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Multiple organs damage in *Channa punctatus* when chronically exposed to mercuric chloride

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ABSTRACT

In the present investigation, live specimens of *Channa punctatus* were collected, after acclimatization, they were chronically exposed to sublethal concentrations of mercuric chloride for 15 and 30 days. As compared to the control fish, Mercury treated fish kidney and liver showed marked histopathological changes. After 15 days the alterations detected initially were the widening of space between the capsule and the glomerular capillary network. The effect was dose and duration dependents. After 30 days of exposure, marked shrinkage of the glomerular tuft at all the concentrations were noticed. Further, the effect became so severe that visceral epithelium got ruptured, glomerulus got evacuated and necrosis of kidney tubule was seen. Elemental composition of the normal scales and scales of exposed fishes was also compared and it was found that fishes exposed to mercury, their scales have greatly reduced mineral composition.

***Key words*:** Mercury; LC_{50}; *Channa Punctatus*; Liver; lepidonts; Scale; Kidney

1) INTRODUCTION

An Among the natural resources water is the most vital element for all the living organisms as no life exists without water. However, for the last few decades water quality deteriorates because of addition of pollutants from commercial properties, industrial and domestic waste and pollutants of varied origins, where these pollutants are found in their untreated form [1]. As a result of this in India 70% of the avialble water is polluted, out of which 84-92% is polluted by sewage pollution and 8-16% by industrial pollution [2]. Impact on fish scales as indicators of wastewater toxicity from international water channel Tung Dhab drain has been reported [3].
Increase in the concentration of heavy metals (Cd, Cu, Fe, Ni, Mn, Zn, Pb and Hg) has been reported in water of Vasai Creek, Maharastra [4]. Heavy metals salts constitute a serious type of pollution in fresh water and being stable compounds, they are not readily removed by oxidation, precipitation or other processes and affect the activity of recipient organisms [5].
Mercury, one of the heavy metals once released it persists in the environment. It has been extensively used in industries like pesticides, electroplating, medicines and battery manufacturing [6]. Effluents from these sources are ultimately dumped to aquatic ecosystem, where they harm non-target flora and fauna such as fish.
The present study was to study the impact of mercury on the multiple organs like kidney, liver and scale alterations in *Channa punctatus* a freshwater fish.

2) MATERIALS AND METHODS

For the present studies live specimens of *Channa punctatus* were collected. They were given bath in 0.1% $KMnO_4$ for 2-3 minutes. The fishes were acclimatized for 7 days under laboratory conditions. Mercury in the form of mercuric chloride was used for present investigations. The salt is selected because of its uses in industries reported toxicity and water solubility.
LC_{50} value for present study was calculated by Probit analysis as suggested by Finney [7]. Based on the Probit analysis technique, 96h LC_{50} value was found to be 1.21 mg/L by graphical interpolation and arithmetic methods. A stock solution of 1 g/L was prepared in normal tap water. From the stock solution measured aliquots of this was added to each experimental tanks so as to bring the mercuric chloride concentrations to required levels i.e. 0.25 mg/L and 0.55 mg/L. The fishes were exposed to these concentrations for 15 days and 30 days.

3) RESULTS AND DISCUSSION

(A) Impact of Mercury on Kidney

General Structure of Kidney The basic unit of kidney is a nephron that in C*hanna punctatus* consists of renal corpuscle and renal tubule. Renal corpuscle is having a well-developed vascularized glomerulus with an inconspicuous mesangium (Fig. 1 a). The renal tubule consists of initial proximal segment (Proximal I) with prominent brush border, second proximal segment (Proximal II) with numerous mitochondria but less

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developed brush borded (Fig 1 b); distal segment with relatively clear cells and elongated mitochondria and a collection duct system. Distal tubule and collecting duct both devoid of brush border are much sparsely distributed. The renal corpuscles are generally located in close vicinity to renal tubules and blood vessels in the interstitial tissue.

Kidney in the freshwater fishes functions mainly as an excretory organ. This is accomplished by the filtration at the renal glomerulus and the presence of suitable cellular components that help to conserve filtered ions while dilute urine is excreted. Histopathological examination of fish kidney is an authentic method of determining impacts of contaminants in the aquatic environment [8, 9 & 10] during the present study, response of a teleostean (*Channa punctatus*) kidney to the sublethal concentration of Mercury has been studied.

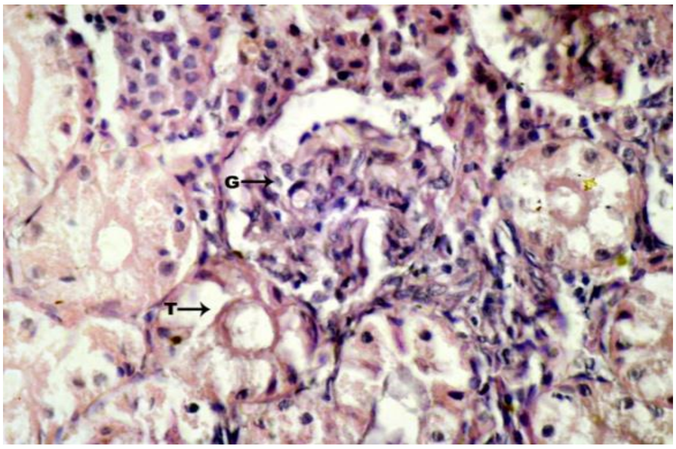

Figure 1(a): Photomicrographs of fish kidney (control), showing glomerulus with parietal and visceral epithelium

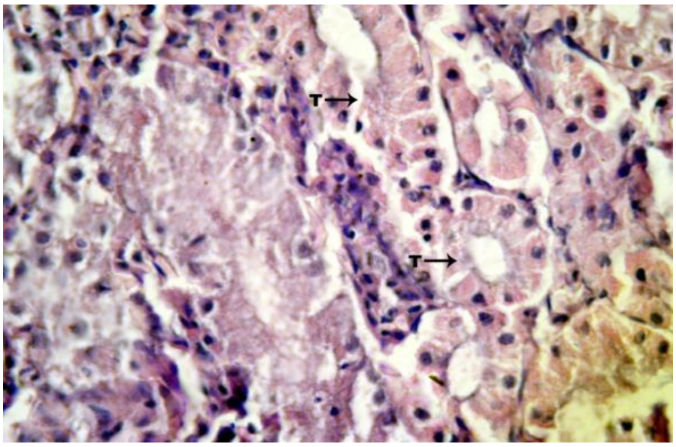

Figure 1(b): Photomicrographs of fish kidney (control), showing kidney tubules. G (Glomerulus)

As compared to the control fish, Mercury treated fish kidney showed marked histopathological changes. The alterations detected initially were the widening of space between the capsule and the glomerular capillary network. The effect was dose and duration dependents. After 15 days exposure, marked shrinkage of glomerular tuft at all the concentrations were noticed (Fig. 2a and 2b; 3a and b).

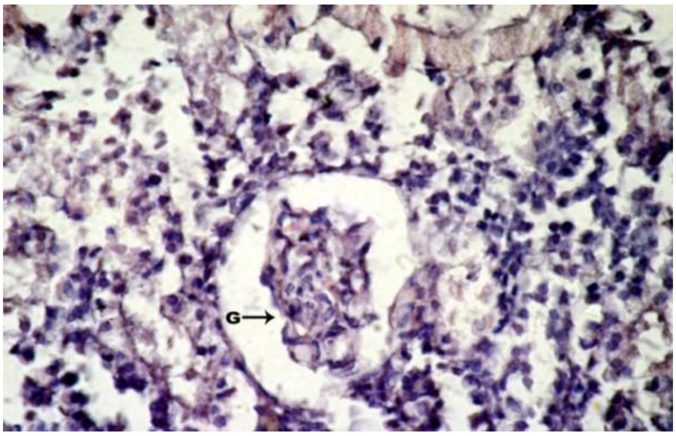

Figure 2(a)

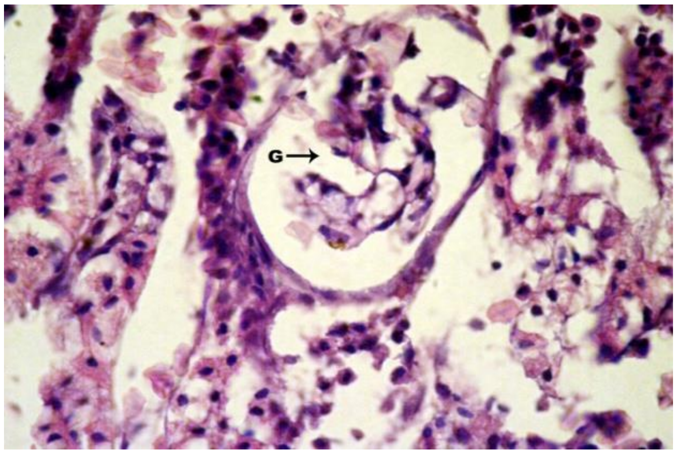

Figure 2(b)

Figure 2: Photomicrographs of fish kidney (Mercury treated) showing shrunken glomerulus (**a-**0.25 mg/L and **b-**0.55mg/L 15 days) G (Glomerulus)

As compared to the control fish, Mercury treated fish kidney showed marked histopathological changes. The alterations detected initially were the widening of space between the capsule and the glomerular capillary network. The effect was dose and duration dependents. After 15 days exposure, marked shrinkage of glomerular tuft at all the concentrations were noticed (Fig. 4a and 4b).

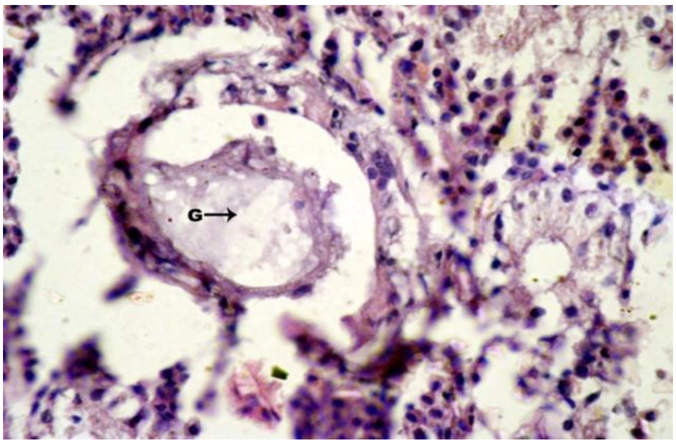

Figure 3(a)

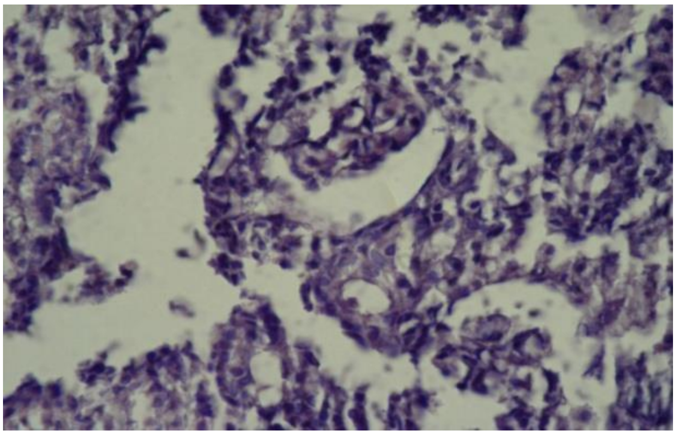

Figure 3(b)

Figure 3: Photomicrographs of fish kidney (Mercury treated) showing evacuated glomerulus (**a**-0.25 mg/L and **b**-0.55 mg/L, 30 days) G (Glomerulus)

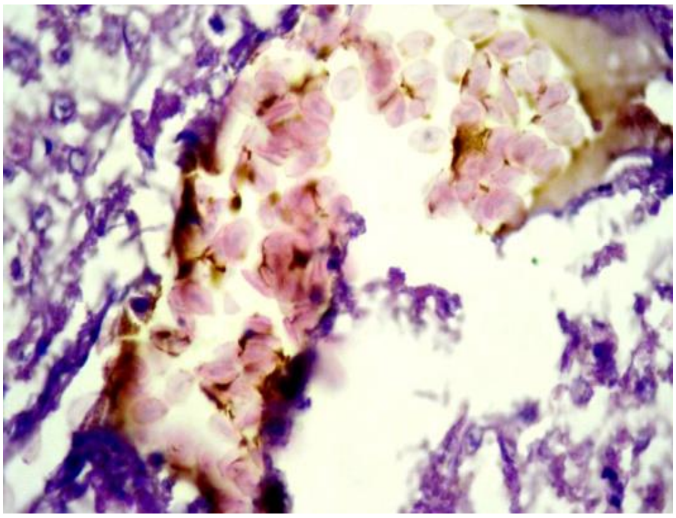

Figure 4(a)

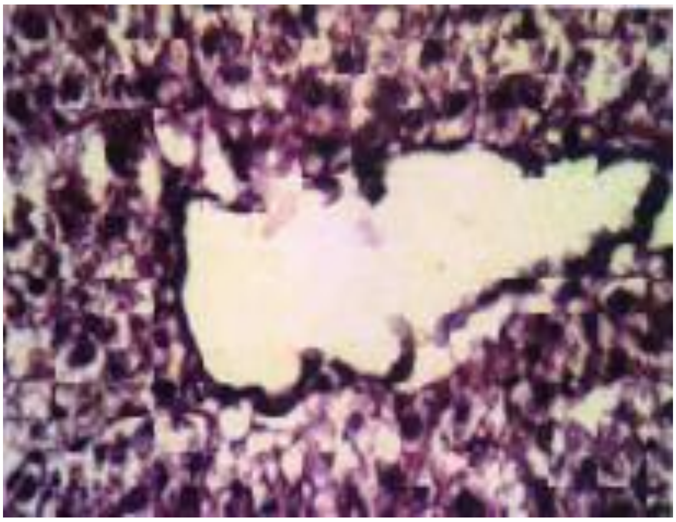

Figure 4(b)

Figure 4: Photomicrographs of fish liver (Mercury treated) showing totally degenerated and evacuated portal region (**a**-0.25mg/L and **b**-0.55 mg/L,30 days)

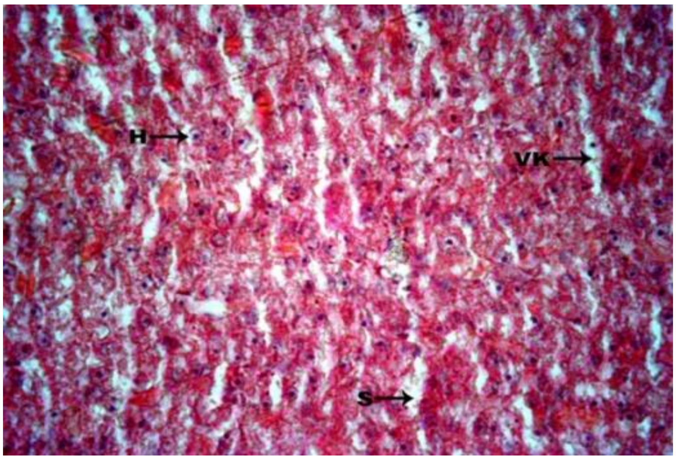

Figure 5(a)

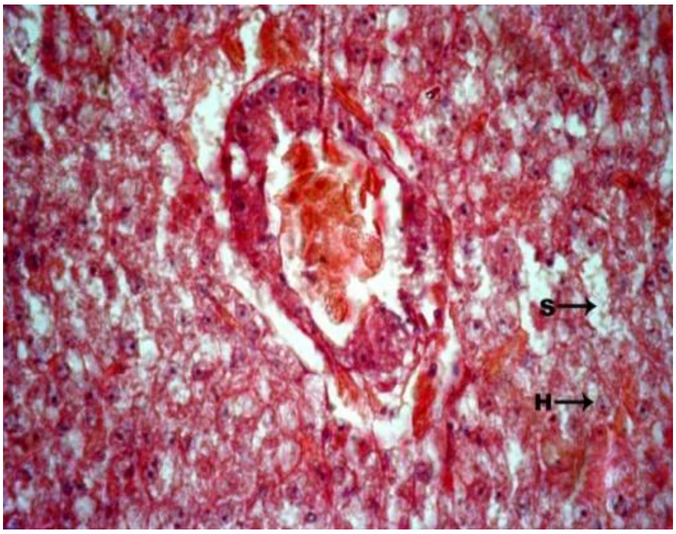

Figure 5(b)

Figure 5: Photomicrographs of fish liver (control) showing (a) two cell thick cords of hepatocytes and sinusoid with Von kupffer cell (b) portal region of the liver along with hepatocytes. Where, H (Hepatocyte), S (Sinusoid), VK (Von kupffer cell)

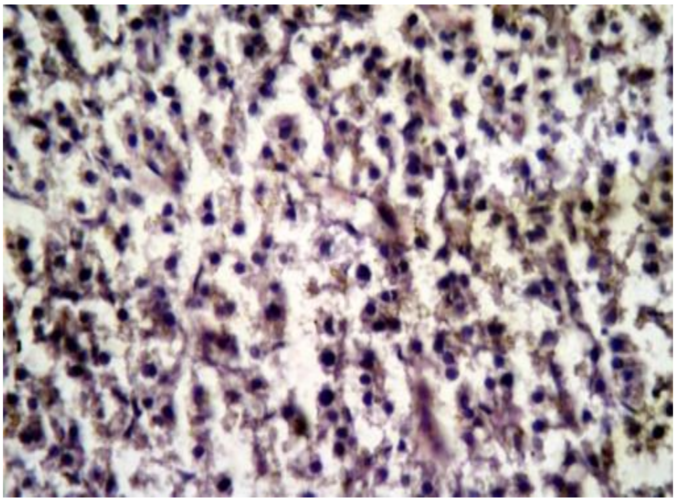

Figure 6(a)

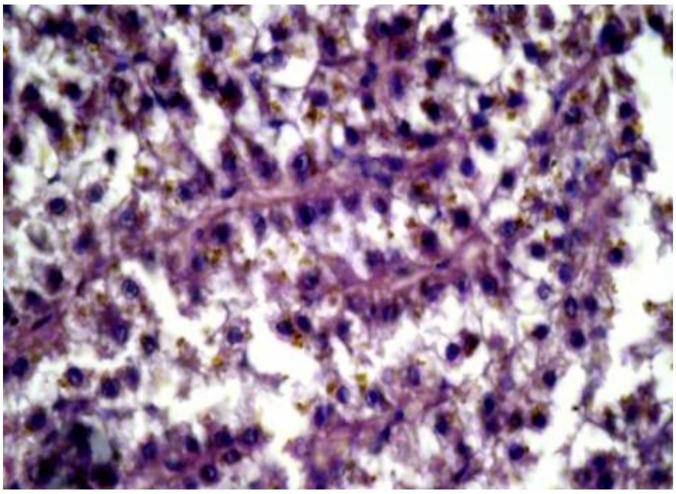

Figure 6(b)

Figure 6: Photomicrographs of fish liver (Mercury treated) showing degeneration of hepatic cell boundaries (**a**-0.25 mg/L and **b**- 0.55mg/L, 15 days)

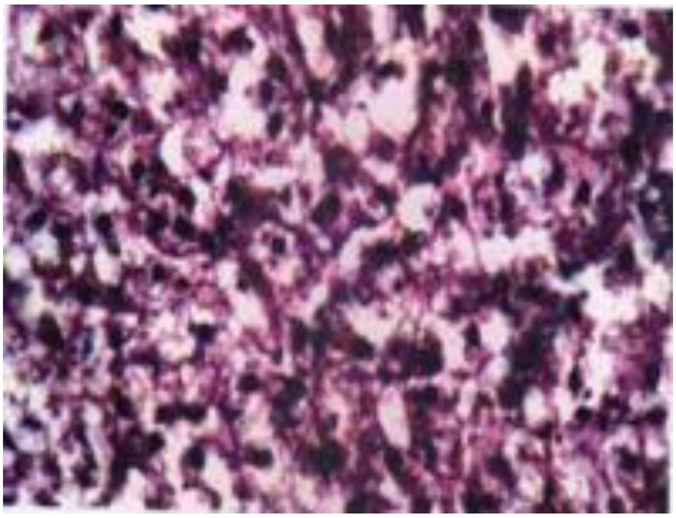

Figure 7(a)

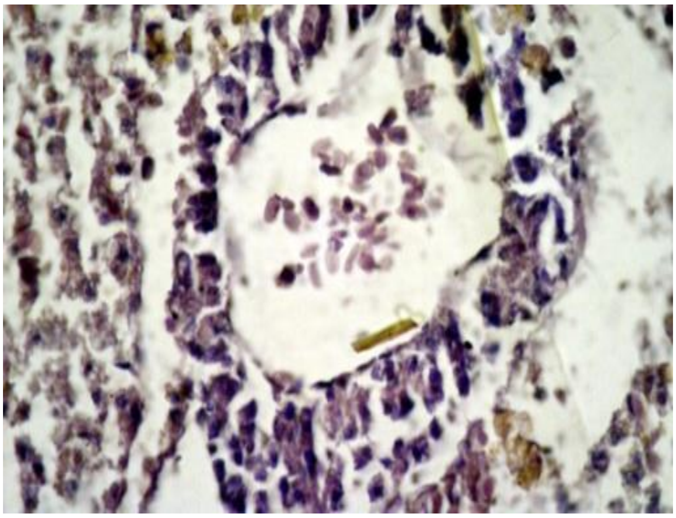

Figure 7(b)

Figure 7: Photomicrographs of fish liver (Mercury treated) showing more degeneration of hepatic cell boundaries and hepatic portal region (**a**-0.25 mg/L and **b**-0.55 mg/L, 30 days)

Similar observations have been reported earlier also upon exposure to various pollutants: Endosulfan [11] Cadmium [12] and *Mylio* macrocephalus (Teleostei) [13], Malathion (*Clarias batrachus* (Linn)- [14] Mercury compound (*Clarias batrachus* (L.)- [15].

(B) Impact of Mercury in Liver

The liver of *Channa punctatus* is made up of hepatic lobules which comprise of hepatic cells having located nucleus which contains a nucleolus and homogenous cytoplasm (Fig.5a). These parenchyma cells contain masses of glycogen, whose formation generally begins in the center of lobules while the secretions of bile occur at periphery. The hepatic cells are arranged in the form of hepatic cords which are two cells thick, but branching and anastmosing of cords often results in four or more cell layers (Fig. 5a). The hepatic cords are pierced with a network of sinusoids blood from interlobular branches of the portal vein. At intervals, in the wall of the sinuses are present conspicuous cells known as satellite cells or Von kupffer (VK) cells (Fig. 5a). These cells lie in the contact with liver cells and are highly phagocytic in nature. They also have a tendency to take in red blood cells which appear to undergo disintegration within them. Bile pigments are formed as a result of this process [20].

On exposure to various sublethal concentrations of Mercury for 15 and 30 days, the hepatic tissue show marked histopathological alterations. Fish showed hepatic congestion and distinct cell boundaries were found to be destroyed, when exposed to higher concentrations (0.25mg/L and 0.55mg/L, 15 days) (Figs. 6a & b). They also showed increases in the size of hepatic sinusoids. These effects were dose and duration dependents. With the increase of exposure period to 30 days the effects of lesions were also increased (Figs. 7a & b). This was probably because of site-specific action of heavy metal thus impoverishing the cell of their various organelles.

The present findings are in conformity with the earlier reports of [16, 17 & 18]. These workers reported vacuolation and hypertrophy in the hepatocytes; pycnosis; lytic necrosis; focal cell necrosis; lipid accumulation and the absence of nuclei in various regions of the liver parenchyma in various fish species, which are exposed to various toxicants. Through SEM, cytopathological alterations of hepatocytes such as irregular nuclei outlines and heterochromatin, fragmentation vesiculation of endoplasmic reticulum, disruption of mitochondria, proliferation of lysosomes with electron dense bodies and lipid inclusions and increased serum metabolic enzyme activity were noticed in *Cyprinus carpio* under the stress of gallium[19], noticed liver tissue necrosis, apoptosis and inflammatory infilitration when treated with Cadmium toxicity in male wistar rats.

(C) Impact of Mercury in Scales

In both the focal and lepidontal regions scales of *Channa punctatus* are composed of four major elements and these are: calcium (Ca); iron (Fe); aluminium (Al); and phosphorus (P). The percentage composition of calcium was recorded to be maximum, followed by phosphorus, aluminium and Iron (Table 1).

Table 1: Elemental composition of scales in focal and lepidontal regions of *Channa punctatus*

EXPOSURE PERIOD	FOCAL REGION	LEPIDONTAL REGION
Normal scales	Ca > P > Al > Fe	Ca > P > Al > Fe
15 DAYS	Ca > Al > P > Fe	Ca > Al > Fe > P
30 days	Ca > Al > P > Fe	Ca > Al > P > Fe

The percentage composition of each element upon exposure to mercury was compared to corresponding control and the deviations were observed in both regions of the scales. When fish was exposed to different sublethal concentrations of mercury for 15d and 30d, an increase was observed in percentage composition of aluminium and phosphorus, whereas reverse trend was noticed for calcium and Iron (Table 1).

For statistical analysis of the data, the values of standard deviation (SD) and standard error mean (SEM) were calculated. The use of these values is based on the fact that greater the values of SD or SEM, irrespective of increasing or decreasing trend, more is the variability in occurrence of the specific elemental due to damage done by the toxicant. This type of quantitative comparison was not possible when the degree of lepidontal damages was considered; hence, the methodology described herein is more practicable. On the basis of the values of SD, the following trends in the focal and lepidontal regions have been observed (Table 2).

On the basis of SD and SEM it is concluded that in all the cases calcium is the most affected mineral followed by phosphorus, Iron and aluminum. Thus, calcium deposition can be termed as a true pollution indicator in quantitative analysis. This is in accordance with the earlier studies [21& 22].

Percentage and the elemental composition of the scale are attributed to the chemistry of the surrounding water in which the fish resides. In the scales of the bony fishes elements like Al, Ca, P, Si, F, Mg, Li, Na, Ca and Bu have been reported by various workers. [23] reported twenty elements (Ca, Cl, Cr, Co, Cu, F, I, Pb, Mg, Mn, Ni, K, Rb, Se, Si, Na, Sr, S, Ti and Zn) in the scale of *Latimaeria chalumnase* (Smith). However, four major elements, which are studied during the study, are of common occurrence [24].

4) CONCLUSION

From all the above observations it can be concluded that mercury has its impact in almost all the organs of the fish and hence it is a potentially harmful chemical for the aquatic life. So, its use should be minimized to protect the aquatic environment.

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Table 4: Percentage composition of major elements in the focal and lepidontal regions of scale of *Channa punctatus*

	Calcium				Phosphorous				Aluminium				Iron			
Control	Focal		Lepidontal		Focal		Lepidontal		Focal		Lepidontal		Focal		Lepidontal	
	15	30	15	30	15	30	15	30	15	30	15	30	15	30	15	30
Control Conc. mg/L	66.53	62.37	62.17	59.34	19.42	20.23	20.57	24.26	8.53	10.01	8.97	10.32	5.52	7.39	8.29	8.08
0.080	62.97	62.56	62.8	58.17	20.29	20.87	21.38	25.14	12.10	10.38	9.42	10.02	4.52	6.19	7.12	6.67
0.10	58.74	61.27	60.19	54.97	19.80	21.87	23.02	23.89	13.47	10.75	9.87	14.68	7.99	6.11	6.92	6.46
0.25	63.17	60.36	59.43	51.33	21.40	22.15	23.61	27.94	11.28	11.17	10.93	15.88	4.5	6.32	6.03	4.85
0.40	57.59	59.83	59.11	49.83	23.80	22.87	22.17	28.76	14.74	11.33	12.89	16.92	3.87	5.97	5.80	4.49
0.55	56.83	55.43	58.07	46.03	23.98	24.42	23.05	29.14	16.94	15.72	14.77	17.46	2.25	4.43	4.11	7.37
Mean	60.97	60.47	60.17	53.27	21.44	22.06	22.30	26.56	12.84	11.56	11.14	14.21	4.71	6.06	6.37	6.32
SB	3.8	2.6	2.6	5.13	2.01	1.49	1.15	2.85	2.9	2.09	2.25	3.31	1.92	0.94	1.41	1.40
S. EM	1.7	1.16	0.71	2.3	0.90	0.64	0.51	1.27	1.3	0.93	1.01	1.43	0.86	0.42	0.63	0.62

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