

Impact of diesel oil effluent in the mucosal surface of the alimentary canal of *Oreochromis nilotica* (Linnaeus): A scanning electron microscopic study

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Abstract: Adverse effects of diesel oil on microanatomical structure of the alimentary canal of *O. nilotica* were studied using SEM observations. The study revealed irregular arrangement of the stratified epithelial cells along with fragmentation of the normal concentric pattern of microridges of the same cells in buccopharynx and oesophagus. The excessive secretion of mucus of buccopharynx and oesophagus were the salient changes caused by diesel oil pollution. The destruction and degeneration of the mucosal folds of stomach and intestine along with their epithelial cells exhibited a concrete hyperactivity resulting in abundant secretion of mucus over the microridges of the epithelial cells. In the intestinal region the columnar epithelial cells showed tumefaction and microvilli of the plasma membrane of epithelial cells get heavily damaged. Disarray of the microridges of epithelial cells, excessive secretions of mucus formation of even cell sheet were the most conspicuous changes in rectum. It was concluded that chronic exposure of diesel oil may hamper the absorption of the nutrients through alimentary canal resulting into ill-growth and production of the fish.

Key words: Diesel oil, Impact, Alimentary canal, *Oreochromis nilotica*, SEM study.

Introduction

Diesel oil, natural petroleum, is a complex mixture of hundreds of different hydrocarbons but its bulk composition is remarkably constant about 85% carbon and 15% hydrogen. Diesel chains have 14 carbon atoms (C₁₄H₃₀). Diesel fuel is heavier and oilier than other petroleum oils. It is obtained from distillation or refining of crude oil *i.e.*, from petroleum products. Diesel requires less maintenance and generates energy more efficiently – with less CO₂ emission, but when uncontrolled, emits harmful particulate matter, nitrogen oxides and a variety of carcinogenic substances. In spite of the lower CO₂ emissions and economically profitable, to-day's diesels are more harmful to human health as well as aquatic life. Petroleum discharge containing hydrocarbons is considered a worldwide problem (Pauluis, 1979). Fishes absorb those hydrocarbons and can carry them (including carcinogenic polynuclear aromatics) to human through food chain. Several authors have studied the chronic effects of petroleum hydrocarbons on feeding, growth, reproduction, and tissue damage of fishes (Haensley *et al.*, 1982; Khan and Kiceniuk, 1984; Kiceniuk and Khan, 1987; Solangi and Overstreet, 1982). It is estimated that in case of water pollution, around 15% of the total petroleum input to aquatic systems is from natural sources and the remaining 85% is due to anthropocentric causes. When a petroleum product is spilled in or on water, a series of changes occur to its physical and chemical properties. Therefore, aquatic pollution by natural petroleum products now a day is one of the most threatened issues for the aquatic organisms particularly to the fishes occupying the surface layer (Khan, 1999). Some chronic effects of diesel oil fractions on gill, liver and spleen were also recorded by Alkindi *et al.*, (1996) and Kohler and Pluta (1995).

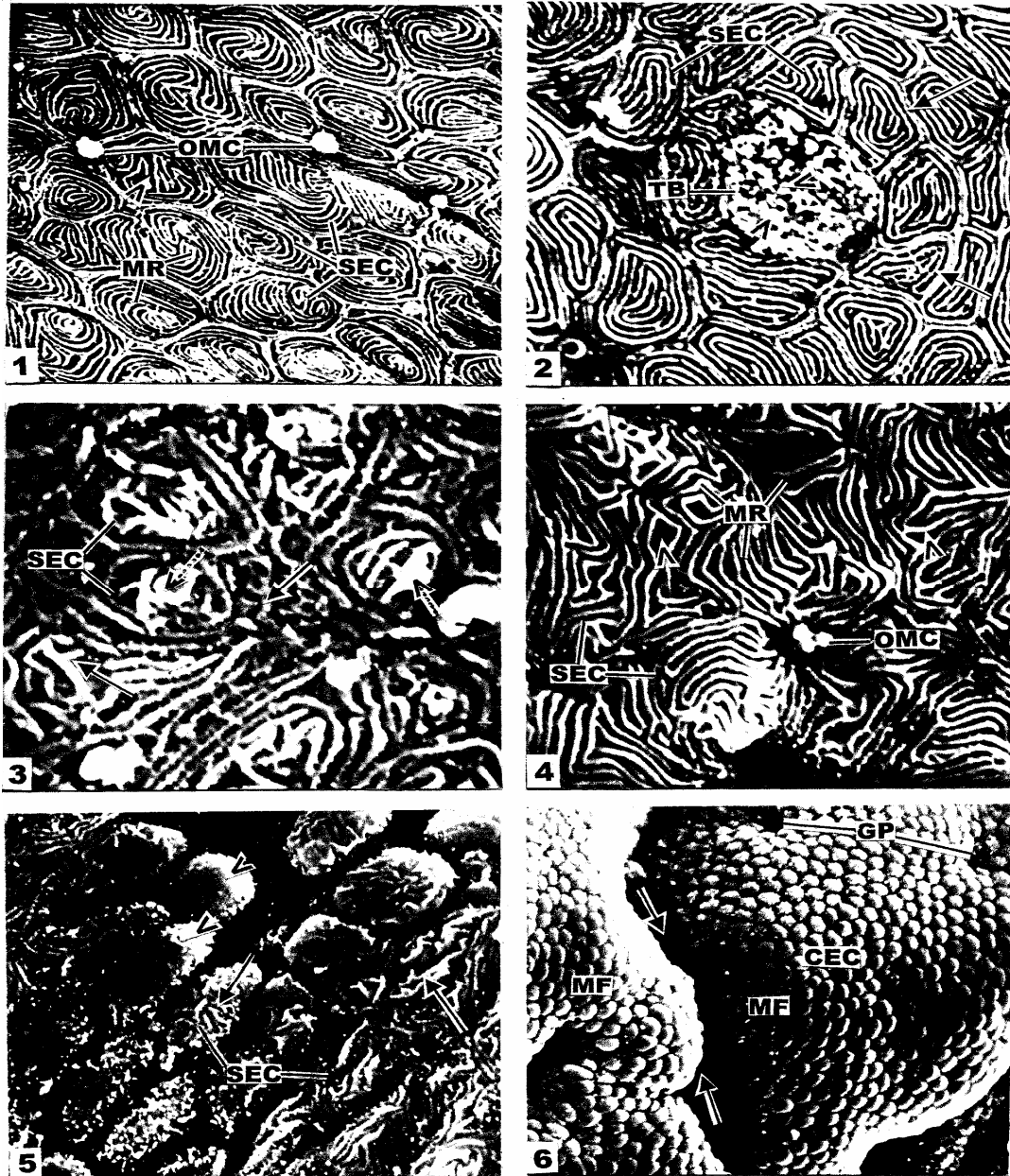
The objective of the present study is to highlight the effect of diesel pollution on the alimentary canal of freshwater teleost *Oreochromis nilotica*, using SEM for histopathological observations.

Materials and Methods

Only adult, healthy *Oreochromis nilotica* (Fam. Cichlidae Peters) were collected from both the nearby control pond and the Loco Tank near Bardhaman Locomotives contaminated with diesel fuel. The average length of the fish is 21.0±2cm and wet weight was 50±5g. Both the control and diesel infested fish were anaesthetized with tricaine methanesulphonate (MS 222) and the representative portions of the alimentary canal *viz.*, buccopharynx, oesophagus, stomach, intestine and rectum were incised longitudinally, spread out and pinned exposing luminal surface upper side on the cork sheets. The adhering mucus of the luminal surface was removed by rinsing in heparinized saline. After rinsing again with 0.1-mol/l cacodylate buffer of pH 7.5, the tissues were fixed in 2.5% glutaraldehyde for 24hrs at 4°C, post-fixed in 1% OsO₄ (osmium tetra-oxide) for 2hrs, dehydrated through graded acetone, and subsequently followed by amyl acetate and subjected to critical point drying (CPD) [Pressure – 72.8kg/cm², Temperature – 31°C] with liquid carbon dioxide. After drying, the tissues were mounted on metal stubs, coated with gold, and were examined under S-530 Hitachi SEM.

Results and Discussion

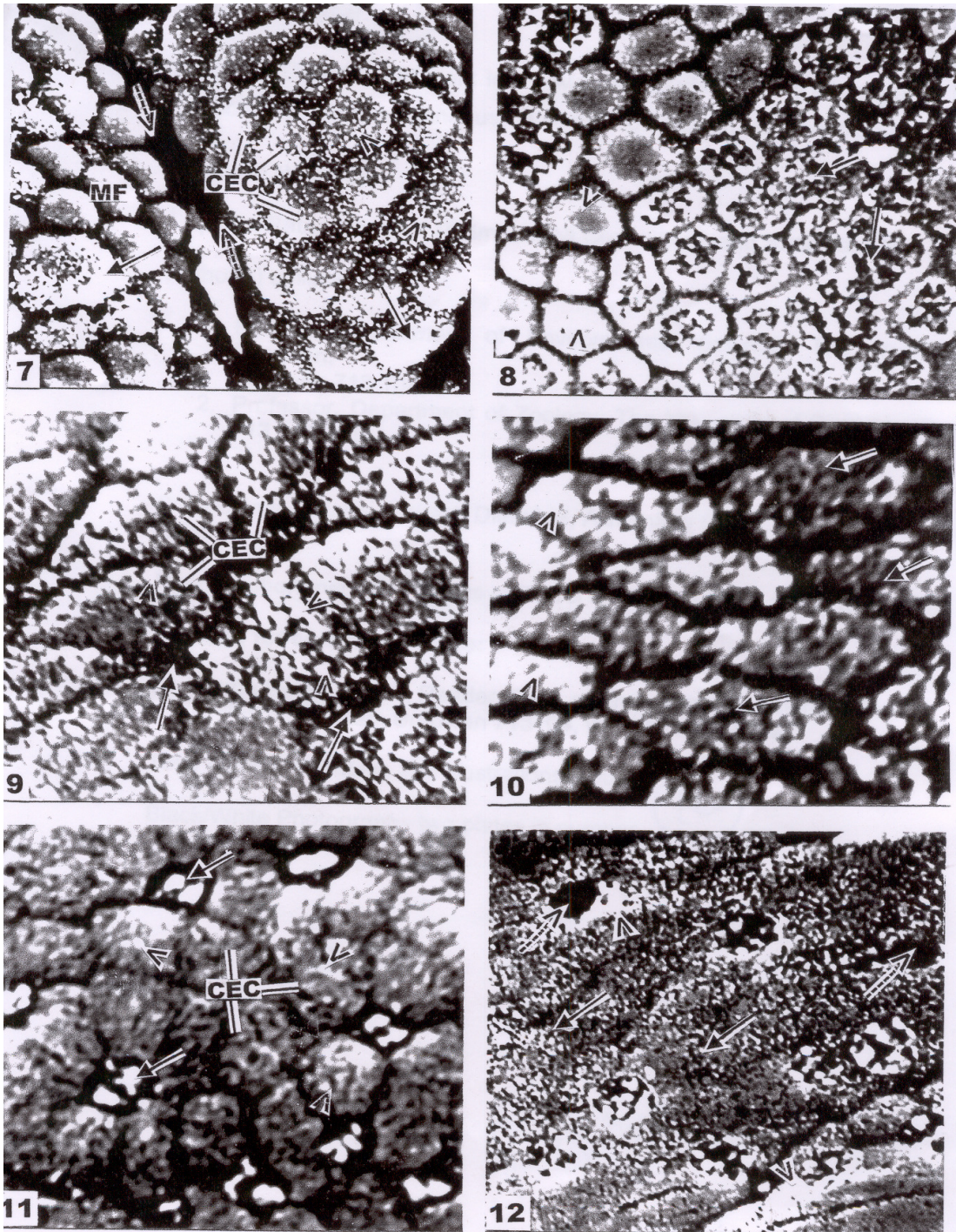
The amount of oil and grease present in the water sample at Loco tank was measured 112ppm (Partition–Gravimetric Method, APHA, 1998) whereas the permissible limit in the surface of inland water is only 10ppm, public sewers



Figs. 1-12 : Scanning electron micrographs of various regions in the alimentary canal of control (C) and diesel oil (Do) treated *Oreochromis nilotica*.

- 1) Mucosal surface showing stratified epithelial cells (SEC) provided with microridges (MR). Arrow heads indicate deep channels between MR. Note the opening of mucous cells (OMC) at the cell junction (Buc) (C)X3200.
- 2) Showing apical view of taste bud (TB) located in between SEC. Note the presence of densely packed projections (arrow heads). Arrows indicate high double ridged structure (Buc)(C)X3200.
- 3) Showing disruption of the regular arrangement of MR in SEC (Solid arrows). Note the excessive deposition of mucus over the SEC (broken arrows) (Buc)(Do)X6400.
- 4) Luminal surface showing SEC provided with wavy and linearly arranged MR. Note the channels in between MR (arrow heads). Note also presence of OMC (Oeso) (C)X4000.
- 5) Showing shrinkage and damage of SEC. Note necrosed MR of SEC (arrows). Arrow heads indicate retention of mucin mass on SEC (Oeso)(Do)X3200.
- 6) Luminal surface showing furrows (arrows) located between mucosal folds (MF). Note the presence of gastric pits (GP) encircled by columnar epithelial cells (CEC) (Sto)(C)X600.

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- 7) CEC provided with stubby MR (arrow heads). Note the presence of mucin deposition over CEC (solid arrows). Broken arrow indicate furrow in between MR (Sto)(Do)X1600.
- 8) Showing fragmentation and loss of MR of the CEC (arrows). Note excessive deposition of mucin over CEC (arrow heads)(Sto)(Do)X1600.
- 9) Elongated or oval elevations provided with prominent microvilli (MV)(arrow heads) representing the apical surface of CEC. Note the presence of OMC (arrows) (Int)(C)X3200.
- 10) Showing erosion and loss of identity of MV over CEC (arrows). White patches indicate secreted mucin over CEC (arrow heads) (Int)(Do)X3200.
- 11) Oval or rounded CEC provided with short and thick MR (arrow heads). Note mucus plug in the OMC (arrows) (Rec)(C)X3200.
- 12) Showing loss of identity of CEC due to disruption of MR over CEC. Note the disruption of MR over CEC. Note the formation of even cell sheet of CEC (solid arrows). Note the deposition of mucin over CEC (arrow heads). Broken arrows indicate opening of empty mucous cells (Rec)(Do)X3200.

20ppm, land for irrigation 10ppm, marine coastal areas 20ppm. This pond is continuously receiving diesel fuel discharged from the diesel shed of the Locomotive. During rainy season, the diesel oil spread all over the pond may be due to wind action, on the other hand, the expelled diesel particularly remain aggregated on the South and Southwest parts of the pond. The rate of production of *O. nilotica* is approximately 30% of the total annual production.

The pH of the pond water is alkaline (pH7.5). Dissolved oxygen concentration (2.1 to 2.5mg/l) always remains below the optimum level, ammonia-nitrogen (NH₃-N) in an enhanced concentration (11.5 to 14.6mg/l) and phosphate-phosphorus (PO₄²⁻-P) content of the water is 0.07 to 0.7mg/l. *O. nilotica* is herbivorous and omnivorous (Panikkar and Tampi, 1954) and feeds mainly on unicellular algae, filamentous algae, and detritus food materials. In the present observations, ultrastructural levels in the different parts of the alimentary canal through SEM have been highlighted.

Buccopharynx: The luminal surface of the buccopharyngeal epithelium is provided with a number of prominent folds comprising of pentagonal and /or hexagonal stratified epithelial cells (SEC). The apical surfaces of the stratified epithelial cells appeared in the form of labyrinth-patterned microridges of 10 - 15 μ in length (Fig. 1). The microridges appear to be regularly spaced leaving long and deep channels in between them (Figs. 1&2). The outermost microridges of particular cell fused with the same of the neighbouring cell forming a thickened boundary (Fig. 2). Few wart-like structures located at cell junctions represent the opening of the mucous cells (Fig. 1). A few discrete areas mark the apical pores of taste buds provided with various projections (Fig. 2).

The major changes observed due to diesel pollution are wrinkling and irregular arrangement of SEC and reduction of the cell size. The lateral connections between the adjacent SECs become indistinct and damaged. The regular arrangement of the microridges is disrupted (Fig. 3). As a result of toxicity, the mucous cells exhibited maximum secretion of mucus which glides over the adjacent stratified epithelial cells (Fig. 3).

Oesophagus: The internal mucosal surface of oesophagus is oriented with regularly spaced elongated pentagonal and / or hexagonal epithelial cells. The luminal plasma membrane of these cells presented thick and linearly arranged microridges leaving long and deep channels in between them to hold more mucin (Fig. 4).

Owing to chronic exposure of diesel oil the oesophageal epithelium exhibits disorientation in arrangement of the epithelial cells. The stratified epithelial cells undergo shrinkage and severe damage in the microridge structures (Fig. 5). The necrosed microridges are evident over the cell surface (Fig. 5). An increased mucous cell activity is demonstrated by the deposition of heavy secretion of mucus from the neighbouring mucous cells (Fig. 5).

Stomach: The topographical study reveals that the gastric mucosa is provided with numerous gastric folds which

anastomose with each other to form irregular depressions (Fig. 6). Few gastric pits surrounded by the epithelial cells have also been detected in this region (Fig. 6). The apical surface of mucous folds exhibits densely packed oval or rounded columnar epithelial cells that are embraced with short but stubby microridges. Mucin mass is also found to be adhered to the epithelial surface (Fig. 7).

In the stomach, the most prominent alterations are disarrangement of mucosal folds. The gastric epithelium undergoes severe damage showing fragmentation of the cells exposing the lamina propria into the lumen. The apical plasma membrane of the epithelial cells shows fragmentation and loss of microridges (Fig. 8). The cell boundary in a number of the epithelial cells gets ruptured and cellular contents have a tendency to move away from the cells into the lumen. Vigorous secretion of the mucus from the epithelial cells spreads over the cell surface covering the microvilli (Fig. 8).

Intestine: The most characteristic feature of the intestine is the presence of irregular, wavy mucosal folds in the fashion of zig-zag pattern enclosing a number of concavities in between them. The internal mucosa of the intestine is supported by regularly packed, elongated or oval columnar epithelial cells measuring 4 - 6 μ in diameter. The apices of these epithelial cells are provided with densely oriented microvilli (Fig. 9), which are actually increasing the cell surface area for necessary absorption. The orientations of the epithelial cells are interrupted in certain areas due to presence of mucous cells (Fig. 9).

Due to effect of diesel oil pollution, orientation of the mucosal folds become totally interrupted resulting into formation of debris of fragmented secondary folds filling the concavities in between the folds. The apical plasma membrane of the columnar epithelial cells of the intestine is severely damaged showing the exposed cell surface. The microvilli of the plasma membrane of the epithelial cells get heavily damaged (Fig. 10). Excess secretion of mucus indicates a severe response of diesel effect (Fig. 10).

Rectum: The internal mucosa of the rectum is provided with many folds anastomose with each other to form a complex reticulated pattern leaving shallow irregular depressions. The luminal surface of the rectum is distinguished into round or oval columnar epithelial cells. The apical surface of the cells exhibits regularly spaced short and thick microridges (Fig. 11). Opening of the mucous cell interrupts the orientation of epithelial cells (Fig. 11). Maximum disruption in the mucosal folds results in the formation of cellular debris. Necrosis of the columnar epithelial cells resulted into formation of mosaic structure leaving a number of openings of the mucous cells. The luminal plasma membrane of these cells is provided with disrupted microridges where the cells become obliterated and degenerated (Fig. 12). In some areas, the microridges of the columnar epithelial cells undergo disintegration resulting into formation of even cell sheet (Fig. 12).

According to oceanographers, in some cases, between 3 and 6 million metric tons of diesel oil are discharged

in the world's oceans each year from both land and sea operations. A significant quantity of oil is also released from various industrial operations viz., petroleum refinery, metal industries, coke plants, meat and fish processing units, woolen textiles, and paint industry. Petroleum products may be spilled, leaked, dumped, pumped or simply thrown away on lands or surface water body. Influence of petroleum refinery effluent was found to be significant on zooplankton, alkalinity and hardness of the polluted waters (Saha and Konar, 1985). They observed that increase of alkalinity and hardness was due to their much higher concentration in the refinery effluent compared to the receiving estuarine water. The freshly spilled oil in the water, after interacting with the prevailing physico-chemical conditions and biological factors causes compositional and chemical changes in the receiving water. In addition to the surface oil, some of the components may settle to the bottom or may be deposited on the edge of the pond. Oil pollution can have both short-term acute and long-term chronic effects on the aquatic environment. Oils affect the organisms in a number of ways depending upon the characteristics of the oil fractions and their concentration in water (Khan, 1999). Oils have smothering (suffocation) effects on most of the aquatic animals including fishes. Oil layers increase the water temperature, which may be critical for several organisms. Besides, microbial degradation of oil in water also consumes substantial quantities of oxygen making the conditions further severe for the very small, microscopic forms of life to the larger marine mammals. Due to floating and spreading nature of petroleum products, it can destroy or damage the phytoplankton and most species of microscopic algae. Zooplankton population particularly the crustaceans, especially the copepods and cladocerans, appear to be highly sensitive to oil pollution. Decrease of plankton in the estuary may affect food chain which may cause reduction in fish production in the long run (Saha and Konar, 1985). At the microscopic level, the 'neuston' are completely destroyed by floating oil. Fishes being the first level of consumers of the food chain, accumulation of aquatic pollutants occurred in fish tissues in highest concentration through ingestion of planktons and other organisms involved in their food chain. Different authors have studied accumulation of number of pollutants like heavy metals, pesticides, etc., in different fishes occupying different trophic levels. The effects of oil on lipids are also manifested in the development of eggs and larvae following oil spills. Oil on the surface of water decreases filter-feeding capacity, survival and fecundity in animals (Alkindi *et al.*, 1996). Most of the oil fractions are normally lipophilic in nature with a very low solubility in water, and tend to accumulate in fatty tissues of the organisms. The absorption mode of the oil may be through respiratory surfaces, the gastro-intestinal tract, and external surfaces. Fishes are efficient in converting HCs they ingest or absorb into metabolites. The benthos will also be destroyed when the oil settles to the bottom or forced to settle through the use of sinking agents.

Effects of the exposure of *Mytilus edulis* to diesel oil (27.4 - 127.7 µg/l) in seawater for a period of 6- and 9-months

have been studied by Lowe and Pipe (1987). Some quantity of diesel oil present in water can enter the alimentary canal of the fish through consumption of food chain organisms and can adversely affect the process of digestion of food materials and absorption of nutrients by the intestine.

The results obtained in the present study showed severe pathological lesions in the various regions of the alimentary canal due to diesel oil. The exaggerated secretion of mucus throughout the length of alimentary tract is caused by exocytosis probably due to change of the luminal environment. The microridge structure of the stratified epithelial cells of the buccopharynx and oesophagus anchors the mucin blanket and plays an important role in the lubrication of food materials, and protection of cell membrane from mechanical rubbing. Disintegration of microridges of the epithelial cells of the aforesaid regions can thus reduce the retention ability of mucus, which may affect the transmission of food to the next region. According to Chakraborti and Sinha (1987), neutral mucopolysaccharide film over the microridges of the columnar epithelial cells of gastric mucosa in carnivorous fish protects the epithelial cells from injuries caused by acid. The present study depicts the deterioration of microridges of the epithelial cell due to diesel oil pollution in the stomach. Such topological alterations concomitantly reduce the protection ability of the gastric epithelium against acid injuries and cell lysis. In the intestine, the regular arrangement of the microvilli possibly perform at least two functions, namely, the anchoring of the mucus film and increasing the surface area for effective absorption of various macromolecules (Yamamoto, 1966; Kremenetz and Chapman, 1975; Sinha and Chakraborti, 1985). Thus, the present observations suggest that, severe alterations in the intestinal columnar epithelial cells may diminish the absorption capacity of nutrients from lumen to cell interior. Crespo *et al.* (1986) also noted the impairment of the intestinal absorption due to the treatment of sublethal concentration of cadmium.

The mucosal folds of the rectum help in the final expulsion of faecal matter and the mucus spread over the microridges provide easy defaecation of the faecal matter. In the present observation, damage in the mucosal folds and microridges of the columnar epithelial cells may also probably cause impairment in the defaecation capacity of the region concerned.

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