 277-281.

Glazebrook, J., 2005. *Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens*. Annual Review of Phytopathology, 43: 205-227.

Glick, B.R., 1995. *The enhancement of plant growth by free-living bacteria*. Canadian Journal of Microbiology, 41: 109-117.

Glick, B.R., 2003. *Phytoremediation: synergistic use of plants and bacteria to clean up the environment.* Biotechnology Advances, 21: 383-393.

Glick, B.R., 2005. *Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase.* FEMS Microbiology Letters, 251: 1-7.

Glick, B.R., Liu, C., Ghosh, S., Dumbroff, E.B., 1997. *Early development of canola seedlings in the presence of the plant growth promoting rhizobacterium Pseudomonas putida GR 12-2.* Soil Biology and Biochemistry, 29: 1233-1239.

Glick, B.R., Penrose, D.M., Li, J., 1998. *A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria*. Journal of Theoretical Biology, 190: 63-68.

Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan J., McConkey, B., 2007. *Promotion of plant growth by bacterial ACC deaminase*. Critical Reviews in Plant Sciences, 26: 227-242.

Gupta, A., Meyer J.M., Goel, R., 2002. *Development of heavy metal resistant mutants of phosphate solubilizing Pseudomonas sp. NBRI4014 and their*

characterization. Current Microbiology, 45: 323-327.

Hall, J.A., Peirson, D., Ghosh, S., Glick, B.R., 1996. *Root elongation in various agronomic crops by the plant growth promoting rhizobacterium Pseudomonas putida. GR 12-2.* Israel Journal of Plant Sciences, 44: 37-42.

Han, H.S., Lee, K.D., 2005. *Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity*. Research Journal of Agricultural and Biological Sciences, 1: 210-215.

Hontzeas, N., Zoidakis, J., Glick, B.R., 2004. *Expression and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the rhizobacterium Pseudomonas putida UW4: a key enzyme in bacterial plant growth promotion.* Biochimica et Biophysica Acta, 1703: 11-19.

Huang, X.D., Alawi, Y.E., Gurska, J., Glick, B.R., Greenberg, B.M., 2005. *A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soil.* Microchemical Journal, 8: 139-147.

Humphry, D.R., Andrews, M., Santos, S.R., James, E.K., Vinogradova, L.V., Perin, L., Reis, V.M., Cummings, S.P., 2007. *Phylogenetic assignment and mechanism of action of a crop growth promoting Rhizobium radiobacter strain used as a biofertilizer on graminaceous crops in Russia.* Antonie van Leeuwenhoek, 91: 105-113.

Jacobson, C.B., Pasternak, J.J., Glick, B.R., 1994. *Partial purification and characterization of 1-aminocyclopropane-1-carbooxylate deaminase from the plant growth promoting rhizobacterium Pseudomonas putida GR 12-2.* Canadian Journal of Microbiology, 40: 1019-1025.

Joner, E.J., Hirmann, D., Szolar, O.H.J., Todorovic, D., Leyval, C., Loibner, A.P., 2004. *Priming effects on PAH degradation and ecotoxicity during a phytoremediation experiment.* Environmental Pollution, 128: 429-435.

Jones, D.L., Hodge, A., Kuzyakov, Y., 2004. *Plant and mycorrhizal regulation of rhizodeposition.* New Phytologist, 163: 459-463.

Karthikeyan, R., Bhandari, A., 2001. *Anaerobic biotransformation of aromatic and polycyclic aromatic hydrocarbons in soil microcosms: a review.* Journal of Hazardous Substance Research, 31: 210-215.

Kathi, S., Khan, A.B., 2011. *Phytoremediation approaches to PAH contaminated soil.* Indian Journal of Science and Technology, 4: 56-63.

Khan, M.Y., Asghar, H.N., Jamshaid, M.U., Akhtar, M.J., Zahir, Z.A., 2013. *Effect of microbial inoculation on wheat growth and phyto-stabilization of chromium contaminated soil.* Pakistan Journal of Botany, 45: 27-34.

Kumar, R., Pandey, S., Pandey, A., 2006. *Plant roots and carbon sequestration.* Current Science, 91: 885-890.

Luepromchai, E., Lertthamrongsak, W., Pinphanichakarn, P., Thaniyavarn, S., Pattaragulwanit, K., Juntongjin, K., 2007. *Biodegradation of PAHs in petroleum-contaminated soil using tamarind leaves as microbial inoculums*. Songklanakarin Journal of Science and Technology, 29: 515.

Maqbool, F., Wang, Z., Xu, Y., Zhao, J., Gao, D., Zhao, Y.G., Bhatti, Z.A., Xing, B., 2012. *Rhizodegradation of petroleum hydrocarbons by Sesbania cannabina in bioaugmented soil with free and immobilized consortium.* Journal of Hazardous Materials, 30: 237-238.

Marcelis, L.F.M., Hooijdonk, J.V., 1999. *Effect of salinity on growth, water use and nutrient use in radish (Raphanus sativus L.).* Plant and Soil, 215: 57-64.

Mishra, S., Jyot, J., Kuhad, R.C., Lal, B., 2001. *Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge contaminated soil.* Applied and Environmental Microbiology, 67: 1675-1681.

Nadeem, S.M., Hussain, I., Naveed, M., Asghar, H.N., Zahir, Z.A., Arshad, M., 2006. *Performance of plant growth promoting rhizobacteria containing ACC-deaminase activity for improving growth of maize under salt-stressed conditions.* Pakistan Journal of Agricultural Sciences, 43: 3-4.

Njoku, K.L., Oboh, B.O., Akinola, M.O., Ajasa, A.O., 2012. *Comparative effects of Abelmoschus esculentus (L) Moench (Okro) and Corchorus olitorius L (Jew Mallow) on soil contaminated with mixture of petroleum products.* Research Journal of Environment and Earth Sciences, 4: 413-418.

Odjegba, V.J., Sadio, A.O., 2002. *Effects of spent engine oil on the growth parameters, chlorophyll and protein levels of Amaranthus hybridus L.* The Environment, 22: 23-28.

Okoh, A.I., 2006. *Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants.* Biotechnology and Molecular Biology Reviews, 1: 38-50.

Parrish, Z.D., Banks, M.K., Schwab, A.B., 2005. *Assessment of contaminant lability during phytoremediation of polycyclic aromatic hydrocarbon impacted soil.* Environmental Pollution, 137: 187-190.

Pena, H.B., Reyes, I., 2007. *Nitrogen fixing bacteria and phosphate solubilizers isolated in lettuce (Lactuca sativa L.) and evaluated as plant growth promoters.* Intersciencia. 32: 560-565.

Peng, S., Zhoua, Q., Cai, Z., Zhang, Z., 2009. *Phytoremediation of petroleum contaminated soils by Mirabilis jalapa L. in a greenhouse plot experiment.* Journal of Hazardous Materials, 168: 1490-1496.

Plohl, K., Leskovsek,H., Bricelj, M., 2002. *Biological degradation of motor oil in water.* Acta Chimica Slovenica, 49: 279-289.

Racine, C.H., 1994. *Long-term recovery of vegetation on two experimental crude oil spills in interior*

Alaska black spruce taiga. Canadian Journal of Botany, 72: 1171-1177.

Rahbar, F.G., Kiarostami, K., Shirdam, R., 2012. *Effects of petroleum hydrocarbons on growth, photosynthetic pigments and carbohydrate levels of sunflower.* J. Food Agric. Environ., 10: 773-776.

Reed, M.L., Glick, B.R., 2005. *Growth of canola (Brassica napus) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons.* Canadian Journal of Microbiology, 51:1061-1069.

Rodecap, K.D., Tingey, D.T., 1981. *Stress ethylene: a bioassay for rhizosphere-applied phytotoxicants.* Environmental Monitoring and Assessment, 1: 119-127.

Saleh, S.S., Glick, B.R., 2001. *Involvement of gasS and pros in enhancement of the plant growth-promoting capabilities of Enterobacter cloacae CAL2 and UW4.* Canadian Journal of Microbiology, 47: 698-705.

Sarkar, D., Ferguson, M., Datta, R., Birnbaum, S., 2005. *Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation.* Environmental Pollution, 136: 187-195.

Shaharoona, B., Bibi, R., Arshad, M., Zahir, Z.A., Hassan, Z., 2006. *1-aminocylopropane-1-carboxylate (ACC)-deaminase rhizobacteria extenuates ACC-induced classical triple response in etiolated pea seedlings.* Pakistan Journal of Botany, 38: 1491-1499.

Shahzad, S.M., Khalid, A. Arshad, M., Kalil-ur-Rehm, 2010. *Screening rhizobacteria containing ACC-deaminase for growth promotion of chickpea seedlings under axenic conditions.* Soil and Environment. 29: 38-46.

Siciliano, S.D., Germida, J.J., Banks, K., Greer, C.W., 2003. *Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial.* Applied and Environmental Microbiology, 69: 483-489.

Tang, J., Lu, X., Sun, Q., Zhu, W., 2012. *Aging effect of petroleum hydrocarbons in soil under different attenuation conditions.* Agriculture Ecosystem and Environment, 149: 109-117.

Tang, J.C., Wang, R.G., Niu, X.W., Wang, M., Chu, H.R., Zhou, Q.X., 2010. *Characterisation of the rhizoremediation of petroleum-contaminated soil: effect of different influencing factors*. Biogeosciences, 7: 3961-3969.

Vasudevan, N., Rajaram, P., 2001. *Bioremediation of oil sludge-contaminated soil.* Environment International, 26: 409-411.

Villalobos, M., Avila-Forcada, A.P., Gutierrez-Ruiz, M.E., 2008. *An improved gravimetric method to determine total petroleum hydrocarbons in contaminated soils*. Water, Air and Soil Pollution, 194: 151-161.

Wang, M., Liu, J., Chen, S., Yan, S., 2012. *Isolation, characterization and colonization of 1-aminocyclopropane-1-carboxylate deaminase-producing bacteria XG32 and DP24*. World Journal of Microbiology and Biotechnology, 28:1155-1162.

Weisman, D., Alkio, M., Colón-Carmona, A., 2010. *Transcriptional responses to polycyclic aromatic hydrocarbon-induced stress in Arabidopsis thaliana reveal the involvement of hormone and defence signalling pathways.* BMC Plant Biology, 10: 1-13.

Wenzel, W.W., 2009. *Rhizosphere processes and management in plant assisted bioremediation (phytoremediation) of soils.* Plant and Soil, 321: 385-408.

Zafar-ul-Hye, M., Zahir, Z.A., Shahzad, S.M., Naveed, M., Arshad, M., and Khalid, M., 2007. *Preliminary screening of rhizobacteria containing ACC-deaminase for promoting growth of lentil seedlings under axenic condition.* Pakistan Journal Botany, 39: 1725-1738.

Zahir, A.Z., Asghar, H.N., Akhtar, M.J., Arshad, M., 2005. *Precursor (L-tryptophan)-inoculum (Azotobacter) isnteraction for improving yields and nitrogen uptake of maize.* Journal of Plant Nutrition, 28: 805-817.

Zahir, Z.A., Akhtar, S.S., Ahmad, M., Saifullah, Nadeem, S.M., 2012. *Comparative effectiveness of Enterobacter aerogenes and Pseudomonas fluorescens for mitigating the depressing effect of brackish water on maize.* International Journal of Agriculture and Biology, 14: 337-344.

Zahir, Z.A., Arshad, M., Frankenberger Jr, W.T., 2003. *Plant growth promoting rhizobacteria: applications and perspectives in agriculture.* Advances in Agronomy, 81: 97-168.

Zahir, Z.A., Ghani, U., Naveed, M., Nadeem, S.M., Asghar, H.N., 2009. *Comparative effectiveness of Pseudomonas and Serratia sp. containing ACC-deaminase for improving growth and yield of wheat (Triticum aestivum L.) under salt-stressed conditions.* Archives of Microbiology, 191: 415-424.