Histological Changes in the Gill & Hepatopancreas of the Marine Crabs *Charybdis lucifera* (Fabricius) & *Scylla serrata* (Forskal) Exposed to Crude Oil Emulsion

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Received 1 July 1983; revised received 6 January 1984

Toxic effects of different concentrations of crude oil from the Bombay High on *C. lucifera* and *S. serrata* were compared. *C. lucifera*, inhabiting deep waters, was much more susceptible to toxicity of oil-in-water emulsions than *S. serrata*, an intertidal species. Histological examination of treated crabs revealed pronounced pathological changes i.e., rupture of lamellae due to heavy accumulation of haemocytes, fusion of lamellae and epithelial damage in gills and zonal tubular damage in hepatopancreas. Tissue damage observed in these species correlates well with the percentage mortality.

The budget of petroleum hydrocarbons released into the marine environment through accidental spillages, normal tanker washings, etc. has on many occasions exceeded the normal limits affecting the ecophysiology of both benthic and pelagic communities, especially those inhabiting coastal waters and estuaries. Increased traffic of oil tankers in the Arabian Sea has resulted in the concomitant rise (0.126 to 2.44 ml l⁻¹) in the concentration of petroleum hydrocarbons in the coastal waters. The bioaccumulation of the petroleum hydrocarbons is found to affect the homeostatic mechanism and histology of both benthic and pelagic species. However little attempt seems to have been made to understand the level of hydrocarbon in the tissue and its impact on the morphology and physiology of various benthic fauna and flora, along Bombay coast. In view of this, studies have been made to evaluate the possible impact of elevated concentrations of crude oil from the Bombay High, on the histological structure of gill and hepatopancreas in 2 species of marine crabs, *Charybdis lucifera* (Fabricius) and *Scylla serrata* (Forskal).

**Materials and Methods**

Static bioassay technique was followed in the present study. Crude oil sample was obtained from Oil and Natural Gas Commission, Bombay. Tests were carried out at room temperature (±29-31°C). Salinity of the water was ±35.53 x 10⁻³ and dissolved oxygen ±5.8 mg l⁻¹.

Test medium (oil-in-water emulsion) was prepared by vigorously stirring a known volume of crude oil with water for 10 min. Six concentrations between 0.1 and 0.8 ml l⁻¹ were tested on *C. lucifera* and 7 on *S. serrata* in the range 1 and 4 ml l⁻¹. These concentrations were chosen considering the reported levels of hydrocarbons in the ambient waters and also the results of pilot experiments. These concentrations of hydrocarbons were the upper limit of petroleum present in the medium into which the animals were introduced. All exposures were run in triplicate. Inter group comparison was statistically assessed using Student’s *t* test.

*C. lucifera* and *S. serrata* were collected from the Bombay coast and acclimated to the laboratory conditions for about 24 h. Crabs in their intermoult stage were selected for the experiments. Ten animals, more or less of the same size, were introduced into each of the test aquaria and were observed for 24 h. No attempt was made to feed the animals during the experimental period. To avoid possible evaporation of soluble aromatics, the test aquaria were not aerated. Those animals which floated and failed to respond to mechanical stimulus were considered dead or moribund. Such animals were removed and rate of mortality was recorded. To record damage to tissues involved in feeding and assimilation mechanism, gills and hepatopancreas were dissected out from both the exposed and control animals and fixed in Bouin’s fixative. Paraffin sections of 8 μ thickness were cut and stained with Ehrlich’s haematoxyline and eosin.

**Results**

Effect of crude oil on survival — *S. serrata* is quite resistant to adverse effects of oil in comparison with *C. lucifera*. Minimum survival is observed at 0.6 ml l⁻¹ in *C. lucifera* (3.33 ± 5.77%) and at 3 ml l⁻¹ in *S. serrata* (43.33 ± 5.77%). In *C. lucifera* percentage survival is more at 0.7 ml l⁻¹ (16.66 ± 5.77), maximum being at 0.8 ml l⁻¹ (37.33 ± 5.77). Similarly in *S. serrata*...
83.33 ± 5.77 and 93.33 ± 5.77% survival is recorded at 3.5 and 4 ml l⁻¹ oil respectively. All the observed percentage survival is statistically different from zero concentration (control) by Student's t test.

**Histological changes**—Gill: In both the species gills are composed of a double row of closely spaced lamellae extending anteriorly and posteriorly from the gill shaft. The lamella is lined by a thin layer of epithelial cells enclosing the central haemocoelic sinus. Pillar cells, specialised epithelial cells, extend into the lamellar sinus at intervals and abut with similar cells extending from the opposite surface. Elongate clumps of these cells are arranged in curved rows presumably facilitating even perfusion of the lamella with haemolymph and preventing detention due to blood pressure. In addition, epithelial cells are supported and separated by pillar cells (Figs 1a and 2a).

Distinct pathological changes could be noticed in the gills of *C. lucifera* exposed to different concentrations of crude oil (Figs 1b to e). At 0.1 ml l⁻¹ conc. disruption of lamellae was observed. Swelling of the lamellae along with rupture of cells could be noticed at 0.2 ml l⁻¹ conc. (Fig. 1b). Accumulation of haemocytes in the lamellae, necrosis and pronounced lamellar damage—forming amorphous mass of disrupted lamellae particularly towards the base of the shaft—were the other changes observed. At a higher concentration, 0.4 ml l⁻¹, extensive epithelial damage and its lifting were observed specially towards the base of the lamellae. Individual cells at the tip of the
lamellae were necrotic showing vacuolation. Swelling and rupture of lamellae were observed due to heavy accumulation of haemocytes. Certain lamellae were necrotic (Fig. 1c). At 0.6 ml.1\(^{-1}\) conc. large number of haemocytes accumulated in the gill lamellae causing its rupture by blocking haemolymph channels and fusion of lamellae was observed (Fig. 1d). Characteristic lumping and swelling of lamellar cells were shown accompanied by an increase in necrotic debris. Animals exposed to 0.7 ml.1\(^{-1}\) also showed similar pathological symptoms (Fig. 1e). Extensive epithelial lifting was noticed and in some cases complete rupture of epithelium. Only few lamellae showed pathological sign in the animals exposed to 0.8 ml.1\(^{-1}\) oil conc. No cellular damage was evident except in the case of few pillar cells resulting in haemocyte accumulation.

In comparison to C. lucifera lesser damage was observed in the gills of S. serrata. Fewer gill lamellae of the animals exposed to lethal concentrations showed haemocyte accumulation (Fig. 2b). No pronounced lamellar disruption or fusion could be observed at any concentration. However, apparent epithelial damage was noticeable at 3 ml.1\(^{-1}\) conc.

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Fig. 2—a & b: Gills of control and treated (3 ml l\(^{-1}\)) S. serrata respectively (× 250). [GF, gill shaft; GL, gill lamellae; E, epithelial cells; P, pillar cells; and H, accumulation of haemocytes in gill shaft and lamellae]. c & d: Hepatopancreas of control C. lucifera and S. serrata respectively (× 250) [A, absorptive cells; F, fibrillar cells; S, secretory cells]. e & f (× 250): Tubular damage (T) in C. lucifera (0.6 ml l\(^{-1}\)) and S. serrata (3 ml l\(^{-1}\)).
Hepatopancreas: Both C. lucifera and S. serrata share a common structure in the case of hepatopancreas. Four different types of cells, viz. embryonic, absorptive, fibrillar and secretory cells, were detected in the digestive glands. In a typical gland hepatopancreas, four different types of cells, viz. tubule measuring about 2 mm in length the terminal 1/20th portion is occupied by embryonic cells with round or oval centrally placed nucleus. The juvenile absorptive cells dominate the succeeding 2/30th portion, but a few fibrillar cells are also present. In the following 1/20th portion of the tube numerous mature absorptive cells and fibrillar cells are localized along with a few juvenile secretory cells. The basal 6/20th portion of the tube is composed of majority of secretory cells and fibrillar cells. Fibrillar cells and secretory cells have basally placed nuclei whereas absorptive cells have subcentrally placed nuclei (Fig. 2c and d).

The digestive gland of C. lucifera exposed to different conc. of oil (0.2, 0.4, 0.6 and 0.7 ml l\(^{-1}\)) showed significant changes. Zonal tubular damage was observed showing amorphous mass of destructed cells in the lumen. Cells had shrunken in size exposing the intercellular space. Both secretory and fibrillar cells decreased in number. No nuclear damage was evident (Fig. 2e). In S. serrata zonal tubular damage was apparent in the animals exposed to 3 ml l\(^{-1}\) conc. Individual cells seemed to be normal except in necrotic region. Fibrillar cells decreased in number (Fig. 2f).

Discussion

The varied sensitivity observed in these species to toxic effects of oil may be attributed to the difference in habitat. S. serrata is an intertidal species and is rather sturdy in contrast to C. lucifera which prefers offshore waters. The higher degree of survival at elevated concentrations of oil can be due to the poor stability of the media. Greater stability of oil-in-water emulsion is reported\(^{10}\) at lower concentrations (0.01 to 0.1 ml l\(^{-1}\)). Solangi and Overstreet\(^{11}\) observed higher mortality rate in the fish Menidia beryllina exposed to 5\% WSF of Louisiana crude oil compared to those exposed to 50\% WSF. In the present study also the rate of mortality in both the species did not increase with the increase in oil concentration.

In Decapoda, hepatopancreas plays a parallel role of vertebrate liver and is the main site of the metabolism of petroleum hydrocarbons\(^{12}\). Significant pathological changes could be noticed in this tissue although it is not directly exposed to the water soluble pollutants unlike the gills. The tubular damage affected the structural integrity of this organ and might have impaired the digestive and assimilatory processes. The damaged respiratory surface (by the distruption of epithelium) together with the epithelial lifting—thus increasing the diffusion distance between water and blood—ultimately result in inadequate gas exchange. It is presumed that the excessive accumulation of haemocytes in the lamellae has probably impaired their function by altering the blood flow pattern. This in turn results in the failure of lamellar circulation and ultimately respiratory collapse. In addition to this oil is found to adhere to the respiratory surface as an effective barrier to the diffusion of gases. These factors further aggravated by the reduced gas exchange between air and water (due to the presence of oil film on the surface) hasten the death of affected animals.

The soluble fractions of crude oil, like phenol are known to damage the gill structure\(^{6}\). Similarly, the low aromatics present in oil are reported to have greater penetrating capacity not only to the whole organism but also into the lipid layers of cell membrane and to interfere with the enzyme systems and other proteins from a variety of marine plants and animals\(^{13,14}\). These observations are of extreme importance since any change in the fundamental structure of the membrane and enzyme system may result in the malfunctioning of the metabolic and homeostatic mechanisms ultimately leading to the expiration of the animal.

Acknowledgement

One of the authors (JPC) wishes to thank Dr B Patel and Dr (Mrs) L George of BARC for their critical comments on the manuscript.

References

1 North W J, Sea Front, 13 (1967) 212.  