RESULTS OF TOXICITY TESTS WITH MARINE ORGANISMS OF KUWAITI COAST

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ABSTRACT

For obtaining background information on the sublethal to toxic effects of the different types of pollutants entering the marine environment of Kuwait, 33 continuous flow bioassays of 96 h duration, one long duration bioassay of 60 days and 4 static assays of 96 h duration were carried out. Toxicants tested were salts of 5 heavy metals, 9 types of mineral oils, 3 types of oil dispersants and mixtures of oils and dispersants. Test animals included fingerlings of the mullet, Liza mauretana (Smith); adults and juveniles of the prawn, Penaeus semisulcatus de Haan; four species of intertidal gastropods (Lunella coronata Gmelin, Monodonta canalifera Lamarck, Tais fuscogutta Dunker and Planaxis sulcatus (Born); the sea urchin Echinometra mathaei and the brine shrimp, Artemia salina. Estimates of LC 50 values of all the tests are presented. They reveal marked species variations in response to the toxicants.

INTRODUCTION

Arabian Gulf had 142 major coastal plants in existence or planned during 1976. In addition to oil refineries, cement plants and desalination-power plants, these included about 8 fertilizer plants also (Walgate 1978). All these industries are adding to the pollution load on an almost enclosed sea which averages only 34 m deep with shore waters less than 10 m deep.

The rate of introduction of waste materials especially crude oil, metallic toxicants and nitrogenous compounds like ammonia and urea into the marine environment of Kuwait (situated at the head of the Arabian Gulf between 28°45' and 30°05' N and 46°30' and 48°30' E with a coast line of about 200 km) by the effluents of industries has increased continuously over the last 10 years (Cremer and Warner 1976, Sharma and Dourgham 1977, Oostdamm and Anderlini 1978). Kuwait is the third largest petroleum producer in the Middle East and the seventh in the world with an average annual export of 120 million m³. The estimated amount of oil spilled in Kuwaiti waters averaged 183 m³ per year and the ratio of the average amount of oil spilled to oil exported is very low compared to the world average. The average tar concentration of beaches
(388 g/m) is high compared to Bermuda (190 g/m) and low compared to west coast of India (4480 g/m). However the possibility of large-scale oil spillage by accidents in the Kuwaiti waters is a frightening possibility.

The effects of industrial effluents, various types of mineral oils, oil dispersants and mixtures of oils and dispersants on the Kuwaiti marine life is little known excepting limited studies conducted by Mohammad (1974), Enamy and Marafi (1977), Helmy et al (1978) and Jacob (1978). To obtain background information on the sublethal to toxic effects of the various pollutants entering the marine ecosystem of Kuwait, 33 continuous flow bioassays of 96 h duration, one long duration continuous flow bioassay of 60 days and 4 static assays of 96 h duration were carried out. In general, estimates of LC 50 values of all tests revealed marked species variations and striking individual sensitivities of the experimental organisms (Helmy and Jacob 1978).

**Material and Methods**

A variety of marine animals were used for the tests based on their distribution, availability and economic importance. Medium sized specimens of the mullets, *Liza macrolepis* (Smith) with average total length of 6.2 cm and mean weight of 2.6 g were used for the tests. *Penaeus semisulcatus* de Haan, commercially the most important prawn of the Arabian Gulf, were collected by trawlers and the average total length of the adults was 18 cm with an average weight of 48 g whereas the average total length of the juveniles was 6.6 cm and mean weight 2.7 g. Four commonly occurring intertidal gastropods used in the tests were *Lunella coronatus* Gmelin (average shell height 2.4 cm, shell diameter 2.9 cm and mean weight 9.1 g); *Monodonta canalifera* Lamarck (average s.h. 1.7 cm, s.d. 1.8 cm and mean weight 2.4 g); *Thais fuscanigra* Dunker (average s.^h. 2.2 cm, s.d. 1.6 cm and mean weight 3.2 g) and *Planaxis sulcatus* (Born) (average s.h. 1.2 cm, s.d. 0.9 cm and mean weight 0.4 g).

The common rock-boring sea urchin, *Echinometra mathaei* used in the tests (average shell diameter 8.1 cm and mean weight 45.3 g) inhabit coral beds of the shallow coastal waters of Kuwait and adjacent areas (Basson et al 1977). Adult specimens of the brine shrimp, *Artemia salina* (average total length 1.3 cm and mean weight 0.009 g) obtained from continuous cultures (Jacob, 1978) were also used in the tests as a representative of zooplankton.

The short term (96 h) and one long term (1440 h) tests were carried out in the continuous flow toxicity testing proportional serial diluters described by Lamke et al (1978) using relatively pure sea water pumped from Salmiya coast (Fig. 1). Continuous flow through systems provide more accurate estimates of absolute toxicity (Burdick 1966, Lichatowich et al 1973). Dissolved oxygen concentrations remained close to saturation. Salinity of the incoming sea water varied from 40.1 to 44.3 % whereas pH varied from 7.6 to 8.2. As the experiments were conducted in an air-conditioned room, fluctuations in temperature of the test medium were minimal.
Stock solutions of the metallic compounds (CuSO₄·5H₂O for Copper, HgCl₂ for Mercury, Pb(NO₃)₂ for Lead, CdCl₂·H₂O for Cadmium and ZnSO₄·7H₂O for Zinc) were prepared in distilled water by dissolving the required weights and were introduced to the proportional diluter through a Mariotte bottle (Lemke et al. 1978). In tests with oils alone the water soluble fraction (W.S.F.) was prepared by saturating sea water with preweighted amounts of oils in a special mixing chamber. The W.S.F. was then drawn out and fed to the serial diluter by a pump. The final concentration was calculated with reference to the original weight of the oil added. In experiments with oils mixed with oil dispersant Enjay the amount of Enjay used was 6% by volume of the oil.
During long term (1440 h) exposure to crude oil and Enjay, three groups of test animals were used. Five sets of animals remained stable (Stable group) in 5 different concentrations (114, 57, 28, 14 and 7 mg/1). Out of the other two groups one started in the lowest concentration (7 mg/1) and was transferred after every 10 days to the next higher concentration (Ascending group), whereas the other group started first in the highest concentration (114 mg/1) and was transferred after every 10 days to the next lower concentration (Descending group).

Out of the 9 mineral oils tested, the most viscous and opaque were Heavy Fuel Oil and Light Fuel Oil and the lightest and colourless were Aviation Turbine Kerosine, Kuwait Natural Gasoline and Kuwait Light Distillate. Crude oil, Diesel oil and Gas oil were moderately viscous. The three oil dispersants used were quick acting and gave fine dispersion of oil in seawater with a high stability and required minimum mechanical energy. All appeared as clear bright yellowish liquids with slight odour and were non-corrosive and soluble in hydrocarbons.

For static experiments with chemical fertilizers, aliquots of the five chemicals tested were added to the bioassay containers, having five litres filtered seawater in each, to achieve the desired concentration before the test animals were introduced.

The number of animals tested in each concentration (one test tank) varied from 10 to 100 depending on the species tested. For all the tests (both dynamic and static) total mortalities in each test container were recorded at 24 h intervals with observations of animals' behaviour. All observations of mortality of the test animals were made visually. After recording mortalities, all the dead animals were removed from the test containers.

The measure of death used was the calculated 96 h 50% lethal concentration (96 h LC 50), which is the concentration of the toxicant causing death in 50% of the exposed animals at the end of 96 hours of exposure. The LC 50 values for long term test with crude oil and Enjay mixture are the concentrations required to kill 50% of the animals at the end of 1440 hours. The procedure used to determine the LC 50 values was that given by Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

Eventhough there are a number of published works on freshwater toxicity (Applegate et al 1957, Mckee and Wolf 1963) there is very limited amount of corresponding data regarding the marine environment. As it is unlikely that the toxicity of a given material would be the same to freshwater and marine fish, separate tests are needed for most toxicants. Portman (1972) have tested over 160 different "pollutants" against some of the marine organisms of the
coastal waters of England and have given the LC 50 values. But the method he used is static bioassay which has a number of weaknesses compared to dynamic bioassays followed here in most of the tests (Lemke et al 1978).

Results of all the tests conducted with metallic toxicants, mineral oils, oil dispersants, mixtures of mineral oils and dispersants and chemical fertilizers are given in Tables 1 and 2 and in Fig. 2. Of the materials tested the most lethal were metallic toxicants like Mercury, Copper and Cadmium. The least lethal were heavy oils like crude oil, Heavy fuel oil and chemical fertilizers.

**Metals**

Five metals (Cu, Hg, Pb, Cd and Zn) all commonly occurring in industrial effluents, were tested against *P. semisulcatus, L. macrolepis, L. coronatus, M. canalifera, T. fusconigra, p. sulcatus, A. salina and E. mathaei*. Mercury and copper proved to be the most lethal (Table 1) confirming the results of Wisely and Blick (1967) who used larval forms of marine animals. The other 3 metals were less toxic.

Lead precipitated out to a noticeable extent and so it is unrealistic to describe the LC 50 in terms of added concentration when this was not the actual concentration in water. However, the precipitates were kept in suspension by the constant water flow and the movement of the animals.

Juvenile prawns (*P. semisulcatus*) were more susceptible to mercury (Table 1) than adults and even less than half the amount of mercury was needed to kill 50% of the young prawns compared with that needed by adults. Helmy et al (1978) observed haemopathological changes attributable to heavy-metal poisoning in blood smears of *L. macrolepis* taken after 96 h exposure in copper, lead and mercury in the present tests. They point out that metallic poisoning of *L. macrolepis* bear resemblance to the pathologic changes that have been demonstrated clinically and experimentally in mammals. Therefore, blood measurements on marine vertebrates may be diagnostic of undesirably high levels of copper and mercury and may thus constitute useful indicators of marine pollution levels. Biochemical investigations of the blood of *L. macrolepis* after exposure to copper, mercury and lead were also indicative of a complex spectrum of response consistently specific for each metal in the present experiments (Helmy et al 1978).

Helmy and Jacob (1978) reported that out of Cu, Hg, Pb and Cd, cadmium had the most retarded effect on all species of molluscs, a feature attributable to the nature of its toxicity mechanisms. Among the 4 species of snails, *L. coronatus* proved to be the most sensitive and *T. fusconigra* the least to metallic toxicants.

Biochemical investigations of the experimental snails (Helmy and Jacob 1978) revealed that *Monodonta canalifera* had 1.5 times more tissue water than
### Table 1. 96 h LC 50 values (mg/l) for different toxicants on marine species

<table>
<thead>
<tr>
<th>Species</th>
<th>Cu</th>
<th>Hg</th>
<th>Pb</th>
<th>Cd</th>
<th>Zn</th>
<th>CO</th>
<th>LFO</th>
<th>DO</th>
<th>ATK</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. semisulcatus</em> (Juveniles)</td>
<td>&gt;1.1</td>
<td>0.1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td><em>P. semisulcatus</em></td>
<td>9.8</td>
<td>&lt;0.03</td>
<td>&gt;31.6</td>
<td>2.7</td>
<td>&gt;8.9</td>
<td>&gt;106</td>
<td>142</td>
<td>&gt;216</td>
<td></td>
</tr>
<tr>
<td><em>L. macrolepis</em></td>
<td>1.4</td>
<td>0.4</td>
<td>&gt;12.9</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>&gt;114</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td><em>L. coronatus</em></td>
<td>0.2</td>
<td>0.05</td>
<td>&gt;31.6</td>
<td>&gt;14.4</td>
<td>&gt;8.9</td>
<td>&gt;106</td>
<td>&gt;114</td>
<td>&gt;204</td>
<td>&gt;216</td>
</tr>
<tr>
<td><em>M. canalifera</em></td>
<td>0.2</td>
<td>0.05</td>
<td>&gt;14.6</td>
<td>&gt;14.4</td>
<td>...</td>
<td>...</td>
<td>&gt;106</td>
<td>&gt;114</td>
<td>&gt;204</td>
</tr>
<tr>
<td><em>T. fusconigra</em></td>
<td>0.2</td>
<td>0.2</td>
<td>&gt;31.6</td>
<td>12.8</td>
<td>&gt;8.9</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td><em>P. sulcatus</em></td>
<td>0.1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td><em>A. salina</em></td>
<td>&lt;0.6</td>
<td>&lt;0.03</td>
<td>&gt;14.6</td>
<td>&gt;14.4</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>20.3</td>
<td>&gt;216</td>
</tr>
<tr>
<td><em>E. mathaei</em></td>
<td>&lt;0.6</td>
<td>&gt;0.03</td>
<td>&gt;31.6</td>
<td>5.1</td>
<td>&gt;8.9</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>


*T. fusconigra* and the least content of lipid. *P. sulcatus* was very rich in fat. The changes of carbohydrate, fat or nitrogen content in molluscs attributable to cadmium exposure was the least of all the 4 metals (Cu, Hg, Cd and Pb) tested.

Besides species-specificity, metals varied in their effects in the case of gastropods. The relationships of dry tissue weight to shell length, an indirect indicator of general health was not significantly altered by exposure to metals. This indicates that mortalities were caused by metabolically specific mode of poisoning rather than by general weakening of the animals.

It has been observed that heavy metals Hg, Cu and Pb are toxic to aquatic life, but the degree of toxicity depends upon the type of the organism, water temperature, hardness, turbidity and carbon dioxide content (Steeman-Nielsen and Wium-Andersen 1970, Mc Kee and Wolf 1963).

Oil, oil dispersants and mixtures of oils and oil dispersants

The results of the present tests with 9 types of oils and 3 types of oil dispersants alone are not very lethal even in considerably high concentrations (Table 1). However mixture of lighter oils (Kuwait Natural Gasoline, Prefractionated Plate formate oil, Kuwait Light Distillate, Aviation Turbine Kerosine, Diesel Oil and Gas Oil) and the oil dispersant Enjay proved to be lethal to most of the test organisms (Table 1).
The results of the long-term (1440 h) tests of crude oil mixed with the oil dispersant Enjay (6% by volume) show that even though crude oil is not lethal for shorter durations it can completely wipe out entire communities if it is present over a long span of time (Table 2). All test animals of the ascending and descending groups were dead by the end of the test.

Crude oil is an extremely complex mixture of hydrocarbons. Although compounds made up of hydrogen and carbon predominate, small traces of sulphur, nitrogen and oxygen also are present. The gross composition of the Kuwait crude oil, as determined by silica-gel fractionation, was 39% paraffinic, 40% aromatic, 6% asphaltic with an overall recovery of 85% (Dalla Venezia and Fossato, 1977). There is general agreement that the toxicity of crude oil increases along the hydrocarbon series: from paraffins to napthenes and olefins, to aromatics. Ryan (1977) observed that within each series of hydrocarbons, the smaller molecules are more toxic than the larger. Even though many of the toxic compounds are removed during the refining process by treatment with sulphuric acid, in many cases the final product is still toxic.

Venezia and Fossato (1977) reported that long-term effects on exposure of the harpacticoid copepod *Tisbe bulbisetosa* to suspensions of Kuwait crude oil and dispersant Corexit 7664, on number of eggs produced, number of nauplii and hatching for females of the third and fourth generations were
negligible compared with controls. Mohammed (1974) noted that the survival of the tube dwelling polychaete *Pomatoleius kraussii*, an important fouling organism of the Kuwaiti coast, decreases as the area coated with oil increases. He found that although the annual ranges of water temperature (12-13°C) and oxygen content (3-5 ml) are favourable for biodegradation of oil in Kuwait, approximately 33% of the individuals of *P. kraussii* were smothered by the tarry residues after 52 weeks exposure at one station. Atema (1977) found that the lobster *Homarus americanus* react in a variety of abnormal ways when exposed to different concentrations of petroleum hydrocarbons like La Rosa crude oil and No. 2 fuel oil.

The actual effects of oil pollution on marine organisms are still controversial. Although it is known that oil pollution causes severe short term damages and affects the aesthetic value of beaches, the long term effects are not fully known. Research sponsored by the American Petroleum Institute (Mertens and Gould 1976) shows little evidence of lasting effects. However oils adversely affects the benthic and intertidal community because of the persistence of oil in the unconsolidated substratum.
As assessment of the seriousness of hydrocarbon pollution is made difficult by the analytical problems and as a result there are very few works on the petroleum content of ocean waters, organisms or sediments (Goldberg, 1976).

**Chemical fertilizers**

The results of the 96 h static toxicity tests with 5 chemical fertilizers are presented in Fig. 2. The LC50 value with Urea for *L. macrolepis* was 6166 mg/1. For *Ambassis gymnocephalus* and *Puntius bimaculatus* two species of freshwater fishes 96 h LC50 values for Urea have been found to be 22500 mg/1 and 18500 mg/1 respectively (D’Silva and Verlencar 1974). This proves that *L. macrolepis* fingerlings are less tolerant to Urea than these freshwater species. Compared to other chemicals used, Urea appears to be more toxic.

**Table 2.** 1440 h LC 50 values for Crude Oil with Enjay on marine animals of Kuwait using the continuous flow proportional diluter.

<table>
<thead>
<tr>
<th>Species</th>
<th>Start of mortality (after h)</th>
<th>Initial mortality (%)</th>
<th>Final mortality (%)</th>
<th>LC 50 (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Penaeus semisulcatus</em> (juveniles)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable group</td>
<td>216</td>
<td>26</td>
<td>98</td>
<td>&lt;7</td>
</tr>
<tr>
<td>Ascending group</td>
<td>216</td>
<td>89</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Descending group</td>
<td>96</td>
<td>86</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>Lunella Coronarum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable group</td>
<td>360</td>
<td>4</td>
<td>57</td>
<td>25</td>
</tr>
<tr>
<td>Ascending group</td>
<td>360</td>
<td>13</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Descending group</td>
<td>168</td>
<td>12</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td><em>Monodonta candifera</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable group</td>
<td>264</td>
<td>6</td>
<td>98</td>
<td>&lt;7</td>
</tr>
<tr>
<td>Ascending group</td>
<td>360</td>
<td>13</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Descending group</td>
<td>144</td>
<td>24</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>Thais fuscomitra</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable group</td>
<td>264</td>
<td>2</td>
<td>54</td>
<td>24</td>
</tr>
<tr>
<td>Ascending group</td>
<td>1176</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Descending group</td>
<td>384</td>
<td>12</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>Echinometra mathaei</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable group</td>
<td>288</td>
<td>8</td>
<td>38</td>
<td>45</td>
</tr>
<tr>
<td>Ascending group</td>
<td>1056</td>
<td>25</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Descending group</td>
<td>408</td>
<td>24</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Among the four test animals the intertidal gastropod *T. fusconigra* was the least tolerant to Urea. However for sodium nitrate, *T. fusconigra* was the most tolerant while other species were almost equally tolerant to it. In general, results show that the resistance of the organisms decreased with increase of experimental time.

**CONCLUSION**

Static and dynamic bioassay results cannot be considered to be perfect. They are affected by many factors like size, age, photoperiod, exposure to visual stress, temperature, acclimatization, water chemistry, pH, dissolved oxygen and many other factors. Consequently the LC50 values obtained for different bioassay tests or for groups of the same species of the test organism will not give identical results. Furthermore LC50 values cannot be considered safe for very long exposures, because continued action of less toxic or even non-toxic materials may eliminate aquatic species indirectly as is evidenced from results of the tests with the mixture of crude oil and Enjay (Table 2). The objective of marine bioassays usually is to determine a pollutant's concentration harmful to organisms, its persistence and degradability, rate of accumulation and loss in organism consumed by others and mode of action on organisms (Coppage, 1976). The data obtained through bioassays are only general guidelines to establish water quality standards.

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