

Effects of Oils and Dispersant on the Red Tide Organism *Cochlodinium Polykrikoides*

Sam-Geun Lee, Eun-Seob Cho, Wol-Ae Lim and Young-Sik Lee

Aquaculture Environment Center, South Sea Fisheries Research Institute, National Fisheries Research and Development Institute, Kyeongnam 650-943, Korea

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Oil spill caused severe effects on the marine fauna and flora due to direct contact of organisms with the oil and even in regions not directly affected by the spill. This study was conducted to understand the effects of the oil spill accidents and the use of dispersant on the red tide of *Cochlodinium polykrikoides*. Crude oil produced in Kuwait, bunker-C, kerosene and diesel oil, and a chemical dispersant produced in Korea, were added with a series of 10 ppb to 100 ppm in the f/2-Si medium at 20°C under a photon flux from cool white fluorescent tubes of 100 mol m⁻² s⁻¹ in a 14: 10 h L:D cycle for the culture of *C. polykrikoides*. In low concentrations of ≤1 ppm of examined oils no impact on the growth of *C. polykrikoides* was recorded, while in high concentration of ≥10 ppm, cell density was significantly decreased with the range of 10 to 80% in comparison with the control. The growth of *C. polykrikoides* after the addition of the dispersant and the mixtures combined with oils and a dispersant of ≥10 ppm appeared to decrease, whereas the growth of *C. polykrikoides* exposed to ≤100 ppb showed little serious impact. However, almost all the *C. polykrikoides* cells were died regardless of a dispersant and combined mixtures within a few days after the addition of high concentrations.

Key Words : Red tide, *Cochlodinium polykrikoides*, Oil spill, Dispersant, Algal growth

1. Introduction

Oil pollution, which sometimes occur in coastal areas, has become one of the most serious problems we face nowadays. The use of dispersant can scatter in large amount of oil from the sea surface into the water column by dispersing^{1~2)}. Chemical pollution by the spilled oil harms the marine ecosystem, although the amount of damage may depend on the amount and quality of the spilled oil. Chemical surfactants are often dispersed over the surface of the oil-polluted water to control oil pollution and to enhance decomposition of petroleum hydrocarbons by bacterial action. Much research has already been done in bacterial action field in the laboratory^{3~9)}. Many studies have developed quantitative structure activity relationship to predict both the occurrence and potency of

photo-induced toxicity for polyaromatic hydrocarbons^{10~12)}. However very little information is available about *Cochlodinium polykrikoides* as a toxicological subject; this organism is frequently occurred in Korean coastal waters and is well known as a harmful red tide organism¹³⁾.

Recent massive oil spill accidents in the waters off Kwangyang, Yeosu and Busan in Korean peninsula, have led to the use of a great deal of dispersants¹⁴⁾. In 1995, the greatest amount of fish deaths caused by *C. polykrikoides*, and the massive oil spill caused by the tanker "Sea Prince", attracted great public attention in Korea¹³⁾. We should find ways to resolve this problem by certain precautions or to discover ways to save polluted places. In the present study, bioassay and toxicity tests were conducted to investigate the effects of various concentrations of crude oil, bunker-C, diesel oil, kerosene, oil spill dispersant and mixtures of these oils and the dispersant on the growth of *C. polykrikoides*.

Corresponding Author : Sam-Geun Lee, Aquaculture Environment Center, National Fisheries Research and Development Institute, Kyeongnam 650-943, Korea
Phone: +82-55-641-2142
E-mail: sglee@nfri.re.kr

2. Materials and Methods

2.1. Culture

The dinoflagellate *C. polykrikoides* was isolated from the waters off Tongyong in 2005. After a clonal culture was established, it was maintained in a f/2-Si medium¹⁵ using at 20°C 14:10h light/dark cycle with a cool white 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity.

2.2. Oils and dispersant

The test oils used in this study were crude oil produced in Kuwait, bunker-C, kerosene and diesel oil obtained from a Korean Oil Refinery Company (Sun Kyung), and a chemical dispersant, Seagreen 805A (Korean Chemical Industry Company) were applied. Samples of oils and dispersant were diluted in a series between 10 ppb to 100 ppm with sterilized sea water to provide nominal stock solutions of 1,000,000 ppm for the concentration of test oils and chemical dispersant, and kept in plastic bottles until required. The oils were almost suspended on the surface of mediums, but the dispersant and oils plus dispersant mixtures were well mixed with mediums.

2.3. Test organism conditions

Experiments were done in duplicate, with 10 mL culture medium in 50 mL test tubes. Each tube was inoculated with approximately 300 cells mL^{-1} and grown at 20°C in 12:12 light-dark cycles of white fluorescence illumination, providing a photon flux density of about 70 $\text{mol m}^{-2} \text{s}^{-1}$. Cell numbers were determined by direct cell counts at an interval of 2 days for 20 days, using an inverted Carl Zeiss MC80.

Table 1. Composition of f/2-Si medium

NaNO ₃	75 mg
NaH ₂ PO ₄ · H ₂ O	5 mg
Na ₂ · EDTA	4.36 mg
FeCl ₃ · 6H ₂ O	3.15 mg
MnCl ₂ · 4H ₂ O	0.18 mg
CoCl ₂ · 6H ₂ O	0.01 mg
CuSO ₄ · 5H ₂ O	0.01 mg
ZnSO ₄ · 7H ₂ O	0.022 mg
Na ₂ MoO ₄ · 2H ₂ O	0.006 mg
B ₁₂	0.5 μg
Biotin	0.5 μg
Thiamine · HCl	0.1 mg
Sea water	1 ℓ

3. Results and Discussion

3.1. Effect of oils

The effect of crude oil on the growth of *C. polykrikoides* for 20 days is shown in Fig. 1. In low concentrations of ≤ 1 ppm no impact of growth was recorded. At higher concentrations of ≥ 10 ppm, cell density significantly decreased, with a range of 10% to 80%. Such reduction is probably due to the competitive inhibition of the enzymatic activity caused by the polyaromatic hydrocarbons¹⁶. The volatility of polyaromatic hydrocarbons has been shown to limit their toxic effects in an aquatic environment¹⁷. After incubating marine alga *Phaeodactylum tricornerutum* in the presence of crude oil, Lacaze et al.¹⁷ observed a

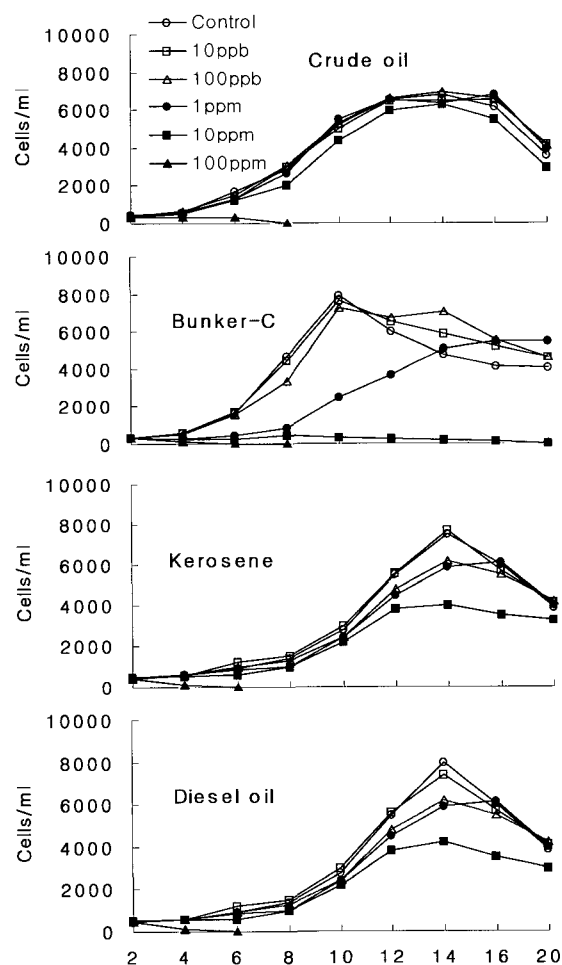


Fig. 1. The growth of *Cochlodinium polykrikoides* for 20 days after treated with a different concentration of oils.

decreased biomass within 48 h, after which growth has been recovered.

The variations of cell density for those exposed to bunker-C, kerosene and diesel oil were found to be similar as the concentrations of crude oil (Fig. 1). Generally, crude oil are known to be more toxic than bunker-C, because crude oil contain potentially toxic compounds such as benzene, toluene and xylene, which are carcinogenic substances. Our results are also in accordance with general theory, because the maximum growths of *C. polykrikoides* in the concentrations added crude oil are recorded the least value compare with the other oils. Djomo et al.¹⁸⁾ reported that the green alga, *Scenedesmus subspicatus* was exposed for 7 days to a series of polyaromatic hydrocarbons of increased molecular weight from two to five rings (naphthalene, anthracene, phenanthrene, pyrene and benzopyrene). The toxicity measured as population growth inhibition by individual polyaromatic hydrocarbon to the *S. subspicatus* followed the order: BaP>Pyr>Ant>Phe>Nap¹⁸⁾. These results confirmed that the toxicity potential of polyaromatic hydrocarbons seems to be strongly influenced by their physico-chemical properties and the conditions of algae culture (light, presence of nitrate ions, etc.)¹⁸⁾. Bunker-C oil contains less acute toxic compounds and contains a great deal of non-dissolved substances which can persist in water for quite a long time compared with crude oils¹⁴⁾. In a laboratory test from Paixão¹⁹⁾, *Tetraselmis chuii* was exposed to gasoline formulations and their components at a range of concentrations from 0% to 100%, for 96 h, it were found to be harmful to organisms. Additionally, some studies have suggested that concentrations above 100 ppm of diesel oil inhibited protoplast fission and nuclear division for algae and metabolism for marine organisms^{20,21)}. Inhibition of photosynthesis was more severe than that of respiration in *Anabaena doliolum* exposed to Assam crude oil, furnace oil, petrol, diesel, and kerosene. Diesel and furnace oil, due to greater concentrations of aromatics, were more toxic than other oils²²⁾. This result indicates that high concentrations of tested oils may significantly suppress the growth of *C. polykrikoides*.

3.2. Effect of dispersant and mixtures

Use of chemical dispersant for oil spill remediation increases petroleum hydrocarbon concentrations in sea

water, while exposing marine organisms to potentially toxic concentrations of dispersant. The use of dispersant can scatter in large amount of oil from the sea surface into the water column by dispersing. In most cases the dispersant Seagreen 850A appeared to be toxic, extremely decreasing the growth of *C. polykrikoides* after the addition to ≥ 10 ppm (Fig. 2).

Similarly, when the cells were exposed to a higher concentration of the dispersant, it made a great impact on the growth of green alga *Chlamydomonas reinhardtii*^{25,26)} and much more toxic to clams²⁷⁾. Burrigge and Shir²⁸⁾ reported that chemical dispersants are sometimes more toxic to marine organisms than the oil itself. However Yoshida et. al.⁹⁾ suggest that the chemical dispersant may be used as a nutrient source by some bacterial groups.

The combined effect of crude oil produced in Kuwait and the dispersant Seagreen 850A to *C. polykrikoides* was seen in Fig. 3. At concentrations of 10 ppm, the cell numbers were decreased by 20% in comparison with the control after 16 days, whereas the growth of *C. polykrikoides* exposed to ≤ 1 ppb showed little serious impact. However, *C. polykrikoides* almost died within a few days regardless of types of mixtures after the addition to high concentrations of 100 ppm. The toxicity of a mixture of the other types of oils and the dispersant appeared to have similar variations shown in Fig. 3. Since the dispersant consist of a mixture of a surfactant and a stabilizing agent, their toxicity may depend on the kind of solvent and surfactant²⁹⁾. Charles et al.³⁰⁾ reported great reduction of mangrove at the site treated with oil and dispersant when compared to the site treated with whole oil.

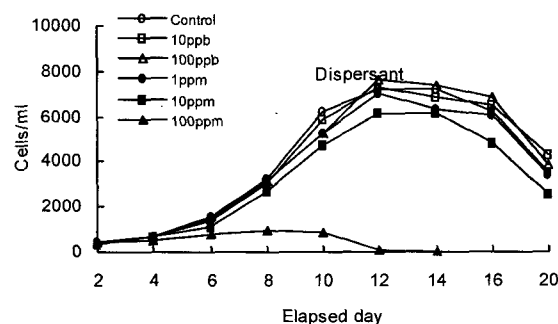


Fig. 2. The growth of *Cochlodinium polykrikoides* for 20 days after treated with a different concentration of a dispersant, Seagreen 850A.

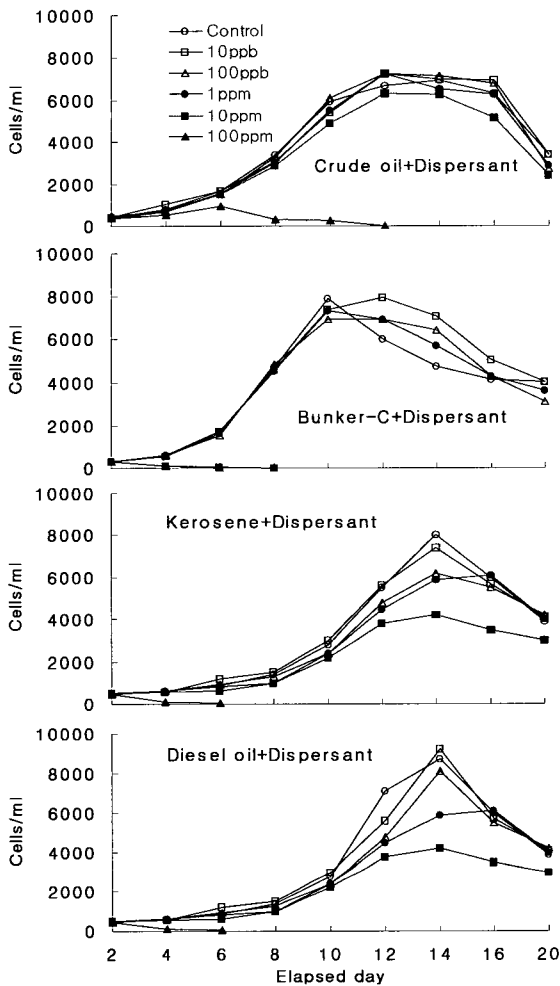


Fig. 3. The growth of *Cochlodinium polykrikoides* for 20 days after treated with a different concentration combined with oils and a dispersant.

4. Conclusions

C. polykrikoides frequently have formed red tide in Korean coastal waters and caused the massive fisheries damages. Oil spill accidents and red tide events are sometime occurred in coastal waters simultaneously. However effects of oil spill and dispersant on the growth of microalga are poorly understood. The results of this study indicate that the high concentrations of ≥ 10 ppm of oils (crude oil, bunker-C, kerosene and diesel oil), and a chemical dispersant and even mixtures made a negative effect to the growth of *C. polykrikoides*, whereas their low concentrations of ≤ 1 ppm had little correlation with the growth. We future expect that the effects on the growth of micro-

alga are performed by adding a low concentration of oils and a dispersant decomposed by bacterial action.

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