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# Effect of Hydrogen Sulphide on Two Species of Penaeid Prawns *Penaeus indicus* (H. Milne Edwards) and *Metapenaeus dobsoni* (Miers)

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Hydrogen sulphide produced by the decomposition of organic effluents from industries and by the anaerobic decomposition of organic materials is found in the sediments of the aquatic environment. The anoxic condition caused by the presence of $H_2S$ is harmful to aquatic organisms. Shigueno (1972) has studied the toxic effect of $H_2S$ to Penaeus japonicus whereas Chen (1985) and Law (1988) have worked on the safe level of $H_2S$ for Penaeus monodon culture. Oseid and Smith (1974) and Boyd (1989) have stated that the toxicity of H S varies with pH due to the presence of un-ionized hydrogen sulphide which is more toxic to the aquatic organisms. In this paper, variation in the toxicity of H S to two species of shrimps, Penaeus indicus (H. Milne Edwards) and Metapenaeus dobsoni (Miers) at three different pH ranges (6.0 to 6.3, 7.0 to 7.3 and 8.1 to 8.3) is reported.

## MATERIALS AND METHODS

Penaeus indicus of the size ranges 20-25 mm, 35-40 mm and 85-90 mm and M. dobsoni of 20-25 mm and 35-40 mm were exposed to different concentrations of H S for 96-hr $LC_{50}$ experiments in seawater. The seawater had a salinity of 32/33 ppt and temperature 28/29°C. These test animals, which were in the intermoult stages were collected from the Kayamkulam estuary and maintained in separate acclimating tanks for 2 wk. To study the effect of pH on hydrogen sulphide toxicity, both species of shrimps in the size ranges of 20-25 mm and 35-40 mm were exposed to different concentrations of H S at three pH ranges, 6.0 to 6.3, 7.0 to 7.3 and 8.1 to 8.3. The experiments were conducted in a flow-through apparatus similar to the one described by Adelman and Smith (1970). The $H_2S$ stock solution was prepared by dissolving a known quantity of analar grade $Na_2S$, $9H_20$ in 1 L of oxygen - free distilled water. Hydrogen sulphide in the water was estimated calorimetrically (FAO 1975). At higher concentrations of sulphides, the samples were

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diluted with oxygen - free distilled water before measuring optical density. Un-ionized hydrogen sulphide was calculated based on pH. The concentration of $H_2S$ in the water flowing out of the test chamber was monitored every hour and the desired $H_2S$ level was maintained by adjusting the flow of the stock solution through the regulator.

Experiments with each $H_2S$ concentration were repeated twice for every size group. Ten specimens were used for each experiment. Every experimental run was accompanied by a control run in which the same number of shrimps of the same size were kept, through which $H_2S$ - free seawater was made to flow through. The behavior of the shrimps in the animal chamber was closely observed throughout the experimental period (96 hr).

**RESULTS AND DISCUSSION**

The $H_2S$ toxicity bioassays conducted with different size groups of *P. indicus* and *M. dobsoni* in sea water have shown that the 96-hr $LC_{50}$ declined with increase in size of the shrimps. The calculated 96-hr $LC_{50}$ values with 95% confidence limit for different size groups of *P. indicuus* and *M. dobsoni* exposed to different concentrations of hydrogen sulphide is given in Table 1. In the shrimp, *P. indicus*, the 85-90 mm size group was highly sensitive, followed by 35-40 mm and 20-25 mm group. The 96-hr $LC_{50}$ values show that the sensitivity of large size animals (85-90 mm) of this species to $H_2S$ is greater than that of the small size (20-25 mm) animals. Similarly for *M. dobsoni* the $LC_{50}$ between 35-40 mm and 20-25 mm size groups showed variation. For the former size group, $LC_{50}$ was 0.340 mg/L while for the latter the $LC_{50}$ was 0.378 mg/L.

The sensitivity to $H_2S$ also varies between species. *P. indicus* was more sensitive to $H_2S$ than *M. dobsoni*. It is also evident that smaller size groups of shrimps are more tolerant to H S toxicity than larger groups. In this context, the work of Shigueno (1972) is worth mentioning. He reported that adult *Penaeus japonicus* lost equilibrium when exposed to hydrogen sulphide at 0.1-2.0 ppm and instantly succumbed to a concentration of 4.0 ppm, which seems to indicate that the adult penaeids are in fact more sensitive to H S than their juveniles and post-larvae, as observed in the present study.

The toxicity of $H_2S$ is highly influenced by the pH of the medium. Because of the presence of greater proportion of un-ionized $H_2S$ at low pH, the toxicity is higher. The 96-hr $LC_{50}$ value for two groups, 35-40 mm and 20-25 mm of *P. indicus* and *M. dobsoni*, exposed to different concentrations of $H_2S$ at three pH ranges (8.1 to 8.3, 7.0 to 7.3 and 6.0 to 6.3) is given in Table 1.

The reduction in $LC_{50}$ at lower pH levels recorded during the present experiments is in agreement with the results obtained by earlier workers (Longwell and Pentelow 1935: Jacques 1936; Jones

1948; Bonn and Follis 1967; Colby and Smith 1967; Groenendal 1980, 1981) who attributed the greater toxicity of sulphides at lower pH to the fact that total hydrogen sulphide (dissolved sulphide) at low pH levels exists mainly as un-ionized $H_2S$ which is more toxic to animals than the ionized forms; $HS^-$ and $S^-$. These ionized forms can penetrate cell membranes only with difficulty because of their electric charge, while, un-ionized $H_2S$ can move freely across the membranes (Jacques 1936). At pH 9 only 1% exists as un-ionized $H_2S$ while at pH5, 99% is present as un-ionized $H_2S$(Smith and Oseid 1974).

In the first set of experiments, the sea water used had a pH of 8.1 to 8.3, at which almost 25% $H_2S$ was in the un-ionized form. Thus the 96-hr $LC_{50}$ determined for the 20-25 mm, 35-40 mm and 85-90 mm size groups of P. indicus if expressed with reference to un-ionized $H_2S$ will be approximately 0.085 mg/L, 0.070 and 0.032 mg/L. Similarly for 20-25 mm and 35-40 mm size groups of M. dobsoni the concentration of un-ionized $H_2S$ will be 0.094 and 0.085 mg/L. In the experiment of pH 6.0 to 6.3 about 65% $H_2S$ was in the un-ionized form. Thus 96-hr $LC_{50}$ for post-larvae (20-25 mm) and juveniles (35-50 mm) of P. indicus, if expressed in terms of un-ionized $H_2S$ will be approximately 0.076 mg/L and 0.041 mg/L, whereas for the same size group of M. dobsoni the un-ionized $H_2S$ will be 0.081 mg/L (20-25 mm post-larvae) and 0.077 mg/L (35-40 mm juveniles).

It is clear that there are comparabilities between the overall lethal toxicity of different size groups with respect to un-ionized $H_2S$ and that the low pH increases the toxicity of $H_2S$ to shrimps. But even at low pH, the smaller size groups are more resistant than the bigger size groups. There is also prominent variation in the tolerance levels between the two species of penaeid shrimps. At all pH levels tried in the present experiments, P. indicus was found to be more sensitive to $H_2S$ than M. dobsoni. Colby and Smith (1967) and Adelman and Smith(1970) found that the toxicity of sulphide increased in fishes (Salmon) with the lowering of oxygen levels from 6 ppm to 2 ppm. Because of the oxidation reaction of hydrogen sulphide, the dissolved oxygen level goes down and causes oxygen depletion. In the present study for all the experiments, the dissolved oxygen level in the test water was maintained at 2.5 to 3 mL/L. P. indicus and M. dobsoni being oxyconformers, the dissolved oxygen level is not lethal (Kuttyamma 1980). Hence in this context the influence of low dissolved oxygen on the toxicity cannot be considered, as the amount of dissolved oxygen present was within the safe level.

Oseid and Smith (1974), estimated 96hr $LC_{50}$ of hydrogen sulphide for Gammarus pseudolimnaeus as 0.059 mg/L which is higher than the $LC_{50}$ observed for shrimps. On the other hand the $LC_{50}$ for penaeid shrimps was lower than that of the $LC_{50}$ for isopod Assellus militaris which was calculated as 1.07 mg/L. They also determined the 96-hr $LC_{50}$ of amphipod Crangony richmondensis laurentianus to $H_2S$ (0.089 mg/L) which showed a higher value than that of the

Table 1. 96-hr $LC_{50}$ values with 95% confidence limit in mg/L for different size groups of *P. indicus* and *M. dobsoni* exposed to different concentrations of hydrogen sulphide at three different pH ranges.

| pH range | Size range (mm) | *P. indicus* $LC_{50}$ (mg/L) | 95% confidence limit | *M. dobsoni* $LC_{50}$ (mg/L) | 95% confidence limit |
|---|---|---|---|---|---|
| 8.1 - 8.3 | 85 - 90 | 0.144 | 0.128 - 0.163 | - | - |
| | 35 - 40 | 0.281 | 0.248 - 0.320 | 0.340 | 0.306 - 0.378 |
| | 20 - 25 | 0.342 | 0.305 - 0.383 | 0.378 | 0.346 - 0.413 |
| 7.0 - 7.3 | 35 - 40 | 0.119 | 0.099 - 0.142 | 0.147 | 0.130 - 0.167 |
| | 20 - 25 | 0.189 | 0.152 - 0.235 | 0.219 | 0.179 - 0.268 |
| 6.0 - 6.3 | 35 - 40 | 0.063 | 0.054 - 0.073 | 0.077 | 0.063 - 0.054 |
| | 20 - 25 | 0.117 | 0.099 - 0.139 | 0.125 | 0.109 - 0.143 |

shrimps. Two species of Ephemeroptera, Ephemera simulans and Hexagenia limbata showed $H_2S$ toxicity similar to that of shrimps.

From these observations it is evident that the benthic groups are more tolerant to hydrogen sulphide toxicity than other non-benthic forms. Among the benthic groups like polychaetes, isopods, amphipods and decapods, the shrimps are less tolerant to $H_2S$.

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