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CONTENTS

Characterization of contamination, source and degradation of petroleum between upland and paddy fields based on geochemical characteristics and phospholipid fatty acids

Juan Zhang[1], Renqing Wang[1,2,3], Xiaoming Du[4], Fasheng Li[4], Jiulan Dai[1,*]

1. Environmental Research Institute, Shandong University, Ji'nan 250100, China
2. Institute of Ecology and Biodiversity, School of Life Sciences, Shandong University, Ji'nan 250100, China
3. Shandong Provincial Engineering and Technology Research Center for Vegetation Ecology, Shandong University, Ji'nan 250100, China
4. Laboratory of Soil Pollution Control, Chinese Research Academy of Environmental Science, Beijing 100012, China

Abstract

To evaluate contamination caused by petroleum, surface soil samples were collected from both upland and paddy fields along the irrigation canals in the Hunpu wastewater irrigation region in northeast China. N-alkanes, terpanes, steranes, and phospholipid fatty acids (PLFA) in the surface soil samples were analyzed. The aliphatic hydrocarbon concentration was highest in the samples obtained from the upland field near an operational oil well; it was lowest at I-3P where wastewater irrigation promoted the downward movement of hydrocarbons. The Hunpu region was found contaminated by heavy petroleum from oxic lacustrine fresh water or marine deltaic source rocks. Geochemical parameters also indicated significantly heavier contamination and degradation in the upland fields compared with the paddy fields. Principal component analysis based on PLFA showed various microbial communities between petroleum contaminated upland and paddy fields. Gram-negative bacteria indicated by 15:0, 3OH 12:0, and 16:1(9) were significantly higher in the paddy fields, whereas Gram-positive bacteria indicated by i16:0 and 18:1(9)c were significantly higher in the upland fields ($p < 0.05$). These PLFAs were related to petroleum contamination. Poly-unsaturated PLFA (18:2ω6, 9; indicative of hydrocarbon-degrading bacteria and fungi) was also significantly elevated in the upland fields. This paper recommends more sensitive indicators of contamination and degradation of petroleum in soil. The results also provide guidelines on soil pollution control and remediation in the Hunpu region and other similar regions.

Key words: aliphatic hydrocarbons; geochemical indices; phospholipid fatty acids; soil; wastewater irrigation

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Introduction

Aliphatic hydrocarbons constitute the bulk of petroleum hydrocarbons. The components of aliphatic hydrocarbons analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) can provide information on the source, degradation, and depositional environment of petroleum. Research efforts on the presence of aliphatic hydrocarbons in the soil, atmosphere, and water due to petroleum contamination have being increasing. N-alkane distribution indices, namely, nC_{17}/pristane (Pr), nC_{18}/ phytane (Ph), and unresolved complex mixtures (UCM) can be used to identify biogenic and petrogenic sources and petroleum degradation (Colombo et al., 1989; Tran et al., 1997; Zheng and Richardson, 1999). Various oils and their products (gasoline, fuel oil, motor oil and so on) can also be distinguished by the chromatographic distributions of aliphatic hydrocarbons (Abdullah, 1997; Latimer and Quinn, 1998).

Terpanes and steranes are ubiquitous in petroleum and its products. They exhibit resistance toward biodegradation. Therefore, they are complementarily used in the assessment of the contamination level and in the identification of pollution sources of petroleum. Analyses of terpanes and steranes showed the biogenic and anthropogenic inputs of aliphatic hydrocarbons to the Santos Bay and Estuary (Medeiros and Bicego, 2004) and to Lake Taihu (Qu et al., 2007). Other researchers classified crude oils from Egypt (El-Gayar et al., 2002) and Hokkaido, Japan into source-related types (Waseda and Nishita, 1998).

Phospholipid fatty acids (PLFA) technique can be used to elucidate the microbial community composition that is sensitive to petroleum hydrocarbon contamination in the soil (Piotrowska-Seget and Mrozik, 2003; Schafer et al., 2009). Bundy et al. (2002) suggested that stimulating the growth of indigenous microbiota was more effective for hydrocarbon removal than the addition of hydrocarbon-degrading microbes. Therefore, determing soil PLFA composition is important for ecological remediation.

The Hunpu region is one of the largest and typical

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* Corresponding author. E-mail: daijiulan@sdu.edu.cn

wastewater irrigation areas in China. The region has been irrigated with water from the Dahuofang Reservoir, Hunhe River, and Xihe River for more than 40 years. The Xihe River is the major drainage channel for the industrial and municipal sewage of Shenyang, which includes petroleum contaminants. Consequently, the Xihe River and its river-bank groundwater are badly contaminated by petroleum hydrocarbons (Song et al., 2007). Wastewater irrigation, atmospheric deposition, and local oil wells would cause petroleum contamination in farmland soils in the region.

The objective of this study is to evaluate effectively contamination and degradation as well as to identify the sources of petroleum hydrocarbons in the soil of upland and paddy fields by analyzing concentrations, chromato-graphic distributions, and geochemical indices of aliphatic hydrocarbons. This research also aims to study various mi-crobial responses to petroleum contamination by assessing PLFA in the typical wastewater-irrigated region.

1 Materials and methods

1.1 Soil sampling

In October 2009, soil samples were collected at the Hunpu wastewater-irrigated area located at the southwest of Shenyang City, Liaoning Province, China. The Hunpu region has an area of 410,000 ha with a typical temperate monsoon climate. The annual mean temperature is 7.8°C, and the annual mean precipitation is 734.4 mm. Although the north wind is dominant during winter, the predominant wind direction all year is toward the south. The main soil type is sandy loam and black soil. Irrigation water is delivered by the main canal and the Xihe River canal, both of which draw water from the Hunhe River. The main canal has some sub-main canals and connects to the Xihe River canal through its fourth sub-main canal.

Considering the effect of wastewater, this study selected six soil units (paddy fields: 1P, 3P, and 5P; upland fields: 1U, 4U, and 6U) along the strike of the irrigation canals for pollutant monitoring (Fig. 1). Five 100 m × 100 m blocks (each block included single or multiple fields) were randomly selected at each unit, and all blocks were at least 200 m away from the roads. Each surface soil sample (0–10 cm below ground surface) was homogeneously mixed with five diagonally sampled subsamples in each block. A portion of each sample was filled into a pre-cleaned aluminum box and transported to the laboratory at a temperature of 4°C. The samples were freeze-dried and ground to 1 mm for petroleum hydrocarbons and PLFA analyses. The other sample portions were air-dried and ground to 2 mm for soil particle composition, then to 1 mm for soil pH and conductivity, and finally to 0.125 mm for organic matter (OM) analyses.

1.2 Aliphatic hydrocarbon analysis

For extraction of petroleum hydrocarbons, a soil sam-ple (ca. 15 g) was soxhlet extracted with 300 mL dichloromethane for 24 hr (USEPA, 1996). The resulting extract was then cooled and concentrated to 2 mL using a rotary vacuum evaporator (35°C). The aliphatic hydrocar-bons were obtained through eluting with approximately 20 mL n-hexane after purification with an alumina and silica gel chromatography column and concentrated to 1 mL for qualitative and quantitative analyses (Richter, 2000; Van De Weghe et al., 2006).
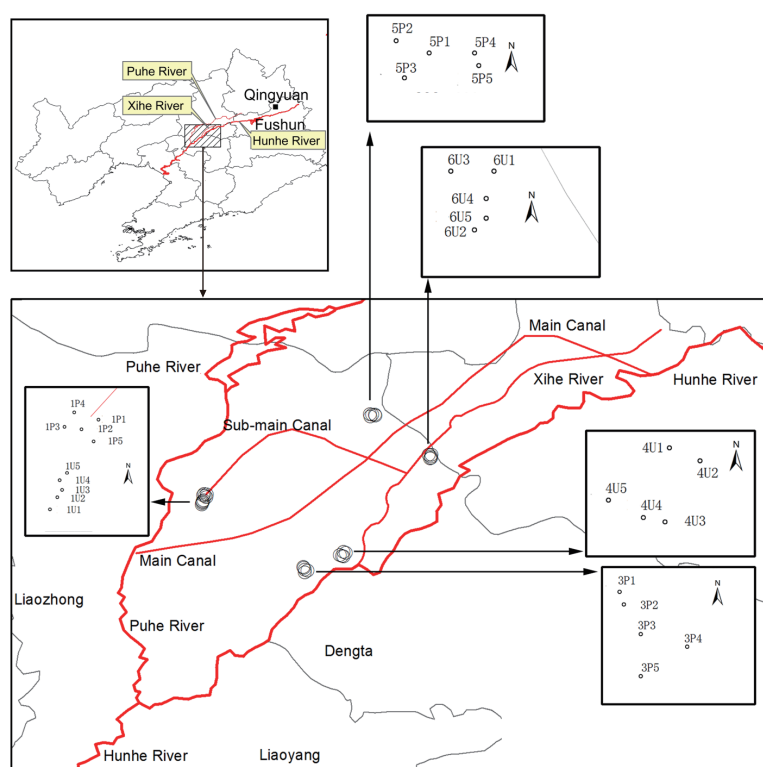
Fig. 1 Sampling sites in the Hunpu wastewater-irrigated region, China. Three soil units from paddy fields: 1P, 3P, and 5P; three soil units from upland fields: 1U, 4U, and 6U.

The aliphatic hydrocarbon fraction was analyzed by an Agilent 7890A GC with a HP-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 mm film thickness). A 2 μL volume was injected in the pulsed splitless mode. Oven temperature was initially at 60°C for 2 min and then increased to 320°C at 8°C/min for 10 min. The flame ionization detector (FID) was at 330°C. The carrier gas was N_2. N-alkanes (nC_{10} to nC_{40}), phytane, and pristane were quantified using the external standard (Accustandard, US) method. UCM was quantified by assuming a response factor of 1.0 based on nonadecane. The value of $\sum$n-alkanes was the sum of all n-alkanes from nC_{10} to nC_{40}. Total aliphatic hydrocarbons (TAH) value was the sum of $\sum$n-alkanes and UCM. The GC/MS analysis was conducted with a Shimadzu GC-2010 (identical column, injector at 300°C, initial oven temperature at 70°C for 1 min and then increased to 150°C at 15°C/min isothermal for 1 min and to 290°C at 4°C/min for 20 min) interfaced directly to a Shimadzu GCMS-QP 2010 Plus (ion source temperature at 200°C, interface temperature at 250°C, 70 eV electron energy and scanned from 40 to 550 Da). Terpanes and steranes were identified by mass fragmentography and quantified by assuming a response factor of 1.0 based on nonadecane (Aboul-Kassim and Simoneit, 1995; DOE, 1991).

The analytical procedure was rigidly evaluated. Calibration graphs were constructed by plotting the peak area against the reference material concentration every two days. A linear relationship with $r^2 > 0.99$ was obtained. The recovery test was performed with a reference material-spiked soil. The reference material is mixed standard containing n-alkanes (nC_{10} to nC_{40}), phytane, and pristane (Accustandard, USA). A control soil sample (ca. 15 g) was spiked with three kinds of reference material solutions with concentrations of 2, 6, and 10 μg/mL, respectively. The spiked sample was analyzed after being left to stand for 30 min at room temperature. The procedure was repeated three times. Recoveries for nC_{10} to nC_{40}, phytane and pristane were 60%–120% with 3.4%–17.0% RSD.

1.3 PLFA analysis

For extraction of PLFA, a soil sample (ca. 32 g) was extracted with single-phase extraction system (75 mL methanol, 37.5 mL chloroform, and 30 mL 0.05 mol/L pH 7.4 phosphate buffer) by shaking for 2 hr. After extraction, the solution was separated from the soil by centrifugation. The organic fraction was obtained by a separatory funnel for liquid-liquid extraction with 75 mL chloroform/water (1/1, V/V) and then was concentrated. Phospholipids were

obtained by elution with 10 mL methanol after purification with the silica gel column (Agilent, 500 mg, 6 mL; preconditioned with 5 mL methanol/chloroform at 1/4, V/V, successively eluted with 10 mL chloroform and 10 mL acetone to remove neutral lipids and glycolipids).

The phospholipids were re-dissolved with 2 mL toluene/methanol (1/1, V/V) and 2 mL 0.2 mol/L methanolic KOH, then incubated for 30 min at 37°C for methyl esterification. Subsequently, they were cooled and extracted with 4 mL hexane/chloroform (4/1, V/V), 400 μL acetic acid (1 mol/L), and 4 mL distilled water. After repeating the process three times, the solvent of the organic phase was changed to 1 mL hexane (Salomonováa et al., 2003).

PLFA was analyzed by an Agilent 7890A GC equipped with a HP-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 mm film thickness). A 1-μL volume was injected in the pulsed splitless mode. The initial temperature was at 120°C for 4 min and rose to 250°C at 2°C/min. FID was at 270°C. The carrier gas was N_2. PLFA was identified by the external standard (Sigma-Aldrich, US) method and quantified by assuming similar response factors for the fatty acids.

1.4 Soil basic properties analysis

Soil pH was measured using a pH meter (PSH-3C, Leici, China) with the ratio of water to soil of 2.5:1 (V/m). Potassium dichromate volumetric method was used to determine OM. Particle composition was determined by a micro-pipettes method (Miller and Miller, 1987). Soil conductivity was measured using a conductivity meter (DDS-12A, LIDA, China) with the ratio of water to soil of 5:1 (V/m) (Table 1).

1.5 Statistical analysis

Simple statistical analysis was done with SPSS 13.0. To compare the PLFA composition of different soil sample units, principal component analysis (PCA) was conducted using the software CANOCO 4.5 bundled with CanoDraw for Windows (Lepš and Šmilauer, 2003).

2 Results and discussion

2.1 Distribution of n-alkanes, UCM, and TAH in the surface soil samples

2.1.1 Concentrations of $\sum$n-alkanes, UCM, and TAH

The Kruscal-Wallis ANOVA results showed that the concentrations of $\sum$n-alkanes, UCM, and TAH of the six soil units were significantly different ($p < 0.05$). The

Table 1 Basic properties (standard error of the mean, $n = 5$) of the soil samples collected from the Hunpu region

Sample	Particle composition (%)			pH	Organic matter (%)	Conductivity (mS/m)
	Sand	Silt	Clay			
1P	44.7 ± 1.8	54.9 ± 1.8	0.37 ± 0.08	5.1 ± 0.0	2.5 ± 0.0	15.4 ± 1.4
1U	42.2 ± 0.9	57.3 ± 0.9	0.46 ± 0.07	5.3 ± 0.1	2.0 ± 0.1	12.1 ± 1.9
3P	22.6 ± 3.6	77.1 ± 3.7	0.35 ± 0.07	6.2 ± 0.2	3.7 ± 0.2	21.1 ± 2.3
4U	29.5 ± 3.2	70.3 ± 3.2	0.14 ± 0.03	5.2 ± 0.1	3.5 ± 0.3	27.8 ± 2.9
5P	42.0 ± 2.1	57.6 ± 2.0	0.33 ± 0.07	6.0 ± 0.1	4.2 ± 0.2	21.2 ± 3.6
6U	40.2 ± 2.8	59.6 ± 2.8	0.15 ± 0.02	5.3 ± 0.1	3.2 ± 0.1	28.9 ± 3.1
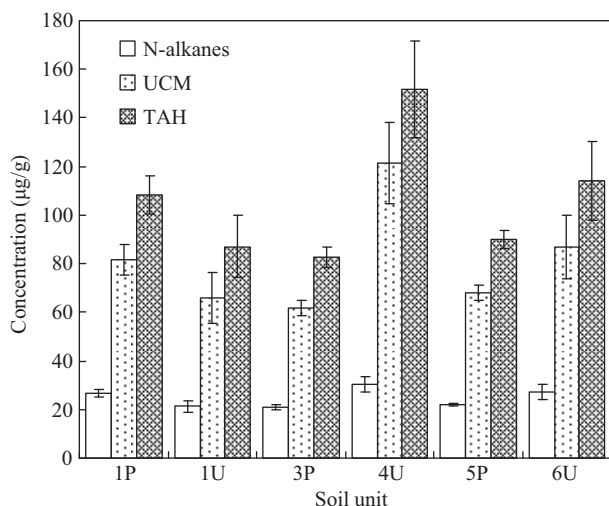
Fig. 2 Concentrations (standard error of the mean, $n = 5$) of $\sum$n-alkanes, unresolved complex mixtures (UCM), and total aliphatic hydrocarbons (TAH) in the surface soil samples of six soil units.

concentrations were the highest in 4U ($\sum$n-alkanes: 30.4 ± 3.2 μg/g, UCM: 121.5 ± 16.8 μg/g, TAH: 151.9 ± 19.9 μg/g) and the lowest in 3P ($\sum$n-alkanes: 20.9 ± 1.0 μg/g, UCM: 61.9 ± 3.2 μg/g, TAH: 82.8 ± 4.2 μg/g) (Fig. 2). The higher hydrocarbon concentration in 4U may be attributed to the nearby operating oil wells and the Xihe River, whereas the lower hydrocarbon concentration in 3P may be explained by the large amount of sewage from the Xihe River and the moisture, both of which promote the downward migration of organic pollutants due to hydrodynamic force (Acher et al., 1989; Muszkat et al., 1993). The Nemenyi test results showed that the differences of the hydrocarbon concentrations between 4U and 1U/3P were significant ($p < 0.05$).

The TAH values in all samples were much higher than the value (10.0 μg/g) earlier reported for the concentration

of total petroleum hydrocarbons (TPH) in unpolluted soil (Adeniyi and Afolabi, 2002). They were also higher than 50 μg/g, the generic and above 2 feet TPH cleanup level of soil proposed by the Oklahoma Department of Environmental Quality (DEQ, 2004). However, they were below the Canada-wide remedial standard (450 μg/g) for petroleum hydrocarbons contaminated soil (CCME, 2008).

2.2 N-alkanes geochemical indices and source apportionment

Biogenic and degraded petrogenic hydrocarbons were identified by geochemical indices (Table 2). The presence of UCM and the ratio of UCM to n-alkanes (U/N = 3 or 4) indicated petroleum pollution and degradation (Colombo et al., 1989; Harji et al., 2008). The long-term petroleum contamination resulted in the accumulation of UCM at high concentrations (Zheng and Richardson, 1999). Additionally, a separately revised carbon preference index (CPI) was used to evaluate the contribution of plant wax (Marzi et al., 1993). CPI_{13-22} equal to or near 1.0, CPI_{22-35} (1.2–1.7) and CPI_{13-35} (1.1–1.5) slightly higher than 1 confirmed predominant petrogenic hydrocarbons and minor biogenic hydrocarbons in the soil in the Hunpu region (Harji et al., 2008; Zheng and Richardson, 1999). Meanwhile, the low ratio of pristane to phytane (Pr/Ph < 1), nC_{17}/Pr, and nC_{18}/Ph near or lower than 1.0 represented degraded rather than fresh petroleum contamination (da Silva and Bicego, 2010; Gao et al., 2007).

The abundance of high carbon number hydrocarbons and scarcity of low carbon number hydrocarbons indicated heavy petroleum contamination and degradation (Colombo et al., 1989). The chromatographic distribution of aliphatic hydrocarbons in the Hunpu region was similar to that in the petroleum-contaminated sediments in the Santos Bay and Estuary (Medeiros and Bicego, 2004). The C_{16} ratio,

Table 2 Distribution of n-alkane geochemical indices of soil samples collected in the Hunpu region

Sample	U/N	CPI			Isoprenoid			C_{16} ratio
		CPI_{13-35}	CPI_{13-22}	CPI_{22-35}	Pr/Ph	nC_{17}/Pr	nC_{18}/Ph	
1P max	3	1.5	1.0	1.7	0.9	1.2	1.0	49
1P mean	3	1.4	1.0	1.5	0.8	1.1	0.9	42
IP min	3	1.3	1.0	1.5	0.6	1.0	0.8	27
1U max	3	1.4	1.0	1.5	0.6	1.0	0.7	63
1U mean	3	1.3	1.0	1.5	0.6	0.9	0.7	35
IU min	3	1.3	0.9	1.4	0.5	0.8	0.7	26
3P max	3	1.4	1.1	1.5	0.8	1.1	0.9	47
3P mean	3	1.4	1.1	1.5	0.7	1.0	0.8	38
3P min	3	1.4	1.0	1.4	0.6	0.9	0.7	30
4U max	4	1.3	1.0	1.4	0.7	1.2	0.8	24
4U mean	4	1.2	0.9	1.3	0.6	1.0	0.7	33
4U min	3	1.1	0.9	1.2	0.5	0.7	0.6	43
5P max	3	1.4	1.0	1.6	0.9	1.1	1.0	33
5P mean	3	1.3	0.9	1.5	0.8	1.0	0.9	30
5P min	3	1.3	0.9	1.5	0.8	0.9	0.8	24
6U max	4	1.5	1.3	1.2	0.7	0.7	0.7	58
6U mean	3	1.1	1.0	1.0	1.0	0.9	0.8	42
6U min	3	1.6	1.4	1.2	0.8	0.7	0.7	33

U/N: the ratio of unresolved complex mixture to the sum of n-alkanes; CPI_{13-35}, odd to even carbon preference index from C_{13} to C_{35}; CPI_{13-22}, odd to even carbon preference index from C_{13} to C_{22}; CPI_{23-35}, odd to even carbon preference index from C_{23} to C_{35}; Pr, pristanes; Ph, phytanes; C_{16} ratio, the ratio of the sum of all n-alkanes to hexadecane. $CPI = \dfrac{\left(\sum\limits_{i=n}^{m} C_{2i+1}\right) + \left(\sum\limits_{i=n+1}^{m+1} C_{2i+1}\right)}{2 \times \left(\sum\limits_{i=n+1}^{m+1} C_{2i}\right)}$, where, C: carbon; m, n, i: carbon number.

defined as the ratio of the sum of all n-alkanes to nC_{16}, is usually high (i.e., 50) for biogenic hydrocarbons and low (i.e., 15) for petroleum-contaminated samples (Colombo et al., 1989; Tran et al., 1997). The values varied from 24 to 63, with the mean value 36, further proving the joint effects of petroleum pollution, minor biogenic hydrocarbons, and degradation.

The Kruscal-Wallis ANOVA results showed that the values for U/N, CPI, and Pr/Ph of the six soil units were significantly different ($p < 0.05$). U/N in 4U was higher than that in other soil units, whereas the values for CPI and Pr/Ph in 4U were lower than those in other soil units. Thus, petroleum contamination was heavier in 4U than in other units, as shown by the concentrations of ∑n-alkanes, UCM, and TAH. The Mann-Whitney results showed that the values for CPI_{13-35}, CPI_{22-35}, Pr/Ph, nC_{17}/Pr, and nC_{18}/Ph in the paddy fields were significantly higher than those in the upland fields ($p < 0.05$), indicating significantly higher petroleum contamination and degradation in the upland fields than in the paddy fields.

2.3 Distribution of terpanes and steranes in the surface soil samples

The Kruscal-Wallis ANOVA results revealed that ∑tricyclic terpanes and ∑pentacyclic terpanes of the six soil units were significantly different ($p < 0.05$). The Nemenyi test results showed that the ∑pentacyclic terpanes were significantly different between 1U and 4U ($p < 0.05$). In any case, the concentrations of terpanes and steranes were the highest in 4U, whereas the concentrations were the lowest or the second-lowest in 3P (Fig. 3). These results were consistent with ∑n-alkanes, UCM, and TAH. The Mann-Whitney results revealed that ∑tricyclic terpanes were significantly higher in the upland fields than in the paddy fields ($p < 0.05$), which again indicated heavier petroleum contamination in the upland fields. A significantly positive correlation was discovered
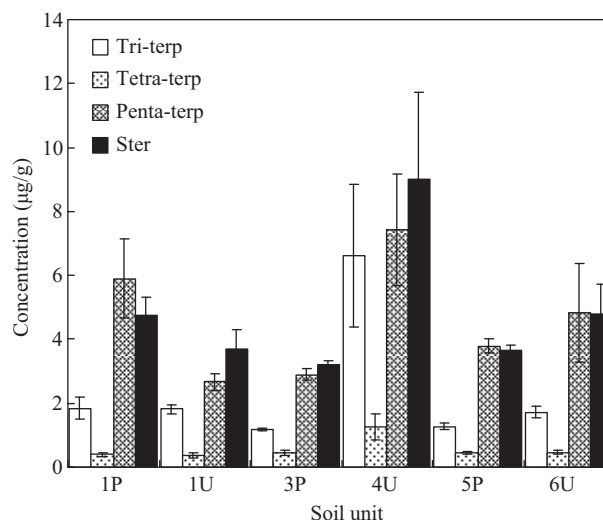
Fig. 3 Concentrations (standard error of the mean, $n = 5$) of ∑tricyclic terpanes, ∑tetracyclic terpanes, ∑pentacyclic terpanes, and ∑steranes in the surface soil samples of six soil units.

between each two groups (∑n-alkanes, UCM, TAH, ∑tricyclic terpanes, ∑tetracyclic terpanes, and ∑pentacyclic terpanes, and ∑steranes), and the correlation coefficients were greater than or equal to 0.4 ($p < 0.05$), indicating that the terpanes and the steranes came from the same sources in each sample site.

2.4 Terpane and sterane geochemical indices and source apportionment

Geochemical information based on terpanes and steranes is presented in Tables 3 and 4, respectively. The concentrations of the αβ-hopanes (C_{30}–C_{34}) decreased with increasing carbon number and extended αβ-hopanes (C_{31}–C_{34})/homohopanes with 22R and 22S configuration supporting the petroleum inputs (Shi et al., 2008). When biologically generated, these hopanes had a 17β(H), 21β(H) 22R configuration (da Silva and Bicego, 2010).

Table 3 Distribution of terpane geochemical indices of the soil samples collected in the Hunpu region

Sample	Triplet ratio	Ts/Tm	C_{29}/C_{30}	22S/(S+R)				HomoR/totalR (%)				HomoS/totalS (%)			
				C_{31}	C_{32}	C_{33}	C_{34}	C_{31}	C_{32}	C_{33}	C_{34}	C_{31}	C_{32}	C_{33}	C_{34}
1P max	3.7	2.9	0.9	1.0	0.5	0.7	0.7	43.3	53.8	23.7	30.0	59.9	29.2	25.4	11.9
1P mean	2.0	2.2	0.9	0.7	0.4	0.6	0.6	27.6	47.7	14.8	9.9	51.6	24.0	17.3	7.1
1P min	0.9	1.4	0.8	0.6	0.3	0.4	0.4	1.8	44.4	6.8	1.3	39.2	18.4	12.7	1.9
1U max	2.8	2.6	1.2	0.9	0.5	0.8	0.6	48.6	60.1	7.8	16.9	82.3	26.9	10.2	2.6
1U mean	1.8	1.9	1.0	0.7	0.4	0.7	0.4	39.5	50.0	4.6	5.9	69.4	21.0	8.0	8.7
1U min	1.2	1.1	0.8	0.6	0.3	0.6	0.2	34.5	40.8	2.9	1.0	61.5	12.3	4.2	1.1
3P max	3.9	2.7	0.9	0.7	0.4	0.9	0.7	49.6	57.9	14.0	5.7	64.1	28.1	21.4	5.7
3P mean	2.9	2.3	0.8	0.6	0.4	0.7	0.7	40.2	47.9	9.7	2.2	55.9	24.3	16.8	3.1
3P min	2.1	1.8	0.8	0.5	0.3	0.6	0.5	31.5	38.5	2.6	0.8	48.2	20.6	12.8	2.0
4U max	3.8	2.1	1.1	0.7	0.5	0.9	1.0	36.1	56.9	23.9	11.9	57.6	33.5	30.1	10.5
4U mean	2.4	1.7	0.9	0.7	0.4	0.6	0.6	30.3	49.6	13.7	6.4	47.4	23.6	21.6	7.4
4U min	1.5	0.03	0.6	0.6	0.3	0.0	0.5	23.1	35.4	6.0	0.4	38.5	15.7	0.0	1.1
5P max	3.5	2.9	0.9	0.8	0.4	0.9	0.7	41.1	58.9	13.8	7.8	62.6	26.7	21.5	8.8
5P mean	2.6	2.5	0.8	0.7	0.4	0.7	0.6	36.6	49.7	9.0	4.7	54.3	24.7	16.0	5.0
5P min	1.5	2.1	0.8	0.6	0.4	0.6	0.5	29.1	42.6	3.3	1.5	45.8	22.6	11.8	1.8
6U max	3.0	1.8	0.9	0.7	0.5	0.7	0.7	39.0	51.0	17.6	15.7	53.3	30.2	27.1	7.7
6U mean	2.1	1.3	0.9	0.6	0.4	0.6	0.5	35.2	44.6	14.7	5.5	47.8	28.3	19.7	4.1
6U min	1.3	0.9	0.8	0.6	0.4	0.6	0.3	25.1	39.4	11.8	1.3	37.7	27.0	15.3	1.2

Triplet ratio, the ratio of C_{26} tricyclic terpane (R+S) to C_{24} tetracyclic terpane; Ts/Tm, the ratio of 18α(H)-22,29,30-trisnorneohopane to 17α(H)-22,29,30-trisnorhopane; C_{29}/C_{30}, the ratio of C_{29} 17α (H)-norhopane to C_{30} 17α(H)-hopane; HomoR/totalR, the ratio of each 22R homohopane to the total 22R homohopanes for C_{31}–C_{34}; HomoS/totalS, the ratio of each 22S homohopane to the total 22S homohopanes for C_{31}–C_{34}.

Table 4 Distribution of sterane geochemical indices of the soil samples collected in the Hunpu region

Sample	$\alpha\alpha$R/total$\alpha\alpha$R (%)			$\alpha\alpha$S/total$\alpha\alpha$S (%)			$\beta\beta$(R+S)/total$\beta\beta$(R+S) (%)			$C_{29}\alpha\alpha$S/(S+R)	D/S	$C_{29}\beta\beta/(\alpha\alpha+\beta\beta)$
	C_{27}	C_{28}	C_{29}	C_{27}	C_{28}	C_{29}	C_{27}	C_{28}	C_{29}			
1P max	25.7	28.0	52.1	35.7	14.5	77.1	60.9	44.8	18.6	0.5	1.1	0.3
1P mean	23.5	25.0	51.5	26.0	11.1	62.9	50.9	34.6	14.5	0.4	0.7	0.2
1P min	20.3	22.8	50.7	16.8	6.2	50.2	43.8	20.6	11.4	0.3	0.5	0.2
1U max	47.6	45.6	45.4	46.1	22.3	69.5	50.6	45.1	23.2	0.9	4.6	0.4
1U mean	33.6	33.5	32.9	33.6	14.1	52.3	47.4	37.0	15.6	0.4	1.8	0.3
1U min	26.3	21.9	7.4	21.6	8.8	37.5	41.6	27.9	12.2	0.2	0.9	0.2
3P max	29.9	25.6	52.0	34.4	28.9	71.8	53.7	37.1	16.5	0.5	1.2	0.3
3P mean	25.8	24.5	49.7	23.8	16.8	59.4	51.2	33.8	15.0	0.4	0.8	0.2
3P min	23.4	23.0	47.1	13.2	10.1	36.7	48.8	29.8	14.2	0.2	0.5	0.2
4U max	40.7	30.4	50.6	58.1	17.1	81.0	66.4	38.4	16.2	0.3	4.1	0.2
4U mean	31.6	24.4	44.0	30.2	12.4	57.4	56.5	33.5	10.0	0.3	2.0	0.2
4U min	24.7	16.2	28.9	1.9	7.0	34.9	45.5	30.3	1.8	0.2	0.2	0.1
5P max	32.9	26.6	55.9	37.9	26.4	59.4	55.3	40.4	17.9	0.4	0.9	0.3
5P mean	24.3	24.4	51.3	29.6	15.9	54.5	50.3	33.7	16.0	0.3	0.7	0.2
5P min	21.3	22.8	44.3	16.1	8.4	46.7	45.9	27.2	13.6	0.2	0.4	0.2
6U max	29.9	26.9	52.6	31.9	11.9	76.1	50.9	48.2	13.9	0.5	2.8	0.2
6U mean	24.3	25.3	50.4	23.1	9.3	67.6	45.6	41.3	13.1	0.4	1.0	0.2
6U min	20.5	23.1	45.5	18.4	5.5	56.3	38.8	35.4	11.3	0.3	0.4	0.2

$\alpha\alpha$R/total$\alpha\alpha$R, the ratio of each $\alpha\alpha$20R sterane to the total $\alpha\alpha$20R steranes for C_{27}–C_{29}; $\alpha\alpha$S/total$\alpha\alpha$S, the ratio of each $\alpha\alpha$20S sterane to the total $\alpha\alpha$20S steranes for C_{27}–C_{29}; $\beta\beta$(R+S)/total$\beta\beta$(R+S), the ratio of each $\beta\beta$ sterane to the total $\beta\beta$ steranes for C_{27}–C_{29}; D/S, the ratio of C_{27} $\alpha\beta$diasteranes to C_{29} $\alpha\alpha$steranes.

The ratios of 22S/(S+R) for C_{31}, C_{33}, and C_{34} were above 0.6 (equilibrium value), indicating high maturity, whereas the ratio for C_{32} was below 0.5, which was similar to the distribution pattern of the oil source rock sample YHS2 reported by Pan et al. (2008). The C_{27}–C_{29} steranes commonly found in mature sediments and crude oils were also identified in the present samples. Recently formed C_{29} 20R steranes, with "biological" configuration depressing two sterane parameters, led to an underestimation of their maturity (Yunker and Macdonald, 2003). Therefore, the sterane ratios were much lower than the corresponding equilibrium values ($C_{29}\alpha\alpha$S/(S+R): 0.53 and C_{29}20R$\beta\beta$/($\beta\beta+\alpha\alpha$): 0.80).

The triplet ratio is dependent upon the sources, depositional environment, and maturity. The values were 3.8–5.9 for the tar balls, 1.2–2.2 for the Exxon Valdez oil (an Alaska North Slope crude) and its residues, and 1.6–7.5 for the oil products used for construction and pavements from the shorelines of the Prince William Sound (Kvenvolden et al., 1995). The triplet ratios varied from 0.9 to 3.9 for the soil in the Hunpu region.

The ratio of 18α(H)-22,29,30-trisnorneohopane to 17α(H)-22,29,30-trisnorhopane (Ts/Tm) is also a source facies parameter. The values were within the range of 0.9–2.9 in the region investigated in the present study; the values were similar to those for the Thailand oils and source rocks (Lawwongngam and Philp, 1993). The values were below 1.0 in the shorelines of the Prince William Sound (Kvenvolden et al., 1995). Ts/Tm values below 1.0 indicate a lacustrine, saline, marine evaporitic or marine carbonate depositional environment, whereas the values above 1.0 indicate lacustrine freshwater or marine deltaic environment (Barakat et al., 1997; Ibrahim and Abdullah, 2002). Ts/Tm can decrease quite late during maturity (Roushdy et al., 2010).

The low concentration of homohopanes relative to C_{29} 17α(H)-norhopane and C_{30} 17α(H)-hopane, as well as the high concentration of diasteranes, reflected a more oxic depositional environment in the Hunpu region (Burgan and Ali, 2009; Ibrahim and Abdullah, 2002). The values of C_{29} 17α(H)-norhopane/C_{30} 17α(H)-hopane (C_{29}/C_{30}, 0.8–1.2) also indicated contamination of the oil from oxic lacustrine freshwater or marine deltaic rather than anoxic carbonate or marl source rocks; these values point toward enhanced maturity in the region (Gülbay and Korkmaz, 2008; Pan et al., 2003). The Mann-Whitney results showed that Ts/Tm in the paddy fields was significantly higher than that in the upland fields, whereas C_{29}/C_{30} in the paddy fields was significantly lower than that in the upland fields ($p <$ 0.05). This trend was consistent with the results from other parameters and proved heavier petroleum contamination in the upland fields.

For different homohopanes and steranes in various media, the biodegradation rates were different. Aboul-Kassim and Simoneit (1995) differentiated petroleum contaminants derived from sewage or from direct input by boating using the ratios of each 22R/S homohopane (homo) to the total 22R/S homohopanes (homos) for C_{31}–C_{34}, each $\alpha\alpha$20R/S sterane (ster) to the total $\alpha\alpha$20R/S steranes (sters), and each $\beta\beta$ sterane to the total $\beta\beta$ steranes for C_{27}–C_{29}. The values of C_{33} R homo/total R homos, C_{33} S homo/total S homos, C_{34} S homo/total S homos, and C_{29} $\alpha\alpha$R ster/total $\alpha\alpha$R sters in 1U were lower than those in other soil units, whereas the values of C_{31} S homo/total S homos and C_{27} $\alpha\alpha$R ster/total $\alpha\alpha$R sters in 1U were higher than those in other soil units.

The main pollution source was an oil well abandoned more than ten years ago, which resulted in a higher level of hydrocarbon biodegradation in 1U, as indicated by the geochemical indices above. Additionally, the Mann-Whitney results showed that the ratios of C_{27} $\alpha\alpha$R ster/total $\alpha\alpha$R sters, C_{29} $\alpha\alpha$R ster/total $\alpha\alpha$R sters, and C_{29} $\beta\beta$ster/total sters in the upland fields were significantly higher, lower and lower than those in the paddy fields, respectively ($p <$
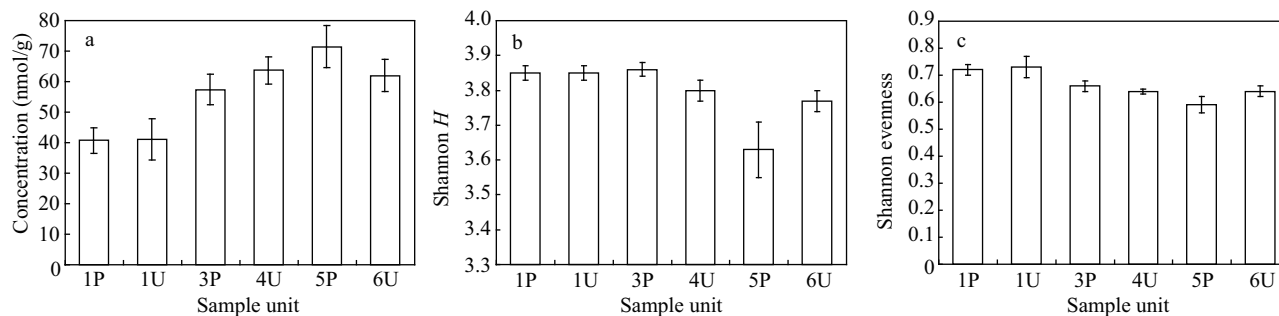
Fig. 4 Concentrations (standard error of the mean, $n = 3$) of total PLFA (a), Shannon H diversity (b), and evenness (c) based on PLFA concentrations (standard error of the mean, $n = 3$) in the surface soil samples of six soil units.

0.05), confirming the higher hydrocarbon biodegradation level in the upland fields.

2.5 Biomass and species diversity based on PLFA in the surface soil samples

The total PLFA, Shannon H diversity, and evenness (Fig. 4) did not change with the aliphatic hydrocarbon concentrations (Figs. 2 and 3). Syakti et al. (2006) reported that hydrocarbon-degrader biomass dose not always change with petroleum concentration. The total PLFA of the six soil units varied from (10.65 ± 1.17) to (18.78 ± 1.92) μg/g dw (40.78 ± 4.17 to 71.44 ± 6.90 nmol/g dw), which were from ($2.41 \times 10^9 \pm 0.25 \times 10^9$) to ($4.21 \times 10^9 \pm 0.41 \times 10^9$) cells/g dw based on the published values for *Escherichia coli* at 5.9×10^{12} cells/g dw and 100 μmol PLFA/g dw (Polymenakou et al., 2005; Syakti et al., 2006).

The total PLFA in the soil near the Xihe River Reach and Shenyang City were obviously higher than those from areas far from Shenyang City and the Xihe River, whereas the abundance and evenness of the species were adverse. Pollution of the Xihe River and Shenyang City led to the increase of microbial biomass and the decrease of microbial diversity in surface soil. However, the total PLFA, Shannon diversity, and evenness were not significantly different between paddy and upland fields ($p > 0.05$).

The differences in the PLFA compositions of the six sample units are shown in Fig. 5. PLFA compositions in the upland fields were obviously different from those in the paddy fields, and the PLFA compositions in the upland fields were more similar than those in the paddy fields, especially in 1U (near an abandoned oil well more than ten years ago) and in 4U (near an operational oil well). The Mann-Whitney results showed that the concentrations of 15:0, 3OH 12:0, and 16:1(9) were significantly higher in the paddy fields, whereas the concentrations of i16:0, 18:1(9)c, and 18:2(9, 12)/18:2ω6, 9 were significantly higher in the upland fields ($p > 0.05$).

Microbial communities in the oiled samples contain significantly more monounsaturated PLFA compared with the unoiled samples (Bundy et al., 2002; Piotrowska-Seget and Mrozik, 2003). The PLFAs 15:0 and i16:0 also associated with the oil contamination (Aries et al., 2001; Bundy et al., 2002; Syakti et al., 2006). The significantly higher 15:0, 3OH 12:0, and 16:1(9) PLFA (indicative of Gram-negative bacteria) in the paddy fields, while i16:0 and 18:1(9)c
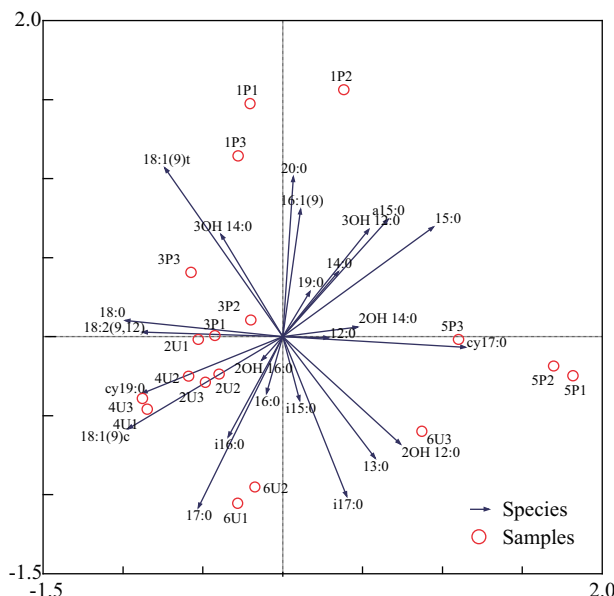
Fig. 5 Biplot of species based on PLFA concentrations and soil samples from principal component analysis.

PLFA (indicative of Gram-positive bacteria) in the upland fields, possibly indicated the different strategies employed by microorganisms to adapt to petroleum contamination and environmental stress (Bundy et al., 2002; Kozdrój and van Elsas, 2001; White et al., 1998).

The concentration of poly-unsaturated PLFA (18:2ω6, 9) in the upland fields was significantly higher than that in the paddy fields ($p < 0.05$). Bundy et al. (2002) reported that the addition of diesel could result in an increase of 18:2ω6, 9. This PLFA is abundant in fungi but has also been reported to occur in certain bacteria (e.g., *Marinobacter hydrocarbonoclasticus* strain 617) that grows on either pure n-alkanes or on n-alkane mixture, and in a consortium composed of marine hydrocarbon-degrading bacteria growing on crude oil (Syakti et al., 2006). Therefore, the 18:2ω6, 9 can measure the level of petroleum contamination in the soil.

Additionally, the concentrations of 18:1(9)t, 3OH 14:0, and 20:0 were higher in 1P near the abandoned oil well. The concentrations of 2OH 12:0, 13:0, and i17:0 were higher in 5P and in 6U near Shenyang City and the middle reaches of the Xihe River. The concentrations of 2OH 14:0 and cy17:0 were higher in 5P than those in other sample units. The 2OH 14:0 is identified as the main fatty acid in *Sphingomonas*, which can measure

the level of contamination in soil polluted by polycyclic aromatic hydrocarbons, as these bacteria preferentially degrade hydrocarbons (Bundy et al., 2002; Zelles, 1999). White et al. (1998) also discovered a small increase of cy17:0 (indicative of Gram-negative bacteria) influenced by hydrocarbons.

More abundant hydroxyl PLFA (mainly from Gram-negative bacteria) in the paddy fields (significantly higher concentration of 3OH 12:0 in the paddy fields, higher concentration of 3OH 14:0 in 1P, and higher concentration of 2OH 14:0 in 5P) indicated that hydroxyl PLFA was positively related with the soil moisture. Similar results were also reported in a previous study where hydroxyl PLFA decreased in desert habitats from rainy to dry periods (Zelles, 1999).

3 Conclusions

The petroleum pollution level in the surface soil of the Hunpu region was moderate. The aliphatic concentrations were the highest in 4U near an operational oil well, whereas the aliphatic concentrations were the lowest at 3P due to the downward migration of organic pollutants as a result of wastewater irrigation.

According to geochemical analysis, heavy petroleum hydrocarbons were discovered dominant in the soil. Light petroleum hydrocarbons, as well as biogenic hydrocarbons, were also identified in this study. The distributions of homohopanes and diasteranes, C_{29} norhopane/C_{30} hopane, and Ts/Tm reflected a lacustrine freshwater or marine deltaic and more oxic depositional environment in the study area. The characteristics of petroleum contamination were most obvious in 4U. The contamination and degradation of hydrocarbon in the upland fields were significantly heavier than those in the paddy fields.

PCA results showed that PLFA compositions in the upland fields were obviously different from those in the paddy fields, especially in 1U (near an oil well abandoned more than ten years ago), which was closely grouped together with 4U (near an operational oil well). The concentrations of 15:0, 3OH 12:0, and 16:1(9) were significantly higher in the paddy fields, whereas i16:0, 18:1(9)c, and 18:2 (9, 12)/18:2ω6, 9 were significantly higher in the upland fields.

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