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# Comparative toxicity of water-soluble fractions of three oils in the Marine clam, *Donax faba*, Gulf of Mannar

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**Introduction:** Oil in the marine environment affects organisms on all systematic levels; microscopic plankton, invertebrates such as crustaceans, mollusks and benthic worm and vertebrates. Oil consists of a wide variety of compounds that are toxic to organisms, the worst being the Polyaromatic Hydrocarbons (PAHs). Toxicity of oils in marine organisms can be determined by acute toxicity tests. The parameters of short-term mortality have been the most common measure of toxicity. The introduction of more than one substance at a time into environment causes combined effects, which are qualitatively and quantitatively different from that of the effects of individual substances. Therefore, it is important to evaluate the interaction between two or more substances in a mixture, the consequences of their toxicity and their mode of action in combination. While making studies on toxicant mixtures (crude oil and its by product) it is essential to know whether the substances influence each other in what ways and with what effects they influence each other as well as the extent of the effects. The present study is aimed to investigate the acute toxicological effects of the water-soluble fractions(WSF) of oils in the marine bivalve, *Donax faba*. It also seeks to provide some toxicological data on products of crude oil in commercial use.

**Materials and Methods:**

The bivalves of the family Donacidae: *Donax faba* were collected from the intertidal sandy beaches of Dhargavalasai coast, Gulf of Mannar. The collected *Donax sp* was transported to the laboratory fiber tanks. Undamaged individuals were acclimatized to laboratory condition for five days in natural seawater, before use in the bioassay test. Mixed phytoplankton culture was given as feed to the test animal. The shell length, of the test animal was measured using Vernire caliper. The field parameter such as salinity, pH and Dissolved oxygen was noted and the same was settled in the acclimization tank. Aeration was provided by gently bubbling air through disposable Pasteur pipettes connected via plastic tubes to air pumps.

**Test solution oils and Preparation of water-soluble fractions:**

The test solution Diesel oil, Kerosene and Petrol were purchased from commercial grade market**.** Water-soluble fractions (WSF) were prepared by adding one part of oil to nine parts of filtered, autoclaved seawater (1:9 ratio) in a volumetric flask. The flask was tightly capped with a stopper and covered with aluminum foil so, as to reduce evaporation of volatile petroleum hydrocarbons. Mixing was done by stirring at a slow speed with magnetic stirrer for 20hrs at room temperature. After mixing, oil and water phases were allowed to separate for 8hrs in a separating funnel and then the aqueous phase was drained out. Since the concentration of WSF decreases with time, fresh solutions were prepared for every set of experiments. This preparation was treated as 100% stock solution. The toxicity of three oil to the *Donax sp* is investigated, similarly the toxicity of combined oil is also done, were 1:1 water soluble fractions of Diesel and Kerosene, Petrol and Kerosene, these are prepared in equal percentage ratio. Different percentages of WSFs were prepared from the stock solution. For all the oil combinations, test solution was prepared starting with lower percentage such as 0.01, 0.1, 1, 10 and 100 % (wide range). A preliminary range finding experiment was conducted for 24, 48, 72 and 96 hrs. Based on the wide range $LC_{50}$ value, definitive range percentage concentration obtained and $LC_{50}$ was calculated using Probit analysis software (Finney, 1971). For each series of percentage concentrations, control was maintained in which WSF was not added. Duplicate test was done. For each experiment 20 individuals of the desired shell length were placed in each test chamber.

**Petroleum Hydrocarbon estimation in WSF:**

For the estimation of petroleum hydrocarbons, 150 ml of WSF was extracted with 25ml doubled distilled hexane, the hexane fraction was separated using a separating funnel and collected in stopper test tubes. The hexane extract was then dried using anhydrous sodium sulfate, which was cleaned earlier with hexane and oven dried. The final volume of hexane extract was adjusted to 25ml and analyzed in Fluorescence spectrophotometer. Double distilled hexane was used as blank and test oil was used as standards. Fluorescence spectrophotometer has been used to quantify aromatic hydrocarbons both through the examination of extracts and through direct water sample examination. It has the potential to rapidly differentiate 2 and 3 ringed aromatic structures from compounds with a greater

number of aromatic rings. Fluorescence emission of the blank, sample and standard were measured at 360nm, when excitation was at 310nm (Gordon *et al.*1974). The concentration of the hydrocarbons in WSF, corrected for blanks, was expressed in terms of standards. All the extractions were done in duplicate.

**Results**

**Petroleum Hydrocarbons of water-soluble fractions of stock solution:**

The petroleum hydrocarbon concentrations were analyzed in each 100% stock solution, using spectrofluorometer. During sample analysis, the PHs value in the solution showed over range concentration in Fluorescence spectroflurometer. When the concentration exceeds µg it will show over range concentration. Then the samples are diluted to 10ml. 10% sample was prepared by mixing 9ml of Hexane and 1ml of 100% stock solution. Each water-soluble fractions of stock solution was analysed. In kerosene, PHs concentration was 196µg/l; petrol-384.4µg/l; diesel oil and kerosene- 442.2µg/l; petrol and kerosene- 721.6µg/l; diesel oil and petrol-1118.7µg/l and combined three oils- 434.8µg/l respectively. As the concentrations are obtained from dilution, percentage (%) range was selected. Based on percentage value Probit analysis was performed and the toxicity of WSF of oils of individual was obtained.

The physico chemical parameter at the initials of the test was noted. In order to avoid the stress to the animals in the experiment due to various physico chemical parameters, same parameters of seawater were maintained in all the experiments including duplicate test. The average length of the test animal-*Donax faba* (Adult) was $2 \pm 0.5$ cm; width $1.3 \pm 0.2$ cm; Weight of the animal with shell-1.96; without shell- $0.76 \pm 0.2$ gm. The Salinity of the test seawater medium was 34 $\%_0$; pH -7.5; and Temperature – $26^oC$.

In each control and five toxicant added test chambers, 20 animals were introduced. A total of 240 test animals were subjected to the experiment including duplicate test. The results of wide range concentration experiment $LC_{50}$ value for Diesel oil- 2.758%; Kerosene-29.793%; Petrol-8.151%; Diesel oil and Kerosene-3.185%; Petrol and Kerosene-4.415%; Diesel oil and Petrol- 1.417%; Diesel oil, Kerosene and Petrol- 0.579. The test animals were also exposed to 500 and 1000%, but the animal showed immediate response, mortality was observed at the $4^{th}$ hour itself. Based on the Wide range result definitive test was done.

The definitive concentration for Diesel oil was 0.6895, 1.379, 2.758, 5.516 and 11.032% respectively. Similarly for kerosene - 3.724, 7.448, 14.90, 29.793, and 185.586%; Petrol – 2.038, 4.076, 8.151, 16.302 and 32.604%; Diesel oil and kerosene combined concentration was 0.796, 1.592, 3.185, 6.37 and 12.74%; Petrol and Diesel oil combined was 1.104, 2.208, 4.415, 8.83, and 17.66%; Diesel oil and Petrol – 0.354, 0.7085, 1.417, 2.834 and 5.668%. All combined oil was 0.145, 0.290, 0.579, 1.158 and 2.316%.

At the beginning of the 24$^{th}$ hour itself the animal started to respond to the test medium containing WSF of Diesel oil. During the duration of 96hrs vast mortality of the test animal was noted. The dead animals were removed then and there. The test animals in the test medium containing WSF of Kerosene in the concentrations 3.724, 7.448, 14.90, 29.793 and 185.586% showed slow mortality response in the initial day, Steady mortality was observed at the end of the 96$^{th}$ hour. The animals showed moderate mortality response in WSF of Petrol, when compared to the animals exposed to the Diesel oil concentrations.

In the concentrations 0.796, 1.592, 3.185, 6.37 and 12.74% of Diesel oil and kerosene showed that the test animals were resistant in this combination, when compared to Petrol and kerosene combination. Very sensitive response was observed in all the three-combined toxicant. The mean lethal concentration for the water soluble fractions of Diesel oil, Kerosene, Petrol, Diesel oil and Kerosene, Petrol and Kerosene, Diesel oil and Petrol and Diesel oil, Kerosene and Petrol are given in Table-1.

**Table -1. Mean $LC_{50}$ Values of *D.faba* exposure to water-soluble fractions of test oils for the 96$^{th}$ hr.**

| Water soluble fractions of test oils | Mean of $LC_{50}$ values in % |
|---|---|
| Diesel oil | 3.916 |
| Kerosene | 13.461 |
| Petrol | 7.837 |
| Diesel oil + Kerosene | 4.631 |
| Petrol + Kerosene | 4.389 |
| Diesel oil + Petrol | 0.848 |
| Diesel oil + Kerosene + Petrol | 0.391 |

**Discussion**

The test animals when exposed in water-soluble fractions of Diesel oil showed mean $LC_{50}$ value 3.916%, but when in combination with kerosene the $LC_{50}$ value was 4.631%. This clearly showed that Kerosene has reduced the toxic effects of Diesel oil. Antagonism effect has occurred. When the second one reduces the effect of one substance is known as antagonism. The individual toxicity of petrol was 7.837%, whereas its combination with kerosene showed synergism. When one substance increases the effect of another one in a mixture of toxicants is referred to as synergism. In all the three-combined toxic concentration, the lethal concentration was 0.391%. The toxic effect was more in all the three-oil combination. The study result also reveals that kerosene acts as a catalyst. It has the capacity to induce and reduce toxicity of other oils in combinations, but in pure exposure of kerosene showed not that much effect. The Correlation of the toxicity of the $LC_{50}$ values of the $96^{th}$ hour showed $\delta = 0.31831$, low degree of correlation. Same toxicological studies have been carried out by Tatem., *et al.* (1978) and Kijhmhold (1980) did the study of toxicity of water soluble fractions of diesel fuel to *O.niloticus* and his findings showed mortality even at low concentrations.

Ahssanullah, (1982) studied the acute toxicity to *Paragrapsus quadridentatus*, of Kuwait light crude oil, BP/AR dispersant and an oil-dispersant mixture was determined. Observed 96-h $LC_{50}$ values average 1555mg $l^{-1}$ for oil added to water. A Statistically valid 96-h $LC_{50}$ value for the dispersant was not obtained but results indicated that a solution containing between 1300 and 22000mg $l^{-1}$ might be expected to produce 50% mortality. A mixture oil and dispersant in the ratio 4:1 gave an observed 96-h $LC_{50}$ value of 96 mg $l^{-1}$, a 16 fold increase in toxicity over oil alone.

During the period of experiment, the test animals exposed to the toxicant showed abnormal behavior. Their siphon and foot movement showed sluggish movement. Some times the animal's shell remained closed due to the irritation of the water-soluble fractions. Johnson, (1977) observed similar response. High concentrations of oil can cause shell closure and narcotisation of ciliary surfaces in bivalves and consequently affects respiration and feeding rates negatively (Johnson. 1977, Bayne *et al.* 1982 and Mageau *et al*. 1987). At low concentration of oil, rates of oxygen consumption are first increased in bivalves, such as the soft-shelled clam (*Mya arenaria),* blue mussel (*Mytilus edulis),* Baltic telling (*Macoma balthica*) and the gastropod (*Littorina littorea*) commonly called as edible periwinkle. Widdows.,

*et al* (1982), Bayne., *et al*. (1982), pointed out that metabolic rates increase due to hydrocarbon association in the body tissues and mucus secretion and excretion increases. As a result energy expenditure increases, while less energy (reduced carbon flux) is available for growth and reproduction.

According to Connell and Miller (1984), toxic effects of Petroleum products are usually due to aromatic compounds. Ballow., *et al.* (1987) found that organisms growing at the intertidal zone are the most affected during oil spills. Stekoll, (1980) stated that bivalves such as clams, mussels and Oysters are indicators of pollution. They provide integrated information about the bioavailability and effects of oil that cannot be determined solely through the chemical analysis of discrete water samples.

A number of toxicity studies have been carried out on exposure of oil to eggs and larvae of bivalve mollusks, Particularly clams, mussels and Oyster (Byrne and Calder, 1977). Wells, (1982) reported that dispersed oil, crude oil and water-soluble fractions have strong effects on fertilization, but embryonic development and larval survival are generally affected only at nominal concentrations exceeding 10mg $L^{-1}$.

**Conclusion:**

Petroleum hydrocarbons concentration varies depending upon the crude oil by products. So, the study reveals that the toxicity of the by products of the crude oil varies. Of the findings revealed that raw crude oil is more toxic when compared to its byproducts. Kerosene is less toxic but its combination with other oils has the effect of inducing or reducing the toxic effect of other oil.

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