JKAU: Met., Env., Arid Land Agric. Sci., **vol. 9,** pp. 137-144 (1418 A.H. / 1998 A.D.)

Effect of Crude Oil and Naphthalene on The Evolution of Oxygen by Three Species of Marine Algae

S.A. KABLI
Biology Department, Faculty of Science,
King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT. The effect of different concentrations of crude oil and naphthalene on oxygen evolution by *Ulva fenestrata* Postels and Ruprecht, *Sargassum crassifolia,* J. Ag., and *Galaxaura fasciculata* Kjellman, were studied. Crude oil (200-800 ppb) and/or naphthalene (20-80 ppb) were introduced into pure cultures of the algae in a $\frac{2}{4}$ factorial design. After 5 hours, oxygen evolution of the three algae was greatly inhibited by crude oil and naphthalene, both singly and in combination, except in case of the lowest level of naphthalene treatment applied alone (20 ppb). Addition of these two pollutants together increased their harmful effects on oxygen evolution.

KEY WORDS: Crude Oil, Naphthalene, Marine Algae.

Introduction

Presently, increasing amounts of crude oil are being transported through the Red Sea. A large number of its aquatic habitats are becoming progressively more contaminated by the discharge of crude oil from ships, and other related activities. The use of algae to monitor crude oil toxicity has increased due to their ubiquity in aquatic ecosystems. In their natural environment algae influence and are influenced by most aquatic processes. Many studies have indicated that oil pollution is potentially hazardous to living organisms at all toxic levels (Blumer, 1969; Cross, 1987; Cross *et al.,* 1987; Singh *et al.,* 1987; Pople *et al.,* 1990 and Roy *et al.,* 1991).

Crude oils are complex mixtures of hydrocarbons of varying molecular weights and structures. These hydrocarbons fall into three main chemical groups: paraffinic, naphthenic and aromatic. Crude oils also contain small amounts of oxygen-, nitrogen-, sulfur- containing compounds, as well as trace amounts of heavy metals (Rossini, 1960). The low molecular weight components of crude oils such as naphthalene and benzene, and their derivatives, are volatile and have relatively higher water solubilities. The lower molecular weight organics are the most toxic components found in crude oil aqueous

extracts (Boylan and Tripp, 1971). The relative amounts of naphthalene compounds in crude oils are used as an index of the toxicity of those polluting oils (Boylan and Tripp, 1971). This approach has not yet been widely accepted, but it has clear implications regarding different crudes and refined fractions.

The purpose of the present study was to estimate the sensitivity of three species of marine algae (*Ulva fenestrata* Postels and Ruprecht, *Sargassum crassifolia* J. Ag. and *Galaxaura fasciculata* Kjellman) to crude oil extracts and naphthalene, and by so doing assess their sensibility as toxicity testing organisms.

Materials and Methods

The marine algae *Ulva fenestrata* Postels and Ruprecht (chlorophyta), *Sargassum crassifolia* J. Ag. (Phaeophyta), and *Galaxaura fasciculata* kjellman (Rhodophyta) were collected from the Jeddah coast and the nearby Obhur creek. These three algae were vegetatively propagated by chopping them into fragments approximately 5 mm in length with a razor blade and introducing the fragments into plexiglass pots containing 1.0 L of aerated sea-water. These unialgal batch cultures were maintained at 25 ± 2ºC, and with a photo flux density of 50-60 μmol photons m^{-2} S^{-1} in a 16.8 light dark cycle.

Saudi Arabian crude oil was obtained from a commercial source. Sea water was collected from a pristine environment of Jeddah south coast, passed through a 0.45 μm filter and sterilized by steaming.

A water soluble extract was prepared from the crude oil using a mixing flask that has been previously described (Maher, 1986), 500 ml of sea water was poured into the flask and 1.5 g of crude oil was added to the surface. The flask was placed into a water bath held at 20 ± 2ºC. A magnetic stirring system was 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. The extraction was performed in the dark. The concentration of oil in colloidal suspension in the sea water extract was estimated by flourescence spectroscopy to be 30 ppm.

Water soluble extract of naphthalene was prepared by adding 3.5 mg naphthalene crystals to 100 ml filtered sea water. According to Bohon and Claussen (1951) this gives 34 ppm solution.

It was found in preliminary experiments that the minimum concentrations of crude oil and naphthalene that affect the rate of oxygen evolution by the study organisms after 5 hours were 200 ppb and 20 ppb respectively.

Appropriate dilutions were made to obtain crude oil solutions of 200, 400, 600 and 800 ppb and naphthalene solutions of 20, 40, 60 and 80 ppb. In the first stage of the experiment these crude oil and naphthalene solutions were used separately. In the second stage of the experiment both crude oil and naphthalene solutions were introduced into the algal cultures in a $\frac{2}{4}$ – factorial design as represented in Table 1.

TABLE 1. Effect of crude oil and naphthalene on oxygen evolution of the three macroalgae (μmol. h^{-1} g^{-1} F.W.).

Experiment number	Crude oil in ppb	Naphthalene in ppb	*Ulva*	*Sargassum*	*Galaxaura*
a Control	0.00	–	12.40	8.50	6.81
a_1	200	–	11.78	7.65	5.71
a_2	400	–	11.20	6.97	5.30*
a_3	600	–	10.04*	6.37*	4.62*
a_4	800	–	8.14*	5.10*	3.88*
LSD at 5%			1.83	1.62	1.23
b_1	–	20	13.64	8.08	6.19
b_2	–	40	11.66	7.22	5.44*
b_3	–	60	10.54	6.54*	4.90*
b_4	–	80	9.05*	5.53*	4.14*
LSD at 5%			1.99	1.46	1.32
1	200	20	11.20	7.31	5.58
2	400	20	10.79	6.30*	4.76*
3	600	20	9.67*	5.78*	4.22*
4	800	20	7.56*	4.59*	3.60*
LSD at 5%			2.17	1.71	
5	200	40	10.42	6.97	4.96*
6	400	40	10.04*	5.95*	4.49*
7	600	40	8.93*	5.35*	3.88*
8	800	40	7.19*	4.25*	3.13*
LSD at 5%			2.19	1.90	1.77
9	200	60	9.92	6.12*	4.62*
10	400	60	9.47*	5.35*	4.42*
11	600	60	8.43*	4.93*	3.41*
12	800	60	6.32*	3.82*	2.72*
LSD at 5%			2.5	1.99	1.72
13	200	80	8.90	5.69*	4.15*
14	400	80	8.06	4.84*	3.74*
15	600	80	7.07	4.08*	3.13*
16	800	80	5.21	3.23*	2.04*
LSD at 5%			3.24	2.32	2.00

*Significant different as compared with control.

Oxygen Evolution

Algae were adapted for 5 hr to different crude oil and/or naphthalene levels followed by determination of photosynthetic rate. A clark-O_2-probe (Hansatech Ltd.) was used in a temperature controlled (20ºC) closed cuvette containing 5 ml media. Algae were positioned in the middle of the cuvette with a polyamide thread. A halogen lamp connected to a quartz fibre optic cable provided a photon fluence rate of 800 μ mole m^{-2} S^{-1} inside the cuvette. This light intensity is thought to exceed the requirement for maximum photosynthesis for most intertidal algae. The electrode was calibrated at each crude oil and/or naphthalene level with air-saturated sterilized sea water, and the oxygen content

was calculated according to Truesdale *et al.* (1955). The rate of photosynthesis was measured until constant rate of oxygen production was recorded (ca. 10-15 min).

Five replicates were used for measuring oxygen evolution

Results and Discussion

The effects of both pollutants on oxygen evolution are shown in Fig. 1 and Table 1. Oxygen evolution of the three algae was greatly inhibited by crude oil and naphthalene, both singly and in combination. Inhibition was proportional to the concentrations of the pollutants. However, at lowest level of naphthalene treatment applied alone (20 ppb) there was a relatively small stimulation in oxygen evolution of *Ulva* (Table 1). The interaction between crude oil and naphthalene has a significant harmful effect. This harmful effect increased with increasing crude oil and naphthalene levels.

Based on the reduction in oxygen evolution relative to the control at varying concentrations of crude oil and/or naphthalene, the relative pollutants tolerance of these algae was as follows: *Ulva* > *Galaxaura* > *Sargassum* respectively (Table 2).

Different species of algae have shown different sensitivities to crude oil and its fractions. Sun and Wang (1990) studied the effects of crude oils added with the dispersant Corexit 9527 (an organic nitrogen containing compound) in continuous culture of *Phaedoctylum tricornutum* and *Dunaliella.* They found that the effect of the oils was strengthened in the presence of the dispersant, but that extent of this strengthening was different with the difference of species of algae and crude oil. Cyanophyta were the dominant algal group in effluents from the Mutheira Oil Refinery; the relative abundance of the group decreased following removal of pollutants (Singh *et al.,* 1987). Aqueous extracts of various crude oils were in most cases inhibitory to *Chlamydomonas angulosa* as tested although these effects were less severe than those obtained with naphthalene using different crude oils (Soto *et al.,* 1974a). Mironov (1970) found that some diatoms are killed within 24 hours by 100 µl/L of kerosene or fuel-oil. Lower concentrations of those fuels, such as 0.1 µl/L, are able to retard the rates of cell division and growth of the most sensitive species.

Ibrahim (1982) found that a crude oil extract at a concentration of 500 ppb decreased the primary production of *Nephrochloris salina* to 60 percent of controls. In the presence of the polychlorinated biphenyl PCB_3 primary production of *Nephrochloris salina* decreased to 65 percent. Acting together this crude extract and PCB_3 reduced primary production of *N. salina* to 38 percent of that in controls (Ibrahim, 1982). Soto *et al.* (1974 a,b) found that the addition of 100% naphthalene to *Chlamydomonas angulosa* cultures caused an immediate and almost complete loss of photosynthetic activity. However, there was a decrease rather than loss of photosynthetic capacity when cells were incubated in media containing saturated aqueous crude oil extracts. Singh and Gaur (1990) showed that inhibition of photosynthesis was more severe than that of respiration in *Anabaena doliolum* exposed to petroleum oils, and their paraffinic and aromatic fractions. Roy *et al.* (1991) found that photosynthetic rates of phytoplankton declined after contamination by dispersed oil.

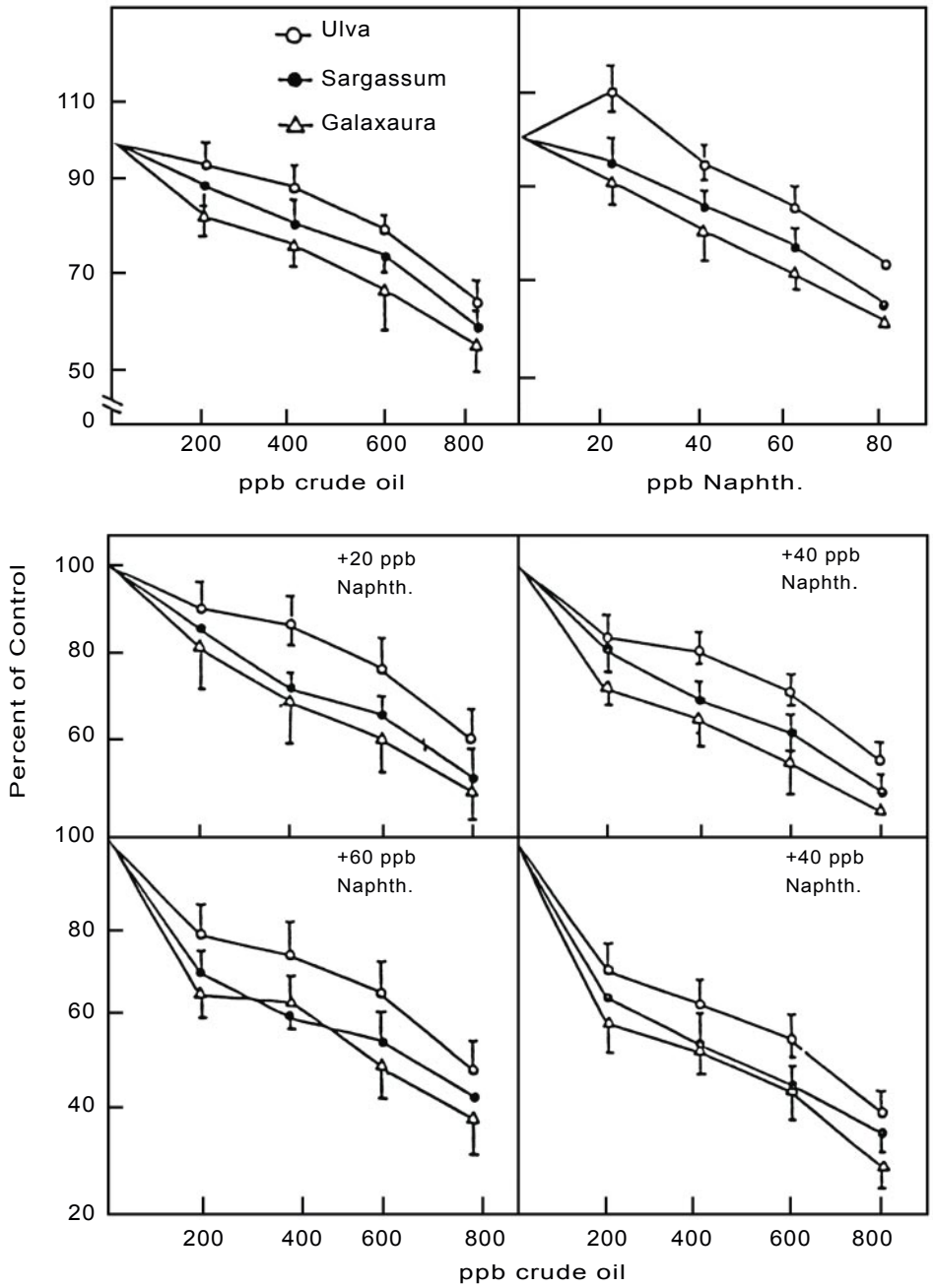

FIG. 1. Percent of oxygen evolution of macroalgae as influenced by crude oil and/or naphthalene.

TABLE 2. Percent of oxygen evolution of macroalgae as influenced by species, averaged over crude oil levels with different levels of naphthalene.

Algae	Naphthalene (ppb)				
	0.0	2.0	40	60	80
Ulva	86.4[a]	83.2[a]	79.0[a]	75.0[a]	67.2[a]
Sargassum	81.4[b]	76.2[b]	73.0[b]	67.6[b]	62.0[b]
Galaxaura	77.4[c]	73.4[b]	68.4[c]	64.8[b]	58.4[c]
S E	2.72	2.80	2.98	2.95	2.35

[a-c] Numbers within a column followed by the same letter do not different at 5% probability level.

Although the crude oil and naphthalene showed a marked inhibitory effect on the oxygen evolution of the three organisms tested in this study, they appeared more tolerant than those test organisms used in other studies (Ibrahim, 1982; Mihnea, 1983; Singh *et al.,* 1987; Sun and Wang, 1990; and Roy *et al.,* 1991). However, addition of these two pollutants together increased their harmful effects on oxygen evolution.

References

Blumer, M. (1969) Oil pollution of Ocean. *Ocianus.* **15:** 2-7.

Bohon, R.L. and **Claussen, W.F.** (1951) The solubility of aromatic hydrocarbons in water. *J. Am. Chem. Soc.* **73:** 1571-1580.

Boylan, D.B. and **Tripp, B.N.** (1971) Determination of hydrocarbons in sea water extracts of crude oil and crude oil fractions. *Nature* **230:** 44-47.

Cross, W.E., Wilce, R.T. and **Fabijan, M.F.** (1987) Effects of experimental releases of oil and dispersed oil on Arctic nearshore macrobenthos 3. Macroalgae. *Arctic* **40:** 211-219.

Cross, W.E. (1987) Effects of oil and chemically treated oil on primary productivity of high Arctic ice algae studied in situ. *Arctic* **40:** 266-276.

Ibrahim, A.M.A. (1982) Effect of crude oil and polychlorinated biphenyls on the primary production of *Nephrochloris salina. Bull. Fac. Sci. K.A.U.* **6:** 61-66.

Maher, W.A. (1986) Preparation of water soluble fractions of crude oils for toxicity studies. *Bull. Environ. Contam. Toxicol.* **36:** 226-229.

Mihnea, P.E. (1983) The effect of BS-SIDER crude oil on the metabolism of marine unicellular algae. *Cercet Mar. Rech. Mar.* **16:** 255-262.

Mironov, O.G. (1970) The effect of oil pollution on the flora and fauna of the Black Sea, *FAO Tech. Conf. Mar. Pollut., Rome pap.* E. 92.

Pople, A., Simpson, R.D. and **Cairns, S.C.** (1990) An incident of southern ocean oil pollution: Effects of a spillage of diesel fuel on the rocky shore of Macquarie Island (Sub-Antarctic). *Aust. J. Mar. Freshwat. Res.* **41:** 603-620.

Rossini, F.D. (1960) Hydrocarbons in petroleum. *J. Chem. Educ.* **37:** 554-561.

Roy, S., Siron, R. and **Pelletier, E.** (1991) Comparison of radiocarbon uptake and DCMU-fluorescence techniques in evaluating dispersed oil effects on phytoplankton photosynthetic activity.*Water Res.* **25:** 1249-1254.

Singh, A.K. and **Gaur, J.P.** (1990) Effect of petroleum oils and their paraffing asphaltic aromatic fractions on photosynthesis and respiration of microalgae. *Ecotoxicol. Environ. Saf.* **19:** 8-16.

————, **Singh, M.P.** and **Gaur, J.P.** (1987) Seasonal variations in periphyton composition and diversity in oil refinery effluents. *Acta Hydrochim. Hydrobiol.* **15:** 401-408.

Soto, C., Hellebust, J.A. and **Hutchinson, T.C.** (1974a) Effect of naphthalene and aqueous crude oil extracts on the green flagellate *Chlamydomonas angulosa.* II, Photosynthesis and the uptake and release of

naphthalene. *Can. J. Bot.* **53:** 118-126.

————, ————, **Hutchinson, T.C.** and **Sawa, T.** (1974b) Effect of naphthalene and aqueous crude oil extracts on the green flagellate *Chlamydomonas angulosa.* I. Growth. *Can. J. Bot.* **55:** 135-142.

Sun, L. and **Wang, X.** (1990) The effects of crude oils and dispersant on cellular and extracellular organic nitrogen culture. *Mar. Sci. Haiyiang-Kexue* **3:** 22-25.

Truesdale, G.A., Downing, A.L. and **Lowden, G.F.** (1955) The solubility of oxygen in pure water and seawater. *J. Appl. Chem.* **5:** 53-62.

تـأثيـر الزيت الخـام والنفثالـين على تصاعـد الأكسيجـين بثلاثـة أنـواع من الطحـالب البحريـة

صالح عبد اللّه كابلي

قسم الأحياء ، كلية العلوم ، جامعة الملك عبد العزيز

جـــــدة – المملكة العربية السعودية

المستخلص . دُرس تأثير تركيزات مختلفة من الزيت الخام والنفثالين على تصاعد الأكسيجين بثلاثة أنواع من الطحالب البحرية : أولفا فينيستراتا وسرجاسم كراسيفوليا وجالاكساوا فاسيكيولاتا . وكانت تركيزات الزيت الخام والنفثالين المضافة إلى مزارع الطحالب الثلاثة النقية : ٢٠٠–٨٠٠ جزء في البليون ، ٢٠–٨٠ جزء في البليون على التوالي بتصميم (4^2 Factorial design) .

بينت النتائج بعد خمسة أيام أن تصاعد الأكسيجين من الطحالب الثلاثة تم تثبيطه بدرجة كبيرة بالزيت الخام والنفثالين ، كل على حده أو مجتمعين ماعدا في حالة التركيز الأقل من إضافة النفثالين بمفرده عند تركيز ٢٠ جزء في البليون . كما أظهرت النتائج أن إضافة المادتين سويًا زادت من تأثيراتهما الضارة على تصاعد الأكسيجين .