

# Naphthenic Acids in Coastal Sediments after the *Hebei Spirit* Oil Spill: A Potential Indicator for Oil Contamination

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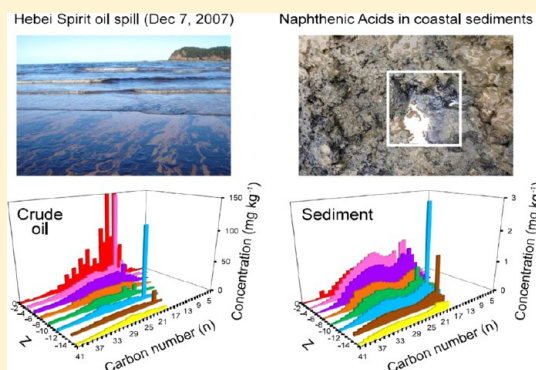
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## Supporting Information

**ABSTRACT:** Naphthenic acids (NAs) as toxic components in most petroleum sources are suspected to be one of the major pollutants in the aquatic environment following oil spills, and the polarity and persistence of NAs make it a potential indicator for oil contamination. However, the contamination and potential effects of pollutants in oil spill affected areas remain unknown. To investigate NAs in oil spill affected areas, a sensitive method was first established for analysis of NAs, together with oxy-NAs in sediment samples by UPLC-QTOF-MS. Then the method was applied to determine the NA mixtures in crude oil, weathered oil, and sediments from the spilled sites after the *Hebei Spirit* oil spill, Taean, South Korea (Dec. 2007). Concentrations of NAs, O<sub>3</sub>-NAs, and O<sub>4</sub>-NAs were found to be 7.8–130, 3.6–44, and 0.8–20 mg kg<sup>-1</sup> dw in sediments from the Taean area, respectively, which were much greater than those measured in the reference sites of Manlipo and Anmyundo beaches. Concentrations of NAs were 50–100 times greater than those (0.077–2.5 mg kg<sup>-1</sup> dw) of PAHs in the same sediment samples, thus the ecological risk of NAs in oil spill affected areas deserves more attention. The sedimentary profiles of oil-derived NAs and background NAs centered around compounds with 21–35 and 12–21 carbons, respectively, indicating that the crude-derived NA mixtures originating from the 2007 oil spill were persistent. Acyclic NAs<sub>n=5–20</sub> were easily degraded compared to cyclic NAs<sub>n=21–41</sub> during the oil weathering processes, and the ratio of oxy-NAs<sub>n=21–41</sub> relative to NAs<sub>n=21–41</sub> could be a novel index to estimate the degree of oil weathering in sediments. Altogether, the persistent oil-derived NAs<sub>n=21–41</sub> could be used as a potential indicator for oil-specific contamination, as such compounds would not be much affected by the properties of coastal sediments possibly due to the high sorption of the negatively charged compounds (NAs) in sediment.



## INTRODUCTION

As energy demands grow worldwide, oil spill accidents have become a global problem.<sup>1</sup> A historical review shows that over one billion liters of oil have spilled into U.S.A. marine waters since the early 1960s,<sup>1</sup> and the recent explosion of the Deepwater Horizon oil platform in 2010 released several million barrels of gas and oil at the seafloor of the Gulf of Mexico from the Macondo well.<sup>2</sup> The huge quantities of spilled oils have released various pollutants, including aliphatic/aromatic hydrocarbons, polar compounds, volatile organic compounds, and heavy metals to the aquatic environment.<sup>1,3–7</sup>

The oil-derived pollutants are resistant to weathering and hence persistent in sediment, leading to long-term ecotoxicological effects to aquatic organisms and possibly human health.<sup>1,2</sup> Thus, proper characterization of spilled oil in complex environmental samples is extremely important for long-term monitoring and environmental assessment of oil spilled-affected area.<sup>1,8</sup>

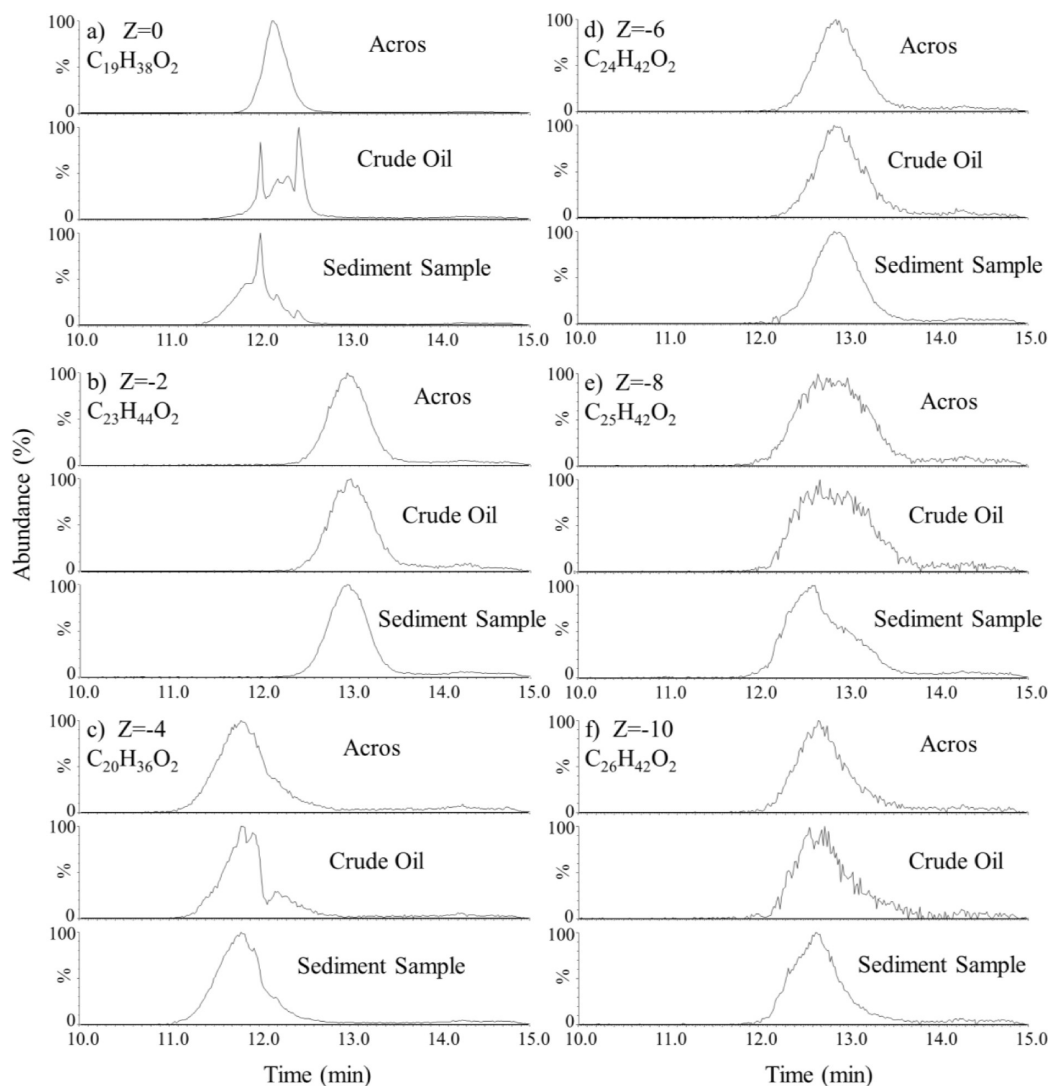
Among various pollutants in an oil spill, those causing the greatest concern with regard to environmental risk would be the polycyclic aromatic hydrocarbons (PAHs),<sup>1</sup> which are assumed to be the primary determinant for oil petroleum toxicity.<sup>9</sup> Various developmental abnormalities including deformation and growth inhibition have been reported in fish embryos after exposure to crude oil or oil-contaminated sediments, posing major threats to fish health and population vitality.<sup>10,11</sup> However, it has been reported that toxic effects of an oil spill could not be linked exclusively to PAHs but also to other components capable of entering the aquatic environment.<sup>9</sup> Naphthenic acids (NAs) are a group of chemicals with formulae of C<sub>n</sub>H<sub>2n+Z</sub>O<sub>2</sub>, where “n” means carbon number and “Z” refers cylinder number. “Z” is zero or a negative, even

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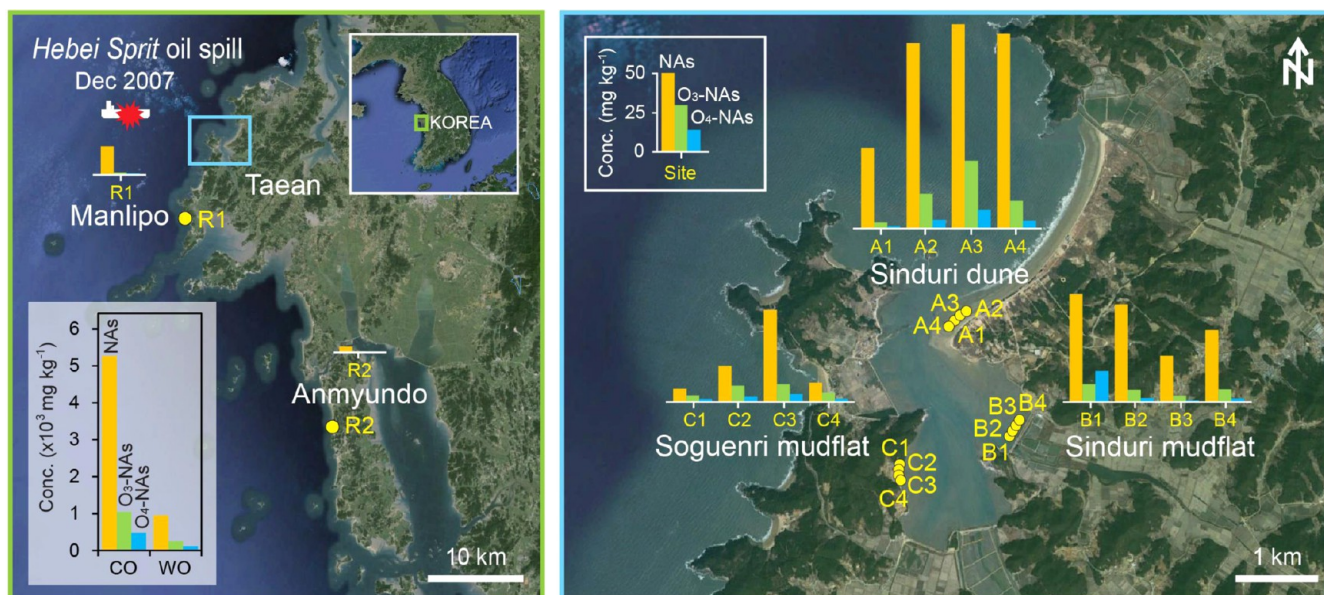


**Figure 1.** UPLC-QTOF-MS chromatograms of naphthenic acid mixtures in commercial mixtures (Acros), crude oil and sediment samples.

integer that specifies the hydrogen deficiency resulting from ring formation. NAs are believed to be some of the most toxic compounds in wastewaters from the petroleum industry, and reported to be acutely toxic to a broad range of aquatic organisms, including plant, invertebrates, fish, rats, and bacteria.<sup>12</sup> Apart from the acute toxicities, NAs have also been identified as xeno-estrogens and antiandrogens *in vitro*,<sup>13</sup> and can modulate sex steroid synthesis production in the H295R Steroidogenesis Assay.<sup>14</sup> Since NAs accounted for up to 3% of petroleum by weight and are quite soluble in neutral or slightly alkaline waters compared to PAHs,<sup>12</sup> these compounds are suspected to be one of the most toxic components in aquatic environment following oil spills.<sup>15</sup> While previous studies mainly focused on hydrophobic compounds (e.g., total PAH) as the major concerned pollutants after oil spill, the potential impacts of the large-scale releases of water-soluble NAs remains unclear.

In addition, oil spill monitoring programs usually measure alkanes, volatile aromatic hydrocarbons (VOCs), total petroleum hydrocarbons (TPHs), and polycyclic aromatic hydrocarbons (PAHs) in sediments.<sup>16</sup> The residence times of alkanes and VOCs are often short, and TPHs provide little source information,<sup>16</sup> thus sedimentary residues of PAHs are common

proxies to detect oil pollution, oil weathering, and source identification at the oil spill impacted areas.<sup>1,16–18</sup> However, the debate still exists regarding contributions of nonoil-derived PAHs to the oil contamination,<sup>8,19</sup> since PAHs have many other sources such as forest fires, agricultural burning, coal, and bacteria biosynthesis and so forth, which could affect the inherent fingerprint of PAHs for spilled oil.<sup>8,20</sup> Previous studies have reported that various environmental processes can cause substantial variability of PAHs in the emission and degradation of individual compounds, potentially undermining the application of PAH ratios as reliable source apportionment tools,<sup>21</sup> and compound-specific isotope analysis was applied for discriminating PAH sources in the environment.<sup>22</sup> Alternative fingerprinting techniques would be of great importance to properly determine and manage the oil spill contamination.<sup>8</sup> Recently, Aeppli et al. reported that the presumably recalcitrant oxygenated fraction made up >50% of the mass of weathered oils released by the *Deepwater Horizon* spill.<sup>23</sup> An oxygenated fraction of petroleum has been examined for the purposes of “environmental forensics” using a variety of novel technologies, such as intramolecular carbon isotope analysis,<sup>24,25</sup> ultrahigh resolution and high resolution mass spectrometry,<sup>24,26–28</sup> and multidimensional comprehensive gas chromatography mass



**Figure 2.** Sampling sites and distribution of NAs and oxy-NAs in crude oil (CO), weathered oil (WO), and surface sediments (A, B, C, and R sites) from Taean area, Korea.

spectrometry.<sup>29,30</sup> As a major oxygenated fraction in petroleum, NAs can easily dissolve in water and are subsequently recalcitrant to biodegradation, making NAs a potential indicator for oil contamination in the aquatic environment.<sup>31</sup> However, studies relating to the development of highly selective analytical methods for NAs, fates in sedimentary environments, weathering characterizations, and so forth are still lacking, while a recent investigation comprehensively analyzed NA profiles in natural waters surrounding the Canadian oil sands industry and demonstrated the need for monitoring NAs in oil-polluted areas.<sup>28</sup>

On December 7th, 2007, the Hong Kong registered tanker *M/V Hebei Spirit* carrying about 209 000 tons of crude oil collided with a crane barge on the Yellow Sea ~10 km off the coast of Taean, South Korean, spilling about 10 900 tons of oil.<sup>8,18</sup> The spilled oils were widely and rapidly spread out along approximately >70 km of Taean shoreline within only a week.<sup>18,32</sup> To assess the contamination of NAs in an oil-spilled area and the feasibility of using NAs as an indicator for oil contamination, the present study first developed a sensitive method for simultaneously analyzing NAs together with oxy-NAs (oxygenated NAs) in sediment samples using ultrahigh-pressure liquid chromatography (UPLC) coupled to a quadrupole time-of-flight mass spectrometer (QTOF-MS). Then the concentration, distribution, and composition of NA mixtures were determined in sediments from heavily contaminated areas of Taean coasts three to five years after the *Hebei Spirit* oil spill. The weathering characterization and spatial distribution of oil-derived NAs were finally evaluated and compared with conventional methods using PAHs, establishing a new index for estimating the extent of oil contamination.

## MATERIALS AND METHODS

**Chemicals and Reagents.** Fourteen NAs and three oxy-NAs were used as model compounds (Table S1 of the Supporting Information (SI)): 4-*n*-propylcyclohexanecarboxylic acid (*cis*- and *trans*-)(C<sub>10</sub>H<sub>18</sub>O<sub>2</sub>), *trans*-4-pentylcyclohexane carboxylic acid (C<sub>12</sub>H<sub>22</sub>O<sub>2</sub>), 12-oxochenodeoxycholic acid

(C<sub>24</sub>H<sub>38</sub>O<sub>5</sub>) cyclohexanecarboxylic acid (C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>), 1-pyrrenbutyric acid (C<sub>20</sub>H<sub>16</sub>O<sub>2</sub>), abietic acid (C<sub>20</sub>H<sub>30</sub>O<sub>2</sub>), 1-adamantaneacetic acid (C<sub>12</sub>H<sub>18</sub>O<sub>2</sub>), 2-hexyldecanoic acid (C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>), and 12-hydroxysteric acid (C<sub>18</sub>H<sub>36</sub>O<sub>3</sub>) were purchased from TCI (Tokyo Chemical Industry Co., Tokyo, Japan); 1,2,3,4-tetrahydro-2-naphthoic acid (C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>), dicyclohexylacetic acid (C<sub>14</sub>H<sub>24</sub>O<sub>2</sub>), 5- $\beta$ -cholanic acid (C<sub>24</sub>H<sub>40</sub>O<sub>2</sub>), cyclohexane pentanoic acid (C<sub>11</sub>H<sub>20</sub>O<sub>2</sub>), and 12-hydroxydodecanoic acid (C<sub>12</sub>H<sub>24</sub>O<sub>3</sub>) were purchased from Sigma Aldrich (Oakville, ON, Canada); 1-methyl-1-cyclohexane carboxylic acid (C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>) was purchased from Alfa Aesar (Ward Hill, MA); *trans*-4-*tert*-butylcyclohexanecarboxylic acid (C<sub>11</sub>H<sub>20</sub>O<sub>2</sub>) was purchased from Acros Organics (Morris Plains, NJ); 1-adamantane carboxylic acid (C<sub>11</sub>H<sub>16</sub>O<sub>2</sub>) was purchased from J&K Chemical (Beijing, China). The commercial mixture of NAs was from Acros Organics, and the chromatograms of the commercial NAs were shown in Figure 1. Methanol, acetonitrile, hexane, ethyl acetate (EA), dichloromethane (DCM), and methyl *tert*-butyl ether (MTBE) were obtained from Fisher Chemicals (Fair Lawn, NJ) and ethyl acetate was obtained from J.T. Baker Chemicals (Phillipsburg, NJ). HPLC grade ammonium acetate and formic acid were purchased from Dima-Tech Inc. (Richmond Hill, ON, Canada). Hydrochloric acid and ammonia were purchased from Beijing chemicals. Distilled water was prepared by a Milli-Q Synthesis water purification system (Millipore, Bedford, MA).

**Sample Collection.** On the basis of the previous investigations,<sup>18,33</sup> sediment samples were collected from the heavily contaminated regions of Taean, South Korea in December 2010 (A1, B1, and C1) and January 2012 (A2–A4, B2–B4, and C2–C4) (Figure 2). Two reference sediments (R1, R2) were collected in the outer regions of the Taean coast in 2012 and analyzed for a comparison. Detailed information on sampling sites and samples were present in Figure 2 and Table S2 of the SI. All samples were immediately transferred to the laboratory, and stored at –20 °C until analysis.

**Crude and Weathered Oil Samples.** NAs and oxy-NAs in crude oil, weathered oil, and sediment samples were compared to explore the variations of the pollutants during the weathering

process. Iranian Heavy Crude oil, one of the major spilled oils of the *Hebei Spirit* oil spill, was artificially weathered under controlled conditions using standardized laboratory experiments reported by Environment Canada.<sup>34</sup> The details of the weathering method have been previously reported.<sup>33</sup> In brief, approximately 50 mL crude oil in a glass beaker was heated at 70 °C in a water bath until the weight loss of the crude was greater than 25%. Finally, the weathered oil had a 28.8% weight loss.

**Sample Preparation.** Sediment samples were freeze-dried, homogenized, and sieved through a stainless steel mesh (0.2 mm). Approximately 1 g of dried sediment samples or 0.1 g of oil and weathered oil samples, spiked with 0.1 µg of surrogate standards (1-pyrenebutyric acid and 12-oxocholesterol), were extracted using a Soxhlet apparatus for 24 h with 200 mL of a hexane/MTBE/methanol (1:1:1, v/v) mixture solution. The extracts were rotary evaporated down to approximately 5 mL, and then dried under a gentle stream of nitrogen gas to approximately 3 mL to ensure the complete removal of hexane and MTBE. The residues were dissolved in 30 mL of water, and then extracted with Oasis MAX cartridges (6 mL, 150 mg, Waters, Milford, MA). The MAX cartridge was conditioned with 6 mL of methanol followed by 6 mL of distilled water. The water samples were then passed through the conditioned MAX cartridge at a flow rate of 5–10 mL min<sup>-1</sup>. The cartridge was then further washed with 6 mL of 5% ammonia solution, and dried under a flow of nitrogen gas. The MAX cartridge was first prewashed with 6 mL of methanol which was discarded, and 12 mL of ethyl acetate saturated with hydrochloric acid (2 M HCl/ethyl acetate = 1:10, v/v) was finally used to elute the analytes. The extract was washed with pure water for three times and dried under a gentle nitrogen stream, and reconstituted with 0.1 mL of methanol for UPLC-ESI-QTOF-MS analysis.

**UPLC-QTOF-MS Analysis.** An ACQUITY UPLC system (Waters) coupled to a Xevo QTOF-MS (G2, Waters) equipped with an electrospray ionization (ESI) source was used in the analysis of NAs. Instrument control was performed using MassLynx Software (Waters, software version V4.1). All the model compounds were separated on a Waters ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 × 50 mm). The column was maintained at 40 °C, and the injection volume was 3 µL. The flow rate was 0.2 mL min<sup>-1</sup>. The mobile phases consisting of ultrapure water containing 10 mM ammonium acetate and methanol were used with gradient elution. The initial conditions were 10% methanol for 2 min, ramped to 60% by 3 min, ramped to 70% by 7 min, then ramped to 100% by 13 min, and finally held for 1 min before returning to initial conditions, which were equilibrated for 5 min before injection of the next sample.

The mass spectra of NAs and oxy-NAs were acquired in the negative ion mode. The analysis was performed in full scan mode in the mass range of 80–700 Da with a 1 s scan time. According to our preliminary experiments, the optimized parameters were found as follows: source capillary voltage of 2.0 kV, sampling cone voltage of 45 V, extraction cone voltage of 4.0 V, source temperature 100 °C, desolvation temperature 250 °C, cone gas flow rate 50 L h<sup>-1</sup>, and desolvation gas flow rate 600 L h<sup>-1</sup>. The [M-H]<sup>-</sup> ion of leucine-enkephalin (200 pg µL<sup>-1</sup> infused at 5 µL min<sup>-1</sup>) was used as a reference lock mass (*m/z* 554.2615). The QTOF detector was calibrated with a sodium formate solution to achieve mass accuracy of <3 ppm by using leucine-enkephalin as the lock mass in negative mode.

The accuracy of mass measurement in combination with the retention times in UPLC were used to calculate empirical formulas of NAs and oxy-NAs.

**PAHs Analysis.** Concentrations of PAHs were identified and quantified by use of a method that has previously been validated and described.<sup>18</sup> In brief, about 20 g of freeze-dried sediment samples were Soxhlet extracted with 400 mL DCM for 24 h, and elemental sulfur in the extract was removed by reaction with activated copper. The extracts were concentrated to 1 mL and passed through a 5 g of silica gel (70–230 mesh, Merck, Darmstadt, Germany) column and eluted with 100 mL hexane/DCM (80:20, v/v). The elute was dried under a gentle stream of nitrogen and measured using an Agilent 7890 gas chromatograph (GC) coupled to a model 5975C mass-selective detector (MSD, Agilent technologies, Avondale, PA). The method detection limits (MDL) ranged from 0.1 to 0.5 ng g<sup>-1</sup> dw (dry weight) for PAHs.

**Total Organic Carbon (TOC) Analysis.** Dry sediment (about 5 mg) was put into a small quartz sample boat, which had been heated at 600 °C for about 4 h. Then phosphoric acid (4%) was added into the boat to remove the total inorganic carbon (TIC) in the sample. Finally, the sample was analyzed using a Total Organic Carbon Analyzer (Apollo 9000, Tekmar-Dohrmann Co., Cincinnati, OH).

**Quality Assurance and Quality Control (QA/QC).** All equipment were rinsed with acetone and hexane to avoid sample contamination, and a procedural blank was incorporated in the analytical procedures for every batch of seven samples. NA mixtures in field blank samples were determined to be <1 µg. Recoveries for the entire analytical procedures were estimated by triplicate analysis of field sediment samples spiked with 0.1 mg kg<sup>-1</sup> for all target model compounds. Since the separation method and standards for all the individual NAs were not available, concentrations of NA mixtures were generally semiquantified based on integration of the hump peak of each NA isomers, assuming that the responses for individual compounds in the hump peaks were similar.<sup>35–38</sup> Semiquantification of the analytes was achieved using an internal standard method with calibration against standard solutions of commercial NAs (Acros) previously described.<sup>37</sup> NAs and oxy-NAs were semiquantified in sample extracts relative to 1-pyrenebutyric acid and 12-oxocholesterol, respectively. The analytical procedure described above was validated by analyzing spiked sediment samples, and the absolute recoveries of model NA and oxy-NA compounds in triplicate spiked samples ranged from 66 to 101% (Table 1). Recoveries of 1-pyrenebutyric acid and 12-oxocholesterol were 76 ± 12% and 70 ± 11% in all sediment samples. The method detection limits (MDL) for model NA and oxy-NA compounds were estimated based on the sediment sample enrichment factor of 10 and the peak-to-peak noise of the baseline near the analyte peak obtained by analyzing field samples (spiked with nondetected analyte at 0.1 mg kg<sup>-1</sup>) using three as the minimal value of signal-to-noise. And MDLs of the model compounds ranged from 0.1 to 1.0 ng g<sup>-1</sup> dw in sediment samples (Table 1).

## RESULTS AND DISCUSSION

**Method Development.** While several analytical methods have been reported for analysis of NAs and oxy-NAs in water samples,<sup>35–38</sup> and liquid–liquid extraction was generally adopted for extraction of unprocessed oil sands,<sup>39–41</sup> efficiencies of the method for determining sedimentary NAs

**Table 1. Absolute Recoveries (%) and Method Detection Limits (MDLs, ng/g dw) for Model Compounds by UPLC/QTOF-MS in Sediment**

	model compounds		absolute recovery (mean $\pm$ RSD)	MDLs (ng/g dw)
NAs	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	Z = 0	95.1 $\pm$ 2.0	0.1
	C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>	Z = -2	66.4 $\pm$ 4.1	1.0
	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	Z = -2	68.8 $\pm$ 5.7	0.5
	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub> <sup>a</sup>	Z = -2	84.2 $\pm$ 0.1	0.5
	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	Z = -2	88.5 $\pm$ 0.8	0.4
	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub> -butyl	Z = -2	93.1 $\pm$ 4.7	0.3
	C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>	Z = -2	82.3 $\pm$ 6.2	0.4
	C <sub>11</sub> H <sub>12</sub> O <sub>2</sub>	Z = -10	82.3 $\pm$ 1.3	0.1
	C <sub>14</sub> H <sub>24</sub> O <sub>2</sub>	Z = -4	101.2 $\pm$ 6.9	0.3
	<sup>b</sup> C <sub>20</sub> H <sub>16</sub> O <sub>2</sub>	Z = -14	71.9 $\pm$ 3.5	0.4
	C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>	Z = -10	88.2 $\pm$ 12.3	0.2
	C <sub>24</sub> H <sub>40</sub> O <sub>2</sub>	Z = -8	73.8 $\pm$ 3.2	0.4
	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	Z = -6	76.9 $\pm$ 5.4	0.4
	C <sub>12</sub> H <sub>18</sub> O <sub>2</sub>	Z = -6	91.3 $\pm$ 1.9	0.3
oxy-NAs	<sup>b</sup> C <sub>24</sub> H <sub>38</sub> O <sub>3</sub>	Z = -10	87.4 $\pm$ 5.5	0.2
	C <sub>18</sub> H <sub>36</sub> O <sub>3</sub>	Z = 0	97.4 $\pm$ 1.9	0.1
	C <sub>12</sub> H <sub>24</sub> O <sub>3</sub>	Z = 0	65.5 $\pm$ 1.3	0.3

<sup>a</sup>C<sub>10</sub>H<sub>18</sub>O<sub>2</sub> (4-*n*-propylcyclohexanecarboxylic acid) was a mixture of *cis*- and *trans*- compounds. <sup>b</sup>Surrogate standards. Spiked level was 0.1 mg/kg, *n* = 3.

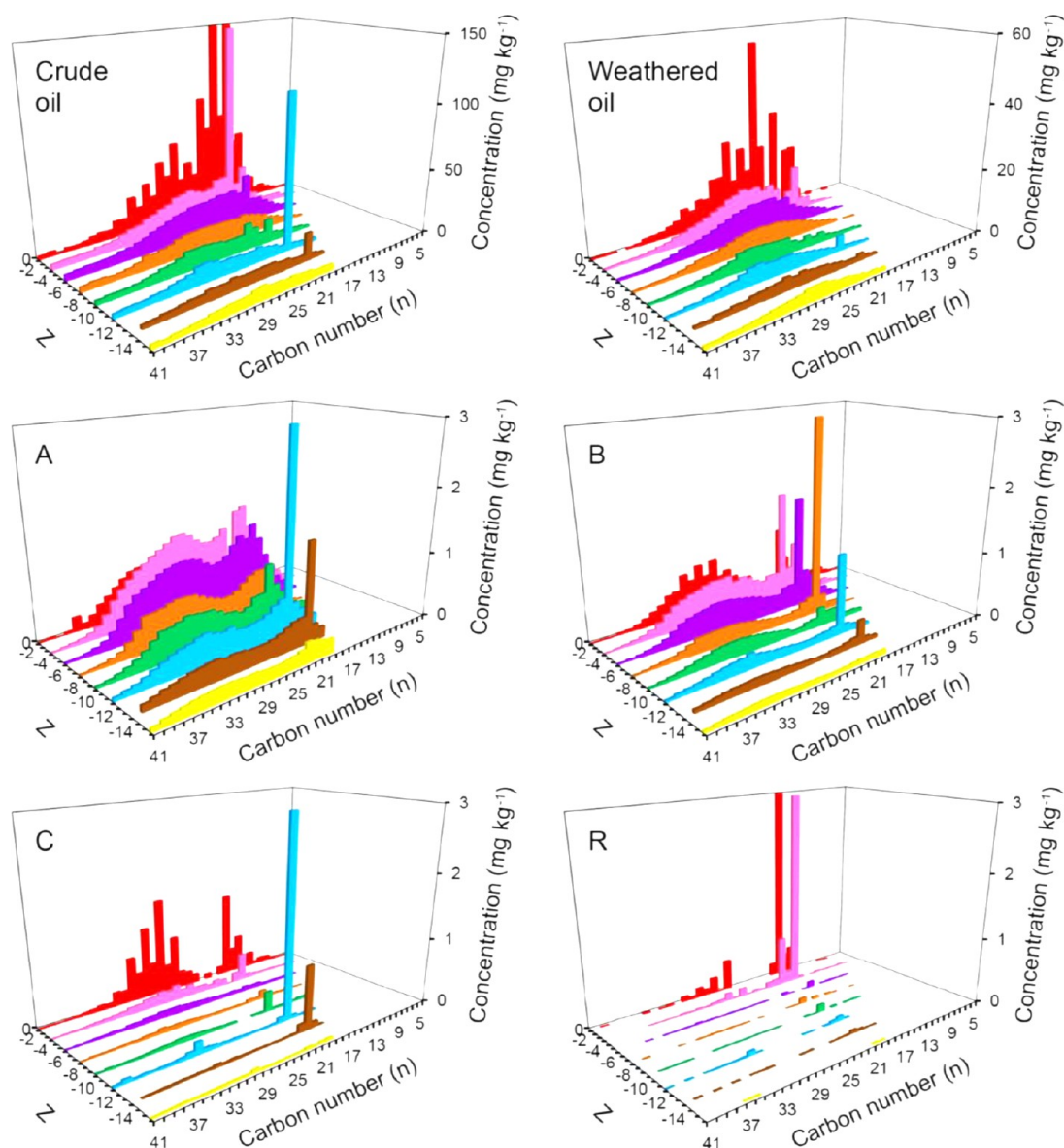
and oxy-NAs has not been fully assessed. In the present study, fourteen model NAs and three oxy-NAs representing a range of *n* and *Z* (SI Table S1) were applied to optimize the simultaneous analysis of NAs and oxy-NAs in sediment samples. By use of oscillation extraction with different solvents including hexane, MTBE, DCM, EA, and hexane/MTBE (1:1), relatively high extraction efficiencies for model NAs (38.1–130%) were achieved when extracted with hexane/MTBE (1:1), but very poor efficiencies (0–51.4%) were observed for model oxy-NAs (SI Table S3). Ultrasonic extraction with hexane/MTBE (1:1) further enhanced extraction efficiencies for target analytes, however extraction efficiencies of C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>, C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>, and C<sub>24</sub>H<sub>38</sub>O<sub>3</sub> were somehow limited to 23–46% (SI Table S3). Meantime, Soxhlet extraction with hexane/MTBE (1:1) for 24 h showed general improvements for most model compounds compared to oscillation and ultrasonic extractions, but it should also be noted that relatively low recoveries were still observed for C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>, C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>, C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>, and C<sub>24</sub>H<sub>38</sub>O<sub>3</sub>. The low efficiencies for low molecular weight NAs and oxy-NAs were possible due to the evaporative losses for volatile NAs during the extraction and concentration procedures.<sup>35</sup> Finally, sediment samples were Soxhlet extracted with hexane, MTBE, and methanol (1:1:1, v/v) for 24 h, and the recoveries of all model compounds including C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>, C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>, and C<sub>24</sub>H<sub>38</sub>O<sub>3</sub> improved greatly (69–121%, SI Table S3), suggesting that methanol could reduce the evaporative loss and/or enhance the extraction efficiencies of the target analytes.

Sample preparation was further developed for minimizing interferences before UPLC-QTOF-MS analysis of NAs and oxy-NAs in sediment extracts. A solid-phase extraction was used for purifying the extracts of sediment. The extracts were dissolved in methanol and diluted with water, which was then passed through the MAX cartridge. The nonacid interferences absorbed in the SPE cartridge due to hydrophobic sorption interactions were removed by a prewash of 6 mL methanol, thus the developed method was more specific for acids with low

matrix effects. The efficiency of the extraction and purification procedure was assessed by spiking the sediment sample (about 1 g dw) with standard solutions of model NAs and oxy-NAs (0.1 mg kg<sup>-1</sup> for each model compound). The absolute recoveries of all model compounds ranged from 65.5  $\pm$  1.3% to 101.2  $\pm$  6.9%, and the relative recoveries ranged from 74.9  $\pm$  1.4% to 129.4  $\pm$  3.3% using 1-pyrenebutyric acid (for NAs) and 12-oxochenodeoxycholic acid (for oxy-NAs) as surrogates (Table 1). To assess matrix effects of purification steps, the percentages of signal intensity of model NAs and oxy-NAs in extracts of spiked sediment samples (0.1 mg kg<sup>-1</sup>) versus the signal of the same concentration in pure solvent (methanol) were calculated. And relatively low signal suppression/enhancement for model NAs and oxy-NAs (-20–17% except for -27% with C<sub>24</sub>H<sub>40</sub>O<sub>2</sub>) were observed.

The optimized extraction and purification procedure coupled to UPLC-QTOF-MS method was applied to the analysis of crude oil, weathered oil, and sediment samples collected from the Taean areas, South Korea. The characteristic humps for each NA isomer class were observed in sediment extracts within the same retention time range as observed for the NAs in the commercial Acros mixtures, as showed for crude oil and sediment samples in Figure 1. Besides NAs, oxy-NAs with shorter retention time were also found in the samples. The MS/MS spectra of NAs and oxy-NAs with the same precursor ion with different *Z* values in MS/MS mode were shown in Figure S1 and Table S4 of the SI (e.g., precursor 323: C<sub>21</sub>H<sub>40</sub>O<sub>2</sub> (*Z* = -2), C<sub>20</sub>H<sub>36</sub>O<sub>3</sub> (*Z* = -4), C<sub>19</sub>H<sub>32</sub>O<sub>4</sub> (*Z* = -6)). Similar to the MS/MS fragmentations of oxy-NAs extracted from petroleum wastewater in our previous study,<sup>37</sup> only molecular ions ([M-H]<sup>-</sup>) were observed for NAs (C<sub>*n*</sub>H<sub>2*n*+*Z*</sub>O<sub>2</sub>), fragment ions of [M-H-H<sub>2</sub>O]<sup>-</sup> and [M-H-CO<sub>2</sub>]<sup>-</sup> were observed for C<sub>*n*</sub>H<sub>2*n*+*Z*</sub>O<sub>3</sub> species, and C<sub>*n*</sub>H<sub>2*n*+*Z*</sub>O<sub>4</sub> species generated one more fragment ion ([M-H-H<sub>2</sub>O-CO<sub>2</sub>]<sup>-</sup>) compared to C<sub>*n*</sub>H<sub>2*n*+*Z*</sub>O<sub>3</sub>, suggesting that oxy-NAs detected in the oil spill affected sediments also mainly composed of hydroxylated NAs or ketonic NAs. While no compound of C<sub>*n*</sub>H<sub>2*n*+*Z*</sub>O<sub>4</sub> was included as model oxy-NAs for O<sub>4</sub>-NAs detected in sediment, a recent study reported that the response factor of (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (C<sub>14</sub>H<sub>18</sub>O<sub>4</sub>) were similar with most model compounds of NAs and O<sub>3</sub>-NAs.<sup>42</sup> To our knowledge, this is the first study to report the occurrence of NA mixtures in sediments.

**Concentrations and Profiles of NA Mixtures.** Since sampling sites targeted in the present study were heavily contaminated areas of Taean coasts affected by the *Hebei Spirit* oil spill,<sup>8,18,33</sup> concentrations of NAs and oxy-NAs in target regions (A–C) were compared with background NA levels from distal open sea areas (reference sites R1 and R2). The geographical distributions of NAs and oxy-NAs are shown in Figure 2. Concentrations of NAs, O<sub>3</sub>-NAs, and O<sub>4</sub>-NAs in sediment samples from Taean coasts were 7.8–131, 3.6–44, and 0.8–20 mg kg<sup>-1</sup> dw, respectively, which were much greater than those in the reference sites in Manlipo (R1) (NAs: 18 mg kg<sup>-1</sup> dw, O<sub>3</sub>-NAs: 1.4 mg kg<sup>-1</sup> dw, and O<sub>4</sub>-NAs: 1.0 mg kg<sup>-1</sup> dw) and Anmyundo (R2) (NAs: 3.8 mg kg<sup>-1</sup> dw, O<sub>3</sub>-NAs: 0.5 mg kg<sup>-1</sup> dw, and O<sub>4</sub>-NAs: 0.3 mg kg<sup>-1</sup> dw). The greatest concentrations of NAs and oxy-NAs were found in Sinduri dune (sites A1–A4), and those of NAs, O<sub>3</sub>-NAs, and O<sub>4</sub>-NAs were 52–130, 4.2–44 and 1.8–12 mg kg<sup>-1</sup> dw, respectively. Relatively low concentrations of NAs and oxy-NAs were observed in the Sinduri mudflat (Sites B1–B4, NAs: 30–69 mg kg<sup>-1</sup> dw, O<sub>3</sub>-NAs: 3.9–11 mg kg<sup>-1</sup> dw, and O<sub>4</sub>-NAs: 0.9–20



**Figure 3.** Three-dimensional plots of concentrations ( $\text{mg}/\text{kg}$ ) versus carbon number ( $n$ ) and Z-series for NAs in extracts of crude oil, weathered oil, and surface sediment samples (A, B, C, and R) analyzed by UPLC-QTOF-MS. The NAs'  $n$  is from 5 to 41, and Z is from 0 to -14. MS/MS spectra of NAs,  $\text{O}_3$ -NAs, and  $\text{O}_4$ -NAs, with some predominant precursor ions shown in SI Figure S1.

$\text{mg kg}^{-1}$  dw), and lowest concentrations were found in Sogreni mudflat (Sites C1–C4, NAs:  $7.8$ – $58 \text{ mg kg}^{-1}$  dw,  $\text{O}_3$ -NAs:  $3.8$ – $11 \text{ mg kg}^{-1}$  dw, and  $\text{O}_4$ -NAs:  $1.7$ – $4.5 \text{ mg kg}^{-1}$  dw). The spatial distribution of NAs and oxy-NAs revealed that the average total concentrations of NA mixtures in oil spill affected regions (A–C) were found to be 3–11 folds greater than those in the reference sites, indicating the high impacts of the oil spill to the inner regions of Taean.<sup>8</sup> In Iranian Heavy Crude oil, one of spilled oils from *Hebei Spirit* oil spill, concentrations of NAs,  $\text{O}_3$ -NAs, and  $\text{O}_4$ -NAs were determined to be 5300, 1000, and 480 in  $\text{mg kg}^{-1}$  crude oil and 1000, 250, and 120  $\text{mg kg}^{-1}$  in weathered oil, respectively (Figure 2). A recent study observed the formation of oxygenated residues in sediment due to oil weathering after the *Deepwater Horizon* disaster, and biodegradation and photooxidation likely contributed to the oxygenation.<sup>23</sup> Since oxy-NAs were detected in the crude oil, oxy-NAs found in Taean sediments could be originated from the spilled oil and/or generated from the weathering processes

of NAs in environment. The occurrences of NA mixtures both in sediment and oil samples indicated that NAs together with oxy-NAs were persistent in the oil spill-affected areas even three to five years after the oil spill.

Three-dimensional plots of NAs in oil and sediment samples from four sites (A, B, C, and C) are shown in Figure 3. For the crude oil sample, the most abundant ions were for the  $Z = 0$  series (56%), followed by  $Z = -2$  (13%),  $Z = -4$  (9.4%),  $Z = -6$  and  $Z = -10$  (5.9%),  $Z = -8$  (4.9%),  $Z = -12$  (3.0%),  $Z = -14$  (2.4%), and the most abundant ions for the weathering oil sample were  $Z = 0$  series (32%), followed by  $Z = -2$  (17%),  $Z = -4$  (15%),  $Z = -6$  (9.8%),  $Z = -8$  (8.1%),  $Z = -10$  (7.4%),  $Z = -12$  (5.3%), and  $Z = -14$  (4.9%). The profiles with relatively high abundance of NAs with 16–35 carbons were different from those of the oil sand extraction waters in Canada or China, in which the most abundant compounds were one to three ring NAs with 11–16 carbons.<sup>12,35–38</sup> In the reference site (R), the most abundant ions were  $Z = -2$ ,  $n = 16$  (36%),

which is consistent with the profile of background natural fatty acids with abundant C16, C18, and C20 unsaturated fatty acids previously reported.<sup>28</sup> It is interesting to note that although the abundant NAs in crude oil and weathered oil were compounds with  $n = 18-30$ , the sedimentary profile of oil-derived NAs and background NAs centered around compounds with  $n = 21-35$  and  $n = 12-21$ , respectively, suggesting that  $\text{NAs}_{n=21-35}$  were relatively persistent in sediment and could be potential oil-originated indicators for oil contamination. In sediment samples collected in oil contaminated sites of A, B, and C,  $\text{NAs}_{n=21-35}$  were detected with relatively high abundance (49.4–64.4% of total NAs), and the oil-derived NAs with  $Z = -14$  to  $-4$  and  $n = 25$  to  $35$  (14.7–38.5% of total NAs) were mostly under detection limit in the reference sites. The profile of oil derived NAs in sediment samples from oil spill-affected area further confirmed that the crude-derived NA mixtures originated from the Taean spill were persistent in the oil spill-affected coasts.

For understanding the potential impacts of NAs in oil spill affected sediments, the concentrations of PAHs were also measured for all the sediment samples, and we found that the total PAHs concentrations (0.077–2.5 mg kg<sup>-1</sup> dw) were 50–100 times lower than those of NAs (7.8–130 mg kg<sup>-1</sup> dw) in the area. Considering the high solubility of NAs compared to those of PAHs, concentrations of NAs in water samples would be much higher than those of PAHs in oil spill affected areas. It has been reported that NAs can cause hatching deformities (optic-cephalic abnormalities, spinal deformities, and circulatory distress) of yellow perch (*Perca flavescens*) and Japanese medaka (*Oryzias latipes*) at 1–10 mg L<sup>-1</sup>.<sup>43</sup> And embryo exposure to individual PAH isomers at similar concentrations (1 mg L<sup>-1</sup> for pyrene, 2 mg L<sup>-1</sup> for chrysene and 10 mg L<sup>-1</sup> for naphthalene, fluorene, dibenzothiophene, phenanthrene, and anthracene) resulted in a characteristic suite of abnormalities including cardiac dysfunction, edema, spinal curvature, and reduction in the size of the jaw and other craniofacial structures in zebrafish fish embryos.<sup>44</sup> Thus, the large-scale releases of water-soluble NAs would also pose a high exposure risks to aquatic organisms (e.g., fish) in the areas. These findings would also help to explain the deformations of fish embryos observed after exposure to crude oil or field-collected sediments in oil spilled areas (yolk-sac edema and cardiac morphology),<sup>10,11</sup> since the toxic effects of an oil spill were reported to be not exclusively associated with PAHs based on in vivo study with Japanese medaka (*Oryzias latipes*).<sup>9</sup> Therefore, the ecological risks of NAs to aquatic organisms in oil spill affected areas deserve more attention.

**Weathering Characteristics of NA Mixtures.** The spilled oil in marine environment is subject to change through weathering processes, such as evaporation and biodegradation, etc., and the processes ultimately determine the fates and impacts of oil-derived pollutants.<sup>8</sup> In the present study, the applied weathering method mainly reflected the consequences of evaporation. The relative percentages of compounds with  $Z$  values of  $-2$ ,  $-4$ ,  $-6$ ,  $-8$ ,  $-10$ ,  $-12$ , and  $-14$  were very constant between crude oil and weathered oil, the percentage for  $Z = 0$  series decreased from 56% in crude oil to 45% in weathered oil (Figure 3). And the percentages of low molecular weight  $\text{NAs}_{n=5-20}$  also decreased from 63% to 46% after the weathered processes, suggesting that acyclic  $\text{NAs}_{n=5-20}$  were relatively easy to be evaporated compared to cyclic  $\text{NAs}_{n=21-41}$ . Preliminary investigations about acidic compounds in crude oil and sediments from biodegraded reservoir also found that low

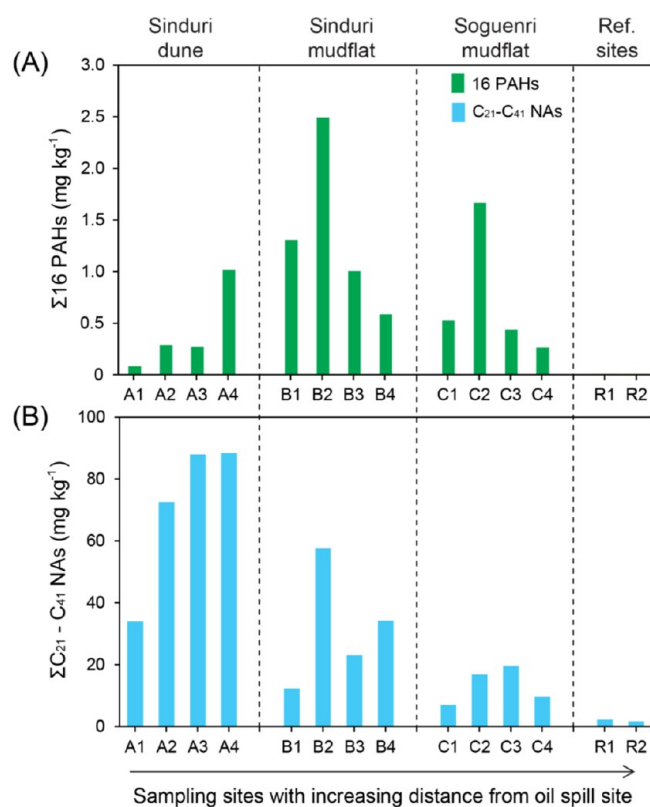
molecular weight  $\text{NAs}_{n=10-20}$  were rapidly biodegraded compared with higher molecular weight acids ( $>C20$ ), and cyclic acids were more recalcitrant than acyclic acids.<sup>31,40,41</sup> Kim et al.<sup>45</sup> proposed a biodegradation index to estimate the biodegradation degree of oil based on the ratio of acyclic  $\text{NAs}_{Z=0}$  to cyclic  $\text{NAs}_{Z=-4,-6,-8}$  (eq 1), and the  $A/C$  ratio decreased as the biodegradation degree increased.<sup>41,45</sup>

$$A/C \text{ ratio} = \frac{\sum \text{NAs}_{Z=0}}{\sum \text{NAs}_{Z=-4,-6,-8}} \quad (1)$$

In this study, the  $A/C$  ratio was found to be 2.76 and 0.95 in the crude and weathered oil, respectively. The results indicated that composition changes of NAs in oil were similar, either due to the biodegradation or evaporation. The  $A/C$  ratio was then used to assess the weathering degree of residual crude oil in surface sediments (A, B, and C sites). Relatively high  $A/C$  ratio were found in site C (1.46–3.66) compared to those in site A (0.18–0.53) and B (0.31–0.53), suggesting that samples from Sinduri dunes and Sinduri mudflats were more weathered than those of Sogenri mudflats. Consistently, conventional methods using the C2 Phe+Ant/C3 Phe+Ant ratios, which were adapted from Sauer et al. to estimate the weathering degree of crude oil in sediment,<sup>18,46</sup> were relatively high in sediments from site C ( $1.07 \pm 0.27$ ) compared to those from site A ( $1.03 \pm 0.02$ ) and B ( $0.90 \pm 0.26$ ). Altogether, the results indicated that samples collected inside the Taean area were slightly weathered compared to those along the beach, which was consistent with the low weathering degree inside the enclosed bay previously reported.<sup>18</sup>

Oxy-NAs, as a potential marker for the degradation of NAs in the environment, were also applied to the assessment of weathering characteristics of NAs in the given area. Due to the natural sources of  $\text{NAs}_{n=5-20}$ , the ratios of oxy- $\text{NAs}_{n=21-41}$  relative to  $\text{NAs}_{n=21-41}$  ( $(\text{O}_3-\text{NAs}_{n=21-41} + \text{O}_4-\text{NAs}_{n=21-41}) / \text{NAs}_{n=21-41}$ ) were calculated. The ratios decreased from 0.64 in crude oil to 0.47 in weathered oil, suggesting that oxy- $\text{NA}_{n=21-41}$  were weathered with a rapid rate than  $\text{NAs}_{n=21-41}$ . In sediment samples, the ratios in samples collected from site C ( $0.55 \pm 0.06$ ) were higher than those in samples at sites A ( $0.28 \pm 0.15$ ) and B ( $0.26 \pm 0.20$ ), suggesting the slowest weathered degree in the samples from site C, which is consistent with the results obtained by  $A/C$  ratio, as discussed above. Thus the weathering processes could contribute to the relatively low percentage of oxy- $\text{NAs}_{n=21-41}$  in sediments inside the Taean area, and  $(\text{O}_3-\text{NAs}_{n=21-41} + \text{O}_4-\text{NAs}_{n=21-41}) / \text{NAs}_{n=21-41}$  could be a new index to estimate the weathering degree of oil in environment.

**Potential Indicator of Oil Contamination.** To evaluate the extent of oil contamination after an oil spill, conventional monitoring programs generally measured the concentrations of PAHs, the hydrophobic components of oil.<sup>16-18</sup> The greatest concentrations of PAHs were found in samples collected in sites B (0.58–2.5 mg kg<sup>-1</sup> dw), and relatively low concentrations of PAHs were detected in sites A (0.077–1.0 mg kg<sup>-1</sup> dw) and C (0.26–1.6 mg kg<sup>-1</sup> dw) (Figure 4a). The concentrations of PAHs did not show any trend with the increasing distances from the location of the Hebei Spirit oil spill. Relatively great concentrations of PAHs in site B were possibly due to high TOC content in sediment samples from the corresponding site, since the TOC-corrected concentrations in site B were similar to those in other locations (SI Table S2). Meanwhile, the present study detected NAs with the



**Figure 4.** The concentration of (A) PAHs and (B) NAs<sub>n=21-41</sub> in sediment samples. The NAs' *n* is from 21 to 41, and *Z* is from -2 to -8 (bicyclic to tetracyclic/monaromatic NA).

greatest concentrations in sediment samples affected by the Hebei Spirit Oil Spill, and profile of oil-derived NAs centered around compounds with  $n = 21-35$ , of which compounds were relatively recalcitrant in the weathering processes. The result indicated that NAs with carbon numbers of 21-41 could be a potential indicator for long-term monitoring and environmental management of oil spill-affected areas. After the exclusion of the natural NAs with carbon number <20, the total concentrations of NAs with carbon numbers of 21-41 showed a distinct decreasing trend with increasing distance from the oil spill sites (Figure 4b). While the type of sediment samples collected in sites A1-A4 was sand with low TOC content, TOC corrected concentrations of NAs in site A were still significantly higher than those in other sites (Figure 4b), suggesting that hydrophobic interactions would not be the major factor leading to the high sorption of NAs in sediment. It has been reported that positive charges in sediment could originate either from rupture of planes of the structural units and the resulting edge charges or of iron and aluminum oxides.<sup>47</sup> Therefore, negatively charged NAs would be absorbed by the positive charges in the sediment through anion exchange, since  $pK_a$  values for naphthenic acids range from 5 to 6 and the pH of seawater is generally in the range of 7.5-8.4.<sup>48</sup> Therefore,  $\sum NAs_{n=21-41}$  could be an indicator for spatial distributions of oil-derived pollutants. Of NAs<sub>n=21-41</sub>, the predominant compounds were found to be NAs with  $Z = -8$ - $-4$  and  $n = 26-30$ . While many ions of NAs can be simultaneously detected by QTOF-MS compared with quadrupole mass, some predominant isomers of NAs<sub>Z=-8-4, n=26-30</sub> could be selectively determined by LC-MS or derivatization coupled to GC-MS for routine quantifications of the chemicals in environmental sample, and detection of the

selected compounds would be an indication of oil contamination in investigated samples.

Overall, a sensitive method was established for simultaneous determination of oxy-NAs, together with NAs in sediment samples. NAs and oxy-NAs were detected in sediment samples from the Hebei Spirit oil spill-affected areas with similar profiles with those in the spilled crude oil. Much higher concentrations of NAs compared to PAHs in sediment samples from the areas indicated the potential impacts of NAs and thus more attention is deserved for other types of pollutants in oil spill affected areas. Acyclic NAs<sub>n=5-20</sub> were easily degraded compared to cyclic NAs<sub>n=21-41</sub> after weathering processes, and the ratio of oxy-NAs<sub>n=21-41</sub> relative to NAs<sub>n=21-41</sub> could be a new index to estimate the weathering degree of oil in environment. The total concentrations of persistent oil-derived NAs<sub>n=21-41</sub>, which do not appear to be affected by the properties of the sediments, can be used as a potential indicator for evaluating oil contamination along coasts.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Figures, and tables addressing (1) name and structures of model compounds; (2) description, location, sediment types, and some information regarding sampling sites in the Taeon area, South Korea; (3) comparisons of recoveries of model compounds in different extraction methods; (4) MS/MS fragment ions of NAs, O<sub>3</sub>-NAs, and O<sub>4</sub>-NAs in MS/MS mode of QTOF-MS; and (5) MS/MS spectra of NAs, O<sub>3</sub>-NAs and O<sub>4</sub>-NAs with precursor ions of 227, 323, and 387 in sediment extract. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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## Supporting Information

for

### **Naphthenic Acids in Coastal Sediments after the *Hebei Spirit* Oil Spill: A Potential Indicator for Oil Contamination**

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Table S1. Name and structure of model compounds in UPLC-QTOF-MS analysis.

Name	CAS No.	Molecular Weight	Structure
12-hydroxysteric acid	106-14-9	$C_{12}H_{24}O_3$ , MW= 300.2664	
12-hydroxydodecanoic acid	505-95-3	$C_{18}H_{36}O_3$ , MW= 216.1725	
12-oxochenodeoxycho lic acid	2458-08-4	$C_{24}H_{38}O_5$ , MW= 406.2719	
2-hexyldecanoic acid	25354-97-6	$C_{16}H_{32}O_2$ , MW= 256.2402	
cyclohexanecarboxylic acid	98-89-5	$C_7H_{12}O_2$ , MW= 128.0837	
1-Methyl-1-cyclohexane carboxylic acid	1123-25-7	$C_8H_{14}O_2$ , MW= 142.0994	
4-n-propylcyclohexanecarboxylic acid ( <i>cis</i> - and <i>trans</i> - mixture)	943-29-3	$C_{10}H_{18}O_2$ ( <i>cis</i> - and <i>trans</i> -), MW= 170.1307	
<i>trans</i> -4-tert-butylcyclohexanecarboxylic acid	5962-88-9	$C_{11}H_{20}O_2$ -butyl, MW= 184.1463	
cyclohexane pentanoic acid	943-29-3	$C_{11}H_{20}O_2$ , MW= 184.1463	
<i>trans</i> -4-pentylcyclohexane carboxylic acid	38289-29-1	$C_{12}H_{22}O_2$ , MW= 198.1620	
1,2,3,4-tetrahydro-2-naphthoic acid	53440-12-3	$C_{11}H_{12}O_2$ , MW= 176.0837	

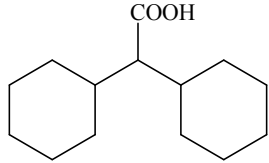
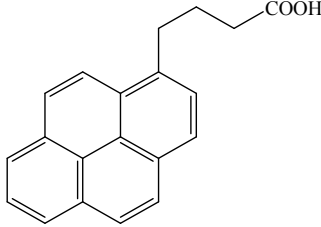
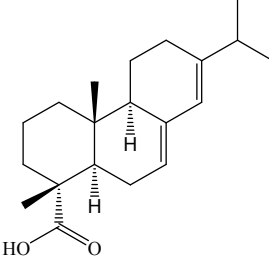
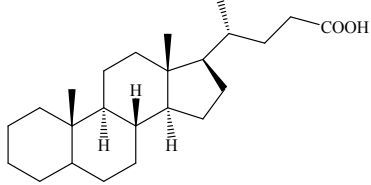
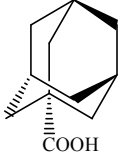
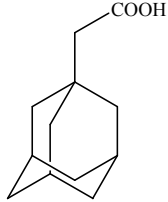
dicyclohexylacetic acid	52034-92-1	$C_{14}H_{24}O_2$ MW= 224.1776	
1-pyrenebutyric acid	3443-45-6	$C_{20}H_{16}O_2$ MW= 288.1150	
abietic acid	514-10-3	$C_{20}H_{30}O_2$ MW= 302.2246	
5-beta-cholanic acid	546-18-9	$C_{24}H_{40}O_2$ MW= 360.3028	
1-adamantane carboxylic acid	828-51-3	$C_{11}H_{16}O_2$ MW= 180.1150	
1-adamantaneacetic acid	4942-47-6	$C_{12}H_{18}O_2$ MW= 194.1307	

Table S2. Description of sampling sites and sediment samples including sediment type, water content, and TOC (total organic carbon), collected in Taean oil spill area, Korea.

Sites	Locations	Latitude (°N)	Longitude (°E)	Sediment types	Water contents (%)	TOC (%)
A1	Sinduri Dune	36° 50'4"	126° 10'45"	Sand	22.4	1.22
A2	Sinduri Dune	36° 50'6"	126° 10'48"	Sand	22.9	3.15
A3	Sinduri Dune	36° 50'5"	126° 10'46"	Sand	21.0	3.64
A4	Sinduri Dune	36° 50'3"	126° 10'44"	Sand	19.0	8.54
B1	Sinduri Mudflat	36° 49'18"	126° 11'12"	Mud	37.8	21.63
B2	Sinduri Mudflat	36° 49'20"	126° 11'13"	Mud+gravel	21.0	3.91
B3	Sinduri Mudflat	36° 49'22"	126° 11'14"	Mud+gravel	27.3	7.64
B4	Sinduri Mudflat	36° 49'24"	126° 11'15"	Mud+gravel	32.6	5.88
C1	Sogenri Mudflat	36° 49'9"	126° 10'17"	Mud	27.9	4.89
C2	Sogenri Mudflat	36° 49'8"	126° 10'15"	Mud	32.9	4.98
C3	Sogenri Mudflat	36° 49'6"	126° 10'14"	Mud	30.8	4.54
C4	Sogenri Mudflat	36° 49'5"	126° 10'13"	Mud	32.8	4.39
R1	Manlipo beach	36° 47'11"	126° 8'4"	Sand	5.4	0.27
R2	Anmyundo beach	36° 31'30"	126° 19'48"	Sand	4.4	0.38

Table S3. Efficiency of different extraction solutions and methods to model compounds of NA and oxy-NA from sediment samples (about 1 g dried sediment spiked with 0.1 µg of standard for each compounds).

Chemicals	Oscillation extraction				Ultrasonic extraction			Soxhlet extraction		
	Hex×3	MTBE×3	DCM×3	EA×3	Hex/MTBE (1:1)×3	Hex/MTBE (1:1)×3	Hex/MTBE (1:1), 2% FA×3	Hex/MTBE (1:1)	Hex/MTBE/ACN (1:1)	Hex/MTBE/MeOH (1:1)
C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	82.5	86.4	81.5	59.9	109.8	106 ± 6.0	104 ± 1.4	106 ± 5.7	100 ± 8.5	113 ± 12
C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>	16.9	41.5	32.9	10.8	44.3	42 ± 9.7	46 ± 3.6	49 ± 3.3	54 ± 37	84 ± 5.1
C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	44.0	50.0	49.4	25.6	66.6	36 ± 12	45 ± 0.4	66 ± 2.2	56 ± 51	88 ± 8.0
C <sub>10</sub> H <sub>18</sub> O <sub>2</sub> <sup>a</sup>	49.0	58.2	41.2	21.6	68.3	71 ± 3.0	87 ± 9.2	79 ± 7.7	78 ± 30	88 ± 1.3
C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	57.5	68.4	47.8	22.9	79.4	93 ± 9.8	100 ± 8.1	78 ± 10	81 ± 25	91 ± 2.9
C <sub>11</sub> H <sub>20</sub> O <sub>2</sub> -butyl	49.1	63.1	49.3	15.1	63.6	78 ± 4.3	89 ± 2.8	74 ± 8.0	81 ± 19	98 ± 14
C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>	58.0	68.6	44.8	14.0	71.6	86 ± 16	96 ± 7.1	73 ± 4.2	82 ± 15	92 ± 9.5
C <sub>11</sub> H <sub>12</sub> O <sub>2</sub>	9.3	59.5	22.5	8.1	38.1	94 ± 8.6	90 ± 7.1	83 ± 4.3	83 ± 15	102 ± 9.5
C <sub>14</sub> H <sub>24</sub> O <sub>2</sub>	85.0	95.0	84.4	63.9	130.0	105 ± 12	114 ± 0.7	63 ± 8.2	93 ± 30	102 ± 2.7
C <sub>20</sub> H <sub>16</sub> O <sub>2</sub>	2.5	72.1	18.0	5.9	23.6	71 ± 15	75 ± 8.3	95 ± 8.5	84 ± 13	108 ± 8.1
C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>	66.6	84.4	67.9	35.4	93.9	107 ± 1.6	93 ± 5.9	52 ± 9.1	70 ± 1.6	121 ± 30
C <sub>24</sub> H <sub>40</sub> O <sub>2</sub>	70.4	62.0	50.2	25.9	79.9	97 ± 1.7	80 ± 12	79 ± 18	70 ± 3.8	69 ± 1.1
C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	52.1	69.7	44.5	21.5	71.5	81 ± 1.2	84 ± 2.4	60 ± 1.0	79 ± 26	90 ± 3.1
C <sub>12</sub> H <sub>18</sub> O <sub>2</sub>	55.9	72.7	61.6	31.2	80.9	78 ± 0.2	87 ± 5.7	79 ± 10	77 ± 18	85 ± 3.7
C <sub>24</sub> H <sub>38</sub> O <sub>5</sub>	0.0	0.7	0.5	0.3	0.3	37 ± 9.3	23 ± 0.2	0.5 ± 0.2	28 ± 2.6	88 ± 8.1
C <sub>18</sub> H <sub>36</sub> O <sub>3</sub>	9.0	51.4	34.5	12.9	43.6	99 ± 8.0	101 ± 4.0	67 ± 8.7	82 ± 15	101 ± 3.1
C <sub>12</sub> H <sub>24</sub> O <sub>3</sub>	0.2	39.1	18.0	5.2	18.0	88 ± 1.4	90 ± 0.5	33 ± 2.5	64 ± 11	87 ± 9.3

Table S4. Precursors, MS/MS fragment ions and retention time of NAs, O<sub>3</sub>-NAs and O<sub>4</sub>-NAs generated in MS/MS mode of QTOF-MS in sediment samples from Taean coasts.

Precursor ion	Collision energy (eV)	Retention time (min)	Compound	Z	Mass fragment ions			
					[M-H] <sup>-</sup>	[M-H-H <sub>2</sub> O] <sup>-</sup>	[M-H-CO <sub>2</sub> ] <sup>-</sup>	[M-H-H <sub>2</sub> O-CO <sub>2</sub> ] <sup>-</sup>
227	15-25	10.5-11.8	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	0	227.2015 (1.8 ppm)	-	-	-
		5-8	C <sub>13</sub> H <sub>24</sub> O <sub>3</sub>	-2	227.1637 (-4.4 ppm)	209.1549 (3.3 ppm)	183.1741 (-4.4 ppm)	-
		4-5	C <sub>12</sub> H <sub>20</sub> O <sub>4</sub>	-4	227.1274 (-4.0 ppm)	209.1172 (-2.9 ppm)	183.1401 (8.7 ppm)	165.1284 (3.0 ppm)
323	20-30	13-14.4	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	-2	323.2944 (-1.9 ppm)	-	-	-
		10-13	C <sub>20</sub> H <sub>36</sub> O <sub>3</sub>	-4	323.2580 (-1.9 ppm)	305.2482 (0.3 ppm)	279.2680 (-2.9 ppm)	-
		6-9	C <sub>19</sub> H <sub>32</sub> O <sub>4</sub>	-6	323.2234 (3.7 ppm)	305.2119 (0.7 ppm)	279.1761 (4.3 ppm)	261.2231 (5.0 ppm)
293	15-25	12-14	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	-4	293.2478 (-1.0 ppm)	-	-	-
		8-11.4	C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>	-6	293.2114 (-1.0 ppm)	275.2003 (-2.9 ppm)	249.2215 (-1.2 ppm)	-
		5.8-6.4	C <sub>17</sub> H <sub>26</sub> O <sub>4</sub>	-8	293.1755 (0.7 ppm)	275.1635 (-4.6 ppm)	249.1847 (-3.2 ppm)	231.1750 (0.4 ppm)
291	20-30	12-14	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	-6	291.2320 (-1.4 ppm)	-	-	-
		7-11	C <sub>18</sub> H <sub>28</sub> O <sub>3</sub>	-8	291.1963 (1.0 ppm)	273.1844 (-4.0 ppm)	247.2065 (1.2 ppm)	-
		2-3	C <sub>17</sub> H <sub>24</sub> O <sub>4</sub>	-10	291.1589 (-2.4 ppm)	273.1511 (7.3 ppm)	247.1682 (-6.5 ppm)	229.1585 (-3.1 ppm)
387	20-30	13.5-15	C <sub>26</sub> H <sub>44</sub> O <sub>2</sub>	-8	387.3256 (-1.8 ppm)	-	-	-

	11-13	C <sub>25</sub> H <sub>40</sub> O <sub>3</sub>	-10	387.2888 (-2.8 ppm)	369.2748 (-12.5 ppm)	343.2984 (-5.0 ppm)	-
	8-9	C <sub>24</sub> H <sub>36</sub> O <sub>4</sub>	-12	387.2550 (3.9 ppm)	369.2422 (-2.2 ppm)	343.2621 (-4.7 ppm)	325.2518 (-4.0 ppm)
	14-15.5	C <sub>31</sub> H <sub>52</sub> O <sub>2</sub>	-10	455.3882 (-1.5 ppm)	-	-	-
455	20-30	OH-C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	-12	455.3529 (0.9 ppm)	437.3440 (4.6 ppm)	411.3616 (-2.7 ppm)	-
	10-12	C <sub>29</sub> H <sub>44</sub> O <sub>4</sub>	-14	455.3170 (2.0 ppm)	437.3051 (-1.1 ppm)	411.3263 (0.0 ppm)	393.3147 (-2.5 ppm)

Mass errors of fragmentation ions of 183.1401, 369.2748, 273.1511, 247.1682 were higher than 5 ppm possible due to the low abundance of the compounds.

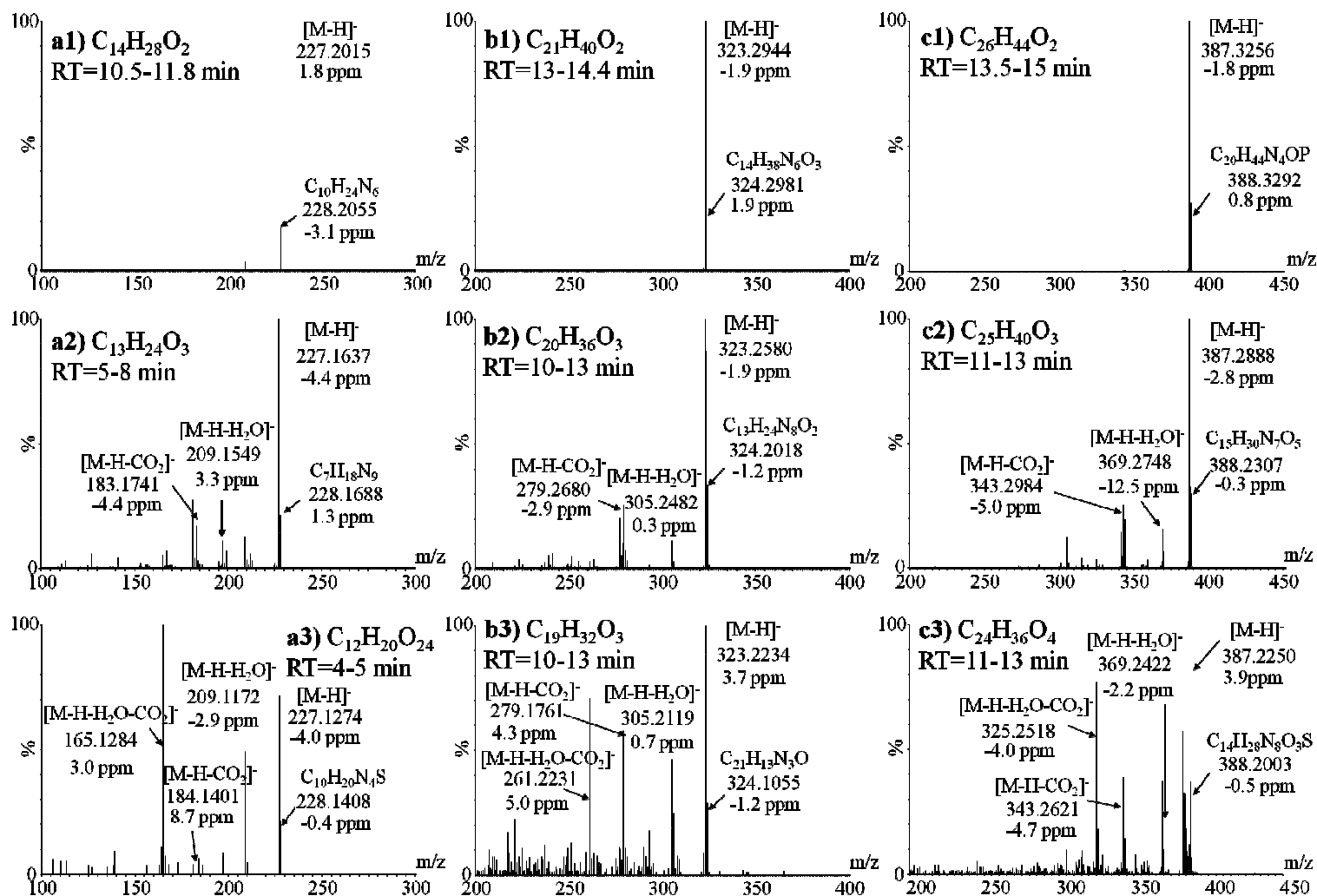


Figure S1. MS/MS spectra of NAs, O<sub>3</sub>-NAs and O<sub>4</sub>-NAs with precursor ions of 227 (a1-a3), 323 (b1-b3) and 387 (c1-c3) in extracts of sediment.