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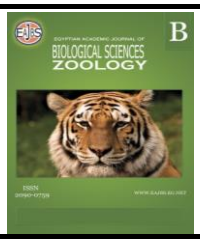

Histological Responses of The Freshwater Clam, *Caelatura nilotica* (Cailliaud, 1827) to Different Environmental Degrees Of Mixed Pollution

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ABSTRACT

Mixed pollution of the freshwaters is a worldwide problem; and the scientific evidence showed its impacts on the bivalves` biological processes. In the present study, the freshwater clam, *Caelatura nilotica* was collected from two water habitats with different degrees of pollution. Examination of gills and digestive gland using routine histology, scanning (SEM), and transmission (TEM) electron microscopy was done. In SEM investigations of the gills, deformation and erosion of cilia, clogging of gills by mucous and ghost gills were observed in the clams collected from a more polluted site. Histological structure of gills proved the appearance of ciliates between the gill filaments. Also, inflammatory reactions appeared and the epithelia lost their integrity and desquamated. The digestive gland showed haemocytes infiltrations, necrosis in some digestive tubules, and granulocytomas was observed. A full description of the digestive gland was done using TEM. In addition, more abnormalities were recorded in different types of the haemocytes, digestive and basophilic cells, flagella, microvilli, lysosomes, residual bodies, mitochondria, nucleus and chromatin with blebbing in most of them. In conclusion, mixed pollution compromised the functions of the gills and digestive gland of the clam, *C. nilotica*, under the effect of synergistic and/ or antagonistic actions of inorganic and organic contaminants with reference to the more severe effects of the non-point source than the point-source pollution in water pollution impacts.

INTRODUCTION

Molluscs (especially bivalves) are cosmopolitan organisms in the aquatic environments and commercially and ecologically important biota on the global level. They can accumulate different types of contaminants and showing significant pathological signs (see Bigas *et al*. 1997; Mouneyrac *et al*. 2003; Sheir *et al.* 2010; Sheir and Handy 2010; Morley 2010; Sheir *et al*. 2013; Bendell *et al*. 2020). Bivalves (mussels and oysters) have been widely used to evaluate distribution trends of contaminants as in the Program mussel watch (O'Connor 2002). Histological studies were considered as one of the most appropriate possible biomarkers as it measures the response of short and long terms of exposure, use several tissues, discernible, and can be efficient (Handy *et al*. 2003). Special attention was paid to the effect of pollution on the digestive gland as their lysosomes were involved in the uptake of pollutants. Gills and digestive gland were considered the main target organs for

pollution accumulation and impacts as discussed by (Bigas *et al*. 1997).

Several pathological signs were pointed out in the literature on the effect of pollutants on bivalves. An increase in the activities of antioxidants and antioxidant enzymes have been detected in molluscs inhabiting polluted areas with metals (Rodriguez-Ariza *et al*. 1993). Organic pollutants such as microplastics caused impairment in the process of water filtration which could affect the food intake in the freshwater crustacean, *Daphnia magna* (Jemec *et al*. 2016), and inflammatory responses in the intestine of the insect, *Drosophila melanogaster* (Capo *et al*., 2019). TEM technologies have been proved to understand the impacts of pollution on subcellular levels. Barka and Cuoc (2019) defined mitochondria, rough endoplasmic reticulum, and nucleus as the target organelles for metals pollution of the marine crustacean copepods, *Tigriopus brevicornis.* Or, mitochondria, Golgi bodies, and granular endoplasmic reticulum in the hepatopancreas of the terrestrial isopod, *Porcellio scaber* was described as targets of metals exposure (Žnidaršič *et al*., 2003). Increased water contamination can increase the susceptibility of bivalves to parasitism, especially metal pollution (Minguez *et al*. 2011). Several genera and species of ciliates were reported as one of the pathogens of the freshwater bivalves belongs to family Unionidae (Carella *et al*., 2016).

The objective of this work was to assess and compare the histological responses as a biomarker of the freshwater clam, *Caelatura nilotica* as a bioindicator of mixed types of pollution (organic and inorganic) in different habitats with different pollution degrees.

MATERIALS AND METHODS

Sites:

Sites were chosen according to the best and worst from the pollution point of view, as site #2 was the best in invertebrates` biodiversity and least in concentrations of metals and pesticides than site #1 as reported by Sheir (2018). Sites are located in Shebeen Elkoom city (coordinates 30°33`31` N, 31°00`36 E). Site 1, is located behind a residential house and is devoid of garbage and no visible pollution (non-point source pollution). Site 2, is behind an administrative building, where garbage represented in domestic wastes was found (point source pollution). The sediment of the sites consisted mainly of silt and the sides were muddy and covered with vegetations. The average depth and width were about 1.5 and 4 metres, respectively (Sheir, 2018).

Environmental factors of water were estimated as temperature, pH, salinity, total dissolved solids, electrical conductivity, total and free chlorines (see Sheir, 2018). Heavy metals (Al, Cd, Cr, Cu, Fe, Pb, Mn, Hg, and Zn) of water samples were analyzed using ICP-OES (Model Agilent 720), at Central Laboratories Sector, the Ministry of Petroleum and Mineral Resources, Egypt (Sheir, 2018). Pesticide residues (organophosphorous and organochlorine) concentrations in sediment samples were carried out according to QuEChERS method (EN 15662:20090-02, Payà *et al.*, 2007). The analysis was done at the Central Agricultural Pesticides Laboratory, Agricultural Research Centre, Ministry of Agriculture & Land Reclamation, Egypt (Sheir, 2018).

Clams:

The freshwater unionid clam, *Caelatura nilotica* (Cailliaud, 1827) was used in the present study. It was collected from different sites (sites #1 and #2) of Bahr Shebeen Nilotic Canal, Menoufia government, Egypt for a year (September 2014 till August 2015, see Sheir, 2018). Clams were maintained in well-aerated aquaria filled with de-chlorinated tap water and fed on silt from their natural environment, under laboratory temperature for few days until dissection and further examination. Adult clams (4-5 cm) from each site were collected for histological investigations.

Histology:

For histological examination, clams were selected randomly from each site. Clams were rinsed in distilled water and plot to dry, and then shells were opened to drain internal field water. One gill plate and the whole digestive gland were dissected and removed from the shells. Dissected organs were immediately fixed in neutral formaldehyde (10 %) for 24 hours. Samples were dehydrated in an ascending series of alcohol, cleared in xylene, and embedded in melted paraplast at 60 ˚C. Serial sections were cut at 6-8 µm thickness, then stained with Ehrlich`s Haematoxylin and counterstained by Eosin (Romeis, 1989). The sections were mounted on slides with glass covers. Histological sections were photographed at desired magnifications using Olympus b x. 41, Japans` microscope and photo-automated camera.

Scanning Electron Microscopy (SEM):

For scanning electron microscope studies, gills from different sites were fixed immediately in 4 Formalin: 1Glutraldehyde (pH 7.4). Then, tissue was post-fixed in 1% osmium tetroxide for 1h at 4ºC and rapidly washed in cacodylate buffer. The samples were dehydrated in graded ethanol at room temperature, critical point dried, and gold-coated according to standard procedures of Felgenhauer (1987). The prepared samples were examined and photographed at the desired magnifications by JSM-IT200 series, Electron Microscope Unit, Faculty of Science, Alexandria University.

Transmission Electron Microscopy (TEM):

For transmission electron microscope studies, small blocks ($1mm^3$) of the digestive gland from different sites were fixed immediately in formalin-glutraldehyde fixative (4 Formalin: 1Glutraldehyde, pH 7.4). The post-fixation was carried out using 1% osmium tetroxide for 1-2 hours at 4°C. After fixation, the tissues were dehydrated through graded ethanol series. Infiltration was carried out using a series of propylene oxide and Epon® raisin mixture. Embedding and polymerization were carried out in the oven at 58 °C. Ultra-thin sections (60-70 nm thick) were cut and fixed on 200 mesh copper grids. Grids were stained with uranyl acetate and lead citrate according to the procedure of Reynolds (1963). Sections were examined and photographed at desired magnifications using JEM-1400 Plus, Electron Microscope Unit, Faculty of Science, Alexandria University.

RESULTS

Effect of Pollution on the Gills of *Caelatura nilotica:*

1. SEM

The clams collected from site #2 showed almost a normal structure of gills. The gill filaments generously had the typical three types of cilia. The 1st one was the frontal cilia, which is the shortest type. The 2nd type was the laterofrontal cirri, which was longer and stiffer than the frontal one and ramified. And the 3rd type was long and called lateral cilia but not shown because of the crowdedness of the laterofrontal cirri. Some samples showed bare and unramified cirri (Fig. 1a, b & c).

The clams collected from site #1 showed abnormal organizations of the gills. Short and eroded cirri and long singles or bundles of cilia connecting adjacent filaments appeared. In addition, eroded ciliary junctions with water tubes in between appeared because of much less crowded cilia of the filaments. At some examined gills, cilia were covered and entangled completely with mucous and particle-like structures. Other gills appeared as ghost filaments covered by one short type of cilia and bare focus (Fig. 1d-h and Table 1).

2. Histology

The histological structure of the clams` gills collected from site #2 showed typical architecture. The gill filaments epithelia lined by a layer of columnar epithelia at the free end. They had the typical three types of cilia, the frontal, the laterofrontal, and the lateral cilia. Deeper in the gill lamellae, the filaments lined by cuboidal epithelia. The branchial vessel containing haemocytes was surrounded by the epithelia. Ciliary junctions were connecting every two adjacent filaments. Some samples exhibited some light signs of pathology as disappearance of some cilliary junctions, deformation in the filaments shape, decrease in frontal cilia, haemocytes invasion of the filaments epithelia with necrosis in some parts (Fig. 2a, b & c).

Clams` gills collected from site #1 showed necrosis and desquamation in the epithelial layer of the filaments and presence of ubiquitous ciliates in the inter-filamentar spaces. Also, inflammatory responses recorded in the branchial vessel and the filaments` epithelia with claviform filaments of some clams. Fibrous tissue appeared in the gill filaments with increased presence of ciliates. The ciliates themselves were disintegrated in the inter-filamentar spaces. The appearance of undulating gill filaments dominated some gills, bare gill filaments, deformed ciliary junctions that were documented in some filaments. Thickened epithelial basement membrane (basal lamina) was documented and appeared as skeletal tissue. Moreover, lysis of cuboidal epithelia beside eroded ciliary junctions was approved (Fig. 2d-f & Fig. 3a-f and Table 1).

Effect of Pollution on the Digestive Gland of *Caelatura nilotica:*

1. Histology

The digestive gland of clams collected from site #2 recorded nearly normal digestive tubules lined by a layer of columnar epithelia surrounding a central lumen. The typical two types of epithelia were digestive and basophilic cells. The tubules connected by connective tissue, and sections of the stomach and the intestine and some haemocytic infiltrations (nodule) were recorded. Some specimens exhibited inflammatory responses as focus, necrosis in the intestinal epithelia, and fusion of some digestive tubules (Fig. 4a, b & c).

In site #1, clams` digestive gland showed increased basophilic cells in some clams` digestive tubules. Lysis of connective tissue and digestive tubules also was detected. In addition, fibrotic tissue and increased intra-epithelial haemocytes infiltrations in the digestive tubules were recorded. Haemocytes infiltrations, especially granulocytomas and fibrous tissue were observed in some clams (Fig. 4d-h and Table 1).

2. TEM

In the transmission electron micrographs of clams collected from site #2, the digestive tubules composed of two types of epithelial cells, the digestive and the basophilic (secretory) cells. The digestive cells characterized by rectangular, columnar shape, large nucleus located toward the basement membrane with the nucleolus, many electron-dense, and light lysosomes, endoplasmic reticulum, and several residual bodies, which little were deformed in shape, lots of mitochondria and occasional Golgi bodies and lined with packed microvilli on their free end directing toward the tubule lumen for food ingestion. Mitochondria were crowdedly packed under the origin of microvilli. Microvilli appeared in cross-sections as a single membrane of oval rings filled with matrix and in longitudinal sections as glove fingers filled with matrix.

The basophilic cell characterized by the pyramid shape, nucleus almost oval, and located in the middle of the cell near the narrow end of the cell, nucleolus, electron-dense, and light lysosomes. The nucleus was surrounded by lots of rough endoplasmic reticulum (which gives the cytoplasm its basophilic properties) and far from it with the smooth endoplasmic reticulum. Intact oval and round-shaped mitochondria were located beneath the

cilia roots and covered by cilia projecting into the tubule lumen. The structure of cilia was (9+2) configuration, 9 peripheral and 2 central tubules embedded in the matrix, and surrounded by plasma membrane in cross-section and in longitudinal section appeared as several long tubules in the matrix and surrounded with the plasma membrane (Fig. 5). Granulocytes were recorded in the connective tissue between the tubules. It consisted of a round nucleus, several lysosomes, sometimes a vacuole with or without several pseudopodia (Fig. 6a-f).

Clams from site #1 showed responses represented in alterations of cellular and subcellular organizations of the digestive gland. The granulocyte incorporated ingested pollutants within a primary phagosome by a pseudopodium or had a bizarre shaped nucleus and nucleolus and other haemocytes showed irregular nucleus and intracellular vacuoles (Fig. 6g & h). Digestive cells with an apoptotic nucleus, irregular basement membrane, intercellular spaces; or others with several fat droplets and apoptotic nucleolus were detected. The subcellular components of the digestive tubules` cells exhibited deformed shape and increased number of the residual body, lysed vesicles of Golgi bodies, and bizarrely shaped lysosomes with precipitations of particles inside them and leaky end mitochondria (Fig. 7). Also, the microvilli of the digestive cell showed a zigzag pattern of their outer membrane with less density of mitochondria beneath the base of microvilli, ribosomes of rough endoplasmic reticulum lost their integrity on the cristae and became loose between the cristal space. The most obvious deformities appeared in the nucleus, which showed chromatin aggregations, finger-like projections, and dilation of the rough endoplasmic reticulum sheets with giant nucleolus. Some clams` basophilic cells recorded the sunken nucleus to the wide end of the cell. Cilia appeared with blebbing and deformation in organization in transverse and longitudinal sections (Fig. 8 and Table 1).

Table 1. Summary of cellular alterations detected in the gills and digestive gland of *C. nilotica* collected from different polluted sites

Organs	Alteration type	Site 1	Site 2
Gills	**Gill cilia**	Erosion, connecting cilia and dominance of one type of cilia	Unramified cilia
	Gill filaments	Covered with mucous, precipitations and folded	Some were folded
	Gill epithelia	Necrotic, fibrotic and inflamed	
	Cuboidal epithelia	Mostly necrotic	Normal
	Ciliate presence	Present	Absent
Digestive gland	**Digestive tubules**	Necrotic, fused, inflamed	Rarely fused
	Connective tissue	Necrotic and fibrotic	Normal
	Inflammatory responses	+++	+
	Granulocytoma	++	+
	Granulocytes	Contain pollutants	Not detected
	Flagella	With blabbing and irregularity	Normal
	Microvilli	Irregular in shape	Normal
	Residual body	Irregular in shape and increased in number	Normal
	Golgi bodies	Lysed vesicles	Normal
	Mitochondria	Lysed ends and	Normal
	Lysosomes	Irregular in shape	Normal
	Fat droplets	Increased	Not detected
	Endoplasmic reticulum	Loos ribosomes between RER sheets	Normal
	Nucleus	Folded and with blebs	Normal
	Nucleolus	Large	Appeared normal
	Chromatin distribution	Aggregated in the centre	Normally distributed

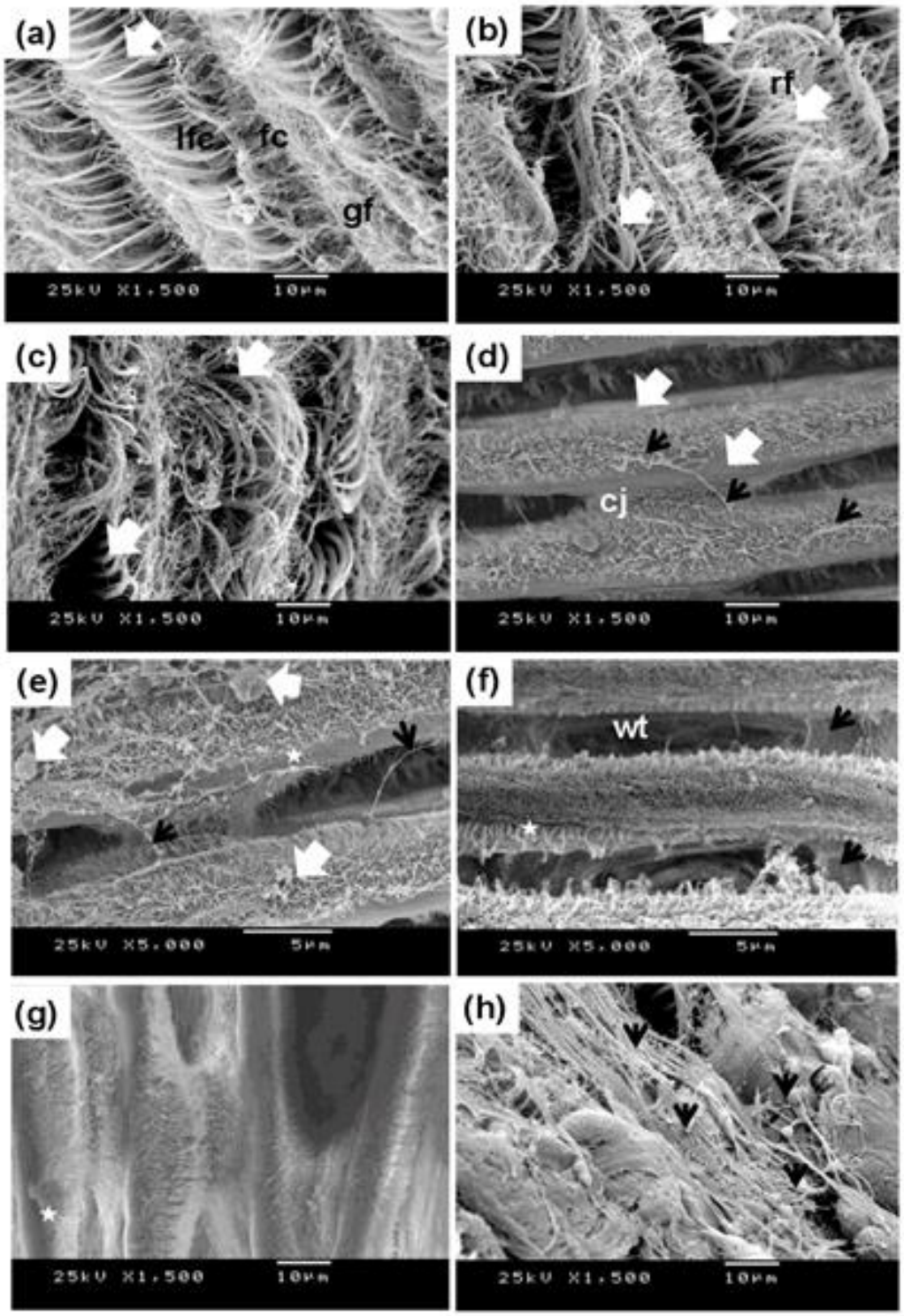

Fig. 1. Scanning electron micrographs of *C. nilotica* gills collected from less polluted site showing **(a & b)** upper view of gf, gill filaments with fc, frontal cilia, lfc, latero-frontal cilia, and rf, ramify cirri (white arrows); **(C)** bare and unramified cirri (white arrow); *C. nilotica* gills collected from more polluted site showing **(d)** eroded cirri, cj, cilliary junction, long connecting cilia (black arrows); **e)** erroded cilia; predicated mucous and particles (white arrows), eroded lateral cilia (star) and long connecting cilia (black arrows); **(f)** wt, water tubes, long bundle of cilia (black arrow), eroded lfc (star); **(g)** ghost filaments covered with one type of cilia, bare focus (star) and **(h)** clogged filaments with mucous/particles-like structures (black arrows).

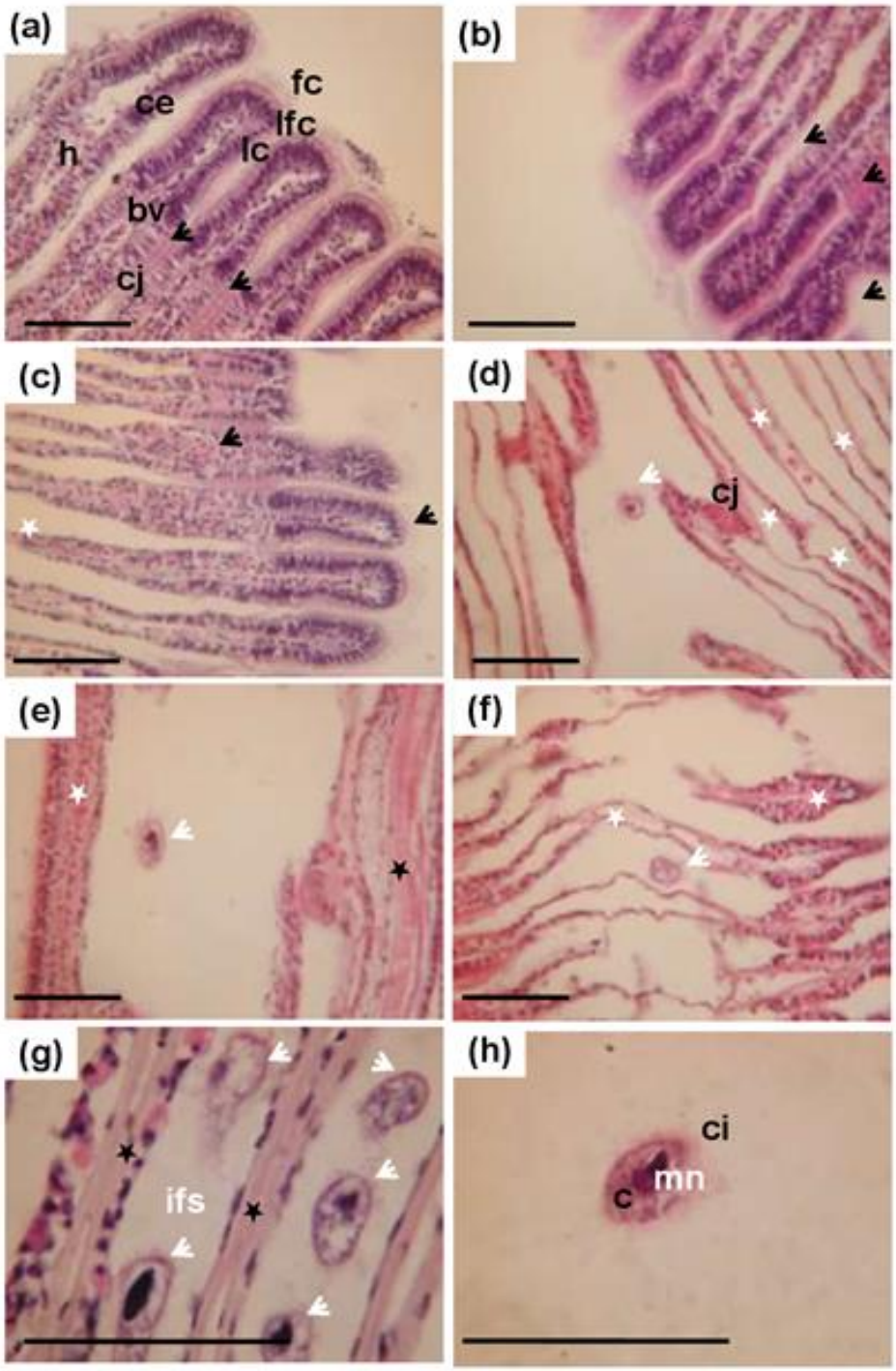

Fig. 2. Light micrographs of sections through *C. nilotica* gills collected from less polluted site stained with H & E. **(a)** showing fc, frontal cilia, lfc, laterofrontal cilia, lc, lateral cilia, e, epithelia, cj, ciliary junction, bv, branchial vessel, h, haemocytes, cb, cuboidal epithelia; **(b &c)** disappearance of some cilliary junctions, deformation in the filaments, decrease in frontal cilia, haemocytes invasion of the filaments epithelia with necrosis in some parts; *C. nilotica* gills collected from more polluted site showing **(d)** necrosis and desquamation of gill epithelia (stars) and interfilamentar ciliate (white arrow head); (**e)** haemocytes infiltrations (star) fibrosis (black star); **(f)** haemocytes infiltration in epithelia (stars); **(g)** necrosis and fibrosis (black stars) with damaged ciliates (arrow heads),), interfilamentar space, ifs and **(h)** showing high magnification of the ciliate, ci, cilia; mn, macronucleus; c, cytoplasm. Scale bar 100 µm.

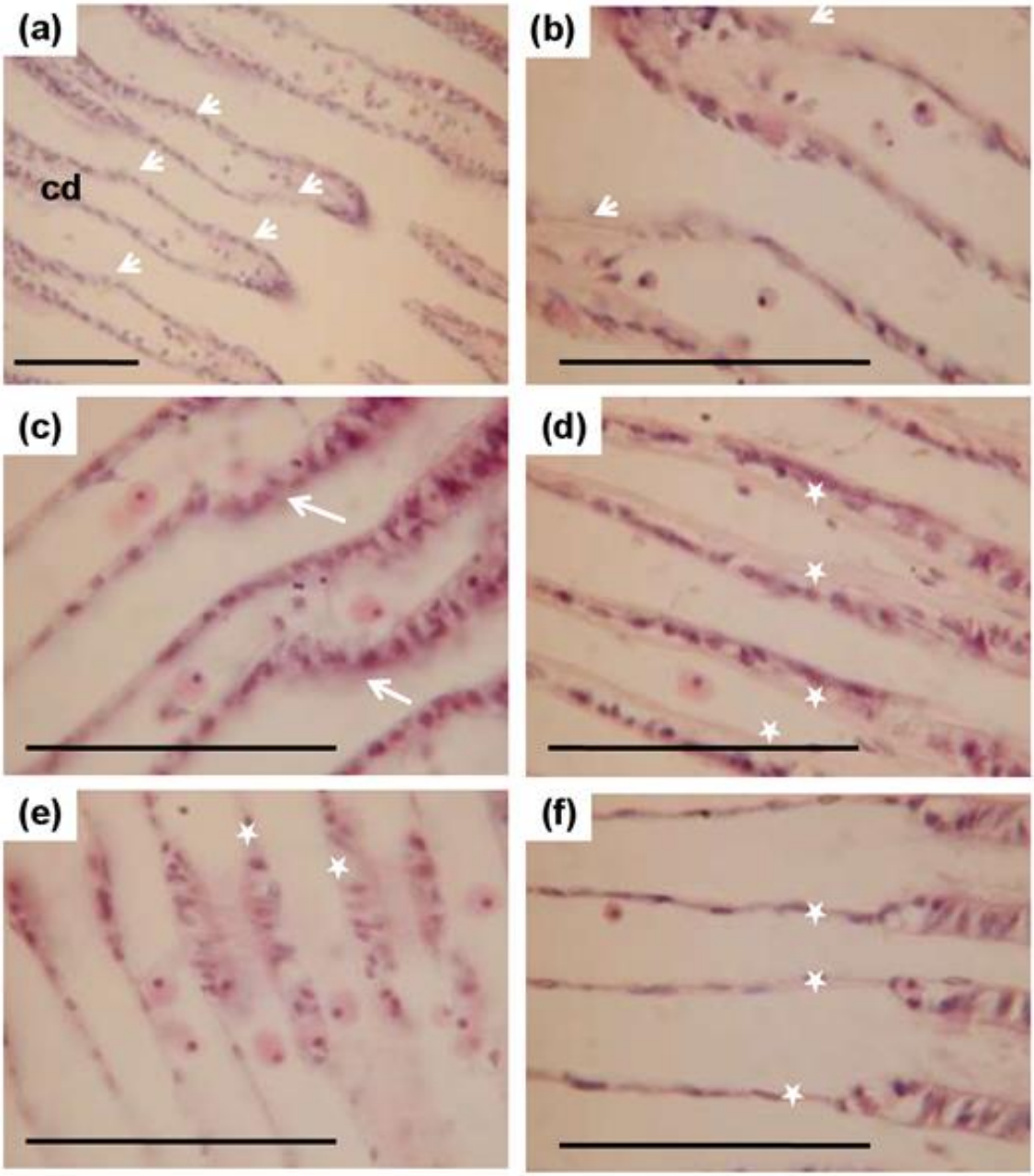

Fig. 3. Light micrographs of sections through *C. nilotica* gills collected from less polluted site stained with H & E. **(a)** showing undulating gill filaments (white arrow heads); **(b)** bare gill filaments; **(c)** folded free end of the gill filaments (white arrows); **(d)** thickened epithelial basement membrane; **(e)** deformed epithelia baring ciliary junctions, cj, (stars) and **(f)** necrosis of cuboidal epithelia (stars). Scale bar, 100 µm.

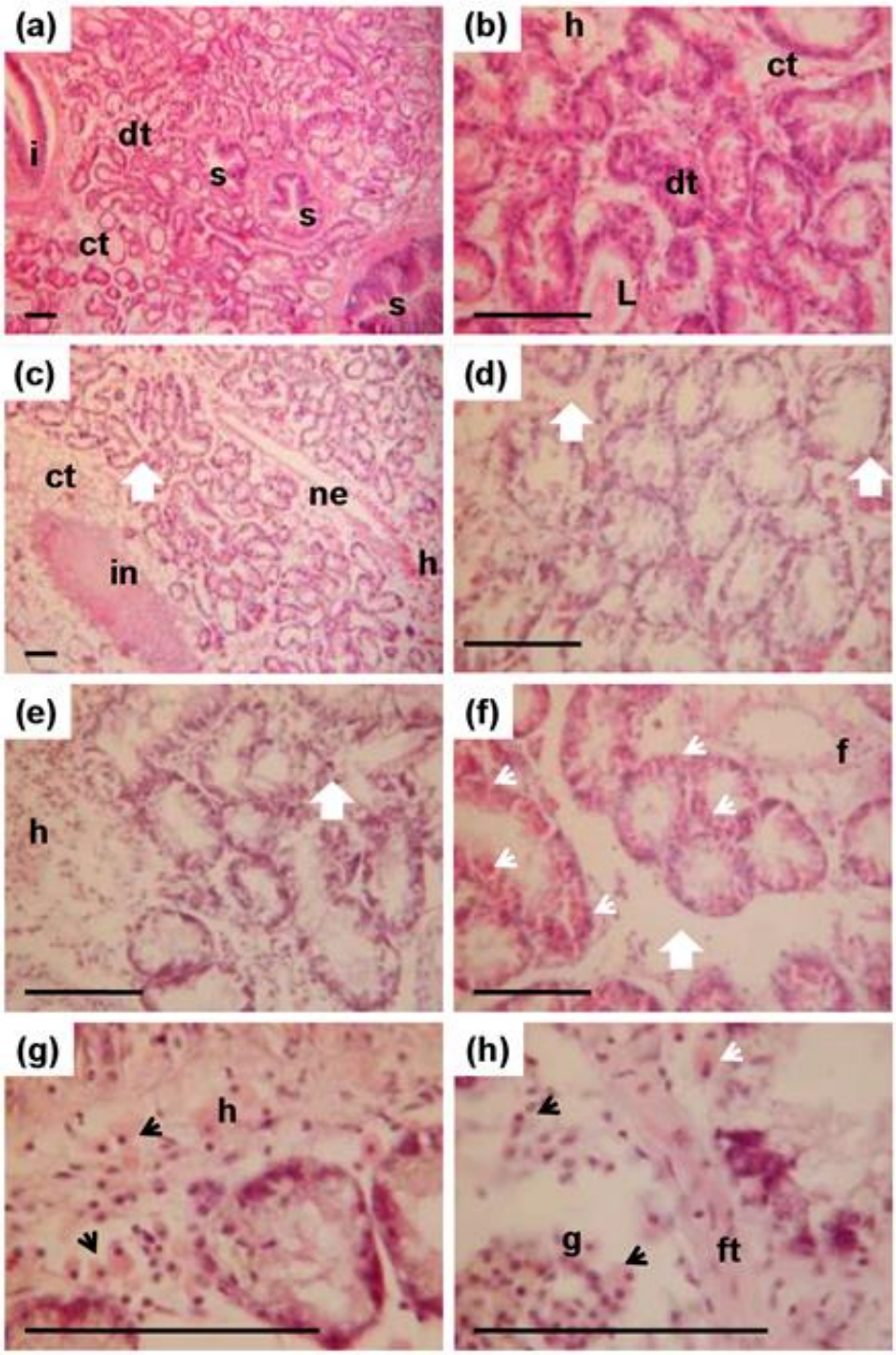

Fig. 4. Light micrographs of sections through *C. nilotica* digestive gland collected from less polluted site stained with H & E showing **(a, b & c)** dt, digestive tubules, ct, connective tissue, s, stomach, I, intestine, h, haemocytes, l, lumen, some specimens exhibited in, inflammation, h, haemocytes infiltration, ne, necrotic epithelia and fusion of some digestive tubules (white arrows); *C. nilotica* digestive gland collected from more polluted site showing **(d)** necrotic connective tissue and digestive tubules (white arrows); **(e)** h, haemocytes infiltration, fusion of digestive tubules (white arrow); **(f)** necrotic connective tissue (white arrows) and haemocyte infiltration, h; **(f)** fibrosis, haemocytes infiltration (arrow heads); **(g)** h, haemocytes infiltration, g, granulocytoma (arrow head) and **(h)** ft, fibrous tissue, nc, neoplastic cells-like aggregations (black arrow heads) with haemocytes infiltration (white arrow heads). Scale bar, 100 µm.

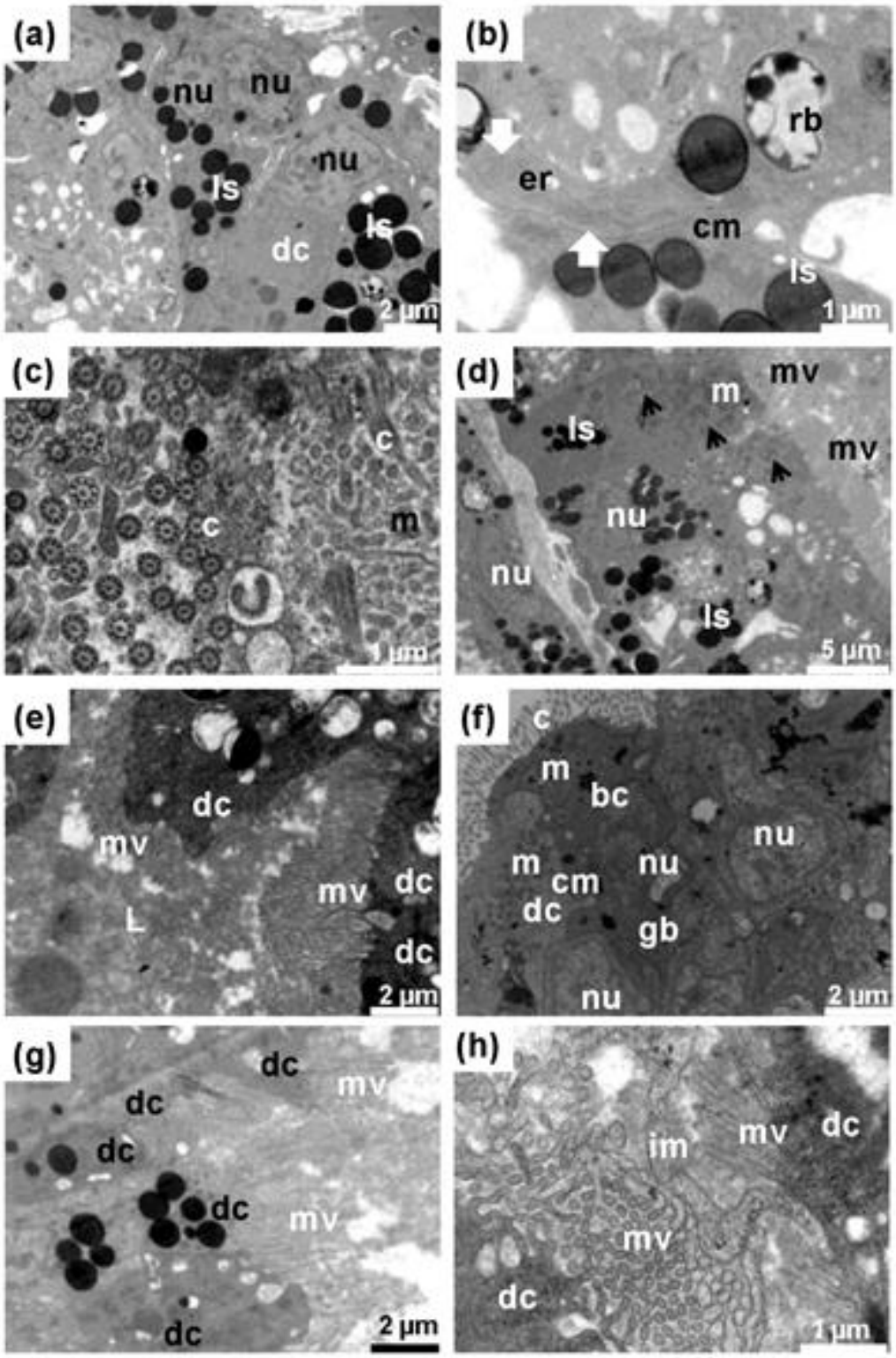

Fig. 5. Transmission electron micrographs of *C. nilotica* digestive gland collected from less polluted site showing **(a)** digestive cell, nu, nucleus, ls, lysosomes; **(b)** cm, cell membrane, er, endoplasmic reticulum (white arrows), rb, residual body; **(c)** c, cilia transverse (TS) and longitudinal (LS) sections, m, mitochondria; **(d)** dc, digestive cell, mv, microvilli, mitochondria (arrow heads); **(e & g)** free end of digestive cells, microvilli, l, lumen; **(f)** bc, basophilic cell, cilia; nucleus; gb, golgi bodies; digestive cell, m, mitochondria, cell membrane; **(h)** digestive cells with TS and LS of mivrovilli, im, ingested material contained dark precipitations.

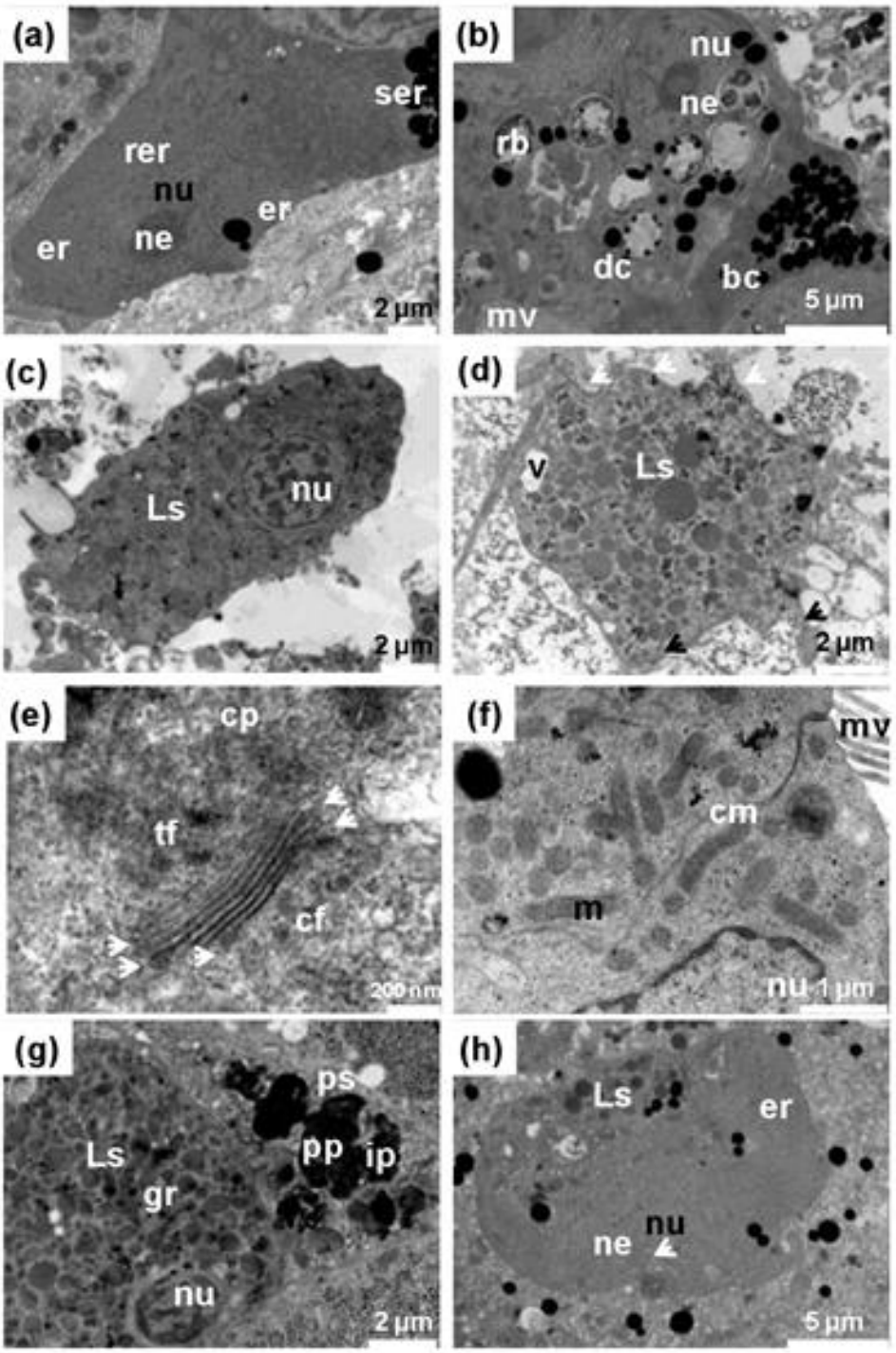

Fig. 6. Transmission electron micrographs of *C. nilotica* digestive gland collected from less polluted site. **(a)** showing basophilic cell with nucleus, nucleolus and lots of rough and smooth endoplasmic reticulum, rer, ser; **(b)** digestive cell with microvilli, nucleus, nucleolus, several residual bodies, basophilic cell with lysosomes; **(c)** granulocyte with nucleus, lysosomes; **(d)** granulocyte with lysosomes and ps, pseudopodia (arrow heads), v, vacuole; **(e)** golgi bodies in cp, cytoplasm with vesicles (arow heads), tf, trans face and cf, cis face; **(f)** mitochondria of digestive cell, cell membrane, microvilli; *C. nilotica* digestive gland collected from more polluted site **(g)** showing gr, granulocyte containing lysosomes, nucleus, ps, peusodopodium, ip, ingested pollutants, pp, primary phagosome; **(h)** haemocyte with edoplasmic reticulum, lysosomes, intracellular vacuoles, v and irregular nucleus.

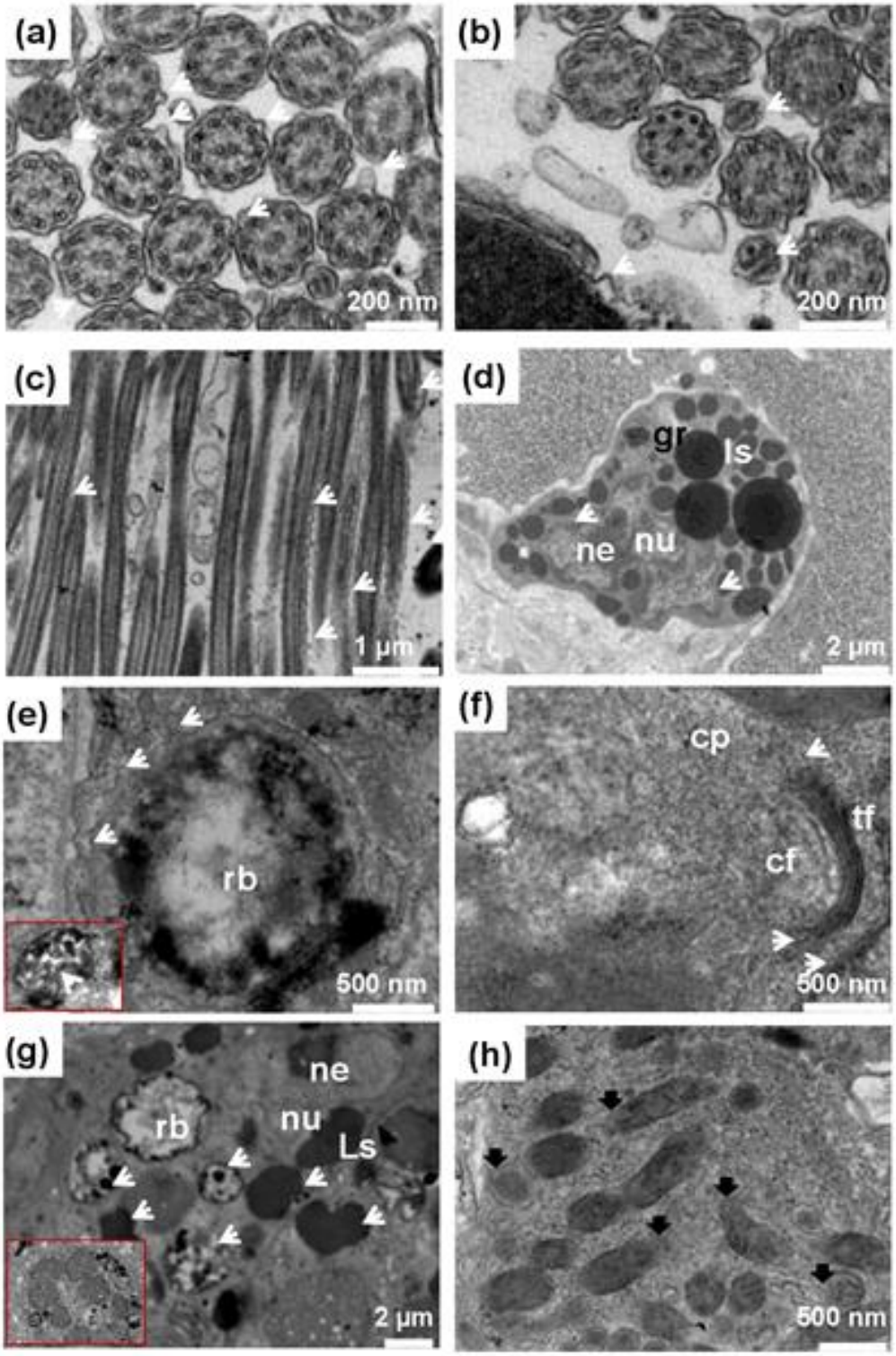

Fig. 7. Transmission electron micrographs of *C. nilotica* digestive gland collected from more polluted site. **(a, b, & c)** showing TS and LS of cilia with blebbing and deformation (white arrows) **(d)** deformed granulocytes with nucleus and nucleolus; **(e)** deformed shape of residual body (arrow heads); **(f)** lysed vesicles of gb, golgi bodies; **(g)** digestive cell with deformed residual body, lysosomes, nucleus and nucleolus (white arrows) and precipitations of particles inside lysosomes; **(h)** lysed ends of mitochndria (black arrows).

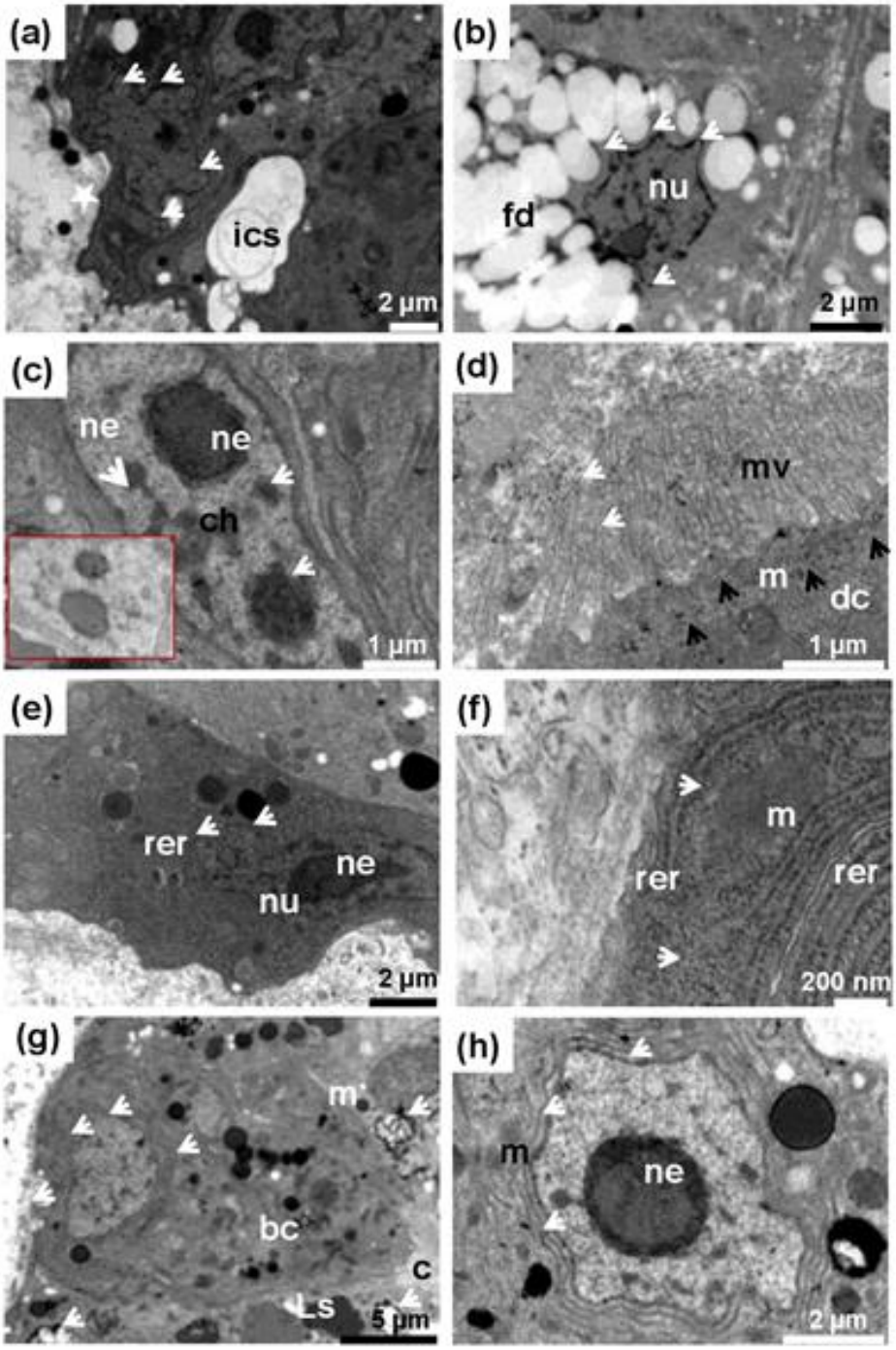

Fig. 8. Transmission electron micrographs of *C. nilotica* digestive gland collected from more polluted site. **(a)** appoptotic nucleus (white arrows), irregular basement membrane (star), ics, intercellular space and **(b)** digestive cell with fats and apoptotic nucleus and nucleolus; **(c)** showing nucleolus with abnormal ch, chromatin aggregations and chromatin mass (arrows); (d) irregular outer membranes of microvilli of the digestive cell; **(e)** irregular shaped nucleus and nucleolus; **(f)** lose ribosomes of rough endoplasmic reticulum (arrow heads) and degenerated mitochondria; **(g)** basophilic cell with irregular basement membrane and rough endoplasmic reticulum surrounding the nucleus (white arrows) and **(h)** the nucleus was surrounded with dilated sheets of rough endoplasmic reticulum and giant nucleolus

DISCUSSION

Clams collected from the site with a higher concentration of metals and pesticides recorded more pathology in the gills and digestive gland than the lower polluted site. Some of these pathologies were considered as a protective strategy of the clams to the stressors in their habitats. However, others were considered as toxicity signs which led to cell death.

Many alterations, morphologically and histologically were recorded in clams` gills collected from a more polluted site (Figs. 1, 2 & 3). The epithelial cells erosion and rupture of the gills were noted by Bigas *et al.* (1997) after exposure of European flat oyster, *Ostrea edulis* to Hg. As explained by them. Also, the damage observed in the plasma membranes of the epithelial cells was confirmed by the results of Viarengo *et al*. (1993), who found that copper strongly stimulates the lipid peroxidation damage of the gill plasma-membrane, and inhibited Ca 2+-ATPase activity. Mohammadein and Desouky (2000), reported inhibition of filtration rate in *Venerus aurea* clam after short-term exposure to cadmium. In addition, shorten and coagulated cilia caused a loss of gill structure architecture under the effect of barite on the gill tissues of the suspension feeder, *Cerastoderma edule*, and the deposit feeder, *Macoma balthica* mussels (Barlow and Kingston 2001). Sometimes pollution could modulate gene expression and impaired muscle contraction and relaxation processes. Rodriguez-Ortega *et al.* (2003) recorded changes in muscle proteins, as the upregulation of putative isoforms of tropomyosin and light chain of myosin and downregulation of actin in the clam, *Chamaelea gallina* under pollution effects. Moreover, Medler and Silverman (2001) proved that during smooth muscle contraction of gills, interfilamntar spaces and water tubes decrease in size. This could explain the responses of the present clams to control the entrance of the polluted water into the gills as a protective method. Changes in the shape of the filaments and the epithelial membrane were recorded in the present study. Also, Claviform gill filaments and thickened basal lamina of the gill epithelia were symptoms of endosulphan (pesticide) exposure in the freshwater prawns, genus *Macrobrachium* (Bhavan and Geraldine 2000). Pesticides found to regulate genes responsible for protein expression of NADH dehydrogenase, ATP syntase beta and alpha, Tubulin alpha and beta, Ribosomal proteins, and Actin cytoplasmic A3 (Tanguy *et al*., 2003). Besides that, they discussed the overproduction of NO in the cell caused cell cycle arrest and apoptosis under the effect of pesticides. Some of the present clams exhibited skeletal tissue instead of the basal lamina in the more polluted site. This result was pointed out by Nogarol *et al*. (2012) who described the presence of this tissue was to support structural maintenance of the gill filaments underneath the epithelia as a protective response to desquamation. Some samples showed cumulated mucous and particles on the filaments` tips. Hamza (2019) pointed out that the increased production of mucous by the gills was a protective response of the freshwater clam, *Tapes decussates* to control the contaminants entering through the gills. This could happen because of the rejection/sorting process of the unwanted particles toward the gill surface with the stimulation of mucous production to capture it and prevent it from entering with the water current back again. However, that response will seriously compromise the filtration efficiency of the gills. Ciliates were observed between the interfilamentar spaces of the current work without obvious effects. The presence of microorganisms in the gills of the clams collected from the polluted area was reported in several studies (Neff *et al*. 1987; Minguez *et al*., 2012; Carella *et al*., 2016). They discussed that pollution could compromise the animals` defence system and make it susceptible to parasites and invading organisms. Some researchers confirmed the neglected effect of ciliates on the gills of bivalves and considered it as a symbiont (Mladineo 2008). Carella *et al*. (2016) discussed the presence of parasitic ciliates in the freshwater bivalves belongs to family Unionidae in the gills and palps without showing any pathological symptoms. Minguez *et al*. (2012) recorded a decrease in

lysosomal activity and an increase in ROS production under the combined effect of parasitism and metal contamination. Morley (2010) reviewed the adverse effects of pollution on increased susceptibility of molluscs to infection with parasites by compromising their immune responses. Also, Anderson *et al*. (1998) discussed the combination of two stressors (tributyltin+hypoxia) which caused a synergetic effect to increase infection rates in the oyster, *Crassostrea virginica.*

The digestive gland of the current study revealed several pathological signs ranged between some inflammations to apoptotic to necrotic responses of the cells and their organelles. Inhibition in the antioxidant enzyme activities in the digestive gland of the marine mussel, *Mytilus galloprovincialis* after exposure to metals under field and laboratory conditions (Regoli and Principiato 1995) could explain the cell reactions to metal toxicity. Inflammation as granulocytomas, necrosis, fibrous tissue, and neoplastic lesions was a characteristic feature of the marine clam, *Mya truncata* gills and digestive tract collected from the polluted site and much less pathology which did not affect the function of the organs of clams collected from low levels of polluted sites` with oil spill (Neff *et al*., 1987). da Silva Souza *et al*. (2011) discussed the causes of necrosis process in the cells as oxidative stress, hypoxia, and high concentrations of metals which lead to disturbance in membrane integrity, impaired cellular organelles and ionic imbalance. Increased accounts of basophilic cells in some digestive tubules of the present clams were observed. Molluscs found to respond to different types of pollution by increasing the number of basophilic or calcium cells of the digestive tubules (Cajaraville *et al*., 1990) and this will consequently decrease the digestive cells count which will impair intracellular digestion. The ultrastructural alterations of subcellular contents were documented in many organisms subjected to pollution environmentally and experimentally. Wang *et al.* (1991) recorded the reason for mitochondrial abnormalities after exposure of gastropods to bromoacetamide was accompanied by a decrease in the action of mitochondrial enzymes such as citric acid synthase and ornithine carbamyl-transferase. In addition, changes in ER as a decrease in cristal sheets and concentric aggregations were documented by Neff *et al.* (2003). Lysed connective tissue between the digestive tubules, lipid storage and increased the residual body numbers were noted by Bigas *et al*. (1997) after exposure of European flat oyster, *Ostrea edulis* to Hg. This was due to disturbance in glycogen and fat metabolism and cellular compartments membrane stability. Distortion of the lysosomal shape was reported in the invertebrates (earthworm) gut chronically exposed to metals in their habitats (Mouneyrac *et al*., 2003).

Pesticides were considered as one of the most toxic organic pollutants because it modulates organisms` hormones and cellular compartments. Bhavan and Geraldine (2000) mentioned the histopathlogical alterations in the gills of the prawn, *Macrobrachium malcolmsonii* exposed to endosulphan was expressed in swelling, fusion, and hyperplastic lamellae, along with haemocytic infiltration. Also, tissue damage in the hepatopancreas such as haemocytic infiltration in the interstitial sinuses, thickening of basal laminae, and necrosis of the digestive tubules was reported. Pesticides residues (Diazinon, Chlorpyrifos, Methoxychlor, Endosulfan, Endrin, Dieldrin, Aldrin, Heptachlor, DDD, and DDE) were detected in over permissible levels in the muscles of fishes collected from the same present study sites at Bahr Shebeen Nilotic Canal (Khallaf *et al*., 2018). Lysosomal membrane destabilization always a target for environmental stressors. Short term exposure to petroleum hydrocarbons generated a decrease in the size and numbers of the lysosomal compartment of the digestive gland cells. While, long term exposure caused an increase in the lysosomal compartments size and decrease in numbers (Cajaraville *et al*., 1995). They discussed the reason for these alterations that it might be due to a disintegration process of the cells and subsequently loss of lysosomes. However, in the second case, lysosomal changes could be

caused by the fusion processes of lysosomes generating bigger ones, as a general stress response. Perry and Lynn (2009) affirmed that apoptosis increased with decreased concentrations of pesticides and decreased with high concentrations as the threshold of apoptosis was no longer effective for protection when the damage is too extreme to be repaired. The inflammatory responses and haemocytes infiltrations in the digestive gland of the clams from polluted sites in the present work could be due to the role of the haemocytes (especially granulocytes) to help in the intracellular digestion of pollutant material or for the recovery processes as the digestive cells process dissolved food by pinocytosis.

Conclusion

Mixed pollution compromised the functions of the gills and digestive gland of the clam, *C. nilotica*, under the effect of synergistic and/ or antagonistic actions of inorganic and organic contaminants. This was highlighted by morphological and cellular investigations of both organs obtained from sites with different degrees of contamination. Although the 2nd site seemed clean of garbage and the 1st was covered with domestic wastes, the metals and concentrations of pesticides were higher and zooplankton diversity was lower in the former than the later (previous work). This also was confirmed in the present study as more cellular damage was detected in the clams collected from the 1st site (non-point source) than the 2nd one (point source).

Conflicts of interest/Competing interests:

The author declares no conflict of interest in the present study.

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