

ORIGINAL RESEARCH ARTICLE

Studies on the Effect of Tannery Effluent and Chromium Accumulation in Common Crop *Tilapia mossambica*S.F. Lesley Sounderraj*¹, Nancy Lesley², P. Senthil Kumar³¹Post Graduate and Research Department of Zoology, Voorhees College, Vellore – 632001, Tamil Nadu, India²Department of statistics, presidency college, Chennai – 600015, Tamil Nadu, India³National Environmental Engineering Research Institute (NEERI), CHZI, CSIR – Complex, Taramani, Chennai – 600113, Tamil Nadu, India

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ABSTRACT

Effect of lethal and sub lethal concentration of tannery effluent (35 PPM and 3.5 PPM) in haematological and histopathological conditions were studied in freshwater common crop *Tilapia mossambica*. Tannery effluent toxicity resulted in significant decrease in haematological parameters in lethal and sub lethal concentration and maximum at 35 ppm (RBC (-47), haemoglobin Content (-77), ESR (-75) and PCV% (-68).

Key words: Tannery effluent, lethal and sub lethal toxicity, parts per million (ppm), haematology histopathology, common corp.

INTRODUCTION

Leather production is a major industry in India which makes a significant contribution to the country's foreign exchange earnings. It is estimated that 30-35 L of water is used per kilogram of leather processed, generating about 680×10^6 L of effluent daily. During the process of leather making, several chemicals like Cr (SO₄)₃, NaCl, Ca(OH)₂, H₂SO₄, etc., are extensively used. Therefore the resultant effluent is enriched with Chromium (Cr) and salts (NaCl and SO₄). Chromium present in effluent is primarily in the less toxic trivalent form Cr (III). When this effluent is discharged into the soil, due to varying environmental condition, Cr (III) is oxidized to toxic hexavalent form which seldom remains as Cr (VI).

Heavy metals present in effluents discharged by the leather industries constitute the most common group of toxic and non-degradable substances. On reaching the aquatic ecosystem, they pose a serious threat to the biotic living there in especially fish by altering the physiochemical characteristics of water and production of fish food organisms (Joir *et al.*, 1991).

Fishes have a great significant in the life of mankind, being a most important source of protein and providing other useful products. The need for fish arises from the need for protein in human diet. In this pretext, the present study was carried

out to evaluate the efficient accumulation of chromium from the tannery effluents by the common corp available in the local area, namely exotic *Tilapia mossambica*.

And the toxicity tests haematological and histopathological studies were carried out to measure the effects of tannery effluents on *Tilapia mossambica*. Tests were conducted for different length of time, but LC 50 value for 96 hrs.

MATERIALS AND METHODS**Collection of Sample**

The test fish of *Tilapia mossambica* (15± 2cm in length and weight 60± 3.0gm) were collected from Vellore fort moat and acclimated to the laboratory conditions for two weeks and were fed with liver meal during the completed period of study.

Experimental Setup

The control and experimental fishes were maintained for 15 and 30 days to evaluate the long term effect of tannery effluent. The medium was changed once in two days and no mortality of fishes was recorded during the period of investigation.

Collection of Effluent

The tannery effluent for the present study was collected from a tannery at Ranipet, Vellore. Only the filtered effluent was used for the study. Various parameters of the tannery effluent and the

water sample from the laboratory were also analyzed.

Chromium Estimation (Wet Oxidation Method)

Principle:

In this method, chromium in sample of the respective specimen were oxidized with an oxidizing mixture containing perchloric acid, nitric acid, sulphuric acid to hexavalent chromium.

Procedure:

About 0.1gm of sample was taken in an Erlenmeyer flask. Oxidizing mixture was added in the ratio of 2.5: 5.0:11.5. The digestion was performed till the color of the digest turns green to orange. The digest was allowed to cool and 40% NaOH was added until the fizzling stops. The content was made up to 50ml and the diluted digest was read at 372nm.

Calculation:

$$\text{Amount of Chromium as Cr (VI)} = \frac{\text{OD at 372 X Dilution Factor X 52}}{4830 \text{ X } 20}$$

$$\text{Amount of Chromium as Cr (III)} = \frac{\text{OD at 372 X Dilution Factor X 52 X 152}}{4830 \text{ X } 20}$$

Haematological Study

After exposing the fishes in lethal 35ppm and sub lethal 3.5ppm concentration, certain basic haematological parameters like RBC, WBC Count, Haemoglobin content, Erythrocyte sedimentation rate and packed cell volume were recorded and compared with untreated control nearly for 15 and 30 days, by routine methods (Dacie. V, Lewis S. M. 1975); with Neubaur crystalline counting chamber; hemoglobin content was estimated by acid haematin method and ESR was calculated by the wintrobe's method, (3000 rpm/hr) and westergen's tube method. Blood for haematological parameters was obtained by severing the caudal ends of fishes.

Determination of LC₅₀

The filtrated effluent was mixed with tap water in appropriate dilution to get wide range of concentration of the effluent with that pilot experiments were done to get the suitable range. Within the range of 12 concentrations were selected, and in each concentration 10 fishes were introduced approximately of equal size. The mortality in the each concentration was recorded after 24, 48, 72, and 96 hours exposure.

Histopathology

Fishes of similar size (50 ± 70 grams live weight) were taken from the stock and five fishes were out in each concentration namely 3.5 ppm and 35 ppm for fifteen days. The organs - gills, liver, kidney and intestine were dissected out after exposure and fixed in bouins fluid for 24 hours, they were

then passed through graded series of alcohol and xylene and serial sections of these organs were prepared and stained with haematoxylin and counter stained with eosin. The changes in the tissues of the treated fishes were observed comparing with the control.

STATISTICAL ANALYSIS

Student t-test was applied to find out if there is any significance difference between the control and treated groups for the various hematological parameters studied. Data's were expressed as means ± SE and where analyzed using ANOVA. A "P" value <0.05 was considered as statistically significant.

RESULTS

The physico-chemical parameters of the experimental water were measured following the procedures of APHA (1981) and were given in the (Table 1). The concentrations of heavy metal chromium accumulation in selected tissues viz., liver, muscle, gills, intestine and kidney of *Tilapia mossambica* were analyzed and represented in (Table 2), after fifteen days treatment in sub lethal concentrations. The maximum accumulation of chromium was in the liver 1.65 mg/kg in the intestine 0.04 mg/kg and the gill was 0.16 mg/kg. The concentration of chromium in the muscle was 0.64 mg/kg that is nearer to liver and in the kidney was 0.09 mg/kg. The maximum total uptake of chromium by the fish *Tilapia mossambica* was 2.58 mg/kg.

Toxicity Test

Toxicity tests were carried out to measure the effects of tannery effluents on *Tilapia mossambica*. Tests were conducted for different length of time, but LC 50/96 hrs, test was conducted in the present evaluation according to Cremlin, 1978. (Table 3) shows the effect of tannery effluent on the percentage mortality of the fish for 24, 48, 72 and 96hrs. There was no mortality in concentration 10 and 20 ppm for 96hrs. At 30 ppm 20% mortality in 72hrs, out in 96hrs the mortality rate was 40%, at 40 ppm the mortality rate was 10%, 30%, 50% and 60% in 24, 48, 72 and 96hrs respectively. Natarajan (1981) found that the LC 50/48hrs was 5 mg/l for *Metazytos*. Santhosh (1989) also found that the LC 50/96 hrs was 15.96 ppm the tannery effluent treated fish *Cirrhinus mrigala*. The report of Sudha Singh *et. al* (1998) elucidates that 28 ppm and 30 ppm represented LC 50/96 hrs and LC 100/96 hrs respectively, where the murrel was exposed to endosulfan.

In the present study the toxicity test confirmed that the 35 ppm of tannery effluent is the Lc 50 value for 96 hrs. Sub lethal concentration (3.5 ppm) as taken for the rest of the experiments such as haematological and histopathological studies.

Haematological Study

In recent years much important is given to haematological studies in assessing the health conditions of fishes. The complete haematological picture is placed in (Table 4) and it reveals that the decreased level of red blood corpuscle count, percentage of haemoglobin content and the percentage of erythrocyte sedimentation rate and packed cell volume were due to increased concentration of tannery effluent. The RBC count decreased considerably 2.26 control to 2.0 (3.5ppm), 1.8 (3.5ppm) in 15 and 30 days treated groups respectively. RBC count is reduced to 1.2 in experiment carried out for 15 days treatment in lethal concentration (35ppm) when compared to control. The Haemoglobin content also showed a steady decline from control to that of tannery effluent exposed fishes (control – 0.85%: treated exposed groups 0.6% 0.4% (35ppm) in 15 and 30 days treatment, 0.2% in (35ppm) 15days treatment. PCV% (Control 4.5% and 3.5PPm

treated groups for 15days and 30days exposure showed 3.3%, 2.5 % respectively. In lethal groups PCV reduced to 1.5% in 15days treatment.

The ESR also reduced from 8.2% (Control) to 7.0, 4.2% in sub lethal concentration (3.5ppm) for 15 and 30 days exposure, 2.8% in 35ppm for 15days exposure. Whereas the WBC ($\times 10^3/\text{mm}^3$) Showed an increasing trend from 41.62 (Control) to 47.5, 55.0 in (3.5ppm) treated for 15 and 30 days respectively and 63.8 in 35ppm in treated groups for 35 days. This reduction in RBC and Haemoglobin content supports the earlier observation made on *Oncorhynchus kistuch* by Mc ley (1973). The reduced RBC count may be due to destruction of the RBC or inhabitation of RBC production. A significant decrease in RBC, Haemoglobin content, ESR, PCV and increased trend in WBC have been observed earlier in fishes exposed to heavy metals and pesticides by Banerjee and Varma (1987), Natrajan (1981).

This observation is in accordance with the previous findings made in effect of various pesticides on fresh water fishes. By Seth N Saxena (2003), Park *et.al* (2004) Ramesh *et.al* (2008) and Palanisamy *et.al* (2011). Due to effect of Lindane, Fenvalerate and Chorpyrifos pesticides

Table 1: Concentration of different physico chemical parameters in test sample

Sites	EC	pH	Turb	TDS	Cl	ALK	TH	Ca	Mg	Fe	NH ₃	NO ₃	F	SO ₄	PO ₄	Cr	Tidy's
			NTU														
Lab Tap Water	450	7.2	0	315	74	232	196	45	20	0.1	0	0	0.6	32	0.2	0	0.04
Moat Water	2580	7.9	5	1805	420	516	860	192	91	2.	14.4	0.9	53	143	1.4	0.03	1.36
Tannery effluent	4350	8.9	125	3045	785	670	1780	496	130	4.6	24.2	1.2	75	328	2.6	45.4	14.4

Table 2: Concentration of chromium in different organs of the fishes

Sample No	Genus	Tissue type	Cr conc Mg/Kg	Mg %Cr
1	<i>Tilapia</i>	Liver	1.65	33.266
2	<i>Tilapia</i>	Muscle	0.64	14.767
3	<i>Tilapia</i>	Gills	0.16	2.532
4	<i>Tilapia</i>	Intestine	0.04	5
5	<i>Tilapia</i>	Kidney	0.09	4.787
Total			2.58	60.352

Table 3: Mortality rate of *Tilapia mossambica* exposed to different concentration of Tannery Effluent

Concentration (PPM)	Total Mortality				Percentage of Mortality
	24 hrs	48 hrs	72 hrs	96 hrs	
Control	0	0	0	0	0
10	0	0	0	0	0
20	0	0	0	1	10
30	0	1	2	4	40
40	1	3	5	6	60
50	2	4	5	8	80
60	3	7	9	10	100
100	4	8	10	-	100

* Values are more of 10 individuals

Table 4: Haematological Parameter of *Tilapia mossambica* exposed to Tannery Effluent

Haematological parameter	Control	Experimental			
		3.5PPm		35PPm	
		1.5 Days	30 Days	15 Days	30 Days
Red Blood corpuscles	2.26±0.0571	2.0±0.3365 (-12)*	1.8±0.4182 (-20)**	1.2±0.4778 ***** (47)	-
White blood Corpuscles ($\times 10^3/\text{MM}^3$)	41.62±0.3102	47.5±0.3165 (+14)***	55.0±0.482 (+33)***	63.8±0.7348 (+55)	-
HB/	0.85±0.316	0.6±0.0716 (-30)***	0.4±0.644 (-56)***	0.2±0.0326 (-77)***	-
ESR%	8.2±0.2449	7.0±0.3265*** (-17)	4.2±0.2499*** (-56)	2.8±0.1642	-
PCV %	4.5±0.3265	3.3±0.2449 (-27)***	2.5±0.3355 (-45)***	1.5±0.1534 (-68)***	-

Values are expressed in mean ± SE for 5 observations;

Number in parenthesis represent the percentage change from control value, significant at $P < 0.001$ ***, $P < 0.01$ ** , $P < 0.05$

Histopathology

Gills

The normal gills are finger like filaments attached to cartilaginous gill bar. Numerous delicate, leaves like structures, the lamella, project from each filaments and these consist of minute capillaries covered by single layer epithelial cells. The epithelial layers of filaments are separated by cells called a pillar or pilaster cell which gives support to the lamella (**Plate 1**). The fishes, exposed to 3.5ppm, fusion of the adjacent secondary lamella was showed. The necrosis of the epithelial layer resulted in the erosion of the respiratory epithelial is often seen. Some of the secondary lamella showed clubbing at their tips due to the toxic effect of the effluent. Hypertrophic nuclei were seen and cell showed hyperplasia (**Plate 2**).

In the gills exposed to 35ppm of tannery effluent for 15 days, hyper plastic cells were seen and the nuclei were hypertrophic clubbing of the secondary lamella was clearly seen (**Plate 3**) and there was erosion of the respiratory epithelium. Some of these changes were noticed in fishes under the exposure to different pesticides by Gandner Yevich (1970), Chakravarthy and Konar (1974), Moses Giriya (1985) and Santhosh (1989).

Liver

In (**Plate 4**) control liver compared to the liver of the fish exposed to 3.5ppm (**Plate 5**) of tannery effluent showed the following changes. The hepatocytes had lost their polyhedral shape and hence the gabs between the cells had increased. There was aggregation of the hepatocytes also noted. Some of the cell showed lysis leading to binucleated conditions. Liver exposed to 35ppm showed lose of the shape of the hepatocytes, vacuolization among the hepatocytes and cells were hyperplasic. The cell membrane was lyses and the cytoplasmolysis had occurred was exposed. The nuclei were hypertrophy and some of the cells showed displacement of the nuclei. An aggregation of the hepatocytes was also seen in (**Plate 6**). Some of the hepatocytes had ruptured leading to denucleated cells. This observation is accordance with the previous findings made in effect of pesticides on fishes Backthavathsalan *et.al*; (1984), and Santhosh (1989).

Intestine

In (**Plate 7**), the control intestine of a fish has 4 layers (tunica) namely the tunica mucosa, tunica sub mucosa, tunica muscularis and tunica

external. The tunica mucosa consists of columnar epithelial cells attached to a basement membrane. Above the basement membrane lies a layer of connective tissue. The epithelium of the intestine projects into the lumen in the form of leaf like structures called villi. In (**Plate 8**). The fish exposed to 3.5 ppm of the effluent for 15 days, the tunica externa showed necrosis. There was a vacuolization of the epithelial cells and shortening of the villi. In the intestine of the fish exposed to 35 ppm of the effluent showed there was vacuolization of the tunica mucosa. There was necrosis of the cells leading to formation of gaps in between the cells. The cells showed hypertrophic nuclei shortening of villi was seen in these concentrations (**Plate 9**). The same observation of reported by Santhosh (1989) due to effect of tannery effluent on teleost fish *Cirrhinus Mrigala*

Kidney

In (**Plate 10**), the kidney of a normal healthy fish comprises of numerous functional excretory units, the nephrons. The nephrons consist of renal corpuscles, a coiled uriniferous tubule and interstitial hematopoietic tissue. The renal corpuscles are made up of a glomerulus and Bowmen's capsule. The Bowmen's capsule comprises an interlayer of visceral epithelial cells which are squamoidal nature and the outer parietal layer of cuboidal cell. The haemopoietic tissue occupies intertubular space. The cells are parenchymatous in nature, round to polygonal in shape with distinct nuclei in the centre and is beset with numerous red blood corpuscles.

The kidney exposed to 3.5ppm tannery effluents for 15 days showed more vacuolization of the interstitial cells. The tubular cells showed necrosis. The cells showed hyperplasic condition and there was aggregation of the cells also. The nuclei showed hypertrophy and some of them were picnotic. There were degenerations of the cells membrane also.

In (**Plate 12**), the kidney exposed to 35ppm of effluents for 15 days showed more vacuolization and hyperplasia. There was hyperplasic growth of cells and the nuclei of the cells were displaced to the periphery. The cells of the renal corpuscles showed cytoplasmolysis. The nucleus of cells showed lysis leading to gap in between the cells. Due to lysis of cell membrane of adjacent cells, there was aggregation of the nuclei leading to suncytial condition. In the kidney exposed to 35ppm of tannery effluent drastic changes were

noted. There were large vacuoles found in the interstitial cells. Most of the cells showed cytoplasmolysis in the multinucleated condition. Abnormality in histology of treated kidney is due to toxic stress of some heavy metal and pesticides have been reported by Natarajan and Manimegalai (1988).

Plate 1: T.S. Through Normal Gills

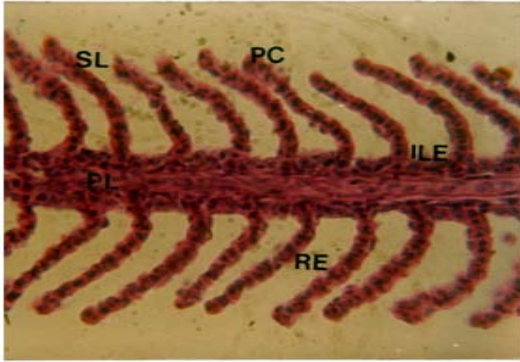


Plate 2: T.S. of gills exposed through 3.5ppm of tannery effluent

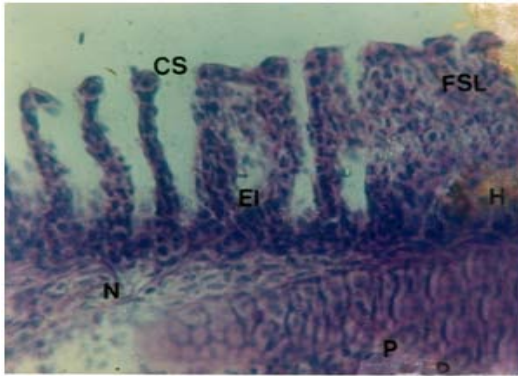


Plate 3: T.S. of gills exposed through 35ppm of tannery effluent

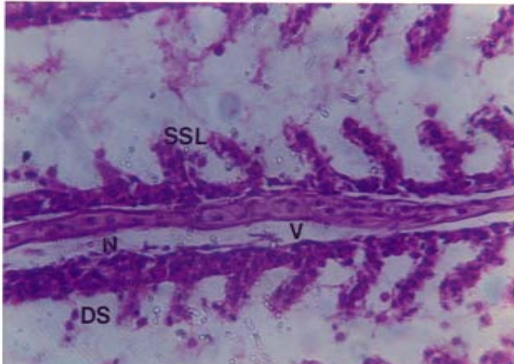


Plate 4: T.S. of normal liver

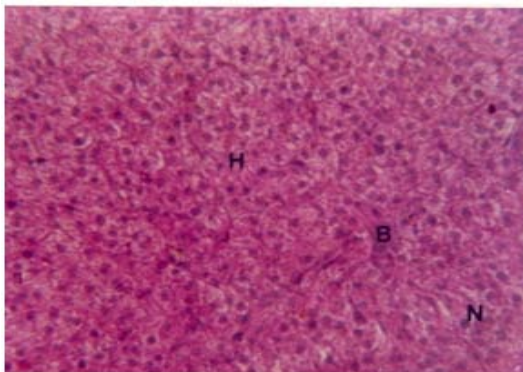


Plate 5: T.S. of liver exposed to 3.5ppm of tannery effluent

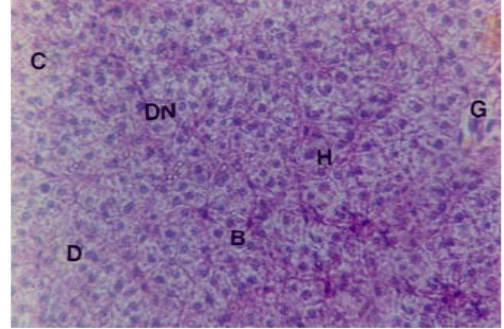


Plate 6: T.S. of liver exposed to 35ppm of tannery effluent

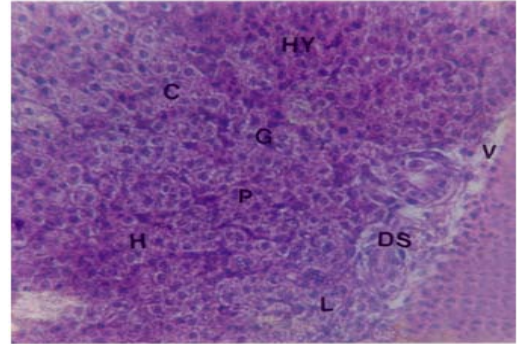


Plate 7: T.S. of normal intestine

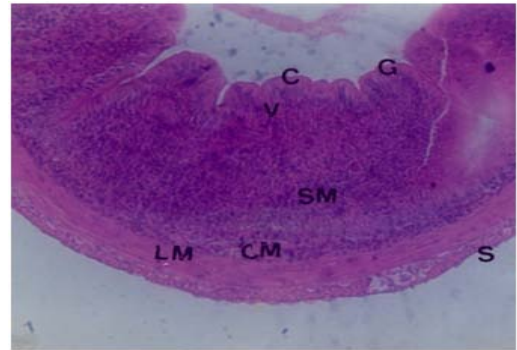


Plate 8: T.S. of intestine exposed to 3.5ppm of tannery effluent

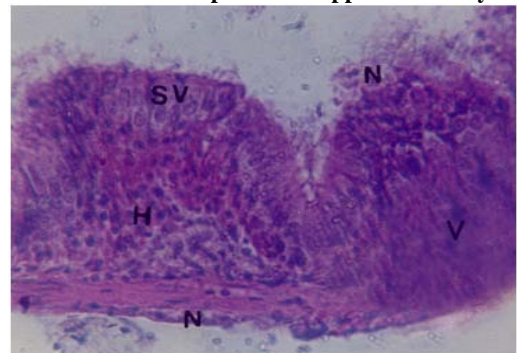


Plate 9: T.S. of intestine exposed to 35ppm of tannery effluent

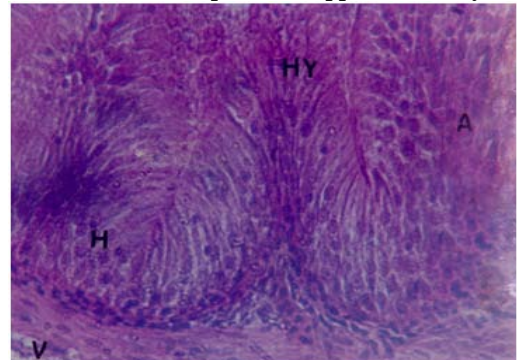


Plate 10: T.S. of normal kidney

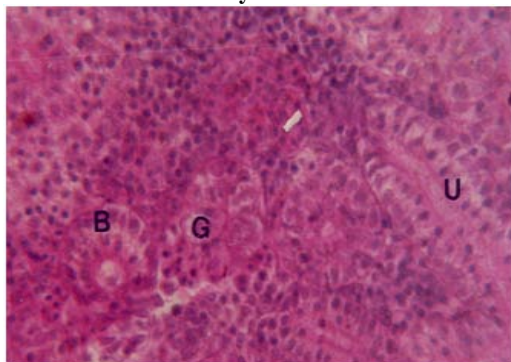


Plate 11: T.S. of kidney exposed to 3.5 ppm of tannery effluent

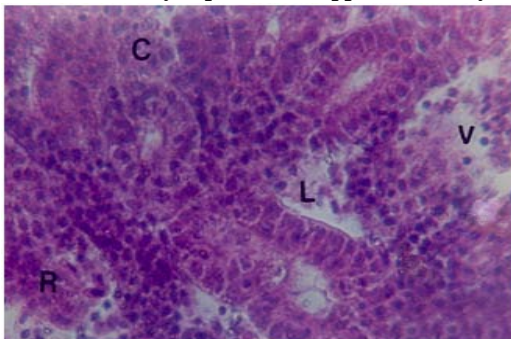
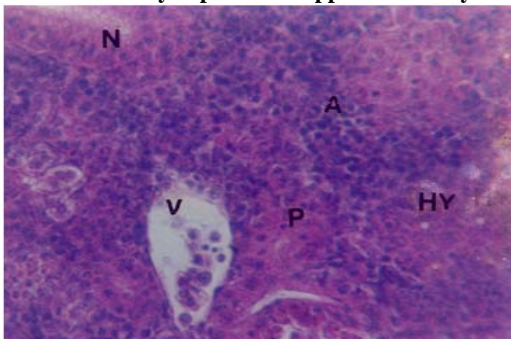


Plate 12: T.S. of kidney exposed to 35ppm of tannery effluent



DISCUSSION

The moat water is green in appearance due to the presence of bacteria and algae. The water was having a fishy odour. The pH of the moat water was 7.85, which indicates that the water is slightly alkaline. This is evident of the fact that the bicarbonates and the carbon dioxide in the water are in equilibrium. Singh (1995) found that the pH of river water contaminated with industrial waste effluent was slightly alkaline. Dewis and freitar (1970) recorded a higher value of pH in effluent mixed water at Edward victor's bridge in Madurai.

In our present investigation, uptake of heavy metal chromium in selective tissues like liver, gills, kidney intestine and muscles were observed in *Tilapia mossambica* exposed period of 15 days. Liver acts as a tolgate for circulatory system whatever the toxic substance enter in the blood and it is carried to the liver first and then it reaches other organs hence the accumulation of chromium more in liver compare to other organs.

Chromium accumulated more in ascending order with increase in the exposure period of 24, 48, 72 and 96 hours respectively. Similar pattern of accumulation was reported by Geetha *et al* (1996) that the fish in the tissue with an increase in exposure time. The correlation between the rate of accumulation and period of exposure was also indicated by Viarengo *et al* (1988). It was reported that heavy metals predominantly found in gills liver, intestine and kidney of fish (Brown *et al* 1986).

The presence study revealed that the accumulation of chromium is within the normal level (ISI tolerance limits) in the tissues. The data of harmless concentration reported in the present study helps in determining the acceptable concentration causing on adverse effect on human beings biota. The observation on bioaccumulation of chromium in different tissue of the fishes liver, muscle, gills intestine, ovary and kidney suggesting the possibility of heavy metal chromium present in the effluents entering the food chain, which includes the humans. It's therefore suggested that periodical monitoring programme is necessary to warn and preserve this important part of the traditional diet.

Gills are a primary site of Osmoregulation and respiration is first and probably main target for the aquatic toxicant and all the most seriously affects organs (Wood watt *et al* 1983). Similar is the case with fish, these four organs gills, liver, intestine, and kidney are taken after exposure such studies are of significant importance in predicting the early changes caused by the toxicant. Several other parameters such as mucous cell membranes and chloride cell membranes are also studied for exploring the mechanism of the toxicity pathomorphological response may be correlated with the chronic toxicity of chemicals at other levels of biological organization also.

SUMMARY

The present study was carried out on the physicochemical characterized of the Vellore fort moat and the estimation of heavy metal chromium in the water and the selected tissues of the fish like *Tilapia mossambica*. In the physiochemical parameters, chromium in the water samples were found to be within the ISI Tolerance limits. The concentration of heavy metal chromium was within the tolerance limits 0.250. Accumulation of heavy metal (chromium) in a fresh water fish *Tilapia mossambica* was 2.58. All the values obtained are the normal tolerant levels. Haematological studies in assessing the effect of

tannery effluent on fishes reveals that the decreased level of RBC Count, percentage of haemoglobin content erythrocytes sedimentation rate percentage and PCV was due to effect of increased concentration of tannery effluent. The decreased erythrocyte count and haemoglobin in content observed in the study may be due to the disruptive action on the erythropoietic tissue, which in turn effected the cell viability. The increase in WBC count can be correlated with an increase in antibody production which help in survival and recovery of the fishes exposed to toxicant. Distinct histopathological changes were observed in the gills were fusion of adjacent secondary lamellae, necrosis of respiratory epithelium, clubbing of the tips of the secondary lamellae, the prevalence of hypertrophic nuclei, cytoplasmolysis and hyperplasic growth of cells. The changes in the liver noted were loss of the shape of hepatocytes binucleated condition, vacuolization and lysis of cell membrane. The changes noted in the intestine were necrosis of the intestinal walls, shortening of villi, hypertrophic nuclei, hyperplasia of cells and vacuolization. The changes in the kidney were necrosis of tubular cells, hyperplasia, hypertrophic nuclei, picnotic nuclei and vacuolization.

Under this toxicity study it is concluded that exposure to lethal and sublethal concentration of tannery effluent results in a significant alteration in different haematological and histopathological structures. This kind of changes may directly affect the survivability of this fishes in their natural habitat.

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