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HISTOPATHOLOGY OF THE GILL, LIVER AND KIDNEY TISSUES OF THE FRESHWATER FISH *TILAPIA MOSSAMBICA* EXPOSED TO CADMIUM SULPHATE

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ABSTRACT
An investigation on the effect of the heavy metal, cadmium sulphate ($CdSo_4$) on the gill, liver and kidney of the *Tilapia mossambica* was carried out in the laboratory. Sixty fishes were exposed to continuous exposure to sub-lethal concentrations (0.084mg/l) of cadmium sulphate for a period of 20 days. The gill, liver and kidney of fish were removed for histological examination. The results showed that the degree of distortion of the gill, liver and kidney was proportional to the exposure periods and concentration of the metals was found to be dose and time dependent.

KEY WORDS: Cadmium sulphate, *Tilapia mossambica,* Histopathology, gill, liver and kidney.

INTRODUCTION

In the modern world the urbanization and Industrialization have boosted the man kind's economy through various means and ways. But at the same time pollution of aquatic resources has become a huge challenge and a serious threat. Recent years have witnessed significant attention being paid to the problems of environmental contamination by a wide variety of chemical pollutants, including the heavy metals (EI-Demerdash and Elegamy, 1996). Trace metals can be accumulated by fish, both through the food chain and water (Hadson, 1998). Fish living in the polluted water may accumulate toxic trace metals via their food chains. (Tarrio *et al.*, 1991). Different environmental pollutants are likely to affect biological systems in different ways according to their chemical properties. In some of physiological changes created by particular pollutant is likely to be characteristics of that pollutant. Thus by observing the effects of pollutant on a set of physiological parameters, it might be possible to establish specific responses of that pollutant and may make it possible to identify a pollutant on the basis of its physiological effect pattern. Heavy metals due to their potential toxicity produce biochemical changes in the organs of animals and continuous exposure may alter genetic composition (Mohanraj Ebenezer, 2003). Among the various heavy metal pollutants, cadmium merits special attention due to its potential hazards to aquatic biota (Mayer *et al*., 1991; Barber and Sharma, 1998) as well as to human beings (Groten and Van bladeron, 1994; Vanderpool and Reeves, 2001). This heavy metal is a common aquatic pollutant and is known to be highly toxic to most organisms, even at small concentrations in natural waters (Lovert *et al*., 1972).

Cadmium (Cd^{++}) is a highly toxic heavy metal commonly used in environmental studies. In general, cadmium is a biologically non-essential, non-biodegradable, persistent type of heavy metal and its compounds are known to have high toxic potentials. Further, continuous, low level cadmium exposure may have a gross biological impact comparable to that of recurring exposures of much greater intensity. In fresh water fish, cadmium uptake is taking place mainly through three routes namely, gills, skin and also from food via the intestinal wall (Karlsson-Norrgran and Runn, 1985). Cadmium exposure leads to pathological conditions in various tissues including liver, testes, brain, nervous system, kidney, spleen and bone marrow. This study evaluates the impact of the short-term cadmium exposure on gills, kidney and liver function of the freshwater fish *Tilapia mossambica*.

MATERIALS AND METHODS

In recent years *Tilapia mossambica* has served as a bioindicator and integrator of contaminants on various reasons, viz., wide distribution in the fresh water environment, free swimming nature, ability to respond against environmental pollution and importance as an economic food source for human beings (Pelgrom *et al*., 1995). *Irrespective of sex, healthy specimens of Tilapia mossambica having a body weight from 7.8 to 9.2g* were collected from local lake Otteri, Tamil Nadu, India. In a preliminary experiment, the sublethal concentration of cadmium sulphate for *Tilapia mossambica* over 96 hrs exposures was determined by exposing 10 fishes of *Tilapia mossambica* to different concentrations of cadmium sulphate separately. After acclimatization to the laboratory conditions, acute toxicity study was carried out by following the standard EPA/ROC (1998) guidelines to determine the lethal (LC_{100}), median lethal (LC_{50}) and safe sublethal (LC_0) levels of cadmium for *Tilapia mossambica*. The 96-h LC_{50} value of mortality for each exposure concentration was recorded and tested by probit analysis as described by Finney (1971). The lethal, median

lethal and sublethal concentration were found to be LC_{100} (200 to 300 mg/l), LC_{50} (250 mg/l) and LC0 (300 mg/l) and LC_0 (0.10mg/l). *After acclimatization the total number of 60 fishes were collected and* were grouped into 6, each tub (20 litre capacity) containing 10 fishes along with control group at room temperature. *Each group of fishes was treated with increased concentration of cadmium sulphate i.e. 0.200, 0.220, 0.240, 0.260, 0.280 and 300mg/l. respectively. After 96 hrs. of exposure in cadmium sulphate*, the mortality rate was determined by using the standard method (Saptami Moitra and Verma, 1997).

Histological study

The study of histopathological changes of the tissue sample like gill, liver and kidney were carefully removed from both control and experimental group at 0^{th}, 10^{th} and 20^{th} day. The tissues were immediately washed in 0.9% NaOH remove the adherence of mucous and blood. It was kept on the blotting paper to drain the moisture. The tissue samples were processed for logical observation. The gill, liver, kidney and muscle of the fish groups were fixed in physiological saline solution for 24 hrs. Using tetra hydrofuron as a dehydrading and clearing agent. The section of 6µ thickness were selected to observe the changes in the gill, liver and the kidney by adding haematoxylin and Eosin counter stain (Humason, 1972). Results were expressed in as photomicrograph.

Metal analyses

Aqueous cadmium concentrations were determined using a Graphic Furnace Atomic Absorption Spectrophotometer (GFAAS) equipped with a graphite tube atomizer (Perkin-Eloner, Simaa 6000) and an auto sampler Perkin - Eloner model As-72). Measured cadmium concentration, were consistently within the certified range for each element.

Biological tissues

The aliquot taken from the whole tissue homogenate and the various centrifugation pellets were freeze dried and then weighed. The dried material was first digested at room temperature with nitric acid directly in the centrifuge tubes for 24 h, to limit sample loss and metal adsorption onto the tubes. Dig estates were then transferred in to Teflon containers and a hot - digestion was performed in an autoclave for 3 hr (120 - 125 C). The cooled digests were diluted with ultra - pure water before analysis. The supernatants from the differential centrifugation procedure were also digested in an autoclave in an equal volume of nitric acid, Cadmium concentration were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP - AES) (Varian, Vista AX). Analytical procedural blanks and standard reference materials were analyzed during each run.

TABLE 1: Mortality rate of fresh water fish *Tilapia mossambica* at different concentration of Cadmium compound at 96 hrs exposure

Sl. No	Concentration of $CdSO_4$ (mg/l)	No. of fishes exposed	No. of fishes dead	Percentage of mortality (%)
1	0.200	10	0	0
2	0.220	10	1	10
3	0.240	10	3	30
4	0.260	10	5	50
5	0.280	10	8	80
6	0.300	10	10	100

TABLE 2: Accumulation of Cadmium compound (µg/g dry wt) in fish organs and tissues at 96hrs exposure

Gills	0.023 ± 0.011 (0.004 – 0.064)
Liver	0.60 ± 0.21 (0.11 – 2.05)
Kidney	1.66 ± 0.26 (0.08 – 2.64)

Values are mean ± one standard error.

TABLE 3: Concentration of protein in selected tissues of the *Tilapia mossambica* exposed to cadmium compound

Treatment	Organ	Total protein content (mg/g)		
		0^{th} day	10^{th} day	20^{th} day
Control	*Muscle*	13.3 ± 0.4	11.86 ± 0.9	15.80 ± 0.8
	Liver	11.61 ± 0.4	12.00 ± 0.4	12.90 ± 0.2
Exposed with CdSO4	*Muscle*	12.50 ± 0.8***	9.64 ± 0.5*	7.8 ± 0.6*
	Liver	10.28 ± 0.5	8.41 ± 0.7**	6.00 ± 0.9

TABLE 4: Effect of cadmium sulphate on feeding energetics parameters in *Tilapia mossambica*

Conc. (mgL^{-1})	Reading rate	Absorption rate	Growth rate	Metabolic rate	Absorption efficiency (%)	Conversion Efficiency (%)	
Control	58.25 ± 8.3	55.64 ± 5.1	6.27	42.14 ± 1.4	98.64 ± 0.28	18.0 ± 1.6	17.3 ± 1.5
$CdSo_4$ treated	20.46 ± 3.95***	28.69 ± 2.6**	2.43	27.0 ± 3.63**	66.84 ± 0.4*	1.83 ± 0.5*	2.61 ± 0.5**

RESULTS AND DISCUSSION

Gills: The gills, which participate in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants. (Camargo, M.M. and C.B. Martinez, 2007). The histology of gill in control fish Tilapia mossambica is given in the plate I and (fig. 1a). In control fish the structure of the gill bearing four pairs of gill lamellae and both the sides were supported by bony structure and primary lamellae. The secondary lamellae showed numerous channels of blood capillaries, each separated by single layered pillar cells when observed in vertical section. The laminar epithelium was thick followed by basement membrane below which the pillar cells enclosed blood spaces, large number of mucous cells were present on the epithelial gill rackers, where as primary lamellae had comparatively small and less number of mucous cells. The histopathology of experimental fish gill is given in Plate 1 and (fig. 1b). The gill showed slight damage in 10th day of treatment with sub lethal concentration of Cadmium Sulphate. The gill shows lesion in the epithelial layer, hypertrophy if mucous cells and vacuolation in gill membrane. The histopathology of Cadmium exposed fish gill (20 days) is given in Plate 1 (fig. 1c). The Cadmium exposed gill showed marked edema and active secretion of mucous, increased in size but decreased in number and most of them were either vacuolated or almost empty. The secondary lamellae are also showed destruction of either epithelial cells or few lamellae were curled, that leads to congestion and hemorrhage of gills. In experimental fish, the gills became reddish in colour.

FIGURE 1: Mortality rate of fresh water fish *Tilapia mossambica* at different concentration of Cadmium compound at 96 hrs. exposure

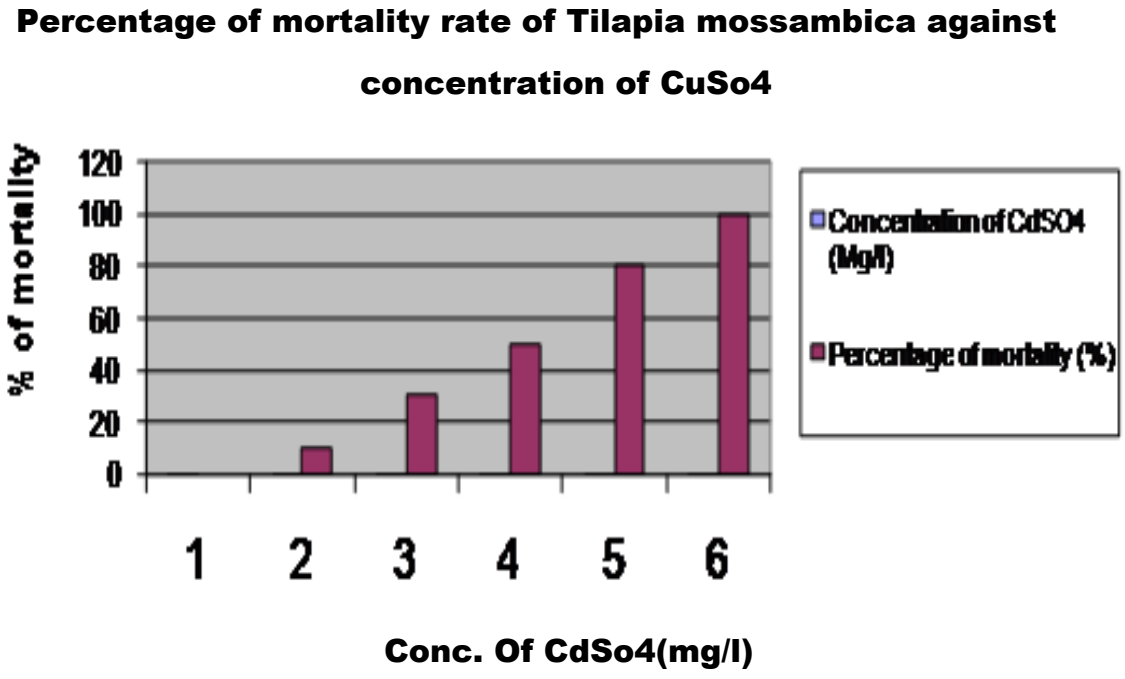
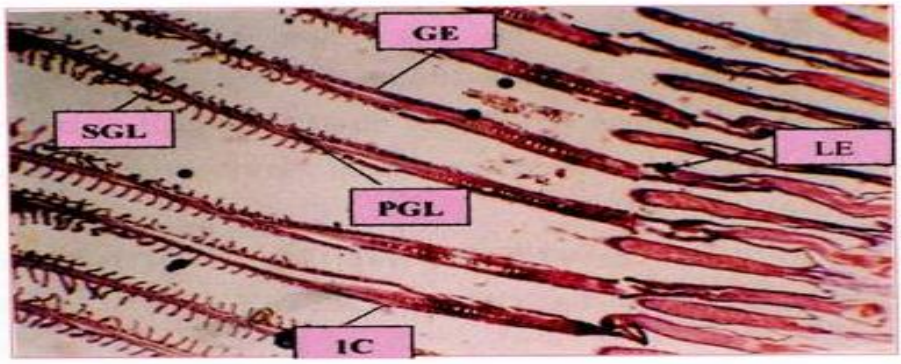


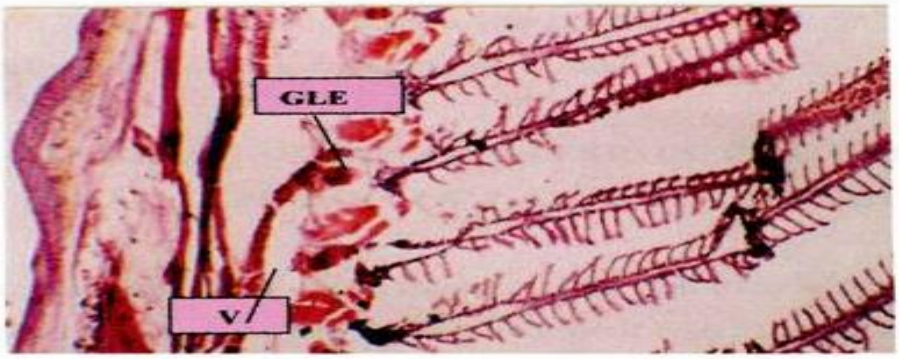
PLATE 1

HISTOPATHOLOGY OF GILL IN FISH (CONTROL)



1a
Gill Epithelium (GE)
Interstitial Cells (IC)
Lamellar Epithelium (LE)
Primary Gill Lamellae (PGL)
Secondary Gill Lamellae (SGL)

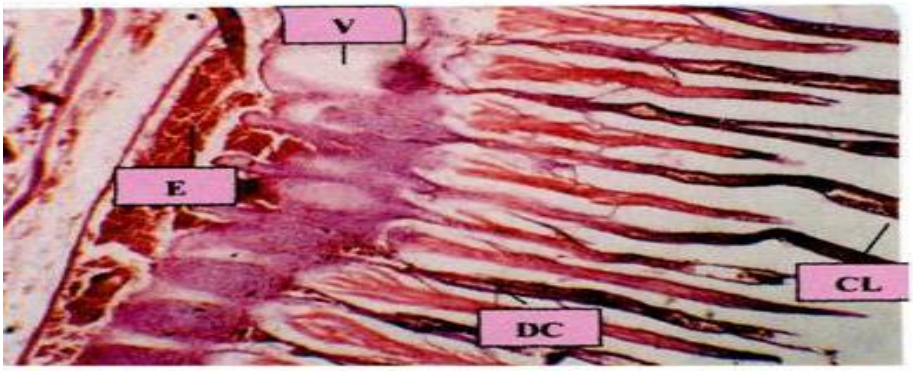
HISTOPATHOLOGY OF GILL WHEN EXPOSED TO CADMIUM COMPOUND (10days exposure)



1b
Lamellar Epithelium (GLE)
Vacuolation (V)

HISTOPATHOLOGY OF GILL WHEN EXPOSED TO CADMIUM COMPOUND (20days exposure)

HISTOPATHOLOGY OF GILL WHEN EXPOSED TO CADMIUM COMPOUND (20days exposure)

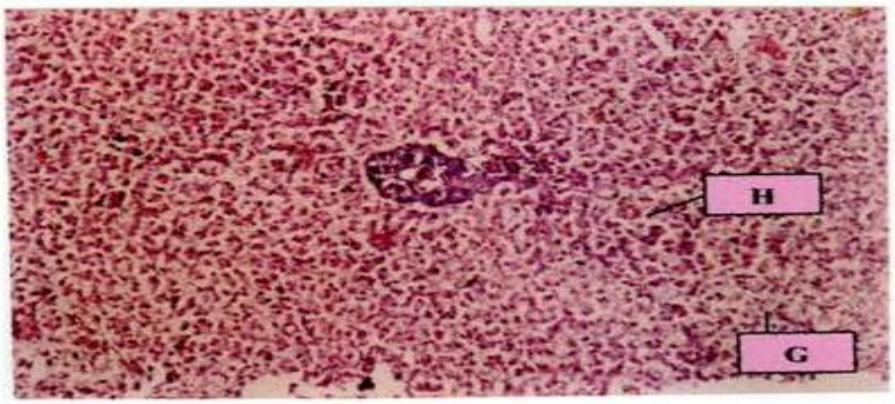


1c
Curled Lamellae (CL)
Damaged Cells (DC)
Damaged Epithelium (DE)
Edema (E)
Vacuolation (V)

Liver: The histology of Tilapia mossambica liver tissue in the control group section is given in the Plate 2(fig. 2a). The liver cells showed normal exo-structure of hepatic cells, the connective tissue of liver expressed normal condition, normal hepatic mass granulation were observed, in the pancreatic tissue no changes was noticed. The histopathology of liver in experimental fish Tilapia mossambica was given in Plate 2 (fig. 2b). In the liver proliferation of ducted cells and small spaces were appeared in between hepatic cords. The position was slightly damaged in 10 days exposure of sub-lethal concentration of $CdSo_4$. The histopathology of liver in experimental fish after exposure of (20 days) was given in Plate 2 (fig. 2c). The 20 day treatment of sub-lethal concentration the liver cells showed severe damage and marked proliferation. The liver tissue was converted into sponge mass and the cells were showed scattered nature. The pancreatic tissue was broken and large vacuoles were seen.

PLATE 2

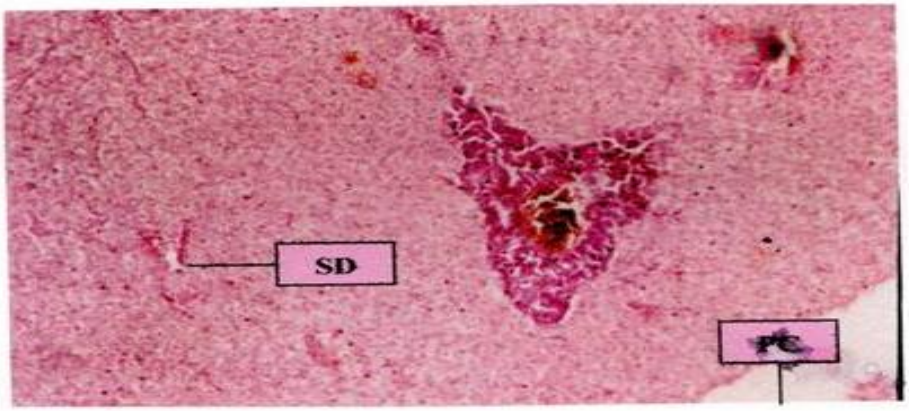
HISTOPATHOLOGY OF LIVER IN FISH (CONTROL)



2a

Normal Hepatic cells (H)
Granulation (G)

HISTOPATHOLOGY OF LIVER WHEN EXPOSED TO CADMIUM COMPOUND (10 days exposure)

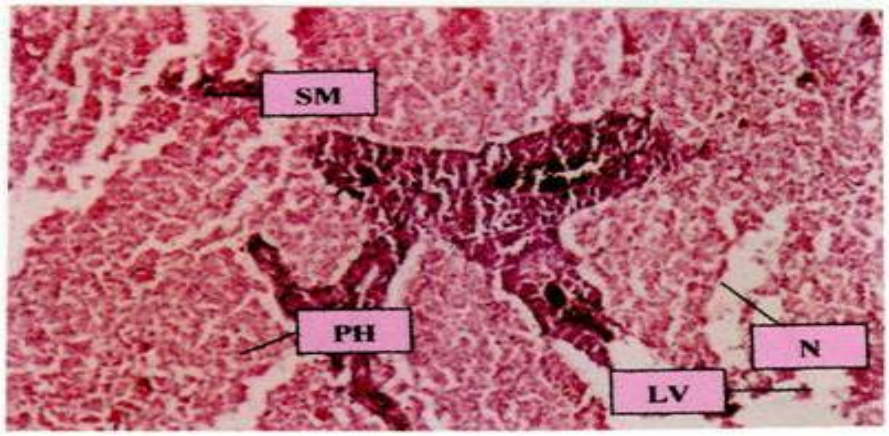


2b

Small Damage in hepatic cells
Proliferation in cells (PC)

HISTOPATHOLOGY OF LIVER WHEN EXPOSED TO CADMIUM COMPOUND (20 DAYS EXPOSURE)

HISTOPATHOLOGY OF LIVER WHEN EXPOSED TO CADMIUM COMPOUND (20 DAYS EXPOSURE)



2c

Large Vacuoles (LV)
Necrosis (N)
Proliferation of Hepatic cells (PI
Sponge mass (SM)

Kidney: The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluids and erythropoieses (Iqbal, F. et al, 2004). The kidney is one of the first organs to be affected by contaminants in the water (Thophon, S. et al, 2003). The histology of kidney Tilapia mossambica in control fish is shown in the Plate 3 (fig. 3a). In the kidney normal architecture was recorded. The glomerular tissue was closely arranged with renal tubules including distal and collecting tubules and intact interstitial cells. The histopathology of experimental fish Tilapia mossambica kidney was shown in the Plate 3 (fig. 3b). The section showed mild edema. The cell size was reduced and the glomerular tissues remained more or less intact, mild interstitial edema and mild damage of renal tubes was found in several areas. The hydrophobic degeneration of renal tubes in the glomerular tissue was

seen. The histopathology of kidney in experimental fish (20 day exposure of Cadmium Sulphate) was given in Plate 3 (fig. 3c). The experimental kidney sections were showed severe damage and disorganization of tubules. The glomerular edema and necrosis were also noticed. All the histopathological observation indicated that exposure to sublethal concentrations of cadmium sulphate caused destructive effect in the gill, liver and kidney tissues of T. mossambica. Gill, liver and kidney histopathological alterations, such as those observed in these studies and findings from previous studies, could result in severe physiological problems, ultimately leading to the death of fish

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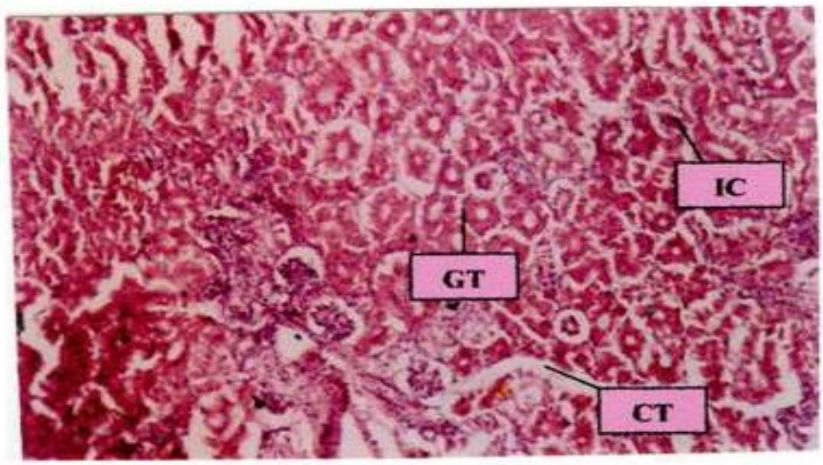
PLATE 3

HISTOPATHOLOGY OF KIDNEY IN FISH (CONTROL)

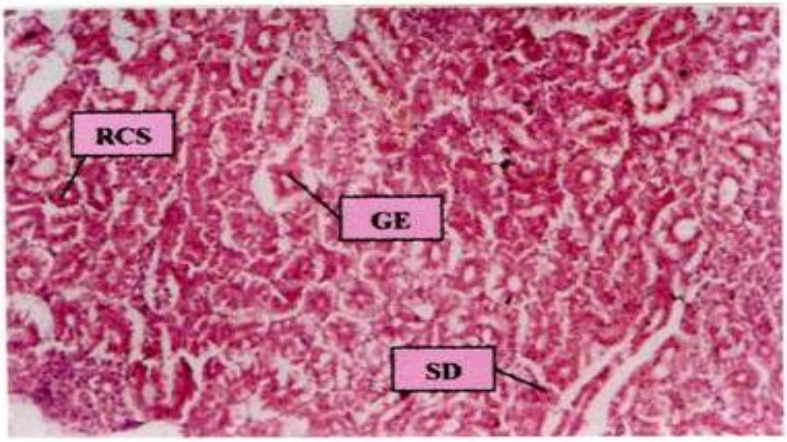


3a

Intestinal cells (IC)
Glomerular Tissue (GT)
Collecting Tubules (CT)

Histopathology of Kidney when exposed to cadmium compound (10 days exposure)

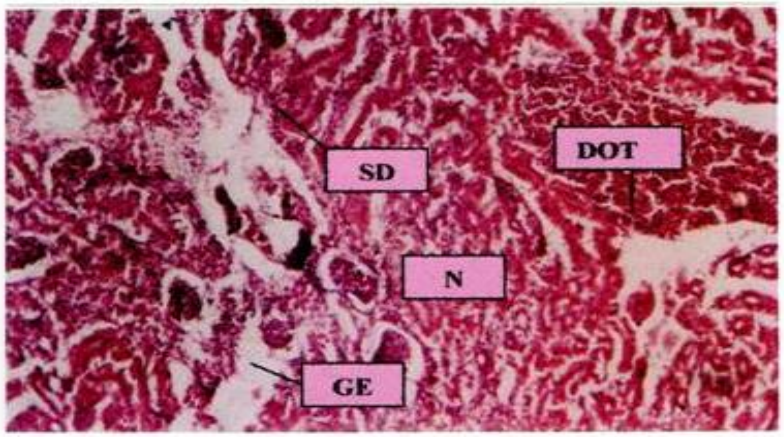
Histopathology of Kidney when exposed to cadmium compound (10 days exposure)



3b

Glomerular Edema (GE)
Renal Cells size reduced (RCS)
Mild Damage Renal Tubules (MDRT)

Histopathology of Kidney when exposed to cadmium compound (20 days exposure)



3c

Disorganization of Tissue (DT)
Glomerular Edema (GE)
Necrosis (N)
Severe Damage (SD)

CONCLUSION

In conclusion the present study showed that histopathology is a useful biomarker for environmental contamination. Metals are stored in different sites in animals depending on the metal and on the animal species. To check the continual introduction of these metals into the food chain, a more cautious application of insecticides and pesticides should be employed and effluents from industries must be treated before disposal.

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