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Phytoremediation of petroleum hydrocarbon contaminated saline-alkali soil by wild ornamental *Iridaceae* species

Lijuan Cheng, Yanan Wang, Zhang Cai, Jie Liu, Binbin Yu, Qixing Zhou*

Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria / Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

*Corresponding author at: Key Laboratory of Pollution Processes and Environmental Criteria at Ministry of Education, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China. Tel.: +86 22 23507800; fax: +86 22 66229562.

E-mail addresses: Zhouqx@nankai.edu.cn, zhouqx523@yahoo.com

Abstract

As a green remediation technology, phytoremediation is becoming one of the most promising methods for treating petroleum hydrocarbons (PHCs) contaminated soil. Pot-culture experiments were conducted in this study to investigate phytoremediation potential of two representative *Iridaceae* species (*Iris dichotoma* Pall. and *Iris lactea* Pall.) in remediation of petroleum hydrocarbon contaminated saline-alkali soil from the Dagang Oilfield in Tianjin, China. The results showed that *I. lactea* was more endurable to extremely high concentration of

PHCs (about 40,000 mg/kg), with a relatively high degradation rate of 20.68%. The degradation rate of total petroleum hydrocarbons (TPHs) in soils contaminated with 10,000 and 20,000 mg/kg of PHCs was 30.79% and 19.36% by *I. dichotoma*, and 25.02% and 19.35% by *I. lactea*, respectively, improved by 10-60% higher than the unplanted controls. Presence of *I. dichotoma* and *I. lactea* had promoted degradation of PHCs fractions, among which saturates were more biodegradable than aromatics. Adaptive specialization within the bacterial community was observed. In conclusion, phytoremediation by *I. dichotoma* should be limited to soils contaminated with ≤ 20,000 mg/kg of PHCs, while *I. lactea* could be effectively applied to phytoremediation of contaminated soils by PHCs with at least 40,000 mg/kg.

Keywords

rhizodegradation; soil contamination; petroleum hydrocarbons (PHCs); wild ornamental; rhizospheric microorganism

1. Introduction

Petroleum hydrocarbons (PHCs) are one of the most common groups of persistent organic pollutants (POPs). Along with the extensive usage of petroleum products, contamination of soil by PHCs has become a major environmental challenge and aroused worldwide concern mainly due to the complicated compositions, biological toxicity and ecological risks of PHCs (Lin and Mendelssohn 2012; Phillips *et al.* 2009; Soleimani, Farhoudi and Christensen 2013; Zhang *et al.* 2015; Zhou and Song 2004).

As an emerging green remediation technology (Peng *et al.* 2009; Xu and Lu 2010), phytoremediation is becoming one of the most promising methods for treating PHCs contaminated soils because of its cost-effectiveness and ecological advantages (Bell *et al.* 2014; Gurska *et al.* 2009b; Kukla, Płociniczak and Piotrowska-Seget 2014). So far, many plant species such as Tall fescue (*Testuca arundinacea* L.) (Gurska *et al.* 2009a), bermuda grass (*Cynodon dactylon* (L.) Pers.) (Hutchinson, Banks and Schwab 2001), *Impatiens balsamina* L. (Cai *et al.* 2010) and *Pharbitis nil* L. (Zhang *et al.* 2010) have been found to have the ability of degrading PHCs in soils. Besides, some native plant species such as *Axonopus compressus* (Bordoloi *et al.* 2012), *Cymbopogon ambiguus*, *Brachiaria decumbens* and *Microlaena stipoides* (Gaskin and Bentham 2010), were also screened out for remediation of petroleum contaminated soils. However, plants applied for phytoremediation are mostly gramineous grasses (Ikeura *et al.* 2016; Zhou *et al.* 2011), the phytoremediation potential of wild

ornamentals has been rarely reported. Besides, contamination of PHCs in previous studies is generally low enough to guarantee the seed germination and plant growth. Many soils from oilfields are also salinized and alkaline, posing extremely adverse challenges to biomass accumulation and root growth (Liu *et al.* 2014). Therefore, in addition to grasses, ornamental plant species that are suitable for saline-alkali soils and capable of degrading PHCs are still needed.

Wild ornamentals, with extensive fibrous root systems, rapid biomass production and high ornamental characters, can not only possess the traditional characteristics (for example, adaption to the local environment, and degradation capability of contaminants) of phytoremediation grasses, but also beautify our environment at the same time (Cheng and Zhou 2014). Particularly, because of broad adaptability and widespread distribution in nature, wild ornamentals are easier to cultivate and manage, therefore present a huge engineering and economic advantages for phytoremediation at the scale of fields.

Among the various mechanisms involved in phytoremediation of PHCs contaminated soils, rhizodegradation is of major significance for the enhanced removal of PHCs from soil (Zhou *et al*. 2011; Zhou and Song 2004). In the process of rhizodegradation PHCs are broken down through the microbial activity which is enhanced by metabolites from the metabolism of plant roots, such as carbohydrates, amino acids, fatty acids, organic acids, putrescines, sterols and vitamins (Kang *et al.* 2010). In other words, stimulation of microbial degrading activity of

PHCs is driven by root exudates, oxygen and favorable redox conditions offered by roots, thereby leading to a reduction of the PHCs concentration (Kirk *et al.* 2005). The activity of soil microorganisms in the rhizosphere, in turn, could benefit the remediation of plants by supplying amino acids, vitamins, cytokinins and auxins that stimulate plant growth and lead to enhanced degradation of PHCs. During this process, uptake and degradation rate of PHCs are mainly restricted by hydrophobic and toxicological nature of the contaminants, as well as the synergy existing between plant roots and soil microorganisms (Wang *et al.* 2011). However, most researchers had only focused on the efficiency of phytoremediation (Ferrera-Rodríguez *et al.* 2013; Liu et al. 2014; Megharaj *et al.* 2011; Michael *et al.* 2007). Hence, it is urgently necessary to make a systematical exploration on effects of plant growth and associated microbial activities on removal of hydrocarbons, and the synergy between plant species and rhizosphere microorganisms.

According to the principles of phytoremediation, our preliminary study has been conducted by an elementary screening-out of wild ornamentals to pick out those that are salt-tolerant, adapted to the local environment, and most of all, survive under heavy contamination of PHCs. A total of 29 wild ornamentals from 5 genera were tested through the pot-culture experiment, among which *Crassulaceae* (Cheng and Zhou 2014), *Asteraceae* and *Iridaceae* species, were found to exhibit differently under saline-alkali stress and petroleum contamination. The pot-culture experiments in this study were conducted then to further

examine the potential of *Iridaceae* species, including the two typical candidates *Iris dichotoma* Pall. and *Iris lactea* Pall., in remedying low (1%), moderate (2%) and high (4%) concentration of petroleum contaminated soils. This might be the first study on phytoremediation of petroleum contaminated soil by *Iridaceae* species, and our objective is to offer a preferable alternative to efficient phytoremediation.

2. Materials and methods

2.1 Experimental sites and soil preparation

The experiments were conducted in a greenhouse of the Taida branch, Nankai University, Tianjin, China. The temperature in the greenhouse was between 18 ℃ to 25 ℃, and the natural day light time was about 16 h.

Clean surface soils used in the experiments were collected from the Dagang District, Tianjin, China. The petroleum contaminated soil samples were collected from the surface (0-20cm) of No.4 Oil Production Plant, the Dagang Oilfield. Total petroleum hydrocarbon (TPH) concentration in the contaminated soil was 51,411 mg/kg. Both the contaminated and uncontaminated soils were air-dried and passed through a 4 mm sieve so that the soil homogeneity could be ensured. Small amount of soils were used to determine its physical and chemical properties.

2.2 Pot-culture experiment

In order to determine the phytoremediation and microbiological effects of PHCs by

Iridaceae plants, three treatments with low (10,000 mg/kg), medium (20,000 mg/kg) and high (40,000 mg/kg) concentrations of PHCs were designed in the pot-culture experiment. The PHCs contaminated soils were mixed by hand with the clean soils at certain ratios to obtain the designed gradient PHCs concentrations. The practical concentration of PHCs in soils after the artificial mixture was 11,874, 20,075 and 38,986 mg/kg, respectively. The treatment with clean soil was regarded as the control (CK). Then, 1.5 kg mixed soils were filled in per cylindrical plastic pot according to its size (D=230 mm, H = 180 mm), and equilibrated for 2 weeks. After that, the value of pH in diluted soil was in the range from 7.5 to 7.8 (no significant difference). And the value of organic matter was between 27 and 52 g/kg.

Two different species of *Iridaceae*, namely *Iris dichotoma* Pall. and *Iris lactea* Pall., were selected for the phytoremediation study after screening-out and confirmatory experiments. These two plants are ubiquitous ornamentals growing wild and widely distributed throughout China. For each species, three transplants of weed seedlings with the similar age and height were planted in the pots with treatments CK, T1, T2 and T3. Each treatment was set using three replicates. A total of 36 pots were nurtured in the greenhouse, including 12 pots of the corresponding unplanted controls. All the pots were watered daily using tap water to sustain 80% of the water holding capacity. The plants were harvested after a 90-day culture.

2.3 Measurement of biomass

The harvested plants were washed using running water, rinsed with distilled water and

then carefully dried by using filter paper. The shoot weight and root weight were measured first. Roots and shoots were scanned with the EPSON Scanner. Then the digitizing results of scanning image were given in the form of shoot height and root length through the use of WinRHIZO analysis software.

2.4 Determination of TPHs and various components

Determination of TPHs and various components followed the US EPA Method 3550c. The soil samples gathered from the rhizosphere of plants were dried by using the vacuum freeze-drying technique to avoid volatilization of hydrocarbons. About 5 g dried soil samples wrapped with filter paper were put into a Soxhlet extractor, completely immersed in 125 mL of dichloromethane and heated to 54 degrees for 12h. The extraction was at first concentrated by the rotary evaporation, and then dried under nitrogen to a constant weight.

After the gravimetric determination, the residual TPHs were redissolved in 1 mL of hexane, and subsequently filtered and separated by means of silica gel column chromatography. The saturated and aromatic hydrocarbons were successively eluted using 20 mL of hexane and 75 mL of a mixed solution of hexane and dichloromethane with the volume ratio of 1:1. The eluents were concentrated by the rotary evaporation, dried under nitrogen and then dissolved in 10 mL of chromatographically pure hexane for GC-MS analysis.

2.5 Enumeration of PHCs degraders in soils

Enumeration of alkane-degrading and PAH-degrading microorganisms in soil was

achieved through the five-tube MPN methods using separate 96-well microtiter plates. One gram of each soil sample was diluted in 9 mL of sterile water, and ten-fold serial dilutions from 10^{-2} to 10^{-9} were prepared. Before inoculation with 20 μL of the dilutions, the plates were injected with 180 μL of the Bushnell Haas (BH) mineral salts medium. For alkane degraders, the selective growth substrates were n-hexadecane and Number 2 fuel oil. A mixture of four PAHs (10 g/L phenanthrene, 1 g/L anthracene, 1 g/L fluorine and 1 g/L dibenzothiophene, all dissolved in pentane) were served as the selective growth substrates of PAH degraders. Each specific concentration of dilutions was added in one of the twelve rows of the five parallel wells. A sterile control was prepared simultaneously.

All the microtiter plates were incubated in digital biochemical incubator at 25 ℃ stationary temperature. For alkane degraders, 50 μL of iodonitrotetrazolium violet (INT) was added to each well after two weeks of incubation. In positive wells, INT would degrade to a red precipitate within one day. As for PAH degraders, the incubation time was three weeks, and the positive wells would turn turbid or yellow. The number of hydrocarbon degraders in each category was calculated according to the published MPN tables.

2.6 PCR-DGGE analysis

The microbial DNA was extracted from 1.0 g of the rhizosphere soils using the OMEGA E.Z.N.A. Soil DNA Soil Kit, and the experimental procedures were followed by the manufacturer's manual.

Amplification of the 16s-rDNA genes from most bacteria was completed by the use of PCR technique. Two sets of primers, 16s-Forward (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG-3') and 16s-Reverse (5'-ATTACCGCGGCTGCTGG-3'), were designed to amplify an approximate 230 bp sequence. The PCR reaction conditions were initial denaturation at 94℃ for 5 min, followed by 10 cycles of denaturation (94℃ for 1 min, 55~65℃ for 1 min and 72℃ for 1 min) and 25 cycles of annealing (94℃ for 1 min, 55℃ for 1 min and 72℃ for 1 min), and finally ended with an extension at 72℃ for 1 min.

DGGE of the PCR products was carried out using the Dcode ^{TM} System of the Bio-Rad Company. Electrophoresis was performed at 200 V and 60 ℃ for 3.5 h, with the denaturing gradient of 20 to 50%. The DGGE gels were photographed and observed under a ultra-violet transmission analysator. The electropherogram was digitized and analyzed by the gel imaging system and the quantity one software.

2.7 Statistical analysis

Data were processed by the use of SPSS 20.0 and Origin 8.0. The experimental results were represented in the form of arithmetic mean ± standard deviation. All the samples were prepared in triplicate in order to decrease the experimental errors. The significant testing was conducted with the LSD test of one-way analysis of variance ($p < 0.05$).

3. Results and discussion

3.1 Tolerance of plants to PHCs

In this study growth of the two different seedlings, *I. dichotoma* and *I. lactea,* was slightly inhibited in the contaminated soils during the early growing stage. After a 20-day exposure and pot-cultivation, the plants tended to be adapted to the toxic environment, which could be seen from the increases in the biomass and the number of plant tillers. However, the two iridaceous plants responded differently under PHCs stress after then.

Based on the results (Table 1) of *I. dichotoma,* it was found that the shoot height and shoot biomass decreased gradually as the concentration of TPHs increased. Shoot height and shoot weight of *I. dichotoma* in treatment T1 did not reduce significantly ($p>0.1$) when compared with those in the control, whereas a significant ($p<0.05$) reduction was observed in treatment T2. Plants in treatment T3 got chlorosis and died after a 1-month exposure. However, a different result was observed from plant roots. The root length reached the highest level in treatment T1, and then declined. Besides, there was no significant ($p>0.1$) decrease found in root length and root weight between treatment CK, T1 and T2.

The impact of PHCs contamination on growth of *I. lactea* was different from that of *I. dichotoma* (Table 1). *I. lactea* survived in all contaminated treatments with no visible adverse symptoms such as wilting, lodging and defoliation before harvest, indicating a strong endurance to both low and high concentration of PHCs. The plant parameters in contaminated treatments

reduced at different levels compared with those in the control. Generally speaking, the inhibitory effects mainly occurred in the shoots rather than the roots, and reduction in shoot weight was the most significant ($p<0.05$). Surprisingly, the biomass of *I. lactea* in treatment T3 was much higher than that in treatment T2.

Generally biomass of plants (especially the roots) is regarded as indicator of plant tolerance to soil contamination (Bramley-Alves *et al.* 2014). Our study suggested the law that the inhibitory effects of PHCs on the two iridaceous plants were mainly occurred in the shoots rather than the roots. Actually, there was either slightly inhibited or even stimulated root growth in the presence of PHCs contamination. As the roots play the vital role in improving soil structure, secreting exudates and enriching the rhizosphere microbial flora (Young and Crawford 2004), which are all crucial for plant tolerance and phytoremediation efficiency, deep and extensive root systems might indicate a beneficial potential for further phytoremediation.

The presence of PHCs had typical phytotoxic effects on shoot growth of the two *Iridaceae* species, such as reduction in the height, biomass and high rate of plant mortality, which had also been proved by the study of Peng *et al*. (Peng et al. 2009) and Brandt *et al*. (Brandt *et al.* 2006). Generally these reactions occur when there is exogenous contamination or variation in soil-derived resources (Merkl, Schultze-Kraft and Infante 2005). In the present study, these symptoms were obviously derived from the PHCs stress. Surprisingly, the biomass of *I. lactea* in treatment T3 was much higher than that in treatment T2. The relatively high increase in shoot

biomass must be a reflex of the stimulation on root growth, which effectively reduced the mobility, bioavailability and phytotoxicity of PHCs to the whole plants through the rhizosphere effects. Although the intricate mechanisms involved are still unclear, the large plant biomass is definitely beneficial for successful phytoremediation.

3.2 Degradation and phytoremediation of PHCs

As the concentration of PHCs increased, the degradation rate in all treatments including the planted group and the natural attenuation control reduced gradually (Fig. 1). The general reduction of TPHs in the planted soils was higher than that in the unplanted control. As wild ornamentals for phytoremediation of petroleum contaminated soil, the two *Iridaceae* species had some differences in degradation rate of TPHs and the various components.

The degradation rate of TPHs by *I. dichotoma* in treatments T1 and T2 was 30.79% and 19.36%, respectively (Fig. 1), whereas it was 21.50% and 17.84% in the corresponding control. The degradation by plants was improved by 61% in treatment T1 and merely less than 10% in treatment T2, compared with that in the control, respectively. Apparently, *I. dichotoma* was more suitable for phytoremediation of soils contaminated with less than 10,000 mg/kg of PHCs.

As for the *I. lactea* planting group, a different trend of degradation was observed. The degradation rate of TPHs by *I. lactea* was in a very small range from 19 to 25% in soils contaminated with 10,000 mg/kg to 40,000 mg/kg of PHCs, all higher than that in the corresponding control, which might indicate that *I. lactea* could improve the removal of both

low and high concentration of PHCs in soil. Strangely, the degradation rate in treatment T2 was only 19.35%, showing no significant ($p>0.1$) increase over that in the corresponding control. The degradation rate of TPHs in treatments T1 and T3 was 25.02% and 20.68%, significantly ($p<0.05$) higher than that in the corresponding control (19.10% and 14.70%, respectively). Besides, there was no significant ($p>0.1$) reduction in TPH degradation between treatments T2 and T3, which further confirmed that the extremely high concentration of PHCs might have very limited adverse effects on phytoremediation by *I. lactea*.

When evaluating efficiency of phytoremediation, the promoted reduction of TPHs concentration by plants was always regarded as the primary index. Based on this, lots of researches had shown that specific plants could promote degradation of oil. Gao *et al*. found a 43.8% TPH reduction (only 1.09 times more than that by natural attenuation) by *Suaeda salsa* in petroleum contaminated soils in 2 months (Gao *et al.* 2014). Dong et al. showed that TPH degradation was less than 40% during 3 months of oat cultivation (Dong *et al.* 2014). Nevertheless, no study on phytoremediation of PHCs contaminated soil using *Iridaceae* plants has been conducted. Besides the different plant species, another factor that differentiated our study from the similar studies was the soil. The soil used in our study was originally polluted with aged oil, which meant that it contained a large fraction of high molecular weight hydrocarbons. As high molecular compounds were less degradable due to the poor hydrophilicity and low bioavailability, phytoremediation of aged oil contaminated soil was

commonly more difficult than that of the fresh contamination. Even so, our study proved that both *I. dichotoma* and *I. latea* could definitely promote the removal of aged oil from soil, with a relatively high TPH degradation rate of 19.35-30.79%, improved by 10-60% than the corresponding unplanted controls.

After a 3-month cultivation, a significant difference in efficiency of phytoremediation between the two *Iridaceae* species was observed. Simply, *I. dichotoma* could remediate soils contaminated with no more than 20,000 mg/kg of PHCs, and cause a relatively high petroleum dissipation in soils contaminated with low concentration of PHCs (no more than 10,000 mg/kg), which was approximately 1.43 times more than that of the unplanted control. As for phytoremediation using *I. latea*, the concentration of PHCs in soils could increase to at least 40,000 mg/kg. The differences in phytoremediation efficiency between *I. dichotoma* and *I. latea* could be explained by the rhizosphere effects. Compared with *I. dichotoma* rhizomes, *I. lacteal* had slighter fibrous root systems, which could effectively increase adaptability of plants and rhizospheric microorganisms to the contaminated environment. In other words, the high efficiency of phytoremediation might be attributed to suitable plant growth (especially root growth), on the basis of which, functional soil microorganisms and root exudates that were essential for transformation and degradation of the contaminants, were stimulated. Furthermore, presence of plant roots could improve soil properties (such as oxygen content, permeability, moisture and so on) in the root zone, and ultimately facilitate the elimination of toxic pollutants

(Bramley-Alves et al. 2014). Specifically for the present study, *I. lactea* had a stronger fibrous root system than that of *I. dichotoma* in the extremely high concentration of PHCs contaminated soil, thus developing a wider and deeper root system and offering more advantages for degradation of PHCs. Similarly with *I. dichotoma*, *I. latea* did not show a significant effect on phytoremediation efficiency in soils contaminated with 20,000 mg/kg of PHCs. This might indicate that poor rhizosphere of *I. dichotoma* and *I. latea* under the medium contamination had little effect on phytoremediation efficiency. That is, reduction in TPH concentration in this planted group is largely attributed to natural attenuation such as volatilization, oxidation, photodegradation and microbial decomposition (Peng et al. 2009).

3.3 Component changes after phytoremediation

After a 3-month exposure or pot-culture experiment, changes of residual PHCs components in soils corresponded to TPH reduction both in the planted treatments and the unplanted controls (Fig. 2).

Undoubtedly, presence of *I. dichotoma* and *I. lactea* had improved the degradation of saturated and aromatic hydrocarbons when compared with that in the unplanted soils. The remaining saturates in treatment T1 had a significant reduction ($p<0.05$), while the aromatic residuals did not reduce significantly, compared with that in the corresponding control, respectively. The residual PHCs components in the planted soils in treatment T2 had an insignificant reduction compared with that in the control. Strangely, in the highest concentration

(40,000 mg/kg) of PHCs contaminated soil, *I. lactea* could still promote the degradation of saturated and aromatic hydrocarbons, leading to a significant ($p<0.05$) reduction in residual hydrocarbon fractions, which was consistent with the decline of TPHs.

Our study has proved that the high-molecular-weight PAHs were harder to be degraded compared with that of alkanes. This result was in agreement with that of Zhang et al., which pointed out that saturates were the most easily degradable fractions in the four oil components, including saturates, aromatics, asphaltenes and resins (Zhang et al. 2010). PAHs with longer chains (>C40) were less likely to evaporate or leach than that of saturates because of the poor water solubility and low vapor pressure. Besides, because most PAHs had a log KOW (Octanol-Water Partitioning coefficient) > 4, they were tightly absorbed onto soil and hardly transported to the plant. Regardless of the chain lengths, another factor that caused such differences between the degradation rate of the PHCs components and especially between the two plant species was the type of PHCs bacteria in the rhizosphere, which is discussed in the next section.

3.4. The number of PHCs degraders

In our study, the number of PHCs degraders in soils was cultivated and enumerated after phytoremediation. Obviously, plant species and PHCs contamination had distinct impacts on the growth of alkane degraders and PAH degraders.

The number of alkane degraders and PAH degraders in contaminated soils planted with *I.*

dichotoma was all greater than that in the unplanted soils (Fig. 3). Apparently, low concentration of PHCs could increase the activity and proliferation of degraders in the rhizosphere, leading to a significant reduction in saturated and aromatic hydrocarbons described in the above section. This trend could be proved by Brandt et al., who found that the treatments planted with *Axonopus compressus* showed a significant increase in the population of petroleum-degrading bacteria after a 360-days exposure. However, the difference was that the number (log (MPN g^{-1} soil)) in petroleum contaminated treatments in the present study changed from 6 to 8, much greater than the previous counts (between 3 and 6). This might indicate that roots of *I. dichotoma* could provide better rhizosphere effects in enhancing growth and reproduction of the degrading bacteria. The alkane degraders and PAH degraders in soils under treatment T2 decreased significantly ($p<0.05$), which well explained the little loss of PHCs components from soils.

Curiously, the number of petroleum degraders in *I. lactea* planting soils had some different variations, which was inconsistent with the TPH degradation rate. Commonly, degradation by microorganisms in the rhizosphere was thought to be one of the most important mechanisms of petroleum removal in phytoremediation systems (Euliss *et al.* 2008)，and hydrocarbon degraders in the planted soils were generally much more than those in the unplanted control (Khan *et al.* 2013). But our study proved some exceptions. The number of the alkane degraders and PAH degraders in the *I. lactea* planted treatments was smaller than that in the unplanted

control when soils contaminated with no more than 20,000 mg/kg of PHCs. The reduction in the number of degraders might be due to that despite of the excreted substances from plant roots that provided as carbon source and energy, there was also hazardous organics that acted as growth inhibitors for rhizospheric microorganisms (Soleimani *et al.* 2010). However, reduction of TPHs and the PHCs fractions in all planted treatments were still higher than that of the control. The possible reason might be that phytostabilization, phytodegradation, phytoaccumulation and phytovolatilization, based on the interactions of endophytes, root exudates and mycorrhizal characteristics, also contributed to the degradation rate of TPHs, except for the degradation by rhizosphere microorganisms in the soil. Actually, the effectiveness of phytoremediation in some previous studies was mainly depended on endophytic bacteria (Hardoim, Overbeek and Elsas 2008; Li *et al.* 2012), which lived inside plant tissues and were capable of degrading organic contaminants, rather than the microorganisms that only had the ability to degrade hydrocarbons in soils. In *I. lactea* planting soils with 20,000 mg/kg of PHCs, the above mechanisms such as phytodegradation (based on root secretion and endophyte) and phytoaccumulation, were very likely to be predominant amongst all the mechanisms. Reduction in the number of degraders had very little influence on the TPH degradation than that of the other degrading effects. There was significant ($p<0.05$) increase in the number of alkane degraders and PAH degraders in treatment T3 compared with that in treatment T1, treatment T2 and the unplanted control. In fact, the number of alkane degraders and PAH degraders in

planted soils in treatment T3 was approximately 100 times more than that in the unplanted control. Due to this spectacular increase, degradation of TPHs by rhizosphere microorganisms was significantly promoted then.

3.5 Community structure and diversity of soil microorganisms

The DGGE image of 16S rRNA (Fig. 4) reflecting the bacterial community structure in the different treatments over the phytoremediation process was displayed. Regularly, shift in bacterial community was probably in response to the different contamination and host plant species (Chen *et al.* 2014; Moreno *et al.* 2015). Similarly in our study, three clear clusters between the planted group of *I. dichotoma*, *I. lactea* and the unplanted controls (Fig. 4) were distinguished according to the Canonical Correspondence Analysis (CCA). The direction represented the relationship between the DGGE gel banding patterns and the environmental factor, and revealed a strong positive correlation between the PHCs contamination and plant species on the microbial community composition and structure.

As the degradation rate of TPHs increased, the Shanon diversity (Table 2) decreased gradually. Furthermore, the *I. lactea* and *I. dichotoma* planted treatments had lower microbial diversity (Shanon index) and higher Simpson diversity than that in the control. This fact might reveal that some types of bacterial specialization occurred due to the selective pressure of PHCs contamination. Similar results were concluded by Viñas (Viñas *et al.* 2005), who found that specific bacterial phylotypes were strongly associated with different phases of PAH degradation

in the PAH contaminated soil. Plants had the ability to secrete specific exudation that selectively enhanced the reproduction of degrading bacteria species, and thus made the degrading bacteria become the dominant species. Specifically in our study, the dominant microbes might be responsible for resistance to the PHCs stress. In return, due to the effective removal of PHCs from soil, the degrading bacteria provided an environmental condition that could benefit the growth of remediation plants and the elimination of toxic contaminants. Based on this, targeted genes and strains with varying capacities for pollutant degradation and/or transformation, and the interactive activities of remediation plants and functional microorganisms, should be further identified and explored.

4. Conclusions

The study has shown the rhizodegradation effects of two representative *Iridaceae* species and the associated microorganisms for PHCs contaminated saline-alkali soils. Compared with *I. lactea*, *I. dichotoma* had obvious phytotoxic symptoms to extremely high concentration (up to 40,000 mg/kg) of PHCs and died after a 1-month exposure. Although the plant height and biomass were significantly inhibited in the contaminated soil, root growth was reduced insignificantly ($p<0.05$). The average degradation rate of TPHs by *Iridaceae* species was up to 19.35-30.79%, improved by 10-60% than that in the corresponding unplanted control. Noticeably, the efficiency of phytoremediation by the two plants for 20,000 mg/kg of PHCs contaminated soil was the lowest compared with that in the unplanted control, indicating that

Iridaceae plants were less suitable for phytoremediation of soils contaminated with the medium concentration of PHCs. Undoubtedly, presence of *I. dichotoma* and *I. lactea* had improved the degradation of PHCs fractions, among which saturated hydrocarbons were more biodegradable than aromatics. The number of hydrocarbon degrading bacteria in the rhizosphere varied greatly in response to the plant species and PHCs concentration. High plant-to-plant variations in the number of PHCs degraders and the microbial community structure and diversity among treatments with different PHCs concentrations were detected. Also, adaptive specialization within the bacterial community related to the PHCs degradation and plant species was observed. In conclusion, phytoremediation by *I. dichotoma* should be limited to soils contaminated with no more than 20,000 mg/kg of PHCs, while *I. lactea* could be effectively applied to phytoremediation of at least 40,000 mg/kg of PHCs contaminated soil.

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Table 1 The biomass of *I. dichotoma* and *I. lactea* under different PHCs treatments

Biomass	*I. dichotoma*			*I. lactea*			
	CK	T1	T2	CK	T1	T2	T3
Shoot weight	15.13± 4.77	9.86± 1.12	3.16± 0.88	3.92±0.71	2.82±0.63	1.41±0.37	2.10±0.38
Shoot height	30.73± 3.32	27.43± 2.51	15.55± 1.06	33.87±1.69	28.43±5.70	21.30±2.66	26.40±1.56
Root weight	9.60± 1.68	8.40± 3.84	6.89± 2.50	0.74±0.22	1.51±0.67	1.26±0.18	1.74±0.08
Root length	10.33± 2.94	10.57± 3.36	9.10± 1.84	11.53±2.37	10.89±1.54	10.00±2.97	10.00±0.99

Table 2 Richness, Shannon index, Simpson index and Evenness of soil bacteria

	I. dichotoma		*I. lactea*			Control		
	T1	T2	T1	T2	T3	T1	T2	T3
Shannon (*H'*)	2.59	2.66	2.51	2.58	2.50	2. 65	2.80	2.72
Evenness (E)	0.95	0.94	0.95	0.94	0.94	0.94	0.94	0.94
Simpon's (D)	0.08	0.07	0.09	0.08	0.09	0.07	0.07	0.07
Richness (R)	14	15	13	14	13	15	17	16

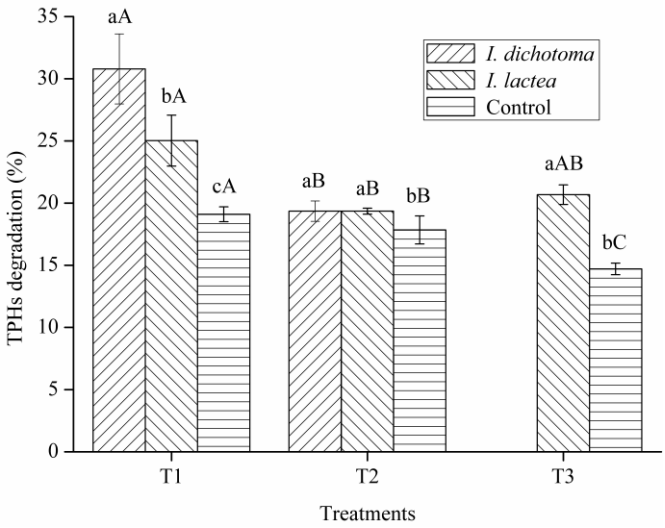

Fig. 1. The degradation rate of TPHs by *I. dichotoma*, *I. lactea* and natural attenuation (The means within same PHCs treatment followed by the different letter (a and b) were significantly different. The means among different PHCs treatments followed by the different letter (A, B and C) were significantly different)

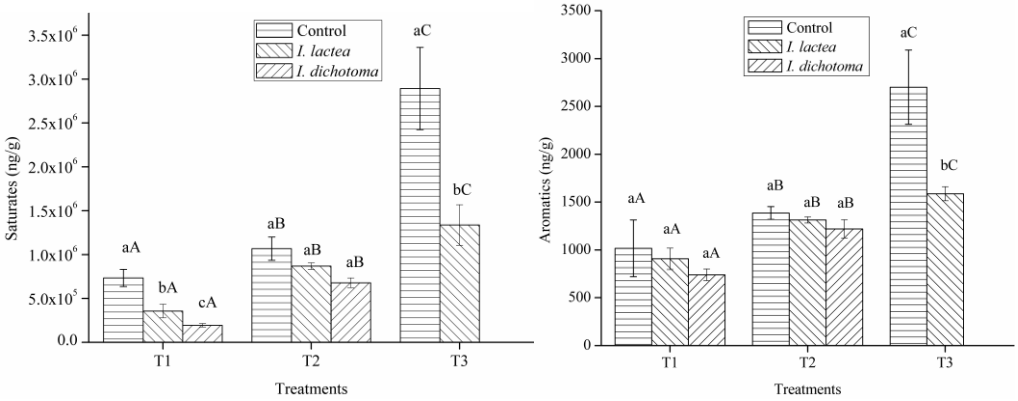

Fig. 2. The concentration of the residual saturates and aromatics (The means within same PHCs treatment followed by the different letter (a and b) were significantly different. The means among different PHCs treatments followed by the different letter (A and B) were significantly different)

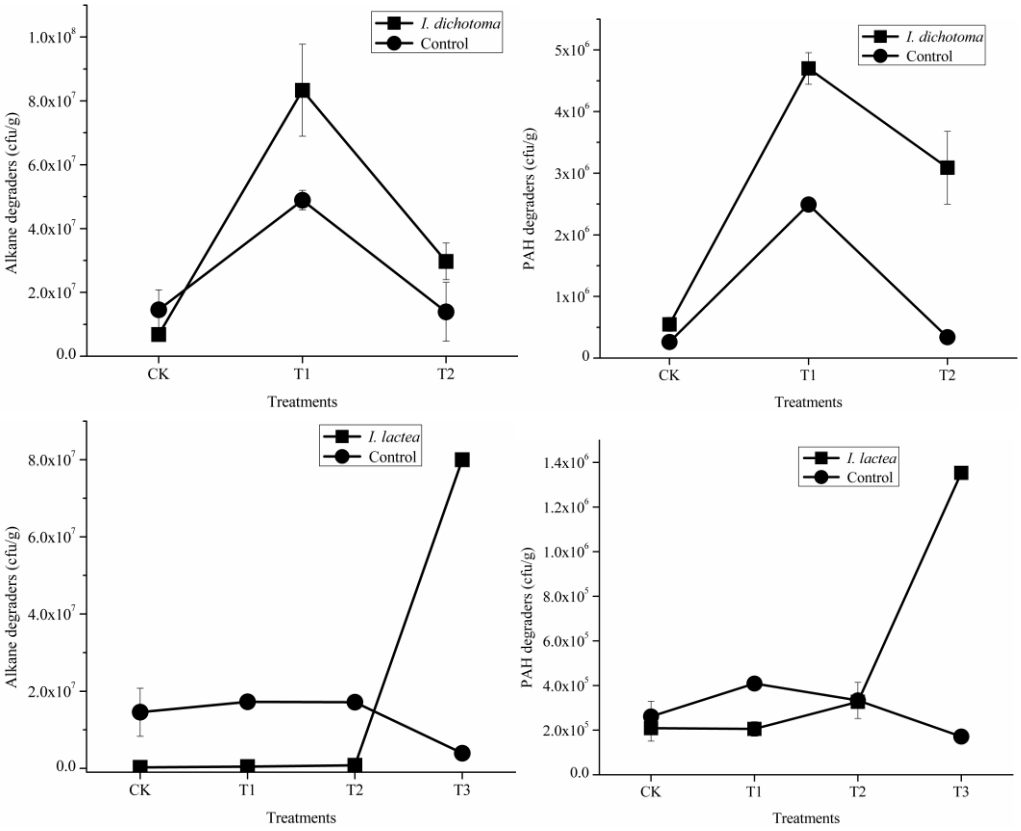

Fig. 3. The number of alkane degraders and PAH degraders in rhizosphere soils

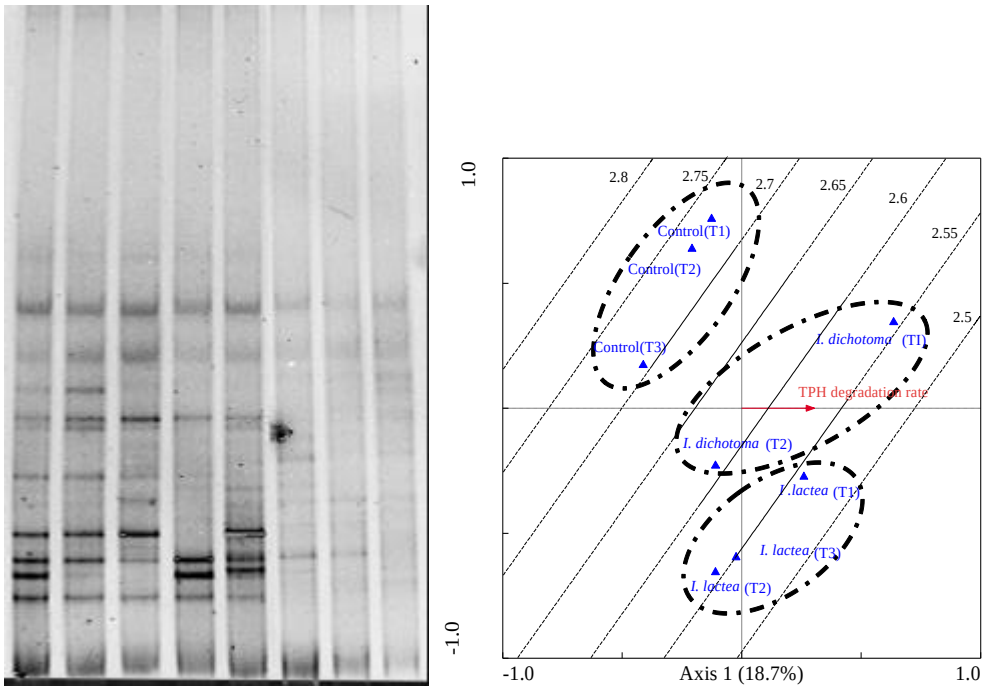

Fig. 4. Image of DGGE bands showing comparison in the soil of unplanted controls and rhizosphere of *I. dichotoma* and *I. latea*. From left to right: *I. lactea* (T1), *I. lactea* (T2), *I. lactea* (T3), *I. dichotoma* (T1), *I. dichotoma* (T2), Control (T1), Control (T2), Control (T3), and CCA biplot analysis of DGGE gel banding patterns belonging to different PHCs treatments