Water-Soluble Components of Four Fuel Oils: Chemical Characterization and Effects on Growth of Microalgae

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Abstract

Approximately 50% of the compounds in the water solubles from 4 fuel oils have been identified via gas chromatography and mass spectrometry. In addition to the well-described types of compounds (naphthalenes, benzenes) expected in water-soluble extracts we have found phenols, anilines, and indoles. Of these classes of compounds methyl, dimethyl, and trimethyl derivatives are present in relatively high concentrations. The water solubles from the 4 fuel oils showed considerably different inhibitory effects to growth of 6 microalgae, 2 blue-greens, 2 greens, and 2 diatoms. Two of the fuel-oil extracts, Baytown and Montana, were lethal to blue-green algae. This was in part traceable to their content of p-toluidine which was found to be toxic to *Agmenellum quadruplicatum*, Strain PR-6, 1 µg in the algal lawn-pad assay and 100 µg/l in liquid culture. The water-soluble fraction from New Jersey fuel oil was lethal to the 2 green algae, with lesser effects on the 2 blue-greens. The 2 estuarine diatoms used as test organisms were not greatly inhibited by Baytown, Montana, or New Jersey fuel-oil water-soluble extracts. However, earlier work with an American Petroleum Institute fuel oil and the diatom *Thallassiosira pseudonana* (3H) showed that 3H was a very sensitive organism. Water solubles from the Baton Rouge fuel oil were almost without effect on the growth of all 6 microalgae. On the basis of the work herein and earlier work, a very cautious viewpoint is advisable in generalizing on the toxicity or lack thereof of a given fuel oil on the growth of different kinds of microalgae. On the other hand, with water solubles from toxic fuel oils such as Baytown or New Jersey the data clearly suggest that their potential for environmental damage is high, either through selective or enrichment effects on natural populations or through a lowering of total primary production.

Introduction

We have reported that water solubles from a fuel-oil sample obtained from American Petroleum Institute showed considerable toxicity to growth and photosythesis of representative types of microalgae (Pulich *et al.*, 1974). This work was done with only one fuel oil, and the question remained as to the toxicity of fuel oils in general. Herein we have expanded the observations on the chemical composition and toxicity of water solubles from 4 fuel oils tested against 6 microalgae — 2 blue-greens, 2 greens, and 2 diatoms. We have purposely used an open-type growth system, i.e., test-tube cultures continuously bubbled with 1% CO_2-in-air. In this type of growth system, lower molecular-weight volatile compounds will not persist for long and, unless their effect on the algae is rapid, they should not contribute to the results described here. The observations reported herein are then, in our opinion, caused by the relatively nonvolatile, potentially environmentally persistent, aromatic compounds in the water solubles from fuel oils.

Materials and Methods

Organisms and Growth Conditions

Strains PR-6 (*Agmenellum quadruplicatum*) and 17a (*Coccochloris elabens*) are coccoid blue-green algae, they are isolates of this laboratory. Strains DUN (*Dunaliella tertiolecta*) and 580 (*Chlorella autotrophica*) are green algae and were originally obtained from R.L. Guillard, Strains N-1 (*Cylin-*

drotheca sp.) and AMP-1 (*Amphora* sp.) are estuarine diatoms recently isolated into pure culture in this laboratory by J.C. Morgan. PR-6 and 17a were grown on medium ASP-2 (Van Baalen, 1962) containing 8 µg/l of vitamin B_{12}, DUN and 580 on medium ASP-2 plus 8 µg/l vitamin B_{12} and 1mg/l vitamin B_1, AMP-1 was grown on medium ASP-2 containing 8 µg/l vitamin B_{12}, 1mg/l vitamin B_1, and 250 mg/l $Na_2SiO_3 \cdot 5H_2O$; and N-1 on the same medium as used for AMP-1 but with the usual $NaNO_3$ nitrogen source of ASP-2 supplemented with 100 mg/l NH_4Cl.

All liquid culture work was done in a water bath at 30°C ± 0.1C° under continuous illumination from 20W Daylight fluorescent tubes, two on each side of the bath, 7.5 cm from the lamp center to the position of the tubes in the bath. The growth tubes were Pyrex 22x175 mm test tubes fitted with bubbling tubes, through which was passed continuously 1±0.1% CO_2-in-air. This general procedure is an adaptation of the method of Myers (1950).

With each organism, the inoculum was pre-conditioned to the above growth conditions. The inoculum size used to start each growth run was approximately 10^5 cells/ml. Specific growth-rate constants were determined turbidimetrically using a Model 402-E Lumetron Colorimeter. For convenience in data presentation, the specific growth-rate constants were converted to generations/day.

The conventional algal lawn technique was used to test pure compounds. Exponentially growing cells (Final concentration 5 to 10,000 cell/ml) were added to agarized ASP-2 medium (1% Difco agar, 0140) held at 42°C; 20 ml were then immediately distributed to plastic Petri dishes. The test materials, using absolute ethanol as solvent, were presented to the algal cells embedded in the agar by absorbing them on antibiotic sensitivity discs (12.7 mm) and placing the discs directly on the agar surface. The plates were then sealed with Scotch tape and incubated in the light for 3 to 7 days at 28° to 30°C. The experimental endpoint was the zone size of growth inhibition around the pad, judged visually and microscopically. No inhibition was seen in ethanol controls.

Fuel Oils and their Water-Soluble Extracts

The 4 fuel oils used herein were kindly provided us by Exxon Corporation. We are indebted to Dr. C.B. Koons of Exxon Production Research Company, Houston, Texas, for his help in obtaining these samples. The fuel oils are referred to by refinery location, Baytown (Texas), Baton Rouge (Louisiana), Montana (Billings) and New Jersey (Linden). The water solubles from each oil were prepared by addition of 1 part oil to 8 parts of culture medium in a bottle containing a Teflon-covered magnetic stirring bar. The bottle was sealed with aluminum foil and the water stirred at room temperature (ca. 25°C) for 24 h at a rate which avoided formation of an emulsion. The water was allowed to stand undisturbed for several minutes before being removed by means of a stopcock at the base of the bottle.

Chemical Characterization

The relative paraffinic, aromatic and asphaltic contents of the 4 fuel oils used were determined by silica-gel column chromatography. A description of the procedure followed has been previously reported (Pulich *et al.*, 1974).

An all-glass continuous liquid-liquid extractor was used to extract organic compounds present in the water-soluble fraction. Two liters of oil-equilibrated ASP-2 growth medium (or distilled water) were extracted with benzene for 5 h in the apparatus. The benzene extract was evaporated to 2 ml under a stream of filtered air prior to gas chromatographic analysis.

Distilled water was substituted for growth medium in water-soluble fractions prepared for gravimetric analysis of total organics. Benzene extracts of these water-soluble fractions were transferred to weighed vials and allowed to evaporate at room temperature. The vials were weighed hourly, the final value being taken as the vials approached constant weight.

A Perkin-Elmer 900 gas chromatograph with flame ionization detectors was used for all quantitative analyses. Peak areas were determined by an Infotronics 204 integrator. Peak areas calculated by the integrator were occasionally found in error due to an inability to track the baseline properly. Chromatograms were scrutinized and some peak areas were recalculated by planimetry. Analyses were made on 6' x 1/8" stainless-steel columns packed with 80/100-mesh Gas Chrom Q. The stationary phase was either 5% FFAP or 4% Apiezon L.

A Dupont 21-491 mass spectrometer interfaced to a Varian 2700 gas chromatograph was used for qualitative analysis.

Results

Chemical Characterization

The gross composition of the 4 fuel oils, as determined by silica-gel fractionation, were quite similar (Table 1). Table 1 values are also similar to values reported for the American Petroleum Institute No. 2 fuel oil (Pulich *et al.*, 1974).

Analyses of the water-soluble fractions prepared from the oils yielded the results presented in Fig. 1 and Table 2.

Table 1. Paraffinic, aromatic, and asphaltic composition of test oils as determined by fractionation on silica gel

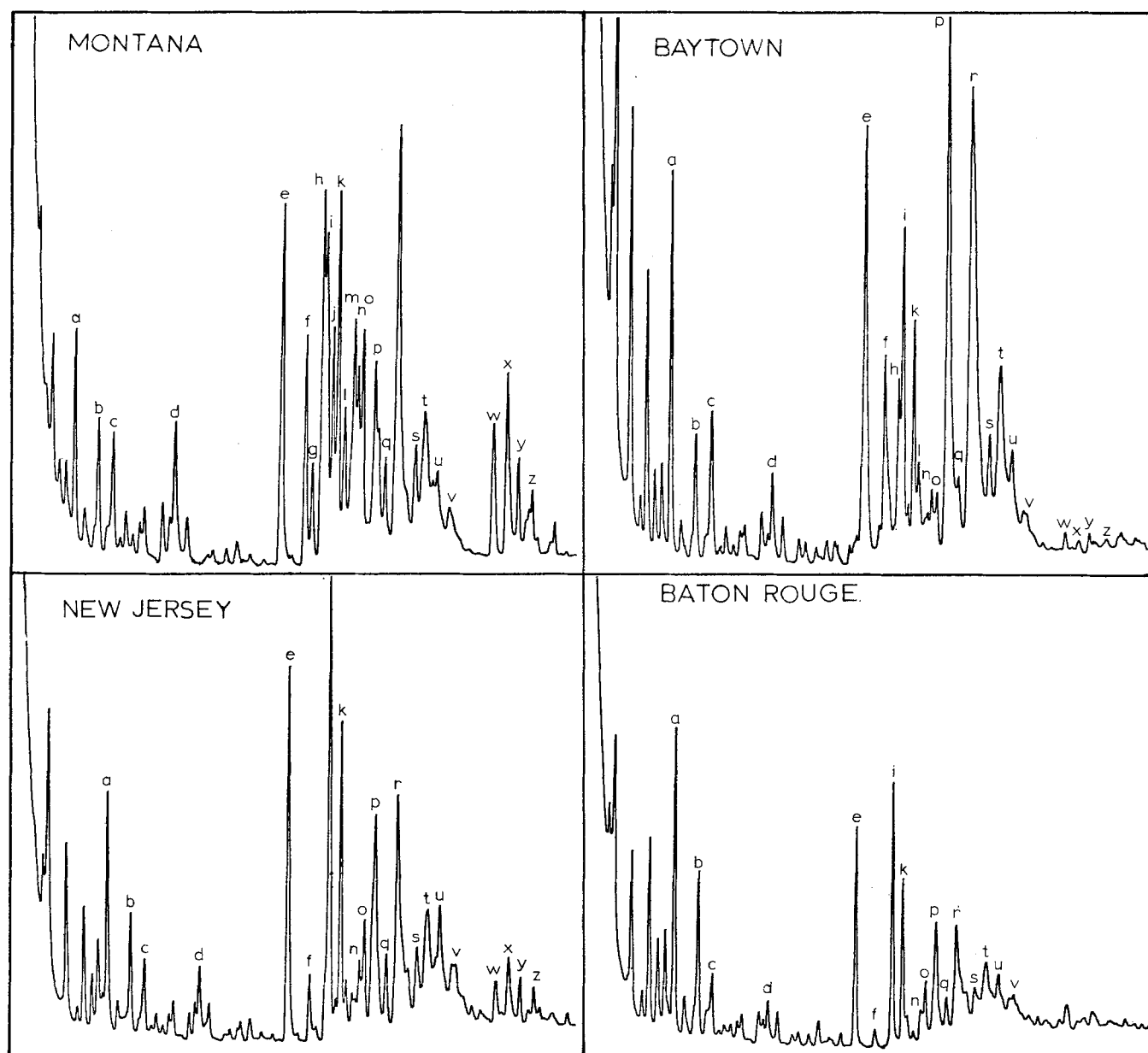| Oil	% paraffinic	% aromatic	% asphaltic	% recovery
Montana	57	33	0.3	90
New Jersey	51	38	0.6	90
Baytown	53	38	0.3	91
Baton Rouge	57	38	0.3	95

Fig. 1. Gas chromatograms of water solubles prepared from the 4 test oils. Column: 1/8" x 6' 5% FFAP on 80/100-mesh Gas Chrom Q; flow rate: 20 ml/min helium; temperature: 60° to 270°C at 6C°/min

Table 2. Identification and concentration of major components in the water-soluble fractions

Peak	Major components	Montana ppm[a]	RC[b]	Baytown ppm	RC	New Jersey ppm	RC	Baton Rouge ppm	RC	Minor components
a	1,2,4 trimethyl-benzene	.37		.56		.42		.50		
b	C$_3$-benzene[c]	.23		.23		.21		.29		C$_4$-benzene
c	Indan		1		1		1		1	
		.22		.26		.13		.11		
c	C$_4$-benzene		2		2		2		2	
d	Methylindan	.25		.15		.13		.07		
e	Naphthalene	.64		.75		.66		.39		Aniline
f	o-toluidine	.37		.34		.12		.04		Thianaphthalene
g	p-toluidine	.14		Included in h		.02		-		
h	m-toluidine					Included in i		Included in i		
		.53		.24						
h	2,6 dimethylaniline									
i	2-methylnaphthalene	.33		.51		.84		.48		
j	2,4 dimethylaniline	.24		-		-		-		
k	1-methylnaphthalene	.20		.30		.46		.28		
k	2,5 dimethylaniline	.30		.04		.03		-		
l	2,6 dimethylphenol		1		1					1
		.19		.13		-		.04		
l	C$_2$-aniline		2		2					O
m	3,5 dimethylaniline		1							
		.33		-		-		-		
m	Dimethylnaphthalene		2							
n	2,3 dimethylaniline		1		2		2		O	
n	Dimethylnaphthalene	.26	3	.07	1	.08	1	.04	1	
n	C$_3$-aniline		2		3		3		O	
o	Dimethylnaphthalene		2		1		1		1	
o	3,4 dimethylaniline	.32	1	.08	2	.16	2	.08	O	
o	C$_3$-aniline		3		3		3		O	
p	o-cresol		1		1		2		2	
p	2,4,6 trimethyl-phenol	.42	2	1.16	2	.46	O	.25	O	
p	Dimethylnaphthalene		3		3		1		1	
q	Dimethylnaphthalene	.14		.11		.12		.05		
r	m + p cresol		2		2		2		2	
		.96		1.95		.60		.32		
r	2,4 + 2,5 dimethyl-phenol		1		1		1		1	
s	2,3 dimethylphenol		1		1		1		1	Trimethyl-naphthalene
		.18		.12		.14		.08		
s	C$_3$-phenol		2		2		2		2	
t	3,5 dimethylphenol		1		1		1		1	Trimethyl-naphthalene
		.51		.60		.45		.24		
t	C$_3$-phenol		2		2		2		2	
u	3,4 dimethylphenol		1		1		1		1	
		.06		.15		.21		.13		Trimethyl-naphthalene
u	2,3,5 trimethyl-phenol		2		2		2		2	
v	C$_3$-phenol	.09		.06		.10		.02		
w	Indole		1		1		1		-	
		.24		.03		.07		-		
w	Methylindole		2		2		2			
x	Methylindole		1		1		1			
		.35		.02		.11		.06		
x	Dimethylindole		2		2		2			
y	Methylindole		1		1		1			
		.15		.02		.08		-		
y	Dimethylindole		2		2		2			
z	Dimethylindole		1		1		1			
		.05		.02		.06		.05		
z	C$_3$-indole		2		2		2			
	Total organics by gas chromatography	12.8		12.9		10.5		7.0		
	Total identified organics	8.07		7.90		5.66		3.52		
	Methylnaphthalenes	0.53		0.81		1.30		0.76		
	Dimethylnaphthalenes	0.31		0.24		0.55		0.41		
	Phenols	2.33		4.12		1.96		1.08		
	Anilines	2.57		0.72		0.27		<0.02		
	Total organics by weight	16		19		14		9		

[a] ppm: Parts per million.

[b] RC: Relative concentration in a given peak, O = absent or trace, concentration of compound 1>2>3.

[c] C$_2$, C$_3$ or C$_4$: parent compound plus 2, 3 or 4 additional saturated carbon atoms in side chains of unspecified chain length.

The values in Table 2 are based on water-soluble fractions prepared with distilled water. The batch to batch variation in naphthalene concentration in water solubles from a given oil was about 10%. The values given are averages of three samples from each oil.

Methylnaphthalene and dimethylnaphthalene concentrations were determined by analyses on Apiezon L columns which gave better resolution of these compounds. Concentration of all other compounds in Table 2 were determined by analyses on FFAP.

Extracted water solubles which were evaporated to provide a weight of total dissolved organics were chromatographed before and after evaporation. The chromatograms indicating weights of total organics (Table 2) do not quantitatively include compounds with volatilities greater than 1, 2, 4 trimethylbenzene.

Concentrations of naphthalenes in Table 2 are similar to those reported by Anderson *et al.* (1974) for a water-soluble fraction prepared from the American Petroleum Institute No. 2 fuel oil (1 part oil:9 parts 20% artifical sea water, 20 h). Their values for the concentration of naphthalene, methylnaphthalenes and dimethylnaphthalenes were 0.84, 0.82 and 0.24 ppm, respectively. Anderson *et al.* did not report phenols, anilines, or indoles.

Boylan and Tripp (1971) reported concentrations of petroleum hydrocarbons in seawater extract of a kerosene. Their experimental procedure differed significantly from the procedure followed in this laboratory. They used a lower ratio of oil to water (25 ml:1.5 l), shorter equilibration time (12 h), and reextracted water solubles with pentane.

Although they used only 13% as much oil and half the equilibration time, the concentration of naphthalene in their water solubles was 39% (0.153 mg/l) of a value reported here (Baton Rouge). No phenols, anilines or indoles were reported.

Montana and Baytown fuel oil were each used to prepare seawater soluble fractions from filtered offshore seawater. The concentration of compounds other than phenols in seawater solubles were similar to values for distilled water preparation (Table 2). The concentration of phenols was significantly higher (110 to 190%) in the seawater preparations, probably due to the higher pH of seawater.

In an effort to obtain information about rates of solution, the equilibration time was varied in the preparation of a series of water solubles. The results of this study are presented in Fig.
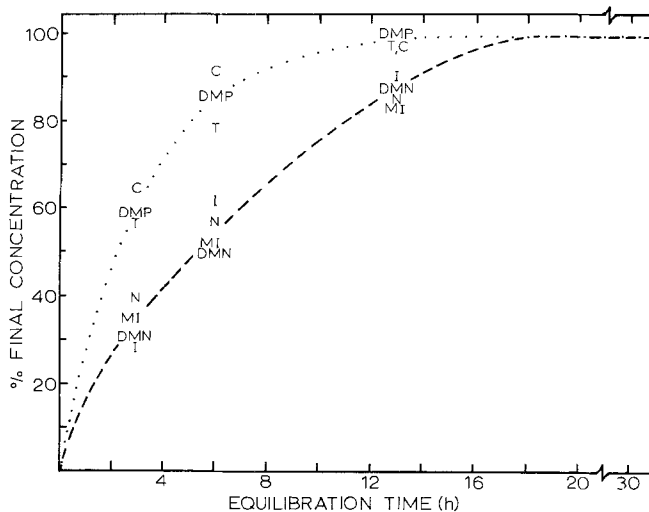

Fig. 2. Rate of solution of water-soluble components. Concentrations expressed as percentage of equilibrium concentration. C: o-cresol + 2,4,6 trimethylphenol; DMP: 2,4 + 2,5 dimethylphenol, m + p cresol; T: o-toluidine; N: naphthalene; MI: methyl indan; DMN: dimethylnaphthalene; I: indole + methyl indole

2. It should be noted that all compounds analysed reach equilibrium concentration in less than 20 h and greater than 80% equilibrium concentration in 12 h. The 3 and 6 h samples demonstrate the significantly faster rate at which the more soluble compounds such as phenols and toluidines approach equilibrium.

Although these compounds are present in relatively high concentration in our water solubles, phenols and alkyl anilines are generally not abundant in whole oils. Another experiment was therefore designed to determine the quantity of water which could be equilibrated with a given volume of oil before these compounds were, for practical purposes, removed from the oil.

For the experiment, a mixture of equal volumes of the 4 fuel oils was prepared to represent an "average" fuel oil. Water solubles from this "average" oil were prepared by the usual method. After the 24 h equilibration the water was removed and replaced with an equal volume of water which was allowed to equilibrate for another 24 h. This procedure was repeated to yield 4 samples of water solubles which had been successively equilibrated with the same oil. Changes in the composition of these samples are

Table 3. Concentration (%) of selected compounds in water-soluble fractions prepared by successive equilibration

Compound	1st equilibration (0 - 24 h)	2nd equilibration (24 - 48 h)	4th equilibration (72 - 96 h)
1,2,4 trimethylbenzene	100[a]	98	99
Naphthalene	100	94	92
2 methylnaphthalene	100	98	102
1 methylnaphthalene	100	99	98
Dimethylnaphthalene	100	104	104
Indole + methylindole	100	106	67
o-toluidine	100	50	11
m-toluidine	100	52	10
2,4 + 2,5 dimethylphenols, m + p cresol	100	57	14
3,5 dimethylphenol + C-3 phenol	100	49	13

[a] Concentration expressed as percentage of the concentration present in 1st equilibration (0 to 24 h).

Table 4. Growth rates (generations/day) of microalgae grown at 30°C in presence of 25 or 50% water solubles from No. 2 fuel oils. Doubling times calculated from turbidimetric measurements of cell number during exponential phase of growth

Strain designation	Controls	Baytown 25[a]	Baytown 50	Baton Rouge 25	Baton Rouge 50	Montana 25	Montana 50	New Jersey 25	New Jersey 50
PR-6	4.6±0.2	NG-7[b]	NG-7	3.4(14)	3.9(31)	NG-7	NG-7	2.3(90)	1.9(150)
17-A	3.6±0.2	NG-9	NG-9	3.8	3.5	NG-9	NG-9	2.5(43)	2.3(65)
DUN	3.3±0.2	3.1(27)[c]	3.5(65)	3.2	3.3	3.0	1.7(33)	3.5(79)	NG-8
580	2.7±0.2	2.8	2.9(55)	2.7	2.7	2.7	2.7	2.9(26)	NG-9
N-1	4.9±0.2	4.3	4.3(16)	4.9	4.9	4.9	1.4(81)	4.8(12)	4.6(4)
AMP-1	3.5±0.2	3.1	2.6(18)	3.5	2.8(14)	3.2	3.1	3.0(12)	3.0(24)

[a] Number indicates percent of ASP-2 medium equilibrated against a given fuel oil (8 parts medium, 1 part oil) added to plain medium ASP-2; e.g. 25% means 5 ml of ASP-2 containing water solubles from a fuel oil plus 15 ml of ASP-2 medium.

[b] NG: No growth, followed by number of days before termination of experiment; e.g. NG-7 means no growth in 7 days.

[c] Number in parentheses indicates lag time in hours in initiation of growth as compared to the zero or short lag times seen in control growth curves.

Table 5. Effect of pure compounds on growth of Organisms PR-6, 580, and N-1 using algal lawn technique. Numbers represent zone of inhibition (mm) out from edge of filter pad. Complete killing results in zone of inhibition of 36 mm on plate

Organism	Amount on pad (mg)	2,3-DMP[a]	2,4-DMP[a]	2,5-DMP[a]	2,6-DMP[a]	3,4-DMP[a]	3,5-DMP[a]	2,3,5-TMP[b]	2,3,6-TMP[b]	2,4,6-TMP[b]	Indole[c]	Meta-cresol	Ortho-cresol	Para-cresol
PR6	10	36	36	36	36	36	36	36	36	36	36	36	36	36
	2	17	36	32	0(36*)	20(36*)	20(36*)	36	36	36	25(36*)	6	14	15
	1	1	0(10*)	11(36*)	0	8	0(8*)	20(36*)	25(36*)	36	14	0	1	8
	0.5	0	0	0	0	0(5*)	0	2	3(36*)	1(36*)	2(6*)	0	0	0
580	10	36	36	36	36	36	36	36	36	36	36	36	36	36
	2	36	34(36*)	10(36*)	0(36*)	17(36*)	25(36*)	36	36	36	36	1(36*)	3(36*)	6(36*)
	1	0(36*)	1(36*)	0(36*)	0	3(36*)	12(36*)	20(36*)	10(30*)	3(36*)	25(36*)	0	1(36*)	0
	0.5	0	0	0	0	0(36*)	0(8*)	2(10*)	1	0	7(36*)	0	0	0
N-1	10	30(36*)	36	36	36	25(36*)	25(36*)	36	36	36	36	36	36	36
	2	15	23(36*)	18(36*)	13	18	15	36	36	12(18*)	20(27*)	12	15	13
	1	8	14	5	2	8	3	15	13	2	12(17*)	3	5	3
	0.5	0	1(4*)	1	0	1	1	3(12*)	3(10*)	0	1(7*)	0	3	1

[a] Dimethylphenol (Aldrich Chemical Co., Milwaukee, Wis.).

[b] Trimethylphenol (Aldrich Chemical Co.).

[c] Chem. Service Inc., Media, Pa.

* Colonies much reduced in size this distance from pad.

Table 6. Effect of toluidines (methylanilines) and dimethylaniline on growth of organisms PR6, 580 and N-1 using algal lawn technique. Numbers represent zone of inhibition (mm) out from edge of filter pad. Complete killing results in zone of inhibition of 36 mm on plate

Organism	Amount on pad		meta-t[a]	ortho-t[a]	para-t[a]	DMA[b]
PR-6	500	μg	36	36	36	36
	100	μg	36	36	36	36
	10	μg	0	12(17*)	36	10(15*)
	1	μg	0	0	6(10*)	0
	0.1	μg	0	0	0	0
580	10	mg	36	36	36	36
	2	mg	9(36*)	10(36*)	36	5(10*)
	1	mg	0	2	4(15)	2
	0.5	mg	0	0	0	0
N-1	10	mg	36	36	36	36
	2	mg	29	30	36	15
	1	mg	12	14	25	6
	0.5	mg	8	5	7	1(15*)

[a]Toluidine (Chem. Service).

[b]2,4-dimethylaniline (Chem. Service).

*Colonies much reduced in size this distance from pad.

given in Table 3. The concentration of phenols and alkyl anilines decreased in successive samples, while the concentration of alkyl benzenes, naphthalenes and indoles remained relatively constant.

Biological Toxicity of Water Solubles from the Four Fuel Oils

With reference to Table 4, there are interesting differential growth responses shown either by a single water soluble on the different microalgae or by a single organism with the 4 extracts. Baytown and Montana, even at 25% concentration, completely suppressed the growth of the 2 blue-green algae. With the green algae and the diatoms, considerable lags in growth and lower final growth rates were found in a 50% concentration. It is evident that the water solubles from these two oils are inhibitory, and especially so to the 2 blue-green algae tested. With water solubles from the New Jersey fuel oil there were long lags with the blue-greens, and with the 2 green algae complete suppression of growth at 50% concentration. With the diatoms this fuel oil also induced lags, but the final growth rates were close to the controls. Baton Rouge caused only a

lag in growth for 1 blue-green (Strain PR-6), and 1 diatom (Strain AMP-1). If Baton Rouge had been the only fuel oil tested, then a misleading picture of the toxicities of water solubles from fuel oils would have been obtained. The same type of argument holds as a function of the organisms tested. If Baytown or New Jersey had only been tested against the 2 diatoms, then these fuel oils would have seemed rather non-toxic.

The data of Table 4 reinforce our previous findings that extreme caution is advised in generalizing on the toxicity of a given oil to a given organism. Water solubles from fuel oils can completely suppress the growth of some organisms, or induce long lags in initiation of growth, or lower the growth rate of an organism. Very simply, water-soluble extracts of fuel oils contain compounds which inhibit the growth of microalgae. Since Baton Rouge fuel oil was so non-toxic, we are allowed to subtract its chromatographic pattern from the three other fuel oils. This then leads to the supposition that it is not necessarily the total amount of water solubles that are critical but rather specific compounds, possibly present in small amount, which are the important toxic agents.

Tables 5 and 6 present toxicity data obtained via the algal lawn-pad assay technique of compounds now identified in the water-soluble extracts. The data in Table 5, with various di- and trimethyl phenols, indole, and cresols are reminiscent of the degree of toxicity seen previously with ethyl and methyl benzenes, naphthalenes, biphenyls, penanthrenes (Pulich *et al.*, 1974). While it may be argued that some of these compounds are toxic, for example, dimethyl naphthalene, 1, 2, 4 trimethyl benzene, indole, 2, 3, 6 trimethyl phenol, none are particularly impressive. We have not yet looked at synergistic effects, and it is possible that combinations of the more toxic compounds may be active at lower levels. On the other hand, the data in Table 6 show that substituted anilines, particularly p-toluidine, are highly toxic for the coccoid blue-green alga, Strain PR-6. In the pad assay 1 μg/pad showed a clearcut effect, and in liquid culture 100 μg/l caused a lag in growth. It is also interesting to note that the toluidines are present in sufficient amounts in the water solubles from Baytown and Montana to be lethal to Strains PR-6 and 17a. We take this then as a model case to support our argument that single compounds, perhaps at low concentration in a fuel oil (or a crude oil), can be highly toxic. The lethality of p-toluidine, 10^{-6}M, ap-

proaches the toxicity of the classic and much-used inhibitor of photosynthesis, DCMU (dichlorophenyl dimethyl urea).

Discussion

The results reported here confirm and extend our previous observations on the toxicity of water solubles from fuel oils to the growth of representative types of microalgae. The chemical analyses of the water-soluble fractions have revealed several classes of compounds not noted before — anilines, indoles, and in addition significant concentrations of alkyl phenols. The data suggest that in the event of a fuel-oil spill, alkyl phenols and alkyl anilines would rapidly be extracted by seawater in contact with the oil. These compounds could be expected to enter the water column even under minimal wave action, and probably before significant loss into the atmosphere.

Of the compounds identified, the toluidines and p-toluidine in particular, were remarkably toxic to the growth of the coccoid blue-green, PR-6. Preliminary observations on filamentous bluegreens, a *Nostoc* sp., *Oscillatoria williamsii* and *Fischerella ambiqua* suggest that these forms are also sensitive to p-toluidine, albeit at a slightly higher concentrations (10 to 100 μg/pad in the algal lawn assay). The biochemical basis for the selective toxicity of p-toluidine towards blue-green algae is being investigated.

The water solubles from New Jersey fuel oil were more toxic to the 2 green algae, and to some extent to the diatoms. However, we have not found single compounds showing a high degree of selective toxicity, as with p-toluidine to blue-greens, towards these types of microalgae. The New Jersey fuel-oil extract also caused long lags and slower growth rates in the 2 blue-green algae, but its toluidine concentration was only 50 and 25%, respectively, of Baytown and Montana. Despite the demonstrated toxicity of toluidines to blue-green algae, this comparison of New Jersey with Baytown and Montana suggests caution in overinterpretating or oversimplifying the basis for toxicity of the water solubles.

In our previous work we used the diatom *Thalassiosira pseudonana*, Strain 3H. This organism was very sensitive to the water solubles from the A.P.I fuel oil (Pulich *et al.*, 1974). In contrast, the 2 benthic diatoms used here were not greatly affected by the water solubles from the 4 fuel oils. The biochemical basis for these very different responses is unknown, and considerably more effort with this important algal group is indicated.

Despite our hesitance in making any broad generalizations on possible detrimental effects of fuel oils on natural phytoplankton and, therefore, primary productivity, it is clear from an experimental standpoint that fuel oils are toxic to the growth and photosynthesis of the microalgae. Their evident differential toxicities will lead to selective or enrichment effects on natural populations. In essence, chronic or indiscriminate addition of fuel oils to the environment is to be avoided.

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