

## Amelioration the Toxic Effects of Cadmium-Exposure in Nile Tilapia (*Oreochromis Niloticus*) by using *Lemna gibba L*

Hussein A. Kaoud<sup>1\*</sup>, Manal M. Zaki<sup>1</sup>, Ahmed R. El-Dahshan<sup>1</sup>, Sherein Saeid<sup>2</sup> and Hesham Y. El Zorba<sup>3</sup>

<sup>1</sup>Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

<sup>2</sup>Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

<sup>3</sup>Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

[ka-oud@link.net](mailto:ka-oud@link.net)

**Abstract:** The effect of cadmium (Cd) toxicity and its impact on histopathology, haematological, and biochemical changes in Nile tilapia (*Oreochromis niloticus*) were studied. Fish (35-45 grams) were exposed to Cd that resulted in significant reduction ( $p < 0.05$ ) of the erythrocyte count (RBCs), haemoglobin content (Hb) and haematocrit value (Hct). Significant increases in plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed in fish exposed to Cd. The obtained results indicate that *Lemna gibba L* weed and their extract are effective in removing Cd from water and reducing Cd bioaccumulation in fish. The addition of *Lemna gibba L*-extract reduced significantly ( $p < 0.05$ ) the Cd level in water and the metal uptake as compared to fish exposed to Cd alone. The Cd concentration in water was  $9.620 \pm 0.198$  mg L<sup>-1</sup> and it decreased significantly ( $p < 0.05$ ). The Cd accumulation in liver and muscles of fish exposed to Cd alone was higher than that of *Lemna gibba L*-extract treatment. The addition of *Lemna gibba L*-extract improves the haematological parameters (RBCs, Hb and Hct) and ameliorates the toxic effect of Cd which indicating the capability of *Lemna gibba L*-extract to chelate Cd from the media. Subsequently, the Cd toxicity was reduced. This study indicates that Cd poisoning cause structural damage in the fish organs. It is also demonstrated that *Lemna gibba L*-extract, weed or the weed plus the extract provided protection against the degenerative action of Cd and increased the chance of tissue regeneration.

**Keywords:** Cadmium; histopathology; *Lemna gibba L*; amelioration; *Oreochromis niloticus*

### 1. Introduction:

Cadmium (Cd) is a well known heavy metal toxicant with a specific gravity 8.65 times greater than water (Lide, 1992). Cadmium is an extremely toxic metal. It is widely used in mining, metallurgical operations, electroplating industries manufacturing vinyl plastics which used in metallic and plastic pipes. Effluents from such activities are sources of cadmium into aquatic environments. Most aquatic organisms have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. Metals interact with legends in proteins particularly, enzymes and may inhibit their biochemical and physiological activities (Passow *et al.*, 1961).

The levels of contamination by cadmium in fish are of considerable interest because fish consumption is an important source of intake cadmium for the general population. Most of the cadmium content in fish or other seafood is highly absorbable in humans; the efficiency of gastrointestinal absorption of cadmium has been reported to be approximately 3–8% of the ingested

load. Cadmium is particularly accumulated in kidney and to lower extent in muscles (ATSDR, 2003).

Bio-enhancement of Cd transfer along a food chain was studied by Seebaugh & Wallace (2005) and fish are reported to be used as biological indicators to assess water pollution (Rashed, 2001). In aquatic systems, as fish occupy the upper trophic level, there are greater chances of transferring Cd to higher organisms particularly to human. The European Commission (2001) established the maximum levels permitted of cadmium in seafood as follows 0.05 mg/kg in fish, 0.5 mg/kg in crustaceans (except crab), 1.0 mg/kg in molluscs and crab. Moreover, the Joint FAO/ World Health Organization have recommended the provisional tolerable weekly intake (PTWI) as 0.007 mg/kg for cadmium (0.420 mg/g/week for a 60-kg person) (FAO/WHO, 2005).

Metal bioaccumulation can occur via complexation, coordination, chelating, ion exchange and other processes of greater or lesser specificity. It is a strictly aggressive process in which metal ions are sequestered by metal binding site in the interior of the cell. The removal of toxic elements from contaminated water has potential advantages over the

conventional treatment process. The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method. The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation (Hiemesh & Mahadevaswamy, 1994).

The use of aquatic plants in water ecosystems and terrestrial plants in hydroponic systems has high potential to clean up the metal contaminated water through phytoextraction and phytostabilization. Phytostabilization utilizes the plant production of compounds, which immobilize contaminants at the entrance of roots. An example of this method is where root exudates cause the precipitation of metals and reducing their bioavailability. Phytodegradation (also known as phytotransformation) is the enzyme-catalysed metabolism of contaminants, typically organics, within plant tissues. The enzymes are usually dehalogenases, oxygenases and reductases (Black, 1995).

Biosorption potential of *Prosopis juliflora* seed powder (PJSP) for Pb (II) from aqueous solution has been investigated by Jayaram & Prasad (2009) where they found that the maximum Pb (II) adsorbed was found to be 40.322 mg/g and the adsorption process was spontaneous and exothermic in nature. Removal of certain heavy metals from waste water by *Lemna gibba L.* has been reported by Kwan & Smith (1991); Buckley (1994); Miranda & Ilangovan (1996) and Wafaa (2007).

In the present study, short and long-term bioassays were designed to evaluate the influence of *Lemna gibba L.* plant and/or its extract on the reduction of cadmium in water as well as to investigate the amelioration effect of *Lemna gibba L.* on some blood parameters, enzymes and histopathological alterations induced by Cd exposure on Nile tilapia (*Oreochromis niloticus*).

## 2. Materials and Methods:

### Fish culture management

Healthy *Oreochromis niloticus* of 35-45 grams were collected during the late August and early September, 2010 from the ponds of the Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, and Sharkia, Egypt (belonging to a single population). They were collected locally and confined to large plastic aquaria bearing tap water for up to 7 days in the laboratory for acclimation.

### Cadmium chloride

Technical grade cadmium chloride (99% purity) was obtained from El-Nasr Chemical

Company (Cairo, Egypt) and prepared in aquatic solution to provide the required concentrations of cadmium. Control test without cadmium was performed.

### Determination of LC<sub>50</sub>

#### Acute Toxicity Assays

Laboratory bioassays were conducted to determine the 24 hrs, 48 hrs, 72 hrs and 96 hrs LC50 values for tilapia exposed to CdCl<sub>2</sub>. The experimental design and calculations for the acute toxicity were based on well-known procedures given by Finney (1978) and Sparks (2000). The tests were carried out in 50 Litres rectangular fibreglass aquaria filled with well-aerated tap water (pH 6.5–7.0). Dissolved oxygen in each tank was maintained at close to saturation by aeration. The temperature in each aquarium was maintained at 25.5–27°C using submerged heaters. The photoperiod was 12 hrs light length/day. The fish were visibly free of any deformities, lesions, or disease and were acclimated in tap water for 1 week prior to the experiment. The nominal concentrations of CdCl<sub>2</sub> tested were 0 (control), 1, 2, 5, 10, 20, 40, and 80 mg/L (Chung, 1983). Gross fish mortality of each concentration was recorded every 1 h for the first 12 hrs and every 2 hrs thereafter for 96 hrs while the dead fish were removed every 3–8 hrs.

Tilapias were not fed throughout the test. The control and each test concentration were tested in duplicate.

LC<sub>50</sub> value of cadmium chloride for *T. nilotica* was determined by the simple graphic method, Probit graphic method and the un-weighted regression method (Finney, 1971).

### The tested weed

The duckweed species used was *Lemna gibba L.* which was taken from Ganabiet-Tersa drain, Giza, Egypt. The duckweed was acclimatized to the laboratory conditions for one week before starting the experiments.

### Plant extracts

Dried plant materials were extracted twice with 50% and 100% methanol as well as 50% and 100% acetone in v/v proportions (200 ml/5g plant) for 2 hrs with constant stirring. The collected filtered extracts were dried in a rotary evaporator (Büchi: Rotavapor-R114 and water bath B-481) at 40°C under reduced pressure (Ghobrial *et al.*, 2009).

### Cadmium reduction

Tilapias were distributed randomly in 120 Litres rectangular fibreglass aquaria filled with well-aerated tap water (pH 6.5–7.0) at a rate of 15 fish /

aquarium. Dissolved oxygen in each tank was maintained at close to saturation by aeration. The temperature in each aquarium was maintained at  $27\pm 1^\circ\text{C}$  by means of thermostats. The photoperiod was 12 hrs light-length/days. These aquaria were divided into five groups with three replicates each per group. The first group was free from Cd and *Lemna gibba* L and maintained as a control. The second groups were exposed to 10 mg of  $\text{CdCl}_2$  only (Equivalent to  $1/4$  96 h  $\text{LC}_{50}$ ). The third, fourth and fifth group were exposed to 10 mg  $\text{CdCl}_2$   $\text{L}^{-1}$  and 0.1, 1 and 0.1 plus 1 g  $\text{L}^{-1}$  extract, plant and extract plus plant of *Lemna gibba* L, respectively (Table I).

Fish were fed frequently on a diet containing 30% crude protein at a rate of 3% of live body weight twice daily for 7 and 25 days. Siphoning three quarters aquariums was done every day for waste removal and replacing it by an equal volume of water containing the same concentration of Cd and *Lemna gibba* L. Dead fish were removed and recorded daily.

Hematological, enzymatic and histopathological investigations

After 7 and 25 days of the experiment, blood samples were taken from three Fish from each aquarium. Fish were not fed for 24 hrs before sampling and were anaesthetized with buffered MS222 (50 mg  $\text{L}^{-1}$ ) and blood samples were taken from the caudal vein of an anaesthetized fish by sterile syringe containing EDTA solution as anticoagulant. These blood samples were used for determining erythrocyte count (Dacie & Lewis, 1984) and hemoglobin content (Van Kampen & Zijlstra, 1961). Haematocrit value (Hct) was calculated according to the formulae mentioned by Britton (1963).

Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and non haemolysed plasma was stored in a deep freezer for further biochemical analyses. After decapitation of fish, samples of gills, liver, spleen, kidney, stomach, intestine and brain were taken and frozen for histopathological investigations. Plasma activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to Reitman & Frankel (1957)

Cd residue

Cadmium residues were measured in water, liver and muscles according to method of Eaton & Stinson (1983). The water samples were preserved by the addition of one mL of concentrated nitric acid per litre until the time of analysis. The water samples were filtered through 0.45 $\mu\text{l}$  membrane filter. The required volume (100 mL) of the filtrate was

collected to measure cadmium levels in water samples by using Air/Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer).

The analysis of tissue sample was represented by one gram of tissues dissected from the liver and muscles, then placed in a clean screw-capped tube and digested according to the method described by Finerty *et al.* (1990). The obtained solutions were then analyzed by using Air/ Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer).

Statistical analysis

The obtained data were subjected to analysis of variance according to Snedecor & Cochran (1982). Differences between means were done at the 5% probability level, using Duncan's new multiple range test (Duncan, 1955).

### 3. Results:

The  $\text{LC}_{50}$  values from the three methods (the simple graphic method, Probit graphic method and the un-weighted regression method) are 40.47 mg/L, 40.99 mg/L and 40.13 mg/L, respectively. Hence the calculated average  $\text{LC}_{50}$  is 40.533 mg/L. The equation for the dose mortality regression line was found to be  $Y = 2.65X + 3.368$ .

Hematological parameters

Table II shows that the RBCs, HB and HCT were reduced in fish exposed to Cd at both periods and they were lower than that of the control ( $p < 0.05$ ). The RBCs count also decreased significantly in fish exposed to Cd at 7 and 25 days. On the other hand, these parameters were return to the normal values and increased significantly in fish exposed to Cd with *Lemna gibba* L- weed and/or its extract for 7 and 25 days. Blood parameters were improved in fish exposed to Cd with different levels of *Lemna gibba* L.

Biochemical parameters

Table III showed that AST activity increased significantly in plasma of fish exposed to Cd alone. The addition of *Lemna gibba* L-extract decreased significantly the AST activity to be lower than that of Cd alone ( $p < 0.05$ ). The AST activity in fish exposed to Cd with *Lemna gibba* L plus their extract became nearly similar to that of control at 7 days and 25 days. The plasma ALT activity increased significantly in fish exposed to Cd alone at 7 and 25 days. The addition of *Lemna gibba* L enhanced ALT activity to be nearly as in the control especially the groups exposed to Cd with *Lemna gibba* L- weed plus their extract at both periods.

### Histopathological alterations

The histopathology of different Tilapia tissues revealed that there are several histopathological changes in different Tilapia organs as shown in our figures.

**Gills** (Figure 1(A)) showed necrosis and atrophy of the gill lamellae. Severe edema, hyperplasia, fusion and focal desquamation of the epithelial lining of the secondary lamellae also were detected. The gill arch showed numerous mononuclear leucocytic infiltration, edema, congestion and the apex of gill filaments showed congestion, hyper activation of the mucous and chloride cells.

**Liver** of tilapia treated with cadmium showed degeneration of the hepatocytes with nuclear pyknosis in the majority of the cells as well as the accumulation of the metal binding proteins in their nuclei. Intravascular hemolysis is seen in blood vessels and sinusoids with necrosed hepatocytes (Figures 1 (B, C, D, E)).

**Spleen** The microscopic examination of Tilapia's spleen revealed lymphoid depletion (LD) associated with congestion and necrosis of splenic sinusoids and activation of Melanomacrophage center (MMC). Large sub-capsular areas of necrosis were also observed (Figure 1(F), 2(A)). In the control fish, liver contains few melano macrophage centers (MMC) and free macrophages.

**Stomach** The sub-mucosal tissues were fully vacuolated with degeneration of the serosal layer, degeneration of columnar epithelium, goblet cell and basement membrane as well as the secretory cells were damaged and fully distorted (Figure 2(B)).

**Intestine** Intestine of tilapia treated with cadmium showing necrosed mucosa, sub-mucosal hemorrhage, muscle fibers were loosely arranged with the degeneration of sub-mucosal tissue and each villus facing the lumen showed cell degeneration and the cells did not show distinct nuclei and cytoplasmic boundaries. There was a distortion of basement membrane of the villi and blood vessel, and lymphocytes were fully distorted and there was a degeneration of columnar epithelium of the intestine (Figure 2 (C, D, E, F)).

**Muscular tissues** degeneration in muscle bundles with aggregations of inflammatory cells (leucocytic infiltration) between them with focal areas of necrosis (Figure 2 (F)). Atrophy and edema of muscle bundles as well as splitting of muscle fibers and hyalinized muscles tissue were seen (Figure 3 (A, B)).

**Brain** edema was the main characteristic histopathological change in the brain tissues. The lesion also characterized by vacuolation of brain tissue in addition to encephalitis that characterized

microscopically by congestion of cerebral blood capillaries associated with neuronal degeneration, severe necrosis and demyelination of brain tissue with extravasations of free RBCs as well as aggregation of EGCs in brain tissue (Figure 3 (C, D, E, F)).

**Kidney** showed hydropic swelling of the renal tubules, sometimes with pyknotic nuclei and many necrotic areas as well as swollen proximal epithelial cells with necrotic nuclei (Figure 4 (A)).

**Abnormal blood cells** Tilapia treated with cadmium showing abnormal blood cells (Aminocytosis) (Figure 4 (B, C)). Gills of tilapia treated with cadmium showing atrophy and necrosis of gill lamellae (arrows) (H & E X 400).

### Cd Bioaccumulation

The highest bioaccumulation of cadmium was observed in the organs mainly implicated in metal intoxication and so it was higher in the liver followed by muscles.

Addition of *Lemna gibba L*-extract to the Cd polluted media reduced significantly ( $p < 0.05$ ) the Cd level in aquarium's water as compared to that of Cd alone, Table I. The Cd concentration in water exposed Cd alone was  $9.620 \pm 0.198 \text{ mg L}^{-1}$  and declined significantly ( $p < 0.05$ ) to  $7.8 \pm 0.276$ ,  $4.58 \pm 0.208$  and  $0.89 \pm 0.105 \text{ mg L}^{-1}$  with 0.1, 1 and 0.1 plus 1 g  $\text{L}^{-1}$  extract, weed and extract plus weed of *Lemna gibba L*, respectively. The highest amount of Cd residue was found in the liver after 7 days of exposure. Table I also, showed that the uptake of Cd in the liver of fish exposed to Cd alone was  $2.350 \pm 0.18$  and  $5.88 \pm 0.540 \text{ mg g}^{-1}$  dry weight for 7 and 25 days, respectively. It declined significantly to  $1.392 \pm 0.095$ ,  $4.500 \pm 0.320$  and  $0.990 \pm 0.015$ ,  $3.792 \pm 0.320$  and  $0.510 \pm 0.021$ ,  $1.850 \pm 0.150 \text{ mg g}^{-1}$  dry weight in fish group exposed to Cd with 0.1, 1 and 0.1 plus 1 g  $\text{L}^{-1}$  extract, weed and extract plus weed of *Lemna gibba L* at 7 and 25 days, respectively. Similar trends were observed in fish muscles.

### 4. Discussion:

The present study reveals that the fish exposed to Cd alone showed significant reduction in RBCs, Hb and HCT than those exposed to Cd with different level of *Lemna gibba L*-extract and plant. The reduction of these parameters in *O niloticus* at sub-lethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of

**Table I:** Changes in cadmium residue in water (mg Cd L<sup>-1</sup>), liver and muscles (mg Cd g<sup>-1</sup> dry weigh) of Nile tilapia (*O. niloticus*) exposed to Cd with and without *Lemna gibba L* plant

Group Days	Water		Liver		Muscles	
	7	25	7	25	7	25
Control (metal free water)	0.032±0.004 <sup>a</sup>	0.038±0.003 <sup>a</sup>	0.040±0.006 <sup>a</sup>	0.021±0.004 <sup>a</sup>	0.018±0.001 <sup>a</sup>	
Cadmium alone (10mg) L <sup>-1</sup>	9.620±0.198 <sup>b</sup>	2.350±0.18 <sup>b</sup>	5.88±0.540 <sup>b</sup>	0.485±0.11 <sup>b</sup>	1.097±0.058 <sup>b</sup>	
Cadmium (10mg)+0.1g ext. L <sup>-1</sup>	7.8 ±0.276 <sup>b</sup>	1.392±0.095 <sup>b</sup>	4.500±0.320 <sup>b</sup>	0.343±0.086 <sup>a</sup>	0.868±0.088 <sup>b</sup>	
Cadmium (10mg)+1g P L <sup>-1</sup>	4.58 ±0.208 <sup>c</sup>	0.990±0.015 <sup>c</sup>	3.792 ±0.320 <sup>c</sup>	0.380±0.098 <sup>b</sup>	0.383±0.077 <sup>b</sup>	
Cadmium (10mg)+ 0.1g ext. L <sup>-1</sup> +1g P L <sup>-1</sup>	0.89 ±0.105 <sup>d</sup>	0.510 ±0.021 <sup>d</sup>	1.850 ±0.150 <sup>d</sup>	0.216±0.086 <sup>a</sup>	0.217±0.076 <sup>a</sup>	

The same letter in the same column is not significantly different at P<0.05.

The first group was free from Cd and *Lemna gibba L* and maintained as a control.

The second groups were exposed to 10 mg of CdCl<sub>2</sub> only (Equivalent to 1/4 96 h LC<sub>50</sub>).

The third was exposed to 10 mg Cd L<sup>-1</sup> and 0.1 extract.

The fourth group was exposed to 10 mg Cd L<sup>-1</sup> and 1 g L<sup>-1</sup> *Lemna gibba L* plant.

The fifth group was exposed to 10 mg Cd L<sup>-1</sup>+ 0.1g extract L<sup>-1</sup> +1 g L<sup>-1</sup> *Lemna gibba L* plant.

**Table II:** Changes in erythrocyte (count x10<sup>6</sup>/mm<sup>3</sup>), hemoglobin content (g 100 mL<sup>-1</sup>) and haematocrit value (%) in the blood of Nile tilapia (*O. niloticus*) exposed to Cd with and without *Lemna gibba L* plant.

Group	Erythrocyte count (RBCs)		Hemoglobin(HB)		Haematocrit value (Hct)	
	7	25	7	25	7	25
Control (metal free water)	1.560 ±0.040 <sup>a</sup>	1.622±0.080 <sup>a</sup>	5.590±0.354 <sup>a</sup>	6.422±0.454 <sup>a</sup>	14.900±0.334 <sup>a</sup>	16.332±0.454 <sup>a</sup>
Cadmium alone (10mg) L <sup>-1</sup>	1.190±0.070 <sup>b</sup>	1.160±0.050 <sup>b</sup>	4.320±0.354 <sup>b</sup>	4.212±0.345 <sup>b</sup>	13.220 ±1.454 <sup>b</sup>	13.026±0.354 <sup>b</sup>
Cadmium (10mg) +0.1g ext. L <sup>-1</sup>	1.482±0.080 <sup>a</sup>	1.370±0.060 <sup>c</sup>	4.440±0.534 <sup>b</sup>	5.340±0.452 <sup>c</sup>	14.110 ±0.432 <sup>a</sup>	15.065±0.765 <sup>c</sup>
Cadmium (10mg) +1g P L <sup>-1</sup>	1.56 ±0.080 <sup>a</sup>	1.677±0.070 <sup>a</sup>	5.270±0.454 <sup>a</sup>	6.112±0.457 <sup>a</sup>	14.320±0.359 <sup>a</sup>	15.688±0.655 <sup>c</sup>
Cadmium (10mg)+ 0.1g ext. L <sup>-1</sup> + 1g P L <sup>-1</sup>	1.678±0.070 <sup>a</sup>	1.625±0.050 <sup>a</sup>	5.476±0.65 <sup>a</sup>	6.450±0.554 <sup>a</sup>	14.800±0.365 <sup>a</sup>	16.112±0.732 <sup>a</sup>

The same letter in the same column is not significantly different at P<0.05.

The first group was free from Cd and *Lemna gibba L* and maintained as a control.

The second groups were exposed to 10 mg of CdCl<sub>2</sub> only (Equivalent to 1/4 96 h LC<sub>50</sub>).

The third was exposed to 10 mg Cd L<sup>-1</sup> and 0.1 extract.

The fourth group was exposed to 10 mg Cd L<sup>-1</sup> and 1 g L<sup>-1</sup> *Lemna gibba L* plant.

The fifth group was exposed to 10 mg Cd L<sup>-1</sup>+ 0.1g extract L<sup>-1</sup> +1 g L<sup>-1</sup> *Lemna gibba L* plant.

**Table III:** Changes in aspartate aminotransferase activity (AST) and alanine aminotransferase (ALT) activity (IU L<sup>-1</sup>) in plasma of Nile tilapia (*O. niloticus*) exposed to Cd with and without *Lemna gibba L* plant.

Group Days	AST		ALT	
	7	25	7	25
Control (metal free water)	50.390±1.62 <sup>a</sup>	88.690±2.343 <sup>a</sup>	30.966±2.532 <sup>a</sup>	41.65±2.688 <sup>a</sup>
Cadmium alone (10mg) L <sup>-1</sup>	96.480±1.822 <sup>b</sup>	117.770±2.564 <sup>c</sup>	50.966±2.854 <sup>b</sup>	73.80±2.966 <sup>b</sup>
Cadmium (10mg)+ 0.1g ext. L <sup>-1</sup>	95.690±1.666 <sup>b</sup>	107.870±2.644 <sup>b</sup>	48.966±2.674 <sup>b</sup>	71.68±2.845 <sup>b</sup>
Cadmium (10mg)+ 1g P L <sup>-1</sup>	71.793±1.688 <sup>c</sup>	95.790±2.865 <sup>a</sup>	35.966±2.727 <sup>a</sup>	52.67±2.754 <sup>c</sup>
Cadmium (10mg)+ 0.1g ext. L <sup>-1</sup> + 1g P L <sup>-1</sup>	53.570±1.995 <sup>a</sup>	90.780±2.986 <sup>a</sup>	34.966±2.568 <sup>a</sup>	42.77±2.876 <sup>a</sup>

The same letter in the same column is not significantly different at P<0.05.

The first group was free from Cd and *Lemna gibba L* and maintained as a control.

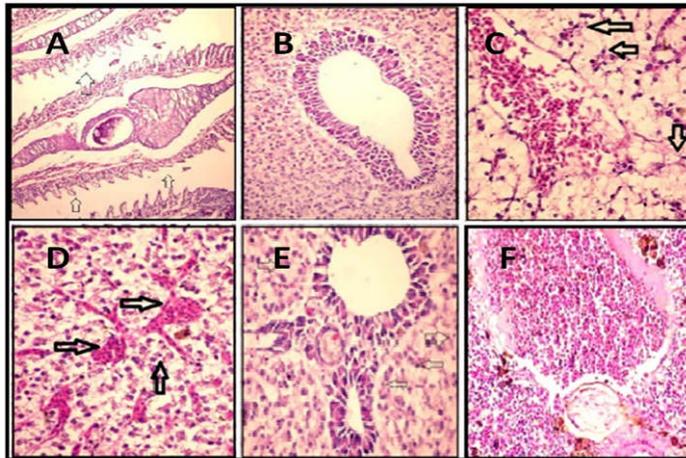
The second groups were exposed to 10 mg of CdCl<sub>2</sub> only (Equivalent to 1/4 96 h LC<sub>50</sub>).

The third was exposed to 10 mg Cd L<sup>-1</sup> and 0.1 extract.

The fourth group was exposed to 10 mg Cd L<sup>-1</sup> and 1 g L<sup>-1</sup> *Lemna gibba L* plant.

The fifth group was exposed to 10 mg Cd L<sup>-1</sup>+ 0.1g extract L<sup>-1</sup> +1 g L<sup>-1</sup> *Lemna gibba L* plant.

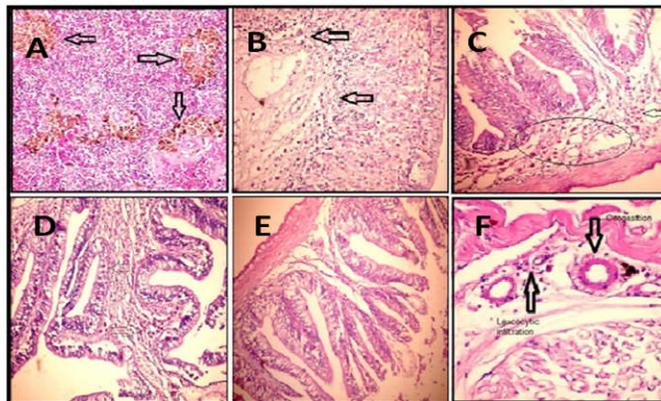
Figure 1



**Figure 1.**

- A. Gills of tilapia treated with cadmium showing atrophy and necrosis of gill lamellae (arrows) (H & E X 400).
- B. Liver of tilapia treated with cadmium and Plant-ext showing apparently healthy liver tissue (H & E X 200).
- C. Liver of tilapia treated with cadmium showing congestion (C) with severely degenerated and necrosed hepatocytes (arrows) (H & E X 200).
- D. Liver of tilapia treated with cadmium showing congested sinusoids with necrosed hepatocytes (arrows) (H & E X 200).
- E. Liver of tilapia treated with cadmium and Plant-ext showing normal portal tract with slightly degenerated hepatocytes (arrows) (H & E X 400).
- F. Spleen of tilapia treated with cadmium and Plant-ext showing apparently normal spleen (H & E X 200).

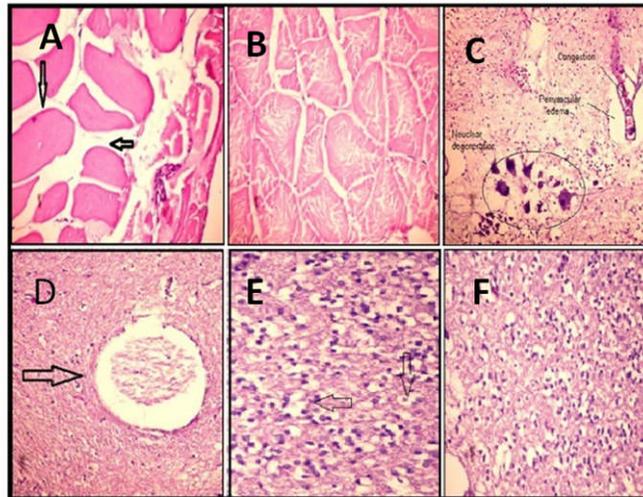
Figure 2



**Figure 2.**

- A. Spleen of tilapia treated with cadmium showing hyperplasia in the melanomacrophage cells (arrows) (H & E X 200).
- B. Stomach of tilapia treated with cadmium showing leucocytic infiltration in the sub mucosa (arrow) together with congestion (C) (H & E X 400).
- C. Intestine of tilapia treated with cadmium showing necrosed mucosa (n), sub mucosal haemorrhage (arrow) (H & E X 200).
- D. Intestine of tilapia treated with cadmium showing higher power of the previous lesion (H & E X 400).
- E. Intestine of tilapia treated with cadmium and Plant-ext showing apparently intestinal tissue (H & E X 200).
- F. Muscles of tilapia treated with showing congestion (C) and leucocytic infiltration (arrow) (H & E X 200).

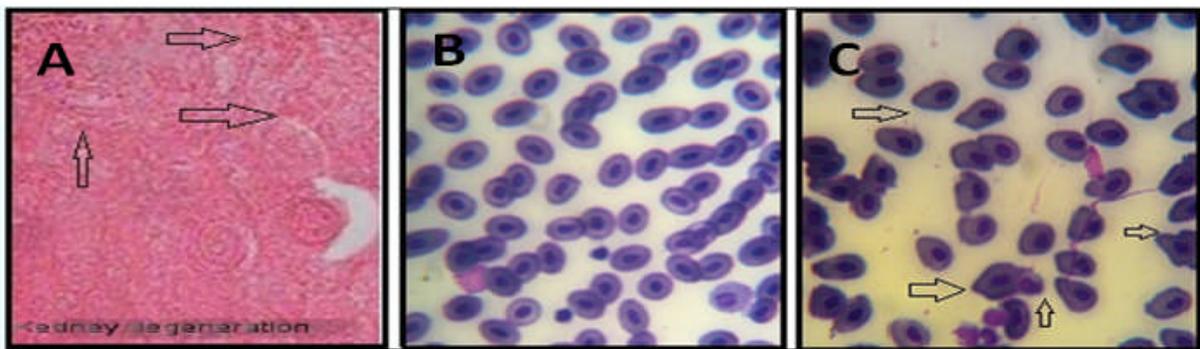
Figure 3



**Figure 3.**

- A. Muscles of tilapia treated with cadmium showing hyalinised muscles tissue (arrow) (H & E X 200).
- B. Muscles of tilapia treated with cadmium and Plant-ext showing apparently healthy muscular tissue (H & E X 200).
- C. Brain of tilapia treated with cadmium showing congestion (C), perivascular edema (e) and neural degeneration (n) (arrow) (H & E X 200).
- D. Brain of tilapia treated with cadmium showing osteomalacia (arrow) (H & E X 200).
- E. Brain of tilapia treated with cadmium showing intracellular brain edema (arrow) (H & E X 200).
- F. Brain of tilapia treated with cadmium and Plant-ext showing apparently healthy tissue (H & E X 200).

Figure 4



**Figure 4.**

- A. Kidneys showed hydropic swelling of tubules with pyknotic nuclei and many necrotic areas as well as swollen proximal epithelial cells with necrotic nuclei.
- B. Tilapia treated with cadmium and Plant-ext showing apparently healthy blood cells (X 1000). Tilapia treated with cadmium showing abnormal blood cells (Amioscytosis) (X 1000).

haem synthesis that is affected by pollutants (Wintrobe, 1978). Also, the decrease in RBCs count may be attributed to haematopathology that results in severe anemia in most vertebrates including fish species

exposed to different environmental pollutants (Khangarot & Tripathi, 1991). The decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in

sever anemia (James and Sampath, 1999). Also Gill & Epple (1993) found a significant reduction in the RBCs, Hb and HCT in American eel (*Anguilla rostrata*) after exposure to 150 ug Cd L<sup>-1</sup>. Karuppasamy *et al.* (2005) found a significant decrease in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, *Channa punctatus* after exposure to sub-lethal dose of Cd (29 mg Cd L<sup>-1</sup>).

The decrease in RBCs, Hb and HCT values may be due to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sub-lethal concentration of pollutants (Moussa, 1999).

The activity of AST and ALT enzymes in blood may also be used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress (James *et al.*, 1991; Svoboda, 2001). In the present study, there were significant changes in AST and ALT activities in plasma of fish exposed to cadmium compared to the control group. The increase in concentration of AST and ALT in blood plasma indicates impairment of parenchymatous organs mainly liver. In addition, the increase of plasma AST and ALT may be attributed to the hepatocellular damage or cellular degradation in liver, spleen or muscles (Yamawaki *et al.*, 1986). These results are in agreement with those reported by Shalaby (1997) who found that sub-lethal concentration of Cd caused significant increases in AST and ALT of Common Carp after 7 and 25 days.

Histopathological biomarkers have been largely used in fish to identify and evaluate the toxic effects of pollutants exposure (Rabitto *et al.*, 2005; Oliveira Ribeiro *et al.*, 2006). The presence of necrosis is in fact one of the most visible damages in tissues affected by a pollutant (Rabitto *et al.*, 2005). According to Manahan (1991) the occurrence of necrosis is also a consequence of enzymatic inhibition, damages in the cellular membrane integrity, and disturbances in the synthesis of proteins and carbohydrate metabolism.

Mallat (1985) reported that the pathological changes of fish gills are induced by elevated heavy metals and low oxygen content in water. The hyperplasia induced by any pollutant may be due to the simple response to cellular necrosis as previously mentioned by Marie *et al.* (1998). Moreover, Shaker *et al.* (2000) reported that the epithelial hyperplasia is known as a protective and defense mechanism of fish gills. In addition to, the congestion in branchial blood vessels may be due to the counter irritation and stimulation of vasodilators (Marie *et al.*, 1998).

Structural damage due to Cd toxicity in fish gills has been reported by Voyer (1975); Karlson-Norrgrén *et al.* (1985) and Kothari & Saxena (1988). Narayan & Singh (1991) observed extensive degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen in liver tissue of *Heteropneustes fossilis* while subjecting them to acute thiodan toxicity. Recent studies have demonstrated the links between exposure to pollutants and the development of hepatic lesions. Stentiford *et al.* (2003) and Stehr *et al.* (2004) observed that toxicopathic liver lesions in fish species are effective biological markers of chemical exposures.

Melano macrophages are pigmented cells that can appear isolated or arranged in clusters forming the MMCs in the spleen. These cells are positive indicator for the presence of neutral carbohydrates (P.A.S. stain) and melanin (Masson-Fontana stain) (Rabitto *et al.*, 2005).

The pathological findings in the intestine included atrophy in the muscularis, degenerative and necrotic changes in the intestinal mucosa and sub-mucosa with necrotized cells aggregated in the intestinal lumen, edema and atrophy in the submucosa. These findings are similar in *Chana punctatus* exposed to Cadmium (Stromberg *et al.*, 1983) and lead (Sastri & Gupta, 1978).

The present results indicate that *Lemna gibba* L weed and extract are effective in removing Cd from water and reducing Cd bioaccumulation in Tilapia fish. The addition of *Lemna gibba* L-extract reduced significantly ( $p < 0.05$ ) the Cd level in water and the metal uptake as compared to fish exposed to Cd alone. The Cd concentration in water was  $9.620 \pm 0.198$  mg L<sup>-1</sup> and it decreased significantly ( $p < 0.05$ ). The Cd accumulation in liver and muscles of fish exposed to Cd alone was higher than that of *Lemna gibba* L-extract treatment group. These results suggest that *Lemna gibba* L weed and/or extract could chelate Cd ions producing a stable complex, thus reducing the chance for metal uptake by tissues. These results are in agreement with Santschi (1988) who reported that any agent that can remove Cd from water helps to reduce the bioaccumulation of this metal in fish.

The addition of *Lemna gibba* L-extract improves the haematological parameters (RBCs, Hb and Hct) and ameliorates the toxic effect of Cd in Tilapia fish which indicating the capability of *Lemna gibba* L-extract to chelate Cd from the media. Subsequently, the Cd toxicity was reduced. These results are in agreement with those recorded by Jayaram & Prasad (2009) who observed the biosorption potential of *Prosopis juliflora* seed powder (PJSF) for Pb (II) from aqueous solution at pH 6.0. Findings in fish also indicated that

degenerative changes were less when *Lemna gibba* L-extract or the weed and their extract were added to the rearing water.

The present study shows that addition of *Lemna gibba* L- weed and/or its extract to Cd contaminated media reduced significantly the Cd level in the water and helped to eliminate metal from the fish body and in turn improved the biochemical parameters as compared to fish exposed to Cd alone.

Finally, we could conclude that Cd poisoning cause structural damage in Tilapia organs. It is also demonstrated that *Lemna gibba* L-extract, weed or the weed plus the extract provided protection against the degenerative action of Cd and increased the chance of tissue regeneration.

#### Correspondence author

Hussein A.Kaoud

Department of Veterinary Hygiene and Management,  
Faculty of Veterinary Medicine, Cairo University,  
Giza, Egypt

[ka-oud@link.net](mailto:ka-oud@link.net)

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