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Toxicity of Produced Water from Crude Oil Terminals to *Photobacterium phosphoreum*, *Chaetoceros* sp., and *Donax faba*

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The primary objective of the study was to compare simple toxicity tests that can be used by two oil companies based in Malaysia, namely Sarawak Shell Berhad (SSB) and Sabah Shell Petroleum Company (SSPC), to routinely monitor the effluents discharged from their crude oil terminals.

Three groups of organisms were used as test organisms for the toxicity experiments. These include the clam, *Donax faba*, the diatom, *Chaetoceros* sp., and the marine luminescent bacteria, *Photobacterium phosphoreum*.

MATERIALS AND METHODS

The effluents tested in this study were samples of produced water which are regularly discharged from the crude oil terminals of Sarawak Shell Berhad in Lutong and Bintulu in Sarawak, Malaysia, and Sabah Shell Petroleum Company's terminal in Labuan, Malaysia, into the nearby coastal areas. The effluent samples were collected by the companies prior to discharge and were air-freighted to the testing laboratory in Penang, Malaysia, in air-tight, 5-gal jerry cans. The samples were then stored in a cool environment until needed. For the preparation of the various dilutions of the test solutions, each can was first agitated gently to disperse undissolved materials present in it, prior to subsampling. The subsamples were then added to the appropriate dilution water, which was subsurface seawater collected approximately 1 mi offshore from the testing laboratory and filtered through a 2-μm filter. This dilution water met the specifications as described by Rand and Petrocelli (1985).

Prior to testing, stock cultures of the diatom which was originally obtained from the phytoplankton collection of Dr. Greta A. Fryxell of the Oceanography Department, Texas A & M University, were grown in f/2 media (Guillard 1975) through several growth cycles. This was necessary because growth responses of phytoplankton are directly related to their nutritional history. Phytoplankton that have been acclimated in this manner tend to give more reproducible results in toxicity tests.

The clams used for the toxicity tests were dug out from the intertidal zone area in the proximity of the laboratory. They were then kept in fiberglass tanks with a flowthrough seawater system and at room temperature for about 4 d and fed a daily diet of *Chaetoceros* sp. until about 24 hr before the tests. In an earlier work, 4-d acclimation of this species of clam was found to be sufficient.

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Toxicity tests using the luminescent bacteria followed strictly the protocol as outlined in the manual that accompanies the Microtox system (Microbics 1988). The assay period, that is , the period of exposure of the bacteria to the effluents, was chosen to be 15 min as this period was found to give less variable results than a 5-min exposure. Using the data collected, the EC_{50} values were calculated using the Microtox Data Capture and Reporting Program (Microbics 1988).

The tests on the phytoplankton and the clam followed the United States Environmental Protection Agency's (USEPA) protocol as described by Rand and Petrocelli (1985). Both the range and definitive tests were carried out. The range test included concentrations of the test effluents covering four orders of magnitude in the following range order: 0.1%, 1.0%, 10%, and 100%. For the definitive test, the organisms were exposed to 4 concentrations of the effluents in a geometric progression. The 4 concentrations chosen for each effluent were based on the estimate of its EC_{50} or LC_{50} as calculated from the range test.Untreated controls were also maintained for each test. Because of the inherent variability of the test, the experiments were designed to include two replicates, in order to permit statistical evaluation of the results. Toxicity tests on the diatom were conducted in 500-mL Erlemeyer flasks, each containing 200 mL of f/2 media, and inclusive of the test effluents. One mL of inoculum containing approximately 1 million diatom cells was introduced into each flask, and these were left to incubate for 96 hr. Cell density was measured using a haemocytometer. Toxicity tests on the clams were carried out in glass containers. Fifteen animals were used in a total test volume of 20 L.

A statistical probit analysis (Finney 1977) was run through the computer to determine the 96-hr EC_{50} values for the diatom and the 96-hr LC_{50} values for the clam. Regression analyses were then run to compare the LC_{50} results of the test using the bioluminescent bacteria with those of the tests using the clam and the diatom.

RESULTS AND DISCUSSION

The results of the toxicity tests on the crude oil terminal effluents using marine bioluminescent bacteria as test organism are shown in Table 1. EC_{50} values for the diatom and the LC_{50} values for the clam are included for comparison. Using the Microtox procedure, the LC_{50} values calculated were 0.2% for the Labuan effluent, 8.9% for the effluent produced from Lutong crude oil terminal (MCOT), and 23.7% for the Bintulu COT effluent. The tests on the diatom and the clam also indicate that among the three crude oil terminal effluents tested, the one from Labuan is the most toxic while the one from Bintulu is least harmful.

The regression analyses showed a positive linear relationship between the LC_{50} results of the test using the bacteria and that using the the clam, with a correlation coefficient (r^2) of 0.96. A positive linear relationship with an r^2 value of 0.94 was also found between the LC_{50} values of the bacteria and the EC_{50} values for the diatom.

The results also show that among the three tests, the one using the diatom as test organism was the most sensitive with LC_{50} values ranging from 0.1% for the Labuan effluent to 4.2% for the effluent produced from the Bintulu crude oil terminal. The test using the bioluminescent bacteria seemed to be least sensitive.

In terms of variability, the test using the diatom had the greatest precision with standard deviation values ranging from 2 - 10%, as compared to the test using the Microtox procedure which resulted in standard deviations of 15 - 38%.

Table 1. LC_{50} values (%) of the crude oil terminal effluents using bioluminescent marine bacteria (Microtox system) as compared with 96-hr LC_{50} values for the clam, *Donax faba* and 96-hr EC_{50} values for the diatom *Chaetoceros* sp.

Effluent	Microtox LC_{50} (%)	*D. faba* LC_{50} (%)	*Chaetoceros* EC_{50} (%)
Labuan COT	0.2 ± 0.03	0.2 ± 0.02	0.1 ± 0.01
Miri COT	8.9 ± 2.8	2.8 ± 0.1	0.6 ± 0.05
Bintulu COT	23.7 ± 8.9	15.3 ± 1.1	4.2 ± 0.1

The results of the toxicity tests on the crude oil terminal effluents using the marine bioluminescent bacteria indicate that in general, the bacteria is not a very good pollution indicator since it was the least sensitive to the effluents when compared to the clam *Donax faba* and the diatom *Chaetoceros* sp.This fact had also been pointed out by several other earlier studies. Jackim *et al.* (1989), working with eight organic compounds, compared results of toxicity tests using the Microtox system to standard toxicity tests using two freshwater species, *Pimephales promelas* and *Daphnia magna*, and found that in most cases, the LC_{50}s using the bioluminescent bacteria as test organism were usually higher, suggesting that the bacteria was less sensitive to the toxicants than the other two species. Miller *et al.* (1985), found that the method using the Microtox system to be somewhat less sensitive to selected toxic metals and insecticides when compared with standard toxicity tests using *Daphnia* sp. and algae. The Microtox test was again found to be less sensitive in a study involving 35 chemicals when compared to tests using fish as test animals (McFeters *et al.* 1983). However, there are cases in which the results of the tests using the Microtox system were found to be comparable in sensitivity to those using standard toxicity tests. One such example is the study conducted by Tarkpea *et al.* (1986).

In addition to generally being less sensitive, the values from the tests using the Microtox system resulted in high variability. In this study, the standard deviations for the LC_{50}s calculated using the Microtox system was as high as 38% as compared to a maximum of 10% for the clam and the phytoplankton.

Despite having low sensitivity and high variability, the toxicity test using the Microtox system has proven to be useful, particularly in effluent toxicity testing. Some of the reasons why this is so are that the procedure is quick, i.e., a test can easily be completed in just a few hours , it is quite easily conducted, and it is not messy, i.e., a wet laboratory, where test animals need to be maintained for test purposes, is not necessary.

The results of LC_{50}s estimated using the Microtox procedure have also been shown to correlate well with other standard toxicity tests. In this study, a good positive linear correlation was found between the LC_{50} values estimated using the Microtox system and the test using clam ($r^2 = 0.96$) and the diatom ($r^2 = 0.94$). Similar conclusions were made in several earlier studies. Jackim *et al.* (1989) found that results from the Microtox tests were linearly correlated ($r^2 = 0.88$) with results from acute toxicity tests using two freshwater species *Pimephales promelas* and *Daphnia magna*. These results were from tests using eight organic compounds. Tarkpea *et al.* (1986), compared toxicity test using the bioluminescent bacteria with that using

a species of harpacticoid copepod for 11 industrial complex mixtures and 16 pure technical chemicals. They found that the two tests correlated with an r^2 value of 0.75 - 0.80 for the pure chemicals and 0.90 - 0.93 for the complex mixtures. Other studies that have shown that results from toxicity tests using the Microtox system compared favorably with other acute tests include that of DeZwart and Slouff (1983), and Qureshi *et al.* (1983).

In general, it can be concluded that the toxicity test using the Microtox system should not be used when absolute toxicity values are required since it has limited value in itself in environmental risk assessment. However, this procedure has proven to be useful for preliminary estimates of the relative toxicity of toxicants including effluents, for looking at relative changes in effluent toxicity, as well as prescreening or range finding in conjunction with other toxicity tests.

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