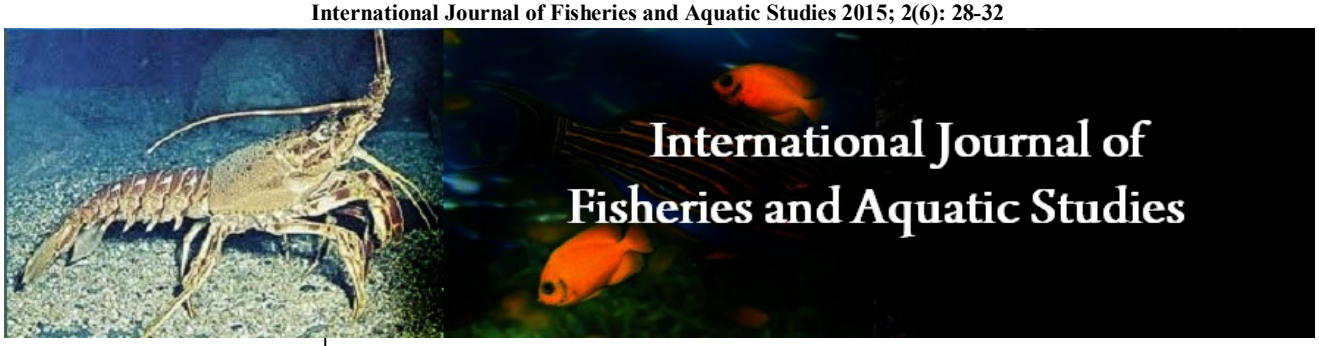

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Histopathological effects of sub-acute lead chloride on the vital organs of the suckermouth sailfin catfish *Pterygoplichthys pardalis* Castelnau

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Abstract
This study investigated the effects of sub-acute (70 mg/L) lead chloride ($PbCl_2$) exposure on the vital organs of the suckermouth sailfin catfish *Pterygoplichthys pardalis*, a species known to thrive in polluted freshwaters. Histological observations in the gills show thickened lamellar epithelium and distinct thrombosed telangiectatic secondary lamella. The liver had the least type of lesions such as fatty change and focal necrosis. The small intestine exhibited massive sloughing of the submucosa and vacuolar degeneration of secretory cells while thinning and disintegration of the submucosa externa, accompanied by pyknotic respiratory cells and necrotic secretory cells were seen extensively in the stomach. The pattern of lead accumulation was in the order of gut> liver>gill>muscle with Pb accumulated significantly in the gut (46.33 mg/L). High accumulation of Pb in the gut, an organ often considered as "end accumulator" could suggest the potential of *P. pardalis* as a "partial regulator" species for Pb contamination.

Keywords: Janitor fish, lead, bioaccumulation, histology

1. Introduction
Many organisms, including fishes have evolved systems and regulation strategies that allow the uptake and selective utilization of essential trace metals and minimize reactive forms of non-essential elements which can be deleterious to the system. In "strong accumulator" organisms, there is a very slow rate of loss such that high concentrations are accumulated significantly in tissues before the rate of excretion matches that of the uptake [1]. Metal bioaccumulation capacities and toxicity are important issues to be addressed when studying sentinel species with undefined accumulation strategies. The ability to regulate metal uptake and loss is necessary to assess a particular species if known to thrive in polluted systems.
Histology is a useful tool to evaluate the degree of pollution, particularly for sub-lethal and chronic effects [2]. Exposure of fish to pollutants is likely to induce lesions in different organs at varying extent. Gills, liver and gut, are suitable organs for histologic examination to determine the effects of pollution, especially in laboratory experiments [3].
The suckermouth sailfin catfish *Pterygoplichthys pardalis* (Castelnau 1855) is a hardy species that often dominate the ichthyofauna where it is introduced [4, 5]. An initial survey of Pb and Cd levels in *P. pardalis* from Marikina River, Philippines show permissible levels of Pb in gills and guts except for the liver [5]. Gills and guts of these field-caught samples were of little pathologic observations except for the high fatty deposition in the liver. The present study aims to determine the pathologic effects of sub-acute $PbCl_2$ on the vital organs of *P. pardalis* to shed light on the histologic response of this species to Pb under controlled conditions.

2. Materials and Methods
2.1 Experimental fish
Healthy *P. pardalis*, (ave. wet weight 153.2 g; total length 26 cm) irrespective of sex, were purchased from a local pet fish supplier. Prior to experimentation, the fishes were acclimatized for eight weeks under laboratory conditions before Pb exposure. The fishes were fed daily with TetraMin fish flakes (2% of total fish wet weight). The experiment set-up consisted of plastic aquaria with vigorously aerated water kept at room temperature.

2.2. In vivo exposure experiment

Formulation of the sub-acute concentration of $PbCl_2$ (Reidel de Hein 11516 Bleichlorid) was derived initially by estimating the 96-hr median lethal concentration (96 h LC_{50}) following the 24-hr renewal bioassay system in mg/L. A sub-acute dose of 70 mg/L was used all throughout the actual in vivo experiment. Fish specimens were assigned randomly to eight plastic tanks, each with 50-L capacity; four tanks for the negative control and treated samples each. Each tank contained eight fish replicates. The fishes maintained in dechlorinated tap water were considered as negative control. Fish specimens were exposed to 70 mg/L $PbCl_2$ for seven days with the change of test water every 24 h. Fish samples were sacrificed at the 7th day of experiment with liver, gills, intestine and stomach isolated for histological processing and analyses. The remaining samples were utilized for the isolation of Pb in the gills, liver, gut and muscles.

2.2. Tissue preparation and observation

Tissues were collected in the following order: gills, liver and gut. These organs were isolated and fixed with Bouin's fluid and processed routinely for histological studies [6]. Sections were viewed under Carl Zeiss Primostar microscope. Gill peculiarities or pathology were quantified by counting 100 secondary lamellae on one gill arch of the samples and getting the percentage of lamellae with lesions. Liver hyperplasia was scored manually by counting (5 counts/ section) the number of nuclei in a liver area of 25,500 μm^2. Frequencies of occurrence of fatty droplets/ adipocytes were quantified by scoring sections with more than 200 adipocytes in a liver area as liver with high fatty deposits.

2.3. Pb measurements

Tissue preparation for Pb analysis followed the AOAC Official method of analysis [7] for Pb isolation in fish samples using Graphite Furnace Atomic Absorption Spectrophotometer (AA-6501F Shimadzu Model). The limit for detection (LOD) was 0.004 mg/L. Wavelength for Pb was 283.3 nm, slit 0.7 nm, Lamp current m/A as 6 and with atomization at 1700ºC. The calibration curve for the determination of lead was prepared using a blank and working standards solution (10 – 60 µg/l). The concentration of Pb was expressed in mg per liter (mg/L) of the sample. All reagents used were of analytical grade. Use of analytical blanks, standards and actual samples were carried out to avoid contamination.

2.4. Statistics

Data was described as means ± SE using column statistics. Pb levels in the vital organs were analyzed using one-way ANOVA and post-test was done using Bonferroni method. F-test was used to establish the homogeneity of distribution. All statistical analyses were done using GraphPad Prism 5.

3. Results

3.1. Gross morphological observations and mortality

Fish from control tanks did not show any respiratory distress while treated fish are distinctly less mobile. There was 0% average cumulative mortality rate (ACMR) in the treated samples during the course of experimentation. Samples were observed to have reduced body weight (data not shown) upon termination of the experiment.

3.2. Histology of gills, liver, and gut

Gills of fish unexposed to $PbCl_2$ exhibited typical injuries in the secondary lamella (<3%; < 5% of filaments with swollen tips; < 3% of the signs of oedema, and 0% hyperplasia). Exposure to $PbCl_2$ resulted in the increase in the incidence of oedema in the secondary lamella, extensive lamellar hypertrophy (48%), and high incidence of telangiectatic secondary lamella (aneurysm) (Figure 1). Telangiectasis was often accompanied by fibrosis and fusion with the adjacent secondary lamellae. The proportions of secondary lamella with oedema (mean percentage ±SEM) were 2.6±1.1 and 48±5.7 for the control and treated group respectively.

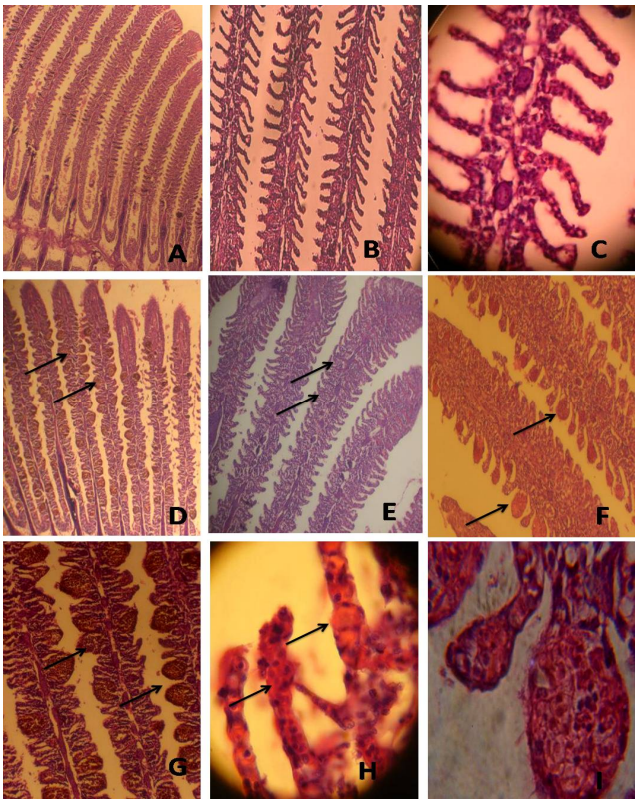

Fig 1: Gill histology in *P. pardalis*. A, B & C are representative sections of the control group showing the outline of primary and secondary lamellae (A, 10x; B 40x) and the details of the secondary lamellae (C; 100x). D & E show outline of gill with lamellar hyperthrophy and thickening (arrowed in E). F & G show thrombosed telangiectatic secondary lamella (arrowed) on a gill showing lamellar hyperthrophy in treated samples. H. Early lamellar hypertrophy (arrowed). G & I are details of D & F respectively (100x). Sections were 8 µm, stained with Hematoxylin- eosin

The liver had the least histologic abnormality observed (Figure 2). The occurrence of fatty change characterized by large vacuolated spaces either devoid of the nucleus or its relocalization near the cell membrane were 3.4±1.7, 52±7.4 (mean percentage ±SEM) for the control and the treated samples respectively. Other lesions observed in the hepatocytes were focal necrosis and thickened periportal area of the liver. Observations in the small intestine of treated samples show the distinct occurrence of pyknotic epithelial cells in the intestinal mucosa (65.2%) (Figure 3). There were also sloughing and thinning of the mucosal lining. Observations on the stomach showed necrosis throughout the stomach wall and destruction of the mucosa. Some respiratory cells exhibited pyknosis while a portion of granular cells appears necrotic. There was also a collapse of the epithelial lining of the stomach in some samples (23%; Figure 4).

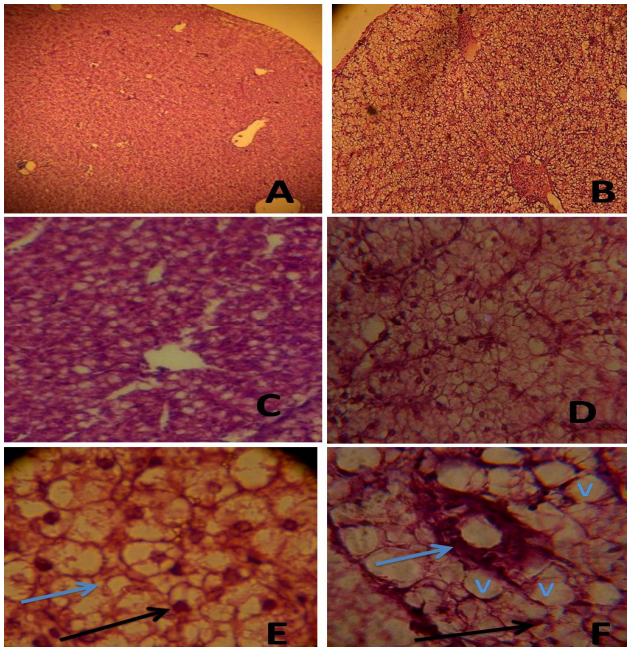

Fig 2: Liver histology in *P. pardalis*. A & C, Livers of control fish show normal histology (A, 10x). Liver of most fish have predominant accumulation of adipocytes or fatty change (D-F; D 40x; E-F, 100x). E show very diffuse hepatocyte nuclei (focal necrosis, black arrows). F. Adipocytes highly vacuolated (V) located surrounding a thickened periportal of liver (blue arrow). Black arrows point to a displaced nucleus in the periphery of adipocytes. Sections were 8 µm, stained with Hematoxylin- eosin

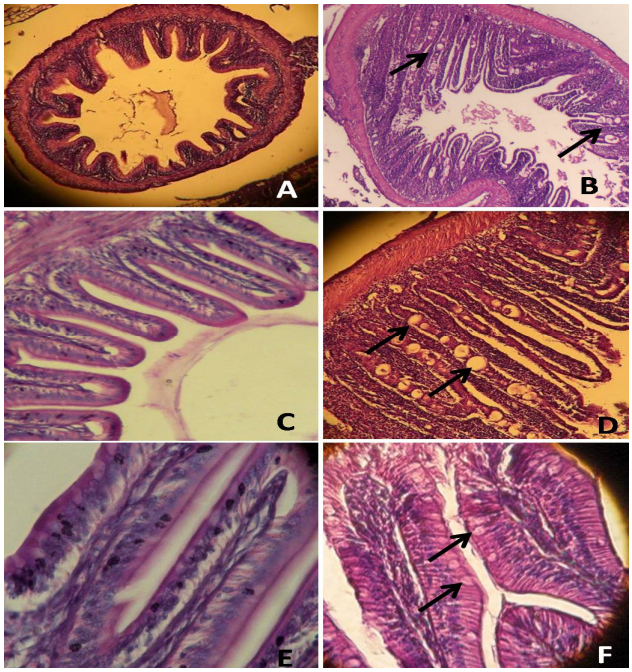

Fig 3: Intestine histology of *P. pardalis*. A. Normal anatomy intestine with an intact thick muscularis externa (10x). B. Section of the small intestine with sloughing between necrotic columnar epithelium underneath the muscularis externa in a fish treated with $PbCl_2$. C & E show intestinal loop lined by simple columnar epithelium and abundance of eosinophilic granular cells (EGC). D show hydropic, vacuolar degeneration, appearing as large hollow swellings inside columnar epithelia in 30% of treated fish. Sections of the intestine also manifest the formation of pyknotic epithelial cells (McKnight cells) in the EGC layer (arrowed in F). Sections were 8 µm, stained with Hematoxylin- eosin

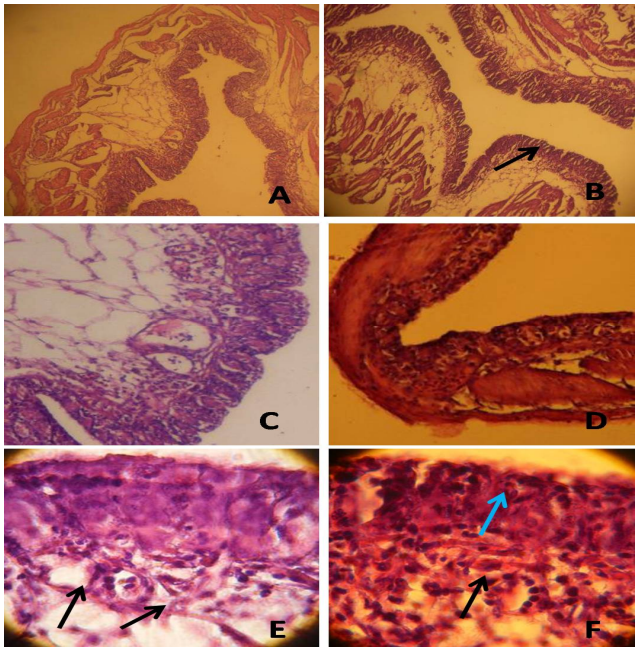

Fig 4: Stomach histology of *P. pardalis*. A. Normal anatomy of mucosa layer devoid of folds. B. tissue necrosis extended throughout the thickness of the stomach wall destruction of the mucosa (black arrow). C. portion of the stomach lined with respiratory cells with the epithelial layer underlain with capillaries. D. Distended portion of a stomach of treated *P. pardalis* showing thin but compact muscularis externa. E, F show details of D showing pyknosis of some respiratory cells in E and necrotic granular cells in the mucosal layer (blue arrow) Sections were 8 µm, stained with Hematoxylin- eosin

3.3. Trace Pb in vital organs

Trace Pb (mg/L) in the vital organs of treated *P. pardalis* were detected after seven days of exposure. Pb was significantly accumulated ($P<0.05$) in highest amounts (Mean 46.31 mg/L) in the gut. Pb concentrations in liver, gill and muscle were not statistically different from each other. Nonetheless, the average pattern of accumulation is in the order of gut> liver>gill>muscle (Table 1). All concentrations accumulated in the treated samples were significantly higher than the control ($P<0.001$).

Table 1: Trace Pb in vital organs of *P. pardalis* after the 7-day exposure to sub-acute $PbCl_2$

Organs	Pb (mg/L)
Gut	46.33±14.74*
Liver	6.783±1.585
Gill	2.408±0.4503
muscle	0.6317±0.1596

*P>0.05

4. Discussion

4.1 Histopathological observations

Aqueous exposure to 70 mg/L $PbCl_2$ apparently caused respiratory toxicity to *P. pardalis*. Gills from the treated group had numerous injuries in the primary and secondary lamella. The types of gill injuries (oedema and thickening the secondary lamella, lamellar hypertrophy) were not unusual, and numerous chemicals can induce these types of injuries [8]. The observation of thickened secondary lamella in treated samples are an indication of increased cell turnover. However, a characteristic pathological change seen in most of the treated samples--lamellar telangiectasis is a typical association with metabolic waste or metal or pollution or chemical trauma [9].

With the increase in telangiectatic lamellae, the respiratory function may be impaired, and prolonged trauma may result in rupture, and fatal haemorrhage may supervene. This pathology was consistent with *Prochilodus scrofa*, exposed to sub-acute copper [10] and sublethal exposure of the air-breathing catfish Hetropneustes fosilis to lead nitrate [11].

Degenerative fatty change of the hepatocytes was the most common pathology in the liver. However, pathology is also common in farmed fishes with unsuitable dietary fats [9]. A previous study on field samples of *P. pardalis* from Marikina River [5] also exemplified frequent observations of degenerative fatty liver change. Focal necrosis and generalized swelling and pyknosis of the hepatocyte nuclei were also observed in this study, often accompanied with cytoplasmic vacuolations. These lesions apparently are common pathologies in toxic conditions.

Massive thinning and high incidence of vacuolar degeneration appearing as large swellings in between columnar epithelium were observed in the gut of treated samples. This lesion is caused mainly by the failure of the sodium pump mechanism at the cell membrane causing intracellular fluid accumulation. Upon severity, vacuolar degeneration could result, and the cytoplasm may be entirely displaced by large clear vacuoles [9].

The stomach, largely functioning as an alternative breathing organ in *P.pardalis* also shows sloughing of the thin muscularis externa though this observation was not general for all treated fish samples. This observed pathology may also compromise the function of the stomach as an air breathing alternative since the health of the gills, as seen through numerous pathologies, is already affected by Pb exposure. The occurrence of pyknosis in the stomach lining is not severe, as compared to the intestine. Nonetheless, degeneration of this lining gravely affects the digestion and respiratory process under stressful conditions. Necrosis and mucosal degeneration may affect the permeability and absorption of substances mediated through this organ [9].

4.2. Trace Pb in vital organs

Levels of lead accumulation in the various tissues studied were in the order of gut> liver>gill>muscle with Pb accumulated significantly in the gut. The gut is considered as a site serving as "end accumulator" involved in metal release instead of serving as "primary accumulator" during metal uptake [12]. Most luminal metals appear to have been released from the cells of the gut (secretory, granular cells), indicating that they have been released by the digestive cells instead. Metals directly incorporated from food materials and also those coming from the other tissues may enter the different digestive cells and could eventually be excreted finally as faeces [13]. Accordingly, exposure to metals in some fishes provokes an increase in faeces deposition [12].

The liver is the main detoxifying organ and the primary target for the accumulation of the majority of metals in fish [14, 15]. However, it is apparent in this study that there is a relatively very little accumulation of Pb in this organ compared to the gut. The accumulation of metals in piscine liver is a very rapid process indicating the presence of a nonsaturable ion channel and the lysosomal system where metals typically arrived coupled to metallothioneins [12]. Metallothioneins (MTs) are metal binding proteins that are upregulated upon exposure to metals. In turn, MTs may attenuate the detrimental effects of the metal to the system of the organism to a certain extent and concentration of exposure [16, 17]. It is possible that the liver of *P. pardalis* has strong detoxifying potential, coupled with the inherent detoxifying capacity of the kidney, which lead to a minimal accumulation of Pb in this organ.

Of the organs studied, the trace Pb was least the muscles. This result is consistent with many reports in other fishes that the muscles are not the primary target for accumulation [18]. The level of metal accumulation in this organ is a vital aspect of assessing the safety of a fish for consumption, especially in the environment potentially polluted with toxic metals. The disparity of metal accumulation between induced, acute conditions, as in this study, and, natural field conditions [5] are influenced by many factors. It is probable that Pb has accumulated in the liver in minute levels in the field throughout the fishes' lifetime. Nonetheless, biotic factors such as age and body weight and abiotic factors such as salinity, temperature, oxygen, co-occurrence of metals and subsequent pre-exposure also mediate metal bioavailability [12]. Long-term, static exposure of $PbCl_2$ in *P. pardalis* is necessary to explore this partial regulating capacity in future studies alongside studies on MTs and potential regulating mechanisms of the gut and kidney.

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6. References

1. Viarengo A, Knot GA. Mechanisms of heavy metal cation homesostasis in marine invertebrates. Comp. Biochem. Physiol 1993; 104c:355-372.
2. Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T. Histopathology in fish: Proposal for protocol to assess aquatic pollution. J. Fish Dis 1999; 12:25-34.
3. Capkin E, Birincioglu S, Altinok I. Histopathological changes in bowtrout (Oncorhynchus mykiss) after exposure to sublethal composite nitrogen fertilizers. Ecotoxicol. Environ Safety 2009; 72:1999-2004.
4. Chavez JM, Dela Paz R, Manoha SK, Pagulayan RC, Carandang JR. New Philippine Record of South American sailfin catfishes (Pisces: Locariidae). Zootaxa 2006; 1109:57-68.
5. Jumawan JC, Salunga T, Catap E. Lipid peroxidation and patterns of cadmium and lead accumulation in vital organs of suckermouth armored catfish *Pterygoplichthys pardalis* Castelnau from Marikina River. J. Appl. Sci. Environ. Sanit 2010; 5(4):375-390.
6. Mahoney R. Laboratory Techniques in Zoology. 2nd Edition, Butterworth Tonbridge England, 1984.
7. AOAC. Official Methods of Analysis for Pb in fish. AOAC, 1990, 257-258
8. Mallat J. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can J. Fish Aquat 1985; 42:630-648.
9. Roberts RJ. The Pathophysiology and Systematic Pathology of Teleosts. In: Fish Pathology, (Roberts RJ (eds) Baillierre Tindall London, 1878.
10. Mason AF, Monteiro EAS, Pinheiro GHD, Fernandes M. Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa.* Braz. J. Biol 2002; 62(4A):621-631.

11. Parashar RS, Banergee TK. Toxic impact of lethal concentrations of lead nitrate on the gills of air-breathing catfish *Hetropneustes fossilis* (Bloch). Ver Arhiv 2002; 72(3):167-183.
12. Soto M, Marigomez I, Cancio I. Biological aspects of metal accumulation and storage. Zoology and Animal Cell Biology Dept., University of the Basque Country, 2003.
13. Louma SN, Rainbow P. Why is metal accumulation so variable? Biodynamics as the unifying concept. Envl Sci. Tech 2005; 397:1921-1932.
14. Grosell MH, McGeer JC, Wood CM. Renal copper and sodium excretion in hepatic copper metabolism in both copper acclimated and non-acclimated rainbow trout Oncorhyncus mykiss Aqua. Toxicol 1998; 40:275-291.
15. Grosell MH, Hogstrand C, CM Wood. Copper toxicokinetics and hepatobiliary excretion in Cu acclimated freshwater rainbow trout *Oncorhyncus mykiss*. Am J Physiol 2001; 280:797-806.
16. Hamilton SJ, Mehrle PM. Metallothionein in fish: Review of its importance in assessing stress from metal contaminants. Trans. Am. Fish. Soc 1986; 115:569-609.
17. Quig D. Cystein metabolism and metal toxicity.Alt Med Rev. 1998; 3(4):262-270.
18. Cinier CC, Rommel MP, Faure RS, Garen D. Cadmium bioaccumulation in carp *Cyprinus carpio* tissue during long term high exposure analysis by inductively coupled plasma mass spectrophotometry. Ecotox Envi Safe 1997; 38(2):137-143.