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Preparation and Characterization of Water-soluble Fractions of Crude and Refined Oils for Use in Toxicity Studies

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The increase in oil contamination of marine and estuarine environments has led to concern about the effects of oil pollution on marine organisms (CONNELL and MILLER 1981).

The most immediate toxic and subtoxic fractions of oils are those soluble in water (HUFFORD 1971), toxicity of oils to organisms usually being related to the content of non-volatile persistent aromatic hydrocarbons (ZIKTO and CARSON 1970; NEFF and ANDERSON 1975).

Accumulated evidence indicates that mixing parameters and other factors may play a critical role in determining the quantity of oil and hence the aromatic hydrocarbons transferred to the water column (BOYLAND and TRIPP 1971; GORDON *et al.* 1973; WINTERS *et al.* 1976). This paper reports the results of an investigation of factors affecting the preparation of water soluble fractions of oils for use in toxicity studies. The use of synchronous fluorescence spectroscopy for the characterization of fractions was also examined.

EXPERIMENTAL

Equipment: All fluorescence measurements were made with a Perkin Elmer Model 3000 spectrofluorimeter.

Materials: A crude oil designated as Arabian Crude and a lubricating oil (MACOMBA 82) were obtained from commercial sources. Seawater was collected from a pristine environment, filtered through pre-combusted Whatman Type F glass fibre filters, sterilized by steaming and stored at 1°C until required.

Preparation of water soluble fractions: Water extracts of oils were prepared using the apparatus illustrated in Figure 1; 100 ml of seawater was poured into the flask and 0.1-1 g of oil layered onto the water surface through the side arm B. The flask was placed into a thermostated shaking water bath and agitated to produce a turbulent layer of less than 0.5 cm depth. The water soluble fraction was removed through the centre tube A.

Analysis: Aliquots (5 ml) of water soluble fractions were extracted with 5 ml of n-hexane using vortex mixing. The organic layers were removed, dried over anhydrous sodium sulphate and the

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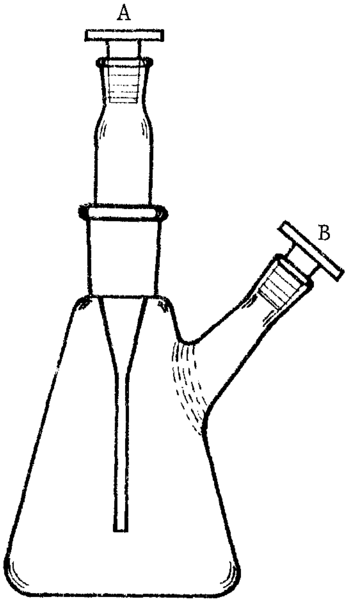

Fig. 1. Apparatus for the preparation of water soluble fractions of oils.

aromatic hydrocarbon contents estimated by fluorescence emission spectroscopy. Solutions were excited at 300 nm, the emission scanned from 310-390 nm and the fluorescence emission intensity of the main peaks measured and reported as equivalents of the respective oils.

RESULTS AND DISCUSSION

Preparation of water soluble fractions: Mixing of oil with seawater by gentle oscillation rather than by turbulent stirring was used in an attempt to simulate the process of the contact of oil slicks with seawater because it has been shown previously that a large fraction of the oil solubilized by turbulent mixing is lost on allowing the mixture to stand (GORDON *et al.* 1973). Also emulsions can be produced during turbulent mixing which may be more toxic to organisms than the water soluble extracts alone.

The aromatic hydrocarbon concentrations of the water soluble fractions were found to be dependent on the time of mixing the temperature and the amount of oil initially added. The aromatic hydrocarbon concentrations showed a gradual increase over several days (Figure 2), after which a slight decrease in concentration occurred. There was no evidence of bacterial activity in the aqueous phase and losses are attributed to adsorption from solution onto glass surfaces. The solution temperature was found to have a profound effect on the dissolved aromatic concentration

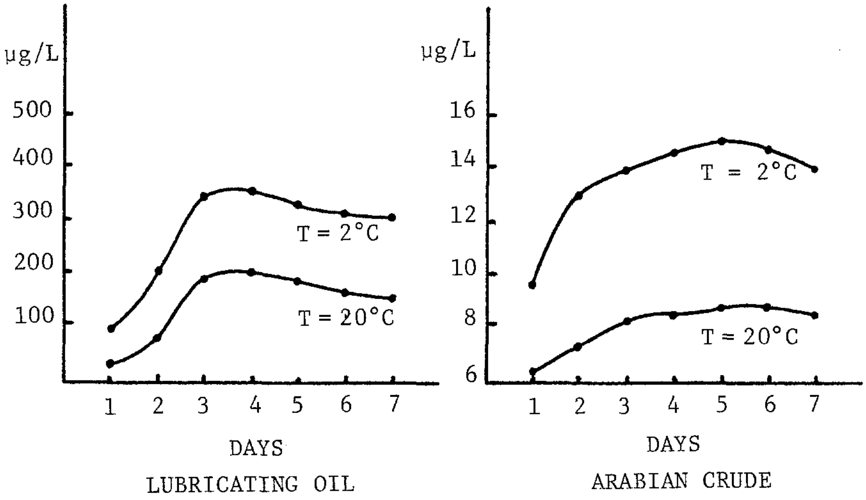

Fig. 2. Variation of total dissolved aromatic hydrocarbon concentration with time and temperature (Salinity 20%S).

(Figure 2). Decreasing the temperature from 20° to 2°C resulted in an 80% increase in concentration. Synchronous fluorescence spectra of the hexane extracts of water soluble fractions revealed no apparent changes in the relative amounts of dissolved aromatic components with temperature. However as the solubility behaviour of the oils was influenced by temperature, preparation of water soluble extracts should be at the temperature at which toxicity studies are to be performed.

Under conditions of constant temperature, salinity and mixing, the dissolved aromatic hydrocarbon concentration was dependent on the type and amount of oil added (Table 1).

Table 1. Relationship of oil added to dissolved aromatic hydrocarbon concentration. (Temperature 20°C; Salinity 20%S).

Oil added (g)	Dissolved aromatic hydrocarbon concentration (µg/L)
Arabian Crude	
0.1	15
0.2	30
0.5	50
Lubricating Oil	
0.1	137
0.2	220
0.3	370
0.5	540

Oils differ in composition, hence the solubilities of the oils will also differ and this was reflected in the different water soluble aromatic hydrocarbon concentrations of the test oils. Increasing the amount of oil added increased the water soluble aromatic hydrocarbon concentration but not in proportion. This was probably due to adsorption of oil onto glass surfaces which was visible at higher oil concentrations. The formation of large oil slicks may have also hindered solubilization of the oil in the aqueous phase.

Filtration of water soluble extracts through glass fibre filters (retains particulates > 0.5 μm) reduced aromatic hydrocarbon concentrations by 40-75% indicating that a substantial proportion of solubilized aromatic hydrocarbons was present as particulate or colloidal material.

Characterization of water soluble fractions

As well as water soluble fractions varying in total aromatic hydrocarbon concentration, variation in aromatic hydrocarbon composition can also occur. The use of synchronous fluorescence spectroscopy (LLOYD 1971; WAKEHAM 1977; VODINH 1978) to characterize the water soluble fractions was examined.

Analysis of hexane extracts of water soluble fractions (Figure 3) revealed compositional differences in the Arabian Crude and Lubricating oil extracts. The Arabian Crude water soluble fraction was enriched in aromatic compounds containing 5 or more rings (λ 380-480 nm) relative to 3 and 4 ring compounds (λ 340-380 nm), while for the lubricating oil the reverse occurred. Compositional differences of petroleum products are further illustrated by the synchronous fluorescence spectra of a water soluble fraction of a marine diesel fuel (Figure 3). The extract was enriched in 2 ring aromatic compounds (λ 280-340 nm) relative to higher ring aromatic compounds.

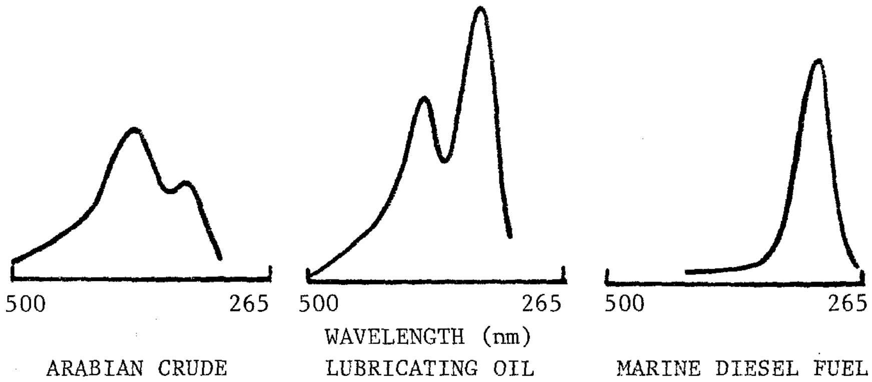

Fig. 3. Synchronous fluorescence spectra of hexane extracts of water soluble fractions.

Although the spectra only give a qualitative analysis of the major types of aromatic compounds present in water soluble fractions, if a water soluble fraction proves to be toxic to a particular organism, the spectra should allow the prediction of the types of toxic components present.

Conclusions

1. This study has shown that the concentration of aromatic hydrocarbons present in water soluble fractions of oils is dependent on the mixing time, temperature and the initial quantity of oil present. Therefore to obtain reproducible water soluble fractions of oils for toxicity studies these variables need to be controlled.

2. Synchronous fluorescence spectroscopy provides a rapid means of broadly characterizing the types of aromatic hydrocarbons present in water soluble fractions of oils.

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