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# Effects of Gramoxone on Survival and Activity of *Perna viridis* (Linn.)

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*Perna viridis* (L.) was exposed to lethal and sub-lethal concentrations of Gramoxone and effects on survival, ventilation rate and oxygen consumption were studied. Gramoxone becomes an acute lethal poison beyond a threshold limit. Exposure to sub-lethal levels of the toxicant for periods upto 12 day enhanced the rate of ventilation and depressed oxygen uptake.

**Key Words: Effects, Gramoxone, Bivalves, Respiration, Ventillation**

## Introduction

Among the pollutants which have received increased attention of ecologists, pesticides rank a very important position. Pesticides and technical organic chemicals released into the marine and fresh waters tend to accumulate and cause long-term effect on the ecosystem as a whole (Kinne 1984).

Pesticides were conceived and developed to kill pests and therefore must be detrimental to other forms of life also. Acute toxicity is probably the most useful method of testing the effects of pesticides on aquatic organisms. Studies on the acute toxicities of pesticides to marine organisms are many (Eisler 1969, 1970, Portmann & Wilson 1971, Hansen et al. 1974, Parrish et al. 1974, Roesijadi et al. 1976, D'Silva 1980, Jacob & Menon 1987). Negherbon (1959) and O'Brien (1966) demonstrated that organophosphate pesticides inhibit competitively and irreversibly the action of several enzymes, especially choline esterases, the chemical mediators of transmission between nerve and effector, resulting in parasympathetic, somatic motor nerve and central nervous system stimulation effects. Generally, the effects of pesticides on respiration and ventilation have received very little attention with reference to molluscs. Menon et al. (1983) proved that Heptachlor is highly poisonous to *Perna viridis* even at very low concentrations and that considerable quantities of mucus secreted as a result of exposure to Heptachlor was found to result in decreased gill irrigation. Reduction in oxygen consumption occurred when *Perna viridis* was subjected to sub-lethal exposure of Malathion. In the case of *Meretrix casta*, Malathion was found to act as a respiratory depressor. Malathion was found to decidedly influence the rate of filtration in *Perna viridis* and *Meretrix casta* (D'Silva 1980). Looking into the effect of organophosphates on the life and activity of the black clam *Villorita cyprinoides* var. *cochinensis*, Jacob and Menon (1987) found that the filtering mechanism of this animal was severly impaired with when exposed to Ekalux and Dimecron. Rita et al. (1987)

observed that biocides like Dimecron, Nuvan and Ekalux had a depressive effect upon the ciliary activity of *Villorita cyprinoides* var. *cochinensis*.

## Materials and Methods

Specimens of *Perna viridis* (shell length 25-30mm) were collected from an unpolluted area of the rocky shore near Narakkal and transported to the laboratory in large polyethylene drums of 50l capacity with sea water from the site of collection. Raw sea water used for the experiments was collected from an unpolluted area in the Arabian Sea off Cochin in polyethylene carbouys, kept for aging (3-4 days) in the dark and filtered by passing through a bio-filter before use. Prior to experimentation, the mussels were acclimatized to the laboratory conditions for 24 hr (temperature: $30 \pm 1°C$, Salinity: $32 \pm 1‰$, Oxygen: 4.8-5.2ml/l, *p*H: 8.3-8.4).

Gramoxone® is manufactured by IEL Ltd. and is a trade mark of the Imperial Chemical Industries Ltd., PLC, London. The commercial formulation contains 24% (w/w) of the active ingredient paraquat dichloride (1, 1'-dimethyl-4, 4'-bipyridilium dichloride) and is water soluble. Stock solution was prepared in double glass distilled water and added to get the desired pesticide concentration.

The method followed by Sprague (1969) was used to conduct the toxicity tests. The animals used for lethality studies were not fed during the period of experimentation. The $LC_{50}$ values and their 95% confidence limits were calculated using probit analysis (Finney 1971).

Sub-lethal toxicity was monitored utilising reactions and activity like ventilation rate and oxygen consumption. Selected specimens were subjected to pesticide insult (1.0-4.0 ppm) for periods upto 12 day. From this lot, batches of 8 individuals were transferred to clean sea water after 1, 4, 7 and 12 day and analysed for the rate of ventilation and oxygen consumption. 3 animals were used for the estimation of ventilation rate and 5 for oxygen uptake. The methodologies used have been described elsewhere (Abraham et al. 1986, Baby & Menon 1986). The animals were fed with the algae *Synechocystis salina* during the course of the experiments.

The data have been analysed using the students 't' test to assess whether there is any significant variation of the sub-lethal response registered in respect of the test animals.

## Results

Experiments were conducted in the laboratory to assess the lethal effects of Gramoxone® on *Perna viridis* by static bio-assay technique with daily replenishment of the medium. The results obtained are presented in figure 1a-d.

None of the animals exposed to 10ppm died within 96hr. Even in 20ppm, the rate of mortality was low. Mortality occurred rapidly after 24hr in concentrations exceeding 60ppm of Gramoxone. The calculated $LC_{50}$ values were $50.3 \pm 4.9$ppm for 48hr and $29.8 \pm 3.6$ for 96hr. The $ET_{50}$ values clearly indicate that increase in concentration had a significant effect on the rate of survival of the test species. (figure 1b). The toxicity curves, the steepness of which exemplify the effects of a pollutant on the rate of mortality of a test organism indicates that Gramoxone becomes on acute poison beyond a threshold limit (See figure 1c & d).

Results of experiment on ventilation rates of *Perna viridis* exposed to the sub-lethal levels of Gramoxone are presented in table 1. The rate of ventilation was similar to that of the control animals by *Perna viridis* exposed to 1.0 and 2.0ppm of Gramoxone for a day. Whereas those maintained under a stress of 4.0ppm of Gramoxone ventilated more

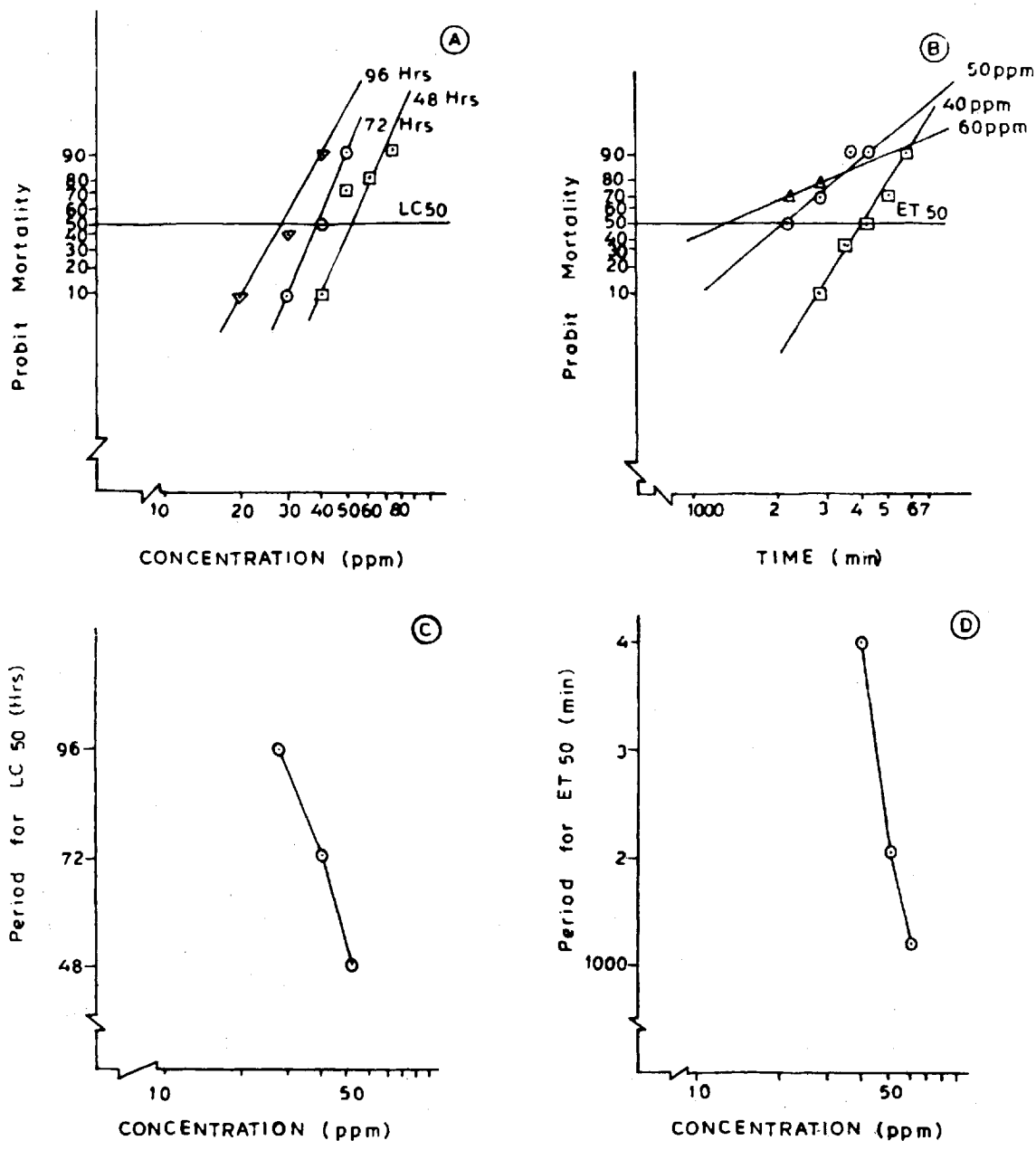


**Figure. 1** *Perna viridis*. Lethal effects of Gramoxone **a**, Progress of mortality against concentration; **b**, Progress of mortality against time; **c & d**, Toxicity curves.

**Table 1** ***Perna viridis:*** *Average ventilation rate (ml $hr^{-1}$ $gm^{-1}$ dry wt) under sub-lethal concentrations of Gramoxone for periods upto 12 days along with the respective standard deviations and percentage performance*

| | Gramoxone (ppm) | Ventilation Rate (ml $hr^{-1}g^{-1}$dry wt) Mean | SD | Percentage performance |
|---|---|---|---|---|
| *Day* 1 | 1.0 | 688.3 | 156.0 | 98.1 |
| | 2.0 | 700.2 | 227.1 | 99.8 |
| | 3.0 | 779.1 | 140.4 | 111.0 |
| | 4.0 | 624.2 | 157.2 | 89.0 |
| | Control | 701.9 | 187.1 | 100.0 |
| Day 4 | 1.0 | 440.3 | 155.2 | 74.7 |
| | 2.0 | 282.1* | 24.4 | 47.9 |
| | 3.0 | 385.6 | 17.9 | 65.4 |
| | 4.0 | 447.4 | 68.0 | 75.9 |
| | Control | 589.4 | 187.1 | 100.0 |
| Day 7 | 1.0 | 1040.1* | 219.2 | 150.1 |
| | 2.0 | 908.7 | 237.2 | 131.1 |
| | 3.0 | 773.7 | 189.7 | 111.6 |
| | 4.0 | 1190.2** | 165.2 | 171.7 |
| | Control | 693.1 | 168.1 | 100.0 |
| Day 12 | 1.0 | 800.2** | 67.2 | 155.9 |
| | 2.0 | 620.1 | 127.4 | 120.8 |
| | 3.0 | 513.4 | 45.2 | 100.0 |
| | 4.0 | 798.1** | 61.5 | 155.5 |
| | Control | 513.4 | 83.7 | 100.0 |

* $P<0.05$; ** $P<0.01$

**Table 2** ***Perna viridis:*** *Average oxygen consumption (mg $O_2hr^{-1}g^{-1}$ dry wt) under sub-lethal concentrations of Gramoxone for periods upto 12days, along with the respective standard deviations and percentage performance*

| | Gramoxone (ppm) | Oxygen Consumption (mg $O_2hr^{-1}g^{-1}$ dry wt) Mean | SD | Percentage performance |
|---|---|---|---|---|
| *Day* 1 | 1.0 | 973.1 | 184.2 | 72.6 |
| *Day* 1 | 1.0 | 973.1 | 184.2 | 72.6 |
| | 2.0 | 1323.4 | 163.5 | 98.7 |
| | 3.0 | 1387.2 | 241.3 | 103.5 |
| | 4.0 | 977.1 | 101.2 | 72.9 |
| | Control | 1340.2 | 231.0 | 100.0 |
| Day 2 | 1.0 | 1713.3 | 212.4 | 82.0 |
| | 2.0 | 1910.3 | 173.3 | 91.4 |
| | 3.0 | 1887.4 | 184.1 | 90.3 |
| | 4.0 | 1737.2 | 154.0 | 83.1 |
| | Control | 2090.7 | 218.1 | 100.0 |
| Day 7 | 1.0 | 1787.9 | 203.0 | 80.0 |
| | 2.0 | 1820.2 | 194.2 | 81.5 |
| | 3.0 | 1987.1 | 174.1 | 89.0 |
| | 4.0 | 623.1* | 203.0 | 72.7 |
| | Control | 2233.2 | 212.2 | 100.0 |
| Day 12 | 1.0 | 1663.3 | 172.1 | 74.6 |
| | 2.0 | 2116.7 | 290.2 | 94.9 |
| | 3.0 | 2763.1 | 184.8 | 123.9 |
| | 4.0 | 1937.2 | 202.7 | 86.9 |
| | Control | 2230.2 | 219.1 | 100.0 |

* $P<0.05$

water than their counterparts in lower concentrations. The general trend was unchanged even after 12 days of exposure. A conspicuous feature of the result was a declension in the rate of ventilation by those animals exposed to 2.0 and 3.0ppm of Gramoxone. The general trend can be described as follows. As a rule Gramoxone exposed animals ventilated much more than the control animals. On chronic exposure (12 days) this was so in the case of 1.0 and 2.0ppm exposed animals.

*Perna viridis* was maintained in test media containing 1.0, 2.0, 3.0 and 4.0ppm of Gramoxone for a period of 12 days along with control. The rate of oxygen consumption was estimated on the same animals after 1, 4, 7 and 12 days. The results are presented in table 2. The rate of oxygen consumption was generally less by the animals exposed to various concentrations. Those test organisms exposed to 4.0ppm of Gramoxone recorded very low rates of oxygen consumption after 12 days compared to those of the controls. In general, the rate of oxygen consumption was less when compared to that of control.

## Discussion

Bivalves inhabiting coastal waters are more exposed to pollutants from diverse sources than their counterparts in other habitats. Trace metals, petroleum hydrocarbons and organic pesticides are the major contaminants of coastal waters. The

indiscriminate use of organic pesticides has resulted in their accumulation in the tissues of organisms in alarming levels and has generated great concern among toxicologists. Toxicity tests are designed for assessing chemical hazard to aquatic life (Cairns et al. 1982).

Analysis of the data revealed that Gramoxone is extremely toxic to *Perna viridis*. Acute angle of the toxicity curves and the asymptotic nature indicate that Gramoxone is highly toxic beyond a threshold concentration. In the case of this species protrusion and withdrawal of the foot are effected by the use of pedal adductor muscles. In all the concentrations employed, the test organisms had protruded foot, which had a flabid texture, indicating total loss of nervous and muscular controls. The fact that even in concentrations where no death was recorded, *Perna viridis* had extended foot, indicates that exposure to such concentrations will have very serious ecological consequences. The secretion of byssus threads which help the animal in moving was totally hampered with. This response is comparable to the animals reaction to toxicity induced by polycyclic aromatic hydrocarbons (Reddy & Menon 1976).

The rate of ventilation estimated by dye clearance technique is a reasonably realistic method to assess activity of bivalves. According to Abel (1976) a test on ventilation rate is based on a response of ecological significance, since bivalves depend on the water for food, oxygen, and removal of waste. Correlations exist between rate of ventilation and valve closure mechanisms. Factors that could affect ventilation rate are frequency of valve closure, rate of ciliary activity and changes in the level of gill irrigation (Reddy & Menon 1979). The quantity of water drawn into the mantle cavity could be significantly affected by variations in valve closure mechanisms and inhibitory effect of ciliary action (Brown & Newell 1972). Reduction in the rate of ventilation is a well established reaction of bivalves esepcially mussels to pesticide and heavy metal toxicity (Murthy 1982, Prabhudeva & Menon 1985, Phillips 1977, Mohan et al. 1986). However, there are instances where heavy metals namely silver was found to induce enhanced ventilation rate. The sub-lethal concentrations of Gramoxone was found to enhance ventilation rate in the case of *Perna viridis*. This pesticide is water soluble and hence is likely to irritate the soft tissues of the bivalves by physical contact. Therefore flushing of water becomes essential to reduce the possible damage to tissues by contact with Gramoxone. It is likely that if the animals were exposed to higher concentrations, the rate of ventilation would have gone down because it was found that high concentrations of this pesticide could distinctly reduce muscle activity of the animal

Rate of oxygen consumption is a useful tool to assess the toxicant stress on aquatic organisms, since it is also an index of energy expenditure to meet the demands of environmental alterations. In bivalves, rhythmic shell movements are closely related to respiration and ventilation rate. Therefore fluctuations noticed in the case of oxygen consumption could probably be due to variations in the quantity of water irrigating the gills, which in turn could have been affected by irregularities in valve movements. Analysis of the data reveals that rate of oxygen consumption increased in *Perna viridis* exposed to the lower concentrations of the pesticide, while in concentrations above 2.0ppm the oxygen consumption decreased sharply. Pesticides are enzyme inhibitors and therefore can function as respiratory depressors. Increased rate of ventilation coupled with decreased rate of oxygen consumption clearly indicate that the chemical processes involved in the gaseous exchange between the surrounding medium and the animal was

hampered. This probably shows that the enzymes involved in the exchange are deactivated; a point which would require further elucidation.

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