

## REMEDICATION OF PETROLEUM CONTAMINATED SOIL USING LITTER FROM AFFORESTATION PLANT SPECIES IN NORTHERN SHAANXI, CHINA

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**Abstract:** This study was undertaken to assess the feasibility of using plant litter resources for the remediation of petroleum-contaminated soil. Twenty-one types of litter from plants used for afforestation in Northern Shaanxi (a main petroleum producing area in China) were collected and mixed with petroleum-contaminated soil to conduct an incubation experiment over 120 days. The improvements of soil chemical and biological properties were detected and analyzed using integrated principal component analysis. Results indicated that litter from *Platycladus orientalis* and *Trifolium repens* resulted in the best integrated remediation effects on contaminated soil, followed by litter from *Caragana korshinskii*, *Medicago sativa*, *Coronilla varia*, *Artemisia argyi*, and *Populus simonii*. These types of litter could be used in the remediation of contaminated soil. In contrast, *Larix principis-rupprechtii*, *Amorpha fruticosa*, *Pinus tabulaeformis*, *Hippophae rhamnoides*, *Quercus liaotungensis*, *Pyrus betulifolia*, *Zanthoxylum bungeanum* and *Robinia pseudoacacia* litter resulted in serious deterioration effects, followed by *Ulmus pumila*, *Armeniaca sibirica* and *Bothriochloa ischaemum* litter. These types of litter should be avoided in the remediation of contaminated soil.

**Key words:** Litter, petroleum, contaminated soil, remediation, biological and chemical properties

### 1. INTRODUCTION

Petroleum is a complex mixture of a large number of toxic components. Petroleum contamination in soils will alter the soil porosity (Andrade et al., 2004), lead to groundwater pollution by leaching and penetration, and cause secondary pollution through soil and water erosion (Zhu et al., 2012). In addition, petroleum contamination will affect the germination, growth and physiological properties of plants, cause plant death, and result in the deterioration of the ecological environment (Zhu et al., 2012; Shan et al., 2014). In recent decades, along with the wide exploitation of petroleum resources, increasing environmental pollution and

ecological environmental destruction have been observed in Northern Shaanxi, one of the main oil-producing regions in China. Thus, it is important to find suitable methods for remediating petroleum-contaminated soils. Within existing remediation technologies, phytoremediation has become a promising method due to its low cost, large biomass, minimum secondary contamination and excellent ecosystem restoration ability (Merkl et al., 2005). Several types of plants can extract, transfer, and degrade petroleum pollutants independently or together with their rhizosphere microbes (Bento et al., 2012; Bramley-Alves et al., 2014; Liu et al., 2014; Liu et al., 2015; Moubasher et al., 2015; Xiao et al., 2015). However, the application of

phytoremediation is still extremely limited by the toxicity of pollutants to the phytoremediating plants. Considering this limitation, utilizing plant residues for the remediation of petroleum contaminated soils could be a feasible method, as revealed in previous studies that indicated lignin (or aliphatic carbon and aromatic carbons), a component of plant residues, can absorb pollutants (Wang et al., 2007; Zhang et al., 2008; Chefetz & Xing, 2009). Furthermore, residue decomposition can accelerate the increase in the population of soil petroleum-degrading microbes (Zhang et al., 2008).

So far, many types of plant residues have been detected for their possible remediation effects on petroleum polluted soil, such as pea and wheat straw, wheat bran, sugarcane bagasse and plant foliar litter (Barathi & Vasudevan, 2003; Rojas-Avelizapa et al., 2007; Zhang et al., 2008; Hultgren et al., 2010; Adetutu et al., 2012; Shahsavari et al., 2013). However, the results observed were generally unsupported (Shahsavari et al., 2013), even when the residues came from the same plant species. As an example, Hultgren et al., (2010) stated that wheat straw does not present a remarkable effect on degrading petroleum pollutants, while wheat bran addition accelerates petroleum degradation (Barathi & Vasudevan, 2003). Hence, it is important to determine the practical effects of plant residues before their large-scale utilization. In addition, most previous studies have mainly assessed the degrading rates of pollutants and the stimulating effects of plant residues on soil petroleum-degrading microorganisms (Adetutu et al., 2012; Shahsavari et al., 2013). It might be better if the remediation effects on other soil properties were also considered when screening available remediating plant residues because soils with low petroleum concentrations and poor biological and chemical conditions remain adverse to ecological restoration.

Plant litter (foliar litter, dead standings) is an accessible resource around petroleum-contaminated areas in Northern Shaanxi. Our previous investigation indicated that tree and herbage litter additions could significantly recover degenerated soil microbial, enzymatic, and chemical properties in artificial pure forests (Liu et al., 2012; Luc et al., 2013). Consequently, plant litter could be used as a material for petroleum contaminated soil remediation. In this study, 21 types of plant litter from tree/shrub/grass species that are widely distributed around the contaminated area in Northern Shaanxi were mixed with contaminated soils collected from local oil fields. Petroleum degradation rates and the soil biological and chemical properties were detected after 120 days to

assess the integrated remediation effects of these types of litter and to select suitable litter for the remediation of petroleum contaminated soil.

## 2. MATERIALS AND METHODS

### 2.1. Sampling of litter and soil

The petroleum-contaminated soil was sampled from an oil deposit in Yan'an, China, in late autumn, 2013. A petroleum contaminated wasteland was chosen and ten 1 m×1 m quadrats were randomly established within it. Soil from the humus layer (0-10 cm, classified as Cambisols in WRB Soil Taxonomy system) was collected and adequately mixed after getting rid of sundries, such as roots, stones and animal debris. Next, the homogenized soil was passed through sieves ( $\Phi=5$  mm) and kept in disinfected plastic bags at 4°C. Simultaneously, different types of litter from 21 plants commonly used for afforestation were collected (foliar litter of trees and dead standings of herbaceous species), including *Pinus tabulaeformis*, *Platyclusus orientalis*, *Larix principis-rupprechtii*, *Robinia pseudoacacia*, *Populus simonii*, *Quercus liaotungensis*, *Ulmus pumila*, *Armeniaca sibirica*, *Ziziphus montana*, *Pyrus betulifolia*, *Hippophae rhamnoides*, *Caragana korshinskii*, *Amorpha fruticosa*, *Ziziphus jujube*, *Zanthoxylum bungeanum*, *Periploca sepium*, *Artemisia argyi*, *Medicago sativa*, *Coronilla varia*, *Trifolium repens* and *Bothriochloa ischaemum*. The collected litter was carefully selected, and the intact litter was quickly rinsed and oven dried at 65°C before crushing and passing through a sieve ( $\Phi=1$  mm).

### 2.2. Remediation experiments

The prepared contaminated soil was divided into 66 parts with weights of 2.5 kg (dry weight). For each treatment, 50 g of each type of litter was mixed with 2.5 kg of the soil sample. Every treatment had 3 replications, and the soil without litter addition was used as the control (CK). Mixed soil samples were placed into plastic pots, and distilled water was uniformly added into the soil using a sprayer to adjust the moisture content to 50% of the field water holding capacity. A plastic film with 4 vents ( $\Phi=1.5$  mm) was used to cover the pots to prevent extreme evaporation and provide air for microorganisms. All of the watered soil samples were cultivated in the lab at 20-25°C for 120 days. During the cultivation period, pots were weighed weekly and distilled water was added according to the mass losses to maintain a constant moisture content.

### 2.3. Determination of indicators

The following methods were used to determine the soil properties. The soil petroleum content was determined using the organic solvent extraction-gravimetric method (Zhang et al., 2013). The organic matter content was determined using the thermal oxidation method with sulfuric acid and potassium dichromate. Alkaline nitrogen was determined using diffusion method, and available P was determined using NaHCO<sub>3</sub> extraction- phosphormolybdate blue spectrophotometry using a UV-Vis spectrophotometer (UV-2450 Shimadzu Corporation, Kyoto, Japan). The available K content was determined by CH<sub>3</sub>COONH<sub>4</sub> extraction and flame photometry using a flame photometer (BMB Technologies UK LTD.). The available Fe, Mn, Cu, and Zn contents were extracted using DTPA and were measured using an atomic absorption spectrophotometer (Z-2000, HITACHI, Tokyo, Japan) (Bao, 1996). The microbial populations were measured using the dilution plating procedure (beef-protein medium for bacteria, Gao-1<sup>st</sup> medium for actinomycetes and PDA medium for fungi, Nanjing Institute of Soil Science, 1985). The soil urease activity was determined using C<sub>6</sub>H<sub>5</sub>ONa-NaClO colorimetry, sucrase was determined using 3,5-dinitrosalicylic acid colorimetry, catalase was determined using KMnO<sub>4</sub> titrimetry, dehydrogenase was determined using triphenyltetrazolium chloride colorimetry, alkaline phosphatase was determined using disodium phenyl phosphate colorimetry, protease was determined using ninhydrin colorimetry and polyphenol oxidase was determined using pyrogallol colorimetry (Guan, 1986).

### 2.4. Data processing

One-way analysis of variance was used to test the significant differences among treatments with a confidence level of 95% using SPSS 19.0. The increment rates ( $R$ ) for each treatment in comparison with the control test were obtained using the following eq. (1)

$$R=(T-CK)/CK\times 100\% \quad (1)$$

where  $T$  was the indicator value obtained in the soil added plant litter and CK was the value obtained from the control testing. In addition, principal component analysis was used to assess the integrated improvement effects of plant litter on the soil biological and chemical properties.

## 3. RESULTS

### 3.1. Initial nutrient contents of the litter

The 21 types of tested litter demonstrated

remarkable differences in initial nutrient contents (Table 1). The initial N concentrations in the litter ranged from 6.78 to 55.42 g kg<sup>-1</sup>; among them, the litter from *C. varia*, *M. sativa*, *T. repens* and *A. fruticosa* had relative higher N contents and the litter from *Q. liaotungensis* and *P. tabulaeformis* had poor N contents. The initial P content of the litter varied from 1.46 to 5.36 g/kg. The litter from *T. repens*, *C. varia*, *A. argyi* and *M. sativa* showed relative higher P contents, while the P contents in the litter from *C. korshinskii*, *Q. liaotungensis* and *P. tabulaeformis* were low. The initial K contents in the 21 types of litter were between 2.76 and 16.93 g/kg, in which *A. sibirica*, *T. repens* and *A. argyi* were K rich while *Q. liaotungensis* and *P. tabulaeformis* had the lowest K contents.

### 3.2. Soil nutrient contents and organic matter contents after incubation

Litter addition resulted in obvious alterations to soil available nutrients and organic matter contents (Table 2). Specifically, all types of litter, except those from *P. tabulaeformis* and *Q. liaotungensis*, led to significant increases in soil alkaline N. The increments ranged from 14.60% to 294.92%, and the most obvious increment was observed in the soil with *Z. montana* litter. All types of litter, except for the *Q. liaotungensis* litter, resulted in significant increases in the available K content (6.73-188.46% increased), in which *T. repens* presented the best effect. Only the litter from *L. principis-rupprechtii*, *Z. bungeanum*, *M. sativa*, *C. varia*, *T. repens* and *B. ischaemum* noticeably ( $P<0.05$ ) contributed to the increase in the available P content, and the addition of all types of litter, except the *C. korshinskii*, *C. varia* and *T. repens* litter, led to a significant increase in the organic matter content, with increments ranging from 13.78% to 37.22% relative to the CK.

For micro-elements, all types of litter, except for the *T. repens* and *Q. liaotungensis* litter, led to appreciable ( $P<0.05$ ) increases in available Fe content (11.78-16.83% increased), in which the *A. sibirica*, *P. betulifolia* and *P. sepium* litter showed the best effects. Remarkable increases ( $P<0.05$ ) were observed in all types of litter added to the soil, while the best effect was observed in the *C. korshinskii* litter treatment, with an increase of 406.64%. The *P. tabulaeformis*, *Q. liaotungensis*, *U. pumila*, *A. sibirica*, *C. korshinskii*, *A. argyi*, *M. sativa*, *C. varia* and *T. repens* litter (especially *A. argyi* litter) caused significant increases in the available Cu content, with the highest increment reaching 45.51%.

Table 1. Initial nutrient contents of the litter

Species	N content g/kg	P content g/kg	K content g/kg
<i>P. tabulaeformis</i>	6.78±1.84p	1.46±0.13 k	1.52±0.08n
<i>P. orientalis</i>	14.69±0.46n	2.34±0.02ij	6.57±0.13kl
<i>L. principis-rupprechtii</i>	19.81±0.16k	3.65±0.10c	6.14±0.14l
<i>R. pseudoacacia</i>	22.43±0.53j	2.22±0.04j	4.24±0.03m
<i>P. simonii</i>	18.75±0.19kl	2.65±0.07ghi	14.58±0.51g
<i>Q. liaotungensis</i>	9.99±0.08o	1.68±0.17k	1.30±0.02n
<i>U. pumila</i>	26.09±0.39i	2.92±0.04fgh	7.82±0.18j
<i>A. sibirica</i>	17.38±0.35lm	3.26±0.05ef	28.28±0.04a
<i>Z. montana</i>	37.23±0.93ef	3.49±0.21cd	16.10±0.05f
<i>P. betulifolia</i>	32.78±1.46g	3.05±0.19ef	18.65±0.50cd
<i>H. rhamnoides</i>	33.00±0.43g	2.53±0.06ij	5.91±0.03l
<i>C. korshinskii</i>	35.36±0.69f	2.19±0.05j	6.94±0.21k
<i>A. fruticosa</i>	42.28±0.57d	3.44±0.10cde	9.46±0.05i
<i>Z. jujuba</i>	28.49±0.17h	3.03±0.09fg	15.22±0.19g
<i>Z. bungeanum</i>	20.11±0.43k	2.58±0.13hij	12.45±0.00 h
<i>P. sepium</i>	22.69±0.24j	2.39±0.32ij	15.17±0.14g
<i>A. argyi</i>	38.04±0.81e	4.40±0.12b	19.35±0.05c
<i>M. sativa</i>	52.81±0.27b	4.35±0.24b	17.98±0.21d
<i>C. varia</i>	55.42±0.63a	4.54±0.01b	17.21±0.51e
<i>T. repens</i>	44.49±0.27c	5.36±0.12a	22.29±0.65 b
<i>B. ischaemum</i>	15.69±0.48mn	3.18±0.03def	12.65±0.07h

Note: Data in the same column with different letters has significant differences,  $P < 0.05$ .

In contrast, *L. principis-rupprechtii*, *R. pseudoacacia*, *P. simonii*, *H. rhamnoides*, *Z. jujuba*, *P. sepium* and *B. ischaemum* litter caused significant decreases in the available Cu contents. All litter additions (except for *T. repens*) led to significant increases in available Mn contents, and the best effect was observed when *P. simonii* litter was added to the soil with an increment of 116.63%.

### 3.3. Soil total petroleum hydrocarbon (TPHs) contents after incubation

After incubation, remarkable degradation of TPHs was observed in all treatments with litter addition (Table 2). In soil without litter addition, the TPH content was 13.69 g·kg<sup>-1</sup> after the remediation experiment and ranged from 5.02 to 11.21 g·kg<sup>-1</sup> when litter was added to the soil samples. The results indicated that the *Q. liaotungensis*, *A. sibirica*, *Z. montana*, *H. rhamnoides*, *C. korshinskii*, *Z. jujube*, *A. argyi*, *M. sativa*, *C. varia*, *T. repens* and *B. ischaemum* litter were the best for degrading TPHs. The TPH degradation rates of the soil samples containing these types of litter (relative to the content in control testing) were greater than 50%. Among these types of litter, the addition of *A. argyi* litter resulted in the highest TPHs degradation rate of 63.33%.

### 3.4. Soil microorganism populations after incubation

The addition of *P. orientalis*, *P. simonii*, *C. korshinskii*, *P. sepium*, *M. sativa* and *T. repens* litter significantly increased the bacterial population by 122.91-688.06%, with the *P. orientalis* litter resulting in the most obvious effect (Table 3). In contrast, the *P. tabulaeformis*, *L. principis-rupprechtii*, *R. pseudoacacia*, *Q. liaotungensis*, *U. pumila*, *A. sibirica*, *Z. montana*, *P. betulifolia*, *H. rhamnoides*, *Z. jujuba*, *A. argyi* and *B. ischaemum* litter resulted in significant decreases in the soil bacterial population. All types of litter resulted in greater fungi populations, including the *P. orientalis*, *P. simonii*, *Z. montana*, *C. korshinskii* and *A. fruticosa* litter, which significantly increased the fungi population by 1567.08%-23792.94% ( $P < 0.05$ ). The *P. orientalis*, *L. principis-rupprechtii*, *R. pseudoacacia*, *P. simonii*, *Q. liaotungensis*, *C. korshinskii*, *A. fruticosa*, *Z. bungeanum*, *P. sepium*, *M. sativa*, *C. varia*, *T. repens* and *B. ischaemum* litter showed significant promoting effects for actinomycetes reproduction, increasing the actinomycetes population by 90.48%-1244.54%. Generally, the *P. orientalis*, *P. simonii*, *C. korshinskii* and *P. sepium* litter noticeably increased the soil microbial populations and positively affected the soil microbial properties.

Table 2. Chemical properties in plant litter treated petroleum-contaminated soil

Species	Contents of organic matters and nutrients								Content of total petroleum hydrocarbon g kg <sup>-1</sup>
	Alkaline N mg kg <sup>-1</sup>	Available P mg kg <sup>-1</sup>	Available K mg kg <sup>-1</sup>	Organic matters g kg <sup>-1</sup>	Available Cu mg kg <sup>-1</sup>	Available Zn mg kg <sup>-1</sup>	Available Fe mg kg <sup>-1</sup>	Available Mn mg kg <sup>-1</sup>	
<i>P. tabulaeformis</i>	70.99±0.06	31.02±0.53	555.00±5.00*	44.35±0.5*	0.63±0.01*	13.61±0.12*	5.82±0.05*	0.88±0.03*	7.81±0.35*
<i>P. orientalis</i>	72.33±0.96*	30.53±1.64	620.00±0.00*	39.19±1.55*	0.59±0.01	17.59±0.11*	8.36±0.05*	0.93±0.05*	7.18±0.28*
<i>L. principisrupprechtii</i>	106.81±1.47*	60.23±14.03*	645.00±5.00*	39.74±0.58*	0.49±0.00*	12.21±0.04*	6.76±0.04*	0.93±0.01*	9.66±1.32*
<i>R. pseudoacacia</i>	99.52±0.56*	41.84±1.12	595.00±5.00*	41.20±0.33*	0.48±0.00*	15.11±0.23*	7.13±0.07*	0.89±0.01*	11.21±0.49*
<i>P. simonii</i>	73.03±0.88*	37.39±2.77	775.00±5.00*	39.72±0.90*	0.55±0.00*	15.59±0.10*	5.86±0.17*	1.50±0.02*	6.51±0.37*
<i>Q. liaotungensis</i>	67.61±2.69	47.64±4.43	530.00±10.00	44.47±1.46*	0.70±0.01*	23.13±0.21*	3.96±0.03	0.65±0.00	5.91±0.41*
<i>U. pumila</i>	170.04±0.56*	33.46±1.37	655.00±5.00*	40.13±1.01*	0.67±0.02*	18.14±0.32*	5.83±0.08*	0.92±0.02*	7.73±0.29*
<i>A. sibirica</i>	106.23±3.54*	38.52±0.67	990.00±0.00*	41.06±2.07*	0.68±0.00*	12.11±0.18*	11.27±0.07*	0.85±0.00*	6.39±0.03*
<i>Z. montana</i>	249.26±1.38*	46.63±3.54	795.00±5.00*	37.22±4.38*	0.62±0.01	7.89±0.07*	5.52±0.08*	1.03±0.00*	6.61±0.25*
<i>P. betulifolia</i>	127.93±1.37*	39.45±4.59	735.00±5.00*	44.10±0.88*	0.63±0.00*	9.46±0.07*	9.47±0.04*	0.88±0.01*	8.63±0.39*
<i>H. rhamnoides</i>	153.88±7.43*	36.66±4.42	610.00±0.00*	41.98±3.15*	0.49±0.01*	22.72±0.06*	7.46±0.06*	0.86±0.03*	6.84±0.20*
<i>C. korshinskii</i>	93.80±0.46*	43.85±3.01	790.00±0.00*	36.70±0.52	0.81±0.01*	23.39±0.11*	4.66±0.04*	0.76±0.01*	5.82±0.40*
<i>A. fruticosa</i>	127.63±0.92*	35.54±0.83	685.00±5.00*	44.21±2.23*	0.58±0.01	13.08±0.17*	6.10±0.08*	0.98±0.00*	8.54±0.74*
<i>Z. jujuba</i>	181.53±0.48*	39.29±2.65	720.00±0.00*	38.61±0.84*	0.51±0.00*	14.62±0.21*	6.67±0.02*	0.85±0.00*	6.63±0.85*
<i>Z. bungeanum</i>	97.18±3.97*	50.21±2.78*	705.00±5.00*	42.81±0.73*	0.60±0.00	10.28±0.16*	4.69±0.03*	1.19±0.01*	6.87±0.11*
<i>P. sepium</i>	98.29±1.47*	43.48±0.96	775.00±5.00*	34.82±0.37	0.50±0.01*	13.07±0.25*	8.90±0.03*	0.78±0.02*	9.98±0.62*
<i>A. argyi</i>	162.23±1.94*	45.61±3.21	890.00±0.00*	40.57±1.09*	0.87±0.01*	16.90±0.19*	5.36±0.05*	0.71±0.01	5.02±0.02*
<i>M. sativa</i>	170.86±6.51*	52.00±7.38*	1110.00±30.00*	38.98±1.21*	0.83±0.01*	12.10±0.13*	4.91±0.05*	0.68±0.02	5.41±0.23*
<i>C. varia</i>	205.74±3.71*	59.21±10.37*	1200.00±20.00*	36.39±1.97	0.79±0.01*	19.15±0.29*	4.39±0.05*	0.72±0.01	6.22±0.16*
<i>T. repens</i>	202.83±4.31*	62.90±10.99*	1500.00±0.00*	31.11±0.66	0.65±0.00*	17.86±0.10*	3.62±0.03*	0.61±0.01*	5.26±0.08*
<i>B. ischaemum</i>	125.53±0.25*	57.95±10.47*	700.00±0.00*	33.53±0.25	0.37±0.01*	10.30±0.09*	5.22±0.07*	0.75±0.01*	6.13±1.05*
Control	63.12±1.99	33.26±0.77	520.00±0.00	32.41±0.14	0.60±0.00	4.62±0.03	3.93±0.00	0.69±0.03	13.69±0.09

Table 3. Microorganism populations in plant litter treated petroleum-contaminated soil

Species	Bacteria $\times 10^8$ CFU g <sup>-1</sup>	Actinomycetes $\times 10^5$ CFU g <sup>-1</sup>	Fungi $\times 10^2$ CFU g <sup>-1</sup>
<i>P. tabulaeformis</i>	2.80 $\pm$ 0.80*	10.40 $\pm$ 1.44	0.80 $\pm$ 0.80
<i>P. orientalis</i>	593.93 $\pm$ 14.18*	17.7 $\pm$ 2.73*	6.69 $\pm$ 0.39*
<i>L. principis-rupprechtii</i>	26.25 $\pm$ 0.72*	41.67 $\pm$ 1.50*	4.17 $\pm$ 1.50
<i>R. pseudoacacia</i>	15.86 $\pm$ 1.41*	17.08 $\pm$ 2.11*	0.81 $\pm$ 0.81
<i>P. simonii</i>	172.83 $\pm$ 7.33*	65.47 $\pm$ 1.77*	17.08 $\pm$ 1.41*
<i>Q. liaotungensis</i>	36.80 $\pm$ 0.80*	42.80 $\pm$ 1.44*	0.80 $\pm$ 0.40
<i>U. pumila</i>	14.23 $\pm$ 1.47*	11.79 $\pm$ 1.47	0.81 $\pm$ 0.47
<i>A. sibirica</i>	5.79 $\pm$ 1.09*	9.92 $\pm$ 1.24	0.00 $\pm$ 0.00
<i>Z. montana</i>	36.79 $\pm$ 1.09*	4.55 $\pm$ 0.83	6.61 $\pm$ 1.49*
<i>P. betulifolia</i>	21.42 $\pm$ 6.34*	1.26 $\pm$ 0.73	3.36 $\pm$ 2.22
<i>H. rhamnoides</i>	30.40 $\pm$ 5.77*	1.60 $\pm$ 0.40	1.60 $\pm$ 0.40
<i>C. korshinskii</i>	197.20 $\pm$ 37.13*	13.60 $\pm$ 1.06*	18.80 $\pm$ 3.27*
<i>A. fruticosa</i>	95.59 $\pm$ 6.66	14.12 $\pm$ 2.25*	94.78 $\pm$ 7.85*
<i>Z. jujuba</i>	23.75 $\pm$ 1.25*	0.42 $\pm$ 0.42	0.83 $\pm$ 0.42
<i>Z. bungeanum</i>	74.83 $\pm$ 2.93	13.42 $\pm$ 1.86*	4.88 $\pm$ 1.41
<i>P. sepium</i>	204.96 $\pm$ 9.76*	16.27 $\pm$ 2.03*	1.22 $\pm$ 1.22
<i>A. argyi</i>	38.00 $\pm$ 7.21*	2.80 $\pm$ 0.80	5.60 $\pm$ 0.40
<i>M. sativa</i>	130.61 $\pm$ 7.21*	74.12 $\pm$ 4.20*	1.98 $\pm$ 0.14
<i>C. varia</i>	60.80 $\pm$ 12.80	18.67 $\pm$ 0.35*	1.60 $\pm$ 1.06
<i>T. repens</i>	168.00 $\pm$ 14.66*	96.00 $\pm$ 2.50*	1.60 $\pm$ 0.40
<i>B. ischaemum</i>	9.81 $\pm$ 2.99*	34.56 $\pm$ 8.71*	0.85 $\pm$ 0.43
Control	75.37 $\pm$ 10.49	7.14 $\pm$ 1.37	0.40 $\pm$ 0.40

Note: \* indicated significant difference between treatment and control values at 0.05 level.

Table 4. Enzyme activities in plant litter treated petroleum-contaminated soil

Species	Catalase mL g <sup>-1</sup>	Polyphenol oxidase mg	Sucrase mg g <sup>-1</sup>	Alkaline phosphatase mg 100g <sup>-1</sup>	Urease mg g <sup>-1</sup>	Dehydrogenase mg g <sup>-1</sup>	Protease mg g <sup>-1</sup>
<i>P. tabulaeformis</i>	8.32 $\pm$ 0.87*	0.0154 $\pm$ 0.00*	39.95 $\pm$ 0.70*	0.41 $\pm$ 0.01*	0.12 $\pm$ 0.01*	0.07 $\pm$ 0.00*	4.52 $\pm$ 0.40*
<i>P. orientalis</i>	13.11 $\pm$ 0.19*	0.0116 $\pm$ 0.00*	68.35 $\pm$ 3.09*	0.26 $\pm$ 0.00*	0.09 $\pm$ 0.00*	0.14 $\pm$ 0.00*	3.24 $\pm$ 0.28*
<i>L. principis-rupprechtii</i>	6.85 $\pm$ 0.13	0.0269 $\pm$ 0.00	28.27 $\pm$ 0.09	0.20 $\pm$ 0.02	0.10 $\pm$ 0.00*	0.19 $\pm$ 0.02*	3.94 $\pm$ 0.05*
<i>R. pseudoacacia</i>	6.77 $\pm$ 0.54	0.0192 $\pm$ 0.00	49.87 $\pm$ 0.56*	0.34 $\pm$ 0.00*	0.13 $\pm$ 0.01*	0.12 $\pm$ 0.01*	5.17 $\pm$ 0.12*
<i>P. simonii</i>	9.60 $\pm$ 0.32*	0.0125 $\pm$ 0.00*	73.90 $\pm$ 3.97*	0.24 $\pm$ 0.01*	0.13 $\pm$ 0.00*	0.08 $\pm$ 0.00*	4.10 $\pm$ 0.14*
<i>Q. liaotungensis</i>	9.90 $\pm$ 0.13*	0.0147 $\pm$ 0.00*	28.60 $\pm$ 0.97	0.35 $\pm$ 0.01*	0.09 $\pm$ 0.00*	0.06 $\pm$ 0.00*	4.08 $\pm$ 0.09*
<i>U. pumila</i>	10.51 $\pm$ 0.63*	0.0154 $\pm$ 0.00*	73.92 $\pm$ 2.39*	0.36 $\pm$ 0.01*	0.12 $\pm$ 0.00*	0.08 $\pm$ 0.00*	5.51 $\pm$ 0.19*
<i>A. sibirica</i>	7.24 $\pm$ 0.22	0.0226 $\pm$ 0.00	70.43 $\pm$ 0.24*	0.34 $\pm$ 0.02*	0.10 $\pm$ 0.00*	0.07 $\pm$ 0.01*	4.79 $\pm$ 0.00*
<i>Z. montana</i>	8.09 $\pm$ 0.23*	0.0197 $\pm$ 0.00	61.93 $\pm$ 1.37*	0.29 $\pm$ 0.00*	0.10 $\pm$ 0.01*	0.05 $\pm$ 0.00	4.2 $\pm$ 0.06*
<i>P. betulifolia</i>	6.36 $\pm$ 0.15	0.0232 $\pm$ 0.00	60.04 $\pm$ 2.34*	0.33 $\pm$ 0.01*	0.12 $\pm$ 0.01*	0.10 $\pm$ 0.00*	4.02 $\pm$ 0.19*
<i>H. rhamnoides</i>	7.95 $\pm$ 0.66*	0.0239 $\pm$ 0.00	37.67 $\pm$ 2.03*	0.23 $\pm$ 0.01	0.08 $\pm$ 0.01*	0.08 $\pm$ 0.00*	4.38 $\pm$ 0.08*
<i>C. korshinskii</i>	12.07 $\pm$ 0.14*	0.0141 $\pm$ 0.00*	71.18 $\pm$ 0.98*	0.58 $\pm$ 0.01*	0.11 $\pm$ 0.00*	0.08 $\pm$ 0.00*	3.57 $\pm$ 0.05*
<i>A. fruticosa</i>	7.30 $\pm$ 0.12	0.0306 $\pm$ 0.00*	41.91 $\pm$ 2.49*	0.22 $\pm$ 0.00	0.08 $\pm$ 0.00*	0.08 $\pm$ 0.00*	5.15 $\pm$ 0.48*
<i>Z. jujuba</i>	13.27 $\pm$ 0.28*	0.0277 $\pm$ 0.00	67.51 $\pm$ 0.30*	0.47 $\pm$ 0.03*	0.12 $\pm$ 0.01*	0.08 $\pm$ 0.00*	2.85 $\pm$ 0.26*
<i>Z. bungeanum</i>	6.38 $\pm$ 0.14	0.0273 $\pm$ 0.00	53.69 $\pm$ 0.91*	0.24 $\pm$ 0.01*	0.11 $\pm$ 0.00*	0.06 $\pm$ 0.00*	3.01 $\pm$ 0.20*
<i>P. sepium</i>	6.76 $\pm$ 0.40	0.0142 $\pm$ 0.00*	68.41 $\pm$ 4.34*	0.29 $\pm$ 0.01*	0.15 $\pm$ 0.00*	0.14 $\pm$ 0.01*	4.11 $\pm$ 0.53*
<i>A. argyi</i>	8.57 $\pm$ 0.39*	0.0207 $\pm$ 0.00	75.97 $\pm$ 0.66*	0.34 $\pm$ 0.02*	0.09 $\pm$ 0.00*	0.17 $\pm$ 0.01*	2.46 $\pm$ 0.19*
<i>M. sativa</i>	9.12 $\pm$ 0.39*	0.0211 $\pm$ 0.00	77.30 $\pm$ 1.36*	0.35 $\pm$ 0.00*	0.09 $\pm$ 0.00*	0.09 $\pm$ 0.00*	3.29 $\pm$ 0.39*
<i>C. varia</i>	8.71 $\pm$ 0.12*	0.0197 $\pm$ 0.00	75.73 $\pm$ 1.05*	0.42 $\pm$ 0.03*	0.10 $\pm$ 0.01*	0.11 $\pm$ 0.01*	4.31 $\pm$ 0.33*
<i>T. repens</i>	7.51 $\pm$ 0.53	0.0174 $\pm$ 0.00*	56.06 $\pm$ 1.71*	0.28 $\pm$ 0.00*	0.10 $\pm$ 0.00*	0.06 $\pm$ 0.01	4.05 $\pm$ 0.19*
<i>B. ischaemum</i>	11.66 $\pm$ 0.09*	0.0250 $\pm$ 0.00	52.64 $\pm$ 2.24*	0.41 $\pm$ 0.01*	0.11 $\pm$ 0.00*	0.18 $\pm$ 0.01*	5.98 $\pm$ 0.03*
Control	6.66 $\pm$ 0.11	0.0235 $\pm$ 0.00	30.11 $\pm$ 0.72	0.20 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.00	1.09 $\pm$ 0.31

### 3.5 Soil enzyme activities after incubation

The addition of litter significantly affected the soil enzyme activities after incubation (Table 4). All of the types of litter significantly increased the urease activity by 111.53-305.90%, including the *P. sepium*

litter, which had the most obvious accelerating effect. All of the types of litter significantly increased the protease activity by 31.57-142.68%, while the *B. ischaemum* litter resulted in the most obvious acceleration.

Table 5. Comprehensive principal component values ( $F$ ) of the remediation effects of litter on the biological and chemical properties of petroleum contaminated soil

Species	$F$ Value	Species	$F$ Value	Species	$F$ Value	Species	$F$ Value
<i>P. tabulaeformis</i>	-0.7715	<i>U. pumila</i>	-0.0174	<i>A. fruticosa</i>	-1.1805	<i>C. varia</i>	0.7631
<i>P. orientalis</i>	1.2247	<i>A. sibirica</i>	-0.1536	<i>Z. jujuba</i>	0.2039	<i>T. repens</i>	1.2245
<i>L. principis-rupprechtii</i>	-1.0479	<i>Z. montana</i>	0.0212	<i>Z. bungeanum</i>	-0.4821	<i>B. ischaemum</i>	-0.2859
<i>R. pseudoacacia</i>	-0.9096	<i>P. betulifolia</i>	-0.6359	<i>P. sepium</i>	0.1419		
<i>P. simonii</i>	0.5322	<i>H. rhamnoides</i>	-0.7965	<i>A. argyi</i>	0.6449		
<i>Q. liaotungensis</i>	-0.4461	<i>C. korshinskii</i>	0.9970	<i>M. sativa</i>	0.9736		

For dehydrogenase, the litter from all species, except *Z. montana* and *T. repens*, showed significant promotional effects. However, the increments of dehydrogenase activity in the soils treated with *L. principis-rupprechtii* and *A. argyi* litter were 327.75% and 376.50%, respectively. All of the types of litter, except for the *L. principis-rupprechtii* and *Q. liaotungensis* litter, significantly increased the soil sucrase activity, among which the litter from *M. sativa* resulted in the best accelerating effect, with an increment of 156.71% relative to CK. The litter from *P. tabulaeformis*, *C. korshinskii*, *Z. jujube*, etc. (18 species) significantly increased the alkaline phosphatase activity by 20.03-191.55%, while the *L. principis-rupprechtii*, *H. rhamnoides* and *A. fruticosa* litter did not present remarkable effects. For polyphenol oxidase, only the *A. fruticosa* litter significantly increased its activity by 30.21%, while the *P. tabulaeformis*, *P. orientalis*, *P. simonii*, *Q. liaotungensis*, *U. pumila*, *C. korshinskii*, *P. sepium* and *T. repens* litter significantly decreased the polyphenol oxidase activity by 25.96-50.64%. The *P. tabulaeformis*, *P. orientalis*, *P. simonii*, *Q. liaotungensis*, *U. pumila*, *Z. montana*, *H. rhamnoides*, *C. korshinskii*, *Z. jujuba*, *A. argyi*, *M. sativa*, *C. varia* and *B. ischaemum* litter remarkably increased the soil catalase activity by 24.81-99.15%, while the other types of litter did not show any noticeable influences.

### 3.6. Integrated effects of different types of plant litter on petroleum contaminated soil

To comprehensively analyze the remediation effects of different types of plant litter on soil properties, the increments of bio-chemical properties and the degradation rate of TPHs in soils amended with litter (relative to the measured values of the CK) were submitted to SPSS 19.0. Comprehensive principal component analysis was completed, and the calculated comprehensive principal component values ( $F$ , eq. (2), Table 5) were used as an indicator for the remediation effects of the different types of litter.  $F$  values greater than one indicated remediation effects,  $F$  values smaller than one indicated deterioration

effects, and the absolute values of  $F$  indicated the degree of impacts.

$$F=0.298 F_1+0.181 F_2+0.130 F_3+0.115 F_4+0.110 F_5+0.087 F_6+0.079 F_7 \quad (2)$$

where  $F_1 \sim F_7$  are the principal components extracted by the SPSS 19.0 software and the coefficients are the ratios of each corresponding eigenvalue of  $F_i$  to the sum of all eigenvalues.

The results revealed that the *P. orientalis* and *T. repens* litter showed the best remediation effects on petroleum contaminated soil, followed by the *C. korshinskii*, *M. sativa*, *C. varia*, and *A. argyi* litter. The remediation effects of the *P. simonii*, *Z. jujuba*, *P. sepium* and *Z. montana* litter were negligible. The *U. pumila*, *A. sibirica* and *B. ischaemum* litter presented slight deterioration effects, and the *P. tabulaeformis*, *H. rhamnoides*, *Q. liaotungensis*, *P. betulifolia*, *Z. bungeanum* and *R. pseudoacacia* litter resulted in considerable deterioration effects. The *L. principis-rupprechtii* and *A. fruticosa* litter strongly aggravated the circumstances.

## 4. DISCUSSION

The results indicated that most of the litter significantly increased the organic matter (OM), available N and K and micro-element contents in the soil, which were correlated with the findings of Luc et al. (2013), because litter can release nutrients during the decomposition process and these nutrients are supplied to the soil. In addition, the allelochemicals released from litter could increase the availability of soil nutrients by influencing the enzymes and microbes associated with nutrient cycling/release, such as urease, protease, azotobacter (free-living nitrogen fixing bacteria), ammonifiers, and phosphate solubilizing bacteria (Niu et al., 2007; Liang et al., 2008; Wang et al., 2015). However, the data presented in table 2 indicated that the addition of litter to soil rarely increased the soil P content and that *P. tabulaeformis* and *Q. liaotungensis* litter did not increase the available soil N and K contents. Noticeably, the types of litter that did not significantly increase the soil available N/P/K contents always

showed significant lower initial contents of the corresponding nutrients (Tables 1 and 2). For instance, the *P. tabulaeformis* and *Q. liaotungensis* litter had the lowest initial N and K contents and the *Q. liaotungensis* litter had the lowest initial P content. The types of litter with low initial nutrient contents could not release a large number of nutrients. On the other hand, because microorganisms need a sufficient nutrient supply (especially N and P) when they decompose/degrade litter or petroleum pollutants (Liu et al., 2007; Berg & McClaugherty, 2014), relatively low nutrient contents will result in the direct utilization (that is, immobilization) of the nutrients released from the litter by microorganisms (Schimel & Bennett, 2004; Schimel & Hattenschwiler, 2007). Consequently, the soil nutrient availability will decrease. Similarly, the degradation of pollutants would cause this process.

For microelements, the addition of litter could increase their contents by releasing available microelements and increase their availability by changing the soil pH. Our results indicated that the addition of litter would significantly reduce the soil pH (unpublished data), which was supported by a previous investigation (Dell'Anno et al., 2009). This decrease in pH could decrease the adsorption and immobilization of microelements in the soil. However, for available Cu and Mn, some types of litter reduced their contents after incubation, which could result from the adsorption of humified litter to these elements and lead to a decrease in their availability. Previous studies demonstrated that the initial quality of litter (humus sources in this study) will influence the properties of humus (Zhang et al., 2014), which would cause differences among the final influences of different types of litter on the available soil microelement contents.

Generally, the addition of litter accelerated the reproduction of actinomycetes and fungi, and some types of litter resulted in significant accelerating effects. However, the influences of litter type on soil bacteria populations were inconclusive and similar to previous findings (Shahsavari et al., 2013). Studies have indicated that petroleum pollutants would remarkably decrease available soil N and P contents (Margesin et al., 2007; Zhang et al., 2013); thus, the sufficient nutrients supplied by different types of litter could significantly accelerate microbial growth. Furthermore, the addition of litter significantly decreased the petroleum content in the soil (Table 2). Consequently, the toxicity of pollutants to microbes decreased and resulted in the recovery or proliferation of microbes. However, several types of litter significantly decreased the bacterial population. The allelochemicals released from decomposed litter

could explain this phenomenon (Wang et al., 2015).

Overall, the results demonstrated that most types of litter significantly increased the enzymes activities in contaminated soil (except for polyphenol oxidase), especially for dehydrogenase, N or P cycling related enzymes and sucrase activities. These results also indicated that the addition of litter significantly increased the ability of N/P cycling and organic matter decomposition in the soil system. One possible reason for these improvements could be that the litter accelerated microbial growth and activity. Second, the release of nutrients from the litter increased the enzymatic activity because there were significant positive correlations among the enzyme activities and nutrient contents in the soil, including the hydrolytic enzyme activities (sucrase, phosphatase and urease) and N content (Tu et al., 2012). Third, the addition of litter provided fresh substrates that could be easily decomposed. Finally, the allelochemicals released from the litter could increase the enzyme activities (Wang et al., 2015). However, some types of litter significantly decreased the polyphenol oxidase activity. This enzyme mainly catalyzes the decomposition of recalcitrant materials in litter and petroleum pollutants (Wang et al., 2009; Berg & McClaugherty, 2014); thus, at the end of incubation, the activity could decrease with a lack of specific decomposition substrates.

Significant increases in petroleum degradation rates were observed in all types of litter-added soil samples. Because microorganisms need suitable C/N/P ratios for degrading hydrocarbons (Ritter & Scarborough, 1995), litter could accelerate this process by providing N and P. In addition, the products released from different types of litter (such as polysaccharides) can stimulate the activities of TPHs-degrading microbes (Zhang et al., 2008). Furthermore, litter could provide co-metabolic substrates for TPHs (Liu et al., 2010) and lead to a higher TPHs degradation rate. However, our results revealed that the degradation rates of TPHs were variable in different treatments. Because the different types of litter showed differences in their nutrient supply abilities and had different effects on the three groups of microbes, they showed different TPHs degrading abilities. For example, Sathishkumar et al., (2008) stated that bacteria is more capable of degrading petroleum pollutants than actinomycetes and fungi, while fungi participates more in the degradation of aromatic hydrocarbons (Franco et al., 2004). In addition, the variable influences on soil biological and chemical properties also resulted in more complex results of the final petroleum degradation rates. Noticeably, our results demonstrated that a single assessment of the

petroleum degradation abilities of different types of litter is not comprehensive because some types of litter with high petroleum degradation ability did not show a favorable intergraded remediation effect (Tables 2 and 5). Thus, it could be better to consider the remediation effects of on the overall properties of the litter to identify suitable plants for the phytoremediation of petroleum contaminated soil.

## 5. CONCLUSIONS

(1) Most of the different types of plant litter significantly increased the alkaline N, available K, Fe, and Mn and organic matter contents in the petroleum contaminated soil. All of the types of litter significantly increased the available Zn contents. Only the *L. principis-rupprechtii*, *Z. bungeanum*, *M. sativa*, *C. varia*, *T. repens* and *B. ischaemum* litter significantly increased the available P contents, and only the *P. tabulaeformis*, *Q. liaotungensi*, *U. pumila*, *A. sibirica*, *C. korshinskii*, *A. argyi*, *M. sativa*, *C. varia* and *T. repens* litter significantly increased the available Cu contents in the contaminated soil.

(2) Generally, the litter treatments accelerated the reproduction of actinomycetes and fungi, and some types of litter showed significant accelerating effects. However, most of the types of plant litter significantly inhibited the reproduction of bacteria. Only the *P. orientalis*, *P. simonii* and *C. korshinskii* litter significantly promoted the proliferation of all three types of microorganisms in the contaminated soil.

(3) Almost all types of litter significantly increased the catalase, sucrase, alkaline phosphatase and dehydrogenase activities in the petroleum contaminated soil. All of the types of litter significantly increased the urease and protease activities, and only the *A. fruticosa* litter increased the polyphenol oxidase activity.

(4) All of the types of litter significantly promoted the degradation of petroleum contaminants, and the *A. argyi* litter resulted in the highest TPHs degradation rate of 63.33%.

(5) According to the integrated analyses of the remediation effects of litter on soil biological and chemical properties and petroleum degradation, the *P. orientalis* and *T. repens* litter showed the best remediation effects in the contaminated soil, followed by the *C. korshinskii*, *M. sativa*, *C. varia*, *A. argyi*, and *P. simonii* litter. These types of litter could be used in the remediation of contaminated soil. In contrast, the *L. principis-rupprechtii*, *A. fruticosa*, *P. tabulaeformis*, *H. rhamnoides*, *Q. liaotungensis*, *P. betulifolia*, *Z. bungeanum* and *R. pseudoacacia* litter showed serious deterioration effects, followed by the *U. pumila*, *A. sibirica* and *B. ischaemum* litter. These

types of litter should be avoided in the remediation of contaminated soil.

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Received at: 20. 05. 2015

Revised at: 19. 11. 2015

Accepted for publication at: 25. 11. 2015

Published online at: 04. 12. 2015