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Toxicity and phototoxicity of water-accommodated fraction obtained from *Prestige* fuel oil and Marine fuel oil evaluated by marine bioassays

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ABSTRACT

Acute toxicity and phototoxicity of heavy fuel oil extracted directly from the sunken tanker *Prestige* in comparison to a standard Marine fuel oil were evaluated by obtaining the water-accommodated fraction (WAF) and using mussel *Mytilus galloprovincialis* and sea urchin *Paracentrotus lividus* embryogenesis bioassays, and copepod *Acartia tonsa* and fish *Cyprinodon variegatus* survival bioassays. Aromatic hydrocarbon (AH) levels in WAF were measured by gas chromatography. *Prestige* WAF was not phototoxic, its median effective concentrations (EC_{50}) were 13% and 10% WAF for mussel and sea urchin respectively, and maximum lethal threshold concentrations (MLTC) were 12% and 50% for copepod and fish respectively. Marine WAF resulted phototoxic for mussel bioassay. EC_{50}s of Marine WAF were 50% for sea urchin in both treatments and 20% for mussel under illumination. Undiluted Marine WAF only caused a 20% decrease in mussel normal larvae. Similar sensitivities were found among sea urchins, mussels and copepods, whilst fish were less sensitive. Unlike Marine WAF, *Prestige* WAF showed EC_{50} values at dilutions below 20%, and its toxicity was independent of lighting conditions. The differences in toxicity between both kinds of fuel could not be explained on the basis of total AH content.

1. Introduction

On November 19th 2002, the tanker *Prestige* sank 209 km off cape Finisterre at 3500 m depth, after 20 h drifting eastwards and 5 d trawling in directions shifting northwest, south and southwest, spreading along its zigzag trajectory some 65,000 t of a high viscosity fuel oil and damaging more than 800 km of the Atlantic coast (Rousseau, 2003). Every year more than 14,000 ships transporting dangerous goods sail across the main routes of international transport among Europe, America and Asia located just a few tens of miles off Galicia (NW Iberian Peninsula). Since the 1950s, some of the most important accidents of oil tankers, such as *Andros Fortune*, *Polycommander*, *Urquiola*, *Andros Patria* and *Aegean Sea*, have taken place in this area. Moreover, the Galician coast is a highly productive fishery zone where shellfish aquaculture bears both social and economical importance. Therefore, in order to protect this marine ecosystem, it is necessary to study the impact of petroleum and fuel oil on representative marine species.

Petroleum affects organisms by physical action (light reduction, asphyxia), by modification of habitat (change in pH, decrease of dissolved oxygen, decrease in food availability) and by toxic effect of soluble components. Most relevant effects of

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fuel oil are attributed to the aromatic fraction, particularly polycyclic aromatic hydrocarbons (PAHs), potentially mutagenic, carcinogenic and teratogenic (Albers, 2003).

According to the recommendations of the European Centre of Ecotoxicology and Toxicology of Chemical Substances, the exposure medium for toxicity tests of low solubility compounds, as fuel oil, should be prepared as a water-accommodated fraction (WAF) (Rufli et al., 1998). WAF is defined as a medium containing only the fraction of petroleum that remains in the aqueous phase once any source of mixing energy has been removed and after a period sufficient for phase separation. WAF is considered to be saturated with petroleum products, mainly hydrocarbons that can be present in true solution or like a stable emulsion (Rufli et al., 1998).

WAF toxicity is commonly assessed by using early life stages of marine organisms, recording embryogenesis, early larval growth, survival or morphological abnormalities as the endpoint. Early life stages are more sensitive than adults and represent a critical period in the life cycle of an organism. Moreover, unlike larger, actively swimming organisms, larvae are part of plankton, and they lack the ability to escape from oil polluted waters. Sea urchins (Falk-Petersen, 1979; Fernandez et al., 2006), molluscs (Pelletier et al., 1997), copepods (Suderman and Marcus, 2002) and fish (Barron et al., 2005) are among the most frequently used organisms in embryo–larval bioassays with oil products.

In the marine environment, solar radiation can increase fuel oil toxicity via phototransformation of its compounds in the water column, creating photoproducts which result more toxic than parental compounds; or via indirect photosensitization of organisms by the activation of chemicals bioaccumulated in tissues (Cleveland et al., 2000). These molecules such as PAHs and heterocyclic hydrocarbons can absorb the radiation energy and transfer it to a molecule of oxygen, creating an oxygen radical capable of damaging DNA or cellular membranes via lipid peroxidation (Arfsten et al., 1996). Although solar radiation effect is not always reflected in the fuel oil toxicity, fundamentally, phototoxicity depends on fuel oil composition, sensitivity of the exposed organism and vital state, methods of exposure to fuel, and intensity, spectrum and quality of radiation (Cleveland et al., 2000). Therefore, to evaluate the fuel oil toxicity in the environment, it is necessary to study WAF toxicity under light conditions that simulate solar radiation in intensity and spectrum. Solar radiation irradiance that arrives at the water column surface depends on cloudiness and incidence angle of solar rays that depends on latitude, season and hour of day. Typically, photosynthetically active radiation (PAR) irradiance at the water surface varies from 2100 to 42 $\mu E\ m^{-2}\ s^{-1}$ (Kirk, 1994). In the water column, solar radiation is attenuated in the first meters based on turbidity and concentration of dissolved organic matter and phytoplankton. Visible spectrum (λ=400–700 nm) includes approximately 50% of the solar radiation that penetrates in the sea, and in addition, it reaches greater depth in the water column whilst ultraviolet (UV, λ=280–400 nm) is dispersed and absorbed rapidly (Barron et al., 2000).

The aim of this work was to analyze the toxicity of *Prestige* fuel oil and Marine fuel oil by obtaining the WAF and using a battery of bioassays with sensitive organisms, including bivalves, sea urchins, crustaceans and fish. The potential phototoxicity under lighting conditions of ecological relevance was also investigated.

2. Materials and methods

2.1. Fuel characterization

The products tested were *Prestige* fuel oil extracted directly from sunken tanker and Marine fuel oil. Fuel transported by the *Prestige* tanker was a heavy fuel oil (type No. 6) characterized by a high density (0.99 kg l^{-1}), viscosity (615 cSt at 50 °C) and persistence. The analyses made by the Spanish National Research Council (CSIC) revealed this fuel was constituted by a 22% of saturated hydrocarbons, 50% of aromatic hydrocarbons, a 28% of resins and asphaltenes and 2.28% of sulphur (CSIC, 2003). Marine fuel was a standard intermediate fuel oil (ISO 8217, IFO 380, RMG 35) a blend of gas oil and heavy fuel oil, characterized by a density of 0.98 kg l^{-1} at 15 °C and a viscosity at 50 °C of 380 cSt (ISO, 2005). Both fuel oils were supplied for scientific research by the Spanish Government through the *Oficina de Vertidos Marinos Accidentales* (Universidade de Vigo, Spain).

2.2. Preparation of water-accommodated fraction

Water-accommodated fraction (WAF) of each fuel was prepared by adding fuel oil (40 g l^{-1}) to 0.22 μm-filtered seawater (FSW) in 500 ml Pyrex® bottles. The content of the closed bottles was magnetically stirred with a Teflon-coated stirring bar of triangular section for 24 h (Singer et al., 2000), in the dark, at room temperature (20±2 °C) and at an arbitrary constant speed (Tsvetnenko and Evans, 2002), but sufficiently high to break the fuel superficial layer in small drops, which maximizes the surface in contact with seawater. WAF was separated by filtration through a glass filter.

WAF dilutions for toxicological testing were prepared with FSW and dosed in 25 ml glass vials with Teflon-lined caps or in 150 ml glass bottles according to the bioassay. Two sets of four replicates per dilution and four FSW controls were assayed for each test. Closed vial sets were incubated in two culture chambers at 18 °C with different conditions of illumination, one set in dark and other set under fluorescent light (70 $\mu E\ m^{-2}\ s^{-1}$ intensity PAR) with a photoperiod of 14 h light:10 h dark. Cool daylight lamps (Osram 15 W/765) were used in order to simulate natural irradiation, the emission spectrum range of these lamps is 380–780 nm. WAF samples for chemical analysis were collected in brown glass bottles and acidified (pH<2) to avoid biodegradation.

2.3. Mussel bioassay

Mature *Mytilus galloprovincialis* were induced to spawn by thermal stimulation in separated beakers with FSW following Bellas et al. (2005). Eggs from a single female were transferred to a 100 ml measuring cylinder and their quality was checked under microscope. Sperm solution was stored at 4 °C until use. Sperm mobility was checked under microscope and a few μl were added to the egg suspension and carefully stirred to allow fertilization. Fertilized eggs (ca. 30 eggs ml^{-1}) were transferred