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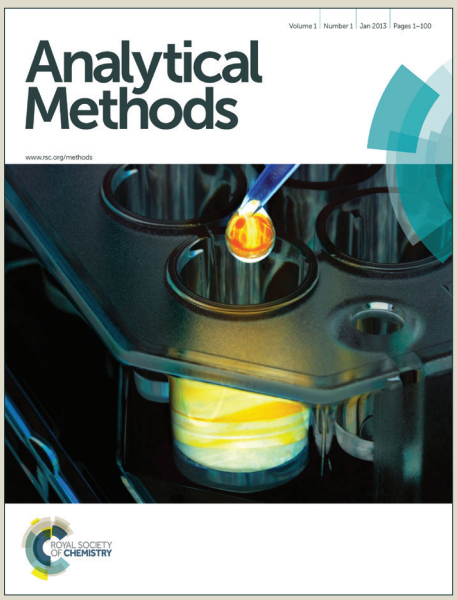

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Quantification and characterization of naphthenic acids in soil of oil exploring area in China by GC/MS

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Abstract

Naphthenic acids (NAs) are a toxic complex mixture of carboxylic acids occurred naturally in petroleum. Based on the serious potential risk of NAs on terrestrial ecology during crude oil exploration and production processes, and the lack of efficient methodologies for extraction and analysis of these compounds, the goal of this study is to detail the development of a routine method for extraction, quantitative and qualitative analysis of NAs in oil contaminated soils. Solid phase extraction using MAX cartridge was employed in combination with GC/MS. Ethyl acetate with 2% formic acid as elution solvent showed the best recoveries of NAs (98.36%-112.35%). Total NAs concentration and NA profiles of oil contaminated soil samples from 4 oilfields in China were examined. High concentrations of NAs (maximum, 132.91 mg/kg) were detected in soils, which implied toxic and estrogenic risk for human and terrestrial organisms. Different profiles of NAs mixtures were observed in soils from 4 different oilfields, and evaporation and biodegradation could influence the compounds of NAs. The authors present the first feasible method for analysis of NAs in soil.

Keywords: Naphthenic acids; soil; oil contamination; SPE; GC/MS

1. Introduction

Naphthenic acids (NAs) are a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids, with the general chemical formula $C_nH_{2n+Z}O_2$, where *n* indicates the carbon number and Z is zero or a negative, even integer that specifies the hydrogen deficiency resulting from ring formation (Fig. S1).[1-4] NAs are natural components of petroleum, and have been considered as the primary toxic component of oil sands process-affected water (OSPW).[5,6] Moreover, NAs are extensively used in a wide range of commercial and industrial applications, such as surfactants and wood preservatives.[3,7] Therefore, not only are NAs a problem associated with the oil sands industry, they are likely to be widespread environmental contaminants.

With carboxylates, NAs are readily dissolved in water at neutral and alkaline pH.[4] Hence, NAs have been detected in OSPW, or petroleum refinery wastewaters, or natural waters surrounding oil sand industry.[8-11] Furthermore, NAs contents in aquatic organisms are also investigated.[12,13] NAs associated with acute and chronic toxicity in different aquatic organisms including bacteria,[14,15]

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phytoplankton,[16,17] fish[18-20] and amphibians,[6,13] and also in mammals[21] have been documented. Recently, NAs have been identified as environmental estrogens and induced the gene for vitellogenin production in *Zebrafish Larvae.*[22-24] However, it is aware of few studies on NAs in oil contaminated soils in terms of concentration and composition, even extracting process from soil.

It has reported that NAs content of crude oil varies geographically and may account for as much as 4% of raw petroleum by weight in fields in Romania, Russia, Venezuela, China, and West Africa.[7] Along with increasing demand for energy, large amount of NAs have been and will be continually entered into soil during crude oil exploration and production processes. Those NAs are serious potential risk for human living around and the terrestrial ecosystem. Therefore, the objective of this study is to develop an efficient extracting method to extract NAs from oil contaminated soil, as well as quantify and characterize the NAs composition in the oil exploring areas of China to support scientific evidence and basic information on soil pollution of NAs from oil contamination.

2. Materials and methods

2.1.Chemicals

Three commercial mixtures of NAs were purchased from Sigma Aldrich, Acros Organics, and TCI Shanghai Organics Chemicals, respectively. The surrogate standard, 9-fluorenecarboxylic acid (9-FCA) and the derivatizing agent, N-methyl-N-(t-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) were purchased from Sigma Aldrich. Methanol, dichloromethane, and ethyl acetate were obtained from Fisher Chemicals. Ammonia, sodium hydroxide and formic acid were purchased from Beijing Chemicals.

2.2.Sample collection

A total 55 soil samples were collected from four oil fields, which were mainly distributed in north of China: Xinjiang (XJ, 10 samples around Kelamayi, and 10 samples around Korla) in northwest China, Daqing (DQ, 15 samples) in northeast China, Shengli (SL, 15 samples) in the Yellow River Delta, and Huabei (HB, 5 samples) in the Huabei Plain (Fig. S2). Soils were collected around crude oil pumping wells where contamination occurred, and kept in cold boxes until transported to the lab. All the soil samples were air dried at room temperature, removed stones and residual roots, sieved through a 40 mesh sieve, and stored in desiccators prior to analysis.

2.3.Naphthenic acids extraction

The method of extracting naphthenic acids from the soils is modified according to Holowenko et al.[15] and Wang et al.[11] Briefly, 10 g soil sample was digested in 100mL 0.1 mol L^{-1} NaOH, and centrifugated to remove solids. The aqueous phase was used for naphthenic acids extraction with MAX cartridges (Oasis MAX, 6 mL, 150 mg, Waters). The MAX cartridge was conditioned with 6 mL methanol followed by 6 mL of distilled water. The aqueous phase was passed through the conditioned MAX cartridge at a flow rate of 1 mL min^{-1}. The cartridge was then further washed with 6 mL of 5% ammonia followed by 6 mL methanol and dried under a flow of nitrogen gas. The sample was eluted from the cartridge with 12 mL of ethyl acetate containing 2% (v/v) formic acid. The extract was take to dryness under nitrogen and dissolved in 0.1 mL of dichloromethane (DCM) and derivatized with 0.1 mL N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) prior to analysis by GC/MS. The derivatized sample was dried under nitrogen and dissolved in 0.5 mL DCM. For the quantitative of naphthenic acids, 5 μg of internal standard 9-fluorenecarboxylic acid (9-FCA, Sigma) was added into each soils before extraction.

2.4.GC/MS analysis

A gas chromatographic analysis of derivatized samples was performed with an Agilent 6890N GC equipped with a 5973I MSD, using a 30-m HP5-MS narrow bore column (0.25 mm × 0.25 μm). The carrier gas was He and was set to maintain an initial flow of 1 mL min^{-1}. The initial temperature of 100℃ was held for 3 min, followed by an increase of 8 ℃ min^{-1} to a final temperature of 300℃. The injector temperature was 290 ℃, and 2 μl of the solution was injected in to the GC/MS with splitless mode. The SCAN mode (m/z: 50 - 550) and SIM mode (m/z = 267) were recorded for each samples.

The mass spectra of derivatived samples were analyzed according to Holowenko et al.[15]. Briefly, peak ion intensity values were averaged over the elution of the NAs hump, generally from retention time 8 min onward. The ‘minimum occurrence’ variable for the averaged data was set at 1%, and the averaged peak intensity values for the desired peak ions were inputted into a Microsoft Excel spreadsheet. These normalized data were used for comparison of carbon numbers and Z-family abundances between the different samples.

For quantitative, Sigma naphthenic acids were used to prepare the calibration curve for GC/MS. 5, 10, 20 ,50, 100, 200 and 500 μg of these acids were dispensed into seven vials, and each portion received 5 μg of 9-FCA and the mixture was made to 100 μl with DCM. These were derivatized with MTBSTFA and analyzed by GC/MS (SIM, m/z 267). The ratios of the integrated area of the naphthenic acids hump to the area of the 9-FCA peak and the amounts of naphthenic acids were plotted for the calibration curve.[25] To assess the minimum concentration of naphthenic acids that could be detected by this method, various concentrations of Sigma NAs (0 – 25 μg) were added to 10 g clean soils. The limit of detection with this method was approximately 5 μg NAs when 10 g soil was extracted (0.5 mg kg^{-1}).

3. Results and discussion

3.1.Extraction and detection of NAs from soils

Before a successful application of chromatographic methods, extraction is typically necessary in order to separate analytes from the interfering matrix components and enrich them.[26,27] Choosing the appropriate sample preparation method is the most important in the qualitative and quantitative determination of target compounds. Solid phase extraction (SPE) is a quite common and widespread sample preparation method, which offers a wide field with numerous applications.[28] And the selection of an appropriate SPE extraction sorbent and elution protocol is the key techniques.[29] Jones et al.[30] used nonaqueous ion exchange SPE to determinate NAs in crude oils, and Bataineh et al.[31] used SPE to extract NAs from water samples, which showed higher extraction efficiency compared to liquid-liquid extraction. In this paper, four SPE cartridges (Oasis HLB and MAX, Waters; SAX and Plexa PAX, Agilent) were chose and the recoveries of three commercial mixtures of NAs (Sigma, TCI, Acros) were tested to develop the suitable process.

Table 1 Recoveries for model commercial naphthenic acids by GC/MS using various SPE cartridges (mean ± SD, n = 4). 10 g clean soils spiked with 50 μg NAs were used.

		Recovery for commercial naphthenic acids (%)		
		Sigma	TCI	Acros
HLB	MeOH	6.12 ± 0.98	5.23 ± 1.19	7.34 ± 0.74
	2%FA MeOH	35.59 ± 2.31	32.36 ± 0.54	32.15 ± 1.94
	2% FA EA	68.97 ± 1.35	53.21 ± 0.95	38.68 ± 1.87
	5% FA EA	72.84 ± 1.68	54.16 ± 1.26	35.74 ± 1.64

MAX	MeOH	0	0	0
	2% FA MeOH	70.59 ± 0.56	67.68 ± 1.98	61.35 ± 2.67
	2% FA EA	98.36 ± 1.26	102.36 ± 2.35	112.35 ± 3.47
	5% FA EA	97.12 ± 2.35	100.42 ± 0.87	103.24 ± 2.17
SAX	MeOH	0	0	0
	2% FA MeOH	12.35 ± 0.15	15.47 ± 0.97	18.56 ± 2.31
	2% FA EA	23.56 ± 0.47	18.26 ± 1.58	20.14 ± 1.65
	5% FA EA	25.69 ± 0.98	19.68 ± 1.64	23.14 ± 0.98
PAX	MeOH	0	0	0
	2% FA MeOH	56.21 ± 1.26	64.29 ± 1.38	32.85 ± 2.98
	2% FA EA	79.62 ± 1.06	76.29 ± 3.54	45.68 ± 1.98
	5% FA EA	78.26 ± 0.98	78.64 ± 1.95	52.19 ± 0.65

Four SPE cartridges worked with different modes of action, reversed-phase for HLB, and ion-exchange for MAX, SAX, and PAX. It is clearly that NAs can be washed away with nonacid composition in the prewash step when HLB cartridge was used. This result was not observed in the other three SPE cartridges. The acids absorbed in the cartridges by electrostatic interactions would be eluted by acidified solvents. Hence, acidified methanol and ethyl acetate by formic acid were chose according to previous research.[11,31] It is illustrated that the recoveries of commercial NAs using the MAX cartridge (98.36%-112.35%) were obviously higher than those eluted from SAX (18.26%-23.56%) and PAX (45.68%-79.62%) cartridge, especially for using ethyl acetate with formic acid as elution solvent. However, the recoveries of NAs did not increase when more formic acid was added into ethyl acetate. Then, the optimized MAX SPE coupled to ethyl acetate with 2% formic acid as elution solvent was applied to analyze NAs in soil samples from the main oilfields in China.

After digestion and extraction, the sample was analyzed by GC/MS SIM (m/z = 267) and a hump with retention time between about 14 and 19 min was collected (Fig. 1). The detection of a hump using m/z = 267 (specific for the t-butyldimethylsilyl esters of $C_{13}H_{22}O_2$ isomers) is consistent with the presence of naphthenic acids.[32,33] Compared with Merlin et al.'s study, the retention time had been shifted, which might be caused by GC/MS programmed with const flow instead of const pressure in Merlin's. This is the first report of an analytical method that detects NAs in soil, although other investigations have quantified the NAs concentrations and characterized the NAs profiles in waters and aquatic organisms.[12,32,33]

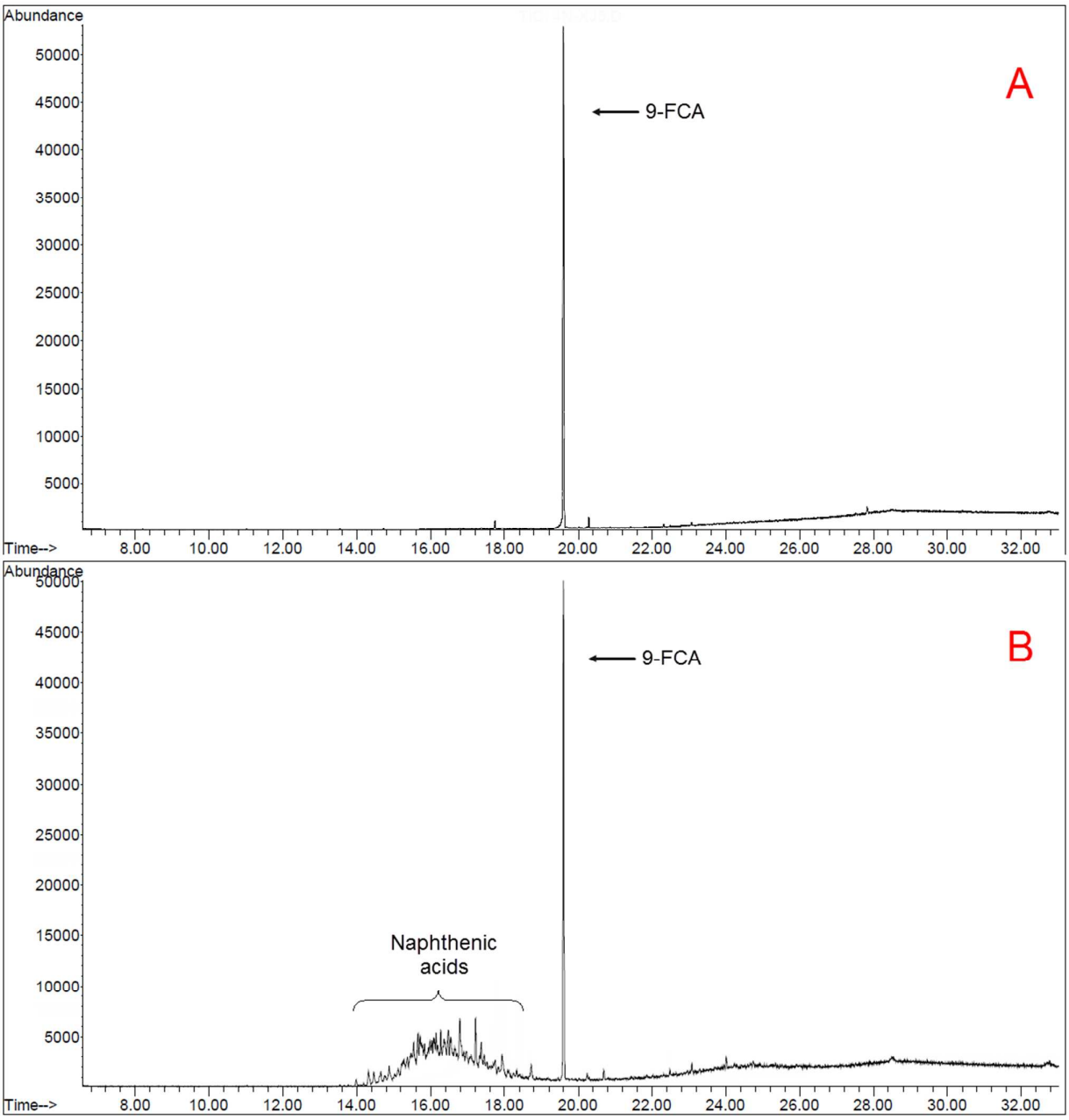

Fig. 1 The GC/MS SIM analysis results of a blank sample (A) and an oil-contaminated sample (B) from DQ oilfield

3.2.Quantification and characterization of NAs in soils from oil exploring area

NAs in 55 oil contaminated soil samples from 4 oilfields in China have been analyzed, and each SIM (m/z = 267) result showed a hump with retention time between 14 and 19 min, while no hump was observed in the extracts of blank control samples. The concentrations of NAs were listed in table 2.

Table 2 Concentrations of NAs in soil samples from 4 oilfields in China

Con. (mg kg^{-1})	DQ	SL	XJ	HB
Min.	3.35	2.32	2.85	2.29
Median	12.23	8.29	12.88	7.38
Max.	132.91	45.91	56.95	27.57
Mean ± SD	40.08 ± 47.67	15.03 ± 15.51	20.34 ± 19.75	10.29 ± 10.31

To quantify NAs concentrations, it is assumed that the commercial mixture used to calibrate the method is representative of the NAs in the mixture being analyzed. And it is also assumed that quantification of the $C_{13}H_{22}O_2$ isomers would be representative of all the isomer classes of NAs from environment. The used commercial NAs were purchased from Sigma-Aldrich, which has been often experimented in other relevant researches.[9,11,34] The calibration curve with $R^2 = 0.9987$ is listed in supplementary data.

Based on the two assumptions, the average concentrations in DQ, SL, XJ, and HB were 40.08, 15.03, 20.34 and 10.29 mg kg^{-1}, respectively. The highest content was found in DQ oil field with 132.91 mg kg^{-1}, and the lowest was in HB oil field with 2.29 mg/kg. For few investigations were processed on the concentrations of NAs in soil, it's difficult to compare the pollution and estimate the ecological risk level in China and around the world. However, it has been reported that on the concentration less than 10 mg L^{-1} extracted from OSPW, NAs can cause deformities and even death of amphibian larvae (*Lithobates pipiens* and *Silurana tropicalis*) and fish embryos (*Perca flavescens* and *Orizias latipes*).[18,20,35] Although the potential toxicity in soil is different from in water, such high NAs concentrations should be given attention due to their toxic and estrogenic risk.

The ions of derivatized naphthenic acids conformed to the empirical formula, $C_nH_{2n+Z}O_2$, should follow the rules: 1) if $Z < 0$, at least one 5-carbon-member ring was present in the molecule; 2) there was one carbon atom available for the carboxyl group; 3) there was at least one carbon atom available for the alkyl *R* group; and 4) structures with > 3 rings ($Z < -6$) could be fused on more than two sides.[36,37] The expected molecular weights were showed in Tab. S1 grouped by carbon numbers and Z families, and the samples from the four oil fields were analyzed in the same way.

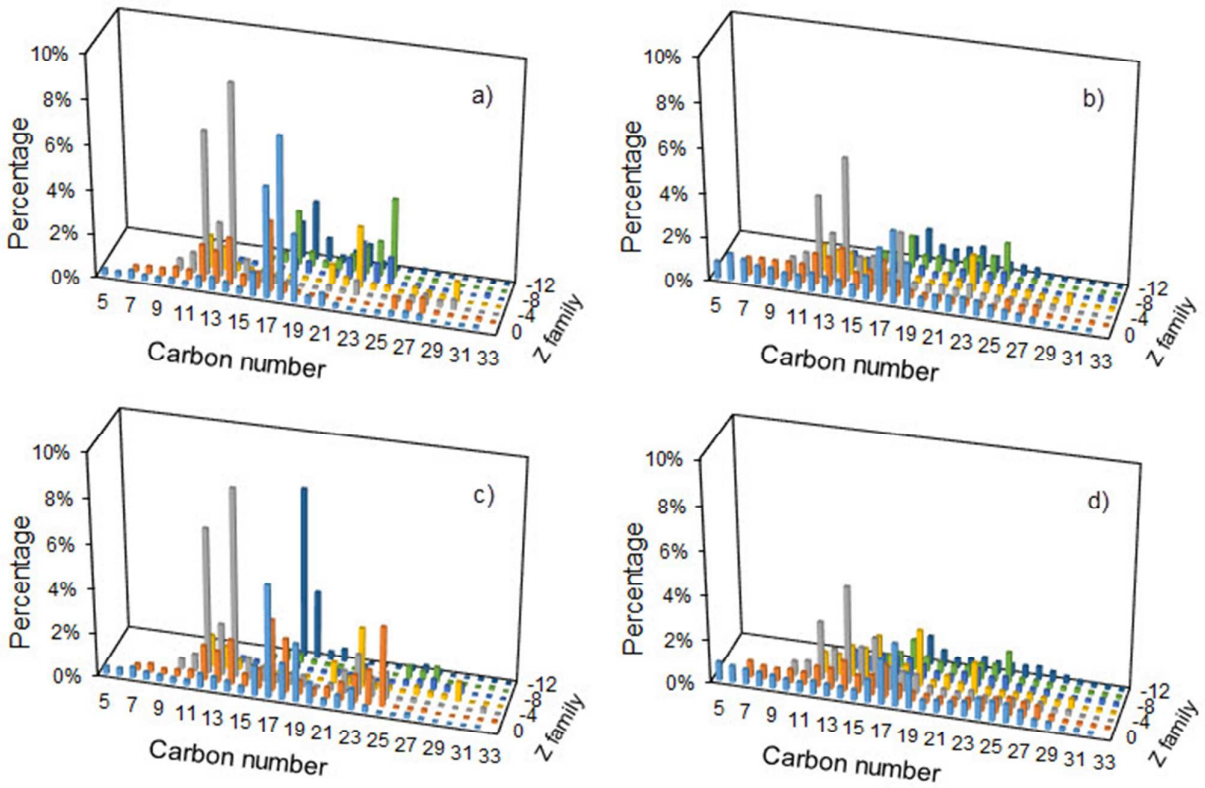

Fig. 2 Three-dimensional plots of carbon numbers and Z families of naphthenic acids from different oil fields (a. SL; b. DQ; c. HB; d. XJ)

The distributions of naphthenic acids were visual in the three-dimensional graphs. The most abundance ions were for the formulas of $C_{12}H_{20}O_2$ and $C_{14}H_{28}O_2$ in samples, except that the relative proportions were shifted. For example, the ratios of $C_{12}H_{20}O_2$ and $C_{14}H_{28}O_2$ in SL sample were 8.8% and 6.5% respectively, and in XJ sample, the ratios were 4.4% and 2.7% respectively. Other ions were also showed different ratios in different oil fields, ion of formula $C_{25}H_{48}O_2$ was 3.6% in HB sample, but the proportions were 0.1%, 0.2%, and 0.7% in SL, DQ and XJ samples respectively. The Z = -4 series accounted the most proportion in SL sample (24.4%), and followed by Z = 0 (22.8%) and Z = -2 series. The same results could be found in DQ sample. However, in HB sample, the second most ions were the Z = -2 series. Previous researches about acidic compounds in crude oil and sediments from biodegraded reservoir have illustrated that low molecular weight naphthenic acids (n < 20) were rapidly biodegraded compared with higher molecular weight acids (n > 20), and cyclic acids were more recalcitrant than acyclic acids. So different proportions of naphthenic acids indicated different degrees of degradation through evaporation or biodegradation. Kim et al.

presented a new degradation index based on the ratio of acyclic NAs (Z = 0) to cyclic NAs (Z = -4, -6, -8), and the A/C ratio ($\sum NAs_{Z=0}/\sum NAs_{Z=-4,-6,-8}$) decreased as the degradation degree increased.[38,39] The A/C ratios were 0.540, 0.537, 0.436, and 0.439 in SL, DQ, HB, and XJ, respectively. Compared with the Biomarker Biodegradation Index,[38] slight degree of biodegradation happened in these samples. The high temperature and drying air caused small A/C ratio in XJ, which enhanced the weathering process of evaporation. Our high throughput sequencing data of microorganism in soil samples showed more abundant bacteria existed in HB soil samples, which may be the reason of small A/C ratio.

4. Conclusions

A routine, quantitative solid phase extraction method has been develop for the analysis of naphthenic acids in soils based on the Oasis MAX cartridge. This method provides suitable samples for qualitative and quantitative analysis of GC/MS. The concentrations of naphthenic acids in soil samples from oilfields were revealed, which implied potential ecotoxicity caused by naphthenic acids. Evaporation and biodegradation could influence the compounds of naphthenic acids. The general characteristics of naphthenic acids could be received by GC/MS. However, the development of analytical methods for studying characterization of naphthenic acids continues to be a formidable challenge.

Acknowledgments

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