Bull. Environ. Contam. Toxicol. (1988) 41:754–758

Environmental Contamination and Toxicology

Sensitivity of Branchial Mucous to Crude Oil Toxicity in a Freshwater Fish, *Colisa fasciatus*

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In the maintenance of respiratory activity of the gills in fishes, mucus secretion is known to have an important function (Munshi and Singh 1968). The normal functioning of the mucous cells is influenced by pathogenic and environmental agents (Falk et al. 1962). Crude oils and their fractions enter the fish tissue by positive transfer via the gills (Corner et al. 1976) thus the mucous cells of gill epithelia act as an obvious site of pathogenic interactions between the fish and the environment. Mucous cell hyperplasia is a general phenomenon associated with crude oil toxicity (Haensly et al. 1982).

Even though a large quantity of crude oil pollutes the freshwater environment annually (Woodward et al. 1981), literatures on the pathogenic effect of oil pollutants on the branchial mucous cells of fishes are limited to the marine and estuarine species (Solangi and Overstree 1982). This investigation has been undertaken to provid a better understanding of the pathogenic effects of crude oil on the branchial mucous of a freshwater fish, Colisa fasciatus. The toxicity assessment is based on the histochemical observations of mucous cells present in the epithelia of gill rakers and filaments.

MATERIALS AND METHODS

Specimens of Colisa fasciatus were collected from fish suppliers and were maintained in the cemented cisterns with continuous water flow. Water of the cistern contained 6.8±0.2 pH at 25°C temperature. Crude oil was obtained from Barauni (Bihar) oil refinery where it is pumped from Naharkatiya (Assam) oil wells through an underground pipe-line. Different fractions of crude oil were analysed (Prasad and Kumari 1987)

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The main components of this crude oil include saturated and aromatic hydrocarbons (Pristane, phytane, benzene, toluene, naphthalene with its alkyl derivatives, phenolic compounds), sulphur, grease and cyanides (in traces) besides water contents. Sublethal (200, 500, 700 ppm) and lethal (1000, 1500 ppm) solutions of crude oil were prepared in the laboratory with borehole water following the methods of Prasad (1987).

Histochemical observations. A group of 6 acclimated fish (average body weight 1.90 g and length 4.1 cm) were exposed to each concentration for 2-24 h in lethal and upto 360 h in sublethal solutions. Each set of experiments was accompanied by a control. Control and treated fishes were killed by an abscission of the spinal cord, just posterior to the head and gills were promptly dissected out for histological and histochemical preparations. Histochemical observations of mucous cells were performed on the paraffin sections (5-8 µm) by using Periodic acid-Schiff (PAS), alcian blue (AB), PAS-AB combinations, toluidine blue (TB) and Mallory's triple stain.

RESULTS AND DISCUSSION

The mucous cells are numerous and more prominent at the epithelial covering of the gill rakers (Fig. 1) and gill filaments (Fig. 2) in control fishes. These cells are strongly PAS and AB positive, staining deep purple in PAS-AB sequence and are stained blue in Mallory's triple.

In 200 ppm, after exposing the fish for 15 days or more, almost no departure in the staining reaction occurs from those of the control fishes though enlargements in the size of mucous cells are apparent. In 500 ppm of crude oil solutions, the mucous cells react strongly with AB. After 96 h, the cells gradually enlarge in dimension and secrete copious amount of mucus (Fig. 3,4) forming a thick layer on the gill surface. In 700 ppm of crude oil, after 48 h of exposure, most of the mucous cells show a moderate intensity of purple-blue and the rest remain either light blue or unstained in PAS-AB. Though these cells are large in numbers, exhibit a comparative decrease in dimensions than that of the fishes kept in lesser concentrations.

AB staining in the lethal dose (1000 ppm) obtains different results, in that the secretory contents of the mucous cells at the gill rakers stain with AB but similar reaction is not discernible at the gill

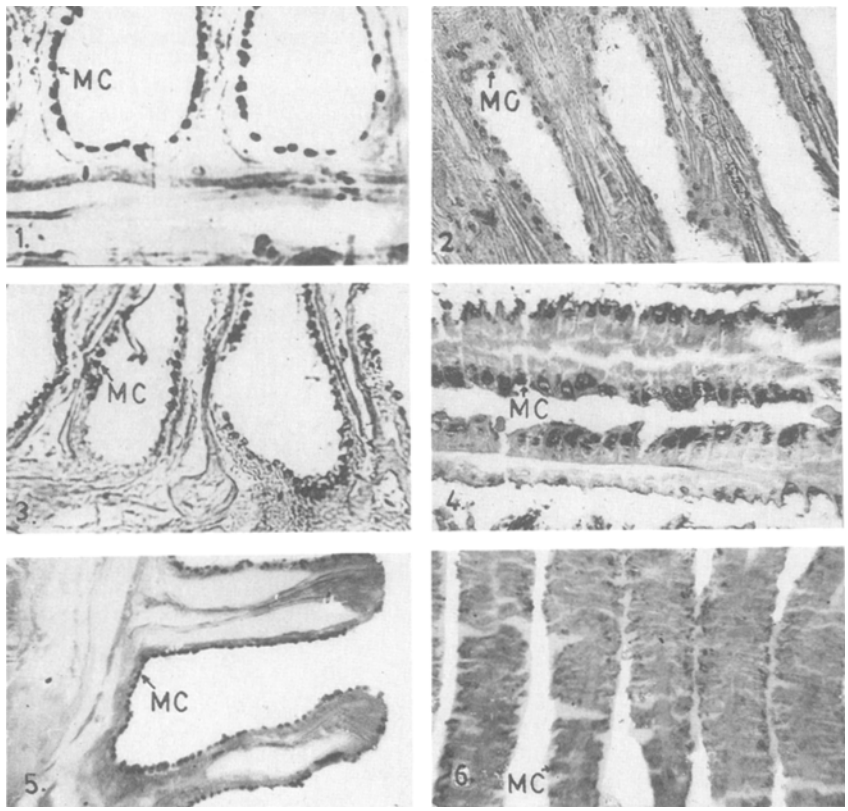

Figure 1. Normal distribution of mucous cells (MC) over the gill rakers of Colisa fasciatus (PAS-AB) X 250.

Figure 2. A part of normal gill arch showing distribution of mucous cells (MC) over the gill filaments (AB) X 250.

Figure 3. Hyperplasia of mucous cells (MC) over the gill rakers after 96 h in 500 ppm crude oil (PAS-AB) X 250.

Figure 4. Increased number and size of mucous cells (MC) in the epithelia of gill filaments after exposing the fish to 500 ppm crude oil for 96 h (AB) X 250.

Figure 5. Decreased size of mucous cells (MC) in the epithelia of gill rakers after 24 h of exposure in 1500 ppm solution of crude oil (PAS-AB) X 250.

Figure 6. Mucous cells (MC) of the gill filaments showing reduced number and size after 24 h of exposure in 1500 ppm crude oil solution (AB) X 250.

lamellae. Lesions in the gill epithelia and curling of the secondary lamellae are marked. In 1500 ppm after 24 h, the epithelia of the gill rakers as well as the lamellae have lost their color in PAS and/or AB. The mucous cells show a sharp decline in their density and dimension, being very small and few in both the epithelia of gill rakers (Fig. 5) and gill filaments (Fig. 6).

In TB, most of the mucous cells show B-metachromasia in normal or polluted (sublethal) environment but these cells in lethal concentrations show Y-metachromasia.

The predominant toxic effects of hydrocarbons on gills have been investigated to be hyperplasia and/or hypertrophy of mucous cells and the squamous epithelial lining, with hypersecretion of mucus (Haensley et al. 1982). Increase in the number of mucous cells and heavy mucus secretion at the gill surface is considered to be a common protective phenomenon in oil exposed gills (Solangi and Overstreet 1982). A thick coating of mucus over the branchial epithelia prevents oil fractions from their further entrance in the gills. However, the excessive coagulation of mucus over the gills impairs the respiratory function causing death by asphyxiation (Jones 1964). Transfer of the fish to crude oil solutions may alter their ionic and osmotic homeostatis (Malins 1982). The increased mucus secretion is helpful in attenuating the osmotic influence of the environmental stress in teleost gills (Jozuke 1966).

Density and dimensions of the mucous cells decrease in the lethal solutions of crude oil. The observed depletion of mucus secretion by different petroleum fractions has been supported by many investigators (Malins 1982). Woodward (1981) demonstrated hypertrophic chloride cells on the gill filaments and degenerating gill epithelium besides other pathological effects in cutthroat trout by higher concentrations of a Wyoming crude oil in water. The increased density of mucous cells approaches to the control fishes after long-term effects of crude oil (Haensley et al. 1982). Such type of depletion of mucous cells may be considered as an accommodative tendency of the fish to get some relief from stimulating agents.

Results obtained here suggest that the mucous cells try to attenuate the stress by increasing their density and secretory products in sublethal concentrations but this physiological adjustment seems transient when the stress is further increased. The inability of mucous cells in restoring PO_2 tension across the respiratory interface is greatly inhanced by the rupture of gill

epithelium and enlargement of the subepithelial space when encountered with the lethal doses of crude oil.

Acknowledgments : Thanks are due to Prof. B. R. Singh for laboratory facilities, Barauni Oil Refinery authorities for crude oil and the UGC, Govt. of India for awarding a Research Associateship.

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Received December 16, 1987; accepted June 16, 1988.