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Research Article

Surface Morphological Changes Induced by Electroplating Industrial Effluent in the Intestine of *Cyprinus carpio*

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Abstract

Background and Objective: Effluents of electroplating industry containing heavy metals put forth a huge impact on aquatic organisms. Heavy metals are hazardous to many organisms even at low concentrations and can stimulate biochemical, histological and morphological changes in fish tissues which can impair fish quality. Hence, the present study aimed to analyze the surface morphological changes in the intestine of the freshwater fish, *Cyprinus carpio* (*C. carpio*) induced by different sublethal concentrations of electroplating industrial effluent. **Materials and Methods:** Healthy fish were exposed to the different sublethal concentrations (0.004, 0.007, 0.010 and 0.013%) of effluent from electroplating industry for 7, 14, 21 and 28 days. After each exposure period, fish were taken out, sacrificed and the intestine was subjected to scanning electron microscope. **Results:** There were significant ultrastructural changes in the intestine like degenerated epithelium, damaged microvilli, disarranged mucosal folds, damaged columnar epithelial cells and excess mucous secretion as evident from Scanning Electron Microscope (SEM). **Conclusion:** The *C. carpio* exposed to the sublethal concentrations of effluent from electroplating industry for 7, 14, 21 and 28 days exhibited surface morphological changes in the intestine which can be used in pollution monitoring.

Key words: Electroplating industrial effluent, scanning electron microscope, intestine morphology, heavy metals, *Cyprinus carpio*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water is an essential constituent of life support systems and its quality plays a pivotal role in the maintenance of aquatic health. Unfortunately, rapid industrialization, population explosion and non-judicious use of natural resources have resulted in many fold increase in water pollution problems. Industrial effluents are the major source of water pollution besides sewage, agricultural runoff and other household residues. Electroplating industrial effluents contain various toxic metals like chromium, nickel, copper, zinc, lead, cadmium and aluminium. These pollutants alter the natural conditions of aquatic medium and cause behavioral changes as well as morphological imbalance in aquatic organisms[1]. Fish are considered to be the most significant biomonitors in aquatic systems for monitoring metal pollution[2,3]. Heavy metals can be taken up by fish either through ingestion of contaminated food via the alimentary tract or through the gills[4,5]. Accumulated heavy metals lead to morphological alterations in the tissues of fish[6].

Previous studies evidenced ultrastructural alterations in fish gills on exposure to nickel and hexavalent chromium[7], cadmium[8,9], cadmium and zinc[10], coal mining effluent[11] and copper[12] but few of them addressed the intestine[13-16]. The histopathological lesions in gills of *Mystus cavasius* [17] and *Oreochromis mossambicus*[18] exposed to electroplating industrial effluent were studied whereas, the reports about effects of electroplating industrial effluent are not available on intestine of fish. Therefore, the objective of the present study was to highlight the effect of electroplating industrial effluent on the surface morphology of the intestine of the fresh water fish, *Cyprinus carpio* using scanning electron microscope.

MATERIALS AND METHODS

Fish maintenance: Healthy *C. carpio*, weighing 25±5 g, irrespective of sex were collected from a local fish farm and were acclimated to laboratory conditions in aerated dechlorinated tap water for 15 days in fibre tanks (150 L capacity).

Test sample: The effluent sample from the direct outlets of an electroplating industry near Choolaimedu at Chennai during normal operation period was collected and transported immediately to the laboratory and stored in a refrigerator. The physico-chemical parameters of the effluent were analyzed (Table 1) adopting standard methods[19]. The median lethal concentration (LC_{50}) values for 24 (0.203%), 48 (0.158%), 72 (0.132%) and 96 h (0.128%) exposure to electroplating industrial effluent were calculated using probit analysis. After assessing the lethal concentration range, sublethal concentrations of 0.004, 0.007, 0.010 and 0.013% of electroplating industrial effluent were selected for further study.

Table 1: Physico-chemical parameters of the electroplating Industrial effluent

Parameters	Electroplating effluent
pH	<1
Turbidity (NTU)	<1
Total dissolved solids (mg L^{-1})	105320
Chlorides (mg L^{-1})	291
Sulphates (mg L^{-1})	3571
Fluoride (mg L^{-1})	400
Sodium (mg L^{-1})	4491.95
Copper (mg L^{-1})	335
Cadmium (mg L^{-1})	<0.05
Chromium (mg L^{-1})	36756
Lead (mg L^{-1})	11.64
Nickel (mg L^{-1})	27562
Potassium (mg L^{-1})	289.35
Manganese (mg L^{-1})	0.94
Iron (mg L^{-1})	16.48
Aluminum (mg L^{-1})	9.84
Calcium (mg L^{-1})	265
Magnesium (mg L^{-1})	107

Experimental design: The experiment was conducted from 1 October-30 November, 2013. After acclimation, 5 groups of fish, one for control and others for 0.004, 0.007, 0.010 and 0.013% of electroplating industrial effluent sublethal concentrations were recruited and treated for 28 days in the laboratory of Zoology Department at Jayaraj Annapackiam College for Women, Periyakulam, India. Each tank housed ten fish (n = 10) and the effluent concentrations in the test chambers were renewed every day. Feeding was allowed for both experimental and control fish throughout the tenure of experiment. After 7, 14, 21 and 28 days of exposure, fish from each concentration as well as control were dissected and the intestine was excised out.

Ultrastructural analysis: For scanning electron microscopic study, fragments of intestine were fixed in 2.5% glutaraldehyde solution for 24 h at 4°C and washed with phosphate buffer (pH 7.2-7.4). The fixed material was dehydrated in a graded series of ethanol and kept in 100% acetone. Following dehydration, the tissues were critically point dried (CPD), mounted on aluminium specimen holders and coated with gold (100A°) in ion sputter unit. These tissues were examined under scanning electron microscope (JEOL JSM-6390 SEM) and were recorded on photographic films at different magnifications (200, 2000, 5000 and 7000X).

RESULTS AND DISCUSSION

The results obtained in the present study showed severe pathological lesions on the surface morphology of the intestine of *C. carpio* exposed to electroplating industrial effluent. The scanning electron micrograph of intestinal tissue isolated from untreated (control) fish showed mucosal folds supported with regularly packed, round or oval columnar epithelial cells. The microvilli of the epithelial cells are short and compactly arranged on the apical surface of the absorptive columnar epithelial cells (Fig. 1).

Intestine is one of the main routes through which toxicants enter into the body and in direct contact with toxicants. The small intestine is the place where terminal digestion of food and absorption of nutrients happen. The nutrients present in the food are absorbed by the cells of the epithelial lining[20]. Any damage to the intestinal lining can be a good indicator of toxicity of the xenobiotic to animals. The effects of different xenobiotics on fish alimentary canal were reported[21-25].

But study on the effects of electroplating industrial effluent on fish alimentary canal is very rare. In the present study, severe pathological lesions and secretion of mucus were observed in the intestine of *C. carpio* on exposure to electroplating industrial effluent in the laboratory.

Disarrangement of mucosal folds was observed in fish exposed to 0.004, 0.007, 0.010 and 0.013% of electroplating industrial effluent after 7 days (Fig. 2, 3, 4 and 5). After 14 days, the toxicity of electroplating industrial effluent loosened the structural arrangement and damaged the columnar epithelial cells (Fig. 6, 7, 8 and 9). Ghosh and Chakrabarti[26] observed similar pathological lesions through scanning electron microscopic investigation on the gut of *Notopterus notopterus* after arsenic exposure. Bose[27] reported damages in different regions of alimentary canal of *Anabas testudineus* with scanning electron microscopic study after lead and cadmium treatment. The effect of

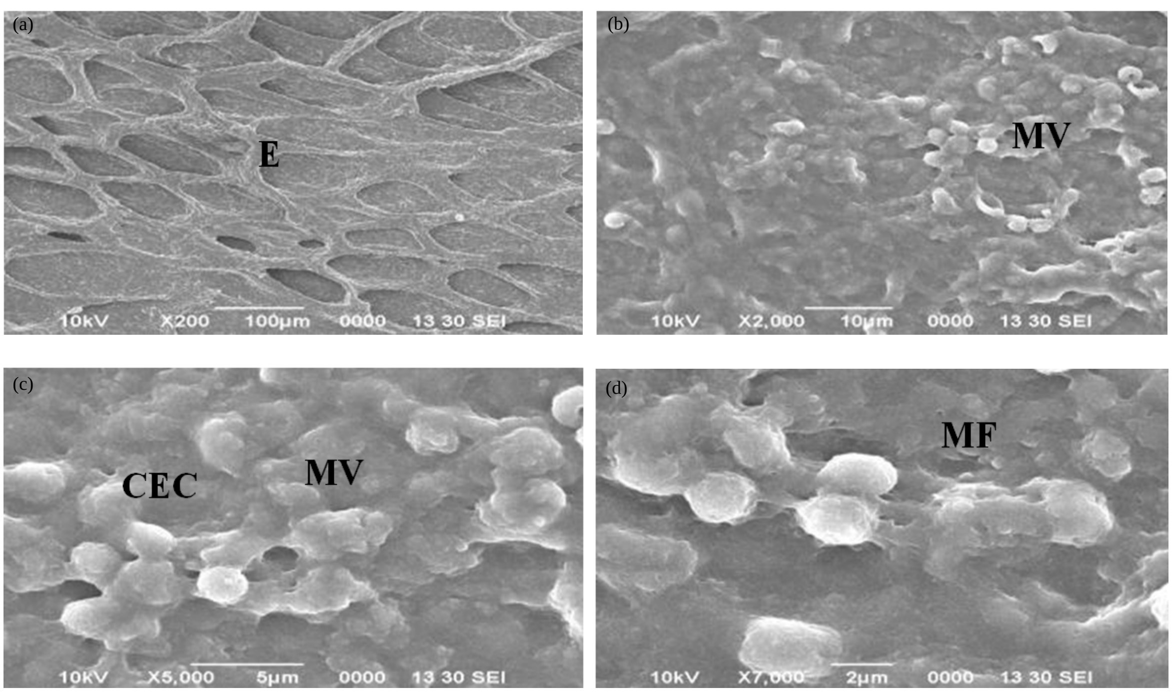

Fig. 1(a-d): Scanning electron micrographs (SEM) of intestine of untreated *Cyprinus carpio* (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

E: Epithelium, MV: Microvilli , MF: Mucosal fold, CEC: Columnar epithelial cell

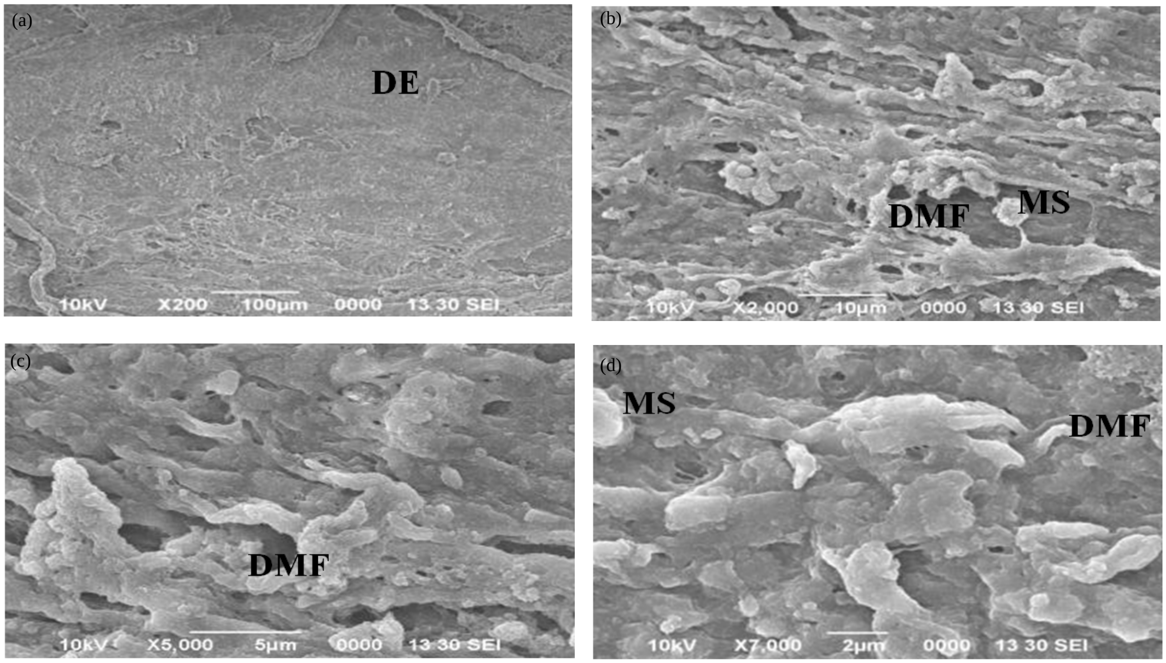

Fig. 2(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.004% electroplating industrial effluent for 7 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X
DE: Degenerated epithelium , MS: Mucus secretion, DMF: Disarranged mucosal fold

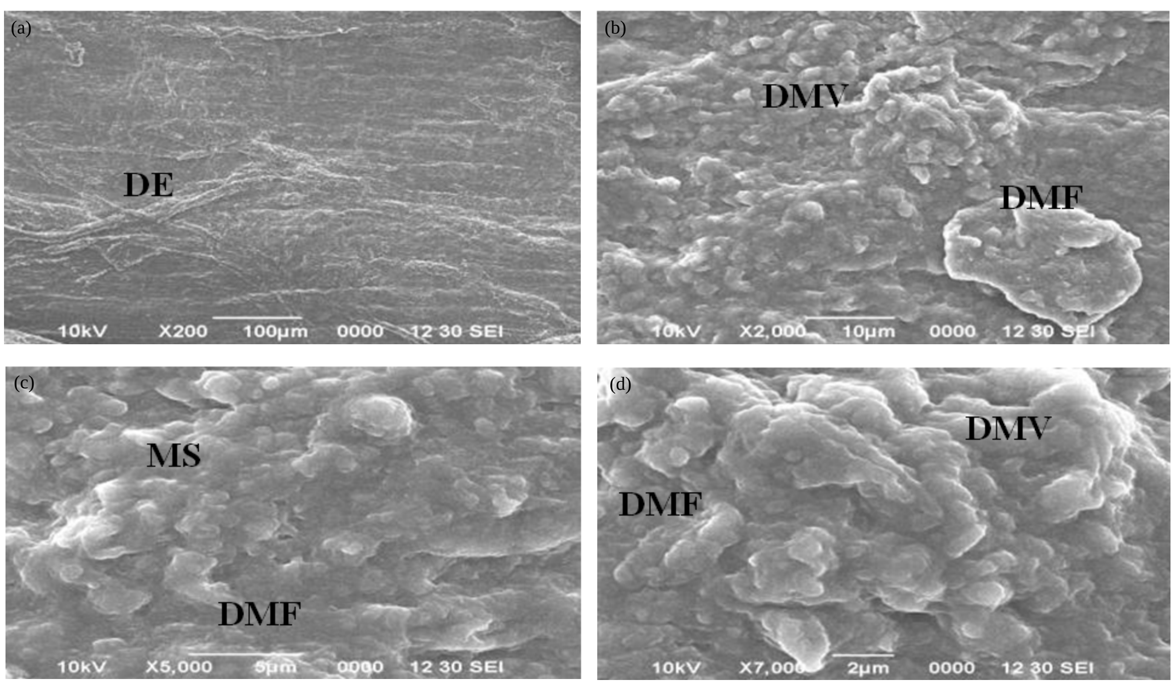

Fig. 3(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.007% electroplating industrial effluent for 7 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X
DE: Degenerated epithelium , MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold

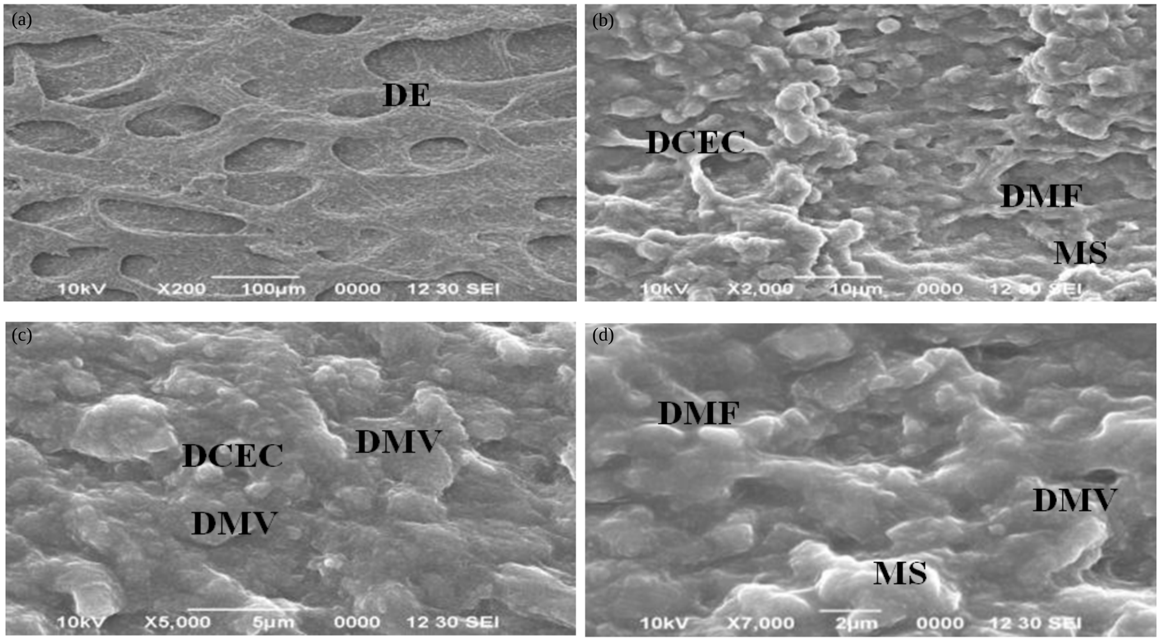

Fig. 4(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.010% electroplating industrial effluent for 7 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

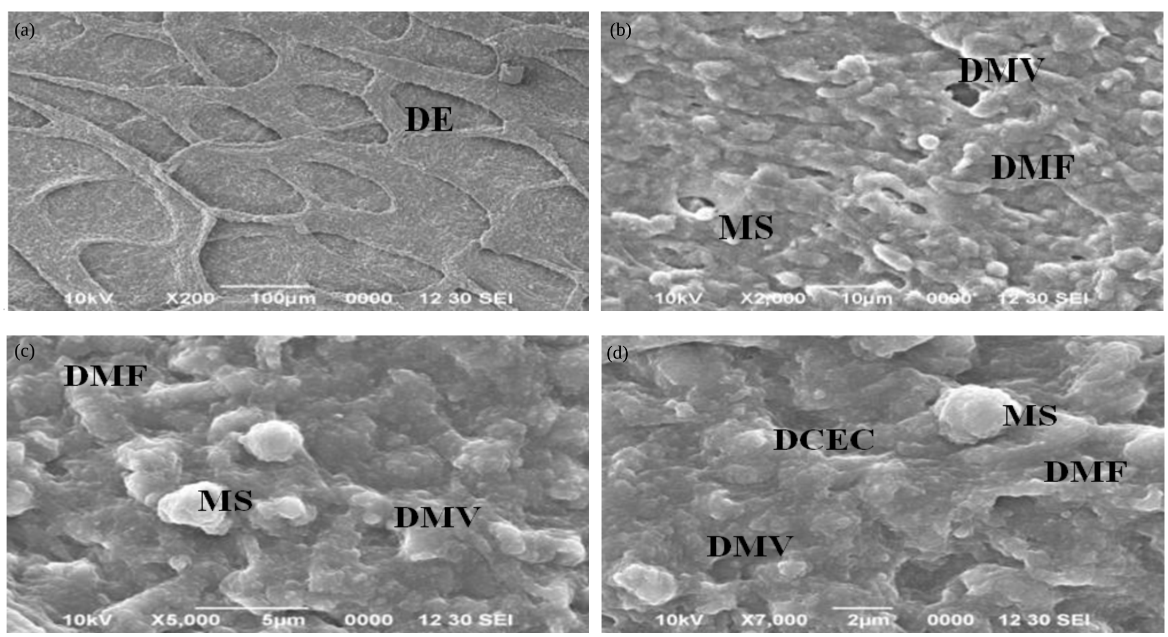

Fig. 5(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.013% electroplating industrial effluent for 7 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

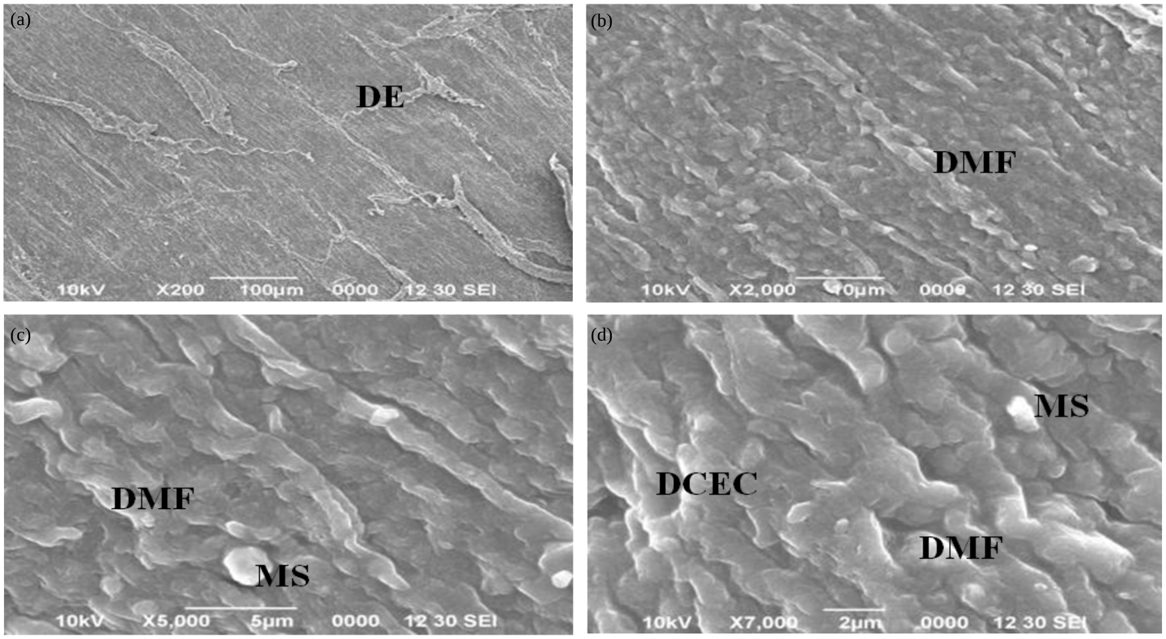

Fig. 6(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.004% electroplating industrial effluent for 14 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

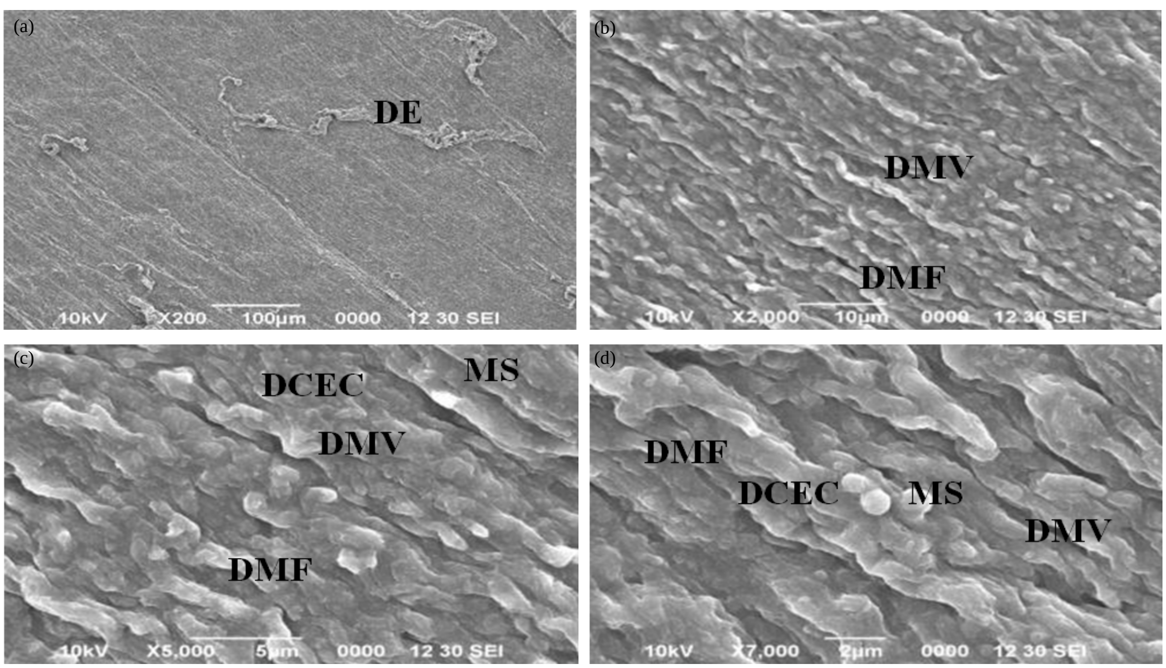

Fig. 7(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.007% electroplating industrial effluent for 14 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

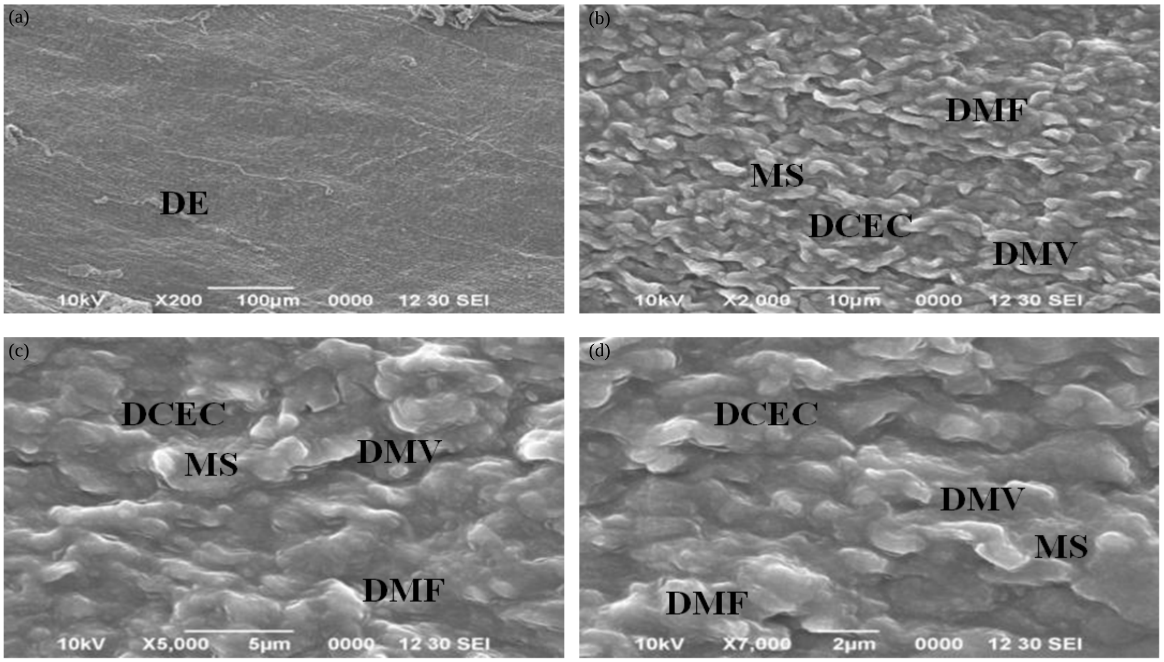

Fig. 8(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.010% electroplating industrial effluent for 14 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell, DCEC: Damaged columnar epithelial cell, SEM: Scanning electron micrograph

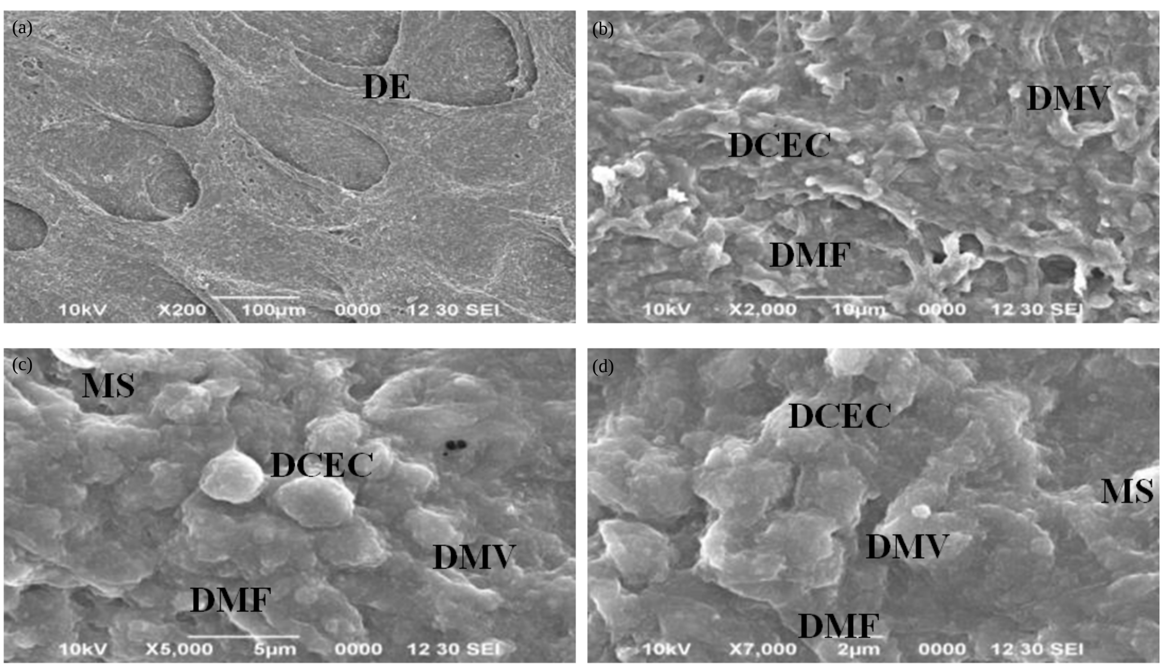

Fig. 9(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.013% electroplating industrial effluent for 14 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

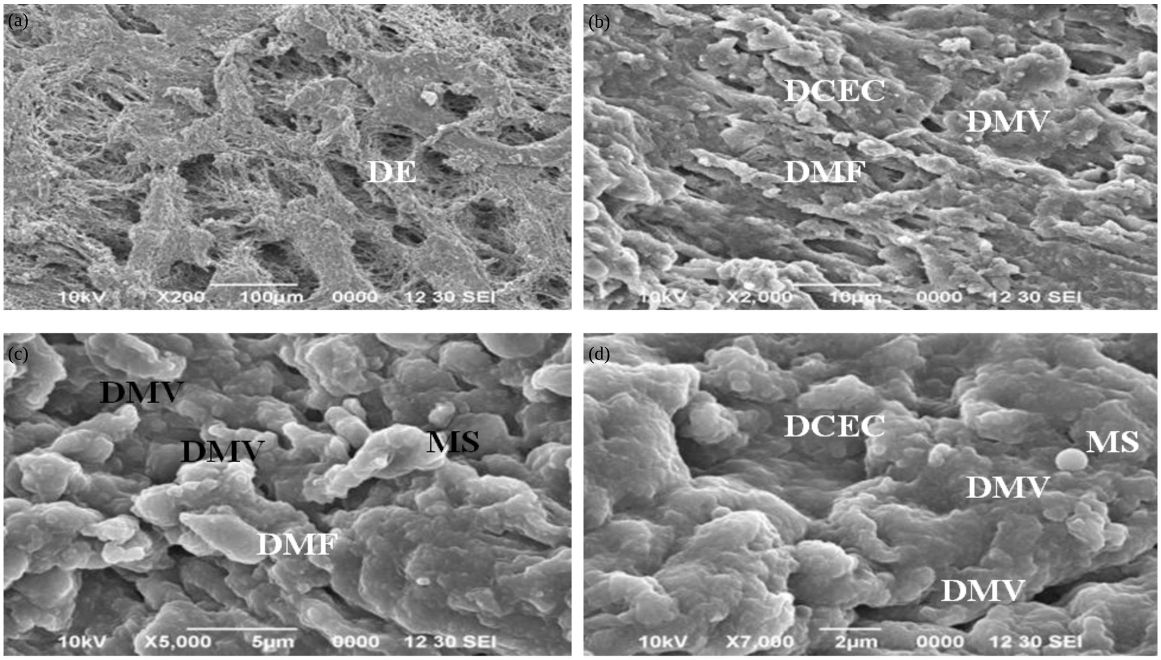

Fig. 10(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.004% electroplating industrial effluent for 21 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X
DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

diesel oil effluent in the mucosal surface of the alimentary canal of *Oreochromis nilotica* (Linnaeus) using SEM was observed[14].

Microvilli of the columnar epithelial cells became disrupted and damaged due to necrosis (Fig. 7, 8 and 9). Mucosal folds in the intestine help to retain the ingested food for a longer time. Microvilli are produced by the sculping of the luminal plasma membrane[28]. Mucosal folds increase the surface area for effective digestion and absorption. Secretion of the mucus by the epithelial cells which serves as a coating over these cells offers protection against chemical injury[29].

Mucus secretion was also observed over columnar epithelial cells on the surface of the intestine of *C. carpio* exposed to electroplating industrial effluent for 14 days. Exposure of electroplating industrial effluent for 21 and 28 days severely damaged the columnar epithelial cells of the intestine (Fig. 10, 11, 12 and 13). The mucosal folds were totally interrupted and damaged (Fig. 14, 15, 16 and 17). Pollutants affect the orientation of the mucosal folds and result in the filling of concavities with debris. Excess mucus secretion is an indication of severe response to contaminant exposure. Excessive secretion of mucus is caused by exocytosis due to change in the luminal environment. The microvilli of the plasma membrane of the epithelial cells got heavily damaged. Excess secretion of mucus indicated a severe response of fish to electroplating industrial effluent. Alterations in the intestinal columnar epithelial cells reduce the absorption of nutrients from lumen to cell interior[14,30]. The intestinal epithelial cells on exposure to contaminants exhibited abnormalities in RER (Rough Endoplasmic Reticulum) with distorted microvilli having deep indented margins. Due to oxidative stress, the antioxidant profile also underwent changes[16]. Villus morphology changes can be used as a rapid prognostic marker for monitoring heavy metal pollution. Exposure to contaminants results in abnormal proliferative and apoptotic processes on the surface of intestine. Fusion of intestinal villi results in thicker epithelium[13].

Thus, the present observations confirm severe alterations in the intestine induced by sublethal concentrations of electroplating industrial effluent. Further studies with other toxicants and effluents on the surface morphology of intestine of fish will help to understand whether these alterations are specific or not.

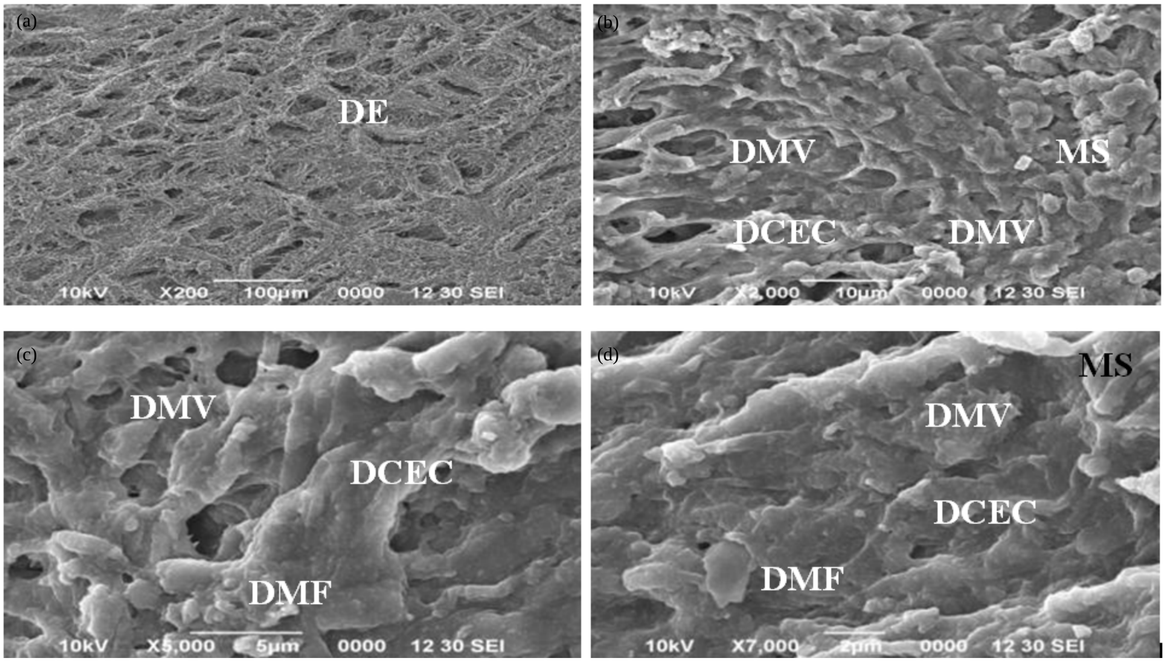

Fig. 11(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.007% electroplating industrial effluent for 21 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

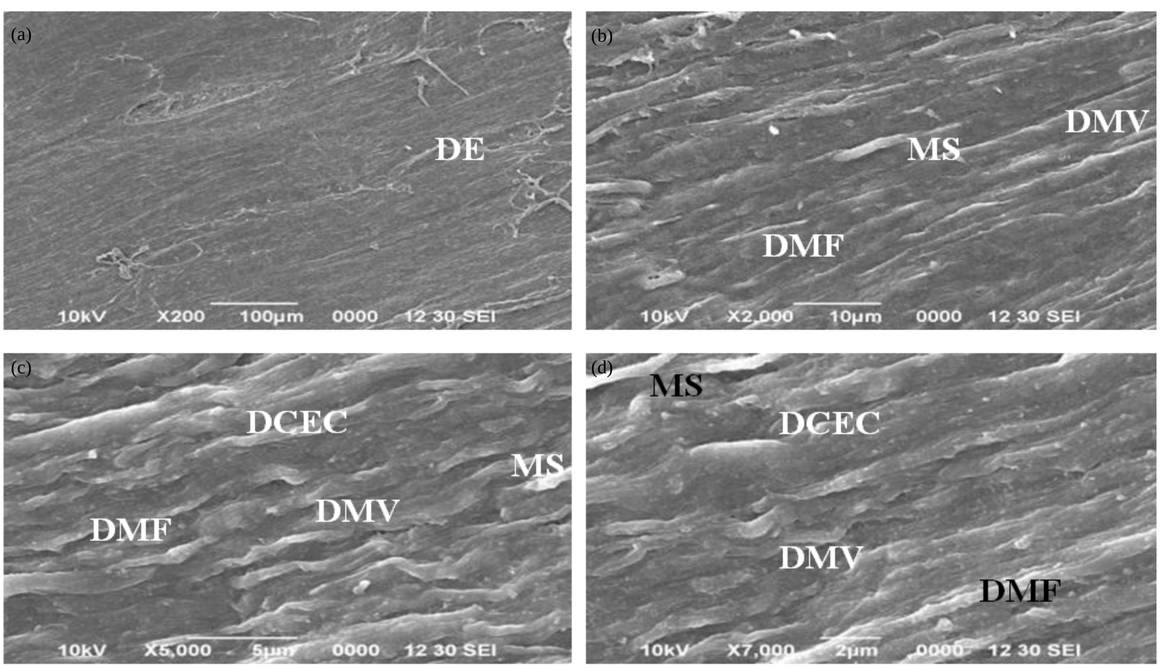

Fig. 12(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.010% electroplating industrial effluent for 21 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

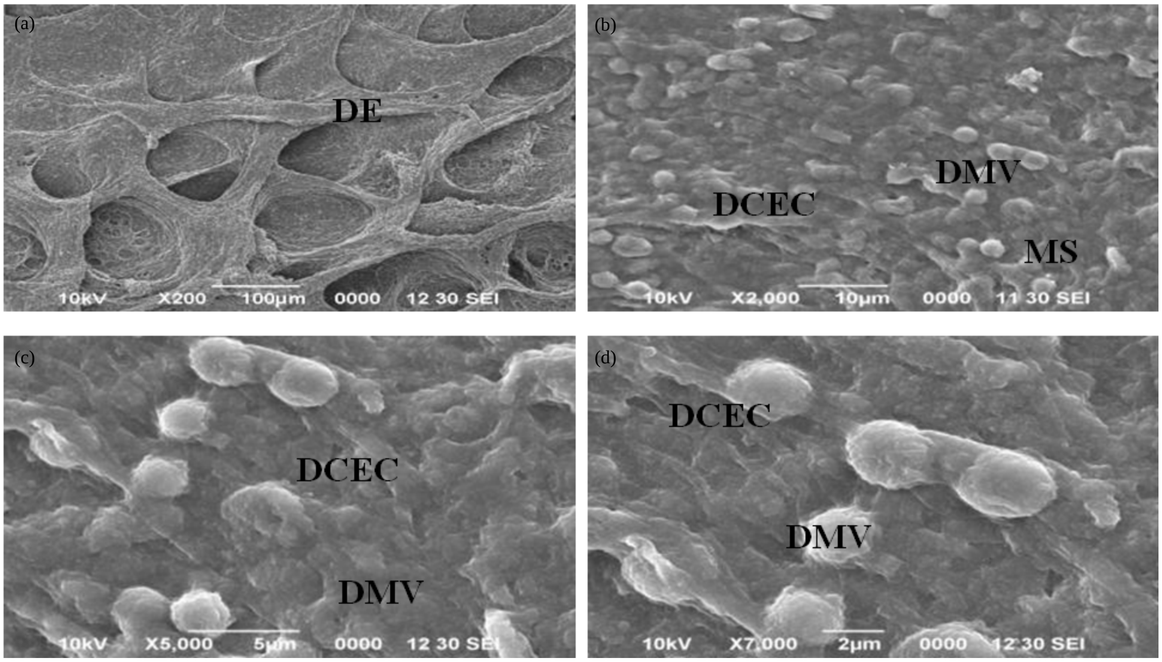

Fig. 13(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.013% electroplating industrial effluent for 21 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

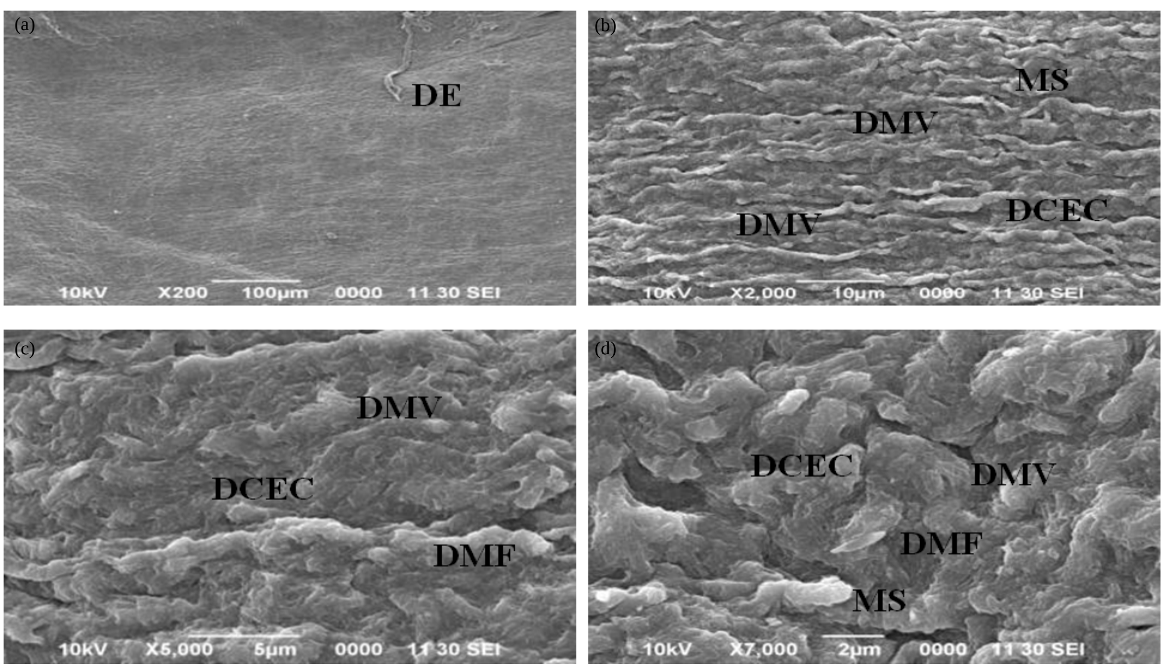

Fig. 14(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.004% electroplating industrial effluent for 28 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

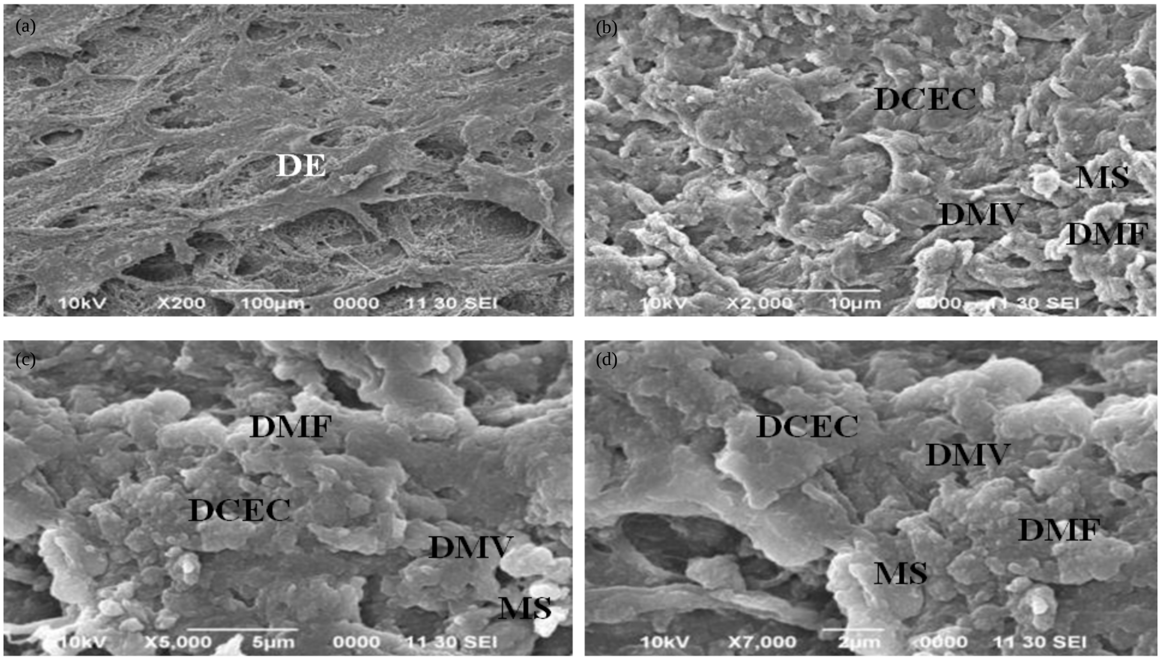

Fig. 15(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.007% electroplating industrial effluent for 28 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

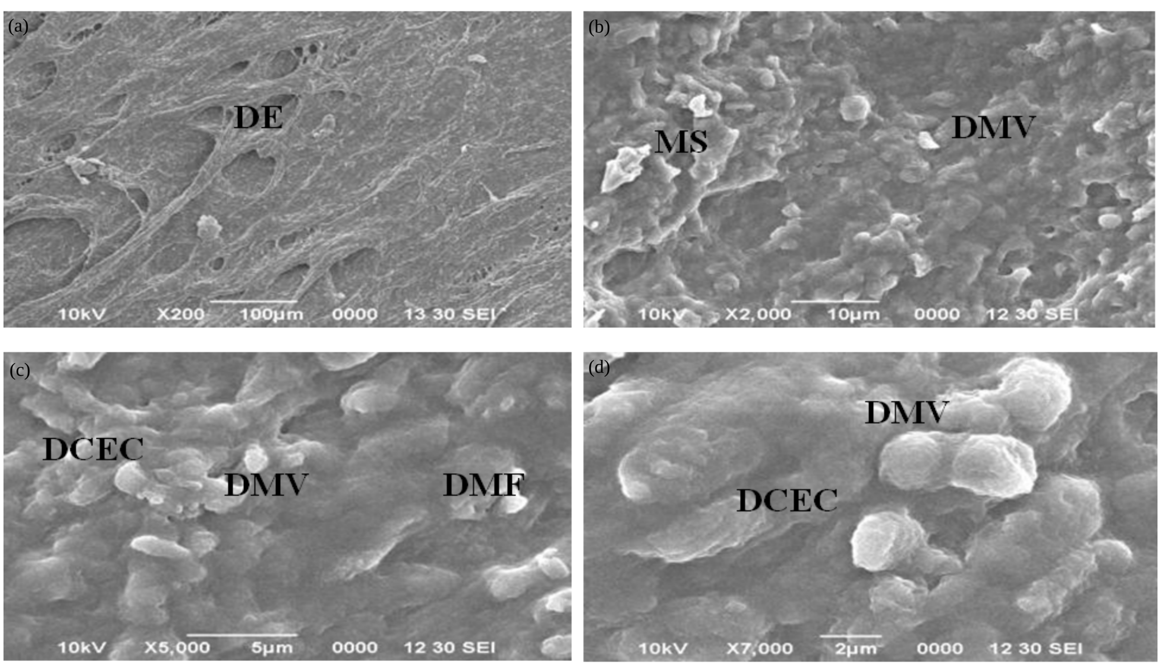

Fig. 16(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.010% electroplating industrial effluent for 28 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X

DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold, DCEC: Damaged columnar epithelial cell

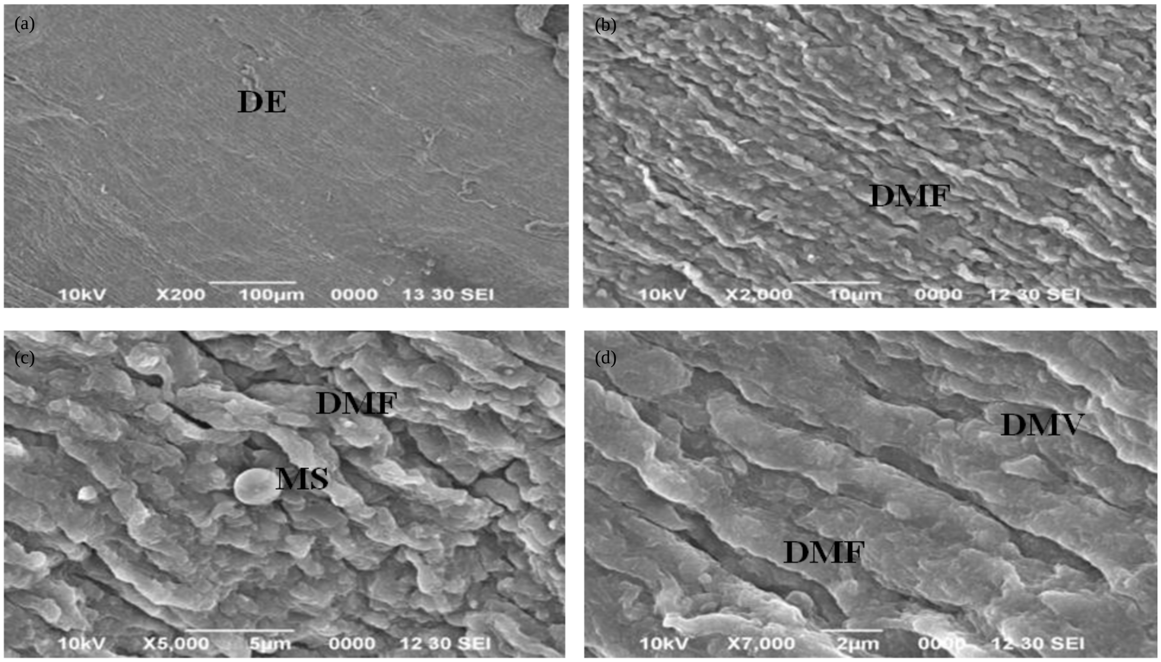

Fig. 17(a-d): SEM of intestine of *Cyprinus carpio* exposed to 0.013% electroplating industrial effluent for 28 days (a) 200, (b) 2000, (c) 5000 and (d) 7000 X
DE: Degenerated epithelium, MS: Mucus secretion, DMV: Damaged microvilli, DMF: Disarranged mucosal fold

CONCLUSION

The present study verifies that morphological changes are valuable biomarkers for field evaluation. Hence, care should be taken to avoid the discharge of effluent above permissible limits. Proper removal of heavy metals in effluent is necessary before their discharge into aquatic systems. Studies of this nature will help in formulating biomonitoring strategies.

SIGNIFICANCE STATEMENT

The changes induced by the sublethal concentrations of effluent from electroplating industry on the surface morphology of the intestine of common carp were discovered using scanning electron microscopy. The results are useful in pollution monitoring programmes. Much study in this line has been carried out with reference to the gills of fish while it was not so, for the intestine of fish. Hence, this study helps to uncover critical areas regarding surface morphology of the intestine of fish exposed to industrial effluents. Toxicants present in industrial effluents acted synergistically and caused damage to the histoarchitecture of the inner wall of fish intestine. Since the study is done with reference to the intestine, it paves way for much research on this line. By this study, the society can realize the damage caused by industrial wastes on fish so that strategies can be devised to alleviate pollution and consumption of such fish can be avoided.

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