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RESEARCH ARTICLE

HISTOPATHOLOGICAL CHANGES OBSERVED IN THE GILL, LIVER AND KIDNEY OF THE FRESH WATER FISH, *CYPRINUS CARPIO* EXPOSED TO SINGANALLUR MUNICIPAL SEWAGE WATER

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ABSTRACT

The Municipal Sewage water are discharged into low level areas, in streams and lands which finally accumulate in the form of large ponds and affects the environment. The sewage sample was collected from Singanallur station of sanganur canal of Coimbatore.1/10th of 72 hour LC50 value was taken as sublethal concentration for sewage water. After the experimental exposure (72hours, 10days, 20days) fishes were sacrificed and organs like gill, liver, kidney used for histopathological studies. It showed degenerative changes in gills, symptoms of general necrosis, cloudy swelling in liver and dilated glomeruli, cellular hypertrophy in kidney.

INTRODUCTION

Industrial effluents are constantly adding up, toxic substances into water bodies at a very high rate, especially in industrial zones. Most of the waste water discharged into the surroundings water bodies disturb the ecological balance and deteriorates the water quality (Francis *et al*., 2010). The chronic toxicity, at sublethal concentration, cause several manifestations in which pathological study is one which is considered as a biological marker. The gills are the most delicate structure of fish. Gills remain in intimate contact with surrounding water and are vulnerable to damage by any pollutant dissolved or suspended in water. The present investigation was carried out to study the acute and chronic effects of municipal sewage on the gills, liver and Kidney of freshwater fish, *Cyprinus carpio*.

MATERIALS AND METHODS

The Sanganur canal, an open drainage in Coimbatore has its origin from the Western Ghats, namely Kuridimalai Hills and flows from west to east and enters Coimbatore city limit at Coimbatore-Mettupalayam Road and flows for about 10 km within the city. It is a major open drainage system which has intricate linkage with storm water supply, domestic sewage and industrial effluent disposal (forming municipal sewage). Sewage and Sullage from adjoining areas, flow in this drain and finally confluences with singanallur pond.

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The municipal sewage sample was collected from singanallur station of sanganur canal (open drainage) of Coimbatore. Plastic containers were used for collection and the samples were immediately brought to the laboratory and refrigerated at 4o C. The fresh water fish, *Cyprinus carpio* were selected as the test animal and healthy specimens of fishes were procured from a local fresh water pond in Coimbatore. The fishes were acclimatized to laboratory conditions for a period of fifteen days at room temperature. During the period of acclimatization fishes were fed regularly with conventional diet (rice bran and oil cake-1:1 ratio). Feeding was stopped one day prior to the start of the experiment.

Fishes of uniform size were taken and static bioassay method was adopted. Desired concentrations of singanallur municipal sewage water was prepared. Pilot study was conducted to find the range of concentrations that resulted in 10-90 per cent mortality. After the range finding tests, 10 fishes from the stock were exposed to each of different concentration of the singanallur municipal sewage water, a control was also run simultaneously. Mortality was recorded at every 12 hours of interval and the dead fishes were removed immediately. The LC50 values were determined for 72 hours by probit Analysis (Finney, 1971).1/10th of 72 hour LC50 value was taken as sublethal concentration for sewage water. Fishes were divided into four groups, each group consisted of 10-15 fishes. After the stipulated period of exposure, fishes of all the four groups were sacrificed and tissues viz gill, liver and kidney were isolated and used for the histopathological studies. Gill, liver and Kidney tissues excised from fishes of the control and experimental groups were fixed in 10 per cent formalin

solution. After proper dehydration by graded alcohols, paraffin blocks were prepared and 4-5µ thick ribbons were cut in rotary microtome and were stained with Eosin and Haematoxylin. The histopathological changes observed were photographed.

RESULTS AND DISCUSSION

Gill histology

Control: Gill histology of the control fish revealed the intact nature of both primary and secondary gill lamellae. The Secondary lamella surface was covered with simple squamous epithelial cells and capillaries separated by mucous cells. Each primary gill lamella was flat leaf like structure. It consisted of double rows of secondary (respiratory) lamellae with the central supporting axis. They were situated laterally on either side of the interbranchial septum. The secondary lamellae on both sides were highly vascularised and covered by a layer of epithelial cells with uniform interlamellar spaces.

Lethal exposure: When the fish was exposed for 72 hours to the lethal concentration of singanallur municipal sewage water, degenerative changes in the secondary lamellae, epithelial lining of gill was observed.

Sublethal exposure-10 days: When the fish was exposed for 10 days to sublethal concentration of singanallur municipal sewage water considerable degenerative changes were observed. The secondary lamellae showed necrosis at the basal region. The formation of the clavatta lamellae were seen.

Sublethal exposure-20 days: Fishes exposed to singanallur municipal sewage water showed Papillary formation which dilated the vasculature. The infiltration was found with chronic inflammatory cells.

Liver histology

Control: Liver consisted of hepatic cells and connective tissues called lattice fibre, which supported the hepatic cells. Hepatocytes were located among the sinusoids and they formed cord like structures, the hepatic cell cords bile canaliculus was centrally located in each cord. Fairly large quantities of lipids and glycogen were observed in the hepatocyte cytoplasm.

Lethal exposure: When the fish was exposed for 72 hours to the lethal concentration of singanallur municipal sewage water the liver showed symptoms of general necrosis and degeneration of hepatocytes.

Sublethal exposure-10 days: Acute and extensive necrosis of liver cells occurred because of the toxic condition. The parenchyma cells showed cloudy swelling. The Glissen's capsules were not thickened.

Sublethal exposure-20 days: In 20 days exposure the pathological changes observed in the liver included degeneration of cytoplasm of hepatocytes, pycnosis of nucleus and formation of vacuoles.

Kidney histology

Control: The kidney consisted of head and body kidneys. Head kidney, the anterior portion consisted of lymphoid tissues. Body kidney composed of many nephrones and interstitial lymphoid tissues. The glomerular capsule was formed of an inner and outer layer of single flattened epithelia. Renal tubules consisted of a single layer of epithelial cells. Mesangium filled the space between the loops of glomerular capillaries.

Lethal exposure: In the 72 hours exposure the kidney showed degenerative changes with dilated glomeruli and Bowman's capsules. Swelling in renal tubules, cellular hypertrophy and granular cytoplasm were evident.

Sublethal exposure-10 days: Renal tissues of the fish under toxic condition showed marked pathological changes. The glomeruli was congested and nephritic changes were seen.

Sublethal exposure-20 days: Severe pathological changes included necrosis, cloudy swelling of renal tubules, disintegration of interstitial tissues and pycnotic nuclei.

Gill: The calculated 72 hour LC50 value for Singanallur sewage water at 95 per cent confidence limit was 35.53 per cent for the fish *Cyprinus carpio*. The short term test like LC50 is useful for quality monitoring and estimating the water quality and for estimating effluent discharge (APHA, AWWA, WPCF, 1985).

Adamu *et al.* (2008). have found that the destruction of gill lamella, epithelial hyperplasia and epithelial hypertrophy. Bulging of tip of gill filament and necrosis of gill tissues led to reduction of respiratory area thereby decreased gaseous exchange and osmoregulatory potential of the fish (Kalita *et al*., 2012). Kakade *et al*. (2013) have found partial degeneration of epithelial cells of secondary gill lamellae and vacuolation in the secondary gill lamellae. Accumulation of blood cells in the middle of the gill filament indicates haemorrhage which is very prominent.

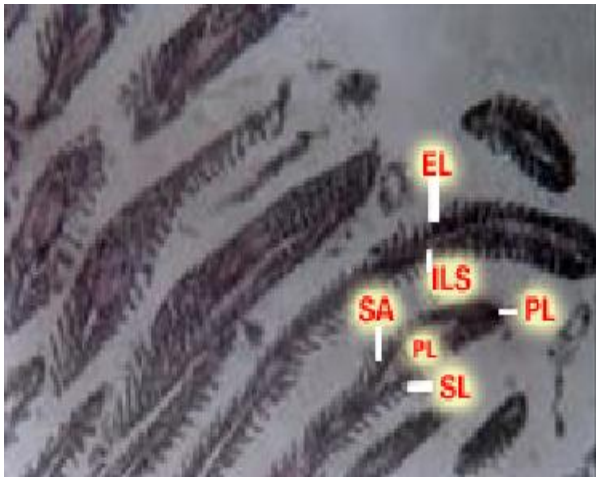
Liver: A number of pathological changes occurred in the liver since it is the first organ to face any foreign molecule that is carried through portal circulation. In the present study, the fish,*Cyprinus carpio* after the exposure to the lethal and sublethal concentrations of the singanallur municipal sewage water showed severe damages in liver cell. Shruti S. Gijare *et al*. (2012) have observed loosening of tissue, distension of cell, hypertrophy of hepatocytes, significant increase of Kupffer cell, pycnotic nuclei and vacuolation of cytoplasm. Degenerative changes like hypertrophy of hepatocytes, prominent vacuolation, necrosis and pyknoticnuclei. (Kakade *et al*., 2013).

Kidney: When the magnitude of the pollutant-induced stress is enough to cause cellular lesions but not the death of the organism, changes may be noticed in light microscopy. Staicu Andrea cristina *et al*. (2008) have found cubic epithelial cells lining renal tubuli were detached from the basal membrane, necrotic renal tubuli appeared, nuclei pycnosys, nuclei hypertrophy, anisokary, contraction of glomeruli and the enlargement of Bowman's space.

HISTOPATHOLOGY OF GILL OF *C.carpio*

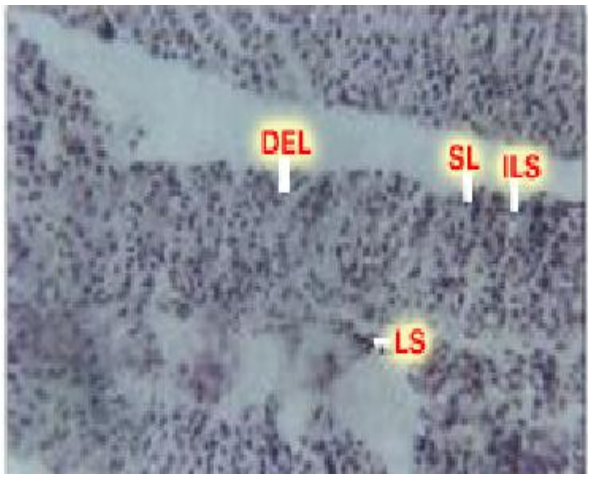
Control Gill section of *C.carpio*

Control Gill section of *C.carpio*



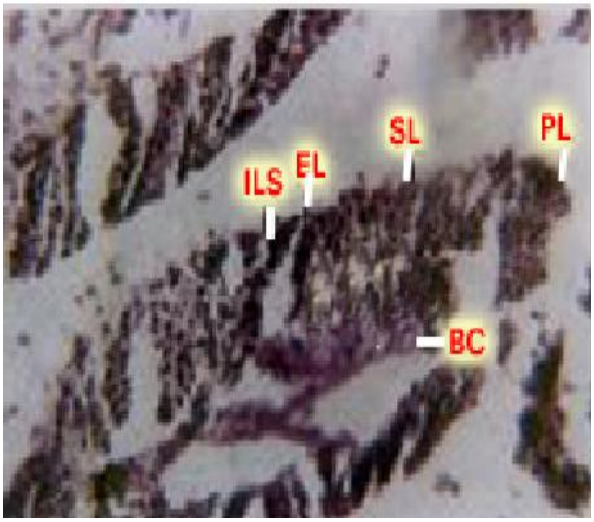
PL – Primary Lamellae
SL – Secondary Lamellae
LS – Lamellar Space
ILS – Inter Lamellar Space
EL – Epthelial lining
SA – Supporting axis

Gill Section of fish exposed to 72 hour lethal concentration of Singanallur water

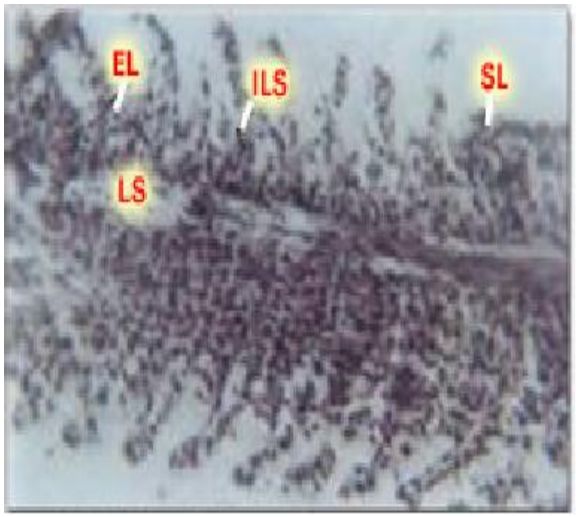


SL – Secondary Lamellae
LS – Lamellar Space
ILS – Inter Lamellar Space
DEL – Degeneration of Epithelial lining

Gill Section after exposure to sublethal concentration of Singanallur water for 10 days



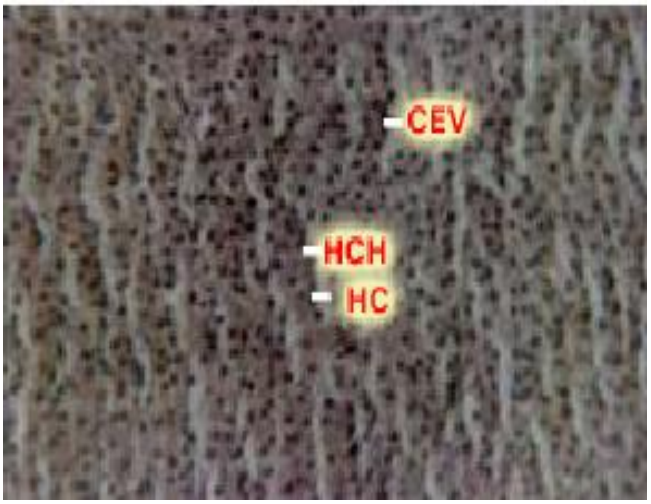
Gill Section after exposure to sublethal



PL – Primary Lamellae
SL – Secondary Lamellae
LS – Lamellar Space
ILS – Inter Lamellar Space
EL – Epthelial lining

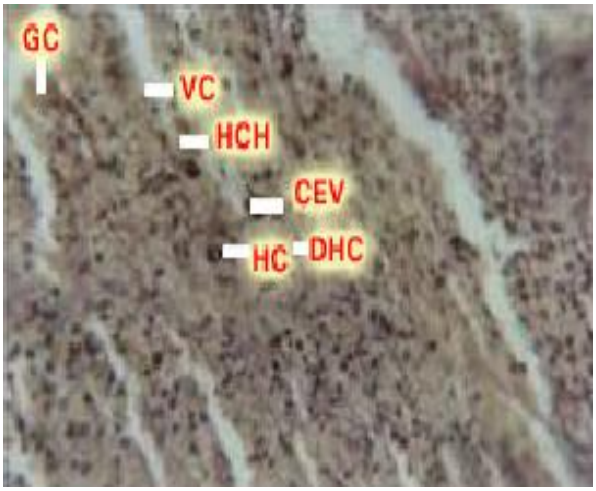
HISTOPATHOLOGY OF LIVER OF *C.carpio*

Control Liver section of *C.carpio*



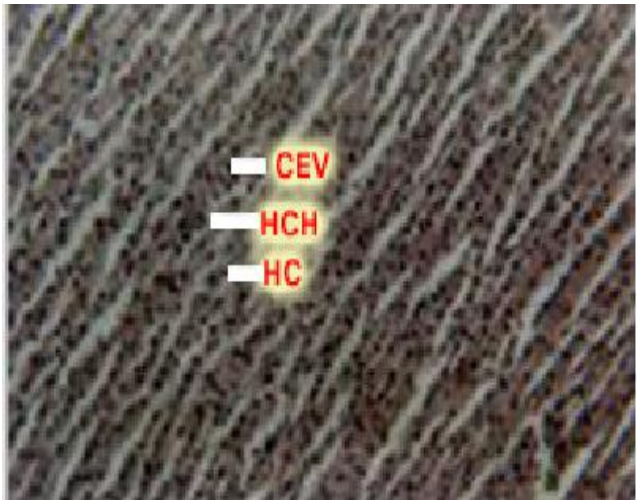
HC – Hepatocyte Cells
HCH – Hepatic Cords
CEV – Central Efferent vain

Liver Section of fish exposed to 72 hour lethal concentration of singanallur water

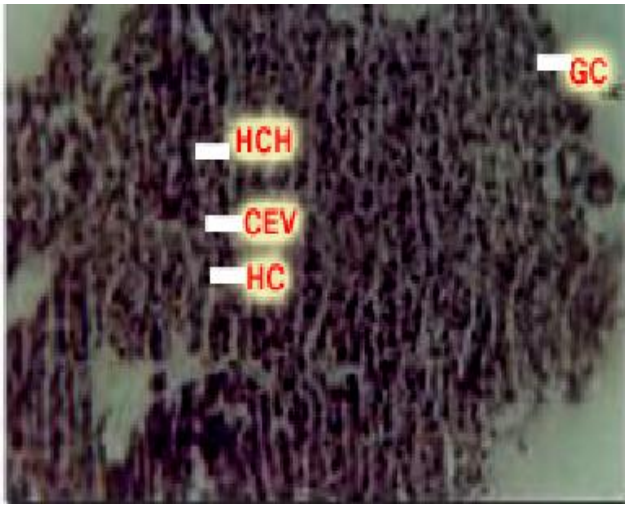


GC-Glissen's capsule
VC – Vacuoles
DHC – Degenerated Hepatocytes Cells
HCH – Hepatic Cords
CEV – Central Efferent vain

Liver Section after exposure to sublethal *concentration of Singanallur water for 10 days*



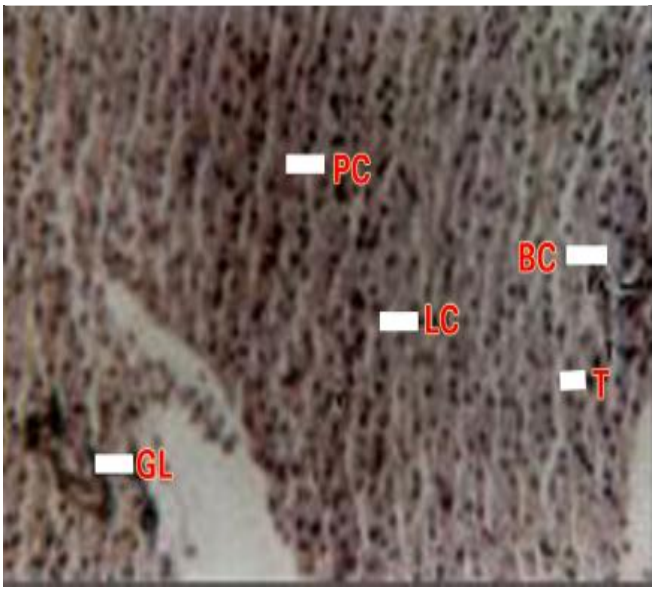
Liver Section after exposure to sublethal *concentration of Singanallur water for 20 days*



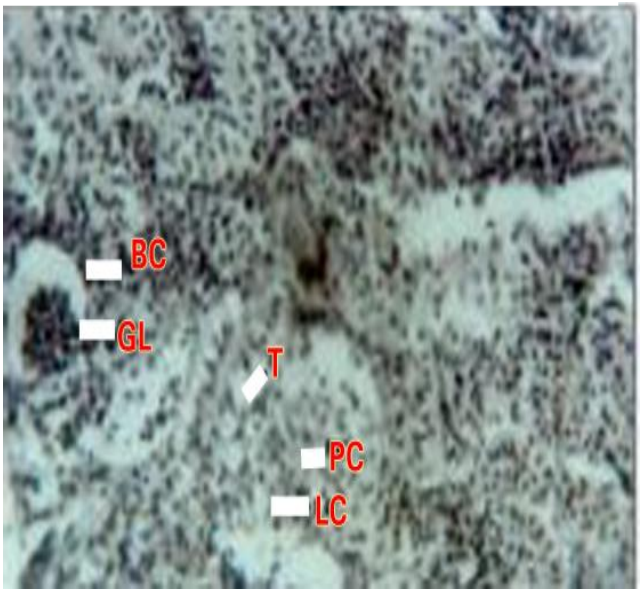
HC – Hepatocyte Cells
HCH – Hepatic Cords
CEV – Central Efferent vain
GC-Glissen's capsule

HISTOPATHOLOGY OF KIDNEY OF *C.carpio*

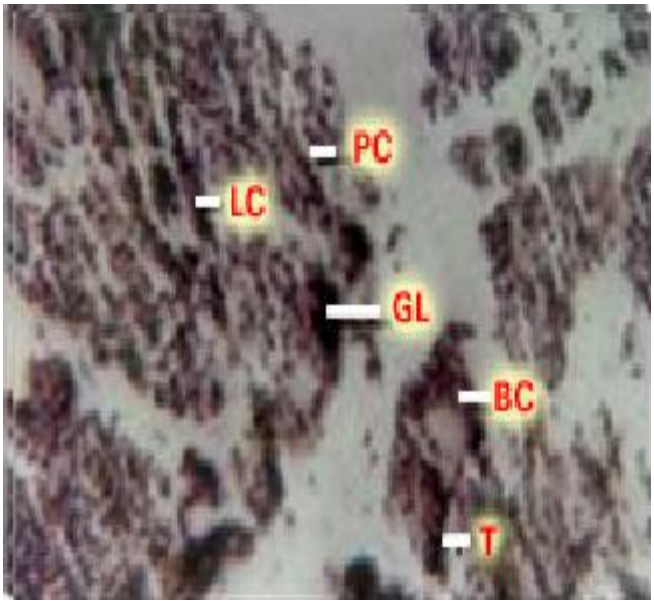
Control Kidney section lethal concentration of Singanallur water



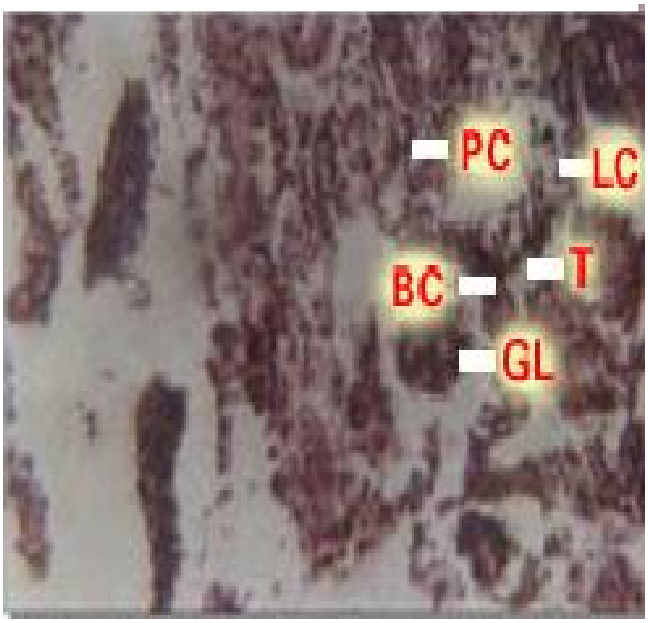
Kidney Section of fish exposed to 72 hour of C.carpio



Kidney Section of fish exposed to Sublethal Singanallur water for 10 days



Kidney Section of fish exposed to Sublethal concentration of concentration of Singanallur water for 20 days



BC – Bowman's capsule
GL – Glomeruli
PC – Parenchyma Cells
LC – Lymphoid Cells
T – Tubule

Increased capsular space, vacuolated cytoplasm, shrunken lumen, shrunken glomerulus and degeneration in glomerulus, renal tubules (Suneetha 2012). The alterations in the structure of kidney on acute exposure showed vacuolation, cell necrosis, hypertrophy of haemopoietic tissue and dilated renal tubules. (Kakade *et al*., 2013).

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