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Acute Bioassays with Benthic Macroinvertebrates Conducted in Situ

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Several methods of toxicity testing using macroinvertebrates in controlled laboratory experiments have been reported (Benfield and Buikema 1980; Maciorowski and Clarke 1980; Reish 1980). Burks and Wilhm (1977) conducted bioassays with natural assemblages of benthic macroinvertebrates exposed to several petroleum refinery effluents. They found that the populations of invertebrates declined after only a few days of exposure. The diversity and number of taxa also declined. Miedecke and Stephenson (1977) found significant declines in benthic macroinvertebrate populations in the vicinity of ocean sewer outfalls.

The objective of the study was to determine the acute toxic effects of discharge water from a petrochemical complex on a natural assemblage of benthic macroinvertebrates. The discharge water consisted of refinery wastewater and sanitary wastewater, as well as brine discharge from a power/desalination plant. The benthic macroinvertebrates were transplanted from a healthy reef area (Figure 1) to the outfall channel receiving the discharge water. The study began on October 7, 1985, and concluded that same week. Any decrease in specific species would indicate that the discharge was toxic to these species. These species could also serve as indicators of toxic conditions at other locations.

MATERIALS AND METHODS

Artificial substrate containers were used to hold the benthic macroinvertebrates during the acute toxicity test. The containers were constructed from 8-L polyethylene water containers with the tops removed.

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The containers had an area of 0.4-m^2 and held a volume of 0.005-m^3 of sediment. The sediment containing the benthic macroinvertebrates was collected by SCUBA divers at a healthy reef area (Figure 1). The sediment was placed in seven artifical substrate containers. One of the containers was analyzed initally for benthic macroinvertebrate species composition and total number for each species. Three of the containers were left at the healthy reef site (control) and the remaining three containers were transplanted at the outfall channel 1-h after collection (Figure 1).

One sampler was collected from each area after 1, 2, and 4 days of exposure. The samples were sieved through 4 and 2-mm mesh screens, respectively. The samples were placed in containers, stained with Rose-Bengal solution, and preserved in 10% formalin. The organisms were sorted after 24 h and identified to the lowest taxa possible (Fauchald 1977; Gosner 1971; *Saudi Arabia Tetra Tech 1982 unpublished)*.

The Shannon-Weiner diversity (H') and eveness (J') indices were determined using the following equations (Hellawell 1978):

$$H' = \sum_{i=1}^{s} - n_i \log_2 n_i$$

s = number of taxa
n_i = number of ith species

$$J' = H' / \log_2 s$$

The Mann-Whitney U statistic was determined following the procedures of Brewer (1977). The Student's t statistic was determined using the following equations (Neudecker 1976):

$$U = \left[\frac{s_1 s_2}{2} \right]$$

s = number of taxa
U = Mann-Whitney statistics

$$t_s = \frac{}{\sqrt{\frac{(s_1 s_2)\left[(s_1 + s_2)^3 - (s_1 + s_2) - \sum_i^M T_i \right]}{[(s_1 + s_2)(s_1 + s_2 - 11)](12)}}}$$

$$\sum_{i}^{M} T_i = (t_i - 1)t_i(t_i + 1)$$ t = number of tied observations

The critical value of Student's t statistic was determined with infinite degrees of freedom using Brewer's table (1977).

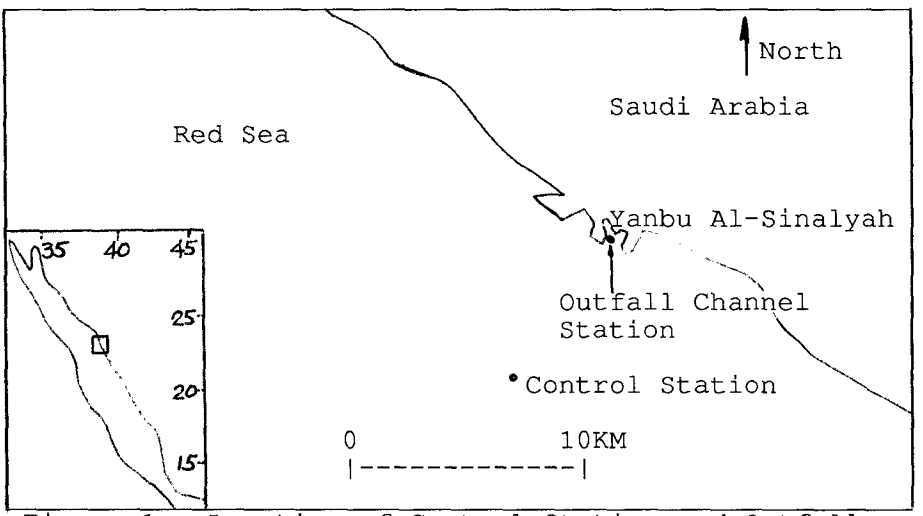

Figure 1. Location of Control Station and Outfall Channel Station.

RESULTS AND DISCUSSION

The total number of organisms per square meter as well as the number of taxa declined significantly over the 4-d exposure (Tables 1 and 2). Student's t statistic was calculated using the initial and the 4-d exposure at the outfall channel species counts. There was a significant difference between the benthic macro-invertebrate populations of the two samples ($t = 3.50$, $p < 0.05$ with infinite degrees of freedom).

The diversity index (H') also declined after the 4-d exposure at the outfall channel station (Table 1). The less tolerant organisms did not survive the 4-d exposure. One type of organism was found to be a possible pollution indicator (Table 2). A species of nematode appeared to be a good indicator of water quality. The eveness index (J') remained relatively constant throughout the test (Table 1).

Table 1. The total number of organisms per square meter (N), the number of taxa (s), the diversity index (H'), and the eveness index (J') of benthic macroinvertebrates exposed to petroleum refinery effluents.

Exposures (Days)	Control Station N	s	H'	J'	Outfall Channel Station N	s	H'	J'
0	2200	28	4.06	0.84	2200	28	4.06	0.84
1	1575	26	4.00	0.85	1025	18	3.89	0.93
2	Sample Lost				1125	16	3.47	0.87
4	1775	26	4.29	0.91	825	18	3.66	0.87

Table 2. Species composition including number of each species for each artificial species container collected.

	Initial	Control Station (days) 1	4	Outfall Channel Station (days) 1	2	4
Annelida						
Oligochaeta						
Unidentified sp.	-	-	1	-	-	-
Polychaeta						
Euniphysa aculeata	3	3	5	3	7	4
Paramarphysa sp.	2	1	2	1	1	1
Schistomeringos sp.	2	2	4	2	-	1
Exogone clavator	20	17	6	6	10	9
Phyllodoce sp.	4	1	2	3	1	1
Lysidice sp	1	-	1	1	-	1
Nothria sp.	4	3	5	3	6	1
Aparaonis sp. 1	3	5	3	-	-	1
Aparaonis sp 2	1	-	-	-	-	-
Ophelia sp.	1	-	-	1	-	-
Diplosyllis sp.	5	1	3	3	4	2
Trypanosyllis zebra	3	3	3	3	3	2
Lacydonia sp.	4	1	2	2	-	1
Eurysyllis sp.	1	-	-	-	-	-
Sabella sp.	-	-	1	-	-	1
Maldanidae #1	4	1	3	1	3	3
Syllidae #1	2	2	3	-	-	-
Syllidae #2	1	-	-	-	1	-
Phyllodocidae #1	2	1	-	1	-	1
Terebellidae #1	1	1	1	-	1	-
Cirratulidae #1	-	1	-	-	1	-
Ampharetidae #1	-	-	1	-	-	-
Nereidae #1	-	-	3	-	3	-

Table 2 continued

Unidentified sp. A	1	1	2	1	1	-
Unidentified sp. B	1	-	1	-	-	-
Unidentified sp. C	-	-	1	-	-	-
Arthropoda						
Crustacea						
Diogeniidae sp.	-	-	1	-	-	-
Galatheid sp.	-	1	-	-	-	-
Echinodermata						
Holothuroidea						
Holothuria sp.	1	-	-	-	1	-
Ophiuroidea						
Unidentified sp.	1	-	-	-	-	-
Mollusca						
Gastropoda						
Nassarius sp.	1	-	1	1	-	1
Olivia sp.	-	-	-	-	1	-
Rissonia sp.	1	-	-	-	-	-
Unidentified sp. A	-	1	-	-	-	-
Unidentified sp. B	-	1	-	-	-	-
Unidentified sp. C	-	1	-	-	-	-
Pelecypoda						
Unidentified sp.	1	-	-	-	1	-
Scaphopoda						
Dentalium sp.	-	-	-	-	-	1
Nematoda						
Unidentified sp.	14	5	12	3	1	1
Cephalochordata						
Branchiostoma						
Unidentified sp.	-	-	-	1	-	-
Total	87	55	69	41	46	32

This 4-d exposure bioassay indicates that the discharge water in the outfall channel is acutely toxic to many of the benthic macroinvertebrates.

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