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SOME EXPERIMENTAL PROBLEMS IN TOXICOLOGICAL STUDIES OF OIL ON MARINE ORGANISMS.

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ABSTRACT

In the field of laboratory toxicological studies of oil on marine organisms, the obtained results and their interpretation and intercomparison are dependent on the experimental conditions, especially the method by which the testing medium is prepared. The medium is usually either oil in dispersion or the water accommodation fraction of oil (WAF).

In this work, the effects of the experimental conditions during the preparation of WAFs of Arabian heavy crude oil are monitored and discussed. Both the ratio of oil to water and the duration of oil-water phases separation are considered. Also, variations in the concentration of WAFs off oil were followed along the habitual time of toxicological experiments: from 1 to 5 days.

The results showed that the working media of WAFs of oil are usually in direct correlation with the above mentioned two factors to the initial concentration of oil in the medium corresponds and never the final one during the experiment. However, most probably, this is why an over all comparative analysis of the available data on the toxicology of oil on the marine organisms has not yet been attempted.

INTRODUCTION

By reviewing laboratory toxicological studies searching on the effects of oil on marine organisms, range of toxic and threshold concentrations of petroleum is exceedingly wide for the majority of the investigated organisms (Mironov, 1972; Anderson et al., 1974 and Patin, 1982). Such variations in obtained results is mainly due to variability in the different experimental methods employed as well as behaviour and conversion of petroleum in the media during the bioassays. In fact, petroleum hydrocarbons in aquatic environment are exposed to instance microbial activity as to physiochemical factors. A major factor is the continuous fractionation of thousands of chemical compounds contained in crude oils. Strictly speaking, the concentration and composition of petroleum products in solution are never constant even under the controlled conditions of toxicological experiment. By consequence, test-organism will be under continuous variable concentration stress. Hence, the final result- at the end of the experiment-should be dependent either upon the magnitude of variations in total

concentration during the experiment or upon certain critical step of petroleum product transformation (for example, conversion of untoxic product to another toxic one). The problem becomes more complicated when the dynamic factor of oil in water is considered resulting in the distribution of petroleum hydrocarbon products between the gas, solution, emulsion and film phases. This is beside the variations in behaviour between different types of oils even variations of the same type in different sorts of natural waters.

Because of the above mentioned problems, the majority of workers were limiting their efforts on searching the effects of water-soluble fraction (WSF) preferably called water accommodated fraction (WAF) because all the petroleum hydrocarbons present in water experiment are not necessarily in a soluble form (Widdows et al., 1982). In spite of unifying the form of oil hydrocarbons in the works reviewed by Anderson (1977), Johnson (1977), Kuhnhold (1977) and Patin (1982), data on the effects of petroleum WAF for different taxonomic groups indicate ambiguous sequence of resistance even when the same type of oil is used. These variations in results are primordially attributed to variations of the followed technique for WAF preparation although the basis is usually the same. The preparation usually includes: adding certain amount of crude oil to water, shaking the mixture mechanically, leaving the produced oil/water emulsion for stabilization and phases separations and withdrawing the lower aqueous layer which could be used directly or after dilution in the bioassay tests.

From local point of view, contamination by Arabian crude oils is the most probable in the marine environment along the Saudian Red Sea coast (Awad, 1988). However, the present work includes a trial to standardize a method for the preparation of WAF from one type of these crude oils (heavy Arabian) to be followed in-in vitro-petroleum toxicological studies. Practical demonstration for the magnitude of effects due to variations in WAF preparative steps on its concentration in the aqueous experimental media is also included.

MATERIALS AND METHODS

Using different ratios of oil to natural seawater, water accommodation fractions (WAF) from heavy Arabian crude oil were prepared. Changes in the produced WAF concentrations with time are followed.

The used preparative steps for WAF could be summarized as follows:

- working crude oil: Arabian heavy (density: 0.887, viscosity: 19.1 cps at 100^{o}F)

- working seawater : natural filtered seawater, salinity: 39.39‰
- Percentages of oil in water: 1%, 2% and 4% (in 2 liters total volume)
- shaking mean: mechanically with 3 glass blades propeller.
  - shaking power: fixed at 300 rpm.
  - shaking duration: fixed for 24 hr.
  - aqueous phase separation time: 3, 6, 12 and 24 hr.
  - intervals of oil-dosing in WAF: 12, 24, 48, 72, 96 and 120 hr (durations covering the traditional toxicological bioassays).

Total hydrocarbons in samples of WAF collected under any of the above mentioned conditions were extracted by carbon tetrachloride, evaporated to dryness at 60°C and re-taken in n-hexane. Detection of concentrations of THC was fixed at 310 nm and the fluorescent wave length at which THC is measured corresponds to 382 nm. The used instrument was Baird fluoripoint spectrofluorometer, Ratiometer RC 200.

RESULTS AND DISCUSSION

Before the discussion of the obtained data concerning the variation of oil-WAF concentration during the bioassay experiments, it is interested to expose firstly the results of a complementary test showing to what extent the working oil-WAF concentration could be affected by changing only one of its preparative steps. However, while shaking power (300 rpm), phases-separation time (3 hr) and ratio of oil to water in the mixture and depth of shaking center (4 cm below the surface) are fixed, the shaking time is varied (3, 6, 12 and 24 hr). The obtained results grouped in Table 1 and represented in Figure 1 show that for the three used initial ratios of oil in water (1%, 2% or 4%), concentration of oil-WAF is usually in gradual decreasing parallel to increase in shaking time. This phenomenon could be attributed to the fact that a part of the produced minute particles of oil in WAF solution tends to leave the aqueous medium and concentrates on its surface (Bishop, 1983). This operation is normally increased by applying more shaking time. However, more shaking time leads to more loss in working oil-WAF. For example, when 1% of oil in water is shaked for 3 hr, the produced concentration is 5.2 ul^{-1}. This value is diminished gradually with increasing in shaking time to reach 3.4 ul^{-1} at the end of 24 hr shaking, losing 34.6% of its initial concentration (Table 1). The results indicate also that the rate of loss in oil-WAF concentration is increased with increasing the starting percentage of oil in oily water mixture. It was found that after 24 hr of shaking, the tested oil/water mixture (1%, 2% and 4% oil in water) have lost respectively 34.6%, 40% and 69.6% of their concentrations in comparison to the corresponding measured concentrations for 3 hr shaking time cases.

The other set of results grouped in table 2 shows the trend of variation oil-WAF concentration during bioassay tests ranging in duration from 12 hr to 5 days. It could be noted that there is a proportional declination in the measured oil-WAF concentration as the duration of bioassay increases. Also, the initial and final concentrations as well as the rate of their loss with time are dependent on the characteristics of the followed preparative steps:

- initial ratio of oil to water
- oil-WAF/oil phases-separation time.
- duration of the bioassay experiment.

For example, when only the ratio of oil to water is successively doubled in the range from 1% to 4% and the other preparative steps are the same (6 hr phases-separation time for 5 days bioassay experiments), oil-WAF concentrations are varied from 3.9 to 1.3, from 11.2 to 3.2 and from 3.1 to 1.6 successively (all expressed in ul^{-1}). Other examples could be concluded by intercomparing any homogeneous set of results in table 2 and followed in Figure 2.

The results in this work could indicate that the followed method for oily aqueous media preparation in one of the main reasons for the actual difficulties faced the intercomparison of the obtained results in the field of toxicology when the crude oils or their products are considered. In fact, organisms in bioassay experiments are exposed to variable concentration of oil contrary to what it is thought. However, their responses to oil contamination are parallely varied during the whole duration of the experiment. In fact, in long-term bioassay, rapid depletion of oil from the medium is the main cause for the apparent organism survival rather than because of their resistance and tolerance to the initial used oil concentration.

CONCLUSION

In the present work, effects of variations in preparative steps of contaminated medium with oil on the oil-WAF concentration during laboratory toxicological studies are monitored. The results showed that unless a standard method for the preparation of contaminated media is established and followed precisely, it well be impossible to intercompare and interpret the obtained results in this field. The results showed that any change or slight modification in even one preparative steps for oil-WAF could be reflected tightly on the actual used concentration, by consequence, on the assessed response and tolerance of organisms under investigation. Also, it should be taken into consideration that the laboratory conditions in which the bioassays are conducted are playing important roles on the final report of toxicological studies using oil as contaminant. In fact, an organic pollutant as oil is usually exposed to loss from the

working medium, mainly through evaporation, biodegradation and photochemical decomposition and structural modification. However, a precise standard procedure for the ambient conditions is also needed as well as for oily contaminated working medium preparation. This is the only way through which an overall comparative analysis of obtained data on the toxicology of oil on the marine organisms could be successfully attempted in the future.

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TABLE 1
Effect of shaking duration on the WAF concentration using different percentage of heavy arabian is seawater.

shaking time	Percentage oil in water 1%	2%	4%
3	5.2	8.5	5.6
6	4.3	6.7	3.9
12	3.5	5.8	2.6
24	3.4	5.1	1.7

TABLE 2
Variations in working WAF concentration in ul l^{-1} using different percentage of heavy arabian oils in water (a) using different phases-separation duration in hr (b) with time in hr (c).

a		1%			2%			4%		
c	b	6	12	24	6	12	24	6	12	24
0		3.9	2.6	1.7	11.2	6.7	5.1	3.1	8.6	4.4
12		2.8	2.0	1.5	7.3	4.4	3.9	2.9	4.4	3.0
24		2.6	1.8	1.5	6.5	3.0	3.0	2.8	4.1	2.8
48		2.3	1.6	1.4	5.9	2.5	2.1	2.7	3.8	2.4
72		1.8	1.3	1.1	5.0	2.3	2.0	2.0	3.7	2.3
96		1.5	1.3	1.1	4.6	2.3	1.7	1.6	0.9	1.8
120		1.3	1.1	1.0	3.2	2.1	1.2	1.6	0.8	1.4

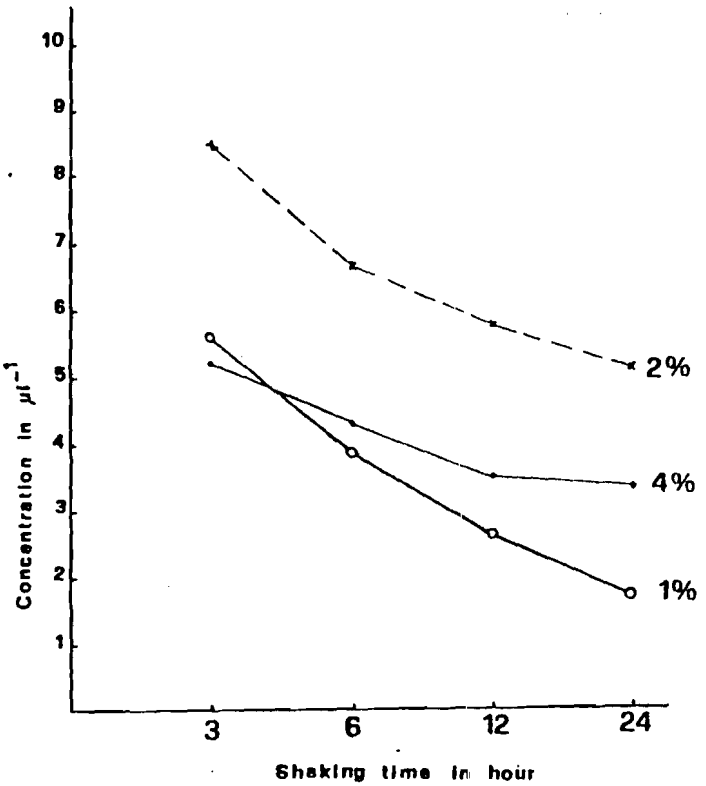

Fig. 1 : Variation of WAF concentration of heavy arabian with increasing of shaking time.

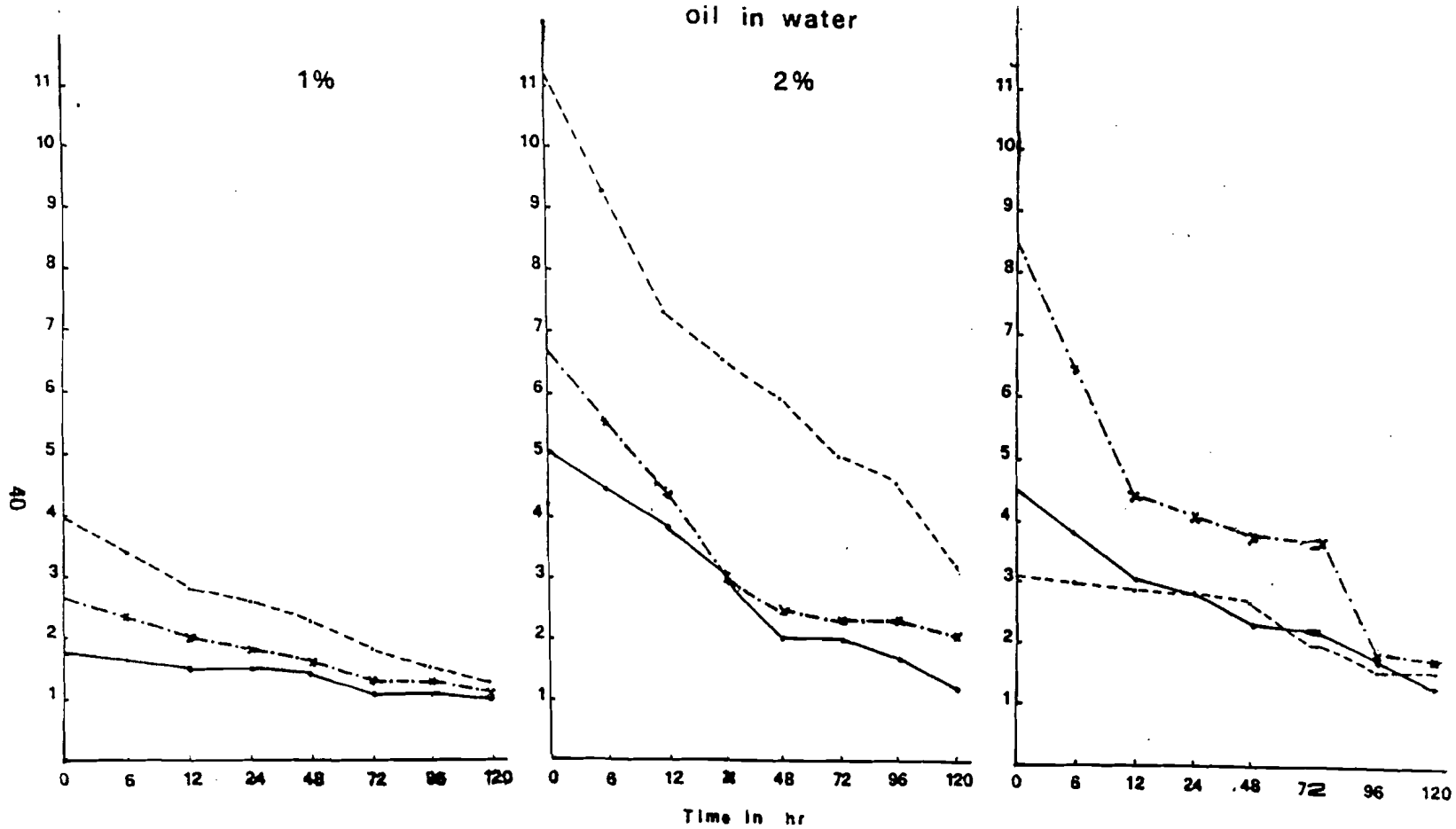

Fig. 2 : Variation of oil - WAF Concentration during bioassay experiments

(Phases - separation time ---- 6hr. -·-·- 12hr, ——— 24 hr.)