



Molecular level determination of water accommodated fraction with embryonic developmental toxicity generated by photooxidation of spilled oil[☆]

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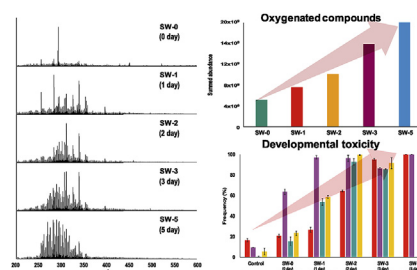
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HIGHLIGHTS

- Molecules generated by photooxidation and contributing to toxicity were identified.
- Polar compounds detected by UHR-MS were increased x 4 in 5 day of photooxidation.
- Toxicity was greatly increased as oils were photodegraded under natural sunlight.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, developmental toxicity was increased as the oil was further degraded under natural sunlight. Detailed chemical composition of the degraded oils was examined by use of gas chromatography (GC) and (–) electrospray ionization ultrahigh resolution mass spectrometry (UHR-MS). Baseline toxicities were estimated based on chemical activities of polycyclic aromatic hydrocarbons, and it was obvious that the predicted chemical activities can not explain increased toxicity alone. However, the ultrahigh resolution mass spectral abundance of polar compounds including O₃ and O₄ class compounds was significantly increased as the photodegradation proceeded. Further examination of double bond equivalence values of the compounds showed that polar compounds with both non-aromatic and aromatic polar structures were increased. Statistical analysis indicates that the increased toxicity can be well

Abbreviations: water-accommodated fraction, (WAF); water-soluble fraction, (WSF); polycyclic aromatic hydrocarbons, (PAHs); gas chromatograph, (GC); flame-ionization detector, (FID); ultrahigh resolution mass spectrometry, (UHR-MS); electrospray ionization, (ESI); dichloromethane, (DCM); double bond equivalent, (DBE); pericardial edema, (PE); tailfin fold defect, (TF); dorsal body axis curvature, (DC); partial least-squares regression, (PLSR).

[☆] Comprehensive study was performed on molecular level identification of compounds in photodegraded oils and evaluation of their embryonic developmental toxicity. Ultrahigh resolution mass spectral abundance of polar oxygenated compounds and embryonic developmental toxicity were increased as oils were further photodegraded under natural sunlight. Partial least squares regression analysis supported the correlation between the abundance of the observed compounds and the toxicity. This work can contribute to improve our understanding on the toxic potency of spilled oil based on detailed chemical information.

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1. Introduction

Despite the ongoing effort to reduce the usage of fossil fuels, petroleum is still one of the most important energy sources for heating, transportation, and electricity generation. It is also an important source for polymers that are heavily used in modern society. Therefore, petroleum is extracted on a large scale and constantly transported throughout the world. This increases the risk of large- and small-scale oil spills. As vividly evidenced by the Deepwater Horizon oil leak, such spills significantly endanger the environment (White et al., 2012; Yim et al., 2012).

A number of studies have proven the toxic effect of spilled oils on marine lives (Neff et al., 2000; Fisher et al., 2016). The spilled oils can be degraded and transformed into different forms, including the water-accommodated fraction (WAF) and water-soluble fraction (WSF). The toxicity of these photo-oxidized products is well documented (Lee, 2003; González et al., 2009; Khursigara et al., 2017). The toxicity of crude oil has been attributed to the aromatic compounds in it (Incardona et al., 2013; Esbaugh et al., 2016). For example, Carls et al. reported damages to fish embryos from dissolved PAHs (Carls et al., 2008), and Esbaugh et al. explained the toxicity of oil based on three-ring PAHs (Esbaugh et al., 2016).

However, a number of reports suggest that there are limitations in explaining the toxicity of weathered oil by aromatic compounds alone. Barron et al. observed that highly toxic oils could have low aromatic contents (Barron et al., 1999). Saco-Álvarez reported that hydroxyl PAHs such as pyrenol and phenanthrol were more toxic than the original oils (Saco-Álvarez et al., 2008). Neff et al. found that the toxicity of WAF in some oils was higher than prediction based on the concentrations of aromatic compounds, and suggested that the unresolved complex mixture and polar compounds could contribute to the toxicity (Neff et al., 2000). Bellas et al. observed the differences in toxicity between fresh and weathered fuels that could not be explained by the aromatic hydrogen content, and concluded that the formation of oxidized compounds plays an important role in the toxicity to the larvae of sea urchins *Paracentrotus lividus* (Bellas et al., 2013). In addition, the toxicity of Canadian oil sands has been attributed to naphthenic acid (Bartlett et al., 2017).

From these previous studies, it is clear that polar compounds generated during the weathering process can play an important role in the toxicity of spilled oils. However, the exact role of polar compounds in the degraded oils' toxicity has not been known. One of the reasons for the lack of information can be attributed to the fact that molecular-level information of these polar compounds is scarce (Bartlett et al., 2017). Of the few studies directly connecting the toxicity to the molecular level analysis of the polar compounds, Melbye et al. found that the toxicity of degraded oil was mainly attributed to the most polar fraction, which was rich in sulfoxide compounds based on GC and comprehensive 2D GC-MS analyses (Melbye et al., 2009).

However, GC is limited in identifying polar compounds, and the ultrahigh resolution mass spectrometry (UHR-MS) technique can expand our knowledge about those compounds. UHR-MS has played an important role in characterizing complex mixtures such as crude oils and natural organic matter at the molecular level (Cho et al., 2015; Kim et al., 2019). Negative mode electrospray ionization

((−) ESI) coupled to UHR-MS has been widely used to study polar compounds generated during the weathering process of oils (Hughey et al., 2007; Islam et al., 2013, 2015, 2016; Vaughan et al., 2016).

In this study, the toxicity of water-accommodated fraction from an oil spill was examined, and (−) ESI UHR-MS and GC-MS were applied to identify these compounds at the molecular level. As far as we know, this is the first study combining the comprehensive analysis of PAHs and polar oxygenated compounds using GC-MS and UHR-MS and the evaluation of their toxicity.

2. Materials and methods

2.1. Sample preparation

On April 3, 2014, an oil spill occurred in the Jangmok Bay, Geoje, Korea. The spiller is not known, but the spilled oil was determined to be diesel oil, based on GC-MS analysis data. The carbon range in the collected spilled oil was C₉–C₃₀, typical of diesel oil (Fig. S1). Pictures of the oil sampling site are provided in Supporting Information (Fig. S2). The spilled oil and seawater were sampled and transferred to 2 L glass bottles with a stopcock on the bottom. The reservoir bottles were placed on the rooftop as shown in Fig. S3, and subjected to photodegradation by sunlight for five days. Seawater was obtained each day from each bottle through the stopcock. The prepared samples were named as "seawater exposed to spilled oil" (SW) and listed in Table 1.

Dichloromethane (DCM) extracts of water column for chemical analysis were obtained by extracting 0.87–1.00 L of seawater sample with 50 mL of DCM. The extraction was repeated for three times for each sample. The obtained fractions were combined in 250 mL round bottom flask, concentrated with a vacuum evaporator (Turbo Storm, SCINCO, Korea), and then transferred to 2 mL vials. The remaining solvent was eliminated under a stream of nitrogen.

2.2. Mass spectrometry analysis

A 15T FT-ICR MS system (Bruker Daltonics, Billerica, MA) at the Korea Basic Science Institute (KBSI, Ochang-eup, Korea) was used in the MS measurements. The sample solutions were infused at a rate of 250 µL/h in the ESI negative mode after dilution with 200 µL of methanol:toluene:dichloromethane (2:1:1, v/v) solution. MS spectra were acquired from *m/z* 180–1700 with a 4 MW transient size, resulting in a resolving power of approximately 400,000 at *m/z* 400. A total of 100 time-domain transients were summed for each sample to enhance the signal-to-noise ratio. The capillary voltage was set at 4800 V, and other experimental conditions were as follows: nebulizer gas pressure 1.0 bar, drying gas temperature 250 °C, drying gas flow rate 3.5 L/min, and ion accumulation time 0.230 s.

The obtained MS data were recalibrated using an internal standard (Agilent ESI tune mix, Agilent Technologies, Santa Clara, CA, USA) in Compass DataAnalysis 4.4 software (Bruker Daltonik GmbH, Germany). After mass calibration, peaks were assigned to chemical formulae within 1 ppm error with the in-house developed software. Spectral interpretation was performed using the Statistical Tool for Organic Mixtures' Spectra (STORMS 1.0) software with

Table 1

Sample information and abbreviation used in this study.

seawater exposed to spilled oil		
oxidation duration (day)	Condition of solar exposure	Sample name
0	Little cloudy	SW-0
1	Clear sky	SW-1
2	Little cloudy	SW-2
3	Clear sky	SW-3
5	Clear sky	SW-5

an automated peak-picking algorithm for more reliable and faster results (Hur et al., 2009; Lee et al., 2014). The conditions for SW samples analysis were as follows: $C_cH_hN_nO_oS_s$; c unlimited, h unlimited, $0 \leq n \leq 5$, $0 \leq o \leq 20$, and $0 \leq s \leq 5$. Double bond equivalent (DBE) values were calculated using Equation (1).

$$DBE = c - \frac{h}{2} + \frac{n}{2} + 1 \quad (1)$$

2.3. Gas chromatography

The contents of saturated compounds and total petroleum hydrocarbon (TPH) were analyzed using an Agilent 7890 gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with a flame-ionization detector (FID) and DB-5 capillary column. PAHs were analyzed using an Agilent 5890 gas chromatograph (GC) equipped with a HP 5972 mass spectrometer (MS) and a DB-5ms column. For *n*-alkanes, C_8 to C_{40} including the selected isoprenoids pristane and phytane were analyzed. For PAHs, the United States Environmental Protection Agency priority 16 PAHs and alkyl-substituted PAHs were analyzed as follows: (C_0 – C_4) naphthalene, acenaphthylene, acenaphthene, (C_0 – C_4) fluorene, (C_0 – C_4) phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, (C_0 – C_3) chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene, and (C_0 – C_3) dibenzothiophene (Table S1). Total PAHs refers to the sum of 16 PAHs and alkylated PAHs.

All the quality control procedures including surrogates recoveries, laboratory blanks satisfied their acceptable ranges. Recoveries of surrogate standards were o-terphenyl ($103 \pm 7\%$) for TPH and naphthalene- d_8 ($70 \pm 2\%$), acenaphthene- d_{10} ($70 \pm 2\%$), dibenzothiophene- d_8 ($84 \pm 3\%$), phenanthrene- d_{10} ($80 \pm 5\%$), chrysene- d_{12} ($81 \pm 1\%$), and perylene- d_{12} ($86 \pm 6\%$) for PAHs. Target PAHs in the blank samples were not detected or their concentrations were lower than the detection limits of the method (MDL). MDL for target PAHs in water samples ranged from 0.09 to 5.67 ng/L according to their physico-chemical properties. Certified reference materials (National Institute of Standards and Technology 1491a) were analyzed to monitor the performance of the analytical methods.

2.4. Toxicity test

Eggs of olive flounder (*Paralichthys olivaceus*) were artificially fertilized in a commercial fishery station (Ihwasangrok Fishery Station, Jeju, Korea). Fertilized eggs were transported to the Korea Institute of Ocean Science and Technology, Geoje, Korea, and acclimated for 24 h at 16°C on a 16 h light/8 h dark photoperiod in the laboratory prior to the experiments. Normal eggs (optic vesicle stage, 33 h after fertilization) were collected and separated under a microscope system, for exposure experiments using the seawater prepared according to the previous section. The exposures were

carried out in 60 mm glass petri dishes with 3 replicates of 50 embryos for each treatment. Embryos of optic vesicle stage were placed on each well containing 20 mL filtered seawater. Six groups were setup, one control group (embryos exposed to filtered seawater) and five experimental groups (embryos exposed to oil-contaminated seawater samples after different durations of sunlight exposure) and incubated at 16°C in dark condition (SANYO incubator Japan). Half of the solution in the dish was replaced with new media 24 h after exposure. Following 48 h of exposure, flounder embryos were examined for developmental toxicity with a Nikon SMZ800 stereomicroscope or a Zeiss Axioplan 2 compound microscope as previously described (Incardona et al., 2004, 2013; Jung et al., 2013). The predominant effects observed after exposure were pericardial edema (PE), dorsal curvature (DC), and tailfin defects (TF). PE, DC and TF defects were defined respectively by the presence of swollen pericardial sac, the typical S-shape of the spine, and contorted tail fin under the microscopy. Frequency percentages were calculated by dividing the number of abnormal embryos (mortality, PE, TF, and DC) with the number of total embryos.

2.5. Chemometric analysis

To carry out multivariate analysis, bucketing of MS data was performed using ProfileAnalysis 2.2 software (Bruker Daltonics, Bremen, Germany). The detailed parameters for bucketing were as follows: data selection and processing, average peak list; normalization, none; baseline subtraction, none; null spectra exclusion, enable; alignment, maximal peak shift, 1 ppm; peak finder, signal-to-noise threshold 4; and relative intensity threshold, 0.01%.

After bucketing, the aligned MS data and toxicity data were treated by partial least-squares regression (PLSR) to uncover the relationship between them. PLSR was performed using the Unscrambler X (version 10.3, Camo Software AS, Oslo, Norway). MS data were normalized, and then the Nonlinear Iterative Partial Least Square (NIPALS) algorithm was implemented for PLSR. To check how well the calibration model works on new data, full cross-validation was used by leaving out only one sample at a time. The optimum number of factors was chosen by two criteria: the coefficient of determination (R^2) and root mean square error of cross-validation (RMSECV) calculated by Equation (2).

$$RMSECV = \sqrt{\frac{\sum_{i=1}^N (y_i - \hat{y}_i)^2}{N}} \quad (2)$$

N is the number of samples used for validation, y_i is the reference measurement value for the i^{th} sample, and $\hat{y}_i$ is the predicted value by the calibration model constructed without the i^{th} sample.

3. Results and discussion

3.1. Identifying the major degradation process

Biodegradation and photo-oxidation were expected as major oil

weathering processes in this study. To identify the biodegradation, the ratio of *n*-heptadecane to pristane or *n*-octadecane to phytane were calculated (Table S2). The decrease in these values have been used to determine the onset of biodegradation of petroleum hydrocarbons (Miget et al., 1969; Prince and Walters, 2016). There were no significant changes for 5 days of exposure, hence the effects of biodegradation are not considered in this study.

To determine the sunlight-induced photooxidation, changes of photosensitive compounds in the samples were examined using GC-MS, and the results are presented in Supporting Information (Fig. S4). The GC-MS trace of C_1 -pyrenes ($m/z = 216$) is known to be susceptible to sunlight degradation (Jacquot et al., 1996). S/N ratios of the peaks assigned to 4- and 1-methylpyrene (4-MPy and 1-MPy) were significantly reduced as the samples were exposed to sunlight for longer periods of time. Therefore, it is concluded that sunlight photodegradation is the major part of weathering process in these samples. Another piece of evidence for the photodegradation is the significant increase of oxidized compounds. The photodegradation (or photooxidation) of spilled oil is well-known to produce oxygen-containing compounds (Chen et al., 2016; Vaughan et al., 2016). Further description on the oxidized products is given in the following sections.

3.2. Toxicity of seawater exposed to photooxidized oil

Fig. 1 shows the developmental toxicity of seawater samples exposed to photooxidized oil at different stages, in terms of the mortality, PE, TF, and DC for olive flounder embryos. The raw data and photos of embryos used to generate Fig. 1 are presented in Supporting Information (Table S3 and Fig. S5). Affected embryos were clearly distinguishable from unaffected ones (Fig. S5). The toxicity parameters significantly increased as the oils were photo-degraded under natural sunlight. For example, the mortality increased from 20 to 64% after exposure to SW-2, and eventually reached 100% with SW-5. Similar trends were observed for other toxicity parameters. In the group exposed to SW-5, TF and DC could not be examined because all embryos were dead. Toxicity of crude oil to developing fish, such as pink salmon (*Oncorhynchus gorbuscha*), Pacific herring (*Clupea pallasii*), olive flounder (*Paralichthys olivaceus*), and spotted seabass have been well documented (Carls et al., 1999; Heintz et al., 1999; Incardona et al., 2006; Jung et al., 2015). Three dominant malformations including PE, TF, and DC in embryos occurring after oil exposure were observed in accordance with the previous studies. In the previous studies, it was reported

that PE was the most sensitive and mortality was less sensitive toxicity parameters. In this study, all defects were increased severely with greater degree of photo-degradation. Interestingly, TF malformations was increased most dramatically with the degree of photo-degradation compared to other parameters. Developmental toxicities showed higher correlation with the polar compounds formed through the photo-degradation than those of PAHs concentrations.

3.3. Chemical composition of seawaters contaminated by degraded oils

To characterize compounds responsible for the observed toxicity, seawater samples used for the toxicity tests were extracted with DCM, and the extracts (refer to Table 1 for their coding) were analyzed by (–) ESI FT-ICR MS. The obtained broadband and expanded spectra are provided in Fig. 2. The time-domain signals are provided in Supporting Information (Fig. S6). It is important to note that typical oil peaks (showing a broad hump ranging between 200 and 1000 m/z) were not abundant in the spectrum of SW-0, as shown in Fig. 2. However, significant abundance in those peaks was observed from the most degraded sample (SW-5). It is generally accepted that (–) ESI is sensitive toward polar oxygenated compounds such as carboxylic acid or ketone. Hence, this observation suggests that polar oxygenated compounds were increased as the oil were further degraded. Expanded spectra presented in the right column of Fig. 2 show that the relative abundance of O_x class compounds, especially O_3 and O_4 class compounds, become increasingly higher from SW-0 to SW-5.

The class distribution plots were generated and are provided in Fig. 3. They confirm that the O_x (especially $x = 3$ or 4) class compounds became increasingly abundant. The summed absolute abundances of O_3 and O_4 class compounds in each sample are presented as the inset in Fig. 3, showing clearly growing abundance as the photodegradation proceeded. This increase of O_x class compounds in degraded oils agrees well with results reported in the previous studies (Kim et al., 2005; Hughey et al., 2007; Chen et al., 2016; Islam et al., 2016; Vaughan et al., 2016).

To further understand the compositional change at the molecular level, DBE was plotted vs. carbon number for O_3 class compounds. As shown in Fig. 4, the plots show an overall increase of abundance across various DBE and carbon numbers. Especially, compounds with DBE = 2, 3, 6, and 7 became more abundant with further degradation. The ones with DBE = 2 or 3 do not have

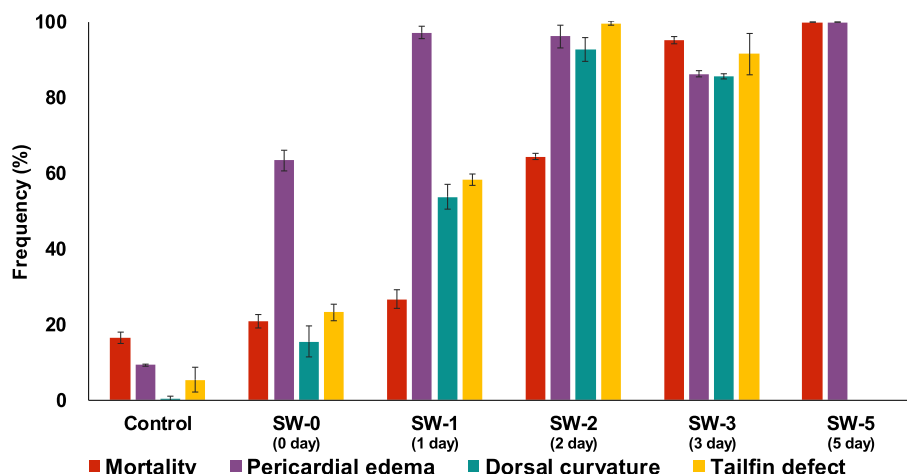


Fig. 1. Toxicity of oil contaminated seawater at different oxidation stages to flounder embryos.

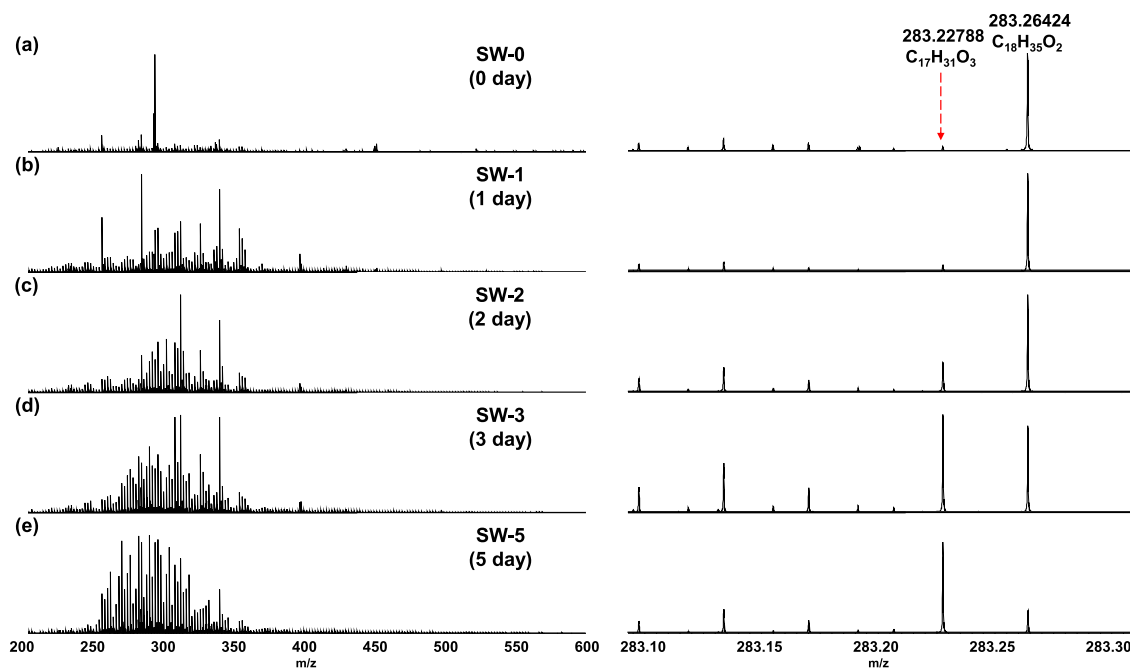


Fig. 2. Broadband (left) and expanded (right) (–) ESI FT-ICR MS data of DCM extracts of seawater samples (SW) contaminated by photodegraded oils after (a) 0, (b) 1, (c) 2, (d) 3, and (e) 5 day(s) of sunlight exposure.

aromatic structures because a benzene ring has a DBE value of 4. The ones with DBE = 3 can be assigned to fatty acids or oxynaphthenic acids. Nonetheless, the data in Fig. 4 clearly show that nonaromatic oxygen-containing compounds (presumably naphthenic acids) were generated during the degradation. The observation of naphthenic acids from degraded oils, especially from oil sand bitumen, was well documented in the literatures (Hughes et al., 2008; Headley John et al., 2016).

It is also notable in Fig. 4 that compounds having DBE = 6 and 7 were increasingly abundant. A previous study showed that insertion of an aromatic ring to non-aromatic compounds results in an increase of DBE value by 4 (Cho et al., 2011). Therefore, adding an aromatic ring to compounds with DBE = 2 would result in

compounds having DBE of 6. The same interpretation can be applied to compounds with DBE = 3 and 7. Therefore, compounds with DBE = 6 and 7 are likely to have an additional aromatic ring compared to those with DBE = 2 and 3, respectively. In summary, the data in Fig. 4 suggest that both non-aromatic and aromatic polar compounds become increasingly more abundant as the degradation proceeds.

The concentration of total PAHs was measured by GC-MS. Their individual concentrations are provided in Supporting Information (Table S4). The summed concentration of total PAHs ranged from 47 to 309 $\mu\text{g L}^{-1}$ in the observed sample. The values were similar or higher than the ones observed from water accommodated and water soluble fractions prepared with crude oil (81–91 $\mu\text{g L}^{-1}$) (Kang

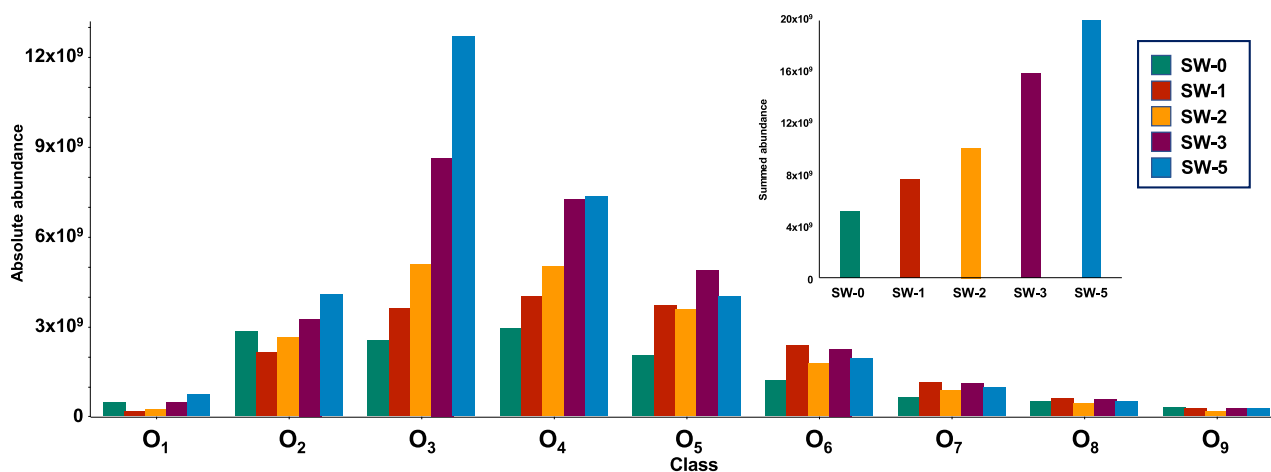


Fig. 3. Distribution of major chemical classes of photo-oxidized products in different degradation stages, detected by negative ion mode ESI. Inset: the summed absolute abundances of O₃ and O₄ class compounds in each sample.

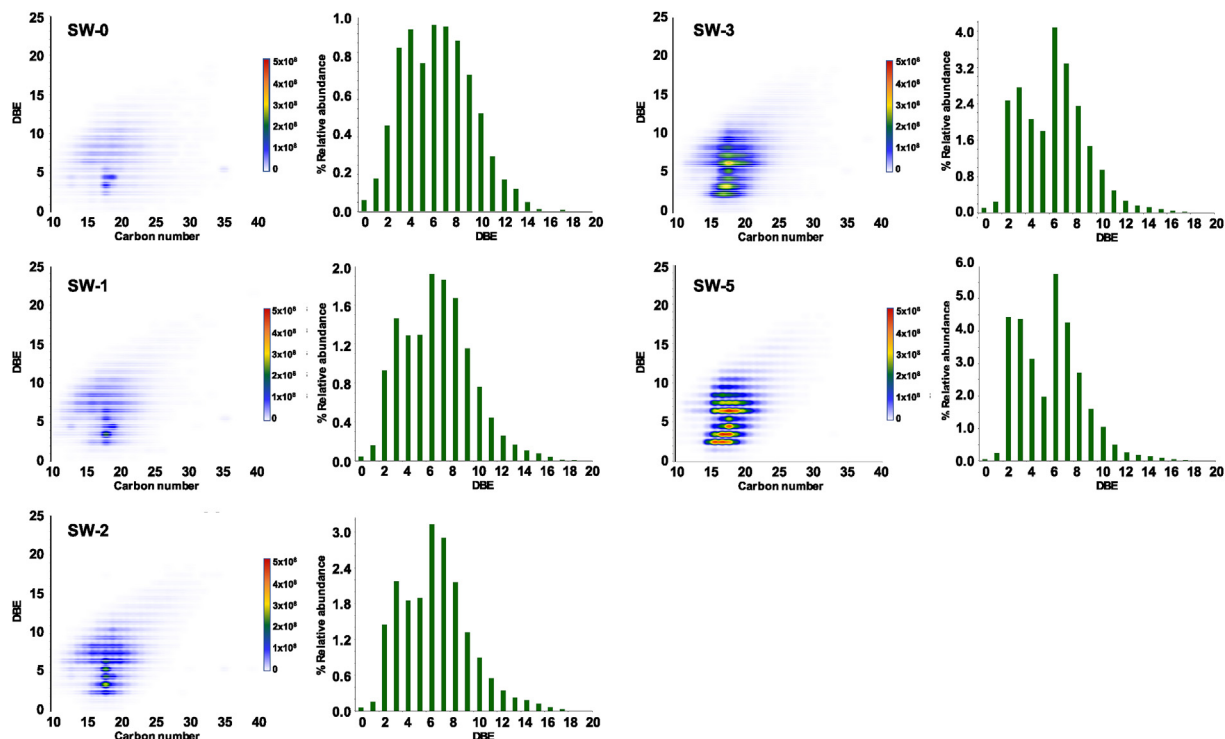


Fig. 4. Double bond equivalents vs. carbon number plots (columns 1 and 3) and double bond equivalent distribution of O_3 class (columns 2 and 4) in each DCM extract of seawater (SW).

et al., 2014). It is notable that the concentration of PAHs in Day 0 sample is significantly higher than those observed in the other days. It can be because the emulsions were not settled and hence they were included in the observed water sample.

3.4. Correlation of compositional and developmental toxicity data

To estimate the contribution to the overall baseline toxicity by total PAHs, their chemical activities were calculated from measured concentration in water and summed as described in the previous study (Table S5, Supporting Information). Ferguson et al. determined that chemical activity could be used as an index of toxicity (Ferguson and Clark, 1939) and it has been reported that baseline toxicity of non-specifically acting chemicals is exerted when the summed chemical activities range between 0.01 and 0.1 and even higher for more hydrophobic organic compounds (Mayer and Reichenberg, 2009; Mackay et al., 2010; Lee et al., 2013; Butler et al., 2016). The summed chemical activities of total PAHs in seawater samples ranged 0.046–0.611. Therefore, it is clear that PAHs contribute to the observed developmental toxicity (Fig. 1 in this study). However, the increased toxicity for degraded samples (e.g. SW-1 and SW-2) displayed in Fig. 1 cannot be explained by chemical activities of PAHs alone.

The comparison of Figs. 1 and 3 shows that the summed abundances of polar oxygenated compounds, such as O_3 and O_4 class compounds, have good correlation with the developmental toxicity. Especially, the mortality is strongly correlated with the summed abundance of O_3 and O_4 class compounds. The percentage mortality of embryos exposed to SW-0 was similar to the control group but increased to 100% after exposure to SW-5. Meanwhile, the summed abundances of O_3 and O_4 class compounds were low in SW-0, and greatly increased in SW-1 to SW-5. The toxicity of polar oxygenated compounds has not been known and hence toxicity can't be estimated like it was done for PAHs. However, the observed correlation

between the abundance of polar oxygenated compounds and increased toxicity strongly suggests that polar oxygenated compounds can do a role in toxicity of degraded oils.

It is important to note that toxicity from SW-0 can be attributed to nonpolar compounds existing in the spilled oil. Especially, the mean incidence of pericardial edema reached over 60% after exposure to SW-0. The cardiac toxicity of PAHs is well documented in previous studies (Incardona et al., 2006, 2011; Carls et al., 2008). Therefore, the overall observed developmental toxicity in Fig. 1 can be attributed to combination of polar and nonpolar compounds.

3.5. Chemometric analysis based on toxicity and abundance of polar compounds

To further investigate the correlation between the polar compounds and the observed toxicity, multivariate statistical analyses were performed. PLSR was used to build a toxicity prediction model. Matrix X was composed of (–) ESI-MS data of SW samples using 15 mass spectra from five samples in triplicates. Matrix Y was designated as the mortality. PLSR analysis was performed with matrices X and Y, and the score and loading plots of the first two factors determined by PLSR are presented in Fig. 5-a and b. The first two factors can explain almost 99% of the Y variance (Fig. 5-c).

In the score plot in Fig. 5-a, each set of triplicate spectra from each sample is gathered together to show good reproducibility. Points representing SW-0 to SW-5 are sequentially located from left to right, showing that Factor 1 can well explain the variation in toxicity. To further identify the chemical compounds responsible for the difference in PLSR, loading plots were examined. In the loading plot (Fig. 5-b), each dot represents a chemical identified in the spectra. It is found that O_3 and O_4 class compounds have the largest effect on Factor 1 (note the elemental compositions in Fig. 5-b). This agrees well with the spectral observation described above, that the O_3 and O_4 class compounds were enriched as the samples

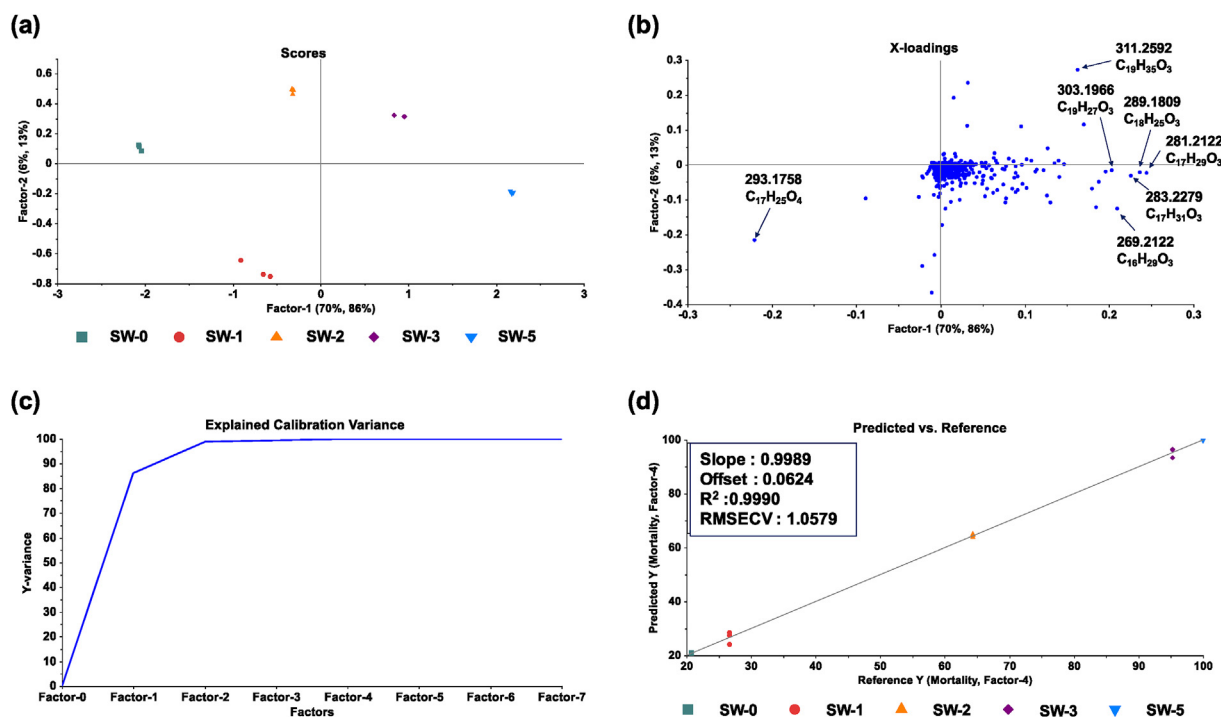


Fig. 5. (a) PLS scores plot. The symbols have the same shape and color as the groups in SW samples. (b) X-loading plot for Factor 1 vs. Factor 2. (c) Plot of the explained Y-variance. (d) Scatter plots of predicted vs. reference mortality (%). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

were further degraded.

Fig. 5-d presents results from PLS regression for mortality. The slope, offset, coefficient of determination (R^2), and RMSECV are listed in the plot. Good correlation ($R^2 = 0.99$) was found between the experimental toxicity factor and the predicted one, based on the abundance of polar compounds observed in (–) ESI-MS.

The data presented here clearly show that the toxicity of spilled oil can be explained by a combination of nonpolar and polar compounds. The initial toxicity before degradation can be attributed to nonpolar compounds, in good agreement with previous studies that relate developmental toxicity to fish embryos with the composition of dissolved PAHs including the tricyclic fluorenes, dibenzothiophenes, and phenanthrenes (Carls et al., 1999; Heintz et al., 1999). A later study used the zebrafish model to compare the composition of individual parent (non-alkylated) tricyclic PAHs to the toxicity of weathered Alaska North Slope Crude Oil in terms of the degree of malformation (Incardona et al., 2006). Increased toxicity was found after the degradation and explained by the polar compounds presumably generated by the oxidation of oil compounds. Kang et al. showed that the increased toxicity of weathered crude oils may be due to increase in the toxic transformation products (such as oxygenated hydrocarbons) in spite of the evaporation loss of smaller molecules. They concluded that polar compounds need to be studied to fill gaps between the observed ecotoxicity and the chemically identified components (Kang et al., 2014). Our findings show that polar compounds generated by degradation can indeed explain the gaps observed by Kang et al.

4. Conclusions

In this work, comprehensive study was performed on molecular level identification of compounds in photodegraded oils and evaluation of their embryonic developmental toxicity. Molecular level identification of polar compounds generated by the photooxidation of spilled oil was performed by FT-ICR MS. The abundance of

identified polar oxygenated compound was significantly increased through the photooxidation of spilled oil. The concentration of PAHs in seawater were measured by GC-MS and baseline toxicities were estimated based on chemical activities. Also, developmental toxicity of seawater exposed to photodegraded oil was examined and it was observed that the toxicity of seawater to the fish embryo was increased as the spilled oil was further photodegraded. PLSR analysis showed that the increased toxicity can be explained by polar compounds generated by photooxidation of spilled oil.

In conclusion, the compounds identified in this study along with PAHs have to be considered together to account for the developmental toxic potency of spilled oil. Therefore, this work contributes to improve our understanding on the toxicity of degraded crude oil.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2019.124346>.

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