D-4.2 The estimation of toxic effects of water-soluble fractions of crude oil on marine organisms

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Abstract The increase in oil contamination of marine and estuarine environments has led to concern about the effects of oil pollution on marine organisms. The most immediate toxic and subtoxic fractions of oils are those soluble in water. The water-soluble fraction of crude oil may produce sublethal effects, such as physiological and biochemical changes, morphological deformations, tissue damage, and abnormal behavior. We examined toxicity of the water-soluble fraction of crude oil and its components. Results are given as under.

1. Main components in water-soluble fraction of Kuwait crude oil were analyzed. Highly volatile compounds like butanes, propanes, pentanes and hexanes were easily lost from seawater. Naphthalenes remained for a longer period. Water-soluble fractions had severe toxic effects on saltwater fish and prawn.

2. 24-h lethal concentrations to red sea bream were 7.8 μl/l(benzene), 12.7 μl/l (toluene), 0.75 mg/l(naphthalene). The values of these chemicals to prawn were 9.7 μl/l, 12.6 μl/l, and 3.98 mg/l, respectively.

3. Dibenzothiophene, which is one of organic sulfur compounds contained in crude oil, was found to remain in seawater for a long time. The lethal concentrations of this component to red sea bream were 0.39 (24 h), 0.28 (48 h), 0.17 (72 h), and 0.15 mg/l (96h) and those to prawn 0.47 mg/l (24-96 h). The bioconcentration factors of dibenzothiophene were 900 in red sea bream and 180 in prawn after exposure for 6 weeks.

Key Words Water-Soluble Fraction, Marine Organisms, Volatile Compounds, Organic Sulfur Compounds, Bioconcentration

1. Introduction

The rapid increase in the demand for and utilization of petroleum and petrochemicals has resulted in a steadily increasing level of petroleum contamination of marine and estuarine waters. The oil spill accident occurred in the Persian gulf caused severe damage to marine environment and the harmful effects possibly continue for long periods. Components of crude oil have various toxic effects on livings. However, the toxicity and accumulation of these hydrocarbons in marine organisms have not been fully understood. Therefore, the study to estimate toxicity and accumulation of the water-soluble fractions of crude oil is further required.

2. Research Objective

(1) The mixing mode to prepare the water-soluble fraction and other experimental factors will influence the physical nature and chemical composition of the extract. Compositions of the water-soluble fractions of Kuwait crude oil are analyzed to estimate their physical and chemical characteristics. Preliminary toxicity tests for the water-soluble fractions are carried out.

(2) Highly volatile compounds are related to acute toxicity to organisms and non-volatile persistent compounds are involved to bioaccumulation. LC50 of aromatic hydrocarbons to the red sea bream and prawn are estimated and organic sulfur compounds contained in crude oil and possibly concentrated in organisms are analyzed.

(3) Dibenzothiophene is one of organic sulfur compounds to remain in seawater for a long period. The acute toxicity and accumulation of this compound in red sea bream and prawn are determined.

3. Method

(1)–a.Monitoring dissolution process

Solutions of Kuwait crude oil were prepared by carefully layering 0.5 *l* oil on top of 4.5 *l* seawater held in a 5–*l* glass beaker and closed with a polyethylene bag. By use of a standard magnetic stirrer run at reduced voltage, gentle stirring was achieved, i.e., no deformation of the oil–water interface was observable. Direct fluorescence spectroscopy of the samples was applied for monitoring the dissolution process. Emission spectra at excitation wavelength 230 nm, favoring the fluorescence of naphthalenes around 335 nm, and at excitation wavelength 265 nm, favoring the fluorescence of phenols around 300 nm, were recorded in a Hitachi MPF–2A fluorescence spectrometer. The typical spectra and the recorded values were designated "naphthalene fraction" and "phenol fraction" here. The fluorescence unit was arbitrarily chosen.

(1)–b. Analysis of major components of the water–soluble fraction

Extract samples were prepared by stirring oil and seawater for 24 h (sample No. 1), stirring oil and seawater for 24 h followed by stirring seawater for 24 h after removal of oil (sample No. 2), and stirring oil and seawater for 240 h. The chemical content of highly volatile components was determined by head–space analysis using GC–MS. Less volatile compounds were extracted with n–hexane and analyzed by GC–MS.

(1)–c. Preliminary toxicity test

Toxicity of sample No. 1 and sample No. 2 (see (1)–b) to girella, *Girella punctata*, (BW: 0.27–0.73 g) and shrimp, *Leander serrifers* Stimpson, (BW: 0.24–0.54 g) was estimated. Ten specimens were held in 4–*l* test solution and monitored the survival rate. The water temperature was 21℃. Fish were not fed during experimental period.

(2)–a. Acute toxicity test

Acute toxicity tests of benzene, toluene, naphthalene, and crude oil were carried out by static bioassays in 5–*l* glass containers. Ten red sea bream, *Pagrus major*, (BW: 0.19–1.08 g) and 6 prawn, *Penaeus japonics*, (BW: 0.06–0.35 g) were housed in each glass container. The test solution was not renewed for 24 h. The concentration of benzene in each test solution reduced to 84.3%, toluene to 41.8%, and naphthalene to 41.1% in average. Aeration was not made. Fish did not receive food during experiments. The salinity, pH, and DO of seawater were 34‰, 7.1–7.7, and 2.5–7.1 mg/*l*, respectively.

(2)–b. Analysis of organic sulfur compounds in the water–soluble fraction

Extract samples were prepared by stirring oil and seawater for 240 h (sample No. 1), stirring oil and seawater for 240 h followed by stirring seawater for 240 h after removal of oil (sample No. 2), and stirring oil and seawater for 480 h. Components with low boiling point was determined by head–space analysis using GC–MS and GC–AED. Components with high boiling point were extracted with n–hexane and analyzed by GC–MS and GC–AED. Results were shown as sulfur concentration in seawater.

(3)–a. Acute toxicity of dibenzothiophene

Red sea bream (BW :0.13–0.51 g) and prawn (BW: 0.045–0.133 g) were used as experimental animal.

(3)–b. Accumulation of dibenzothiophene

One hundred red sea breams (Mean BW: 6 g) and 100 prawns (Mean BW: 0.7 g) were housed in each 60–*l* experimental aquarium. The experimental aquaria were provided with continuous supply of filtered seawater flowing at a rate of 30 *l*/h. Stock solutions of dibenzothiophene were supplied by micro constant flow pumps. After 2, 4, and 6 weeks of exposure, 3 fish and 10 prawns were sampled. The clearance of dibenzothiphene from fish and prawn was determined after exposure for 6 weeks.

(3)–c. Determination of dibenzothiophene

Dibenzothiophene was determined by GC–MS.

4. Results and Discussion

(1) Solutions of Kuwait crude oil were prepared by carefully layering 0.5-*l* oil on top of 4.5-*l* seawater and mixing slowly by a standard magnetic stirrer. The dissolution process of naphthalene fraction (em. 335 nm, ex. 230 nm) and phenol fraction (em. 300 nm, ex. 265 nm) in water-soluble fractions were monitored by direct fluorescence spectroscopy for 13 days. Results are shown in Figs 1 and 2. Content of the phenol fraction in the water phase reached equilibrium after 1 day. Content of the naphthalene fraction increased slowly throughout the experimental period. Reduction in the fluorescence of these fractions was monitored for 10 days after removal of upper oil layer. The fluorescence of the phenol fraction reduced to 50% after 2 days and to 25% after 10 days. The fluorescence of the naphthalene fraction reduced more slowly than that of the phenol fraction. Chemical components in the water-soluble fraction dissolved in seawater were determined by GC-MS. Results are listed in Table 1. Three samples were prepared (see (1)-b in method). Butanes, propanes, pentanes, hexanes, and n-paraffins of alkanes were detected after mixing oil and seawater for 24 h (sample No. 1). Highly volatile compounds like butanes, propanes, pentanes, and hexanes were easily lost by stirring for 24 h without upper oil layer (sample No. 2) or by mixing oil and seawater for 240 h (sample No. 3). Toluenes, benzenes, and naphthalenes were dominant components among aromatics in sample No. 1. These aromatic compounds and alkanes were lost by stirring without upper oil layer. Concentrations of naphthalenes, 2-ethyltoluene, 4-ethyltoluene, trimethylbenzene, and tetramethylbenzene in sample No. 3 were higher than those in sample No. 1. Preparatory toxicity tests to evaluate the toxicity of sample No. 1 and No. 2 to girella and shrimp were conducted. All fish and shrimps exposed to sample No. 1 died within a few minutes. The survival rate of fish exposed to sample No. 2 decreased to 50% after 24 hours and not changed thereafter. The survival rate of shrimps exposed to sample No. 2 was 100% after 8 h but 0% after 24 h. The water-soluble fraction of Kuwait crude oil was thus highly toxic to marine organisms.

(2) 24-h lethal concentrations of oil components to red sea bream and prawn were determined. Results are shown in Table 2. The values of benzene, toluene, and crude oil to red sea bream were almost same as those to prawn. However, the 24-h lethal concentration of naphthalene to prawn was over 5 times of that to red sea bream. Organic sulfur compounds in three samples (see (2)-b in method) of water-soluble fractions of Kuwait crude oil were analyzed. Results are shown in Table 3. Various organic sulfur compounds were detected and could not be identified. The sulfur concentration of organic sulfur compounds with low boiling points in sample No. 1 and 3 were 8.7 and 13.8 μg/*l*, and not detected in sample No. 2. The sulfur concentrations of organic sulfur compounds with high boiling point were 340 μg/*l* in sample No. 1, 292 μg/*l* in No. 2, and 209 μg/*l* in No.3. These results suggest the organic sulfur compounds with high boiling points to remain in seawater for long periods.

(3) The lethal concentrations of dibenzothiophene, which is one of organic sulfur compounds to be contained in crude oil, to red sea bream and prawn were determined. Results are shown in Table 4. These values are much lower than that to freshwater fish, *Oryzias latipes*. The bioconcentration factors of dibenzothiophene were 900 in red sea bream and 180 in prawn after exposure for 6 weeks. These results suggest dibenzothiophene to be a possible pollutant that affects marine life for a long period.

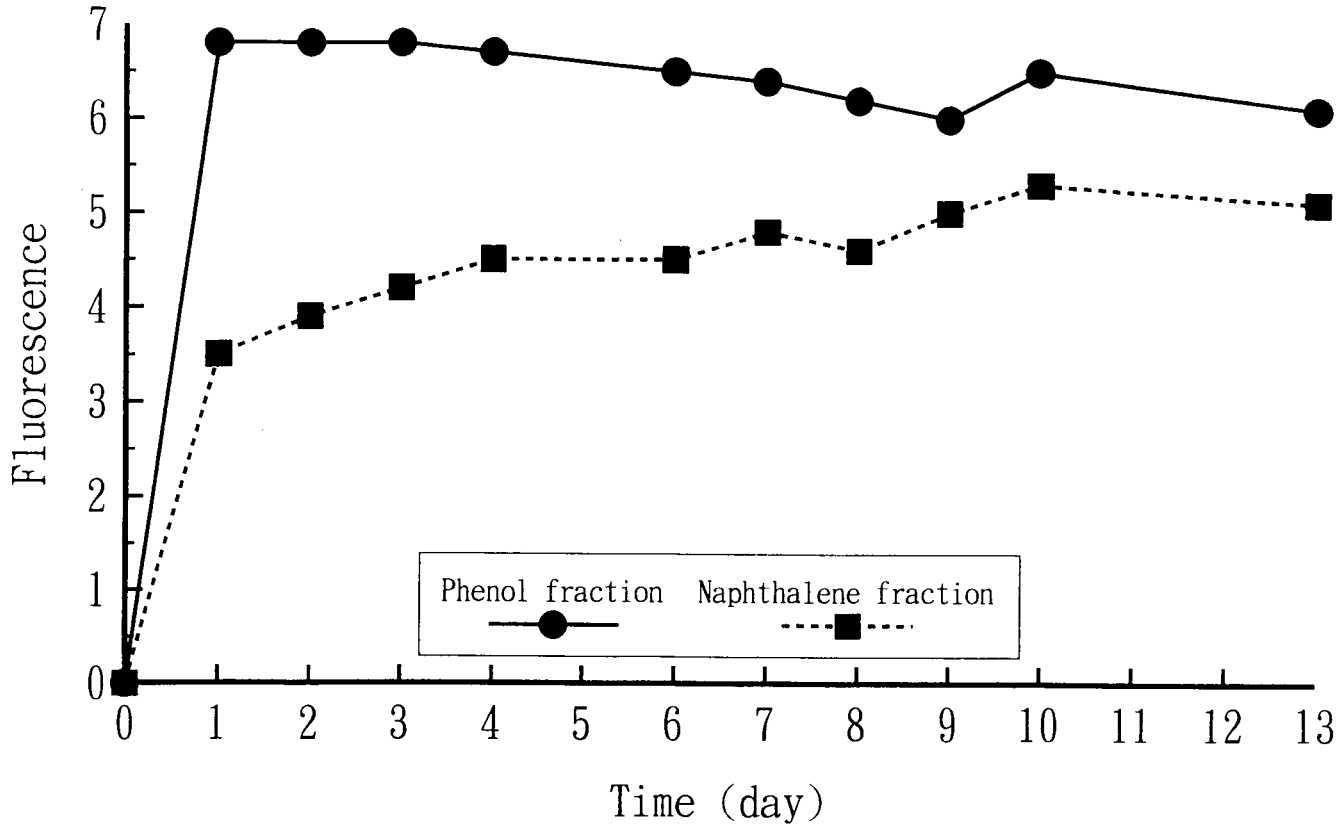

Fig. 1 Dissolution of water-soluble fractions

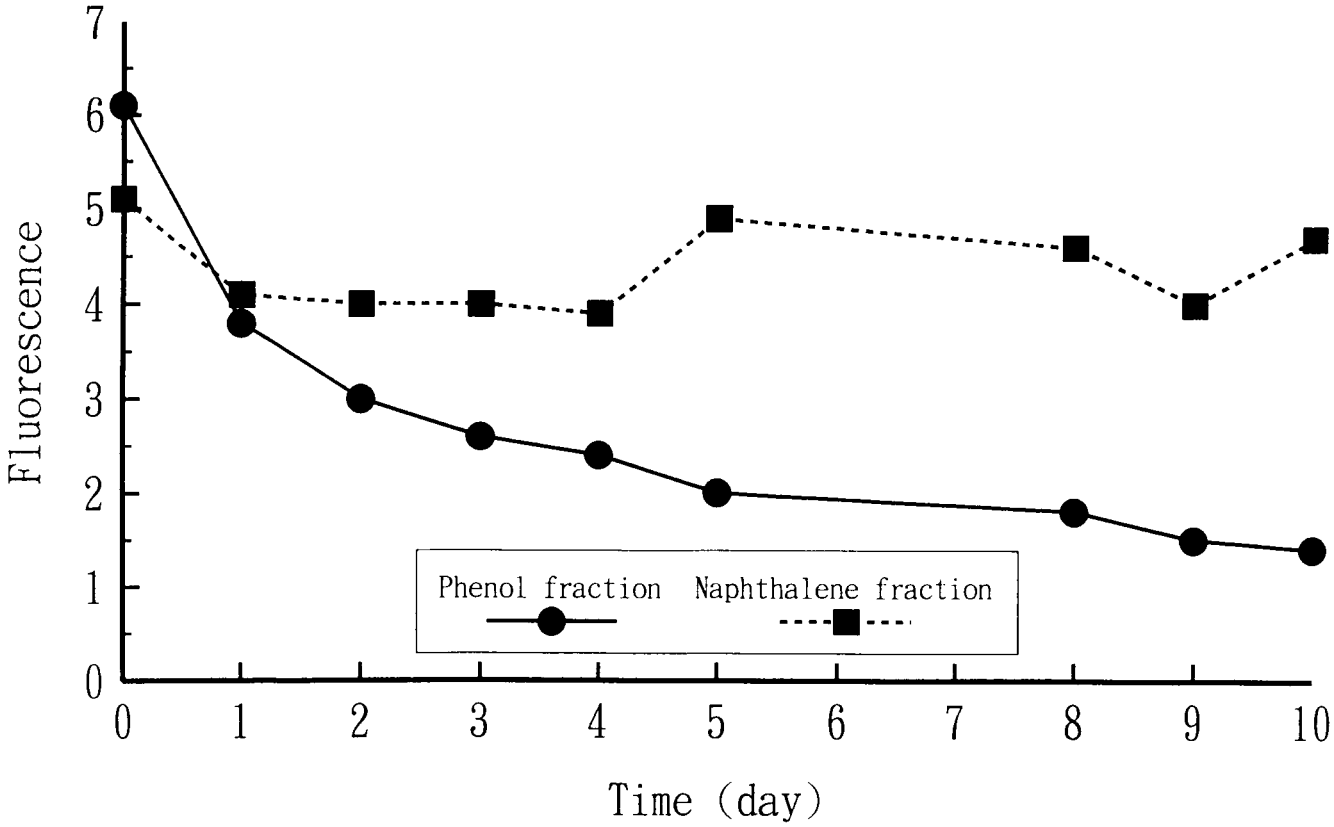

Fig. 2 Disappearance of water-soluble fractions

Table 1. Chemical composition of seawater solutions of Kuwait crude oil stirred for different periods (mg/l)

	Sample No. 1	Sample No. 2	Sample No. 3
Alkanes			
Ethane	0.052	0.002	0.001
Propane	0.28	0.009	N.D.
Isobutane	0.091	0.004	N.D.
Butane	0.43	0.013	0.002
Isopentane	0.18	0.008	0.004
Pentane	0.27	0.010	0.007
Cyclopentane	0.13	0.008	0.007
2-Methylpentane	0.047	0.003	0.005
n-Hexane	0.096	0.003	0.010
Methylcyclopentane	0.073	0.005	0.007
Cyclohexane	0.085	0.005	0.009
Methylcyclohexane	0.037	0.003	0.014
C_{8-34} n-paraffin	0.295	0.073	0.532
Aromatics			
Benzen	0.90	0.056	0.030
Toluene	1.1	0.060	0.34
Ethylbenzene	0.18	0.011	0.14
m,p-Xylene	0.42	0.026	0.36
o-Xylene	0.26	0.023	0.24
2-Ethyltoluene	0.11	0.004	0.12
4-Ethyltoluene	0.082	0.006	0.10
Trimethylbenzene	0.22	0.012	0.26
Tetramethylbenzene	0.12	0.018	0.14
Naphthalene	0.033	0.005	0.044
2-Methylnaphthalene	0.021	0.004	0.031
1-Methylnaphthalene	0.022	0.006	0.031
Dimethylnaphthalene	0.033	0.006	0.052
Phenanthrene	0.004	N.D.	0.007

N.D. : not detectable (<0.001 mg/l)

Sample No.1: Stirred with oil for 24 h; Sample No.2: Stirred without oil for 24 h after stirred with oil for 24 h; Sample No.3: Stirred with oil for 240 h.

Table 2. 24-h lethal concentrations of crude oil components

	Red sea bream	Prawn
Benzene (μl/l)	7.8 (7.1- 8.4)**	9.7 (6.5-14.2)
Toluene (μl/l)	12.7 (11.3-13.9)	12.6 (10.5-14.5)
Naphthalene (mg/l)	0.75 (0.61-0.89)	3.98 (3.7- 4.2)
Crude oil (%)*	20.6 (17.7-23.9)	29.7 (27.4-31.9)

* : Seawater solution stirred with 10% oil for 24 h was arbitrarily set to 100%.

** : 95% confidence limit

Table 3. Sulfur concentration in seawater solutions of Kuwait crude oil stirred for different periods (μg/l)

Sample No.	Volatile	Non-volatile
1	8.7	340
2	N.D.	292
3	13.8	209

Sample No. 1 : Stirred with oil for 240 h.
Sample No. 2 : Stirred without oil for 240 h after Stirred with oil for 240 h.
Sample No. 3 : Stirred with oil for 480 h.
N.D. : Not detectable (<0.1 μg/l).

Table 4. Lethal concentrations of dibenzothiophene (mg/l)

	Red sea bream	Prawn
24-h	0.39 (0.26-0.58)*	0.47 (0.42-0.53)
48-h	0.28 (0.20-0.36)	0.47 (0.42-0.53)
72-h	0.17 (0.03-0.26)	0.47 (0.42-0.53)
96-h	0.15 (0.04-0.22)	0.47 (0.42-0.53)

*95% confidence limit

Table 5. Concentrations of dibenzothiophene in fish and seawater

	Red sea bream	Prawn
Seawater		(ng/ml)
2 weeks	4.6	2.1
4 weeks	3.4	4.9
Fish		(ng/g)
0 week	1	2
2 weeks	970	16
4 weeks	2400	500
6 weeks	3700	640
6 + 2 weeks	66	5
6 + 4 weeks	20	4