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BIOMONITORING OF ATMOSPHERIC POLLUTION: POSSIBILITIES AND FUTURE CHALLENGES

Phytoremediation of contaminated soils containing gasoline using *Ludwigia octovalvis* (Jacq.) in greenhouse pots

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Abstract Greenhouse experiments were carried out to determine the phytotoxic effects on the plant *Ludwigia octovalvis* in order to assess its applicability for phytoremediation gasoline-contaminated soils. Using plants to degrade hydrocarbons is a challenging task. In this study, different spiked concentrations of hydrocarbons in soil (1, 2, and 3 g/kg) were tested. The results showed that the mean efficiency of total petroleum hydrocarbon (TPH) removal over a 72-day culture period was rather high. The maximum removal of 79.8 % occurred for the 2 g/kg concentration, while the removal rate by the corresponding unplanted controls was only (48.6 %). The impact of gasoline on plants included visual symptoms of stress, yellowing, growth reduction, and perturbations in the developmental parameters. The dry weight and wet weight of the plant slightly increased upon exposure to gasoline until day 42. Scanning electron microscopy (SEM) indicated change to the root and stem structure in plant tissue due to the direct attachment with gasoline contaminated compared to the control sample. The population of living microorganisms in the contaminated soil was found to be able to adapt to different gasoline concentrations. The results showed that *L. octovalvis* and rhizobacteria in gasoline-contaminated soil have the potential to degrade organic pollutants.

Keywords Phytoremediation · *Ludwigia octovalvis* · TPH · Gasoline-contaminated soil · SEM

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Introduction

Petroleum products are an integral component of our modern society, especially in industrial and agricultural productions. Fuel transportation has caused environmental risks, such as spills, leaks, and discharges (Peng et al. 2009; Cai et al. 2010; Khan et al. 2013). Petroleum hydrocarbons consist of mixtures of chemical substances containing hazardous chemicals, which pose a serious threat to both humans and ecosystems (Phillips et al. 2008; Park et al. 2011). Gasoline is a complex mixture of liquid organic compounds consisting mainly of hydrocarbons, including monoaromatic compounds, such as benzene, xylene, and toluene, as well as two-ringed phenanthrene (PAH) and naphthalene (Vieira et al. 2007; Freitas et al. 2011).

Characterization of oil contamination is a crucial step in the remediation of oil-contaminated soils, and the chemical composition of the contaminants is used to assess the toxicity and determine the need for remediation (Mao et al. 2009). The susceptibility of organic pollutants, particularly gasoline, to degradation in the field (Tang et al. 2010), as well as the plant bioavailability and amount of the contaminants in the both of plant and soil have been investigated. In addition, specific interactions, such as plant–microbe–hydrocarbons, that

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contribute to soil remediation were also studied (White et al. 2006). Low-molecular-weight hydrocarbons can be transported across plant membranes from the soil and released through leaves via evapotranspiration processes (phytovolatilization), whereas nonvolatile compounds can be degraded by bacteria to nontoxic compounds via enzymatic processes and phytoextraction (Gerhardt et al. 2009). Phytoextraction refers to the ability of plants to remove compounds from the subsurface and transfer to the leaves or other plant tissues (EPA 2006). Other compounds are stable within plants, which are later removed, together with other biomass for incineration (Gerhardt et al. 2009).

Phytoremediation has been incorporated into environmental biotechnology as a green technology that is cost-effective, environmentally friendly, for treatment of contaminated soils, surface water, and sediments (Ghosh and Singh 2005; Kathi and Khan 2011). Phytoremediation efficiency in removing hydrocarbons from the soil depends on the establishment of appropriate environment for both plants and associated microorganisms (Khan et al. 2013). Greenhouse experiments have also been conducted excavated soil to determine how contaminated soils affect plant growth. In this case, rhizosphere can work in tandem to effectively degrade organic compounds to nontoxic, or less toxic, compounds in soil due to is associated with increased root length, which results in increased total bacterial biodegradation of hydrocarbon in crude oil-contaminated soil (White et al. 2006). Plants have been used successfully in phytoremediation in a wide range of contaminated soils, mainly in two ways: by creating favorable conditions for complex interactions involving rhizobacteria and root exudates to degrade of the contaminants in the soil (Kathi and Khan 2011). Furthermore, phytoremediation is strongly influenced by soil chemical properties and soil microorganism populations and activities (Guo et al. 2012).

Bacteria that utilize hydrocarbons in soil systems may decrease plant stress, thereby increasing the plant growth appear to increase the numbers of microbes in contaminated sites (Fernandez et al. 2011). The toxicity of gasoline directly influence on plants via contacted with tissue. Plants respond differently to exposure to petroleum hydrocarbon; some plants are resistant to petroleum hydrocarbon contamination and some tolerant plants might be useful for cleaning contaminated soils (Sharonova and Breus 2012). *L. octovalvis* was chosen for this study since it was one of the plants that could survive at a contaminated site in Malaysia (Rahman et al. 2009). The common name of this plant include Mexican primrose-willow, swamp primrose, water primrose, and yellow willow herb, while in Malaysia, it is locally known as "buyang samalam" and "pujang malam" and grows in wet areas and shallow water with few other plants, and grows up to 1 m/year, but under favorable conditions, as mentioned by Moody (1989). The aims of this study were (1) to determine the tolerance limits of *L. octovalvis* to gasoline at different concentrations (1, 2, and 3 g/kg) in a phytotoxicity test and (2) to assess microbe-plant interactions in the biodegradation of gasoline from soil.

Materials and methods

Propagation of plant species

The plant *L. octovalvis* was propagated from seeds obtained from a parent plant growing at a contaminated site in Malaysia. The seeds were germinated and grown for 8 weeks in the greenhouse in Universiti Kebangsaan Malaysia (UKM). Garden soil and sand were mixed in the following ratio: 0:100, 25:75, 50:50, 75:25, and 100:0. Soil mixture ratio will be chosen for the best growth to improve plant in propagation test. The seeds were planted in plastic crates (37×27×10 cm), with the bulk density of the soil being 100 g soil mixture per 26 mL. All of the plants used in the experiment were 8 weeks old at the beginning of the study.

Experimental design for the phytotoxicity test

The experiment was conducted in a greenhouse located at UKM. Thirteen glass pots were used for the phytotoxicity test to minimize the occurrence of any sticky oils on the walls of the pots. Each pot, with dimensions 60×30×30 cm (*L*×*W*×*D*), was filled with 30 kg soil in the mixture of 50:50 (*w*/*w*) garden soil/sand; this ratio produced the best plant growth in the propagation test (data not shown). The garden soil and sand were sieved using a mesh with the size of 4.75 mm to ensure uniformity and the removal of coarse fragments, since soil can contain organic material that poses problems for plants (Chappel 1997). All of the pots were prepared simultaneously; 1, 2, and 3 g gasoline per kilogram of soil obtained from a local Petronas petrol station were spiked into the 50:50 mixtures (*w*/*w*) of garden soil/sand. Standard gasoline at different concentrations was mixed with acetone (R&M Chemicals, UK) as a solvent in the ratio 50: 50 (*v*/*v*). After spraying the mixture onto the garden soil/sand medium, it was stirred until it became homogeneous and left for a week prior to planting. Soil with each of the gasoline concentrations was planted with the selected plant *L. octovalvis*. The experimental design included three replicates per treated pots (R1, R2, and R3), a pot for the contaminant control without plants (CC), as well as another pot without the gasoline contaminant as a plant control (PC) (Fig. 1). Eighteen healthy *L. octovalvis* plants were transplanted into each aquarium containing different concentrations of gasoline. All experimental plants were watered with deionized water at a fixed calculated volume. The pots were sampled on days 0, 7, 14, 28, 42, and 72.

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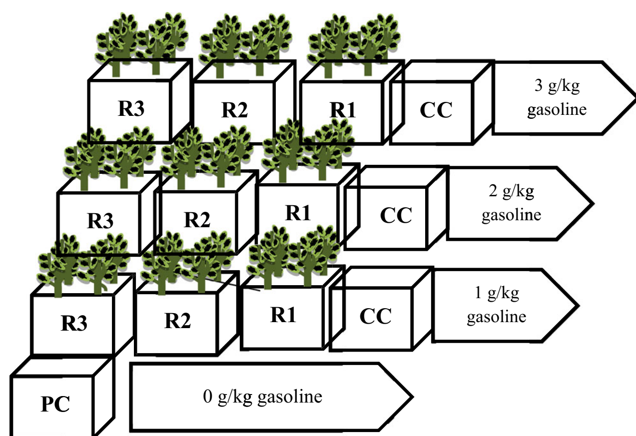

Fig. 1 Experimental design for the phytotoxicity test (*R1, R2, R3* three replicates, *CC* control contaminant without plants, *PC* plant control without the gasoline contaminant)

Determination of the physicochemical properties

During sampling, the physicochemical parameters (temperature (T) (°C), dissolved oxygen (DO) (mg/L), and oxidation–reduction potential (ORP) (mV)) were recorded. The ORP and temperature were recorded using an IQ 150 multi-probe (IQ Scientific Instruments, Spectrum Technologies, Plainfield, USA). The measurement of DO was performed with a dissolved oxygen sensor (GLI International, Model 63, USA).

Plant growth

One plant was harvested on each sampling day (0, 7, 14, 28, 42, and 72) from the three sets of replicates. The plant was rinsed with tap water, and then the water was absorbed using a tissue. The stem height, the root length, and wet weight were measured, and the root length was measured and recorded from the stem buried in the soil to the tip of the longest rootlet. All leaves and stem (upper part) were measured gravimetrically to calculate the biomass using both wet and dry weights (Ogbo et al. 2010). All plant samples were dried in an oven (Memmert, Germany) at 70 °C for 72 h until constant mass was reached in order to obtain the dry weight (Peng et al. 2009).

Microbial plate counts

The microbial population was quantified for the root and soil mixture that was firmly attached to the rhizosphere zone by the serial dilution method. Ten grams of plant root were added to 100 ml sterile distilled water to obtain 10^{-2} dilutions (Prescott et al. 2002). This mixture was shaken at 150 rpm for 1 h to release the adhering microorganisms. Subsequent dilutions of up to 10^{-4}-fold were prepared, and 100 μL of each of three dilutions (10^{-2}, 10^{-3}, and 10^{-4}) was plated on sterile plates containing a nutrient agar medium (tryptic soya agar (TSA)) by the plate pouring method. The plates were inverted and incubated at 37 °C for 24 h before the bacterial colonies were counted on plates with between 30 and was less than 300 colonies. 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 (Peng et al. 2009; Moreira et al. 2011).

Total petroleum hydrocarbon extraction in soil mixture

The TPH in the samples was analyzed using an ultrasonic solvent extraction method (Tang et al. 2012). Three replicates of spiked medium were sampled at each sampling periods. The 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 pot on the same sampling day for all treatments to extract total petroleum hydrocarbon (TPH). Soil samples were dried by mixing with sodium sulfate (Na_2SO_4) and later placed in a 100-mL Schott bottle with 50 mL dichloromethane (DCM) (R&M Chemicals, UK), with the bottle being agitated in an Ultrasonic cleaner (Thermo-10D, USA) 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 2–3 days to allow the solvent to evaporate completely, after which, 1.5 mL DCM was added and the extracts were stored in gas chromatography vials.

Total petroleum hydrocarbon extraction in plant tissue

During the phytoremediation period of 72 days, plants were sampled at 0, 14, 28, 42, and 72 days and then analyzed to determine the degradation removal efficiency of TPH. All of the samples were obtained in triplicate. TPH was analyzed using plant matter by gas chromatography flame ionization detector (GC-FID). TPH in plant tissues was analyzed to determine the fate of the hydrocarbon throughout the phytoremediation process, whether the hydrocarbon is degraded or adsorbed into the plants. To assess gasoline uptake by plants, 1 g of dry upper layer (stems+leaves) and lower layer (roots) of the plants was placed in a 100-mL Schott bottle and mixed with 50 mL of dichloromethane (Merck, Germany) as a solvent. Afterward, the sample in the Schott bottle was extracted using an ultrasonic cleaner (KwunWah International Ltd., China) for 30 min at 50 °C. The samples were then filtered using glass wool, and the extracted solution was poured into a 1.5-mL vial and left under a fume hood for 2 to 3 days to allow traces of water and dichloromethane to evaporate. The extract was then concentrated to a volume of 2 mL and analyzed by GC-FID. The

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concentrations of TPH from GC-FID result is calculated using Eq. (1):

$$\text{TPH concentration (mg/kg)} = \frac{\text{GC-FID result (mg/L)} \times \text{GC vial volume (2 mL)}}{\text{Mass of plant (g)}} \quad (1)$$

Analysis of total petroleum hydrocarbon

The samples extracts were analyzed by gas chromatography with flame ionization detection (GC-FID) (Agilent Technologies, Model 7890A, GC System, UK), with a HP-5 5 % phenyl methyl siloxane column (30 m×0.32 mm i.d.× 0.25 μm) and 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. The percentage of TPH removal on each sampling day was determined using Eq. (2):

$$\%\text{Removal} = \frac{\text{TPH}_0 - \text{TPH}_t}{\text{TPH}_0} \times 100 \quad (2)$$

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

Scanning electron microscopy analysis

Plant parts were analyzed by scanning electron microscopy (SEM). One plant from a high gasoline concentration (3 g/kg) was prepared for analysis by SEM after 72 days of exposure. The plant was washed with tap water and then dried with tissue paper. Slices cut from the stem, leaves, and roots were mounted on metal stubs, coated with gold and examined with a SUPRA 55VP SEM (Germany). The purpose of SEM is to determine the effect of the gasoline on the different parts of *L. octovalvis* (leaves, stem, and root) and to investigate whether the plant can adsorb gasoline via the surface and accumulate it inside the cells.

Statistical analysis

SPSS version 16 (SPSS Inc., USA) was used to determine the significance of the data. All of the experiments were performed in triplicate to compensate for experimental errors and were reported as mean±standard deviation (SD). TPH degradation in mixed soil and the sampling period were analyzed using two-way ANOVA at a 95 % confidence level ($p \leq 0.05$). Plant growth, wet weight, dry weight, and lower layer and upper layer (dependent variables) according to day and concentration (independent factors) were analyzed using the general linear model test, with Duncan's multiple range tests to separate means.

Results and discussions

Monitoring of physicochemical parameters

The physical parameters (DO, temperature, pH, and ORP) of the phytotoxicity test were recorded (Fig. 2). Treatments with planted and unplanted soil mixtures at gasoline concentrations of 1, 2, and 3 g/kg were used to determine the conditions of the experimental study. The results during the entire exposure period showed that temperature varied from 27 to 29 °C. The optimum temperature required for the biodegradation of hydrocarbons in temperate climates generally ranges from 20 to 30 °C (Chan 2011). The results showed that the pH and mean DO value decreased slightly during the exposure period. pH ranged between 4.5 and 7.8 for the planted aquariums and between 3.4 and 5.7 for the unplanted aquariums. Acidic pH conditions were likely observed. Ong et al. (2010) showed that DO and ORP can be measured to determine whether the experimental conditions of the soil are aerobic or anaerobic. The DO ranged from 6.4 to 1.8 mg/L, and from 7.1 to 1.9 mg/L in planted and unplanted aquariums, respectively, this indicated anaerobic conditions.

The presence of hydrocarbons altered the soil pH. The conditions of the phytotoxicity test could be classified as anoxic/anaerobic because the concentration of DO is very limited (Tang et al. 2012). During the 72-day experiment, the ORP of the gasoline concentrations and plant controls indicated anaerobic conditions. The ORP changed during the 72-day period from –50.2 to 115.3 mV in the two treatments. ORP at varying gasoline concentrations in the soil mixture indicated that the soil became more anoxic and anaerobic as gasoline concentrations increased. Faulwetter et al. (2009) reported that high ORP promotes aerobic processes, whereas low ORP favors anaerobic processes. However, redox potential values decrease as gasoline concentrations increase (Lin and Mendelssohn 2009).

Plant response to gasoline contaminant

At each sampling point(s), the plant growth parameters were determined and recorded throughout the 72-day of gasoline exposure, as shown in Fig. 3. After being exposed to gasoline concentrations of 1, 2, and 3 g/kg after 7 days, they showed high growth and compromised physical appearance compared with those in the corresponding control treatments. The dry weights of *L. octovalvis* growing in the soil mixture treated with 1, 2, and 3 g/kg of gasoline were significantly ($p<0.05$) higher than those of *L. octovalvis* in the corresponding control. Results showed that after 14 days, the plants' biomass

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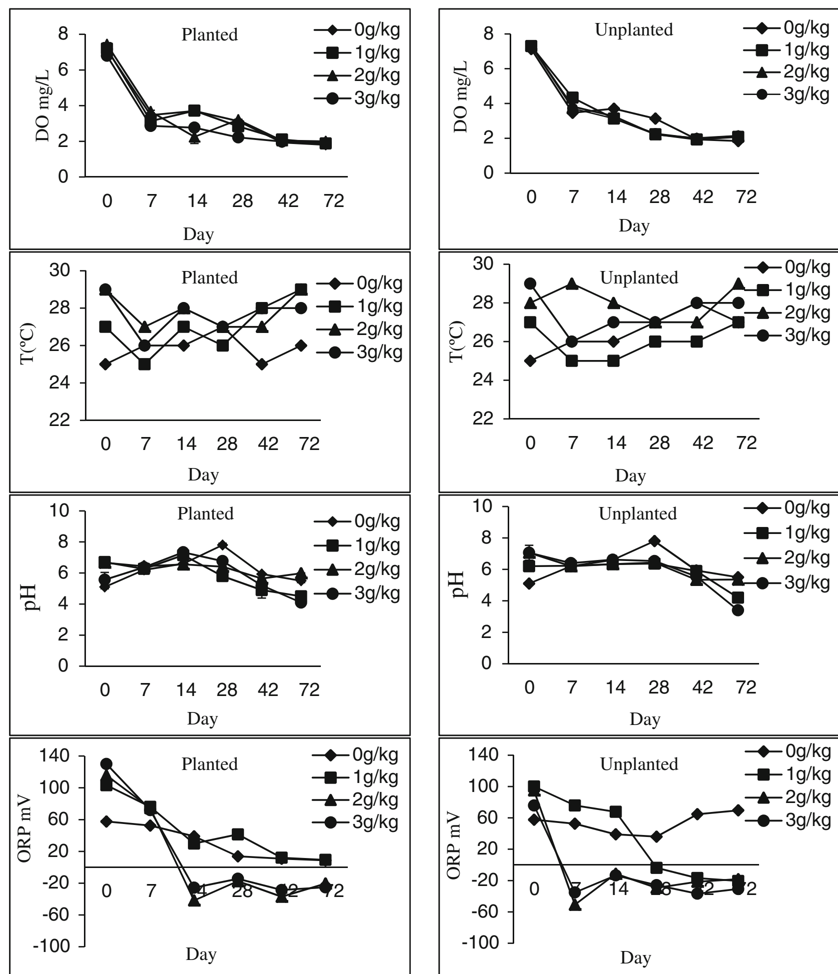

Fig. 2 Physicochemical parameter variations in the phytotoxicity test with *L. octovalvis*, using gasoline as the toxicant

increased until day 42, when the plants started to wither (Fig. 3). Statistically significant ($p<0.05$) difference between the times in all gasoline concentrations was found. After 72 days of experimentation, also the length of the lower and upper plant layer was much higher in the 2 g/kg treatments than at other concentrations in contaminated soils compared to the respective initial values and the control treatment.

The lower and upper plant layers in soil mixtures irrigated with different gasoline concentrations were significantly ($p<0.05$) longer compared to the control sample. The growth of *Scirpus grossus* in contaminated soil at a diesel concentration of 26,100 mg/L was severely affected, indicating that plant growth was inhibited compared to the corresponding control samples (Al-Baldawi et al. 2013). *L. octovalvis* plants in all of the glass containers varied in color from red and green to brown after 14 days. In our results, plants withered when they were exposed to a gasoline concentration of 3 g/kg. Leaves turned red, and the stem changed to brown or yellow. These effects agree with the results of Agamuthu et al. (2010). Water content in the plant tissue was affected by the high stress conditions of contaminated media. It was also possibly related to high temperature and growth requirements of the plants, or might have occurred because the gasoline acts as a physical barrier, preventing or reducing access of the plant to both water and oxygen (Ogbo et al. 2010). The observed symptoms of yellowing and/or degeneration of tissues may be the result of chemical stress at tissue and cellular levels (Meudec et al. 2007).

Rhizosphere microbial count

The population of rhizobacteria in the plant control aquarium without contamination (0 %) was 1.45×10^{2} CFU/mL at day 0, lower than that in the treatments with different gasoline concentrations (Fig. 4). Plants and microorganisms interact with each other to degrade or absorb toxic contaminants from the polluted soil. Similarly, the population of rhizobacteria in treatments with the highest gasoline concentration of 3 g/kg amounted to 1.35×10^{2} CFU/mL at day 0, which was similar with a gasoline concentration of 1 and 2 g/kg, and amounted to 1.39×10^{4} and 1.58×10^{4} CFU/mL, respectively. The rhizobacteria population in the treatment with lowest gasoline concentration of 1 g/kg amounted 1.18×10^{5} CFU/mL during 72 days of

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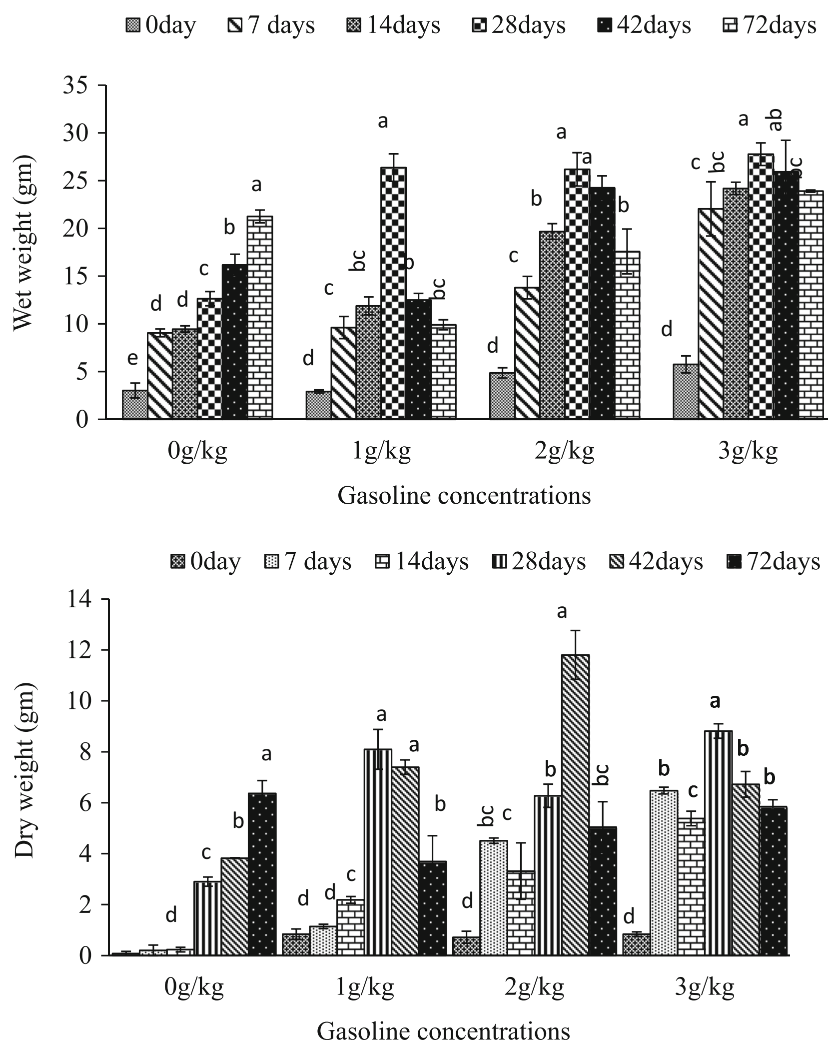

Fig. 3 Growth of *L. octovalvis* including wet weight (**a**) and dry weight (**b**) in different concentrations of gasoline-contaminated soil. *Error bars* indicate the standard deviation ($n=3$). *Different letters* (*a–d*) indicate significant difference between different days within the same gasoline concentration ($p<0.05$)

treatment, which was lower than the other gasoline concentrations of 2 and 3 g/kg. Generally, the rhizobacteria population in the treatments with gasoline concentrations of 1, 2, and 3 g/kg increased until 42 days, and then started to decrease to the end of the 72-day period of exposure. The growth of rhizobacteria with a gasoline concentration of 2 g/kg for 72 days was higher than the other within a range of 2.54×10^5 CFU/mL at 28 days.

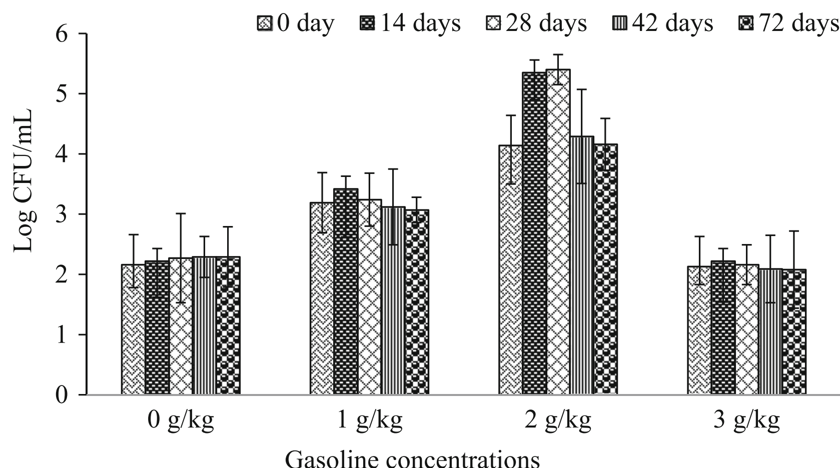

Fig. 4 Total bacterial population during 72 days with the microbial populations for control (0 g/kg) and subsequent gasoline concentrations of 1, 2, and 3 g/kg

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Thus, the growth of rhizobacteria with 2 g/kg gasoline concentration was highly affected and may promote the degradation of petroleum hydrocarbons. In other words, the rhizobacteria population was suitable for the bioremediation of 2 g/kg gasoline-contaminated soil. A significant difference was shown in the microbial populations between the control aquaria (0 %) and those with different gasoline concentrations (1, 2, and 3 g/kg) during the exposure period ($p<0.05$). Bacteria can also assist plants to synthesize several compounds to overcome stress or to provide essential nutrients required for plant growth and development, to improve plant defense system against pathogens, and stimulate contaminant degradation (Khan et al. 2013). Degradation mainly depends on the ability of microorganisms within the rhizosphere to remediate hydrocarbons during the experiment (Tang et al. 2011). Several compounds released by roots act as inducers for microbes in hydrocarbon degradation, and plant roots enhance the tolerance of soil microorganisms to petroleum hydrocarbons (Liu et al. 2011; Cao et al. 2012).

Degradation of total petroleum hydrocarbons in the soil mixture

A reduction in the soil spiked by gasoline concentration was clearly observed during this study. The changes in the concentrations of gasoline under different treatments and the corresponding controls are illustrated in Fig. 5. Some obvious differences in TPH degradation by *L. octovalvis* are poignant between days 0 and 72. There are signs of degradation and gasoline removal over a period of 72 days for the three different concentration treatments (1, 2, and 3 g/kg), either planted or unplanted. The removal efficiency of gasoline contamination in most treatments was significantly different between the three concentrations and sampling days (7, 14, 28, 42, and 72). On day 72 of phytotoxicity testing, all of the concentrations of contaminant decreased. The highest degree of TPH degradation of 79.8 % was observed in the soil mixture exposed to a gasoline concentration of 2 g/kg after 72 days of treatment, whereas the removal in the corresponding control was only 48.6 %. However, the removal of TPH in unplanted pots was lower than in planted treatments because plants help eliminate and reduce TPH and restore contaminated soil (Kirk et al. 2005). These results demonstrated the ability of plants to accelerate the removal of TPH from gasoline-contaminated soil in the first 14 days of exposure; furthermore, the removal of these contaminants slowly increased until 72 days. Figure 6 shows the GC-FID chromatogram profile of gasoline degradation by *L. octovalvis* after exposure to 2 g/kg gasoline, showing a high percentage of degradation between days 0 and 72. The degree of TPH degradation was 71.7 and 70.9 % at 1 and 3 g/kg spiked gasoline, respectively, while the degradation was only 42.8 and 49.5 % at the same respective concentrations in unplanted controls. The removal TPH at all concentrations was significantly ($p<0.05$) different between the planted and unplanted treatments (corresponding control) as depicted in Fig. 5. Hydrocarbons in the planted treatments were metabolized due to rhizobacterial interaction with the plants, but in the unplanted soil mixture, TPH was degraded by volatilization, eluviation, and photolysis (Peng et al. 2009). This clearly indicates that there was less residual TPH in planted treatments compared with the unplanted control (Fig. 5) (Zhang et al. 2010). Taken together, the results indicate the ability of *L. octovalvis* to enhance degradation of TPH and to survive in the three gasoline concentrations in spiked soils. The reduction in total petroleum hydrocarbons in planted soils depends on physical, chemical, and biological processes (Zhang et al. 2010). The factors that decrease the gasoline concentrations in soil might also include

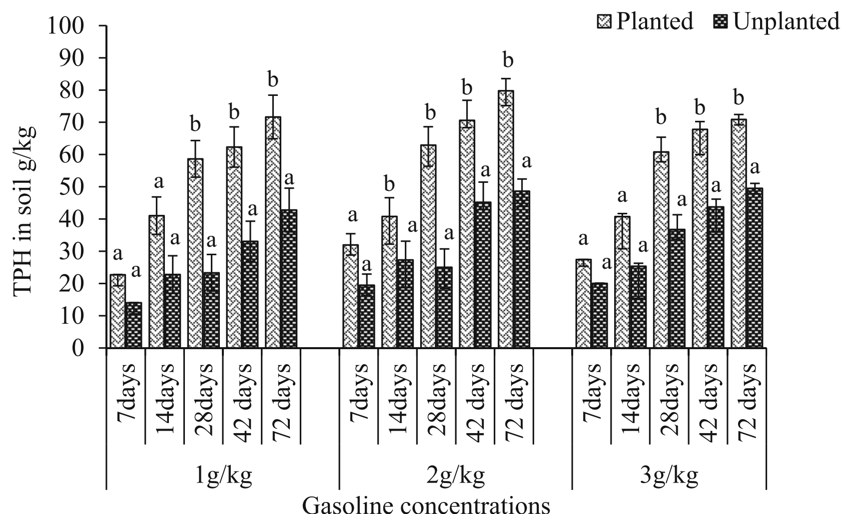

Fig. 5 The percentage degradation in soil mixture extraction by *L. octovalvis* exposed to gasoline contamination at 1, 2, and 3 g/kg. *Bars* indicate the standard deviation of three replicates (n=3). *Similar letters* denote nonsignificant differences; *different letters* denote significant differences in either planted or unplanted treatments ($p<0.05$)

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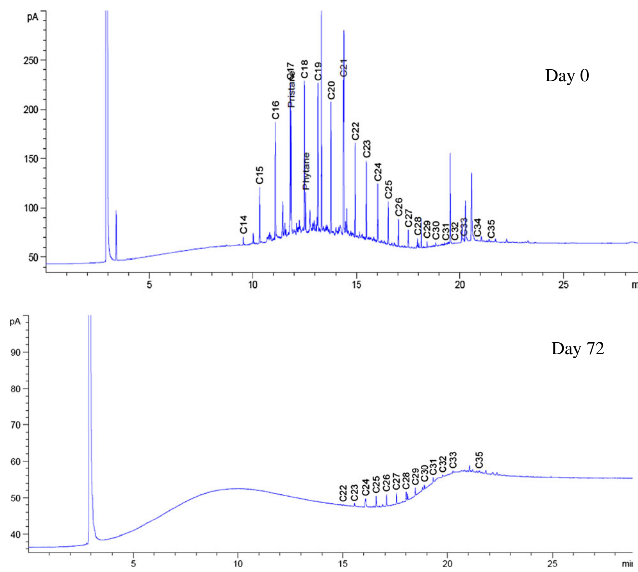


Fig. 6 The GC-FID chromatogram profile of gasoline degradation by *L. octovalvis* after exposure to 2 g/kg gasoline for days 0 and 72

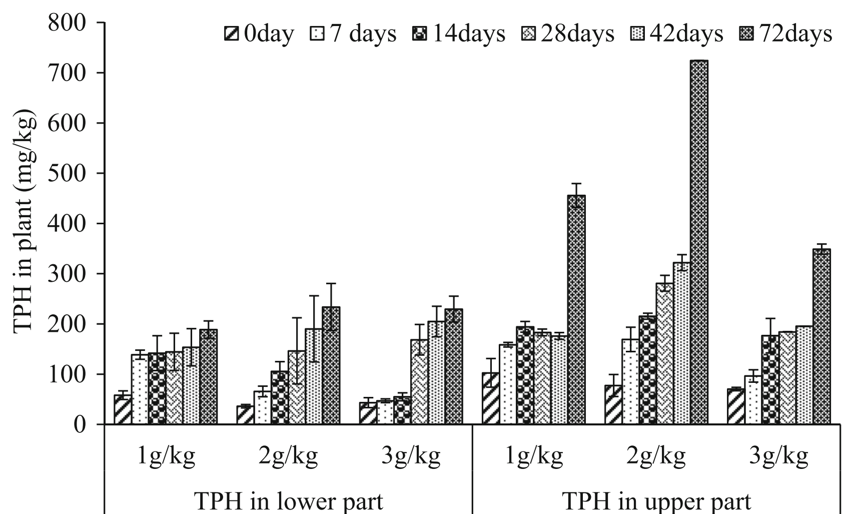
biodegradation by indigenous microorganisms (Peng et al. 2009).

Many studies have demonstrated that microorganisms play an important role in the remediation of petroleum hydrocarbons in contaminated soil (Phillips et al. 2006). It is believed that the plant roots secrete exudates that stimulate the rhizosphere and absorb petroleum, which facilitates the removal of TPH in soils (Cai et al. 2010). For efficient phytoremediation of organic soil contaminants, the soil should provide an appropriate environment for both the plants and associated microorganisms. Previous studies have demonstrated that TPH degradation in soil was improved by the presence of bacteria in the rhizospere (Soleimani et al. 2010; Barrutia et al. 2011). The mechanisms of phytoremediation of petroleum hydrocarbons in sand include volatilization, photodegradation, leaching, plant uptake, biodegradation, and other abiotic losses. However, the main mechanism of TPH phytoremediation in soil contaminants is assumed to be rhizodegradation (Lu et al. 2010), which is the stimulation of bacteria in the rhizosphere to degrade and enhance the removal of TPH (Cai et al. 2010). Siciliano et al. (2003) indicate that applying phytoremediation enhances the degradation of TPH contaminants; furthermore, the rhizosphere provides an ideal microbe habitat for stimulating hydrocarbon degradation. Rhizospheres enhanced the number and activity of microbes. However, in phytoremediation involved many factors on affect TPH degradation in contaminated sites, such as plant type, the physical–chemical parameters of the soil, the amount of contamination, and nutrient availability. Because little is known about how all of these factors affect phytoremediation, more detailed studies are needed (Jagtap et al. 2014).

TPH concentrations in plant tissue and SEM analysis on plant tissues

L. octovalvis upper layer (including leaf and stem) and lower layer (root) were ultrasonically extracted to determine the uptake of hydrocarbons in the plant parts during exposure to different gasoline concentrations of 1, 2, and 3 g/kg. TPH content in plants accumulated in the lower and upper layer, as depicted in Fig. 7. GC-FID analysis of the extract revealed the presence of hydrocarbons in plants in all of the treatments. TPH concentrations in plants varied in each treatment. Furthermore, TPH concentrations in plants increased in different layers of the plant (lower and upper) at 72 days; this result indicated that TPH was adsorbed and accumulated in the root tissues. This phenomenon was observed in plants exposed to

Fig. 7 Percentage of gasoline in *L. octovalvis* after exposure to 1, 2, and 3 g/kg in soil between two parts (lower and upper layers) in plant



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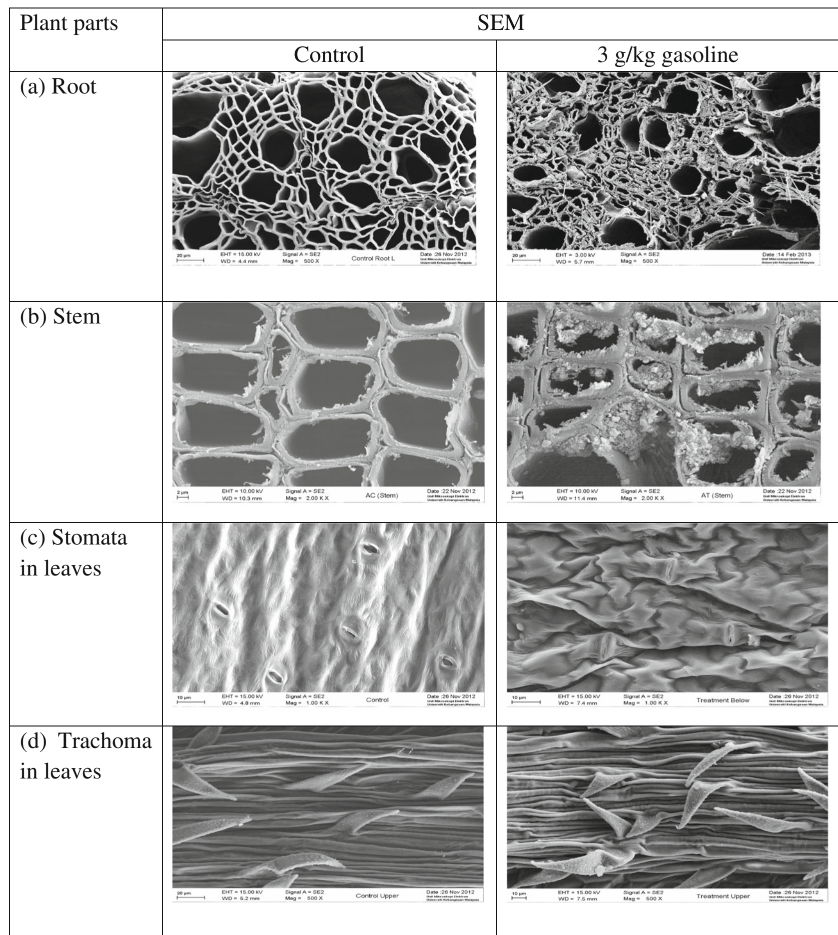

Fig. 8 SEM showing root cross sections at 500× of *L. octovalvis* after 72-day exposure to a gasoline concentration of 3 g/kg and the corresponding control

all of the gasoline concentrations (1, 2, and 3 g/kg). TPH concentrations in the lower layer were lower than those in the upper layer because the upper layer comprises two parts (stem and leaf), whereas the lower layer comprises only the root. The TPH accumulation rate in plant tissues exposed to 2 g/kg from 0 to 72 days was 36.2 to 233 mg/kg in the lower layer and 77.4 to 724.2 mg/kg in the upper layer. Exposure to 1 g/kg gasoline resulted in 188 mg/kg accumulation in the lower layer and 102.5 mg/kg in upper layer, whereas exposure to 3 g/kg resulted in 229.5 mg/kg in the lower layer at 72 days and 348 mg/kg in upper layer. The concentration of TPH was higher in the upper layer (stem+leaves) than in the lower layer (root); thus, gasoline that was absorbed in the upper layer (stem+leaves) was transported from the roots via the transpiration stream (Xia and Ma 2006). This difference may occur partly because shoots performed direct chemical uptake from water.

The presence of hydrocarbons in the soil can inhibit and reduce plant growths (Chaineau et al. 1997). SEM images taken after 72 days of *L. octovalvis* grown in 0 g/kg (control plant) and 3 g/kg gasoline confirm the effect of gasoline on roots, stems, and leaves shown in Fig. 8. The different plant parts were completely withered in comparison to the control samples. This shows that the plant has contributed to the removal of gasoline from soils. Gasoline caused severe damage to roots and stem structures, as illustrated in Fig. 8. However, the effect was less severe on leaf tissues. The shape of the cross-sectional tissues of all parts of *L. octovalvis* (stem and root) after 72 days of gasoline exposure became irregular and folded with shrunken tissues, as illustrated in Fig. 8. Results clearly show the effect of hydrocarbon pollution after penetrating the plant tissues, and damage to tissue or membranes followed by loss of shape due to a reduction in metabolic transport (Xu and Johnson 1995; Chandra and Yadav 2010).

Conclusions

The tolerance of plants to soil contamination by gasoline after 72 days demonstrated that *L. octovalvis* has the ability to survive and provide suitable conditions for rhizobacteria to degrade hydrocarbons at all investigated gasoline concentrations (1, 2, and 3 g/kg). Based on soil extraction, the highest TPH removal rate was 79.8 %, in comparison to the removal rate by the corresponding unplanted controls of only 48.63 %.

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Electron microscopy showed that gasoline contaminants were adsorbed by the plants.

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