HISTOLOGICAL CHANGES INDUCED BY LEAD NITRATES IN THE GILLS OF GRASS CARP, *Ctenopharyngodon idelia* (Val.) JUVENILES

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(Received 31 April 2009, Accepted 6 May 2009)
Key words :Gill, Toxic effect, epithelial cells.

ABSTRACT

The present study showed that the lead had toxic effects on the gill structures of the grass carp (*Ctenopharyngodon idella)* juveniles such as clubbing molting of epithelial cells, epithelial separation, necrosis, fusion of adjacent lamellar epithelium and hyperplasia of secondary lamellar epithelium, destruction of epithelial cells, curved of epithelial, loose in regular shape of epithelium, bite and bleeding tissues through exposure for 48 hrs. All these histological changes depended on lead concentration and exposure period.

INTRODUCTION

Heavy metals is an imprecise term that is generally taken to include the metallic elements with an atomic weight greater than 40, but excluding the alkaline earth metals, alkali metals, lanthanides and actinides. The most important heavy metals from the point of view of water pollution are zinc, copper, lead, cadmium, mercury, nickel, and chromium. In general, the heavy metals may be listed in approximate order of decreasing toxicity as follows: Hg, Cd, Cu, Zn, Ni, Pb, Cr, Al and Co [6], while [12] pointed that cadmium, mercury and lead are toxic to most organisms even in the lowest detectable concentrations.

The environment pollution due to extensive usage of metal without proper managements far reaching effects on survival potential of aquatic animals, some of these toxic chemicals may be persist in the environment for long periods of often unchanged [18]. Heavy metals are serious pollutants in the water environment and are accumulated by aquatic organisms [2,20,25]. Certain metals (such as lead) and its compounds accumulate in the wild fish, particularly in gills and liver tissues [15].

Because lead occurs in nature and it can arise from a variety of sources, it is important to know more about the toxicity of this metal and chronic effect of its compounds in water**.** [28]stated that pollution by lead metal has been recognized for a long time; it does not affect merely aquatic life, as contamination to have occurred everywhere on Earth by industrial and many natural activities.

Fish are relatively sensitive to changes in the surrounding environment including the increase in pollution as showed by previous studies. Early toxic effects of pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance.

Cadmium and lead have a highly toxic for aquatic organisms and are known renal toxicants [29]. Many studies have been carried out on lead toxicity to different aquatic organisms especially on fishes [30,1,4,33,19,9,16,17].

The gills have been considered the important organs in respiration and transport system in the fishes, it is most permeable regions in the body [23], this system gives the animals some ability to survive in different environments by some degrees of regulation of the osmotic concentration of their body tissues [5,11].

Histological tests have been used by many authors to determined the effect levels of water pollutants on the organ structure of fishes [21,26]. histological analysis appears to be a very sensitive

parameter and is crucial in determining cellular changes that may be occur in target organs such as kidney, gills and liver [29].

The present study was aimed to investigate the effects of lead ions on gill structures of grass carp *Ctenopharyngodon idella)* juveniles.

MATERIALS AND METHODS

Specimens of grass carp *(Ctenopharyngodon idella)* juveniles were collected from "Marine Science Centre" (MSC), Garmat Ali during August to October, 2008 (weighted 15 ±1 g.). The specimens were acclimated about 96 hrs. before started, glass aquaria (30X30X60 cm.) have been used for acclimation, specimens were starved for 24 hrs. before treatment.

Health and active individuals were paced in plastic containers (25X20X20 cm.). Ten fishes were placed in each with three replicates of for three concentrations of lead in addition to controls.

An aqueous stock solution of 1,000 parts per thousand (ppt) of metal ion, aqueous lead nitrates [(2H2O . Pb (NO3) 2] were prepared by dissolving 3 gms from aqueous lead nitrates in one liter of distilled water (aqueous solution were prepared each 48 hrs.). Three concentrations (0.5, 1, 3 ppm) were prepared by river water (The range of lead concentrations in the natural waters were 0.1-0.39 as dissolved phase "Wd" [8]. The concentrations were renewed each day for two weeks [14].

Fish groups were exposed for 48 hrs (LT_{50} was determined graphically) at three test concentrations. Gill tissues from controls and experimental fishes were fixed in Bouin`s solution for 24 hrs., then they washed for 2 hrs. in running tap water, then dehydrated in a graded series of alcohol. They were cleared by xylene and embedded in paraffin wax. Sections of 7µ were done using a rotary microtome and they were stained with harris hematoxyline and eosin (h & e) as recommended by [13].

RESULTS

Structure of fish gills:

The gill is composed histologically of two layers, Multilayered filament for epithelium which is responsible the ion exchange, and bi-layered lamellar for epithelium which responsible in the gas exchange (Fig. 1). The position of chloride cells are on the filament epithelium [26].

Histological changes in fish gill:

The exposure fishes to 0.5 ppm lead ions was histological changes represented by:

- Clubbing and fusion in secondary lamellae.
- Hyperplasia (Fig. 2).

- Epithelial separation.
- Destruction of epithelial cells (Fig. 3).

- Fusion of adjacent secondary lamellae.
- Separation of epithelial layer (Fig. 4).

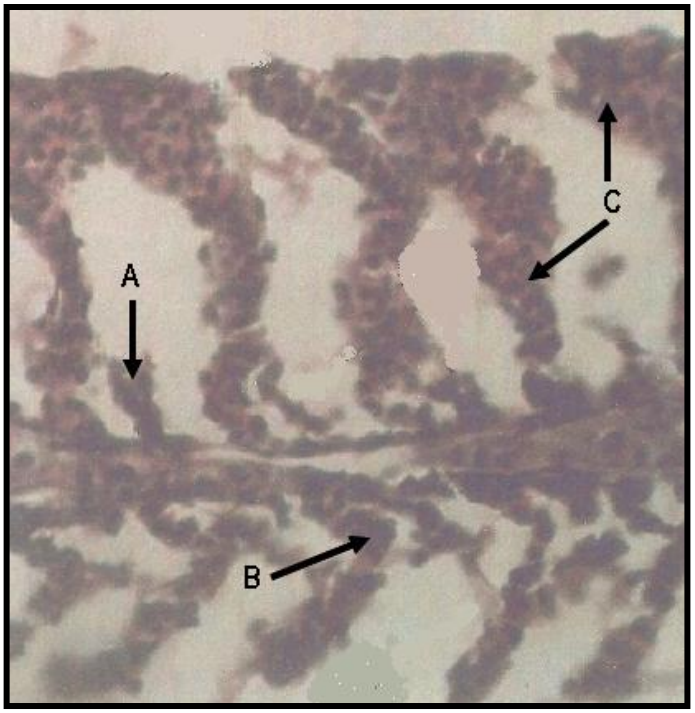

Fig.(1) Longitudinal section in controls gill tissues of *Ctenopharyngodon idella* juveniles showed (A) Secondary lamellae (B) Pillar cells (C) Red blood cells (H&E, 400 X)

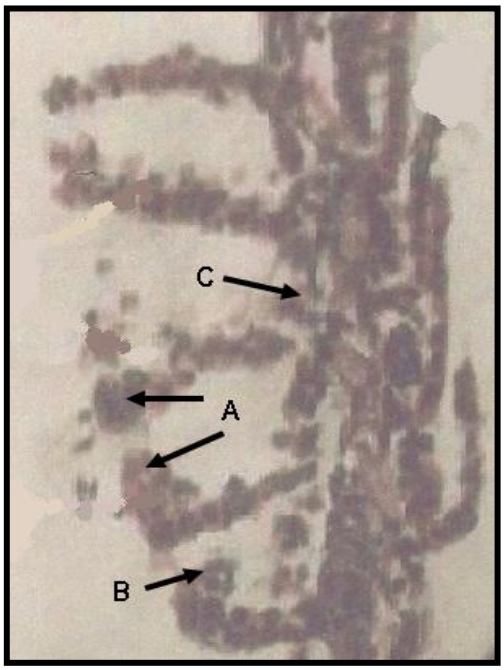

Fig.(2) Longitudinal section in gill tissues of *Ctenopharyngodon idella* juveniles after exposure to 0.5 ppm of lead showed (A) Clubbing in secondary lamellae (B) Fusion of secondary lamellae (C) Hyperplasia (H&E, 400X)

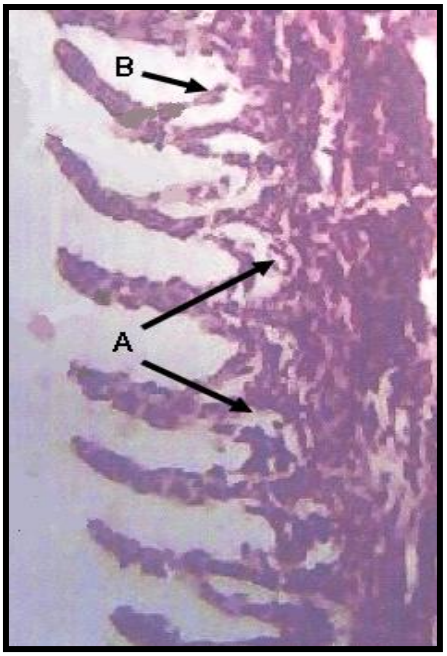

Fig.(3) Longitudinal section in gill tissues of *Ctenopharyngodon idella* juveniles after exposure to 0.5 ppm of lead showed (A) Epithelial separation (B) Destruction of epithelial cells (H&E, 400X)

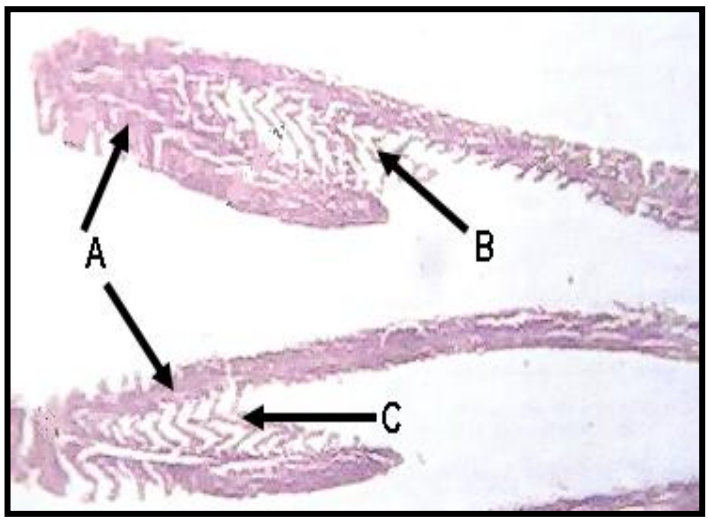

Fig.(4) Longitudinal section in gill tissues of *Ctenopharyngodon idella* juveniles after exposure to 0.5 ppm of lead showed (A) Blood vessels (B) Fusion of adjacent secondary lamellae (C) eparation of epithelial layer (H&E, 400X)

The fish exposure to 1 ppm lead ions, histological changes were showed:

- Curved of epithelial.
- Fusion of secondary lamellae (Fig. 5).
- Clubbing shape in epithelial.
- Loose in regular shape of epithelium (Fig. 6).
- Fusion of secondary lamellae.
- Epithelial separation.
- Bite tissues
- Hyperplasia (Fig. 7).

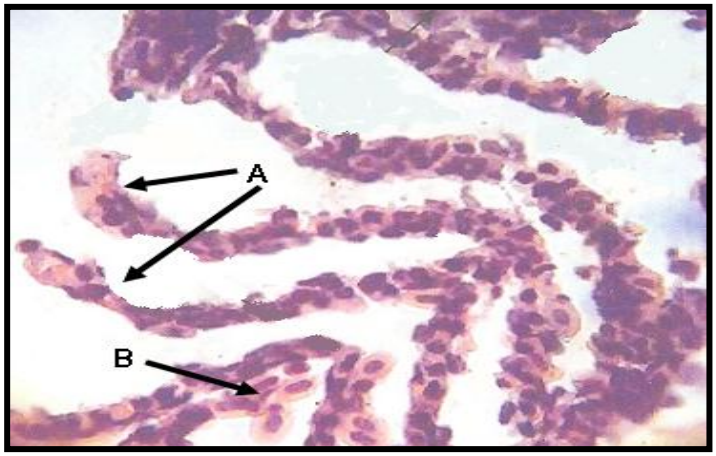

Fig.(5) Longitudinal section in gill tissues of *Ctenopharyngodon idella* juveniles after exposure to 1 ppm of lead showed (A) Curved of epithelial (B) Fusion of secondary lamellae(H&E, 400X)

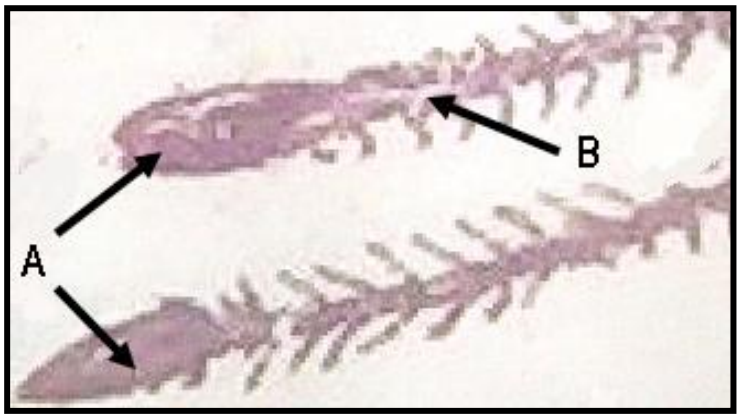

Fig.(6) Longitudinal section in gill tissues of *Ctenopharyngodonidella* juveniles after exposure to 1 ppm of lead showed (A) Clubbing shape in epithelial (B) Loose in regular shape of epithelium (stained by h & e, 400X)

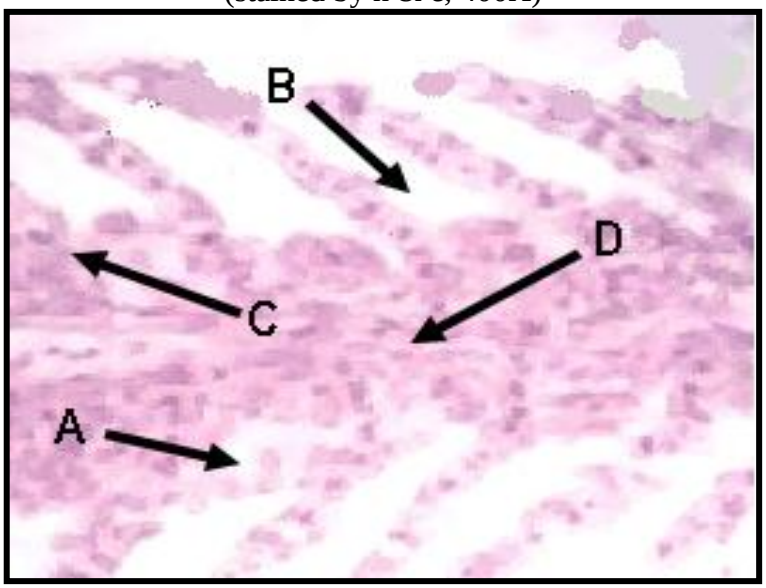

Fig.(7) Longitudinal section in gill tissues of *Ctenopharyngodon idella* juveniles after exposure to 1 ppm of lead showed (A) Fusion in secondary lamellae (B) Epithelial separation (C) Bite tissues (D) Hyperplasia (H&E, 400X)

The fish exposure to 3 ppm lead ions, histological changes were showed:

- Bleeding of tissues.

- Bite tissues.
- Hyperplasia (Fig.8).
- Hyperplasia.
- Separation of epithelial layer.
- Destruction of epithelial cells (Fig.9).
- Separation of epithelial layer.
- Hyperplasia.
- Destruction of epithelial cells (Fig.10).

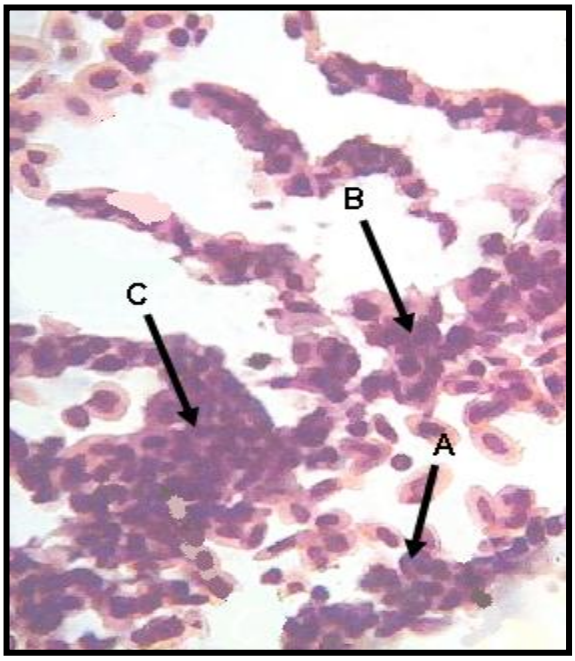

Fig.(8) Longitudinal section in gill tissues of *Ctenopharyngodon idella* juveniles after exposure to 3 ppm of lead showed (A) Bleeding of tissues (B) Bite tissues (C) Hyperplasia (stained by h & e, 400X)

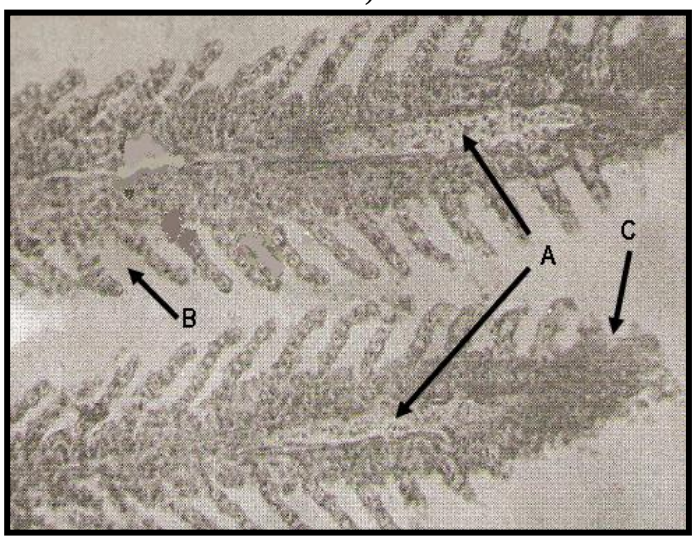

Fig.(9) Longitudinal section in gill tissues of *Ctenopharyngodon idella* juveniles after exposure to 3 ppm of lead showed (A) Hyperplasia (B) Separation of epithelial layer (C) Destruction of epithelial cells (H&E, (400X)

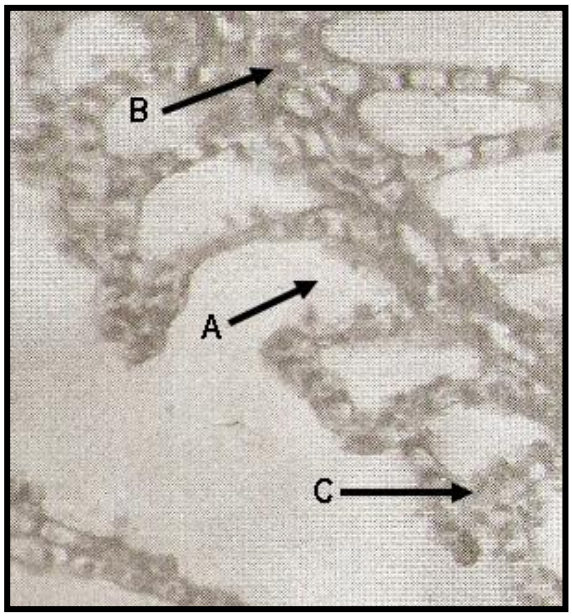

Fig.(10) Longitudinal section in gill tissues of *Ctenopharyngodon idella* juveniles after exposure to 3 ppm of lead showed (A) Separation of epithelial layer (B) Hyperplasia (C) Destruction of epithelial cells (H&E, 400X)

Table (1) illustrated the effects of different concentrations (0.5, 1, 3 ppm) from lead nitrates on gill tissues, it was very clear that the effects increased due to the increasing of lead concentration.

Table (1) showed the effect of different concentrations (0.5, 1, 3 mg/l) of lead nitrates on gill tissues of grass carp, *Ctenopharyngodon idell* juveniles

Concentration mg/l	Clubbing shape	Epithelial separation	Fusion of secondary lamellae	Hyperplasia	Congestion of blood vessels	Sloughing of epithelium	Curved of epithelium	Destruction of epithelial cells	Bite and bleeding tissues	Loose in regular shape of epithelial
0.5	*	**	**	**	**	-	-	*	-	-
1	**	***	***	***	*	*	-	*	*	*
3	-	***	**	**	*	***	*	***	***	*

Control: Normal shape
*** Less effect ** Medium effect *** Acute effect **** Very acute effect**

DISCUSSION

Most of animal tissues were showed histological changes when they are expose to any polluted materials, many studies were pointed that the lead induced histological changes in the gill tissues of fishes [28,4,24,16,7].

After observations during experimental periods in present study, no fish either died or affected in the control containers, mean while, different gills and lamellae showed different degrees of degenerative change depend on metal concentrations and exposure periods such as clubbing and fusion in secondary lamellae, hyperplasia, destruction and separation of epithelial layer, bleeding and bite tissues, each gill lesion tends of vary widely intensity, these results in agree with many studies [28,21,4,24,16,7]. Simple changes were observed in low concentration, however, same observations were recorded for many authors [32,4,24,3,7].

Lead caused pathological alteration in the gill filaments, respiratory lamellae and necrosis of hepatic cell of *Cyprinus carpio* [22], the effects of lead on the tissue structures for some studies were disagreed with present study especially in the low concentrations [27,34,31].

Bioaccumulation is an aspect of sub-lethal toxicity which has received much attention. Pollutants may, over long time periods, accumulate in tissues to levels which may be harmful to the organism. Since many aquatic species are utilized for human consumption, the public health significance of toxic substances accumulated in their tissues is obvious [6].

[15] reported morphological changes in some tissues of trout fishes such as swollen and detection in epithelial layer extensive in intracellular and degenerated epithelial layer after exposure to cadmium and lead, while [4]was found epithelial necrosis and rupture of the gill epithelium are directed responses induced by the action of zinc ions. [10]showed that copper and lead ions were caused hyperplasia and fusion between lamellae of *Carassius carassius* gills.

التغيرات النسيجية في غلاصم صغار الكارب العشبي (*Ctenopharyngodon idella* (Val.) المعرضة إلى نترات الرصاص

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الخلاصة

أوضحت الدراسة الحالية بأن لعنصر الرصاص تأثيرات مرضية كبيرة على التركيب النسيجي لغلاصم صغار اسماك الكارب *العشبي* ***(Ctenopharyngodon idella* (Val.)**، وقد تسبب انسلاخ الطبقة الطلائية واندماج بين الصفائح الثانوية مع تكسر وتنخر في معظم الخلايا وإفراط نسيجي، توسع ونزف في **الأوعية** الدموية الشعرية وكذلك ظهور أشكال بصلية. كما لوحظ بأن ظهور هذه التغيرات النسيجية اعتمدت على تركيز الرصاص وفترة التعرض.

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