Journal of Hazardous Materials 260 (2013) 67–73

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

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

Effects of simulated weathering on the toxicity of selected crude oils and their components to sea urchin embryos

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HIGHLIGHTS

- Effects of weathering (photooxidation, evaporation) on oil toxicity were studied.
- Toxicity of Angolan crude and Heavy Fuel Oil was tested by the sea urchin test.
- Decrease of Angolan oil toxicity with weathering correlated to the aliphatic fraction.
- Aromatic fractions were responsible for most of the toxicity.
- Polar compounds were the second most important toxic components.

ARTICLE INFO

Article history:
Received 2 April 2013
Received in revised form 30 April 2013
Accepted 3 May 2013
Available online 13 May 2013

Keywords:
Oil spill
Weathering
Fraction
Toxicity
Bioassay

ABSTRACT

Artificial weathering of Angolan crude and a Heavy Fuel Oil (HFO) was performed by evaporation and photooxidation. The aliphatic, aromatic, polar and asphaltene fractions of the fresh and weathered oils were isolated. The toxicity of the water accommodated fraction or an oil/fraction dissolved in DMSO was assessed using the sea urchin embryo test. Photooxidation was observed to decrease the aromatics content and increase polar compounds. A slight reduction in the toxicity of Angolan crude was observed following weathering for the water-accommodated fraction and the extract in DMSO, but no effect was seen for the Heavy Fuel Oil. For aliphatic compounds, the toxicity decreased in the order fresh > evaporated > photooxidated for both Angolan crude and HFO. Weathering slightly increased the toxicity of the aromatic and polar fractions of the oil. The aromatic fractions were responsible for most of the toxicity and the polar compounds were the second most important toxic components, despite having less or similar abundance than the aliphatic fraction. The toxic contribution of the aromatic compounds was higher for the HFO than for the Angolan crude. A decrease in the toxicity of Angolan crude following weathering correlated with a reduction in the toxicity of the aliphatic fraction.

1. Introduction

Petroleum consists of a highly complex mixture of organic compounds, predominantly hydrocarbons and can be characterized by the relative content of the fractions of saturated hydrocarbons, aromatics, resins and asphaltenes (SARA analysis) because its physico-chemical properties are determined by the proportions of these major components. Saturated hydrocarbons (paraffins, iso-paraffins and naphthenes) have low aqueous solubility and toxicity. The aromatic fraction has been identified as containing the compounds mainly responsible for the acute toxicity of petroleum, and polycyclic aromatic hydrocarbons (PAHs) as the family of compounds of greater environmental concern [1].

The European Chemicals Agency (ECHA) recommends the hydrocarbon block method to assess the ecological risk of oil [2]. The method is based on the grouping of known compounds of similar properties into blocks to estimate the predicted environmental concentration (PEC), the Predicted No Effect Concentration (PNEC), and subsequently the PEC/PNEC risk ratio of each individual block

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http://dx.doi.org/10.1016/j.jhazmat.2013.05.004

and the whole oil. This requires that the properties of individual compounds related to their fate in the environment (solubility, volatility) and toxicity have to be known or estimated. For this reason, it is solely applicable to those compounds that can be resolved by chromatographic techniques (mainly compounds from the saturated and aromatic fractions) but not so for compounds unresolved by gas chromatography such as the unresolved complex mixture (UCM). The proportion of the UCM in a spilt oil is determined by its origin, degree of weathering and/or biodegradation [3]. The UCM of saturated, aromatic and polar fractions have all been shown to be toxic [4–6], so it is important not to ignore unresolved components just because the exact composition cannot be determined by the available analytical methods [7]. The difficulty in establishing causality between composition and effects has already been reported. For example, PAHs may not be the main cause of toxicity for certain oils [8], and the toxic contribution of the polar organic fraction may also be important [7,9,10]. Recently Bellas et al. [11] have shown that the increased toxicity of certain weathered oils cannot be explained on the basis of the changes in total PAH content.

The term "weathering" involves a series of processes (evaporation, dissolution, dispersion, emulsification, photooxidation, biodegradation, spreading, and adsorption) that alter the physical and chemical characteristics of spilled oil. Evaporation is a crucial process in terms of material balance that results in losses of lighter saturate and aromatic compounds (monoaromatic and light PAHs), which is more marked for light crude oils. For this reason, the proportion of PAHs and their toxic contribution increases with evaporation [12]. The photooxidation of aliphatics and aromatics fractions generates more polar and water soluble compounds such as ketones, aldehydes, carboxylic acids and esters [12].

The present study was aimed at better understanding the effects of weathering on oil composition and toxicity. Artificial weathering of oil was performed by evaporation and photooxidation, and chemical fractionation to characterize compositional changes. The toxicity of the selected oils and their fractions were quantified by the sea urchin embryo test.

2. Materials and methods

The following oils were used: Angolan crude oil (Dalia, API 23.14), Heavy Fuel Oil (HFO, API 11.47) [13].

2.1. Oil weathering

2.1.1. Photooxidation

Photooxidation was performed on the oil samples, to simulate the weathering of oil at sea, using SUNTEST® CPS flatbed xenon exposure system from Atlas (Chicago, USA). It is equipped with 1500B NrB4 Xenon lamp that was operated at a potential of 507.5 W/m^2. An appropriate mass of each oil sample (ca. 0.4 g) was transferred to pre-weighed Petri dishes and spread to obtain uniform layer. Each sample was irradiated for 6 h.

2.1.2. Evaporation

Evaporation was performed to simulate the weathering of oil over the short-term (ca. 2 h) following a spill [14]. Crude oil (1 L) was placed in a pre-weighed crystallizing dish (2 L) in a fume hood (air flow 0.5 m/s) at room temperature for 24 h. Evaporative loss was calculated for each oil.

2.2. Coarse fractionation

Open column liquid solid chromatography was used to fractionate the oils. A glass column packed with silica gel (60–100 mesh; 5% H_2O; 40 g) under alumina (grade 1 neutral; 1.5% H_2O; 20 g) was loaded with oil (1 g), and the column eluted with hexane (2 column volumes), dichloromethane (2 column volumes) and methanol (2 column volumes) to provide aliphatic, aromatic and polar fractions respectively. The obtained fractions were used for toxicity tests.

The oil samples were also analyzed using thin layer chromatography with flame ionization detection (TLC-FID) which is well established as an efficient, fast and cost effective method to obtain quantitative data on the composition of oils, more specifically the relative contents of saturates, aromatics, resins and asphaltenes (SARA analysis) [15,16].

Using a sample spotter SES 3202/IS-02 (Ses GmbH, Nieder-Olm, Germany), 0.8 μL of the DCM oil solution was spotted onto silica-coated quartz rods (ChromaRod®-SIII). A three-step separation was performed using 100% n-hexane to 10 cm, 20:80% n-hexane:toluene to 5 cm and 5:95% methanol:dichloromethane to 2 cm, respectively. All eluents used were analytical grade (Suprasolv grade). After elution, the Chromarods® were dried at 40 °C for 5 min to remove solvents and transferred into a MK-5 TLC–FID Iatroscan® apparatus (Iatron Labs, Tokyo, Japan) where each Chromarod® was scanned with the FID to detect the oil compound classes separated on the silica. The hydrogen flow rate was 160–180 mL min^{-1}, the airflow rate was 2000 mL min^{-1} and the scanning speed was 30 s per Chromarod® burned.

2.3. Preparation of water accommodated fractions and oil extracts

Water accommodated fractions (WAFs) of weathered and unweathered oils (Angolan crude oil and HFO) were prepared by adding 3.5 g of to 87.5 mL of 0.22-μm-filtered seawater (FSW) in a 250 mL bottle with a teflon cap (loading rate, 40 g/L). The mixture was kept in the dark and shaken (150 rpm) for 24 h at 20 °C. The aqueous phase was separated and used to obtain the FSW dilutions to be tested. The experimental concentrations tested (from 1.25 to 1000 mL/L in geometric increments) were obtained by dilution of the WAF in FSW.

DMSO extraction for each fraction or unweathered/weathered oil (Angolan crude oil and HFO) was performed at a ratio 1:9 (m:m) by orbital shaking (150 rpm) for 16 h at 50 °C and dilutions of the extract in DMSO. For the sea urchin embryo test, 1 mL/L of the extract was added to each vial with FSW. The experimental concentrations tested were 0, 10, 20, 50, 100, 250, 500 and 1000 μL/L.

2.4. Sea urchin embryo test

The sea urchin embryo test was performed in accordance with the method of Saco-Álvarez et al. [17]. Gametes of *Paracentrotus lividus* were obtained by dissection of two of the adults and their maturity (ovum sphericity and sperm mobility) checked with a microscope. The ova were transferred to a 100-mL graduated cylinder containing seawater (5–10 ova/μL), a few drops of sperm (30–100 μL) taken from the male gonad were added through a Pasteur pipette, and the mixture shaken gently to facilitate fertilization. The fertilization rate was determined in a Sedgewick–Rafter counting chamber in quadruplicate (n = 100), as the proportion of eggs with a fertilization membrane (>97%). Within 30 min, the fertilized eggs were transferred to vials with 10 mL (WAF) or 4 mL (extracts of oil/fraction in DMSO) of FSW dosed with the oil or fraction to be tested. Each vial received 40 eggs per mL and each dose was performed in quadruplicate.

The eggs were incubated in the dark at 20 °C for 48 h, and the larvae fixed by adding a few drops of 40% formalin. In each vial the maximum length of 35 individuals was measured using an inverted microscope and Leica QWIN image analysis software, version 3.4.0