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A HISTOPATHOLOGICAL CHANGES IN SKELETAL MUSCLES OF *CATLA CATLA* EXPOSED TO THERMAL POWER PLANT EFFLUENT

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ABSTRACT: For the assessment of effect of thermal power plant effluents on the skeletal muscle of fish Catla catla, histological, histochemical and biochemical methods were used and results were compared with control fish. Histologically thermal power plant effluent exposed fish muscles showed marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Pronounced intramuscular oedema with minor dystrophic changes and splitting muscles were also observed in the muscles of fish *Catla catla* exposed to the thermal power plant effluents. The pathological findings included mild lymphocyte infiltration, vacuolar degeneration in muscle bundles and atrophy of muscle bundles. Degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis were noticed. Vacuolar degeneration in muscle bundles and atrophy are also observed in the muscle bundles might be due to the various concentrations of mixture of heavy metals in the thermal power plant effluent. The physiological disturbances and morphological damages in the muscle tissue of fish caused due to thermal power plant effluents pollutants. Histochemically the content of protein and carbohydrate in muscles tissue was found to be decreased with decreased intensity of reaction in thermal power plant effluent exposed to fish as compared to control. Biochemically In the fish exposed to the thermal power plant effluent showed increase in the content of skeletal muscle protein and it was found to be 1.40 mg/gm.

Key words: - Histology, Pathological changes, skeletal muscle, effluent water, fish.

INTRODUCTION:

The adverse input of diverse industrial wastes has aggravated the problem of contamination and industrial disposal has greatly enhanced the addition of heavy metals into the aquatic ecosystem. The contamination of freshwaters with wide range of heavy metals released from domestic, industrial and other man made activities has become a global problem during recent years which may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. Heavy metal contamination has been reported in aquatic organisms (Adham et al., 2002 and Olojo et al., 2005) and trace metal contaminations are important due to their potential toxicity for the environment and human beings (Gueu et al., 2007; Lee et al., 2007; Adams et al., 2008; Vinodini and Narayanan, 2008).

Main anthropogenic sources of heavy metal contamination are mining, disposal of untreated and partially treated effluents which contain toxic metals from different industries. Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms (Storelli et al., 2005). Power generation requirement has been increased in recent decades to meet the demand of increasing population in India. Most of the electricity, about 70% of total is generated by coal based thermal

power plants. So coal supplied to thermal power plant is of poor quality. Coal is the main source used as fuel energy is converted into useful heat energy in Combustion of coal at thermal power plants emits mainly carbon dioxide (CO2), sulphuroxides (SOx), nitrogen oxides (NOx), CFCs.(Vishal Vasistha , 2014)
Major pollutants due to coal based power generation include sulphur, carbon and nitrogen compounds, heavy metals and fly ash. The thermal power plant effluent contains gases, fly ash and traces of heavy metals which can endanger the inhabitants. Coal operated thermal power plant can be a source of pollution, because ash derived from burning of coal containing heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) and zinc (Zn) can contaminate water, presenting a potential hazard to the environment. (Kanungo and Mahapatra, 2000). Histopathological studies have been conducted to help, establish causal relationships between contaminant exposure and various biological responses. Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments. Histopathology provides a rapid method to detect effects of irritants in various organs. The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs. Muscles, Gills, kidney and liver are suitable organs for histological examination in order to determine the effect of pollution (Halis, et. al. 2010).

The biochemical composition of the fish muscles tissue generally indicates the fish quality. Therefore, proximate biochemical composition of a species helps to assess its nutritional and edible value in terms of energy units compared to other species. Variation of biochemical composition of fish flesh may also occur within same species depending upon the fishing ground, fishing season, age and sex of the individual and reproductive status. The spawning cycle and food supply are the main factors responsible for this variation (Love, 1980).

MATERIALS AND METHODS:

The present study was undertaken during the period November 2017 to April 2018 to analyse the histopathology, histochemical and biochemical changes in liver of fish *Catla catla*, exposed to thermal power station effluents.

Experimental animal:

The Indian major carp *Catla catla* was used for the present study.

Collection of Fish:

A live specimen of fully matured *Catla catla* is captured from the Koradi lake with the help of local fisherman. Koradi lake is heavily polluted by the effluents of the Koradi thermal power plants. After collecting live fishes, they were brought to the laboratory for further work of dissection and processing. Block preparation was made. Tissues was fixed, preserved in 70 % alcohol for block making.

Sectioning:

Sectioning was done. Tissues were cut at 7 micron thickness with the help of Rotary Microtome at 25^{o}C to 30^{o}C. The ribbon of the sections were spreads on glass slides which is already coated by Mayer's albumin.

Staining:

a. Histological staining:

The standard double staining method using Haematoxylin and Eosine stains was followed. The cleared sections were mounted in DPX mountant (SRL make) and covered with cover glass of appropriate size, and allowed to dry. Stained sections were observed under compound microscope for histopathological effects.

b. Histochemical Staning:

Routine methods for staining with different chemical were done for carbohydrate and protein localization. Approximately 2-4 stained sections of each were observed under light microscope

(Nikon) and photographs were taken using digital camera (Nikon E8400) at various magnifications.

Biochemical estimation of total protein:

The total protein was estimated by the method of Lowry et al., (1951). The total protein content was expressed in mg/100mg wet weight of tissue.

Observation and Photography:

The stained slides were carefully observed by using BESTO made E13 model light microscope. After careful examination of the slides the microphotographs were taken at different magnifications on the computer attached microphotography unit present in laboratory of Department of Zoology, Sevadal Mahila Mahavidyalaya, Nagpur.

RESULT & DISCUSSION:

a. Histopathological observations:

Histology of skeletal muscles of control fish Catla catla

The muscles of the body of freh water fish Catla catla consist of a double series of muscle segments, the mytomes, in the region of the trunk and tail. The trunk musculature consist of successive segments the myomeres, running along each flank. The muscle fibers are oriented in anterioposterior position in each myotome and are separated from the adjacent ones by stout sheets of connective tissue; the myocommata. The myotomes are bent forward and backward and fit with the adjacent ones by the cone within the cone arrangement. Histologically muscle fibers have peculiar ribbon like myofibrillar bundles and rod edges of the fiber. Muscle fibers are arranged like spokes from a small central sarcoplasmic hub. (Figure 2 A).

Histology of skeletal muscles of fish Catla catla exposed to thermal power plant effluent

On exposure to thermal power plant effluents marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Pronounced intramuscular oedema with minor dystrophic changes was also observed in the muscles of fish Catla catla exposed to the thermal power plant effluents (Figure 2 B & C). Muscles oedema and mild lymphocyte infiltration, vacuolar degeneration in muscle bundles and atrophy of muscle bundles were observed. Oedema between muscle bundles and splitting of muscle fibers were seen. The vacuolar degeneration and atrophy of muscle bundles were also seen (Figure 3A & B). The pathological findings included degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis. Also, vacuolar degeneration in muscle bundles and atrophy that are observed in the muscle bundles might be due to the various concentrations of mixture of heavy metals. The physiological disturbances and morphological damages in the muscle tissue of fish caused due to thermal power pollutants. The degeneration of muscles and its bundles with aggregation of inflammatory cells between them and focal areas of necrosis was observed (Figure 3 C). The photomicrograph of the muscle of fish from control and thermal power station polluted lake shows the presence of normal myotomes with equally spaced muscle bundles. In the muscle of fish *Catla catla* from thermal power plant polluted waters marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Sub lethal concentration of cadmium led to pronounced intramuscular oedema with minor dystrophic changes was also noticed (Figure 3 B & C)

b. Histochemical observations

Protein

In the normal fish Catla catla, muscle fibers were stained with Mercury Bromophenol Blue stain indicating presence of proteins in muscles. Microscopically in exposed fish muscles, following changes were noticeable. Degenerated muscles of exposed fish developed vacuoles in between the muscle fiber. Because of muscle atrophy, muscle fibers were condensed showing progressive decrease in staining intensity to Mercury Bromophenol Blue (Hg-BPB) indicating either reduction in the proteins or loss of proteins. (Figure 4 A, B & C).

Carbohydrates

In the normal fish Catla catla, muscle fibers were stained with PAS stain indicating presence of carbohydrates in muscles as it is the source of energy. Degenerated muscles of exposed fish developed gaps in between the muscle fiber. Because of muscle atrophy, muscle fibers were constricted showing less staining intensity to PAS indicating either loss of the glycogen or more utilization of glycogen for fulfillment of energy. (Figure 5 A, B & C)

c. Biochemical Observations

The total protein content in the skeletal muscles of the fish *Catla catla* in control condition is found to be 1.86 mg/gm. In the fish exposed to the thermal power plant effluent showed and increase in the content of skeletal muscle protein and it was found to be 1.40 mg/gm. (Table 1 & Fig. 1).

In the animal kingdom fish are the most vulnerable to environmental chemicals because they cannot escape from their polluted environment. Muscle tissue come into close contact with pollutants dissolved in water. The muscles of fish showed degeneration in muscle bundles accompanied with focal areas of necrosis as well as atrophy and vacuolar degeneration in muscle bundles. Similar results are studied in tissues of Oreochromis niloticus and Lates niloticus as a result of the accumulated metals (Fatma Mohamed, 2008).

Separation and degeneration of muscles, atrophy of muscle bundles and focal area necrosis was an interesting observation in muscle tissue leading to vacuolar degeneration and splitting of muscle fibers which was seen in present study. The histopathological alteration in the muscle of exposed fish was in agreement with the observations of many investigators who have studied the effect of different pollutants on fish muscle (Sakr and gabr, 1991; Nour and Amer, 1995 and Elnemaki and Abuzinadah, 2003).

The results of many field studies of metal accumulation in fish living in polluted waters show that considerable amounts of different metals may be deposited in fish tissues without causing mortality. Various metals are accumulated in fish body in different amounts. These differences result from different affinity of metals to fish tissues, different uptake; deposition and excretion rates. Metal accumulation in fish depends on pollution, and may differ for various fish species living in the same water body (Jezierska and Witeska, 2001). Generally, the higher metal concentration in the environment, the more may be taken up and accumulated by fish. There seems to be a definite correlation between tissue damage and certain physiological alterations (Olurin et al., 2006).

The total protein content in various tissues in fishes were investigated by various authors and correlated their results with different factors like seasons, habitat differences, processing methods, sex differences, breeding seasons and nonbreeding seasons, size and age differences etc. which are summarized as below. There are very few reports on the variations in the total protein content in the fish tissues with difference in the habitat of fish. Some authors examined the protein level of Mystus

tengara, Mystus cavasius, Mystus gulio at fresh condition were 16.26 %, 15.52 % and 14.80 %. After twenty days of freezing protein level decreased as 14.97 %, 13.91 %, 13.43 %. Amount of moisture for these fishes increased 76.12 % to 78.02 %, 75.35 % to 77.25 % and 76.03 % to 78.23 % respectively.

The presence of pollutants in aquatic environment exerts its effect at cellular or molecular level which results a significant change in biochemical responses and for monitoring of aquatic environment analysis of biochemical methods offer as important biomarkers (Authman et al., 2013b).

In the present investigation the protein and carbohydrate histochemistry showed decrease in the intensity of the stain, hence it the amount of the protein and the carbohydrate in the skeletal muscles of the fish Catala catla may reduce. The reason may be attributed to the fact that due to high metabolism the carbohydrate utilization increases and hence may decrease from tissue cytoplasm whereas protein get degrades from the cytoplasm and enters into the blood to provide further energy for various metabolic reactions.

Bashir and Noory, (2012) also reported depletion in glycogen content of rat liver when treated with lead. Hence it could be reason that heavy metals interfere with the glycolytic activities and reduced the tissue glycogen. Mahran et al., (2011) reported reduction in liver and kidney tissues of rat after exposed to CdCl2 in PAS reaction particularly in degenerative and necrotic areas.

SUMMARY AND CONCLUSION:

The present study is undertaken on fresh water carp Catla catla of Koradi lake to study the effect of thermal power plant effluent on the skeletal muscles. For the assessment of effect of thermal power plant effluents on the skeletal muscle of fish Catla catla, histological, histochemical and biochemical methods were used and results were compared with control fish. Histologically thermal power plant effluent exposed fish muscles showed marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Pronounced intramuscular oedema with minor dystrophic changes and splitting muscles were also observed in the muscles of fish Catla catla exposed to the thermal power plant effluents.

The pathological findings included mild lymphocyte infiltration, vacuolar degeneration in muscle bundles and atrophy of muscle bundles. Degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis. Also, vacuolar degeneration in muscle bundles and atrophy that are observed in the muscle bundles might be due to the various concentrations of mixture of heavy metals in the thermal power plant effluent. The physiological disturbances and morphological damages in the muscle tissue of fish caused due to thermal power plant effluents pollutants.

The photomicrograph of the muscle of fish from control and thermal power station effluent polluted lake shows the presence of normal myotomes with equally spaced muscle bundles. In the muscle of fish Catla catla from thermal power plant effluent polluted waters marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Histochemically the content of protein and carbohydrate in muscles tissue was found to be decreased with decreased intensity of reaction in thermal power plant effluent exposed fish as compared to control.

Biochemically In the fish exposed to the thermal power plant effluent showed increase in the content of skeletal muscle protein and it was found to be 1.40 mg/gm. From above observation it is concluded that the thermal power plant effluent present in water of Koradi lake affect the muscle tissue which ultimately growth of fish. The growth of fish will affect the overall production. Therefore thermal power plant effluent are not favourable for the fishery production in Koradi Lake.

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Table 1: Concentration of Protein in the skeletal muscle of fish Catla catla

Skeletal Muscle	**Protein (mg/gm)**
Control	1.86
Exposed	1.40

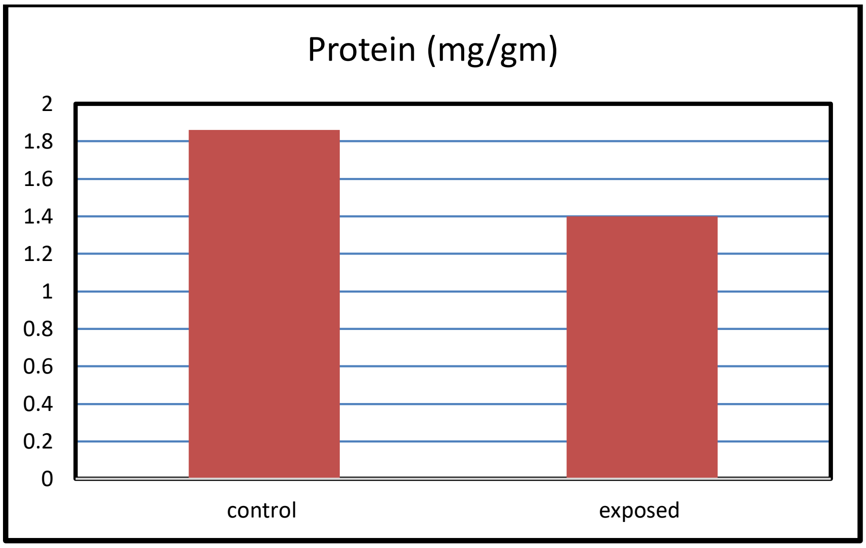
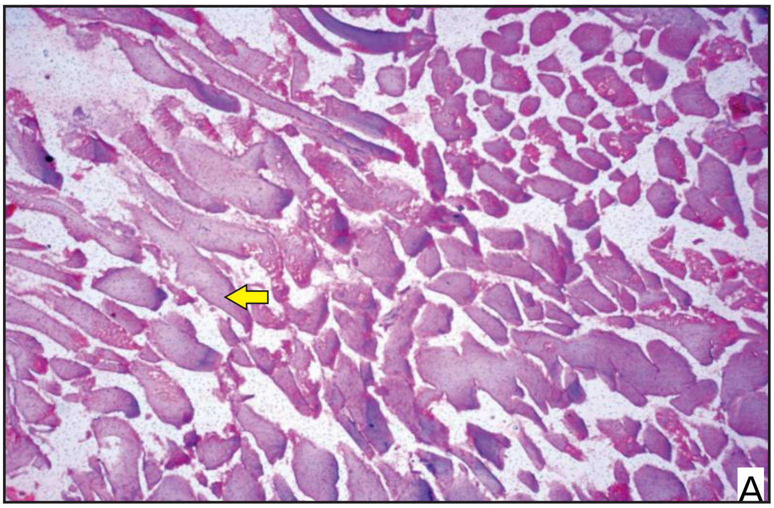
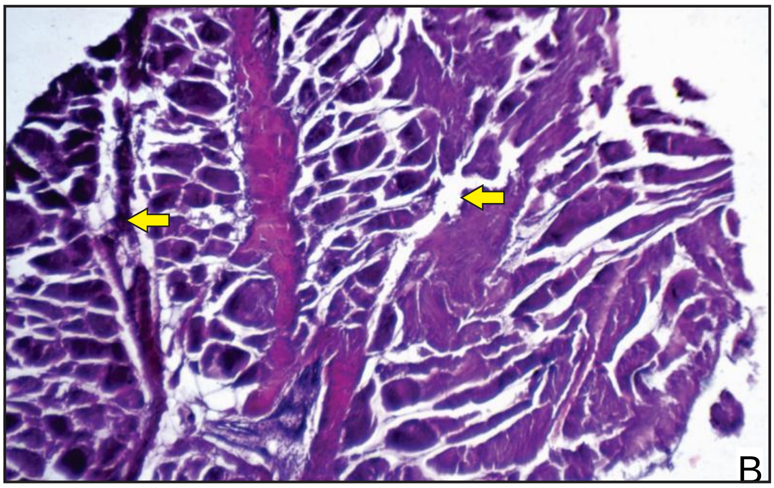


Figure 1: Concentration of Protein in the skeletal muscle of fish Catla catla

ation of Protein in the skeletal muscle of fish *Catla catla*





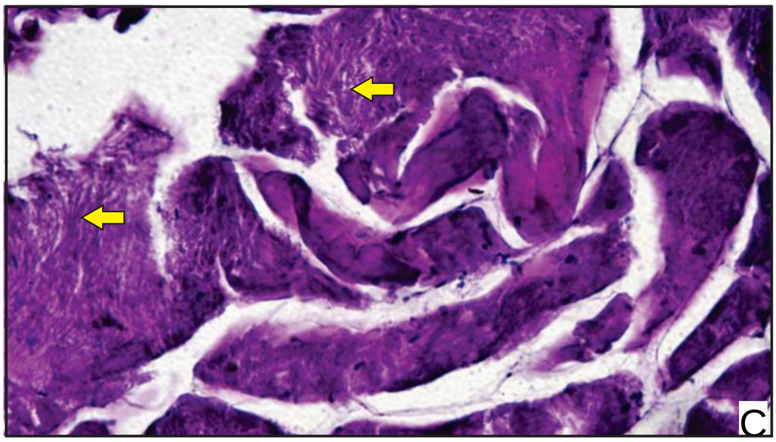
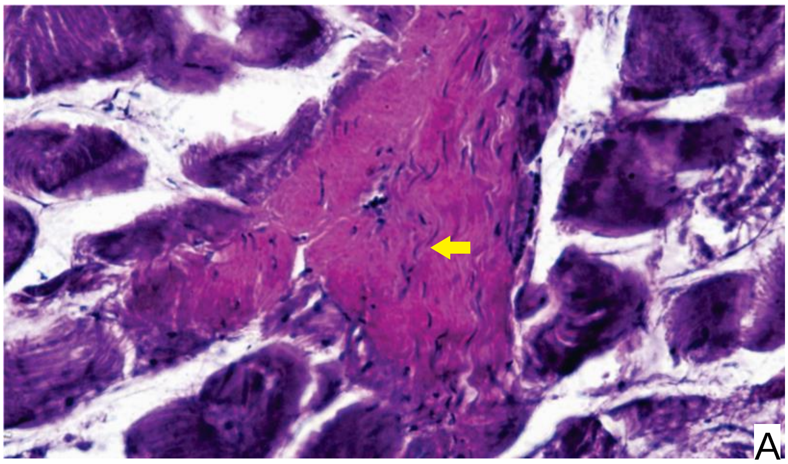
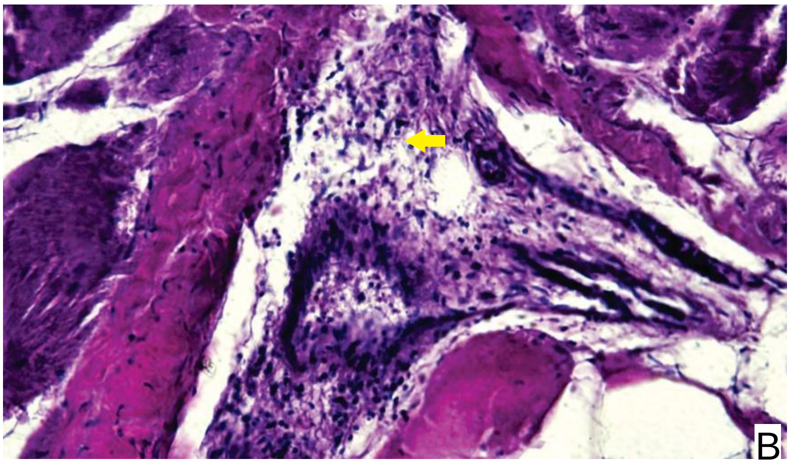


Figure 2 A: T. S. of skeletal muscle of *Catla catla* (Control) showing ribbon like myofibrilar bundles and rod edges of the fibre forming sarcoplasmic hub (Arrow) (HE 400 X)

Figure 2 B: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing oblique muscle fibre got splitting and focal areas of necrosis (Arrow) (HE 100 X)

Figure 2 C: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing shrinkage in the muscle secreting unicellular gland (Arrow) (HE 400 X)





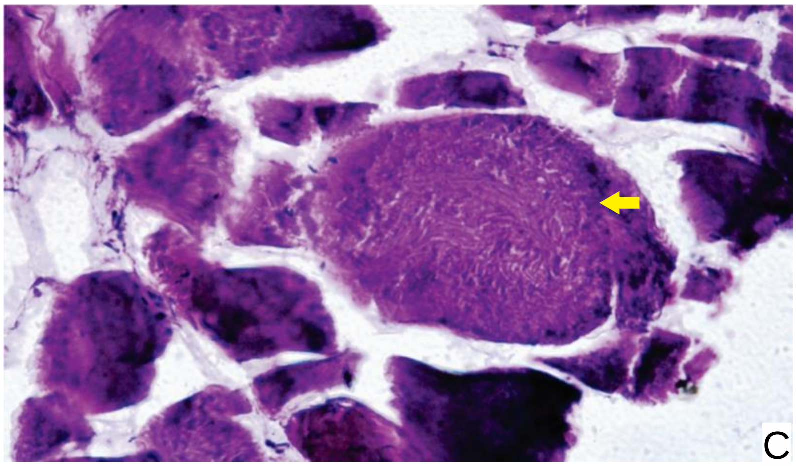


Figure 3 A: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing vascular degeneration and atropy of muscle bundle (Arrow) (HE 400 X)

Figure 3 B: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing muscle atropy (Arrow) (HE 100 X)

Figure 3 C: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing hyaline degeneration and increased empty spaces (Arrow) (HE 400 X)

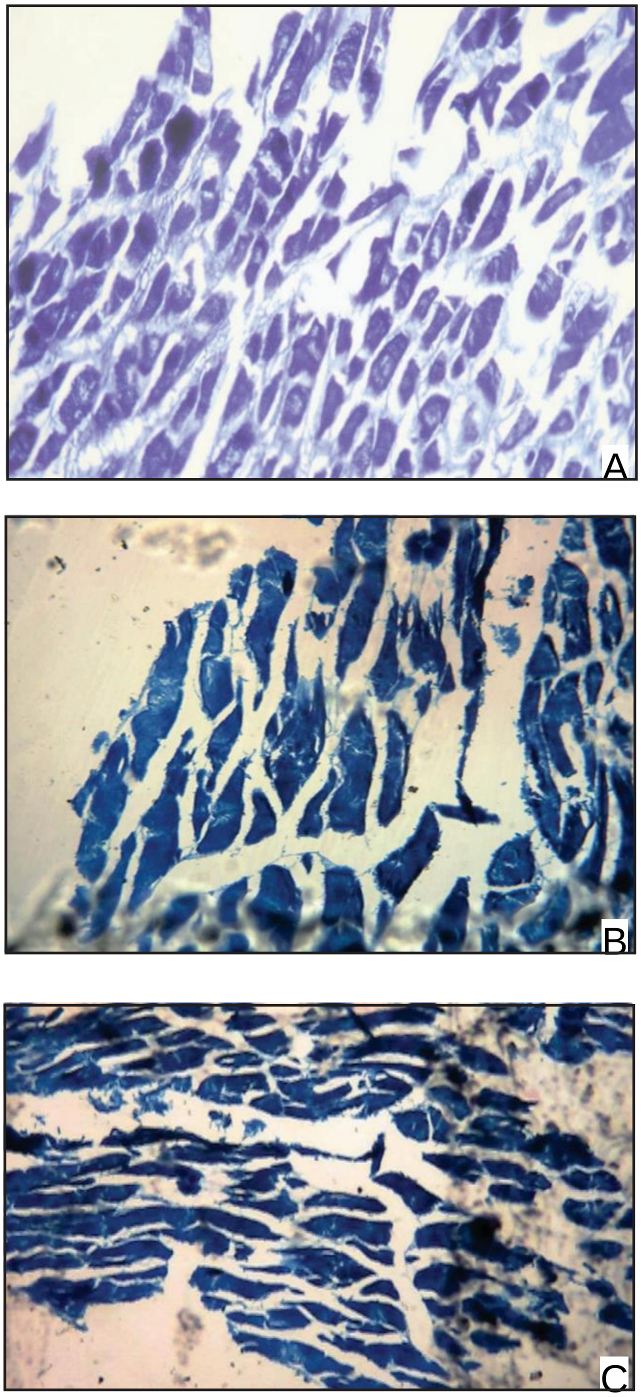
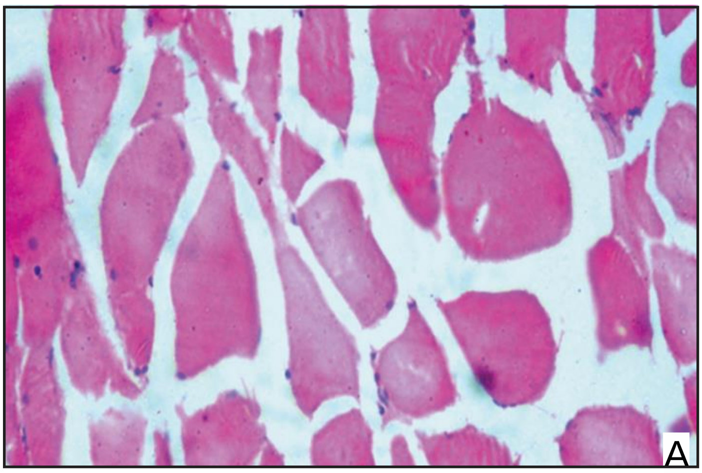
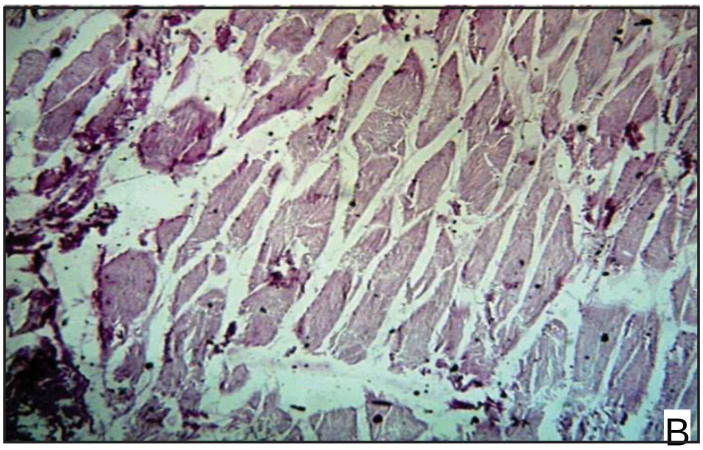


Figure 4 A: T. S. of skeletal muscle of *Catla catla* (Control) showing presence of protein in skeletal muscle fibre (HgBPB 400 X)

Figure 4 B: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing degenerated muscle and progressive decrease in staining intensity (HgBPB 400 X)

Figure 4 C: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing degenerated muscle and progressive decrease in staining intensity (HgBPB 400 X)





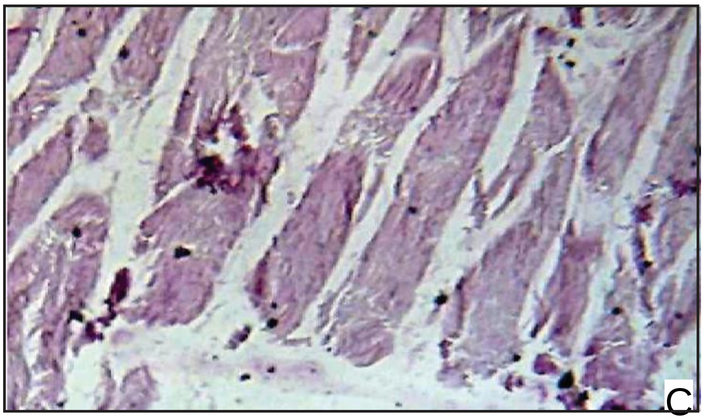


Figure 5 A: T. S. of skeletal muscle of *Catla catla* (Control) showing presence of carbohydrates in muscle fibre (PAS 400 X)

Figure 5 B: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing degeneration of muscle fibre with less quantity of carbohydrate in muscle fibre (PAS 100 X)

Figure 5 C: T. S. of skeletal muscle of *Catla catla* (Thermal power plant effluent exposed) showing degeneration of muscle fibre with less quantity of carbohydrate in muscle fibre (PAS 400 X)