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Toxicity of Mareb Crude Oil on Intertidal Clam *Tivela Ponderosa* and its Effect on Oxygen Consumption under Laboratory Conditions

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Abstract- The impact of chronic exposure of Mareb Crude Oilon the rate of oxygen consumption of the clam, *Tivela Ponderosa* was studied in the laboratory. The bivalve *Tivela ponderosa* is exposed to (0.5, 1, and 1.5) ml/l of Mareb Crude Oil. The oxygen consumption of bivalves *Tivelaponderosa* was controlled hourly at 5th and 10th day (every two and six hours). After 5 and 10 days of exposure to crude oil concentrations, the average oxygen consumption in the clam after 5 days of exposure was (0.131, 0.135, 0.141, 0.319ml O_2 gm^{-1} dry tissue h^{-1} every two hours) and (0.121, 0.124, 0.137, 0.247 ml O_2 gm^{-1} dry tissue h^{-1} every six hours) and after 10 days it was (0.222, 0.561, 0.946, 1.117 ml O_2 gm^{-1} dry tissue h^{-1} every two hours) and(0.126, 0.432, 0.573, 0.632 ml O_2 gm^{-1} dry tissue h^{-1} every six hours). It was observed that the rate of oxygen consumption fluctuated with an increase in the exposure period. The increase in oxygen consumption in the treated clams can be explained by the high metabolic activity of the organisms due to the stress imposed by the pollutant. On the other hand, when increasing the exposure period for pollution substance decrease in oxygen consumption in the clams exposed to toxic substance concentrations. The possible reason for the latter is the insensitivity of the clams especially in high concentration of pollution substances.

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I. Introduction

The rate of oxygen consumption varies with changes in the environmental and physiological conditions. Changes in physiological activities of organisms serve as an indicator of sublethal effects of pollutants on organisms (Sprague, 1971; Swedmark *et al.,* 1971; Hargrave and Newcombe, 1973). Thus, it is an indicator to determine the degree of stress caused by changes in the environment due to various natural and man-made perturbations.

Deshmukh (1979) studies the changes in oxygen consumption by the clam, *Meretrixmeretrix* exposed to various changes in natural conditions (temperature, salinity, etc.).Rate of oxygen consumption has been used as a valuable tool by many workers to assess stress, since it is an index of energy expenditure to meet the demands of environmental alterations (Prabhudeva and Menon, 1986; Mohan *et al.,*1986a,b). Most of the vital activities in bivalves are regulated by neuro-endocrine centers. The respiratory rate data of the animals reflect their general metabolic rate. The aim of this study by using bivalves (*Tivelaponderosa*) as bioindicator to determine the effect of toxic for Mareb Crude Oil on oxygen consumption rate in bivalve mollusks. In addition to study of the condition index of the exposed bivalve.

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II. Materials and Method

The bivalves were collected from Abyan Coast (12°. 48. 485 N, 45°. 02. 381 E) in Aden Governorate. They were collected by hand during the spring low tides in the evening times to avoid higher temperatures and were then kept in open canvas sacs containing wet sand to minimize frictions, desiccation and then transported to the laboratory immediately. They were protected from agitation during the transportation. The clams were cleaned by gentle rubbing in clean seawater to remove the clogged sediment and mucus and kept in aquaria of uniform size, 40cm long, 25cm wide 20 cm height, each containing clean and filtered seawater. Clams of uniform size of (47±1) mm long were used in the study to avoid susceptible size-based variations in response to the test chemicals. At the end of the acclimatization the experimental organisms for 4 days must be in excellent condition to tolerate the experimental conditions. There should be less than 2% mortality during acclimatization (APHA-AWWA-WPCF, 1976). Four clams from each of the control and from the exposed sets were transferred (five and ten days exposure to cured oil concentrations of 0.5, 0.1 and 5.1 ml/l into beakers of 500 ml capacity containing 400 ml filtered seawater. The water columns of the beakers were sealed with a 2.3 - 3 cm layer of inert liquid paraffin to prevent exchange of gas with atmosphere; following the method of Mathew and Menon (1983).

The oxygen consumption was determined for two and six hours in dark chambers to minimize the external stimulations. Then the oxygen contents were determined by Winkler's method for the experimental jars, the initial water and the control water without clams at the end of the experiment. After each experiment, the soft tissues were removed cleaned and dried at 80°C for 24 hours, and dry weights were determined. Standard

deviations were calculated based on 4 determinations in ml O_2 consumed per hour per gm dry weight.

A condition index relating dry tissue weight to shell length was calculated by:

$$\text{Condition index} = \frac{\text{Dry Weigh (gm)}}{\text{Shell length (mm)}} \times 100$$

The soft tissues were shucked off the shells, weighted then dried as above and reweighted to get the dry tissue weights. Then the dry-wet tissue weight ratio in percentage was calculated.

$$\text{Dry - Wet weight ratio} = \frac{\text{Dry Weight}}{\text{Wet weight}} \times 100$$

III. Results

a) Rate of Oxygen Consumption

Table 1.1 and Fig 1.1 illustrate the results of the rate of oxygen consumption of the control and Mareb Crude Oil exposed clams ml O_2/g dry soft tissues/hr for five and days (every two and six hours) of observation. Oxygen consumption in the control was 0.131 and 0.121ml/g dry weight/hr. The oxygen uptake increased gradually in clams exposed to different concentrations of the crude oil, in the low concentration 0.5 ml/l was 0.135 and 0.124 ml/g dry weight/hr, and the medium concentration 0.1 ml/l was 0.141 and 0.137 ml/g dry weight/hr, and the increase reached its peak in the clams exposed to high crude oil concentration 1.5 ml/l with an increase was 0.319 and 0.247 ml/g dry weight/hr, compare control during two and six hours respectively

The results of the rate of oxygen consumption of the control and test substance exposed clams ml O_2/g dry soft tissues/hr for ten days (every two and six hours) of observation. Oxygen consumption rate increased during the ten days compared oxygen consumption rate for five days. In the control was 0.222 and 0.126 ml/g dry weight/hr. During the low, medium and high concentration was {(0.561) (0.432)}, {(0.946) (0.573)}and{(1.117)(0.632)} ml/g dry weight/hr, respectively.

Fig. 1.1 and 1.2 show clearly the difference between the two groups of clams after 5 and 10 days exposure to Mareb Crude Oil toxicity (after two and six hours). The group in the Fig. 1.1 and 1.2 illustrate the rates of oxygen consumed by the control and the exposed clams after they have been transferred to crude oil-free seawater. In the 10 days group the rate of oxygen uptake was generally less than the rate of oxygen consumption in the 5 days group.

Finally, comparison of the charts after 5 and 10 days show clearly the difference between the two groups of clams after 5 days and two groups of clams after ten days of exposure to substance tests toxicity (after two and six hours). The charts illustrate the rates of oxygen consumed by the control and the exposed clams after they have been transferred to chemicals tests-free seawater.

Table 1.1: Average oxygen consumption (ml O_2 gm^{-1} dry tissue h^{-1}) in bivalves exposed to Mareb Crude Oil for five days (after 2 and 6 hours) values are mean ± for 2 determinations

Exposed (to ml/l)	Period of exposure (after two hours) Mean S.D	Period of exposure (after six hours) Mean S.D
0.0 (control)	0.131 ± 0.009	0.121 ± 0.015
0.5	0.135 ± 0.010	0.124 ± 0.070
1.0	0.141 ± 0.020	0.137 ± 0.014
1.5	0.319 ± 0.029	0.247 ± 0.003

Table 1.2: Average oxygen consumption (ml O2 gm-1 dry tissue h-1) in bivalves exposed to Mareb Crude Oil for ten days (after 2 and 6 hours) values are mean ± for 2 determinations

Exposed (to ml/l)	Period of exposure (after two hours) Mean S.D	Period of exposure (after six hours) Mean S.D
0.0 (control)	0.222 ± 0.011	0.126 ± 0.000
0.5	0.561 ± 0.033	0.432 ± 0.000
1.0	0.946 ± 0.000	0.573 ± 0.005
1.5	1.117 ± 0.025	0.632 ± 0.000

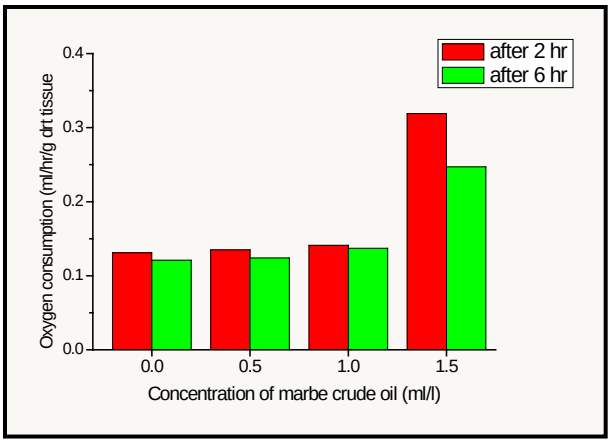

Fig. 1.1: Oxygen consumption by *Tivelaponderosa* after 5 days exposure to Mareb Crude Oil

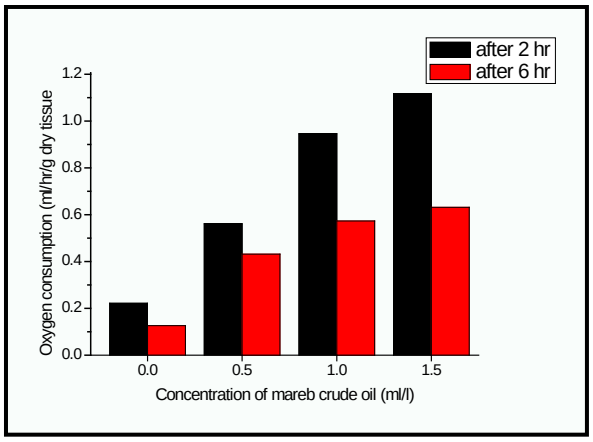

Fig. 1.2: Oxygen consumption by *Tivelaponderosa* after 10 day sexposure to Mareb Crude Oil

b) Condition Index

The observed values of condition index which is the ratio percentage between the dry weight (g) and shell length (mm) are given in Tables 2.1 and Fig. 2.1 which indicate a reduction in condition index of the clams exposed to Mareb Crude Oil for period of 5 and 10 days. The condition index of the control clams was 2.927 after 5 days and 2.751 after 10 days. Where decreasing condition index of the clams exposed to different concentrations Mareb Crude Oil during 5 and 10 days, in the low concentration 0.5 ml/l were 2.587and 2.446 ml/l, while the medium concentration 0.1 ml/l were 2.452, and 2.238 ml/l, the high concentration 1.5 ml/l were 2.396 and 2.198 ml/l.

c) Dry-Wet Weight Ratio

The data of the ratio between dry and wet weights of the control and exposed clams during 5 and 10 days are shown in tables 3.1 and Fig. 3.1. The decreased ratio was more or less inversely proportional to the degree for each test chemicals concentrations compared to the control. The ratio were 25.11 and 25.08 in the control and in the exposed clams to Crude oil compared to the control were {(24.34) (24.24), (22.89)(22.06) and (21.93) (21.12)}.

Table 2.1: Condition index as a function of Mareb Crude Oil concentrations for five and ten days

Concentrations (ml/l)	Condition index for *Tivelaponderosa*	
	concentrations for five days Mean S.D	concentrations for ten days Mean S.D
0.0 (control)	2.927 ± 0.134	2.751 ± 0.232
0.5	2.587 ± 0.326	2.446 ± 0.312
1.0	2.452 ± 0.289	2.238 ± 0.565
1.5	2.396 ± 0.182	2.198 ± 0.203

Table 3.1: Dry-wet weight ratio as a function of Mareb Crude Oil concentrations five and ten days. Values are means ± S.D, n =4

Concentrations (ml/l)	Condition index for *Tivelaponderosa*	
	concentrations for five days Mean S.D	concentrations for ten days Mean S.D
0.0 (control)	25.11 ± 0.44	25.08 ± 0.07
0.5	24.34 ± 0.36	24.24 ± 0.35
1.0	22.89 ± 0.06	22.06 ± 0.06
1.5	21.93 ± 0.44	21.12 ± 0.07

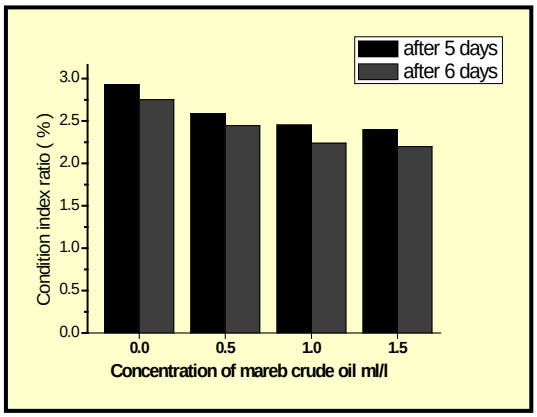

Fig. 2.1: Effect of Mareb Crude Oil concentrations on condition index of *Tivelaponderosa* after five and ten days exposure

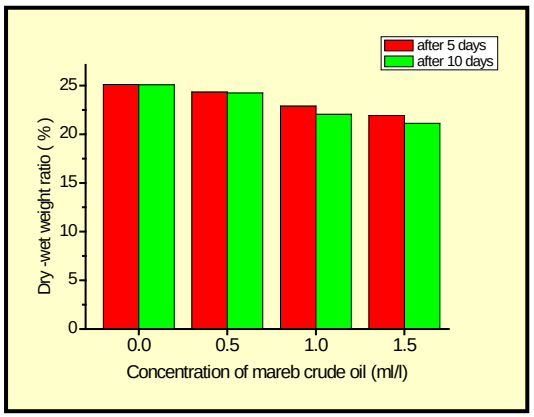

Fig. 3.1: Effect of Mareb Crude Oil concentrations on dry-wet weight ratio of *Tivelaponderosa* after after five and ten days exposure

IV. Discussion

a) Rate of Oxygen Consumption

The concept of assessing ventilation rate and O_2 consumption rate to explain toxicant stress arose out of the knowledge that metabolism and activity are interrelated. Variations in metabolic rate modify the scope for activity and the degree of activity affects metabolic rate. Usually, laboratory determinations of sublethal stress can delineate linear or nonlinear responses. The respiratory rate of aerobic organisms represents the metabolic activity and thus the oxygen consumption is a useful measure of sublethal effects on the animals which has been used to determine the extent of stress by various natural and man-made perturbations. The extent of modifications in oxygen consumption rate may be considered as great as pollutant-produced alterations (Anderson, 1977).

The present study showed a significant increase in oxygen consumption in the Mareb Crude Oil treated clams over that of the control clams. Oxygen consumption increased gradually with the increasing crude oil concentrations. The increase in oxygen consumption in the treated clams can be explained by the high metabolic activity of the organisms due to the stress imposed by the pollutant. The metabolic functions

demand energy and subsequently oxygen is needed for oxidation. It was observed by Percy (1977) that in animals exposed to seawater extracts of the oil, from which particulate oil had been removed, the metabolic rate was significantly stimulated; the degree of stimulation increased with increasing oil concentration. This leads one to explain the increase in oxygen consumption on the basis of oxygen utilization by the organism as due to demand for its high metabolic rate, which involves two distinct metabolic components; the basal metabolism reflects routine maintenance processes of the organism at rest and a further significant fraction of the oxygen demand is attributed to locomotion and other forms of activity (Newell, 1970).

The decline in oxygen consumption in the clams exposed to test crude oil concentrations during 10 days may be due in part, to the narcotization effect of the chemical on the animals and to the disturbance of the overall vital functions of the organisms by the compound. This condition can be attributed to the suppression effect of crude oil concentrations on the clams in addition to the effect of long term exposure. On the other hand, the decrease in oxygen consumption in case of the high hydrocarbon concentration is the result of activity reduction as Percy (1977) hypothetized this situation by stating that, the decrease in oxygen consumption is the result of activity reduction which may mask a general increase in basic metabolic rate.

Struhsakes *et al.* (1974) reported similar finding in fish exposed to benzene and showed that benzene penetrated readily into tissues and stimulated the respiration of fish; they opined that the increased respiration reflected a requirement for more oxygen to metabolize the benzene. Higher concentrations or extended exposure times resulted in a decline in metabolic rate and a possible narcotic effect arising from accumulation of benzene in the tissues.

Several workers reported the effects of hydrocarbons on the respiration of marine organisms but the result varies considerably and in some cases an increase of oxygen consumption is recorded while decrease in others. The variations in the consumption of oxygen may be due to the type of hydrocarbon or the method of preparations or the duration of exposure or due to the environmental conditions of the experiments (Hargrave and Newcomb, 1973; Thomas and Rice, 1979). Avolizi and Nuwayhid (1974) who recorded a depression in the respiratory rates of the bivalves, *Branchiodontes* and *Donax* exposed to light crude oil which showed reversal conditions when exposed to high concentrations of the oil.

The clam *Anadaragranosa* exposed to naphthalene for a short term (96 hrs) exhibiteda reduction in its oxygen consumption (Eapen, 1987). On the other hand, several authors recorded observations similar to those of the present study where low hydrocarbon concentrations increases oxygen consumption and the high concentration decreased the respiratory rates. This is in accordance with Anderson *et al.* (1974b) who recorded an increase in respiratory rates as a function of water soluble fraction (WSF) of No. 2 Fuel. Gilfillan (1975) reported an increase in metabolic rates when bivalves were exposed to low hydrocarbon concentrations and reduction of it when exposed to higher hydrocarbon concentrations. Similar results were obtained by Hargrave and Newcomb (1973); Percy (1977); Tatem (1977); Thomas and Rice (1979).

The clam *Myaarenaria* had doubled its oxygen consumption when exposed to lower oil concentrations and when they were exposed to greater oil concentration showed a depression in the oxygen consumption rate. Stainken (1977) and Neff (1979) stated that the respiratory rate of early and late zoeae and megalops of mud crab were increased by exposure to phenanthrene and naphthalene in which the former had a greater effect than the latter on respiratory rate.

The above mentioned finding by different authors indicates that the metabolic response to hydrocarbons is more complex than the simple unidirectional inhibition or stimulation suggested by some studies (Percy, 1977).

b) Condition Index

This study has shown that both the condition index and the ratio of dry-wet weight tissues in *Tivelaponderosa* were significantly altered by the exposure to Mareb Crude Oil. The condition index parameter is another tool to interpret the growth rate of the animal and the actual energy balance indicating protein, carbohydrate and lipid catabolism to counteract the stressful conditions of pollution. The present results showed a gradual decrease of condition index as the test chemicals concentrations increased.

This condition was also observed by other authors like Roesijidi and Anderson (1979) in *Macomainquinata* and Stekoll *et al*. (1980) in *Macomaballhica*. Both the studies related the decrease in condition index to the negative energy balance which indicates energy utilization rather than storage. Granby and Spliid(1995) recorded a highly significant negative correlation with PAH in the common mussel. According to Anderson (1979) the time factor is necessary in obtaining a good result in condition index.

c) Dry-Wet Weight Ratio

The dry-wet weight ration also showed significant variation from the control with the decrease in values corresponding to the increase in oil concentration. The faster decrease in the dry weight in relation to the wet tissue weight may be explained by the loss of dry weight for using their energy reserves due to oil exposure. This state probably occurred because of various factors such as decreasing in feeding accompanied by the increase in metabolic rate and

reduction in filtering rates. The present results are in agreement with the conclusion recorded by Sophia and Subramanian (1990) who studied the clam *Meretrixcasta* exposed to various Crude and fuel oils whereby they lost dry weight faster than wet weight. Stekoll *et al.,* (1980) reported similar results.

V. Conclusion

The present study showed a significant increase in oxygen consumption in the test substances treated clams over that of the control clams. Oxygen consumption increased gradually with the increasing test substances concentrations. The increase in oxygen consumption in the treated clams can be explained by the high metabolic activity of the organisms due to the stress imposed by the pollutant. On the other hand, when increasing the exposure period for Pollution substance decrease in oxygen consumption in the clams exposed to toxic substance concentrations. The possible reason for the latter is the insensitivity of the clams especially in high concentration of pollution substances.

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