Marine Pollution Bulletin xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

Identification of genotoxic compounds in crude oil using fractionation according to distillation, polarity and K_{ow}

Shin Yeong Park [a], Hyo Jin Lee [a,c], Jong Seong Khim [b], Gi Beum Kim [a,c,*]

[a] Department of Marine Environmental Engineering, Gyeongsang National University, Tongyeong, Republic of Korea
[b] School of Earth and Environmental Sciences, Research Institute of Oceanography, Seoul National University, Seoul, Republic of Korea
[c] Institute of Marine Industry College of Marine Science, Gyeongsang National University, Tongyeong, Republic of Korea

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form 12 October 2016
Accepted 13 October 2016
Available online xxxx

Keywords:
Crude oil
Fractionation
Boiling point
Polarity
Log K_{ow}
Comet assay

ABSTRACT

We examined the degree of DNA damage caused by fractions of crude oil in accordance with the boiling points, polarity and log K_{ow}. Relatively high DNA damage was observed in the aromatic fraction (290–330 °C) and resin and polar fraction (350–400 °C). The resin and polar fraction showed relatively high genotoxicity compared with the aliphatic and aromatic fraction at the 1–4 log K_{ow} range. At the 6–7 log K_{ow} range, the aromatic fraction showed relatively high DNA damage compared with the aliphatic and resin and polar fraction. In particular, every detailed fraction in accordance with the log K_{ow} values (aliphatic and aromatic (310–320 °C) and resins and polar fractions (370–380 °C)) showed one or less than one DNA damage. However, the fractions before separation in accordance with log K_{ow} values (aliphatic and aromatic (310–320 °C) and resin and polar (370–380 °C) fractions) showed high DNA damage. Thus, we confirm the synergistic action between the detailed compounds.

There are tens of thousands of compounds in oil, and the main components are classified as aliphatic, aromatic, and polar hydrocarbons (Morgan and Watkinson, 1994; Sathishkumar et al., 2008 and Shi et al., 2010). Aliphatic hydrocarbons consist of straight and saturated carbon chain starting from C6–C40. Aromatic hydrocarbons are composed of monocyclic aromatic hydrocarbons having a single benzene ring and polycyclic aromatic hydrocarbons having two–six benzene rings, and they are known to have carcinogenicity. Polar compounds consist of resins (compounds with low molecular weight that are combined with nitrogen, sulfur, or oxygen) or asphaltene (compounds with large molecular weight). Due to their very slow biological/physical degradation, they exhibit great toxicity to aquatic organisms.

Offshore oil production and oil spills causes significant quantities of petroleum discharges into the marine environment. Several studies have indicated that oil exposure has a genotoxic effect on aquatic organism, mainly measured as DNA adducts and chromosomal damage in fish. Aas et al. (2000) found that the exposure of Atlantic cod (*Gadus morhua*) to PAHs induced DNA damage by forming bulky DNA adducts. PAHs in the oil may also produce DNA damage through the formation of reactive oxygen species (ROS) (Frenzilli et al., 2001; Regoli et al., 2002). However, oil is a complex mixture and each component in the oil may induce DNA damage through the various mechanisms (Taban et al., 2004). Thus, DNA damage is widely used as a biomarker for assessing the genotoxicity associated with oil (Tice et al., 2000).

Various bioassays have been developed to investigate the genotoxicity of organisms. Comet assay (known as DNA single–cell gel electrophoresis) is a fast and easy technique for investigating the single stranded DNA damage among bioassays and measures the genotoxic damage by using only a small amount of cells regardless of cell types (Seo et al., 2006; Woo et al., 2006; Collins et al., 1997; Singh et al., 1988).

On the other hand, marine pollution by oil arises from various routes, such as natural leakage, vessel navigation, shipwreck, and costal facilities. What becomes the most social issue among the occurrences of the marine pollution is oil spill primarily due to shipwreck. The largest oil spill accident in Korea is the 2007 Hebei Spirit oil spill, resulting in active research on the effects of marine ecosystem and the effects of the oil inflow on marine life (Hong et al., 2015; Jung et al., 2011; Seo et al., 2011). Furthermore, according to the toxicity study in accordance with oil, the toxicity of some compounds in the components constituting oil is confirmed. However, due to the characteristics of the complex components of the oil, how many compounds induce the toxicity has still not been known (Hong et al., 2012; Ji et al., 2011). Therefore, the toxicity test after the detailed fractionation of oil in accordance with various physical and chemical properties are necessary to identify the toxic components among the complex compound of oil. This will help to know which compounds should be monitored when oil spill accident occurs.

* Corresponding author at: Department of Marine Environmental Engineering, Gyeongsang National University, Tongyeong, Gyeongnam 650-160, Republic of Korea.
E-mail address: kgb@gnu.ac.kr (G.B. Kim).

http://dx.doi.org/10.1016/j.marpolbul.2016.10.035
0025-326X/

Iranian heavy crude oil (IHCO), the main oil spilt in the Hebei Spirit oil spill accident in 2007, separated as 33 fractions according to the boiling point (15–50 °C, 50–70 °C, 70–100 °C, and intervals of from 100 °C to 400 °C). According to the result of applying effect-directed analysis (EDA) to the fractions, the distillation fractions at 290–400 °C showed AhR activity and ER–mediated potency (Fig. 1 and MOF, 2014). Therefore, the present study (which targets the distillation fractions showing distinct toxic reactions in oil) further separates crude oil by using its physical and chemical properties (boiling points, polarity, and K_{ow}) and evaluates the potential genotoxicity by using Comet assay on a total of fractions to determine the toxic components in oil that may affect organisms.

We separated Iranian heavy crude oil (IHCO) into eleven samples (F1–F11) at intervals of 10 °C from 290 °C to 400 °C in accordance with boiling point using true boiling point (TBP) test equipment. 1 mL of each distillate fraction was passed through a glass column for separating that was filled with 10 g of the activated silica gel (70–230 mesh, Merck, Darmstadt, Germany) (Khim et al., 1999a, 1999b; Hong et al., 2016). The first fraction (f1), containing aliphatic compounds, was eluted with 30 mL hexane (Burdick & Jackson). The aromatic fraction (f2) was collected by elution with 40 mL of 20% DCM in hexane (v/v). The third fraction (f3) containing resin and polar compound was eluted and separated using 40 mL of 60% DCM-acetone mixed solvent. All aliquots were ultimately concentrated to 1 mL using a rotary evaporator and nitrogen evaporator. In some, the solvents were replaced by dimethyl sulfoxide (DMSO) and used as samples for comet assay. The rest were separated into 10 samples (k1–k10) with the value of log K_{ow}, ranging from less than one to 10 (such as 1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, and 9) again by using reverse phase-high performance liquid chromatography (RP-HPLC). Then, all aliquots were ultimately concentrated to 1 mL using the rotary evaporator and nitrogen evaporator. In the concentrated sample, the solvent was replaced with dimethyl sulfoxide (DMSO) and then used in the experiment for comet assay.

Flatfish (*Paralichthys olivaceus*) was used as the test organism, and approximately 20 of the flatfish blood was collected by a syringe treated with anticoagulant heparin. After diluting the collected blood with Hank's balanced salt solution (HBSS) so that it became 10^5 cells/mL, 50 of the blood apiece was then transferred to a 1 mL centrifuge tube. 10 of the sample separated depending on the physical and chemical properties by using distillation points, silica gel column, and RP-HPLC column, respectively, and 940 of HBSS were added to the tube, thereby making the final volume 1 mL. Then, it was exposed at a 4 °C darkroom for 1 h. After centrifugation (5000 rpm, 5 min), the supernatant was discarded and the pellet part was used in comet assay. Hydrogen peroxide was used as a positive control to verify the comet assay (Fig. S2).

Comet assay experiment was carried out after some modification of the experimental method of Singh et al. (1988). The pellet part was resuspended with 500 μL of 0.65% low melting agarose (LMA) and placed on the slide coated with 30 μL of 1% normal melting agarose (NMA). Then, it was covered with a cover glass. After solidifying this on ice for about 3 min, this was recoated with 30 μL of 0.65% LMA and solidified on ice for 3 min again. The cover glass was removed and the sample was stored in a coplin jar containing lysis buffer solution (2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris-HCl, 10% dimethyl sulfoxide, 1% Triton X-100) for >2 h at 4 °C. Then, the slide was washed in a coplin jar containing the 4 °C distilled water three times every 2 min. The slides were then placed in a Coplin jar containing DNA denaturing buffer (200 mM NaOH, 1 mM EDTA, pH > 13) at 4 °C for 15 min. Then, slides were transferred into electrophoresis chambers filled with DNA denaturing buffer and electrophoresis was carried out for 25 min at 25 V and 300 mA. After the electrophoresis, the slide was washed with 0.4 M Tris-HCl solution (pH 7.5) three times every 2 min. After soaking the washed slide in a coplin jar with ethanol for 5 min, it was dried at room temperature. The dried slide was stained with 20 μL of ethidium bromide solution (20 μg/mL).

DNA strand breaks in cells reflected as extracellular 'comet tails' were determined using a Nikon Eclipse E200 inverted fluorescent microscope (×20 magnification). Cell images were captured using a high-sensitivity charge-coupled device camera connected to a computer. A computerized image analysis system (Komet version 5, Kinetic Imaging Ltd., Liverpool, UK) was used to determine DNA damage as the 'DNA tail moment' (amount of DNA in each tail the tail length). For each experimental condition, 50 cells from each slide were examined randomly. All treatments were repeated three times. Finally, DNA fold induction (the DNA tail moment in a treatment divided by the DNA tail moment in a control) was used for comparing DNA damage levels between treatments.

The genotoxicity of detailed fractions of IHCO (F1–F11), the aliphatic compound (f1), aromatic compound (f2), and resin and polar compound (f3), which was measured by comet assay, is shown in Fig. 1. All fractions except the aliphatic (F5-f1), aromatic (F5-f2), and resin and polar (F5-f3) fractions, all of which had a boiling point of 330–340 °C, showed greater DNA damage than the control (>1-fold induction). In particular, within the F1–F4 fractions with a boiling point of 290–330 °C, the aromatic fraction (f2) showed greater DNA damage than the aliphatic fraction (f1) or resin and polar fraction (f3). When comparing the DNA damage results of these fractions to the chemical composition results (MOF, 2014), F1-f2 fraction have 42% C4-naphthalene and 30% C3-naphthalene; F2-f2 and F3-f2 fractions, 26% and 31% 4-methyldibenzothiophene and 16% and 15% C1-dibenzothiophene, respectively; and F4-f2 fraction have 27% C1-dibenzothiophene and 20% C2-dibenzothiophene. All these compounds represented the highest component in each fraction (Table S1). Through many studies, the aromatic compound of crude oil has been known as the main cause of toxicity (Jeong et al., 2015; Hong et al., 2015), and the present study confirms that the aromatic compound with a boiling point of 290–330 °C causes great genotoxicity. Accordingly, these substances are determined to be the toxic substance mainly responsible for genotoxicity within marine organisms. On the other hand, within the F7–F11 fractions with a boiling point of 350–400 °C, the resin and polar fraction (f3) showed greater DNA damage than the aliphatic fraction (f1) or the aromatic fraction (f2). Although which compound causes toxicity is not exactly known since the chemical analysis of the resin and polar fraction is still impossible, it is predicted that the resin and polar compound with a boiling point of 350–400 °C is the main toxicity-causing substance. In the future, it is necessary to determine the detailed, specific compound that causes the major DNA toxicity through additional analysis of resin and polar compounds.

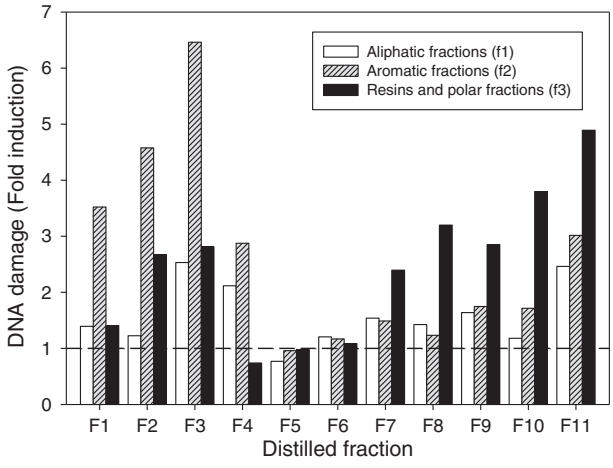

Fig. 1. DNA damage level of IHCO-distillation fractions separated in accordance with the polarity.

Please cite this article as: Park, S.Y., et al., Identification of genotoxic compounds in crude oil using fractionation according to distillation, polarity and K_{ow}, Marine Pollution Bulletin (2016), http://dx.doi.org/10.1016/j.marpolbul.2016.10.035