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SEM Study on the Effects of Crude Oil on the Gills and Air Breathing Organs of Climbing Perch, *Anabas testudineus*

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During last decade, an increasing attention has been paid to the protection of aquatic environment against oil pollution both nationally and internationally. To indicate the degree of oil pollution, the fish has been used widely. Some of the workers (Moles et al. 1979, Woodward et al. 1981, 1983, Haensly et al. 1982, Solangi and Overstreet 1982, Prasad 1988) who studied the histopathological effects of crude oil at light microscopic level observed that the changes associated commonly with the gills were lesions, edema formation and mucous cell hyperplasia.

Ultrastructural studies on the effects of crude oil on the gills are scanty (Nuwayhid et al. 1980, Engelhardt et al. 1981). Recently, Prasad (1989) studied the effect of crude oil on the air breathing organs of striped gourami using scanning electron microscope and observed mucous cell hyperplasia coupled with telangiectasis in the epithelia of air breathing organs. The present investigation has been undertaken to study crude oil toxicity by observing the morphological changes occurring in the epithelia of gills and air breathing organs of climbing perch, Anabas testudineus at SEM level. Since the epithelia of gills and air breathing organs function in two different media, a comparative account of their sensitivity to crude oil solutions would be informative.

MATERIALS AND METHODS

Juvenile and adult specimens of Anabas testudineus were obtained from local suppliers and were maintained and reared in the laboratory aquaria. The fish was fed with tubifex worm and commercially supplied fish meal (Fishtone). The physico-chemical properties of aquaria water was as follows: dissolved oxygen, 7.3 ppm; free carbon dioxide, 2.1 ppm; total hardness as $CaCO_3$, 185 ppm; alkalinity as HCO_3, 104 ppm; pH, 7.9; and temperature, 28 ± 1°C.

Petroleum crude oil was obtained from Barauni (Bihar) oil refinery. The main fractions of this crude oil included hydrocarbons (30%), naphthalene and its alkyl derivatives (5%) and grease (15%), in addition to water contents. Different concentrations (100, 200, 300, 400, 500 ppm) of crude oil solutions were prepared in the laboratory with unchlorinated borehole water. Acetone was used as solvent of the crude oil. Details of the methods for preparation of crude oil solutions are described elsewhere (Prasad 1989). 150 specimens of the experimental fish were subjected to oil solutions. 10 fishes (average body weight 15.2 g) were exposed to each concentration of crude oil in 25-litre glass jars. Each set of the experimental jars was accompanied by two replicas and a control. The control jar received the equivalent amount of acetone which had been used in dissolving the crude oil. Exposure period varied from 12 h to 15 days. Fishes were fed on alternate day throughout the experiment. Test solutions were aerated twice a day for a duration of 30 minutes each time and were changed after every 24 h.

At different exposure periods, gills and air breathing organs were dissected out by killing the fish with a sudden blow on the head and were fixed in 2.5% glutarldehyde in 0.1M Na-phosphate buffer, dehydrated in graded ethanol series, and after critical point drying using CO_2 as the transitional fluid, sputter coated with gold in a gold coating unit. The processed tissues were examined in P-500 Scanning electron microscope operated at 25 kv with a spot size of 640 A°.

RESULTS

In SEM, the architectural pattern of gills of Anabas testudineus is essentially similar to that found in other air breathing teleosts. Secondary lamellae are plate-like projections at right angles to the gill filaments. It lies parallel to the adjacent lamellae and is covered over by a thick and coarse epithelium (Fig. 1). Numerous water pores and mucous cell openings with well developed microridges are discernible at the lamellae. The epithelial cells are large and appear as rounded structures (Fig. 2).

The air breathing organs consist of a suprabranchial chamber enclosing within it a labyrinthine organ borne by the epibranchial tissues of the first gill arch on either side of the pharynx. The lining epithelium of suprabranchial chamber and labyrinthine organ is differentiated into raised vascular areas (islets) and flattened non-vascular areas (lanes) (Fig. 3, 4). Gaseous exchange occurs at the raised vascular areas

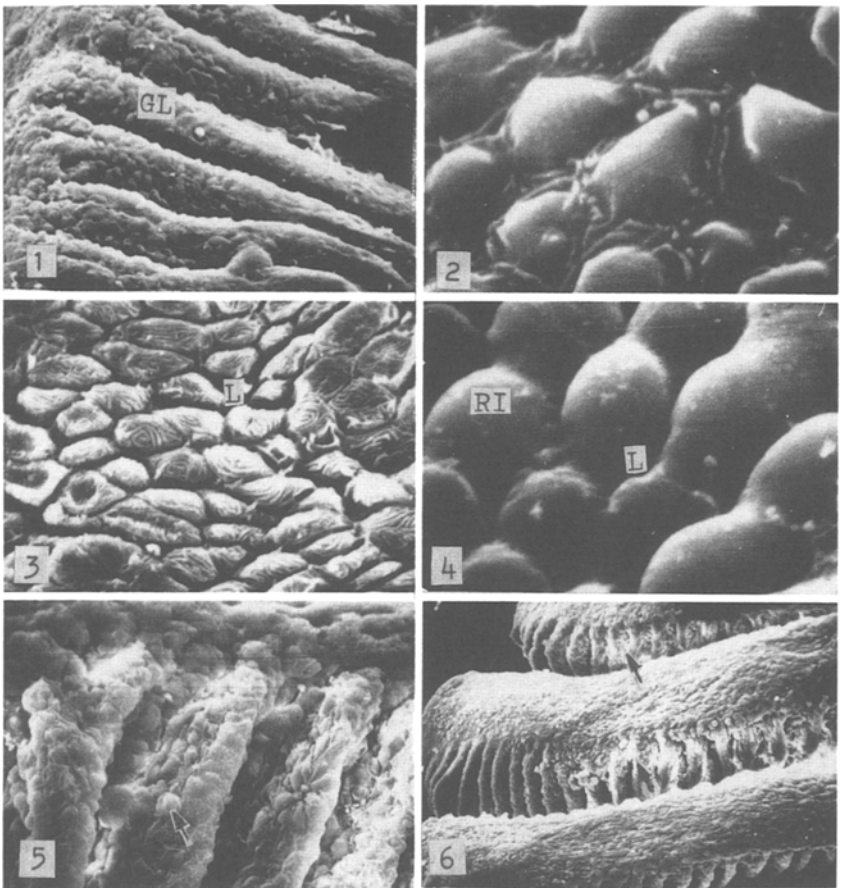

Figure 1. Normal (Control) gill lamellae (GL) with coarse epithelium. SEM X 400

Figure 2. Epithelial cells of the gill (Control), showing microridges. SEM X 6400

Figure 3. Suprabranchial epithelium (Control), showing respiratory islets (RI) and lanes (L). SEM X 3200

Figure 4. Labyrinthic epithelium (Control), showing respiratory islets (RI) and lanes (L). SEM X 6400

Figure 5. Gill lamellae after exposing the fish in 100 ppm crude oil for 48 h. Arrows indicate the swollen epithelial cells. SEM X 800

Figure 6. Gill filaments after 96 h of exposure in 200 ppm crude oil, showing hyperplasia of the epidermal cells (arrows). SEM X 200

which are packed closely in many rows and are perfused with blood from the arterioarterial pathway of the first and second pair of gill arches. The microridges on the vascular areas of suprabranchial chamber are well developed but are feebly discernible at the labyrinthine organs.

Fish exposed to crude oil solutions. The epithelial cells of the gills of fish exposed to 100 ppm solution of crude oil for 48 h, showed swelling of the epithelial cells (Fig. 5). However, the mucus secretion was not more than that in the normal fish. No changes were recorded at this concentration in the epithelia of suprabranchial chamber and labyrinthine organ even though the fish was exposed for a week or more. In 200 ppm, after exposing the fish for 96 h, the epithelial cells showed hyperplasia besides a thin coat of mucus over the gills. Hyperplastic epithelial cells were more pronounced in the middle of the filaments (Fig. 6). The microridges of the epithelial cells were found in degenerating condition (Fig. 7). The epithelia of suprabranchial chamber and labyrinthine organ still appeared to be unaffected except increase in the mucus secretion. After 1 week of exposure in the same concentration, the fish showed an accommodative tendancy by resuming their normal activities.

A thick coat of mucus covered the entire gill filaments and lamellae after 48 h of exposure in 400 ppm solution. The interlamellar space was filled either with hyperplastic epithelial or mucous cells as a result, the secondary lamellae lost their identity and the gill filaments along with the lamellae appeared as finger-like structures composed of spongy and soft substances. At some places, this soft spongy mass was shedding off (Fig. 8). Taste buds enlarged and bulged outwards on the gill arch epithelium (Fig. 9). Microridges present on the epithelium of the suprabranchial chamber disappeared. The vascular islets bulged and their epithelium showed a mild hyperplasia (Fig. 10). Extreme hyperplastic conditions of the mucous and epithelial cells were observed after 1 week of exposure in the same concentration. Gill epithelium was marked by deep lesions and erosion (Fig. 11). Swelling and hyperplasia of the epithelial cells of the labyrinthine organ were also pronounced (Fig. 12).

Gill tissue taken immediately after death of the fish by exposing them for 15 days or more in 500 ppm solution, showed a series of pathological alterations which included: (i) Lesions in the epithelial layer (ii) Hypertrophic mucous cells and sloughing of the epithelial layer and (iii) Vacualization in the substances of the gill lamellae.

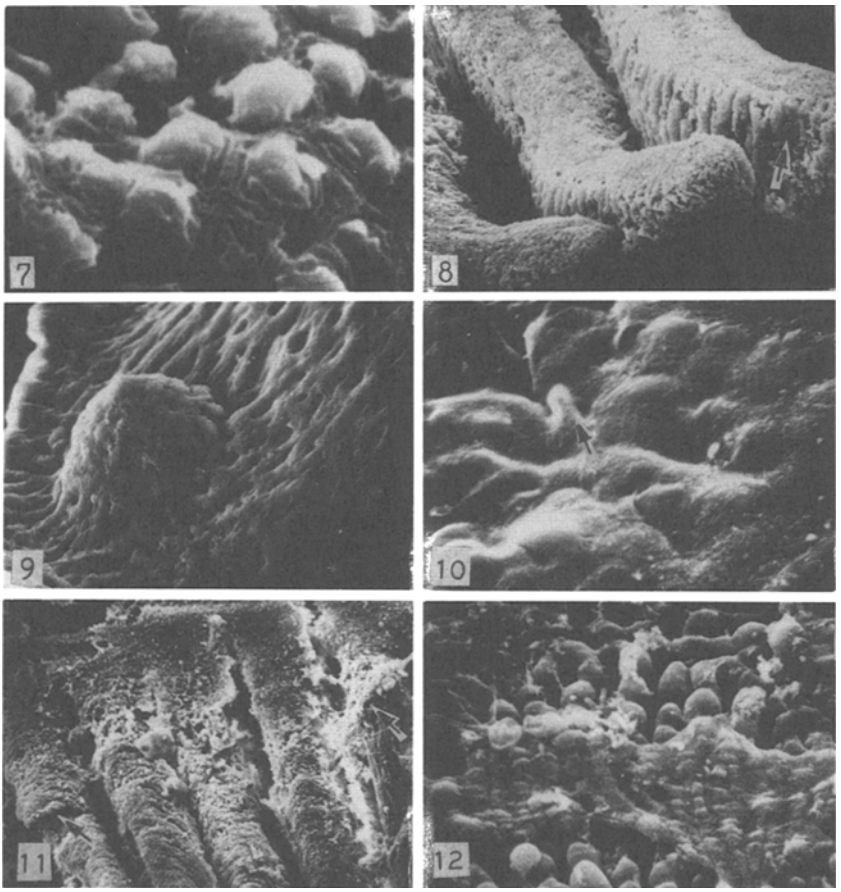

Figure 7. Degenerating microridges on the epithelial cells of the gill (concentration 200 ppm, exposure period 96 h). SEM X 6400

Figure 8. Gill filaments with mucus coat over them. Arrow indicates the sloughing of the epithelium (Concentration 400 ppm, exposure period 48 h).SEM X 200

Figure 9. An enlarged taste bud on gill arch epithelium after exposing the fish in 400 ppm for 48 h. SEM X 400

Figure 10. Suprabranchial epithelium with degenerating microridges and hyperplastic epithelial cells (arrows) after 1 week of exposure in 400 ppm crude oil.SEM X 1600

Figure 11. Gill filaments showing lesions (arrows) in 400 ppm crude oil after 1 week of exposure. SEM X 100

Figure 12. Hyperplasia and swelling of the epithelial cells of labyrinthine organ in the same fish.SEM X 400

DISCUSSION

Gills of fishes are highly susceptible to water soluble toxicants when immersed into it. In the initial hours of exposure, mucus secretion at the gill surface forming a thick coat over it is reported to be a protective device checking further penetration of the toxicants in fish tissue via the gills (Solangi and Overstreet 1982). Alterations in the ionic and osmotic homeostatis of the fish are caused when it is transferred to crude oil solutions (Malins 1982). The increased mucus secretion is also helpful in attenuating the osmotic influence of the environmental stress in teleosts gills.

Wooward et al. (1983) evaluated the toxicity and pathogenic effects of refined oil in cutthroat trout and found that the gills of fish exposed to 183 µg/L for 90 days showed hyperplasia of the gill lamellar epithelium. The concentrations used (24-183 µg/L) in their study were low as compared to the concentrations of present study (100-500 mg/L). In an another study, Woodward et al. (1981) reported hyperplasia, edema formation and fusion of gill lamellae after a 90-day exposure to 450 and 520 µg/L of a Wyoming crude oil. These observations were similar to those reported in this study.

Aromatic fractions of crude oil taken here for study were 30% thus constituting a major portion of the soluble fractions of crude oil. Although the total saturate fraction of Wyoming crude oil was 605 mg/g as compared to less than 1 mg/g in the refined oil, the concentration of major aromatic components - naphthalene and alkynaphthalene was only 18.4 mg/g in the crude oil but was 58 mg/g in the refined oil (Woodward et al. 1983). This seemed to be a plausible reason that why the lower doses of refined oil used by Woodward et al.(1983) showed similar pathological effects as produced by 500 mg/L of crude oil. This is also supported by the results of Anderson et al. (1974) who evaluated the effects of two crude oils and two refined oils and concluded that a higher concentration of naphthalene in refined oil was responsible for its higher degree of toxicity.

In ultrastructural studies made on the effects of crude oil, Nuwayhid et al. (1980) and Engelhardt et al.(1981) reported a damage to the gill epithelium and fusion of gill lamellae in the fish. These observations are comparable to the results obtained after exposing the fish in 100-300 ppm of crude oil. However, sloughing of the gill epithelia and exposure to the blood capillaries in higher doses (400 - 500 ppm) of crude oil confirmed the previous observations made on light microscopic level (Prasad 1988).

Respiratory epithelium of the air breathing organs remained unaffected in the concentrations less than 300 ppm and its sensitivity to crude oil solutions appeared to be less as compared to the gills. In spite of being derived from the gills during early ontogenesis of the fish (Singh and Mishra 1980), the air breathing organs are not in direct contact of the oil solutions as do the gills. The inhalent aperture of the suprabranchial chamber is kept closed by a valve-like structure (shutter) formed from the modified gill rakers of the first gill arch when the fish breathes exclusively through the gills (Singh and Mishra 1980). The opening and closing of the shutter are regulated by certain branchial muscles. In higher concentrations (400 - 500 ppm) of crude oil, functioning of the branchial muscles might be seized due to paralytic action of crude oil. At this stage, crude oil solution is forced into the suprabranchial chamber filling up the entire space and causing death by asphyxiation. In an another air breathing fish, Colisa fasciatus similar suggestions were made by Prasad (1989).

The swelling of epithelial cells and gill lesions are supposed to be associated with hydromineral imbalances when the fish is transferred to crude oil solutions. Hydromineral imbalance is believed to be related primarily to the gill lesions (Malins 1982). Due to hydromineral imbalance, the permeability of epithelial cells increases causing intake of water and increased turgid pressure within the cells. Ultimately, the increasing turgidity of the epithelial cells causes their exfoliation and thus turning into a gill lesion. Sloughing of the branchial epithelium in higher concentrations of crude oil is caused by poor blood supply. By the dissociation of gill epithelium a space is formed between the epithelial layer and underlying blood capillaries leading to scanty blood supply.

In spite of being an active site of uptake of water soluble fractions of crude oil, the gills are also known for elimination and depuration of petroleum fractions. Presence of a large number of metabolites in fish tissue (largely in liver and adipose tissue) help in degradation and elimination of oil fractions(Cravedi and Tulliez 1986). After prolonged exposure in crude oil solutions, an accommodative tendancy of the fish might be due to possession of such type of metabolites.

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