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Assessing the Effectiveness of Depuration of Polluted Clams and Mussels Using the Microtox™ Bioassay

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Hong Kong, being a seaport with a fishing industry, enjoys an abundant supply of seafood of a wide variety. Unfortunately, pollution of the marine environment by effluent streams from industries and sewers is endangering this important food source. Much concern has been raised in the consumption of shellfish, in particular, as it proliferates around the harbor and coastal areas, accumulating toxins, heavy metals, and organics from the surrounding water. Mussels and clams sold in local markets are often submerged in sea water for the purpose of depuration as well as keeping them fresh. It is the purpose of this study to elucidate the efficacy of this method of depuration using the Microtox™ bioassay.

In recent years, rapid toxicity assays such as the Microtox system have been developed using bioluminescent bacteria (King 1984). The Microtox bioassay has versatile applications including the assessment of acute toxicity of contaminants in the aquatic environment (Bulich 1979) and sediment samples (Dutka and Kwan 1988; Lau-Wong 1989), monitoring hazardous waste leachate and detoxification (Calleja et al. 1986; Symons and Sims 1988), and screening bacterial toxicants (work presented by Atkinson DS and Switzenbaum MS at the International Conference on Innovative Biotreatment of Toxic Wastewater in Arlington VA on 26 June 1986), complex effluent (Vasseur et al. 1984), waste drilling fluid (Strosher 1984), biocide (Barton and Delhaize 1986) and biomaterials (Burton et al. 1986). Microtox assay results have also demonstrated good correlation with other bioassay results such as the rainbow trout (Qureshi et al. 1982; Strosher 1984).

MATERIALS AND METHODS

Two samples (about 150 each) of small polymorphic clam (Tapes philippinarum) and two samples (about 80 each) of green-lipped mussel (Perna viridis) were purchased at different markets. The samples of clam and mussel bought at Sai Kung were already under depuration at the shop at the time of purchase and were denoted by CSK and MSK respectively; whereas the clam and mussel samples

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bought at Causeway Bay were untreated by the vendor and were denoted by CCB and MCB respectively.

One-quarter of each sample was reserved for the bioassay, while the rest was depurated in an aquarium filled with clean seawater obtained from distant unpolluted waters. To maintain a constant oxygen supply to the organisms, air was delivered via an aquarium aerator. A quarter portion of the shellfish was removed for assay at 2 day intervals up to 6 days.

The shells of each portion were removed and the tissues were homogenized for 10-15 min and centrifuged at 5000 rpm (3500g) at 15^{0}C for 20 min. The supernatent was aspirated off for testing.

The bioassay was performed using Photobacterium phosphoreum. When challenged by a sample of the shellfish supernatent, the intensity of light diminished was detected with a Microtox Toxicity Analyser Model 2055 (Microbics Corporation, Carlsbad, CA). The assay method was essentially similar to that described in the Microtox System Operating Manual.

The Gamma value Γ is defined as the ratio of light loss during incubation time (t) to the light remaining at time (t). EC50 is the effective concentration of toxicant causing a 50% decrease in bacterial light output under defined conditions of incubation or exposure time (t) and test temperature. EC50 is determined from the plot of log Γ versus log (concentration) where Γ = 1.

Absorption corrections were required for the colored samples. The temperature adopted in the bioassay was 15^{0}C throughout, and readings were taken at incubation periods of 5, 10, 15, and 20 min. Duplicate tests were performed on all samples of various dilutions. Analysis of variance for the data was performed using the SPSS Statistical package.

RESULTS AND DISCUSSION

The effect of depuration treatment on light output by the bioluminescent bacteria was depicted in Fig. 1 to 4. The results for all the samples showed a consistent trend of decrease in acute toxicity as the duration of treatment was prolonged. The Gamma values at 5 min and 20 min incubation times were given in Tables 1 and 2. The data for sample MSK at treatment day 6 were missing, as there was not enough viable samples left for analysis. These missing data were taken into account in subsequent statistical analysis. In analysis of variance, overall F-test showed that the treatment was effective, being highly significant at less than 0.001% for 5 min incubation and 0.7% for 20 min incubation.

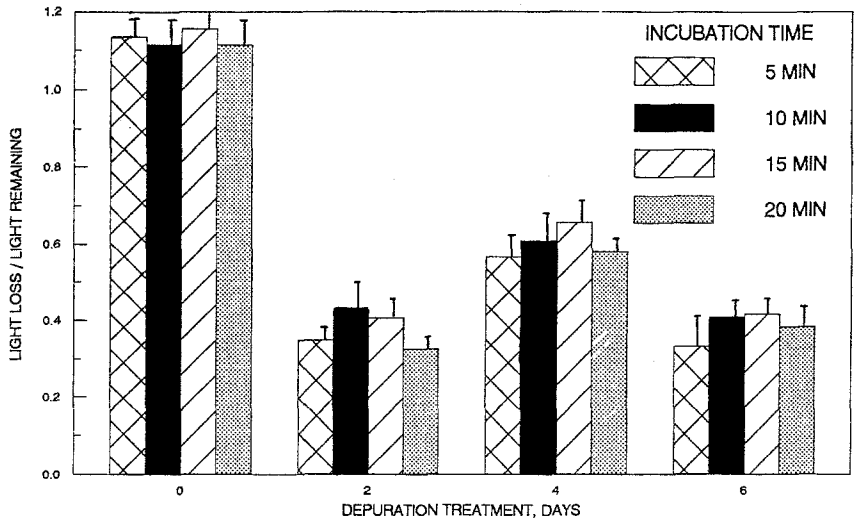

Figure 1 Clam sample CCB -- effect of depuration treatment on light output by bioluminescent bacteria

(CCB - undepurated at the time of purchase)

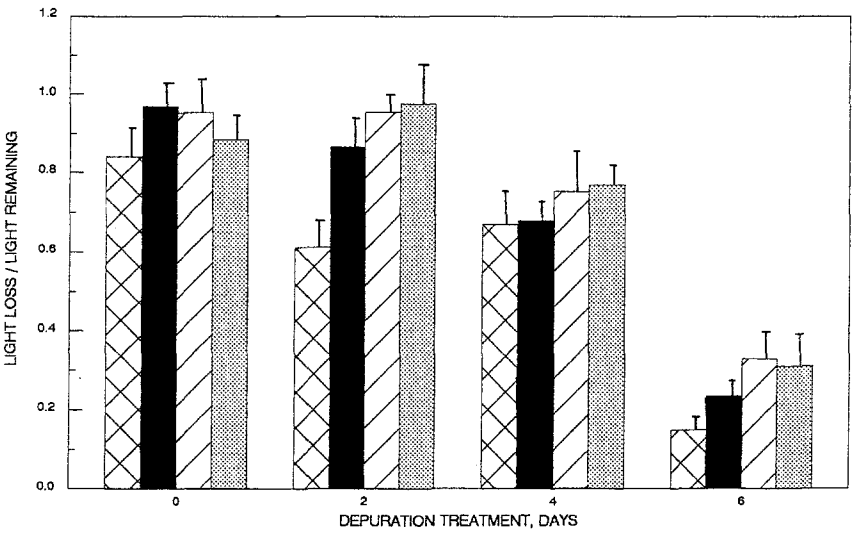

Figure 2 Clam sample CSK -- effect of depuration treatment on light output by bioluminescent bacteria

(CSK - depurated by vendor)

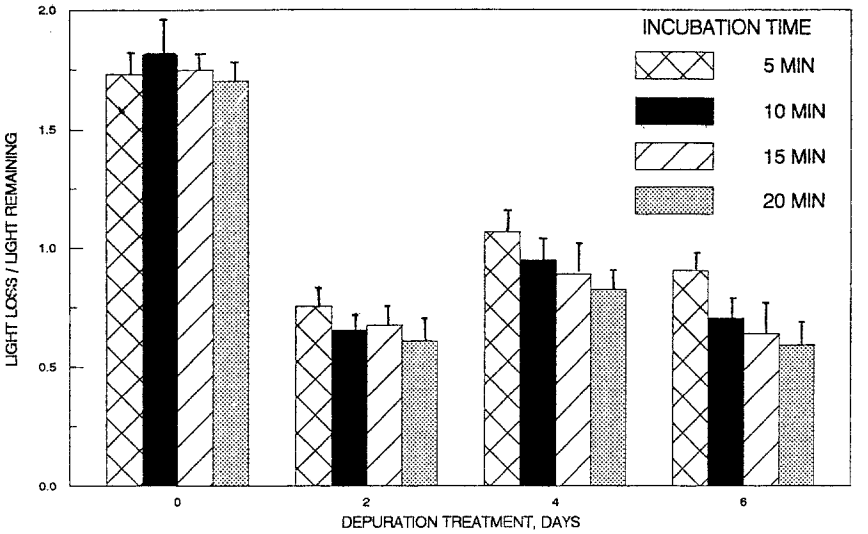

Figure 3 Mussel sample MCB -- effect of depuration treatment on light output by bioluminescent bacteria
(MCB - undepurated at the time of purchase)

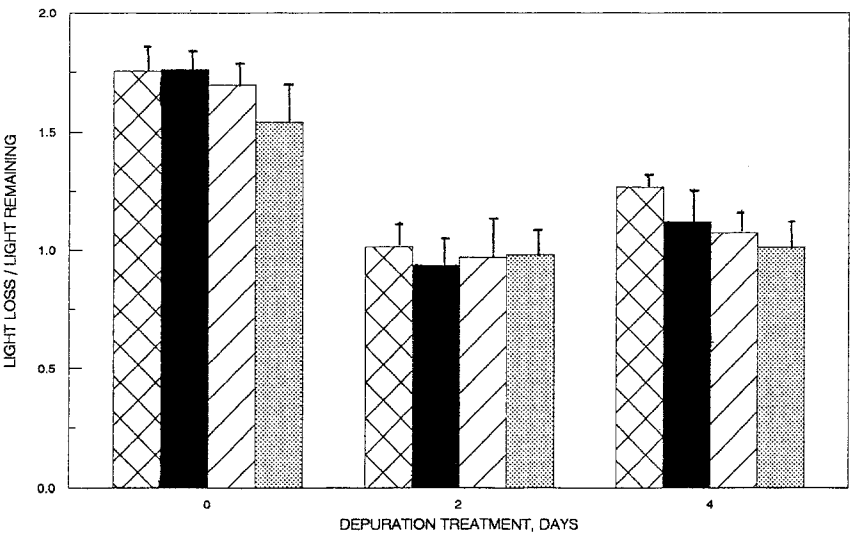

Figure 4 Mussel sample MSK -- effect of depuration treatment on light output by bioluminescent bacteria
(MSK - depurated by vendor)

Table 1. Gamma values at incubation time of 5 minutes

Treatment days	Sample CCB[1]	CSK[2]	MCB[3]	MSK[4]
0	1.094	0.842	1.597	1.650
2	0.362	0.614	0.739	0.942
4	0.566	0.671	1.000	1.213
6	0.333	0.150	0.875	----

---- Data missing for sample MSK since no viable sample was left on day 6.

Overall F-test for the entire treatment scheme: significant at 0.1%.

Table 2. Gamma values at incubation time of 20 minutes

Treatment days	Sample CCB[1]	CSK[2]	MCB[3]	MSK[4]
0	1.075	0.885	1.569	1.433
2	0.340	0.977	0.591	0.910
4	0.584	0.770	0.764	0.965
6	0.383	0.312	0.565	-----

---- Data missing for sample MSK since no viable sample was left on day 6.

Overall F-test for the entire treatment scheme: significant at 0.7%.

[1] CCB Clam sample purchased at Causeway Bay, undepurated at the time of purchase.

[2] CSK Clam sample purchased at Sai Kung, under depuration by vendor at the time of purchase.

[3] MCB Mussel sample purchased at Causeway Bay, undepurated at the time of purchase.

[4] MSK Mussel sample purchased at Sai Kung, under depuration by vendor at the time of purchase.

The differences in the Gamma values obtained for the various incubation times were not statistically significant (significance of F =53%), and this implies that the toxicity effects were experienced at an early stage (at 5 min) and longer incubation times are not necessary for enhancing the sensitivity of toxicants.

The EC50 was defined in this context as the dilution of the

Table 3. Test of significance of the different treatment days

Comparison	5 min significance of F, %	20 min significance of F, %
0 vs 2 days	24	>50
0 vs 4 days	5	22
0 vs 6 days	0.1*	3*
0 vs 2,4,6 days	0.1*	0.5*

* statistically significant at the specified levels.

Table 4. EC50[5] values of clam and mussel samples

Sample	Treatment days	EC50 values 5 min	20 min
CCB	0	0.159	0.145
	2	--	--
	4	--	--
	6	--	--
CSK	0	0.150	0.217
	2	--	0.206
	4	--	--
	6	--	--
MCB	0	0.128	0.131
	2	--	--
	4	0.164	--
	6	--	--
MSK	0	0.116	0.112
	2	0.144	0.154
	4	0.152	0.188

[1] CCB Clam sample purchased at Causeway Bay, undepurated at the time of purchase.

[2] CSK Clam sample purchased at Sai Kung, under depuration by vendor at the time of purchase.

[3] MCB Mussel sample purchased at Causeway Bay, undepurated at the time of purchase.

[4] MSK Mussel sample purchased at Sai Kung, under depuration by vendor at the time of purchase.

[5] Unit being dilution factor of the supernatent from the centrifuged homogenate that could cause a 50% decrease in bacterial light output.

supernatent from the centrifuged homogenates that could cause a 50% decrease in the bacterial light output. The EC50 values obtained for samples depurated by the vendor (CSK and MSK) and those that were not (CCB and MCB) were compared and no significant difference was observed. This suggested that the water used by the vendor for depuration was contaminated, as this is very likely the case since the water was obtained at the adjacent pier.

Interactions of the two independent factors, treatment days and sample type, were also tested. The F-test showed that the interaction term was not significant, at 40% for 5 min and over 60% for 20 min. There is thus no evidence that any combination of treatment and sample reinforces or cancels the effect of each other. In other words, the total effect of any combination of treatment and sample is simply the sum of the effects of the treatment and the sample.

A comparison of the efficacy of treatment at 2, 4, and 6 days was given in Table 3. Significance was detected only in the comparison of 0 versus 6 days (at less than 0.1% at 5 min and 3% level at 20 min). This implies that depuration of shellfish for 2 and 4 days is not sufficient, whereas a 6 day treatment will decrease the toxicity significantly.

The EC50 for the samples at various treatment days were calculated for the 5 min and 20 min incubation times and tabulated in Table 4. It was given in terms of dilution of the supernatent of the original homogenized sample. The lower the EC50, the more toxic the sample. As the treatment duration progressed, the Gamma value decreased, and in some cases to below 1, which is the determining point for EC50. Thus it is impossible to determine EC50 in such cases.

It is demonstrated in this study that the Microtox bioassay is useful in monitoring the treatment of polluted clams and mussels. Depuration is effective when prolonged to 6 days in clean sea water. Contaminants in the shellfish samples may range from metals and organics to toxins from microorganisms and dinoflagellates. As the samples originated in waters suspected of high metal pollution, studies are undertaken to determine the metal contents in treated and untreated shellfish.

One disadvantage of using the Microtox is that the sample has to be applied in liquid form (preferably in solution) in order that the toxic molecules can diffuse readily to the bioluminescent bacteria. In preparation of the sample, the insoluble tissue left behind after homogenization and centrifugation may well contain toxic substances which are only released after human consumption. One likely example is heavy metal bound to tissue and the harboring of red tide toxin in shellfish tissue. In view of recent occurrences of red tide in Hong Kong waters, comparison of the mouse bioassay, which is frequently used for

detection of toxicity in shellfish, with the Microtox bioassay is underway.

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