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PHYSIOLOGICAL SIGNIFICANCE OF ADVERSE EFFECTS OF CITY GARBAGE AND MUNICIPALWASTES ON SKIN AND MUSCLE OFA HILL STREAM FISH OF NORTH EAST INDIA

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ABSTRACT

The hill stream Umkhrah, located in Shillong, the capital city of the state of Meghalaya in the North East India is being contaminated by city garbage and municipal wastes for the last few years. Water quality analysis of the contaminated hill stream revealed remarkably low level of dissolved oxygen, very low to high range of free carbon –di- oxide, fluctuation in pH and high level of total suspended solids (TSS). Histo-pathology, scanning electron microscopy and transmission electron microscopy of skin and muscle of the fish (Channa gachua) used in the study revealed epithelial detachment, proliferation of mucus cells and exfoliation of epidermal cells of skin; atrophy, necrosis and vacuolization of muscle at places; loss of compactness, irregularities in the arrangement of the myofilaments, distortion of the mitochondrial membrane and intense vacuolization of the muscle. The physiological significance of the observed abnormalities in skin and muscle of the fish is discussed with the help of available literature.

Introduction

In the recent years, the awareness of water pollution, besides other types of pollution has grown considerably because of its adverse impacts on the environment. It has become a major global problem requiring evaluation and revision of water resource policy at all levels (international down to individual aquifers and wells). There are numerous sources of water pollution in countries around the world. In developed countries up to 90% of wastewater flows

untreated into rivers, lakes and highly productive coastal zones [1] (WWDR, 2012) and 70% of industrial waste is dumped into waters where they contaminate existing water supplies [2] (UN-Water, 2009). Indian cities have been dumping untreated sewage and partially cremated bodies directly into the river Ganga [3]. Likewise, by an estimate of 2012, Delhi's sacred Yamuna River was found to contain about 7,500 coliform bacteria per 100cc of water. A large number of NGOs, pressure groups, eco-clubs etc have been active in their task to clean the river [4].

The state of Meghalaya, in the North Eastern part of India is endowed with rich water resources, which are gradually being polluted because of anthropogenic causes and unplanned development. The state of Meghalaya is situated at a latitude of 25°35′N and a longitude of 91°53′E, on the Khasi Hills range at varying altitudes of 1400 to 1950 meters above mean sea level. The two main rivers of Shillong are the Umshyrpi and Umkhrah, which join together to form the Wah Ro Ro near Sunapani. The river Umkhrah lies at an altitude of about 1600 meters above mean sea level. It originates from a spring located in the Shillong peak hill range near Demthring, and then it flows in the north-west direction before joining the river Umiam. The river Umkhrah, which was once a very clean water body, has become highly polluted during the last few years. The sources of pollution of the river Umkhrah include both point and non-point sources. Point sources of pollution are the effluents from hotels, restaurants, automobile workshops, hospitals, nursing homes, slaughter houses, vegetable and fish markets situated in the catchment areas. Non-point sources of pollution include indirect discharge of untreated sewage, municipal waste water, dumping of solid wastes and agricultural runoffs.

The hill stream Umkhrah was once known for its large variety of fish species. However, today there is a drastic decline of fish population in the stream, which demands a detail investigation of the stream and the fish species still available in it.

. Keeping these in view, it was considered worthwhile to undertake some studies to assess the water quality of the stream and to analyse the health status of the prevalent fish species, *Channa gachua* inhabiting it.

Channa gachua Hamilton, 1822 was the fish species chosen because this was the only species that was available for study at both the polluted and control sites. *Channa gachua* is a species of dwarf snakehead, some of its other common names being frog snakehead and brown snakehead. It belongs to the family Channidae. It is often referred to as the smallest snakehead, reaching a length of about 17cm [5], rarely exceeding 20cm [6]. It is found in all habitats, from mountain streams to ponds and is tolerant to very stagnant, poorly oxygenated, turbid water. Skin

and muscle were chosen for the study because of their importance in general physiology of the fish.

Materials and Methods

Study area:

A. Control water body: Control water body selected for the study was Rural Resource and Training Centre (RRTC), Umran, Meghalaya, where the ponds are managed by trained attendants to restrict entry of pollutants into them

B. Study sites: Study sites of the stream were *Wah Kaliar* (study site I) and *Jingthang Briew* (study site II). The first sampling station, *Wah Kaliar* is that part of the stream, which is used for washing clothes and vehicles. Besides this, many drains are directly connected to it, which discharge raw sewage from human settlements located along the stream. Further, the wastes generated from the sand and stone quarry located near the stream, and, pesticides and fertilizers from the nearby agricultural fields enter this part of the stream. The second sampling station, *Jingthang Briew* is located further downstream and is surrounded by large human settlements on both the sides. There is a cremation ground near this part of the stream and it is also used for washing intestines of slaughtered animals. In addition, the wastes generated from the hotels, restaurants, vehicle service stations, health centres and hospitals also enter this part of the stream.

Water quality analysis:

The pH and temperature of water of control as well as polluted water body were recorded with the help of DB1046 (Decibel) pH meter and Centigrade (0-110degree) mercury thermometer respectively. Conductivity, dissolved oxygen, free carbon-di-oxide, hardness, alkalinity and total suspended solids were analysed using standard methods [7].

Statistical Analysis

The Student's t-test was used to determine differences between control water body and study sites I and II. The data is presented as average with standard deviation and level of significance.

Fish sample:

Channa gachua (dwarf snakehead) (Fig. 1) was the fish species selected for this study since it was the only fish species found in both the study stations I and II.

Collection of samples:

Mature live fishes were collected with the help of a basket trap made of bamboo (locally called "khoh") from control as well as polluted water body and were brought to the laboratory for further analysis. The skin and muscle tissues were dissected out and were processed for histology, Scanning electron microscopy and Transmission Electron Microscopy.

Fig. 1. Dwarf snakehead (*Channa gachua*)

Histopathology:

Histopathological analysis of the skin and muscle of *Channa gachua* from control water body and polluted sites of the hill stream were analysed following the standard procedure [8]. The dissected tissues were cut into smaller pieces and were immediately fixed in Bouin's fixative for 24 hours. After fixation, the tissues were washed in running tap water overnight till the yellow colour disappears. Dehydration of the tissues was carried through ascending grades of ethanol (30%, 50%, 70%, 90% and 100%) for 15 minutes each. The tissues were then transferred to xylene and were kept in it for 15 to 20 minutes.

The tissues were then infiltrated gradually in rising proportion of paraffin wax mixed with xylene before embedding them in pure wax. The embedded tissues were kept in the oven overnight at 60°C. Blocks were prepared by putting the tissues in melted wax poured in paper boats. After proper cooling, the tissues were trimmed and sectioned with the microtome at 5µm thickness. The obtained ribbons were placed in clean slides

smeared with albumin and stretched using hot water and a hot plate. When the ribbon were fully stretched, the water was drained off from the slide and air dried.

Dried slides were dipped in xylene to remove the wax surrounding the sections and then rehydrated through descending series of ethanol grades (100%, 90%, 70%, 50%, 30% and distilled water for 5 minutes each). Then the sections were stained with aqueous Haematoxylin for 1 minute, washed in tap water to remove the excess stain and then differentiated in acid water. The sections were then dehydrated through ascending series of ethanol grades (30%, 50%, and 70% for 5 minutes each), stained in alcoholic Eosin-Phloxine stain for less than one minute and dipped again in 70% ethanol. Slides were then transferred to 90% and 100% ethanol grades for 7 minutes each, were cleared in xylene and were mounted using DPX . Observations were made in a LEICA, DM-750 image analyser and photographs were taken at X10, X40 and X100 magnifications.

Scanning Electron Microscopy (SEM):

The skin and muscle, tissues of *Channa gachua* from control water body and from the two study sites were cut into small pieces (1 mm x 1 mm) and fixed in 2.5-3% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer (pH 7.2) for 4 hours at 4°C. Following the primary fixation, the tissues were washed in 0.1 M sodium cacodylate buffer for 15-30 minutes, dehydrated in ascending grades of acetone (30%, 50%, 70%, 80%, 90%, 95%, 100% and dry acetone) with two changes of 15 minutes each and were dried in tetra methyl silane (TMS) following the method of Dey *et al.,* (1989) [9].The dried samples were secured horizontally to brass stubs (10mm diameter x 30 mm height) with double adhesive tape connected via a patch of silver paint to ensure charge conduction. A conductive coating of gold was applied to the sample using JFC-1100 (Jeol) ion-sputter coater by establishing a low vacuum (10^{-3} Torr) in the sputtering chamber. The coated samples were examined in JSM-6360 (Jeol) SEM at an accelerating voltage of 15-20 Kv, using the secondary electron imaging mode (SEI).

Transmission Electron Microscopy (TEM):

The skin and muscle tissues of *C. gachua* from control water body and the two study sites were cut into small pieces (1mm x 1mm) and fixed in modified Karnovsky's fixative having the

composition of 250ml of 0.2 M sodium cacodylate buffer, 20g of para-formaldehyde dissolved in it at 60°C, bringing the volume to 480 ml by double distilled water. To this, 20ml of 25% glutaraldehyde and 12.5g of anhydrous calcium chloride were added [10]. After 4 hours in the above primary fixative, the samples were washed thoroughly in 0.1 M sodium cacodylate buffer. Post fixation was carried out in 1% osmium tetroxide in the same buffer for 1 hour at 4°C.

Samples were dehydrated in ascending grades of acetone (30%, 50%, 70%, 80%, 90%, 95%, 100% and dry acetone) with two changes of 15 minutes each. Dehydrated samples were then cleared off acetone by propylene oxide for 30 minutes. Infiltration was carried out gradually in different proportions of propylene oxide with embedding medium {Araldite CY212- 10 ml, DDSA (dodecenyl succinic anhydride) – 10 ml, DMP-30 [Tri- (di-methylaminomethyl) phenol] - 0.4 ml, and di-butyl phthalate-1 ml}. Embedding of tissue was carried out in the araldite embedding medium using beem capsules.

The embedded blocks were kept at 50°C in an embedding oven for 24 hours. The temperature was then raised to 60°C and the embedded tissues were kept for 48 hours to complete polymerization. Ultra-thin sections (600A°-800A°) were cut in an RMC ultra-microtome, MT-X, with a diamond knife. The sections were collected on copper grids and stained with alcoholic saturated solution of uranyl acetate for 10 minutes at room temperature in the dark, followed by lead nitrate for 5 minutes [11]. The stained sections were examined in a Jeol JEM 2100 Transmission Electron Microscope at an accelerating voltage of 100-120 Kv.

Results:

Water quality analysis:

pH: The pH ranges at the two study sites exhibited acidic conditions during the winter months and, alkaline conditions during the summer months (Table1).

Temperature

The recorded temperature ranges in the control water body was 13°C to 30°C whereas in the study sites I and II the recorded temperature ranges were 10°C to 21°C and 10°C to 22°C respectively. **(**Table1)

Conductivity

The conductance values recorded in the control water body ranges from 70 to 110 micro S/Cm^{-1}. Whereas the ranges of conductance values recorded in the study sites I and II were in the ranges of 80 to 130 microS/Cm^{-1} and 180 to 380 micro S/Cm^{-1} respectively. (Table1)

Dissolved oxygen (DO)

The level of dissolved oxygen in the control water body was recorded at the ranges of 6.4 mg/l to 8 mg/l, whereas the ranges of dissolved oxygen in the study sites I and II were recorded as 2.6 mg/l to 5.0 mg/l and 1.2 mg/l to 3.3 mg/l respectively. (Table1)

Free carbon dioxide

In the control water body, the level of free carbon dioxide (CO_2) was recorded between 6 mg/l and 9 mg/l, while the ranges of free CO_2 in the study site I and II were in the ranges of 2.6 mg/l to 8.6 mg/l and 4 mg/l to 16 mg/l respectively (Table 1).

Hardness

Hardness in the control water body was found to be between 90 mg/l to 120 mg/l, whereas in the study sites I and II the level of hardness ranged from 30 mg/l to 125 mg/l and 59 mg/l to 110 mg/l respectively. (Table1).

Total alkalinity

Alkalinity in the control water body ranged from 55 mg/L to 75 mg/L whereas the in the study sites I and II, it ranged from 30 mg/l to 70 mg/l and 28 mg/l to 87 mg/l respectively. (Table1).

Total suspended solids (TSS)

The total suspended solids in the control water body ranged from 7 mg/l to 30 mg/l, whereas in the study sites I and II it ranged from 18 mg/l to 178 mg/l and 42 mg/l to 130 mg/l respectively. (Table 1)

Table 1. Physico-chemical characteristics of water in control and study sites (I & II).

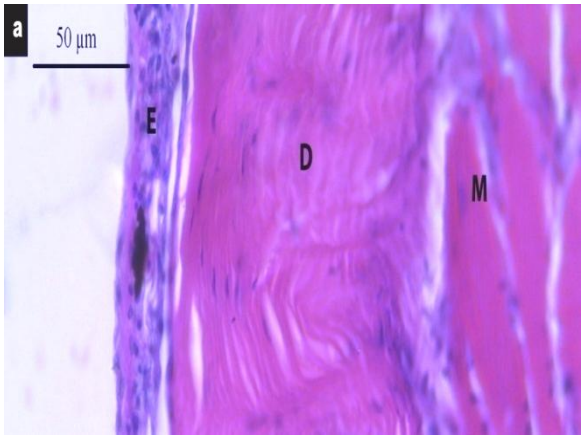
Parameters	Control	Study site I	Study site II	Desirable Range
Temperature. (^oC)	13-30	10 -21	10-22	26-32
p^H	6.5-7.5	6.5 -7.85	6.2 -7.78	6.5-9.5
Electrical Conductivity (micro S /cm)	70-110	80 -130	180-380	100-2000
Dissolved Oxygen (mg/L)	6.4-8	2.6 -5.0	1.2-3.3	7-9
Free Co_2 (mg/L)	6-9	2.6 -8.6	4-16	5-10*
Total alkalinity (mg/L)	55-75	30 -70	28-87	50-150
Hardness (mg/L)	90-120	30 -125	59-110	50-150
Total Suspended Solids (mg/L)	7-30	18 -178	42-130	150

*Desirable range for CO_2 is not fixed but free CO_2 in natural reservoir rarely exceeds 5 to 10 mg/L.

Histopathology

In the control fish, epidermis and dermis of the skin were found to be compact (Fig.2a). In the skin of fish from study site I, on the other hand, distinct gaps between epidermis and dermis were evident (Figs 2b, c.). At higher magnification, proliferation of mucus cells and ruptured epithelial cells were also observed (Fig.2c). Fish collected from study site II, exhibited skin disruption, reduction in skin thickness, exfoliated epidermis (Fig.2d) and massive proliferation of mucus cells (Fig.2e).

In control fish, the muscle fibres showed their natural compactness (Fig.Fig.3a, b). However, in fish collected from study site I, breakage (Fig.3c), disturbance in alignment and atrophy of muscle fibres were observed (Fig.3d). In fish from study site II, intense splitting of the muscle fibres and focal areas of necrosis were observed (Fig.3e). Disturbances in the normal alignment and vacuolar degeneration in certain areas of the muscle fibres were also observed (Fig.3f).



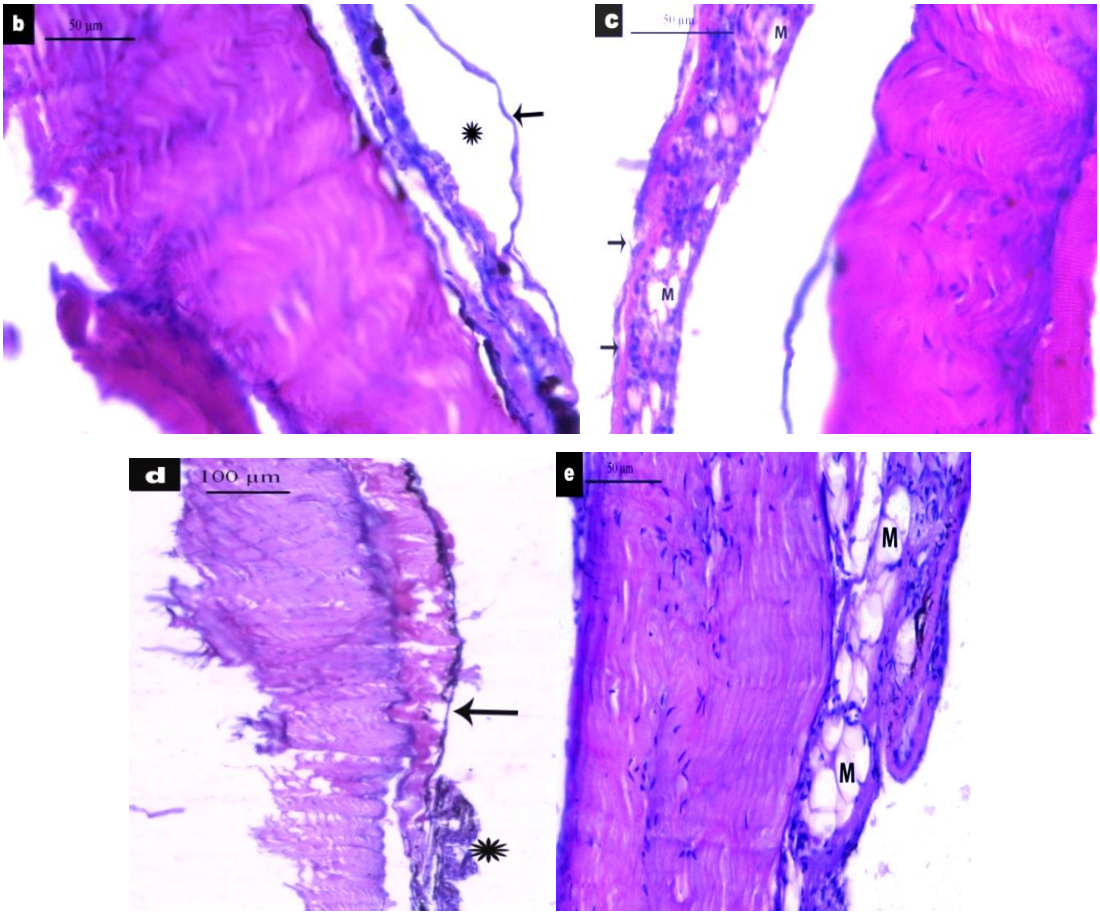


Fig. 2. Photomicrographs of the skin of *C. gachua* (a) **Control**, **E,** Epidermis; **D,** dermis; **M,** muscle layer **(b-c**) ***Tissues of fish*** **from study site I. (b)** Separating epithelial layer (arrow), gaps between the layers (*).. **(c)** Proliferated mucus cells **(M)** and ruptured epithelial cells (arrows), gap between layers.. (**d-e**) ***Tissues of fish from*** **site II. (d)** Reduction in skin thickness (arrow) and exfoliated epidermis (*).**(e)** Proliferated mucus cells **(M),**.

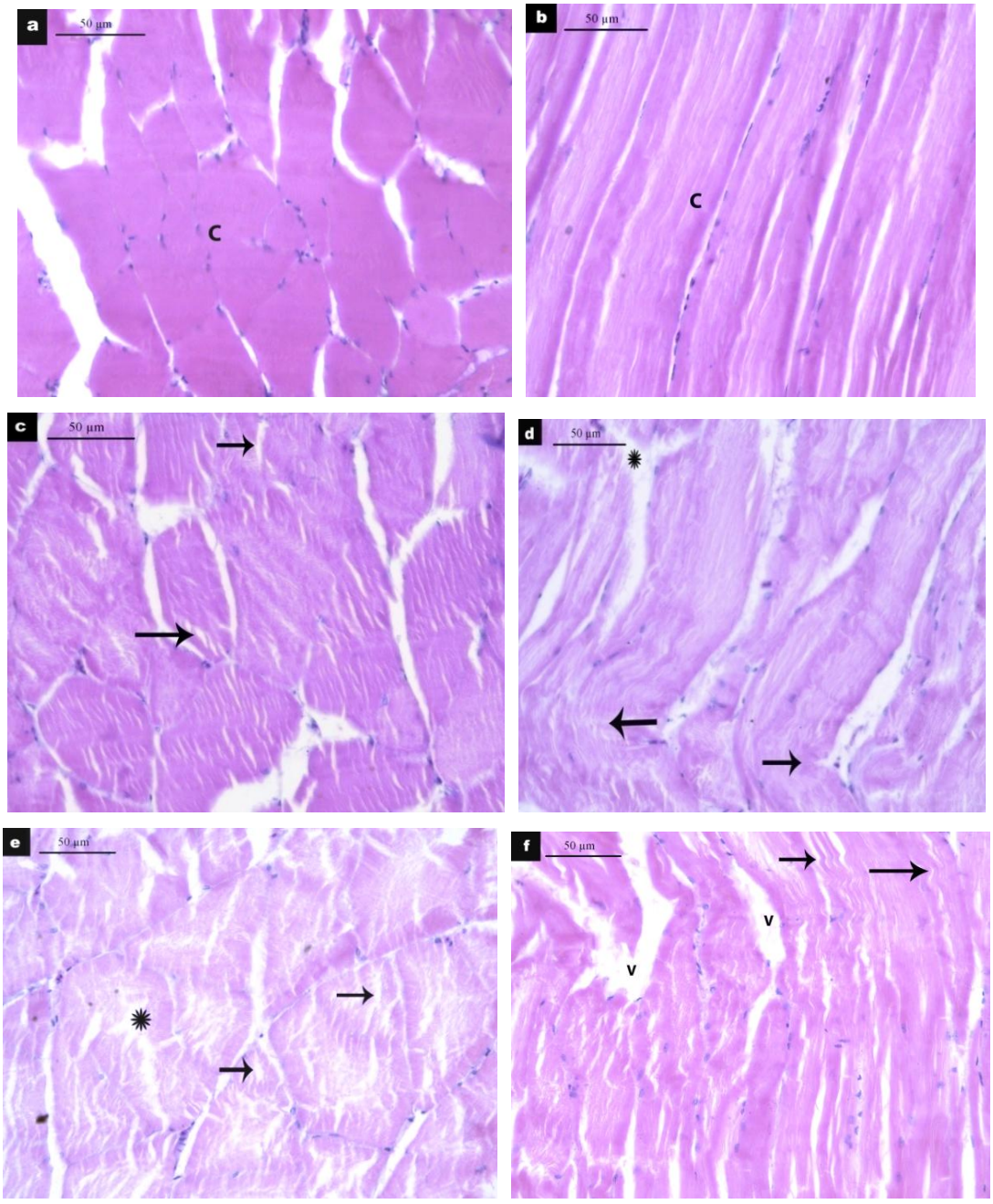


Fig. 3. Photomicrographs of the muscle of *C. gachua* a, b**. Control**. Compactness of muscle fibres, [**C] (b) (c-d**) ***Tissues of fish*** **from study site I. (c)** Splitting of muscle fibres (arrows).**(d)** Distortion (arrows) and atrophy (*) of muscle fibres. **(e-f**) ***Tissues of fish*** **from study site II. (e)** Intense splitting of muscle fibres (**arrows**) with focal areas of necrosis (*).**(f)** Distortion of muscle fibres (arrows) with vacuolar degeneration (**V**).

Scanning Electron Microscopy

The scanning electron microscopy of the skin of *C. gachua* collected from the control site revealed smooth surface features of the epidermal layer (Fig.4a, b). However, lesions and dead tissue debris were observed in the skin of fish collected from study site I (Fig.4c, d). The abnormal features of the skin of fish collected from study site II, included necrosis in the epidermis (Fig.4e) and highly eroded skin surface (Fig.4f) with exposed underlying muscle (Fig. 4f).

Transmission Electron Microscopy

Transmission electron microscopy revealed distinct layers of the skin, normal epidermal nuclei, lysosomes, hemidesmosomes etc (Fig.5a, b).In fish from study site I, on the other hand, breakage of the epidermis with disturbed arrangement of the layers, loss of cellularity (Fig.5c) and degenerating nuclear chromatin in the epithelial cell (Fig. 5d) were observed. In fish from study site II, a very high degree of distortion of the cell membrane, lack of demarcation of different layers and vacuolated cytoplasm in the integument cells were observed (Fig.5e). Distorted shape of epidermal nucleus with condensed nuclear chromatin material as well as breakage of nuclear envelope was also observed (Fig.5f).

Transmission electron microscopy of the muscle of control fish revealed well developed and orderly arranged myofilaments along with the presence of sarcoplasmic reticulum, abundant lipid droplets, well arranged T system and mitochondria with normal arrangement of inner as well as outer membranes (Fig.6a, b). In contrast, muscle examined from fish inhabiting study site I, revealed gaps between the myofilaments, loss of compactness, irregularities in their arrangement and absence of the T system (Fig.6c). Distortion of the outer mitochondrial membrane, reduction in the size of the mitochondria and intense vacuolization (Fig.6d) of the organelles were also evident. Besides these, distortions of myofilaments, T system (Fig.6e) and inner as well as outer mitochondrial membranes (Fig.6f) were exhibited by the muscle of fish from study site II.

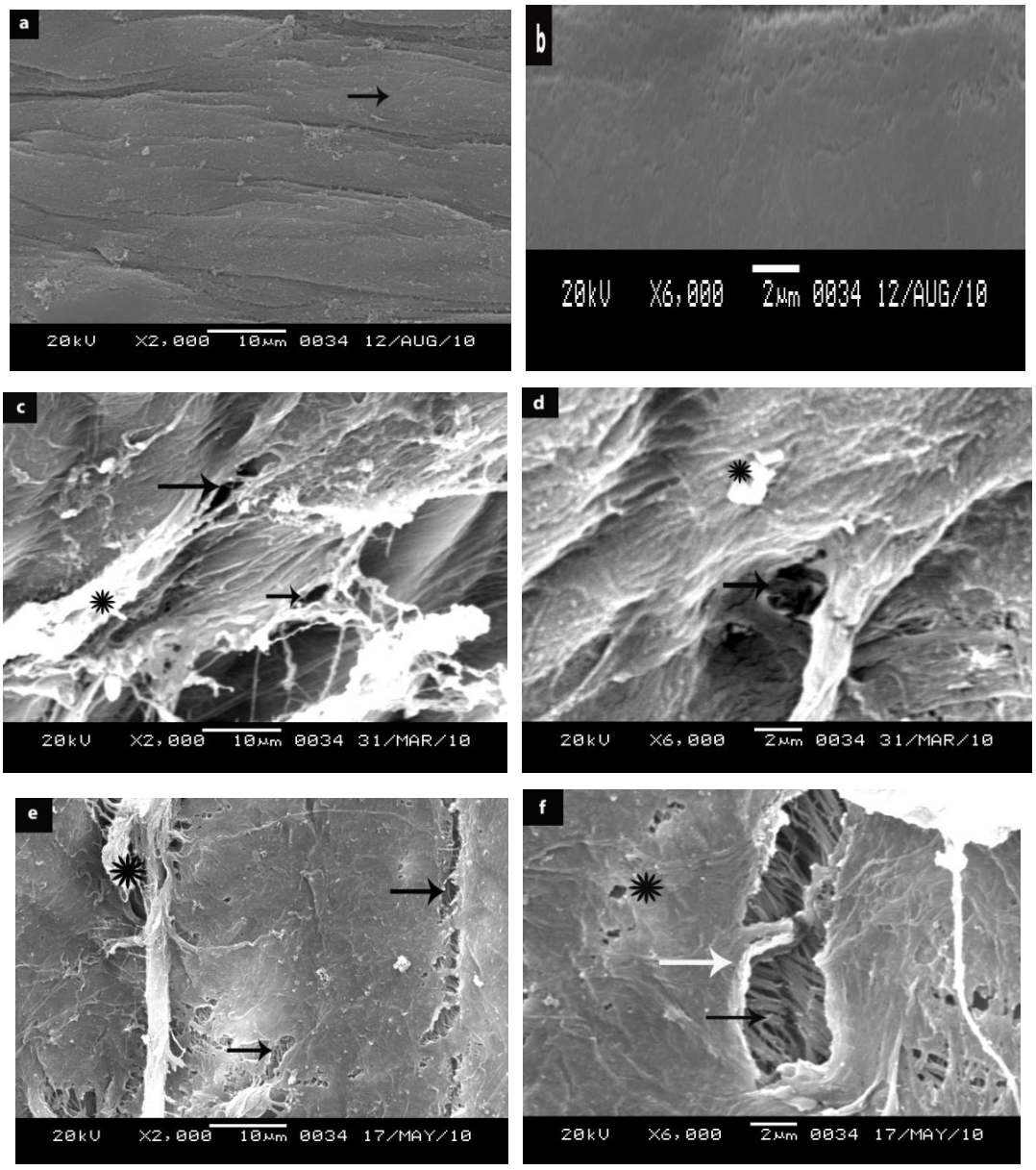


Fig. 4. Scanning Electron Micrographs of skin of *C. Gachua*.

(a-b): Control (a) Smooth surface of epidermal layer (arrow. **(b)** Enlarged view of Fig, a. **(c-d**): Tissues of the fish from study site I. **(c)** Lesions (arrows) and dead tissue debris (*) **(d)** Enlarged view of Fig. 2c, lesion (arrow) and tissue debris (*). **(e-f**): Tissues of the fish from study site II. **(e)** Dead tissue (*) and necrotic regions (arrows). **(f)** Eroded skin (white arrow) with exposed underlying muscle fibres (black arrow) and necrotic regions (*).

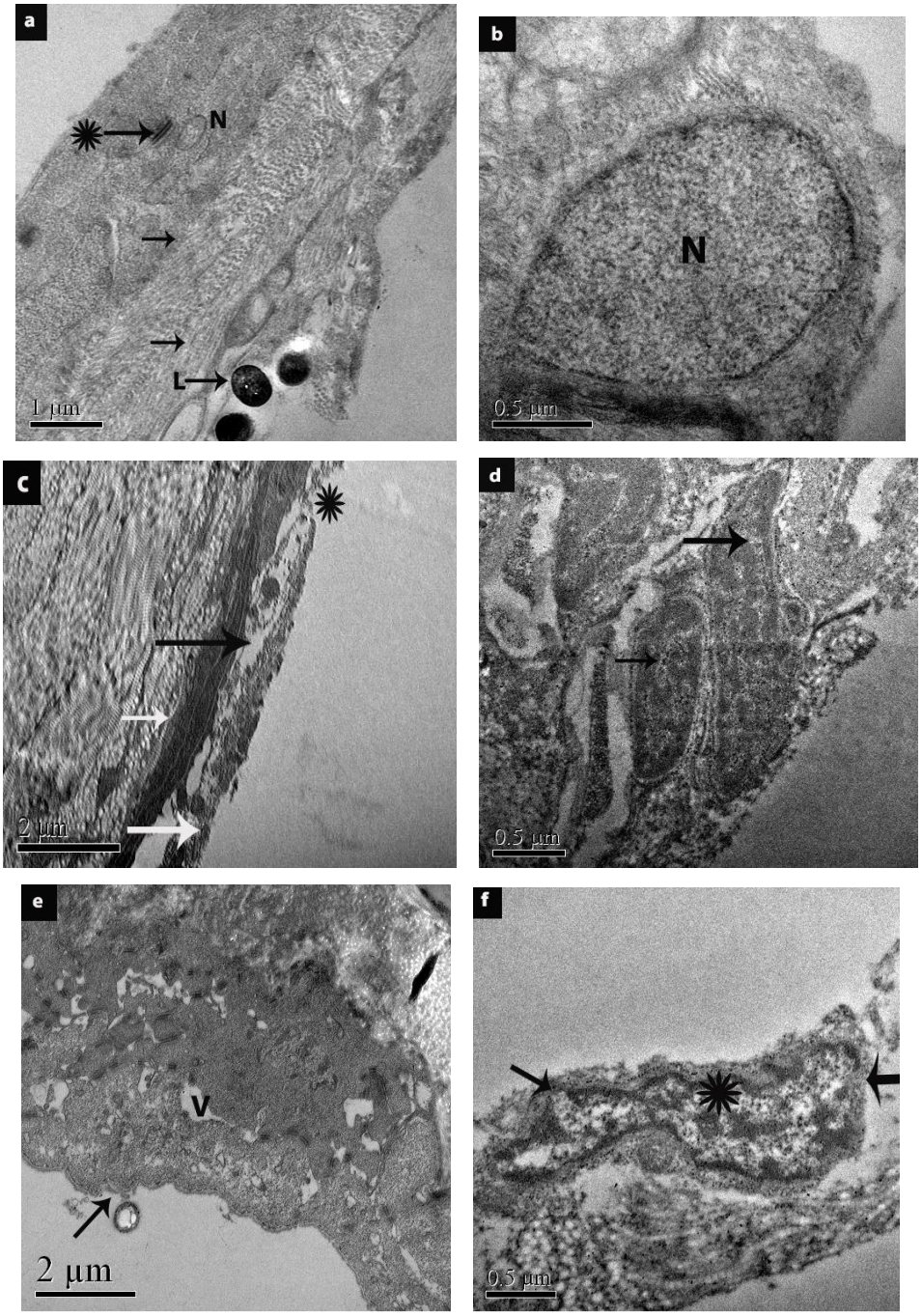


Fig. 5. Transmission Electron Micrographs of the skin of *C. gachua*.

(a-b): Control. (a) Distinct layers (arrows), nuclei (N), lysosomes (L) and hemidesmosomes (*). **(b)** Normal epidermal nucleus (N). (**c-d**): Tissues of the fish from study site I. **(c)** Broken epidermis (*) with disorganised layers (white arrows) and loss of cellularity (black arrow), **(d)** Degenerating nuclear chromatin (arrows). **(e-f**): Tissues of the fish from study site II. **(e)** Distorted epidermal layer (arrow) and vacuolated cytoplasm (V), (**f)** Distorted epidermal nucleus showing condensed chromatin (*) and breakage of nuclear membrane (arrows).

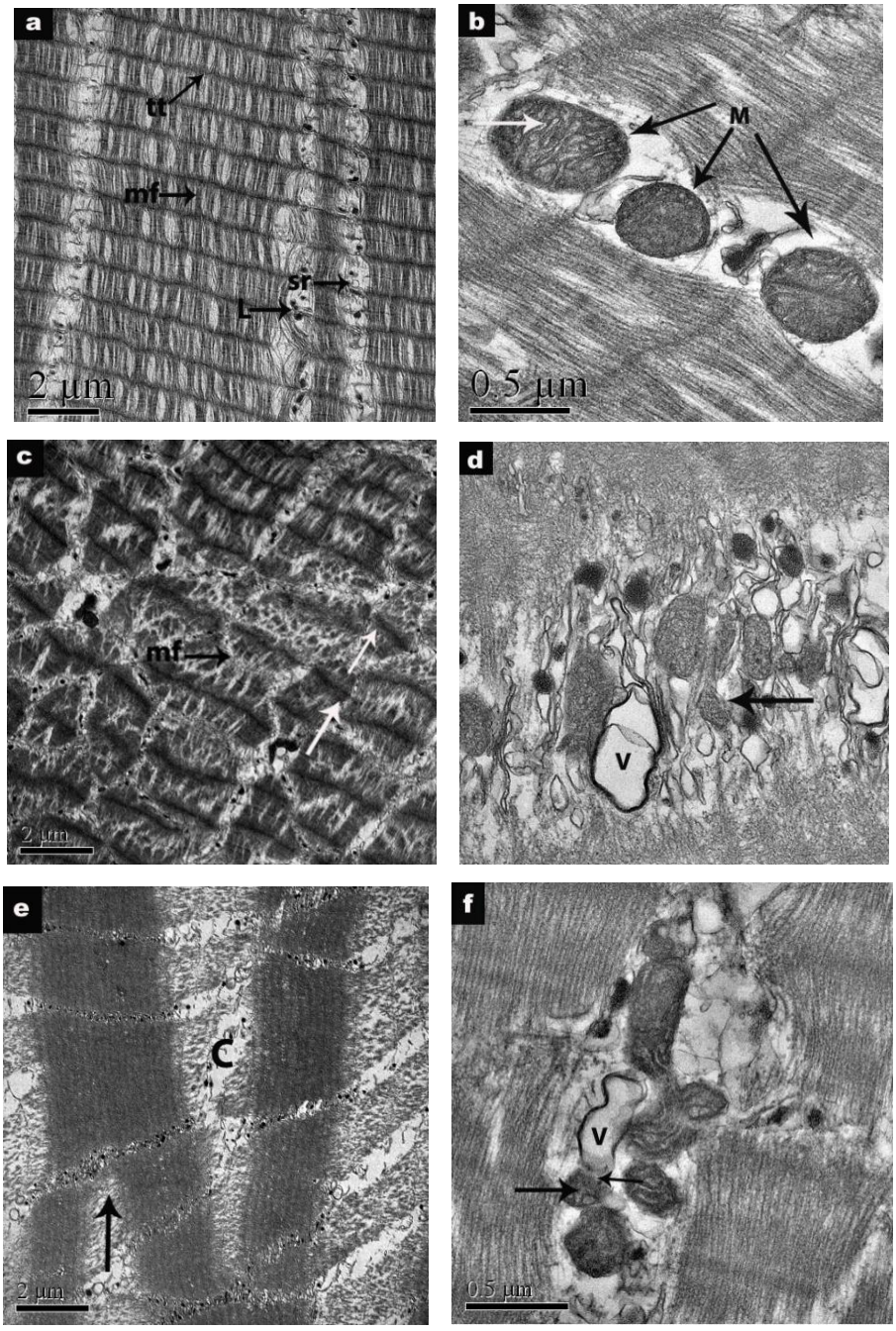


Fig. 6. Transmission Electron Micrographs of the muscle of *C. gachua*.

(a-b): Control. (a) Well developed and orderly arranged myofilaments (mf) with normal sarcoplasmic reticulum (sr), lipid droplets (l) and T system (tt), **(b)** Normal mitochondria (M) with intact membranes (arrows). (**c-d**): Tissues of the fish from study site I**. (c)** Broken myofilaments (mf) & distorted T system (white arrows), **(d)** Distortion of outer mitochondrial

membrane (arrow) and intense vacuolization (V) of mitochondria.(**e-f**): Tissues of the fish from study site II. **(e)** Loss of compactness (C), irregularity in arrangement of myofilaments (arrow) and absence of T system. **(f)** Distortion of inner and outer mitochondrial membranes (arrows), decrease in size and intense vacuolization (V) of mitochondria

Discussion

Water quality parameters:

Abnormal values of some of the water quality parameters in the hill stream Umkhrah as revealed from our present study might have been resulted from contamination by municipal wastes and city garbage, thereby causing adverse effects on the biology and physiology of fish inhabiting it.

The pH ranges at the two study sites exhibiting acidic conditions during the winter months and, alkaline conditions during the summer months appear to be significant. This is because water pH has a significant influence on the toxic action of a number of pollutants such as ammonia, hydrogen sulphide, cyanides, and heavy metals on fish [12]. Heavy metals tend to be more toxic at lower pH because they are more soluble and more bio-available under acidic condition. The concentration of cadmium and lead has been reported to be considerably higher in fish from acidified lakes [13, 14]. Metals such as zinc and lead are most likely to have increased detrimental environmental effects as a result of lowered pH [15]. On the other hand, when pH of fresh water body becomes highly alkaline (9.6), the toxicity of some pollutants increases, exerting adverse effects on fish (*Lenntech.com*) [16]. The more acidic nature of the water at study site II during the winter months may be attributed to the persistence of anaerobic conditions which is due to the higher organic loads from the sewage and municipal dumping. On the other hand, slightly higher alkaline values from the study site I during the summer months may be attributed to the presence of detergents from the washing of vehicles and clothes at this site.

Low dissolved oxygen concentrations at the two study sites throughout the year appears to be due to organic load introduced from municipal waste into the stream[17]. Since oxygen is needed for ATP synthesis in the mitochondria, low dissolved oxygen in the stream causes adverse effects on the synthesis of ATP by the fish population inhabiting the stream. However, in spite of these low dissolved oxygen levels *C. gachua* survives because they are tolerant to poorly oxygenated and turbid water and flourish in waters rendered too toxic to most fishes [18]. Moreover, snakeheads are either obligate or

facultative air breathers and therefore survival in hypoxic waters is not problematic to these fishes [19]. This explains the near absence of most other fish species in the two study sites of the polluted stream.

Never the less, the low dissolved oxygen levels at the two study sites can adversely affect the fish. It has been reported that fish exposed to oxygen deficient water do not take food, collect near the water surface for air, gather at the inflow to ponds, fail to react to irritation, lose their ability to escape capture and ultimately die [20]. Moreover, in oxygen deficient water, the fishes are exposed to a situation where increased amount of toxic substances in the water reaches the gill surface [21]. This is a consequence of increased ventilation rate to compensate for the normal fluctuations of energy demands of the fish due to less oxygen concentration.

The higher values of electrical conductivity at study site II can be explained by greater discharge of municipal wastes and sewage effluents. In this context it is to be noted that increase in conductivity was reported to be due to contamination of water with municipal solid wastes and leachates [22].

The range of CO_2 at both the study sites, which were within permissible limit suggests that it has no adverse effect for fish health [23]. Thus, the recorded free CO_2 levels are unlikely to cause any stress to the inhabiting fishes.

Low water hardness at study site I may be due to increased washing of clothes and cars using laundry detergents, which may include water-softening agents. This may be at times a source of stress to the fishes because toxicity of some metals in fish was reported to be more pronounced in soft waters [24-26].

Total Alkalinity is a measure of the buffering capacity of a solution and is thus an important water parameter. Alkalinity in streams is measured by its bicarbonate content. The greater the amount of bicarbonate, the greater is the alkalinity thus the greater is the resistance to changes in pH. However, high alkalinity results in physiological stress on aquatic organisms and may lead to loss of biodiversity. In the present study, the low alkalinity values in water of the study sites suggests that at times, these water bodies may be susceptible to fluctuations in pH, resulting in stress to the inhabiting fish species.

The presence of suspended particles in the study sites in concentration within the recommended limit suggest that there are no adverse effects of total suspended solids on the fish investigated in the present work.

Histopathology and ultra structure:

Skin:

Skin forms the first line of defence in fish, which is reflected from the fact that, the mucus and epithelium has many antimicrobial factors [27]. However, till now, availability of the skin toxicity data is limited [28].

In the present study, architectural abnormalities in the surface epidermal cells including rupture of the epithelial cells, reduction in epidermal layer, necrosis, proliferation of mucus cells, gaps in between the epidermis and dermis etc. indicate the adverse impact of city garbage and municipal wastes on the skin of *C. gachua* inhabiting the contaminated water body. In this context, it is worth mentioning that brown trout inhabiting polluted river showed a higher prevalence and abundance of multifocal skin erosions and ulcers [29].

The aforementioned defects could be due to the effects of heavy metals present in the city garbage and municipal wastes [30, 31]. This is further supported by reports on hypertrophy of mucus cells and focal epidermal cell necrosis in some fish exposed to domestic and industrial waste water containing cadmium, lead, mercury, zinc, iron and manganese [32]. The vacuoles detected in the epidermal cytoplasm are likely to be due to xenobiotics, estrogens as suggested by some authors [33]. Municipal waste and leakage from septic tanks have been reported to be associated with epidermal necrosis, extensive skin lesions and muscle necrosis [34]. Adverse effects of synthetic detergent on fish skin and epidermal exfoliation has also been reported [35, 36]. The proliferation of the mucus cells observed in the skin of *C. gachua* from both the study sites is significant since the number of mucus cells of fishes is affected by many stressors. In this context, it is worth mentioning that the enumeration of the skin mucus cells of fishes is being used to monitor stress [37]. Toxic and irritating substances can greatly stimulate mucus secretion, increasing the thickness of the mucus blanket that plays an important preliminary role in defence mechanism against metal toxicity [38, 39].

Muscle:

The histopathological observations on splitting, distortion and atrophy of the muscle of the fishes inhabiting the polluted stream could be due to the effects of heavy metals [40, 41).

The disturbances in alignment and distortion of muscle tissues at places in *C. gachua* inhabiting the polluted water body suggest muscular abnormalities in the fish exposed to municipal wastes and city garbage. It is to be noted in this context that muscle of *Anabas testudineus* exposed to bleached sulphite pulp mill effluent has been reported to show loss of alignment of fibres, distortion at places and vacuolization [42]. Further, extensive vacuolization observed in the present study, points to necrotic or apoptotic cell death [43]. The damages to the mitochondrial membranes and decrease in the size of mitochondria in the fish inhabiting the polluted stream could be attributed to the hypoxic conditions in which the fishes are existing [44]. Changes in mitochondrial membrane function in fish exposed to some pollutants are reported to cause decrease in acid phosphatase activity in the muscle [45].

The activation of muscle contraction is a rapid event that is initiated by electrical activity in the surface membrane and transverse (T) tubules. This is followed by release of calcium from the inner membrane system, the sarcoplasmic reticulum [46]. Thus, the distortion and the absence of the T system as seen in the muscles of fish in the present study, suggest that the contractibility of the muscles in the fishes was greatly affected.

The present observation on abnormalities in muscular histology and fine structures suggest that there is an abnormal physiological condition in the fish inhabiting the polluted stream. Moreover, disturbances in horizontal and vertical muscle bands suggest impaired functioning of muscle, since these bands play important roles in muscular performance [47].

Conclusion

The present study reveals that city garbage and municipal wastes entering the stream Umkhrah, has disturbed several important water quality parameters resulting in tremendous stress on fish population inhabiting the water body. In fact, drastic decline in population of a large variety of fish has taken place in the stream except *Channa gachua* which could some how withstand the adverse conditions of the stream and have managed to survive and reproduce till now. However, the histo-pathological and scanning as well as transmission electron microscopy revealed a number of abnormalities in skin and muscle of the fish. Since the aforementioned tissues play vital roles in growth, survival and normal physiological activities of fish, it is quite likely that the fish studied in the present investigation also cannot withstand the deteriorating conditions of the stream caused by dumping of city garbage and municipal wastes any more. Hence, it is necessary to study other vital tissues of the fish exposed to the contaminated stream in order to find out the health status of the fish in the stream. Immediate measures are to be taken to stop polluting the

stream so that the echthyofaunistic diversity of the hill stream is restored. The study further suggests that combined approach involving water quality analysis, histopathology, scanning electron microscopy and transmission electron microscopy is extremely important to address the problems concerning aquatic biota of polluted water bodies.

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