

Plant-microbe interaction of *Serratia marcescens* and *Scirpus mucronatus* on phytoremediation of gasoline contaminated soil

Asia Fadhile Almansoory^{1,4,*}, Mushrifah Idris²,
Siti Rozaimah Sheikh Abdullah³ and Nurina Anuar³

¹School of Environmental and Natural Resource Sciences, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.

²Tasik Chini Research Centre, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.

³Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.

⁴Department of Biology, Science collage, Basrah University, Iraq.

*Corres. Author: asian.almansoory@yahoo.com

Abstract: The aim of this study was to investigate the plant-microbe interaction of *Serratia marcescens* isolates in phytoremediation of gasoline contaminated soil using plant bulrush of *Scirpus mucronatus*. The experiment included control plant and three phytoremediation treatments, with gasoline as a hydrocarbon pollutant model at different gasoline concentrations (0, 5, 10 and 30 g/kg) in soil, each added with 10% (v/v) of *S. marcescens*. The dry and wet weights of the plants slightly increased on exposure to gasoline for 42 days. The highest wet weight in 10 g/kg gasoline was 100.5 g at 72 days while dry weight amount 35.6 g. In the 5 g/kg and 30 g/kg gasoline concentrations, the wet weights were 43.4 g and 10.8 g with 13.1 g and 6.3 g for dry weight respectively. The phytoremediation treatment using *S. marcescens* in 10 g/kg gasoline showed the highest total petroleum hydrocarbon (TPH) removal after 72 days of exposure of 89.5% compared to only 55.0% in the corresponding unplanted control. The maximum TPH removals in the other two phytoremediation treatments (5 and 30 g/kg gasoline) were 84.4 and 83.3%, respectively, while the average removal in the corresponding control treatments were 56.3 and 54.2% respectively. This indicates that the biodegradation process by *S. marcescens* played significant role in the treatment. Hence, the synergy interaction between *S. mucronatus* and the bacteria can be beneficial in remediation of hydrocarbon in gasoline contaminated soil.

Keyword: Phytoremediation, *Scirpus mucronatus*, TPH, gasoline contaminated soil and *Serratia marcescens*.

Introduction

The use of plants and microorganisms associated in rhizosphere to enhance biodegradation of petroleum hydrocarbon pollutants in the soil has gained increasing acceptance internationally as a viable technology to clean up contaminated soils [1,2]. Microbial activity is essential to nutrient cycling in soils, and organic pollutants have effect on soil microorganisms [3]. This interaction between root exudates and the microorganisms populating the rhizosphere has been shown to enhance degradation of hydrocarbon contaminants and could have potential for improving phytoremediation [4, 5, 6]. Growth of plant and development related with the toxicity of hydrocarbons with low molecular weight, furthermore, hydrophobic properties of hydrocarbon reduce the ability of plants and microorganisms to absorb water and nutrients from the soil [7]. Plants and microbes require water and nutrients from the environment [8].

Phytoremediation is efficient to remove hydrocarbon contaminated soil since it can provide the appropriate environment for both plant and associated microorganisms. These properties such as pH, organic matter, cation exchange capacity and structure can effect on plant growth and development [6]. The effect of petroleum hydrocarbon contaminants on plant and microorganisms in soil may be of help in assessing the recovery potential of a soil [3]. Rhizoremediation is a type of microbial assisted phytoremediation, has shown one of the most successful means by which plants can influence the degradation of petroleum hydrocarbon contaminants [9]. According to [4] and [5] the growth of root oxidative degradation of petroleum hydrocarbons can be stimulated by promoting soil aeration that enhances the partnership between root exudates and the microorganisms biodegradation. In the rhizosphere, microbial activity is enhanced by the health of the plant which effects on the health of the rhizosphere and the entire phytoremediation system [8]. The application of successful rhizoremediation is dependent on the capacity of contaminant degraders or plant growth promoting microbes [10]. The microbial removal of contaminants in the soil through two methods of biodegradation and microbial uptake for biodegradation bacteria mediated chemical transformation of organic compound while microbial uptake is the direct removal of the contaminant by adsorbing compounds to the membrane surface [8]. Numbers of microbial increase in the rhizosphere of plant [11]. One of the most important requisite to rhizoremediation of hydrocarbon contaminant soils is that plants are able to germinate and become established in the presence of contaminants [9]. The aims of this study were to determine the plant-microbe interaction of *S. marcescens* in gasoline contaminated soil to enhance gasoline degradation through a good partnership with bulrush of *Scirpus mucronatus* during phytoremediation process.

Materials and Methods

Experimental Design for Phytotoxicity Test

The experiment was conducted in a greenhouse located at Universiti Kebangsaan Malaysia. The glass aquaria were used for the phytotoxicity test. Each aquarium with dimensions of 60 × 30 × 30 cm (L × W × D) was filled with 30 kg soil as a mixture of 75: 25 (w/w) garden soil: sand. All the aquaria were prepared simultaneously and run in parallel. The prepared gasoline concentrations of 5,10 and 50 g gasoline/kg. Soil with each of the gasoline concentrations was planted with 20 plants of the selected plant, *S. mucronatus* which was also sown in a control experiment using soil without gasoline. Three replicates per treated aquarium and another unplanted aquarium for control contaminants (CC), in addition to another aquarium without the gasoline contaminant as a plant control (PC). Standard gasoline obtained from a local Petronas petrol station was mixed with acetone (R&M Chemicals, U.K.) as a solvent in the ratio of 1:1 (v/v) and prepared at different concentrations (5, 10 and 30 g/kg) . After spraying the mixture soil, it was stirred until homogeneous and then left to planting. 20 three-months-old healthy *S. mucronatus* plants were transplanted into each aquarium containing gasoline at different concentrations. All experimental plants were watered with deionised water at a fixed calculated volume. The bulk density of the soil was 100 g soil mixture per 26 mL. Sampling was conducted on day 0, 7, 14, 28, 42 and 72.

Determination of physicochemical properties

To observe the changes in water quality, the physicochemical parameters of dissolved oxygen (DO, mg/L), oxidation reduction potential (ORP, mV) and pH were recorded, using an IQ 150 (IQ Scientific Instruments, UK) multi-probe for pH, ORP and a DO sensor (GLI International, Model 63, USA).

Plant growth

The growth of *S. mucronatus* was observed for 72 days at different gasoline concentrations (0, 5, 10 and 30 g/kg). One plant was harvested on each sampling day (Day 0, 7, 14, 28, 42 and 72) from the three replicates of each gasoline concentrations. The plants were rinsed with tap water and separated into lower part (from the stem buried in the soil to the tip of the longest rootlet) and upper part (leaves and stem). All parts were measured gravimetrically to determine their biomass through both wet and dry weights [12]. All plant samples were dried in an oven (Mettler, Germany) at 70°C for 72 h until constant mass was reached to give the dry weight [4].

Bioaugmentation Bacteria with Soil

This isolate *S. marcescens* was collected from previous students and identified using biochemical and PCR technique. Inoculum at 10% (v/v) of was mixed with bulk density of water in the soil mixture and added to the plant site glass basin containing gasoline contaminated soil. The bulk density of the soil was 26 mL per 100 g of the soil mixture, while the weight of the soil mixture in the glass basin was 30 kg. So, the volume of water in the glass basin is 7800 mL. These bacteria grow in the liquid media containing Total Soy Broth (TSB) shacked for 24 h at 150 rpm and 37 °C. The addition of the bacteria to the soil mixture in an amount of 10% (v/v) bacteria was added to each aquarium.

Microbial plate counts

The microbial population was obtained from the soil that was firmly attached to the rhizosphere zone. The population of live bacteria in the rhizosphere of plants treated with gasoline-contaminated soil was determined through a serial dilution method. Initially, 10 g of rhizosphere soil was harvested and added to 100 mL sterile distilled water to obtain 10^{-2} dilutions [12]. This mixture was shaken at 150 rpm for 1 h to release adhering microorganisms. Subsequent dilutions up to 10^{-4} fold were prepared and 100 μ L of each of three dilutions (10^{-2} , 10^{-3} , and 10^{-4}) was plated on sterile plates containing a nutrient agar medium by the plate-pouring method. The plates were inverted and incubated at 37°C for 24 h before the bacterial colonies were counted and expressed as colony forming units (CFU) per mL. Colonies was counted for plates that have more than 30 and less than 300. The number of colonies counted was multiplied by the reciprocal of the dilution and the amount plated and the results were expressed as CFU/mL [4, 14].

Extraction of total petroleum hydrocarbon in soil mixture

Three replicates of spiked medium were sampled at each sampling period. Collected samples were stored in glass bottles and kept at 4°C prior to analysis. Approximately 10 g of each sample was placed in a 100 mL flask from each aquarium on the same sampling day for all treatments to extract total petroleum hydrocarbon (TPH). The TPH in samples was extracted ultrasonically using a solvent extraction method [15, 16]. Soil samples were dried by mixing with sodium sulphate (Na_2SO_4) and later placed in a 100 mL Schott bottle with 50 mL dichloromethane (DCM) (R&M Chemicals, U.K.) and the bottle was agitated in an Ultrasonic Cleaner (Thermo-10D, U.S.A.) for 30 min at 50°C. The supernatant was filtered through glass wool. The extracts were concentrated and were left in the fume hood for 3–4 days to allow the solvent to evaporate completely, after which, 1.5 mL DCM was added and the extracts were stored in gas chromatography (GC) vials.

Analysis of total petroleum hydrocarbon

The sample extracts were analysed by a gas chromatography–flame ionisation detector (GC–FID, Agilent Technologies, Model 7890A, UK) with a HP-5 5% phenyl methyl siloxane column (30 m \times 0.32 mm i.d \times 0.25 micron) with helium as the carrier gas. The column temperature was programmed to remain at 50°C for 1 min, and then ramped at 15°C per min to 320°C for 10 min. The percentage of TPH degradation on each sampling day was determined by dividing the difference of the current TPH values by the initial TPH value in soil. The percentage of TPH removal in soil on each sampling day was determined using Eq. (1):

$$\% \text{ Removal} = \frac{\text{TPH}_0 - \text{TPH}_t}{\text{TPH}_0} \times 100 \quad (1)$$

Where TPH_0 = total petroleum hydrocarbon on sampling day 0 and TPH_t = total petroleum hydrocarbon on each sampling day.

Results and Discussion

Determination of physicochemical parameters

The selected physicochemical parameters were recorded throughout the phytotoxicity test for each gasoline concentrations in two treatments planted and unplanted (Figure 1). Generally, the pH, ORP and DO decreased slightly during the exposure period. During the 72 days of the experiment, the pH ranged between 6.4 and 7.5 for planted aquaria and between 5.2 and 6.7 for unplanted aquaria. The conditions of experiments, the aerobic or anaerobic, can be distinguished with DO and ORP measurements. For the planted and unplanted treatments, the DO ranged between 1.2 and 7.2 mg/L, the ORP between 7.13 and 111.5 mV. The decreasing DO readings indicate that the treatment environment was anoxic conditions. Furthermore, measurements the ORP for all gasoline concentrations were in the aerobic and anoxic range. [17] Demonstrated that the addition of hydrocarbons to the soil altered the soil pH and the conditions of the phytotoxicity test can be classed as anoxic/anaerobic, as the concentration of dissolved oxygen was very limited.

Plant responses to the gasoline contaminant

The plants growing throughout 72 days in soil mixture irrigated with gasoline contaminated soil interact with bacteria showed obvious differences in appearance compared with those in the corresponding controls (*S. mucronatus* without the contaminant and bacteria) (Figure 2). No plant death was recorded with gasoline concentrations. However, the growth of *S. mucronatus* was increased with the gasoline concentrations, indicating that the plant growth and development was become better due to plants and their associated bacteria interact with each other in planted treatments [6]. The biomass of *S. mucronatus* increased in the gasoline phytoremediation treatment. The wet weight biomass of *S. mucronatus* at the end of the exposure period at concentration 10g/kg reached 100.5 ± 1.7 g and was almost the wet weight biomass of *S. mucronatus* in corresponding control only (36.4 ± 97 g) (Figure 2 i and ii). This situation may have occurred due to the addition of the bacteria that acted and enhanced the growth. However, the plant biomass increased until 42 days in 5 g/kg and 30g/kg for wet amounted were 47.5 g and 15.6 g but for dry weight at 18.9 g and 6.3 g respectively. Several previous studies showed similar results. [18] Reported Pea, cress, and pansy plants have increased amounts of total priority petroleum aromatic hydrocarbon extracted from soil by more than 17% during 68 days. Based on a study by [14] a larger efficiency in the remediation enhanced with the largest growth of bacteria in rhizosphere when attempted plant *Rhizophora mangle* L. and observed a larger growth of plants exposed to contaminated sediments by hydrocarbon after 3 months of phytoremediation.

Microbial plate count

The microbial population in *S. mucronatus* rhizosphere zone was evaluated at different gasoline concentrations (0, 5, 10 and 30 g/kg) as shown in (Figure 3). It was found that the gasoline pollutant enhanced the microbial population and increases its diversity. The degradation of hydrocarbons mainly depends on the capabilities of the microorganisms in the surrounding rhizosphere [5] During the experiment, microbial populations in the control aquarium were significantly lower than those in the aquaria with gasoline concentrations of 5, 10 and 30 g/kg. Generally, the bacterial population in the treatments with different of gasoline concentrations increased until 42 days, and then started to decrease to the end of the 72 day period of exposure.

The population of rhizobacteria in the plant control aquarium without contamination (0 mg/L) was 1.4×10^2 to 1.7×10^2 CFU mL⁻¹ soil at from day 0 to 42, and was obviously lower than that in the treatments with different gasoline concentrations. In other words, the population of bacteria in aquaria irrigated with gasoline-contaminated soil was clearly higher than in the control aquarium. Similarly, the population of bacteria in treatments with the highest gasoline concentration of 30g/kg amounted to 1.6×10^3 to 2.7×10^3 CFU mL⁻¹ soil at from day 0 to 42, which was similar with gasoline concentration of 5 and 10g/kg. The bacterial population in the treatment with lowest gasoline concentration of 5g/kg amounted from 1.5×10^3 to 2.55×10^3 CFU mL⁻¹ soil

during 42 days of treatment. The growth of bacteria in 10 g/kg was higher than other concentrations that amounted 2.4×10^4 to 2.9×10^4 CFU mL⁻¹ soil.

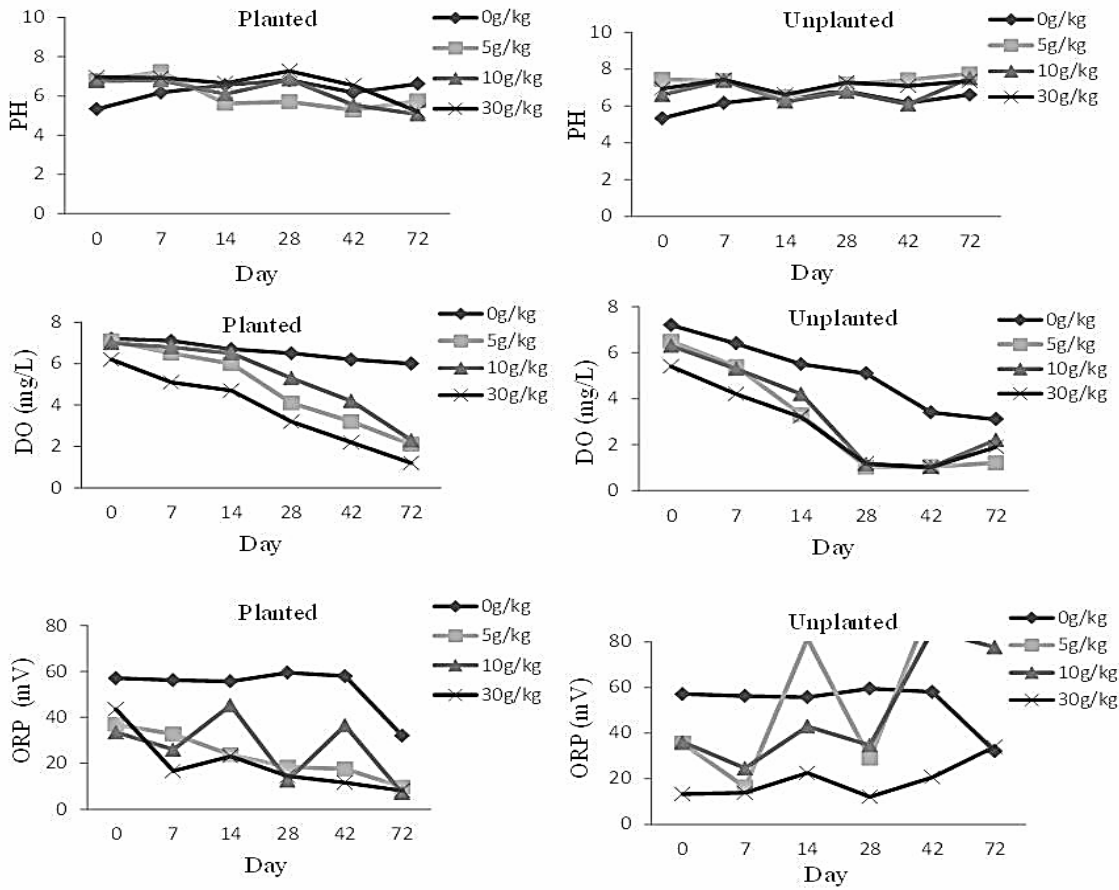
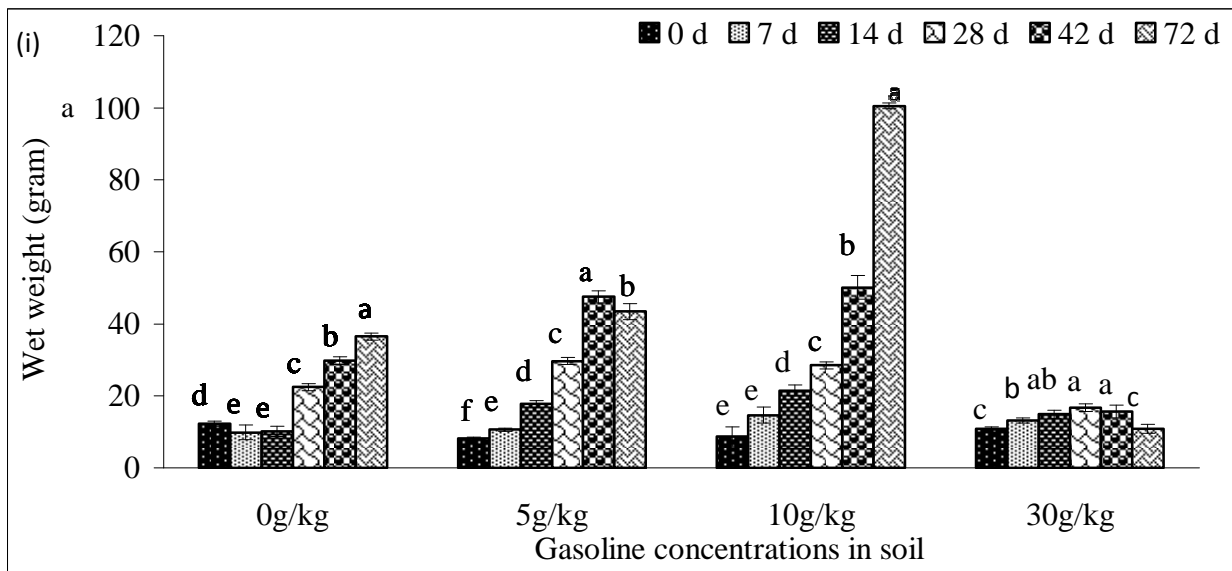


Figure 1. : Physical parameter variations in the phytotoxicity test with *S. mucronatus* using gasoline as the contaminant.



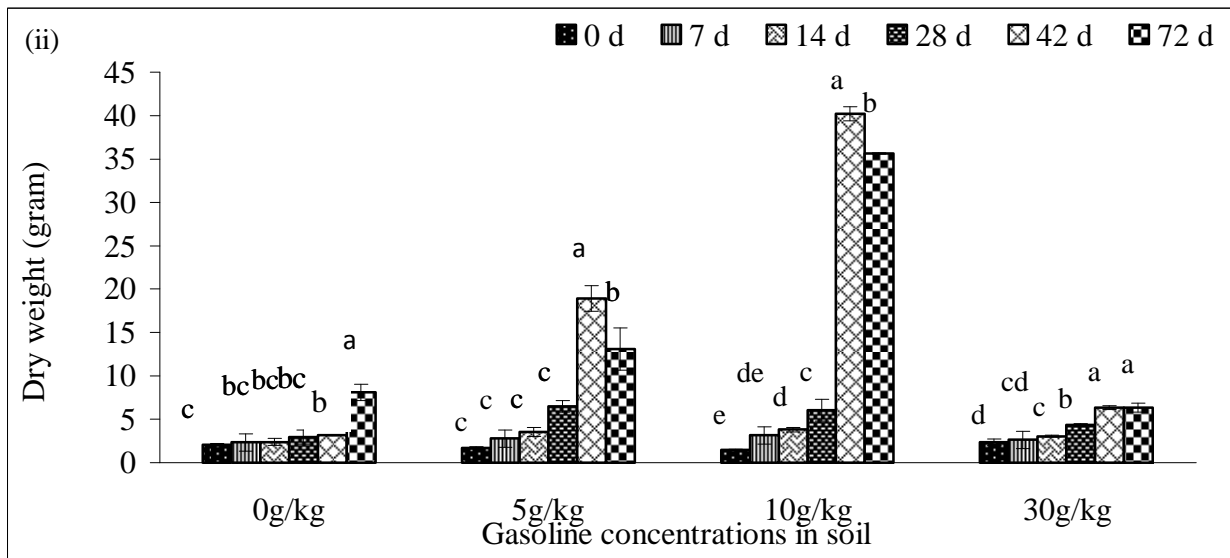


Figure 2.: Growth response parameters: (i) wet weight, (ii) dry weight in the phytotoxicity test of *S. mucronatus* using gasoline as the contaminant. Error bars indicate the standard deviation (n = 3). The means among different gasoline concentrations followed by the same letter (a–e) were not significantly different at $p < 0.05$.

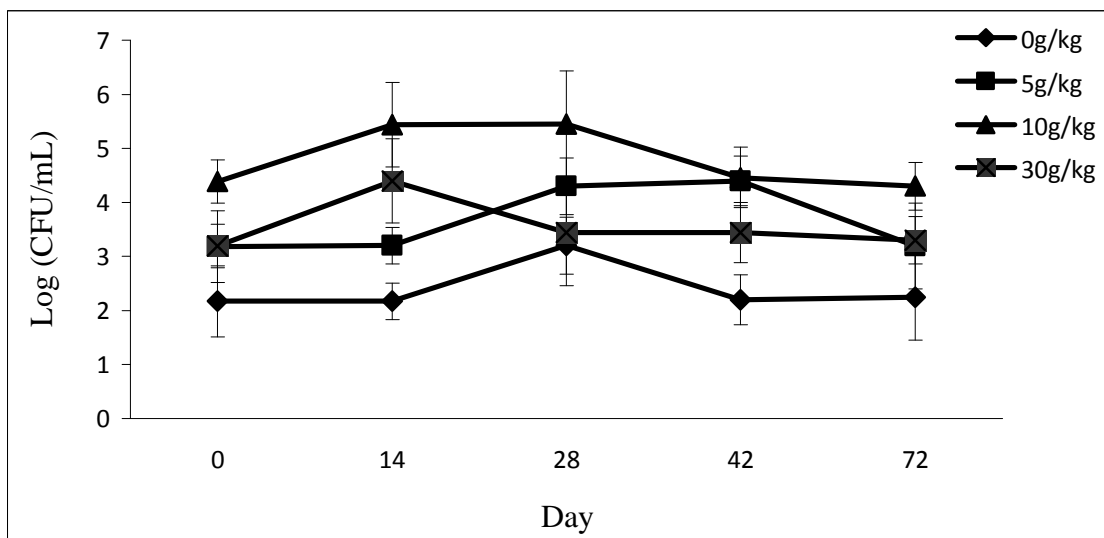


Figure 3. : Total count of the bacterial population during the 72 days of the experiment comparison of the microbial populations in the control and with different gasoline concentrations of 5, 10 and 30g/kg.

TPH Removal in Soil

To obtain more information about degradation of gasoline in the soil mixture was conducted in (Figure 4) shows degradation and removal over 72 days for the three different gasoline concentrations treatments planted and unplanted (5g/kg, 10g/kg and 30 g/kg). The removal efficiency of gasoline contaminant in most treatments was significantly different between all concentrations and sampling days (7, 14, 28, 42 and 72). The maximum TPH degradation removal in soil mixture of 87.5% occurred with gasoline concentration of 10g/kg after 72 days of treatment, while the average removal in its corresponding control treatment was only 55 %. Similarly, the degradation rates with gasoline concentrations of 5 and 30 g/kg were 84.5 and 83.3%, respectively, while the average removal in the corresponding control treatments was 56.3 and 54.2%, respectively. The convergence of results indicates the ability of bacteria *S. marcescens* partnership with plant *S. mucronatus* to enhance degradation of TPH and survive in these three gasoline concentrations.

Statistical analysis was performed between treatments planted and unplanted at each sampling time for all gasoline concentrations as illustrated in (Figure 4). The removal TPH degradation in all concentrations significantly ($p < 0.05$) different between planted and unplanted (corresponding control). Due to the interaction between selected bacteria and plants the hydrocarbons were metabolized. The main mechanism to removal TPH in phytoremediation of contaminated soils is assumed to be rhizodegradation, the stimulation of bacteria in the rhizosphere zone to degrade and enhance removal of TPH [19]. In control soil mixture may also be the consequence of biodegradation by indigenous microorganisms [4] The phytoremediation of TPH, especially in soil on many studies according to [20], the removal of diesel from soil contaminated with 15,000 mg/kg diesel by *Scirpus triqueter* was 67.41 and 72.62% planted and unplanted, respectively. [21] Reported our results show that diverse plant species growing in hydrocarbon contaminated soil with microbial populations, which may impact upon the ability of plants to promote the degradation of specific types of hydrocarbons. The degradation of TPH by *Mirabilis jalapa* and showed that the average efficiency to remove TPH over 127-day culture period was high, up to 41.6–63.2%, while the removal rate by natural attenuation was only 19.7–37.9% [4]. [22] Showed bacterial inoculation in phytoremediation enhances plant resistance to the contaminant stress and increases their acclimation rate and biomass formation. Similarly hydrocarbon degradation by bacteria enhanced plant biomass production and HC degradation [23]. The synergistic action of the plants and bacterial inoculation rhizodegradation of hydrocarbon exhibited more efficient as compared to microbial remediation and phytoremediation [24].

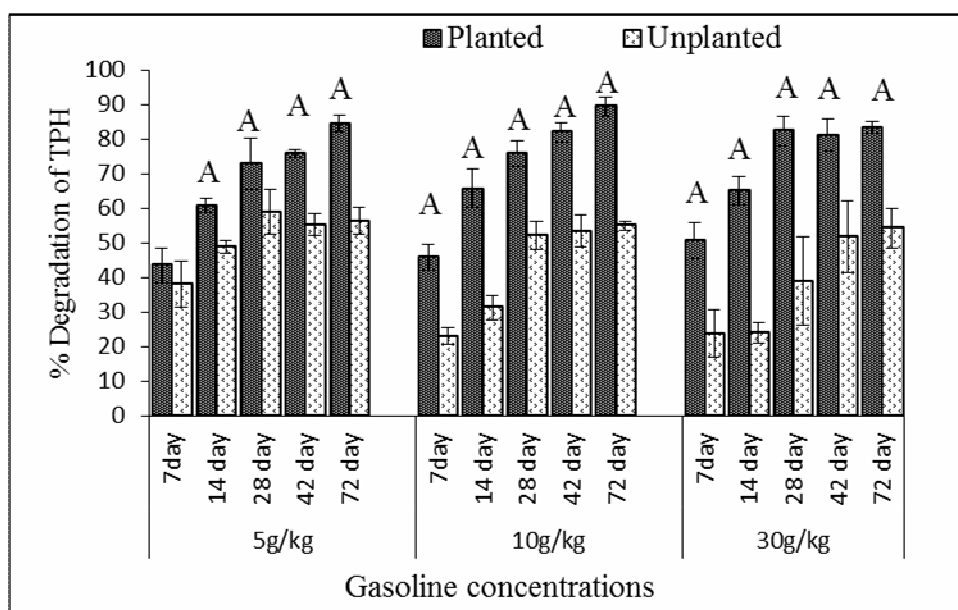


Figure 4.: Degradation percentage in soil mixture extraction by *S. mucronatus* exposed to gasoline contamination at 5 g/kg, 10 g/kg and 30 g/kg. Bars indicate the standard error of three replicates ($n = 3$). Letter A: statistically significant gasoline removal from soil between two treatments planted and unplanted was represented ($p < 0.05$).

Conclusions

The tolerance of plants to soil contamination by gasoline concentrations after 72 d demonstrated that *S. mucronatus* has the ability to survive and provide suitable conditions for rhizobacteria to degrade hydrocarbons at all investigated gasoline concentrations (5, 10 and 30g/kg). The dry and wet weight of the plant slightly increased on exposure to gasoline at 42 d in 5g/kg and 30 g/kg. The highest wet weight in concentration 10 g/kg 100.5 g at 72 d while dry weight amounted 35.6 g. In the 5g/kg and 30 g/kg amounted for 43.4g and 10.8g was wet but dry weight at 13.1g and 6.3g respectively. Based on soil extraction, the highest TPH removal rate was 87.5%, in comparison to the removal rate by the corresponding unplanted controls of only 55%. The removal rates with gasoline concentrations of 5 and 30 g/kg were 84.5 and 83.3%, respectively, while the

average removal in the corresponding control treatments was 56.3 and 54.2%, respectively. This indicates that the biodegradation process played a role in the treatment with 10% rhizobacteria.

Acknowledgement

The authors would like to thank the Universiti of Kebangsaan Malaysia (UKM) and the Tasik Chini Research Centre for supporting this research project under a FRGS grant (FRGS 32-1310063). We also acknowledge the Iraqi Ministry of Higher Education and Scientific Research for providing a doctoral scholarship for the first author.

References

1. Gerhardt, K.E., Huang, X.-D., Glick, B.R., Greenberg, B.M., 2009. Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Sci.* 176, 20–30.
2. Gaskin, S.E., Bentham, R.H., 2010. Rhizoremediation of hydrocarbon-contaminated soil using Australian native grasses. *Sci. Total Environ.* 408: 3683–3688.
3. Labud, V.; Garcia, C. and Hernandez, T. 2007. Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. *Chemosphere* 66: 1863–1871.
4. Peng, S., Zhou, Q., Cai, Z., Zhang, Z., 2009. Phytoremediation of petroleum contaminated soils by *Mirabilis jalapa* L. in a greenhouse plot experiment. *J. Hazard. Mater.* 168, 1490–1496.
5. Lu, S., Teng, Y., Wang, J., Sun, Z., 2010. Enhancement of pyrene removed from contaminated soils by *Bidens maximowicziana*. *Chemosphere* 81, 645–650
6. Khan, S.; Afzal, M.; Iqbal, S.; Khan, Q. M. 2013. Plant–bacteria partnerships for the remediation of hydrocarbon contaminated soils. *Chemosphere.* 90: 1317–1332.
7. Kathi, S., and Khan, A. B., 2011. Phytoremediation approaches to PAH contaminate soil. *Indian Journal of Science and Technology.* 4. ISSN: 0974- 6846.
8. McPherson, A. 2007. Monitoring Phytoremediation of Petroleum Hydrocarbon Contaminated Soils in a Closed and Controlled Environment. Thesis Submitted to the College of Graduate Studies and Research. The Degree of Masters of Science. P206
9. MacKinnon, G. and Duncan, H.J. 2013. Phytotoxicity of branched cyclohexanes found in the volatile fraction of diesel fuel on germination of selected grass species. *Chemosphere* 90: 952–957.
10. Zhang, Z., Zhou, Q., Peng, S. and Cai, Z. 2010. Remediation of petroleum contaminated soils by joint action of *Pharbitis nil* L. and its microbial community. *Science of the Total Environment* 408(22): 5600–5605.
11. Kirk, J.L., Klironomos, J.N., Lee, H. and Trevors, J.T. 2005. The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. *Environmental Pollution* 133: 455–465.
12. Ogbo E.M., Tabuanu, A. and Ubebe R. 2010. Phytotoxicity assay of diesel fuel-spiked substrates remediated with *Pleurotus tuberregium* using *Zea mays*. *International Journal of Applied Research in Natural Products* 3 (2): 12–16.
13. Prescott, L.M., Harley, J.P., 2002. *Laboratory Exercises in Microbiology*, fifth ed. McGraw-Hill Companies, Klein DA.
14. Moreira, I., Oliveira, O., Triguís, J., Santos, A., Queiroz, A., Martins, C., Silva, C., Jesus, R., 2011. Phytoremediation using *Rhizophora mangle* L. in mangrove sediments contaminated by persistent total petroleum hydrocarbons (TPHs). *Microchem. J.* 99, 376–382.
15. Su, Y.-H. and Zhu, Y.-G. 2008. Uptake of selected PAHs from contaminated soils by rice seedlings (*Oryza sativa*) and influence of rhizosphere on PAH distribution. *Environmental Pollution* 155(2): 359–365.
16. Tang, J., Wang, M., Wang, F., Sun, Q. and Zhou, Q. 2011. Ecotoxicity of petroleum hydrocarbon contaminated soil. *Journal of Environmental Sciences* 23(5): 845–851.
17. Tang, J., Lu, Z.; Sun, Q.; Zhu, W. 2012. Aging effect of petroleum hydrocarbons in soil under different attenuation conditions. *Agriculture, Ecosystems and Environment* 149: 109– 117.

18. Liste, H. H. and Prutz, I. 2006. Plant performance, dioxygenase-expressing rhizosphere bacteria and biodegradation of weathered hydrocarbons in contaminated soil. *Chemosphere* 62: 1411–1420
19. Cai, Z., Zhoua, Q., Peng, S., Li, K., 2010. Promoted biodegradation and microbiological effects of petroleum hydrocarbons by *Impatiens balsamina* L. with strong endurance. *J. Hazard. Mater.* 183, 731–737.
20. Liu,T.; Hou. J.; Zuo, Y.; Bi, Z. & Jing, J. 2011. Isolation and characterization of a biosurfactant producing bacterium from Daqing oil-contaminated sites. *Afr. Journal Microbiol. Res.* 5(21): 3509-3514.
21. Phillips, L. A.; Germida, J. J.; Farrell, R. E. & Greer, C. W. 2008. Hydrocarbon degradation potential and activity of entophytic bacteria associated with prairie plants. *Soil Biology & Biochemistry* 40: 3054–3064.
22. Muratova, A.Y., Golubev, S., Wittenmayer, L., Dmitrieva, T., Bondarenkova, A.,Hirche, F., Merbach, W., Turkovskaya, O., 2009. Effect of the polycyclic aromatic hydrocarbon phenanthrene on root exudation of *Sorghum bicolor* (L.) Moench. *Environ. Exp. Bot.* 66, 514–521.
23. Yousaf, S., Afzal, M., Reichenauer, T.G., Brady, C.L., Sessitsch, A., 2011. Hydrocarbon degradation, plant colonization and gene expression of alkane degradation genes by endophytic *Enterobacter ludwigii* strains. *Environ. Pollut.* 159: 2675– 2683.
24. Afzal, M., Yousaf, S., Reichenauer, T.G., Sessitsch, A. 2012. The inoculation method affects colonization and performance of bacterial inoculant strains in the phytoremediation of soil contaminated with diesel oil. *Int. J. Phytorem.* 14: 35– 47.
