

Effect of Petroleum Hydrocarbon on Physiological and Biochemical Parameters in an Estuarine Clam, *Paphia malabarica*

Yasmin Modassir and Azra Ansari

Dhempe College of Arts & Science
Miramar, Goa - 403 001, India

Commercial grade diesel oil was used as a source of petroleum hydrocarbon to study the toxic effect of petroleum hydrocarbons on physiological and biochemical parameters in an estuarine bivalve, *Paphia malabarica*. The results of the investigation revealed the adverse effect of diesel oil on the growth condition index and biochemical constituents. The growth was affected resulting in reduction of about 30% in the average growth rate after 30 days. The impact was more with higher concentration. There was reduction in the values of organic carbon and nitrogen. The biochemical constituents such as protein and carbohydrate also showed slight decline while the lipid content increased marginally. The results suggest that exposure to petroleum hydrocarbon may be deleterious to many marine and estuarine organisms and may change the physiological and biochemical processes, depending on its bioavailability and exposure concentration.

Key words: Petroleum hydrocarbon, toxicity, estuarine bivalve, *Paphia malabarica*

Oil spills are a recurrent problem in the marine environment and is a major source of pollution. Petroleum hydrocarbon (PHC) introduced into the marine environment every year from different sources is estimated to be about 3.2 million tonnes (Doeffer, 1992).

Most of the petroleum hydrocarbons have been reported to modify the physiological and ecological behaviour in the marine organisms (Anderson *et al.* 1974; Patel & Eapan, 1989). Biological effects of oil contamination can be manifested at different levels of organization (Neff, 1979; Capuzzo, 1981). Bioaccumulation of PHC and its effect on physiology and biochemistry of lamellibranchs from the temperate region have been well documented (Patel & Eapan, 1989). But limited information on this aspect is available for tropical species from Indian coastal waters (Sophia, 1987; Das & Konar, 1988). Most of the oils differ in physicochemical properties and hence the study of individual oil component becomes significant. Oil in water dispersions (OWD) is more representative of actual field

conditions following an oil spill. Commercial grade diesel oil has maximum uses and hence the present study was carried out with OWD of diesel oil following static bioassay procedure.

Materials and Methods

The OWD of diesel oil for use in the toxicity bioassay was prepared by the method of Anderson *et al.* (1974a, b). A specific volume of oil (v/v) was added to sea water of 25 ppt salinity, such that the total volume was 500 ml. For a 5% concentration of OWD, 25 ml of oil was added to 475 ml of sea water. The constituents were thoroughly mixed for five minutes at approximately 200 cycles per minute on a shaker platform. Similarly the other concentrations of oil equivalent to 10, 15, 20 and 30% were prepared. The dispersions were allowed to separate for one hour and then the animals were added through the surface oil film by means of a glass tubing. Total PHC concentration of the aqueous phase was estimated (Gordon *et al.*, 1974).

Clams used as the test animals, were collected from the natural bed of Zuari estuary and acclimatized for seven days in the laboratory in holding tanks filled with filtered estuarine water of 25 ppt salinity at room temperature. All animals were fed regularly with algal culture. Animals of approximately same size (8 ± 2 mm) were used in the experiment. The temperature and dissolved oxygen content of the tanks were maintained at $28 \pm 1^\circ\text{C}$ and 4.5 ml/l respectively.

Acute toxicity level of diesel oil was determined first with concentrations of 5, 10, 15, 20 and 30% freshly prepared OWD. Twenty clams were introduced into each concentration and mortality was recorded at 24, 48, 72 and 96 hours interval. Tests were carried out in replicates with a control. During the toxicity test, no feed was provided and slow aeration was given to keep the optimum level of oxygen. The criteria for determining the death were lack of movement and no response to prodding.

The juveniles of *P. malabarica* measuring about 8 ± 2 mm were used for the study of effect of PHC on growth and condition. Fifty clams were introduced into a tank having 10% concentration of OWD. The concentration was selected based on preliminary results of toxicity study. The experiment was run for 50 to 60 days. The condition index (K) was calculated by the formula of Clement *et al.* (1980).

$$K = \frac{\text{Dry weight (g)}}{\text{Shell length (mm)}} \times 100$$

The biochemical constituents like protein, lipid and carbohydrate in the tissue of *P. malabarica* exposed to OWD were estimated. Three concentrations of OWD, 5%, 10% and 15%, were used to study the effect of PHC on the biochemical composition. In each concentration, 20 clams of approximately same size (10 ± 2 mm) were introduced. The salinity of the medium was kept at 25 ppt. All tests were carried out in

replicates with a control for each set of experiment. On the 10th, 20th and 30th days, 5 clams were randomly taken from each tank for the estimation of biochemical constituents. The tissue was dried at 60°C in an oven and kept in desiccator. Organic carbon and nitrogen were estimated on a CHN analyser. Protein was estimated by the method of Lowry *et al.* (1951). The lipid was estimated by the method of Floch & Stanely (1956) and carbohydrate, by the method of Dubois *et al.* (1956). The average values were calculated from replicates.

Results and Discussion

The concentration of PHC in the aqueous phase of the OWD preparation is given in Table 1. The concentrations increased with increasing amount of oil. Toxicity of OWD of diesel was tested at different concentrations. In each concentration dead animals were recorded and a 96 hours LC_{50} value was derived. Based on the 96 hours LC_{50} value, the doses of 5, 10, 15% OWD were chosen and used for the long term exposure. The results of acute toxicity tests (Table 2) indicated no mortality up to 15% OWD. In the higher concentration, however, mortality was recorded after 48 hours. In 5% concentration, the clams had opened out their siphons and had the foot probing about, during the observation period. A low mortality in the clams at lower concentrations suggested an initial accumulatory capacity and the bioavailability of test oil as reported by Anderson *et al.* (1974) and Neff (1979). A reduction in siphon and foot activity as observed in the present study had also been recorded by Clement *et al.* (1980).

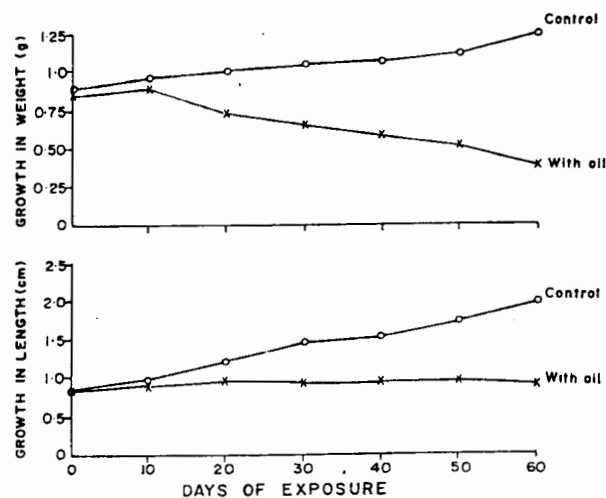
Table 1. Total concentration of petroleum hydrocarbon in aqueous phase of OWD

OWD	Concentration (mg/l)
5%	3.52
10%	7.02
15%	9.91
20%	11.39
30%	13.86

Table 2. Percent mortality of *Paphia malabarica* exposed to different concentrations of OWD of diesel oil

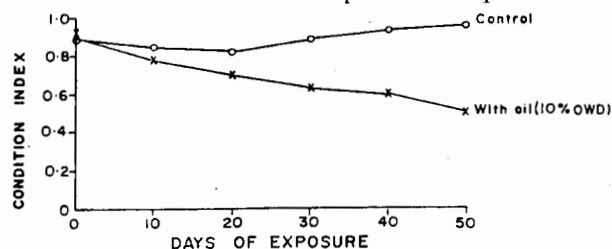
Concentration	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
5%	0	0	0	0
10%	0	0	0	0
15%	0	0	0	0
20%	0	5	10	30
30%	0	10	30	60

The effect of petroleum hydrocarbon on growth (length and weight) is shown in Fig.1. Changes in the length and weight of clams of control and treatment group were significantly different. The growth was significantly lower ($p < 0.1$) in groups treated with 10 and 15% OWD when compared with the control. Clams showed retarded growth after two months. The reduction in growth could be due to intoxication by petroleum hydrocarbon which may result in decrease in rate of feeding and ciliary activity. Similar decrease has been reported in *Anadara granosa* (Patel & Eapan, 1989) and *Mercenaria mercenaria* (Keck *et al.*, 1979). Bioaccumulation of hydrocarbons, in general, has been identified as an important factor affecting the physiological parameters in many shellfish (Anderson *et al.*, 1974).

Fig. 1. Effect of OWD (10%) on growth of *P. malabarica*.

Clams exposed to OWD of diesel registered about 32% decrease in condition index (Fig. 2). Decrease in condition index of clams exposed to petroleum hydrocarbon has been reported earlier also (Stekoll *et al.*, 1980). Patel & Eapan (1989) have observed

that the most plausible reason for this could be secretion of mucus in higher concentration which may affect the kinetics of exchanges of ions and alter physiological processes. In another study, Sophia & Balasubramanian (1992) reported changes in condition index and weight in *Meretrix casta* as a result of effect of exposure to petroleum.

Fig. 2. Condition index of *P. malabarica* exposed to 10% OWD.

Organic carbon and nitrogen are important constituents in the tissue. The concentration of organic carbon in the tissue of the animals exposed to different concentrations of OWD are given in Table 3. The value showed a decreasing trend with increasing concentration of OWD in the environment. The effect was dose dependent. The differences when tested statistically were however, not significant.

Table 3. Percent (dry weight basis) organic carbon and nitrogen in the tissue of *Paphia malabarica* exposed to OWD (% Dry weight basis)

Concentration	Carbon
Control	37.55
5%	35.22
10%	32.02
15%	30.10

The changes in the protein content of the tissue as a result of exposure to OWD are given in Fig. 3. Proportion (dry weight

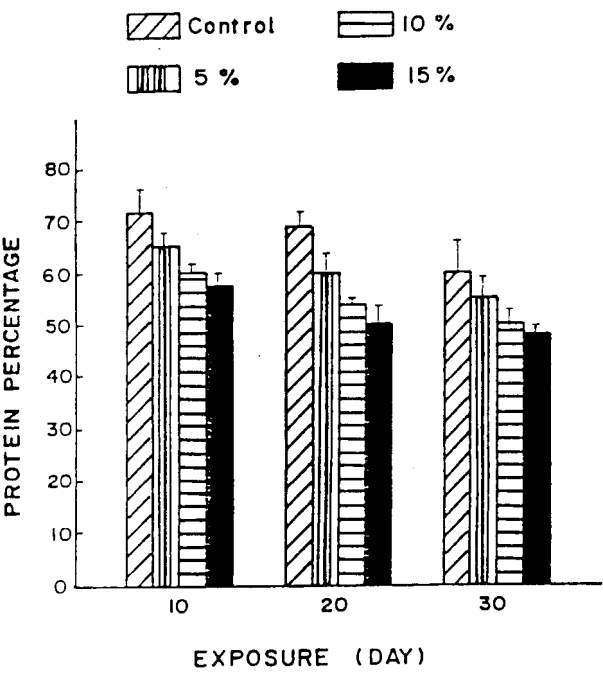


Fig. 3. Protein content of the muscle tissue in *P. malabarica* exposed to OWD.

basis) of protein in the tissue of control animals ranged between 67.4 - 70.8%. On

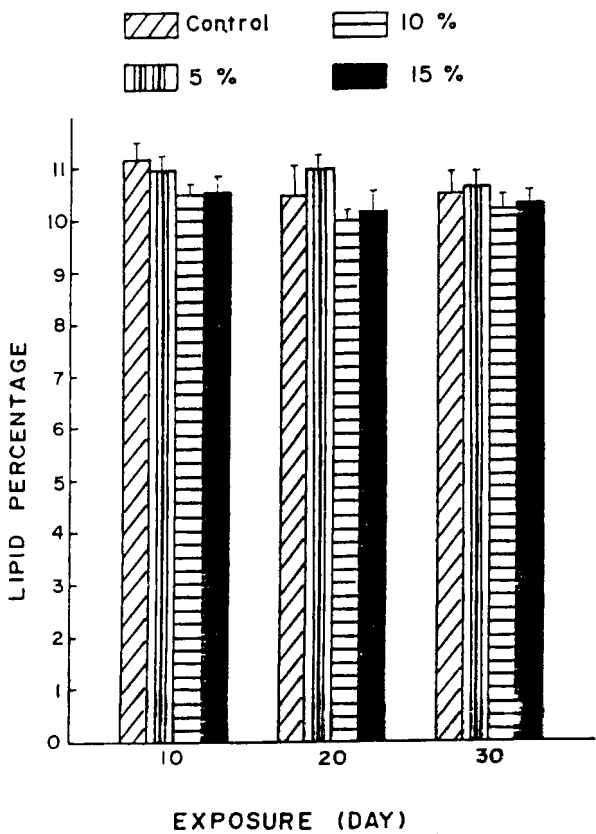


Fig. 4. Lipid content of the muscle tissue in *P. malabarica* exposed to OWD.

exposure to OWD, the protein content decreased marginally. The reduction was in the range of 7- 10%, 9-12% and 15-25% in 5, 10 and 15% OWD, respectively. The decrease in the protein content suggested its probable utilization for metabolic purposes to combat induced stress effect. Fawde *et al.* (1983) reported significant decrease in the protein content on exposure to DDT in white prawn. Reduction in protein level had also been reported in other organisms exposed to petroleum hydrocarbon (Sophia, 1987). It has been reported that during stress condition, animals utilize protein for extra energy (Hochachka & Somero, 1973).

Changes in the lipid concentration of the tissues due to exposure to OWD showed a different trend (Fig. 4). A marginal increase in the concentration was noticed in the animals exposed to OWD. The values however, showed no significant difference after 30 days in the group treated with 10 and 15% OWD. The increase in lipid content in the clams exposed to OWD may be attributed to lypogenic activity. Similar increase in lipid content of clam, *Meretrix casta* (Sophia, 1987), *Scylla serrata* (Kulkarni,

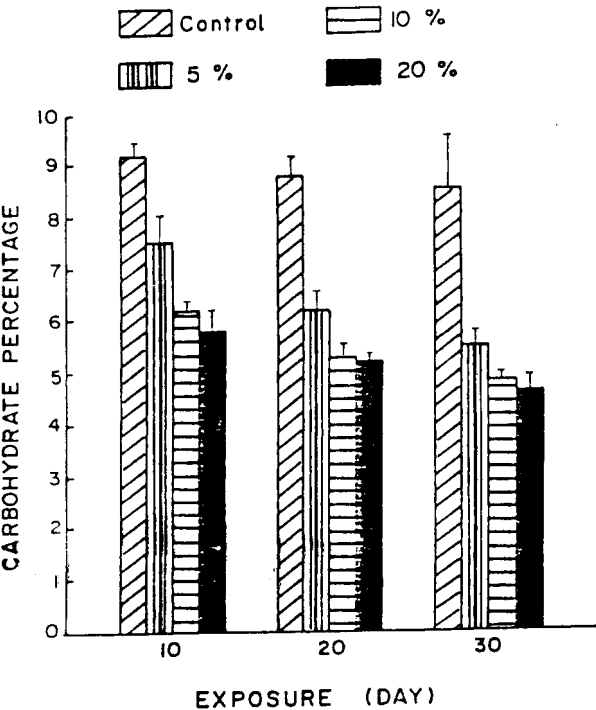


Fig. 5. Carbohydrate content of the muscle tissue in *P. malabarica* exposed to OWD.

1983) and fishes (Brown *et al.*, 1982) on exposure to petroleum hydrocarbons have been reported. Dange (1976) suggested that increase in lipid content may be due to sluggishness, ultimately leading to accumulation of lactic acid which is a precursor for the lipid synthesis.

The levels of carbohydrate in the tissue of control and treated animals are given in Fig. 5. Mean values were 8.5% in control. The values were lower in treated clams. A maximum reduction of 30% was recorded in animals treated with 15% OWD. The lower levels of carbohydrate in OWD treated samples suggested its breakdown to cope with high energy demand caused by the pollutant, since carbohydrate forms major source of energy under stressful condition (Hochachka & Somero, 1973).

Thus the present study on the effect of PHC on the growth of clams showed that even low levels of oil pollutants have measurable effect on the organisms and can alter the normal metabolism. The changes recorded were dose and duration dependent. These changes will lead to malfunctioning of the organisms and poor survival rate.

The authors are grateful to Dr. G.N. Mitra, Principal, Dhempe College of Arts and Science for his keen interest and guidance in the present work. Critical reading of the manuscript by Dr. Z.A. Ansari, Scientist of National Institute of Oceanography is thankfully acknowledged.

References

- Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E. & Hightower, G.M. (1974a) *Mar. Biol.* **27**, 75
- Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E. & Hightower, G.M. (1974b) In *Pollution and physiology of marine organisms* (Vemberg, F.J. & Vemberg, W.B. Ed.) p.285, Academic Press, New York,
- Balasubramanyan, K. (1984) *Studies on Meretric casta from the Vellar estuary, Porto Novo (S. India)*. Ph.D. Thesis, Annamalai University, India, p.135
- Brown, D.A., Cosett, R. & Jenkins, K.D. (1982) In *Physiological mechanism of marine pollutant toxicity*. (Vemberg, F.J., Calabrese, A., Thurberg, F.P. & Vemberg, W.B. Ed.) p.197, Academic Press, New York
- Capuzzo, J.M. (1981) *Oceanus*, **24**, 25
- Clement, L., Stekoll, M.S. & Shaw, D.G. (1980) *Mar. Bio.* **57**, 41
- Das, P.K.M. & Konar, S.K. (1988) *Environ. Ecol.* **6**, 886
- Dange, A.D. (1976) *Some effects of petroleum hydrocarbon on aquatic organisms*. Ph. D. Thesis, University of Bombay
- Doeffer, J.W. (1992) *Oil spill response in the marine environment* p.3, Pergamon Press New York
- Dubois, M.K.A., Gilles, Hamilton, Rebus, J.K. m & Smith, F.S. (1956) *Analytical Chemistry*, **28**, 350
- Fawde, M.M., Machale, P.R., Mane, U.H. & Nagabhushanam, R. (1983) *J. Environ. Biol.* **4**, 81
- Floch, J.M.L. & Stanley, J.H.S. (1956) *J. Biol. Chem.* **226**, 497
- Gordon, D.C., Keizer, P.D. & Dale, J. (1974) *Ocean. Mar. Chem.* **2**, 262
- Hochachka, P.W. & Somero, G.N. (1973) *Strategies of Biochemical Adaptation*, p.24, W.B.Saunders Company, Philadelphia
- Keck, R.T., Heesse, R.C., Wehmiller, J. & Maurer, D. (1978) *Environ. Poll.* **15**, 109
- Kulkarni, B.G. (1983) *Toxicity studies on effect of some petroleum hydrocarbon on some marine animals*. Ph.D. Thesis University of Bombay
- Lowry, O.H., Rosebrough, A.L., Farr, A.L. & Randall, R.J. (1951) *J. Biol. Chem.* **93**, 265
- NAS (1975) *Petroleum in the marine environment* p.107, National Academy of Sciences, Washington D.C
- Neff, J.M. (1979) *Polycyclic aromatic hydrocarbons in the aquatic environment*. Applied Science Publication

Patel, B.P. & Eapan, J.T. (1989) *Mar. Biol.* **103**, 203

Roesijadi, G. & Anderson, J.M. (1979) In *Marine pollution: Functional responses*, (Vernberg, W.B., Thuberg, F., Vernberg, F.J. Ed) p.69 Academic Press, New York

Stekoll, M.S., Clement, L.E. & Sahw, D.G. (1980) *Mar. Biol.* **57**, 51

Sophia, A.J.A. (1987) *The effect of petroleum hydrocarbon on the back water clam Meretrix casta. Chemnitz: Some characteristics relevant to hydrocarbon monitoring.* Ph. D. Thesis, Annamalai University

Sophia, A.J.A. & Balasubramanian, T. (1992) *Arch. Environ. Contam. Toxicol.* **24**, 471