Bull. Environ. Contam. Toxicol. (1986) 36:226–229

Bulletin of Environmental Contamination and Toxicology

Preparation of Water Soluble Fractions of Crude Oils for Toxicity Studies

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Petroleum hydrocarbons (oils) are major contaminants of estuarine and coastal environments (Connell and Miller 1981; Knap et al 1979). Marine organisms accumulate hydrocarbons (Lee et al 1976; Morris 1974) but little is known about the effects of repeated exposures of organisms to low concentrations of hydrocarbons.

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 (Neff and Anderson 1975; Zikto and Carson 1970).

A widely used procedure for producing water soluble fractions of oils for toxicity studies is the slow stirring method of Anderson et al (1974), in which an oil is layered on to water and mixed by means of a magnetic stirring bead. This paper examines the factors affecting the preparation of water soluble fractions of oils by this method. The water soluble fractions were also examined after filtration through membranes of varying pore sizes to establish the importance of particulate matter in the process of solubilization.

MATERIALS AND METHODS

Crude oils were obtained from commercial sources. Sea water was collected from a pristine environment, passed through a 0.45 µm filter and sterilized by steaming.

Water extracts of oils were prepared using a mixing flask that has been previously described (Maher 1982); 400 ml of sea water was poured into the flask and 1-3 g of oil layered onto the surface. The flask was placed into a thermostated water bath (20^0C) and magnetic stirring used to produce a turbulent layer of less than 0.5 cm depth. The water soluble fraction was removed through a tube located below the oil layer. All experiments were performed in the dark.

Aliquots of water soluble fractions (10 ml) were extracted with 5 ml of n-hexane using vortex mixing. The organic layers were

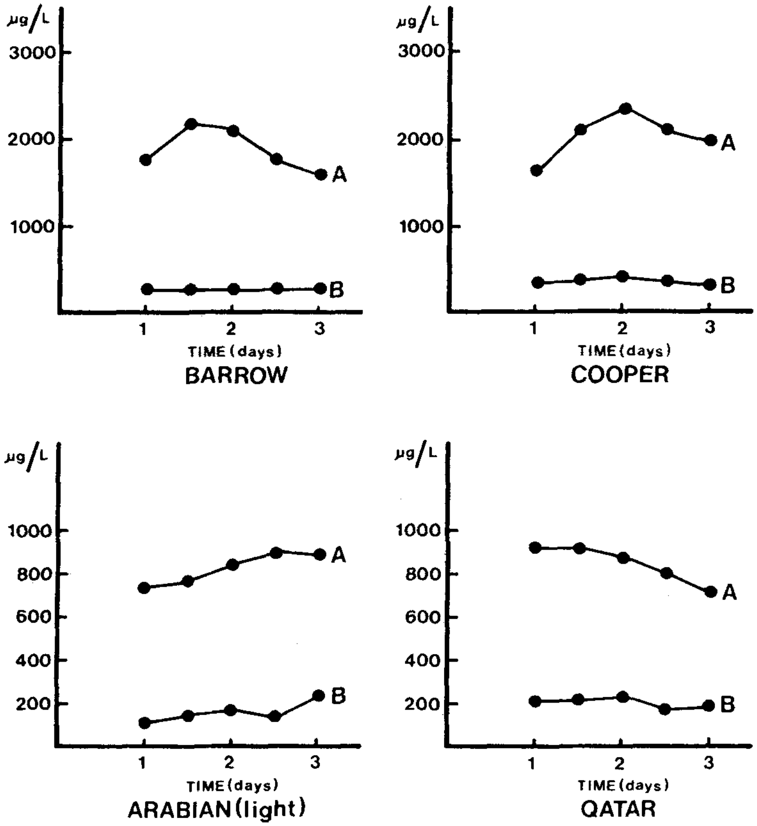

Figure 1. Effect of stirring time on the aromatic hydrocarbon concentrations of water soluble fractions (a) unfiltered, (b) filtered (0.45 µm)

removed, dried over anhydrous sodium sulphate and the 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

The effect of mixing time was examined by stirring separate batches of oil and seawater for different periods of time. The aromatic hydrocarbon concentrations of the aqueous phases gradually increase over 24-48 hours (Figure 1) after which a decrease in concentration occurred. Losses are attributed to the loss of particulate oil from solution as the aromatic hydrocarbon concentrations of filtered solutions (Figure 1) remained constant.

Increasing the amount of oil added increased the water soluble aromatic hydrocarbon concentrations but not in direct proportion (Table 1). Adsorption of oil onto glass surfaces was visible at higher oil concentrations, while the formation of larger oil slicks may have hindered solubilization of oils into the aqueous phase.

Table 1. Effect of filtration on the aromatic hydrocarbon concentration of water soluble fractions

Oil added	Filtration pore size (µm)(1)	Concentration (µg/L)			
		1	2	3	4
Barrow	U.F.(2)	1700	2200	2800	3100
	1.0	530	510	620	680
	0.8	310	360	480	520
	0.45	280	310	415	460
Cooper	U.F.	1000	2055	2452	2610
	1.0	424	642	804	844
	0.8	431	402	603	618
	0.45	391	402	482	603
Arabian light	U.F.	759	880	1030	-
	1.0	220	370	405	-
	0.8	150	282	371	-
	0.45	142	253	341	-
Qatar	U.F.	900	1490	1900	-
	1.0	387	590	670	-
	0.8	215	340	510	-
	0.45	215	380	495	-

(1) Millipore RA, AA and HA filters used.

(2) U.F. unfiltered.

The aromatic hydrocarbon concentrations of water soluble extracts were progressively decreased by filtration through membranes of smaller pore size (Table 1) illustrating the contribution of particular matter to the aromatic hydrocarbon concentrations of water soluble fractions.

Prior to removal of the aqueous phase it was necessary to allow the oil-water mixtures to stand for oil droplets to separate upwards. An interval of 10-15 min. was found to be sufficient to obtain reproducible aromatic hydrocarbon concentrations in the aqueous phase.

This study has shown that the concentrations of aromatic hydrocarbons in water soluble fractions of crude oils are dependent on the mixing time, the oil-water ratio and the particulate oil solubilized. Therefore to obtain reproducible and comparable water soluble fractions of crude oils for toxicity studies these variables need to be controlled.

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Received February 6, 1985; accepted February 11, 1985.