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"Research Note"

# TOXICITY OF WATER SOLUBLE FRACTIONS OF CRUDE OIL TO FISH, *LUTJANUS ARGENTIMACULATUS* AND SHRIMP, *PENAEUS MONODON*[*]

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**Abstract –** The acute toxicity of a Malaysian crude oil to red snapper, *Lutjanus argentimaculatus*, black tiger shrimp and *Penaeus monodon* were investigated. The animals were exposed to water soluble fractions (WSF) of crude oil in a flow-through bioassay system and the toxicants were analyzed by gas chromatography (GC) and gas chromatography and mass spectrometer (GC-MS). The 96 h $LC_{50}$ values of the WSF of crude oil for fish and shrimp were 3.24 ± 0.21 and 8.52 ± 0.89 ppm of WSF of crude oil, respectively. In this investigation, the fish were more sensitive to crude oil than the shrimp, with respect to the similarity in their habitations.

**Keywords –** Crude oil, acute toxicity, fish, shrimp

## 1. INTRODUCTION

Determination of median lethal concentration ($LC_{50}$) is one of the basic tests which provides a better understanding regarding the sensitivity of animals to crude oils. The $LC_{50}$ values of water soluble fractions (WSF) of different crude oils reported previously by Anderson [1] for three species of shrimp and three species of fish, and Neff and Anderson [2] on several life stages of four species of marine shrimp, appeared to be different from each other.

Under tropical (23.5$^o$ south and 23.5$^o$ north) conditions, the $LC_{50}$ values of a Malaysian crude oil to *P. monodon* postlarvae, which have been estimated by Lai and Kessler [3] and Law [4] were different. The differences also exist in estimation of $LC_{50}$ values of a Malaysian crude oil to *P. monodon* postlarvae and seabass, *Lates calcarifer*, fry [3].

The parameters which affect the $LC_{50}$ values may be due to variety in species, age and habitat, duration of exposure, bioassay systems, type of crude oil used in the test, and the analytical methods applied in the assay of the exposure crude oil [1, 5]. This study, designed to get a better understanding of acute toxicity of crude oil to a fish and a shrimp under tropical conditions, based on using the most reliable analytical equipment (ie. GC and GC-MS) and flow-through exposure system.

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## 2. MATERIALS AND METHODS

As an important commercial species in the Indo-Pacific region [6], red snapper, *L. argentimaculatus* (198 ± 13 g) was selected as the test fish. *P. monodon* (5.8±0.84 g) was selected as the test shrimp species. It is widespread in the Indo-West Pacific from Pakistan to Japan, southward to Indonesia and North Australia, East and Southeast Africa [7].

The animals were acclimatized separately for two weeks in flow-through, aerated, filtered (five microns) seawater systems. The fish were fed (3% body weight) once daily with trash fish. The test shrimp were fed with commercial shrimp pellets at 4% body weight three times per day. The feedings were ceased two days in fish tanks and one day in shrimp tanks before they were transferred to the test tanks.

The test oil, a Malaysian crude oil, was used in a flow-through bioassay system which is described by Akbari et al. (8) to conduct the 96 hr lethal toxicity. Five one ton concrete and five 100 l fibreglass tanks were used for the acute toxicity test of fish and shrimp, respectively.

The flow rates of treated and fresh seawater to the experimental tanks were adjusted by using PVC laboratory valves at 3.2 l for each gram of body weight of the animals per day. On the basis of preliminary toxicity studies, five dilutions of WSF of crude oil treated seawater (80%, 60%, 40%, 20% and 10%) and a control (0%) were used to determine the $LC_{50}$ values during a 96 hr period. The experiments had three replicates of five concentrations and an equal number of control tanks. Analysis of hydrocarbons, which consisted of both aromatic (mono, di and three rings) and aliphatic (C10 - C28) components, were performed in different exposure tanks by GC and GC-MS. Water quality parameters (i.e. temperature, dissolved oxygen, pH, ammonia-N and salinity) were checked daily to be in an acceptable range.

To determine the 96 hr $LC_{50}$ values, 10 animals of each species were randomly distributed to five different concentrations of the toxicant. The $LC_{50}$ values, based on WSF of used crude oil to red snapper and black tiger shrimp, were estimated for 12, 24, 36, 48, 60, 72, 84, and 96 hours, using the trimmed Spearman-Karber method as a widely used means to calculate $LC_{50}$ values [9,10]. A regression analysis was preformed to estimate the changes in the slope function of concentration-mortality curves during the acute toxicity test in both animals.

## 3. RESULTS

The analysis of WSF of crude oil prepared from exposure tanks of fish and shrimp are given in Table 1. The $LC_{50}$ values of crude oil to *L. argentimaculatus* based on WSF of crude oil were estimated as 7.16, 6.18, 5.08, 4.59, 4.21, 3.88, 3.53 and 3.24 ppm for 12, 24, 36, 48, 60, 72, 84, and 96 hr. exposure, respectively. The $LC_{50}$ values of the same parameter of crude oil and the same duration of experiment to *P. monodon* were estimated as 16.23, 13.7, 11.88, 10.87, 10.22, 9.5, 8.99, and 8.52. The mean percentage of mortality in fish and shrimp tanks during a 96 hr period are given in Tables 2 and 3, respectively.

Table 1. Concentrations* of water soluble fractions (WSF) in the exposed water, used for estimation of $LC_{50}$ values for *L. argentimaculatus* and *P. monodon.* Concentrations are in mg/l

| Test tanks | WSF of crude oil in fish tanks | WSF of crude oil in shrimp tanks |
|---|---|---|
| C1 | 0 | 0 |
| C2 | 0.572 | 2.664 |
| C3 | 1.048 | 4.690 |
| C4 | 2.037 | 7.848 |
| C5 | 4.451 | 12.622 |
| C6 | 6.389 | 20.166 |

*Each datum represents the mean of three replicates

Table 2. The mortality data of *L. argentimaculatus* exposed to water soluble fraction of crude oil during a 96 hr period

| Exposure con. (ppm) | The mean* of percentage mortality in the fish tanks at 12-96 hr exposure time | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5720 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.6 |
| 1.0476 | 0 | 0 | 0 | 1.6 | 5 | 8 | 10 | 12.3 |
| 2.0372 | 1.6 | 5 | 10 | 10 | 12 | 16 | 22 | 25 |
| 4.4512 | 16.6 | 20 | 28 | 33 | 40 | 43 | 50 | 57 |
| 9.1856 | 68 | 83 | 96 | 100 | 100 | 100 | 100 | 100 |

Note: *= The data are the mean of six replicates

Table 3. The mortality data of *P. monodon* exposed to water soluble fraction of crude oil during 96 hrs

| Exposure con. (ppm) | The mean# of percentage mortality in the shrimp tanks at 12-96 hr exposure time | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.664 | 0 | 0 | 0 | 0 | 0 | 1.1 | 3 | 3.3 |
| 4.6898 | 0 | 0 | 1.1 | 5.5 | 7.7 | 13 | 17 | 17.7 |
| 7.8476 | 13.3 | 17 | 27 | 27 | 30 | 35 | 42 | 42.2 |
| 12.6224 | 31.1 | 34 | 40 | 46 | 50 | 55 | 66 | 68.8 |
| 20.166 | 65 | 85 | 96 | 100 | 100 | 100 | 100 | 100 |

Note: #= The data are the mean of nine replicates

The estimated $LC_{50}$ values of WSF of crude oil to *L. argentimaculatus* and *P. monodon* were plotted separately against their exposure times to obtain the standard $LC_{50}$ curves (Figs. 1 and 2) for both animals. Regression analysis on the percentage mortalities at 12, 48, and 96 hrs showed regular augmentation changes in the slope functions for both test animals. There were differences between the slope functions (Table 4) of the concentration-mortality equations of the fish, with respect to the shrimp at each exposure time. There was no mortality, or any other change for the fish and shrimp in the control tanks within the 96 hr $LC_{50}$ tests.

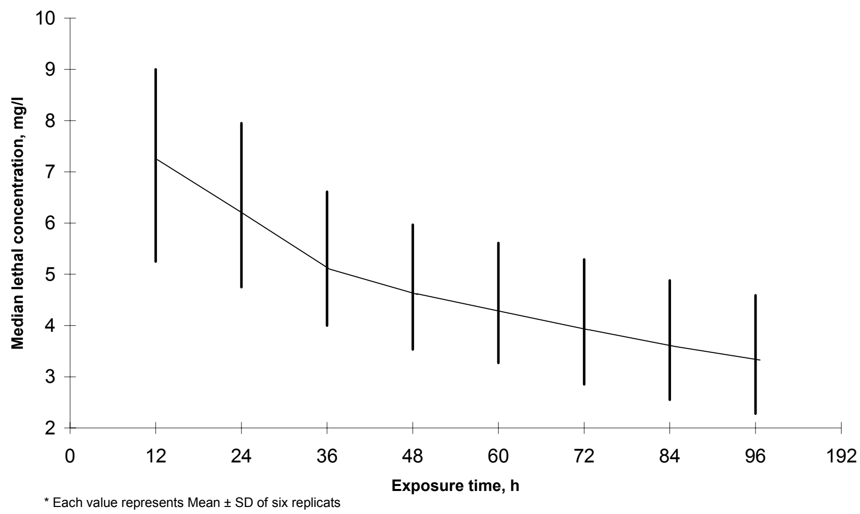


Fig. 1. Standard $LC_{50}$ curve of water soluble fraction of crude oil* for *L. argentimaculatus* during 96 hr

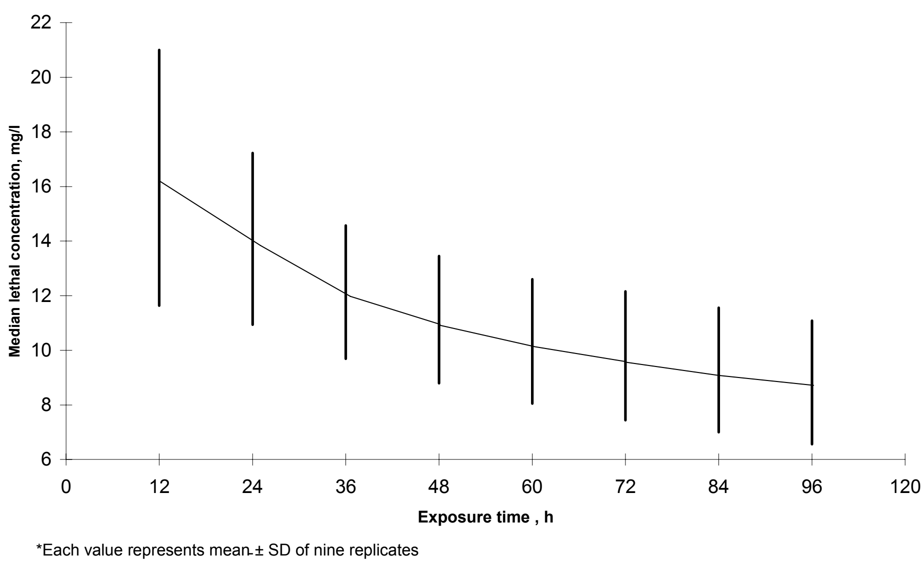


Fig. 2. Standard $LC_{50}$ curve of water soluble fraction of crude oil* for *P. monodon* during 96 hr

Table 4. Slop function of the concentration-mortality of *L.argentimaculatus* and *P. monodon* prepared from regression analysis

| Exposure time (hr) | *L. argentimaculatus* | *P. monodon* |
|---|---|---|
| 12 | 7.56977 | 3.44552 |
| 48 | 11.2006 | 5.16896 |
| 96 | 11.2293 | 5.32268 |

## 4. DISCUSSION

The results of the present study showed that the $LC_{50}$ values of WSF of crude oil exposed to red snapper and black tiger shrimp were different from the values reported by other research [1-4]. Lai and Kessler [3] used a static condition to test the acute toxicity of the same crude oil which is used in this study for black tiger shrimp postlarvae and seabass fry under tropical conditions. They revealed that the acute toxicity (96 hr $LC_{50}$) of WSF of crude oil to black tiger shrimp and seabass were 20.3 and 23.1 ppm, respectively. They scanned their water samples using an IR spectrophotometer. Under tropical conditions, Law [4] also estimated the 96 hr $LC_{50}$ value of WSF of the same crude oil used in this study, but running the experiment in a flow-through system. He estimated the 96 hr $LC_{50}$ for postlarvae of black tiger shrimp at 13.79 ppm. Out of the Law [4] report on $LC_{50}$ value of WSF of crude oil, other reports on estimation of lethal doses of subjected crude oil for aquatic animals under tropical conditions have been based on the use of a static exposure system. Generally, by the use of a flow-through system, it yields the best and most accurate estimate of toxicity [11].

Based on the results of the experiment estimated by Neff et al. [12] and Hyland and Schneider [13], Neff and Anderson [2] found that the relative sensitivity of an animal to oil based on its phylogenic position or its habitat cannot predicted. Citing the Rice et al. [14] work, Bishop [15] revealed that sensitivity to crude oil generally increases from invertebrates to fish, but is actually better correlated with habitat. Thus, Bishop [15] rejected the previous conclusion of Neff and Anderson [2] due to disability in generalizing toxicity of oil with respect to phylogeny or habitat of the animals.

In the case of sensitivity of species to crude oil by the use of a different exposure system, Vanderhorst et al. [11] proposed that marine animals are more sensitive to crude oil in a flow-through or dispersed oil system than a static system. However, crude oil is an extremely complex mixture of different compounds ranging from simple low molecular weight to very complex polynuclear aromatic hydrocarbons containing numerous isomers [16, 19]. According to Neff and Anderson [2], the values of WSF obtained by the IR spectrophotometer are lower for crude oil and higher for refined oil products compared to the values obtained by GC.

The results of the present study showed differences between the 96 hr $LC_{50}$ values for *L. argentimaculatus* (3.24 ± 0.21 ppm) and for *P. monodon* (8.52 ± 0.89). This pattern of sensitivity between invertebrate and fish follows the Bishop [15] principle which was previously discussed. It seems that the differences in $LC_{50}$ values of red snapper as compared to other species [1] in the temperate regions are based on different parameters which can affect the toxicity results, and does not necessarily depend only on temperature. The parameters which affect the $LC_{50}$ values my be due to variety in species, age and habitat, duration of exposure, bioassay system, type of crude oil used in the test, and the analytical methods applied in the assay of the exposure crude oil [1, 5]. However, there is no information available on the acute toxicity of crude oil to red snapper. Sprague [17], who reviewed the effect of temperature on the sensitivity of various aquatic animals to toxicants, could not find any evidence to prove this point.

There are differences between the result of the present study on 96 hr $LC_{50}$ value for black tiger shrimp and those which have been estimated by Lai and Kessler [3] and Law [4], although they used the same crude oil and/or species. These differences may have arisen because of the different life stages of the shrimp used in this experiment (juvenile), and those which are used by the other researchers (postlarvae). The deferential sensitivity to crude oil between the life stages of shrimp species which was previously discussed, has been reported by Hyland and Schneider [13]. Another reason for the differences in the results obtained in the present study when compared to the results obtained by Lai and Kessler [3] and Law [4], could be due to the analytical equipment used for analysis of WSF of crude oil in the exposure water. In the present study, GC and GC-MS were used as analytical equipment compared to the two previous researchers who used the spectrophotometric method. All these factors have contributed to the variability found in the literature.

Comparison between the slope function of percentage mortalities in fish and shrimp at 12, 48 and 96 hr exposure shows that the regression lines of the shrimp are flatter than those for fish. According to Rand and Petrocelli [5] and Rand et al. [18], these differences indicate that shrimp absorb the toxic components of crude oil slower than fish. In addition the effect of toxification in shrimp is slower than that of fish. This could also indicate the higher ability of shrimp to accumulate more toxic components than fish, which has been described by Akbari *et al*. (not published data).

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