Bioremediation the toxic effect of mercury on liver histopathology, some hematological parameters and enzymatic activity in Nile tilapia, *Oreochromis niloticus*.

Hussein A. Kaoud[1], Khaled M.A. Mahran[2], Ahmed Rezk[1] and Mahmoud A. Khalf[1]

[1]Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
[2] Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
ka-oud@link.net

Abstract: The effect of mercury (Hg) toxicity, its impact on liver histopathology, hematological and biochemical changes in Nile tilapia (*Oreochromius niloticus*) were studied. The bioremediation effects of *Spirulina platensis* were investigated through semi-static acute toxicity test developed with mercury chloride ($HgCl_2$). Fingerlings (4.45±0.31 cm and 2.35 ±0.18g) were kept during 96 hours in 5-liter glass aquaria, according to the following mercury concentrations, set up in three replicates: 0.00 (control), 0.05, 0.10, 0.20, 0.30, and 0.40 mg Hg L^{-1}. The value of LC_{50}-96h was estimated in 0.300 mg Hg L^{-1}. Fish exposed to Hg resulted in significant reduction *(P< 0.05)* of the erythrocyte count (RBCs), hemoglobin content (Hb) and haematocrit value (Hct). Significant changes in plasma aspartate aminotranseferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) were observed in fish exposed to Hg. Results also, indicated that *Spirulina platensis* was effective in removing Hg from water. Hg concentration in water was 69.880±0.156 µg L^{-1} and it decreased significantly *(P< 0.05)*. The addition of *dried Spirulina platensis* improves the haematological parameters (RBCs, Hb and Hct) and ameliorates the toxic effect of Hg which indicating the capability of *Spirulina platensis* to chelate Hg from the media.

[Hussein A. Kaoud, Khaled M.A. Mahran, Ahmed Rezk and Mahmoud A. Khalf. **Bioremediation the toxic effect of mercury on liver histopathology, some hematological parameters and enzymatic activity in Nile tilapia, *Oreochromis niloticus*.** Researcher. 2012;4(1):60-69]. (ISSN: 1553-9865). http://www.sciencepub.net. 10

Key words: Mercury**;** hematological, and biochemical changes; Spirulina platensis; Oreochromis niloticus.

1. Introduction

Pollution of aquatic environment with metals is common worldwide and under certain conditions aquatic fauna may concentrate large amount of some metals from water in their tissues (Kaoud and Rezk, 2011).

Accumulation of toxic metals of hazardous levels in aquatic biota has become a problem of increasing concern and could lead to health hazards in man, either through drinking of water and/or consumption of fish (Mathis and Cummings, 1973).

Mercury (Hg) is one of the most toxic metals in our environment including the lithosphere, hydrosphere, atmosphere and biosphere (Barbosa *et al*., 2001). The toxic effects of heavy metals have been reviewed, including bioaccumulations (Adami *et al.*, 2002; Waqar, 2006), and are surrounded with great care and special importance due to their highly toxic effects on fish as they affect survivability, growth and reproduction (Abdel-Tawwab et al.,2004).

Thus, some researchers have been given emphasis to investigations with data for treatment, which is not only curative but also prophylactic (Verlhac and Gabaudan, 1994), such as the case of use of vitamins on the supplementation of commercial diets. Among these investigations, the use of vitamins as A, D, C and E is paramount, because they are closely related to the performance of fish immune system. The need for these vitamins differ according to the species, age and raising period, and the research in this field is still much limited (Abdel-Tawwab et al., 2004).

Metal ions, especially Hg^{2+}, are known to be effective enzyme inhibitors. Mercury is a sulphur-seeking metal that bind to – SCH_3 and –SH groups present in methionine and cysteine. These amino acids are part of the enzyme structure. Often, the sulphydryl (– SH) groups are found on enzyme active site. In such circumstance, attachment of Hg^{2+} on the –SH group would indeed be detrimental to the activities of the enzyme (Manahan, 1979).

Phytofilteration using aquatic plants has promising potential for ex situ clean-up of polluted water. Recent advancements have also proven successful via the addition of matched microorganisms to enhance the break down or removal of contaminants.

Spirulina (*Arthrospira*) is cultured commercially in China (Zhang, 1998; Michael, 1999) and in some other parts of the world as this cyanobacterium has been proved to be a valuable source of food supplement not only for human but for other farm animals too (Lee, 1997). Furthermore, it has been found that microalgae to be very effective biosorbents, as they possess a large surface area and high binding affinity (Roy *et al*., 1993). Cell wall of these microalgae consists of polysaccharides, proteins and lipids having lots of negative groups which are the dominant binding sites of toxic metal cations (Vonshak, 1997).

The aim of this study was to investigate the effect of mercury (Hg) toxicity on liver histopathology, hematological, and biochemical changes in Nile tilapia (*Oreochromius niloticus*) .As well as the ability of *dried Spirulina platensis* –microalgae to reduce the toxic effect of mercury on liver, hematological and biochemical parameters in *O. niloticus*, exposed to mercury for short-term toxicity.

2. Materials and Methods

Fish culture and management

Healthy *Oreochromis niloticus* fingerlings (with a mean weight of 2.35 ±0.18 g and mean total length of 4.45±0.31 cm) were collected in Marsh 2011, from ponds of the Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, and Sharkia, Egypt (belonging to a single population) .They were collected and acclimated in the laboratory 7 days before experimentation.

Mercury chloride

Technical grade mercury chloride (99% purity) was obtained from El-Nasr Chemical Company (Cairo, Egypt) and prepared in aquatic solution to provide the required concentrations of mercury. Control test without mercury was performed.

Calculation of LC_{50}

Acute Toxicity Assays

Toxicity test was conducted according to the standard procedures of FAO (1985). Five concentrations (each with 4 replicates) of Hg were set up ranging between 0.05, 0.10, 0.20, 0.30, and 0.40 mg Hg L^{-1}.

Experimental design

Fresh water was adjusted to the desired parameters as follows: temperature 26.30±2.25 ^{o}C, pH 7-7.8, dissolved oxygen 5-8 mg L^{-1}, salinity 2ppt and hardness 100-150 $CaCO_3$ with a photoperiod (10L: 14D cycle).

A stock solution (370 mg Hg L^{-1}) was prepared by dissolving a calculated quantity of active ingredient (0.5 g $HgCl_2$ in 1,000 mL of dechlorinated tap water). A series of five concentrations of Hg was prepared (0.05, 0.10, 0.20, 0.30, and 0.40 mg Hg $L^{-1)}$ by adding a calculated volume from the stocky solution into test containers, considering the equivalent on mercury (Hg) plus a control (One container was kept as unexposed control group). No food was supplied during the experiment. Test solutions were replaced by fresh ones of the same respective concentrations every 24 h to maintain the definite concentration of Hg for 96 h (APHA, 1998).

Acclimation period was of 7 days, in a 50-L glass aquarium. During this period, fish were fed a dry commercial food (pellets with 25% of crude protein). Afterwards, fingerlings were transferred to 5-L glass aquaria, which were internally covered with a plastic film to prevent contamination by residues from previous experiments. Plastic film was also placed on the top of the aquarium to prevent evaporation. Air pumps and individual air stone diffusers provided aeration. The experiment was carried out at a stocking density of 10 fish/aquarium.

Mortalities were recorded at 24, 48, 72 and 96 h of exposure, and dead fish were removed regularly from the test solutions. The data obtained were statistically analyzed using the Trimmed Spearman Karber method (Hamilton *et al.*, 1977) for estimating the median lethal concentration (LC_{50}), and 1/4 of the LC_{50}-96 h (75 µg Hg L^{-1}) was taken as the safe Hg concentration (Sprague, 1971).

Hematological and enzymatic investigations

After 15 days of the experiment, blood samples were taken from five fish from each aquarium. Fish were not fed for 24 hrs before sampling, blood samples were taken from the caudal vein of 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. Plasma

activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to Reitman & Frankel (1957). Alkaline phosphatase (ALP) was measured by using Diamond diagnostics kits according to the method of Rec (1972). Also acid phosphatase (ACP) activity was determined according to the method of King and King (1954).

Histopathlogical examination

Tissue specimens from fresh Nile Tilapia were taken (liver and muscles) and fixed in 15 % buffered neutral formalin. They were processed to obtain five micron thick paraffin sections then stained with Hematoxylin and Eosin (Bancroft *et al.*, 1996) and examined under light microscope. Hepatossomatic index (HSI) was calculated and the number of hepatocytes nucleus per mm2 of hepatic tissue was obtained according to Figueiredo-Fernandes (2007).

Microalgae

Spirulina platensis was used in this study. It was grown at 25 ± 20 °C in Zarrouk liquid medium (Parada *et al*., 1998), for 8-10 days under white fluorescent light (90 mmol photon m-2s-1) with 14 h illumination. At the exponential growth phase, culture was filtered through filters 47 mm (diameter) (Whatman GF/C). The filter was put in a glass Petri dish in the oven under 35 °C for 3 days (Boussiba and Richmond, 1976).

Hepatossomatic index (HSI) was calculated and the number of hepatocytes nucleus per mm2 of hepatic tissue was obtained in 12 microscopic fields (100x). Means ± standard deviation (SE) were calculated for each experimental group.

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 96 h LC_{50} value for Hg in Nile tilapia, *Oreochromis niloticus*, was calculated by the simple graphic method to be 0.30 mg $HgCl_2$ L^{-1} (Fig.1).

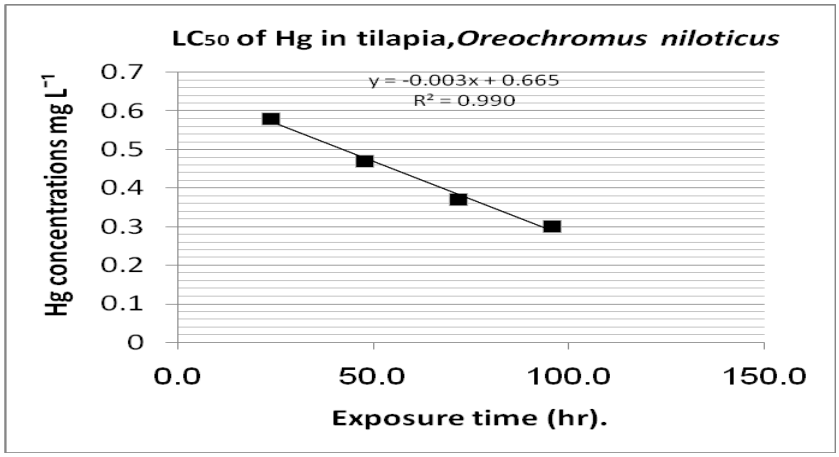

Fig.1.: LC_{50} value for Hg in Nile tilapia, *Oreochromis niloticus*

Hematological parameters

Table 1, showed that the RBCs, HB and HCT were reduced in fish exposed to Hg for 15 days and they were lower than that of the control *(P<0.05).* On the other hand, these parameters were of normal values and increased significantly in fish exposed to Hg with *Spirulina platensis*.

Table I: Changes in Hg residue in water (mg HgL^{-1}) media of Nile tilapia *(O. niloticus*) exposed to Hg (75 µg L^{-1}) (Equivalent to 1/4 96 h LC_{50}). .

Group	Water	Erythrocyte count (RBCs)	Hemoglobin(HB)	Haematocrit value (Hct)
Control (metal free water)	0.032±0.004[a]	1.260 ±0.082	5.950±0.245[a]	13.732±0.442[a]
Hg alone (75 µg L^{-1})	69.880±0.156[b]	1.090±0.170[b]	4.320±0.354[b]	12.441 ±1.652[b]
Hg + *S. platensis* (5mg)	22.92 ±0.426[b]	1. 55±0.110[a]	4.440±0.534[b]	11.910 ±0.322[a]
Hg + *S.platensis* (10mg)	4.95 ±0.085[b]	1.255±0.110[a]	5.440±0.764[b]	13.210 ±0.322[a]

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

Biochemical parameters

Table 11, showed that AST activity increased significantly in plasma of fish exposed to Hg alone. The addition of *Spirulina platensis* decreased significantly the AST activity to be lower than that of Hg alone *(P<0.05).* The AST activity in fish exposed to Hg with *Spirulina platensis* became nearly similar to that of control at 15 days.

The plasma ALT activity increased significantly in fish exposed to Hg alone at 15 days. The addition of *Spirulina platensis* enhanced ALT activity to be nearly as in the control.

Addition of *Spirulina platensis* to the Hg polluted media reduced significantly *(P<0.05)* Hg level in aquarium's water as compared to that of Hg alone, Table I.

Hg concentration in water exposed toHg alone was 69.880±0.156 µg L^{-1} then declined significantly *(P<0.05)* to 22.92 ±0.426 and 4.95 ±0.085 µg L^{-1} by addition of *S.platensis* 5 and 10 mg L^{-1} , respectively.

Table II: Changes in aspartate aminotransferase activity (AST) , alanine aminotransferase (ALT) Alkaline phosphatase (ALP) and Acid phosphatase (ACP) activities (IU L^{-1}) in plasma of Nile tilapia (*O. niloticus*) exposed to Hg (75 µg L^{-1}) (Equivalent to 1/4 96 h LC_{50}).with and without *Spirulina platensis* .

Group	AST	ALT	ALP	ACP
Control (metal free water)	59.588±1.92[a]	29.844±2.632[a]	2.49 ± 0.521[a]	17.4 ± 3.40[a]
Hg alone (75 µg L^{-1})	95.760±1.688[b]	48.655±2.874[b]	1.213[b] ±0.110[b]	32.50 ± 8.60[b]
Hg + *S. platensis* (5mg)	70.782±2.526[a]	40.82±2.424[b]	1.831 ±0.234[b]	19.88 ± 3. 90[a]
Hg + *S.platensis* (10mg)	65.590±1.562[a]	38.933±2.564[a]	2.30[ab]±0.264[a]	18.74 ± 3. 65[a]

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

Liver histopathology

Liver histopathology of control and exposed fish is briefly illustrated in Fig.2. In the control group, the liver exhibited a normal architecture and there were no pathological abnormalities, with hepatocytes presenting a homogenous cytoplasm, and a large central or subcentral spherical nucleus. The hepatic parenchyma of fish exposed to mercury showed lymphocytic infiltration with hepatocellular necrosis (Fig.2_a), hepatocellular dissociation with lymphocytic cell aggregation (Fig.2_b) and increase of cytoplasmatic vaccuolation (Fig.2_c).Also, hepatic parenchyma of intoxicated fish showed; hemorrhages (Fig.2_d), degeneration (Fig.2_e) and congestion (Fig.2_f). The HSI increased in fish exposed to mercury (Table 111). Additionally, the number of hepatocytes nucleus per mm2 of hepatic tissue decreased in fish exposed to mercury (Table 111).

Table 111. Hepatossomatic index (HSI) and number of hepatocyte nucleus per mm of hepatic tissue (Hepat.nucl.mm^2) measured in Nile tilapia *Oreochromis niloticus* exposed to mercury with or without *Spirulina platensis*

Group	HIS	Hepta.nucl.mm^2
Control (metal free water)	1.10±0.0007 [a]	1358.99±0.948[a]
Mercury alone (75 µg L^{-1})	1.80±0.002 [b]	3052.88±1.000[b]
Mercury (75 µg L^{-1}) + *S.platensis (5 mg* L^{-1})	1.40 ±0.0019 [b]	1471.89±0.956[a]
Mercury (75 µg L^{-1}) + *S.platensis (10 mg* L^{-1})	1.18 ±0.0014 [a]	1300.66±0.120[a]

Values are expressed as means ± SE (n=8). Means in the same column with different letters are significantly different *(P< 0.05).*

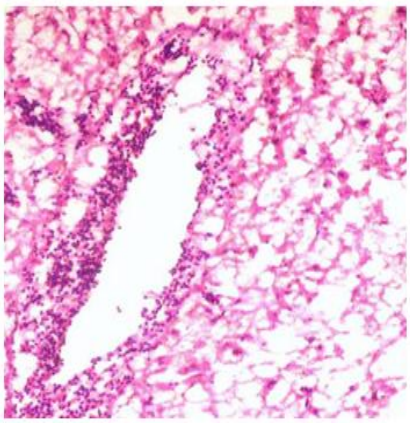

Fig.2a Liver showing perivascular aggregation of mononuclear cells with hepatocellular necrosis (H & E 200 X).

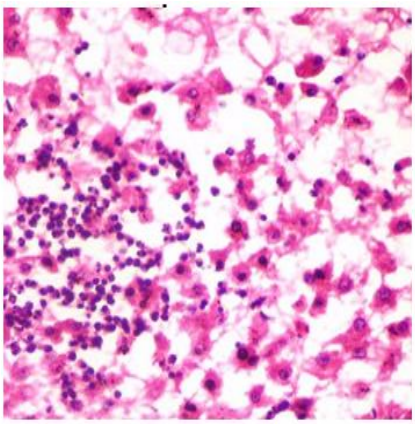

Fig.2b Liver showing dissociation of hepatocytes with focal mononuclear cell aggregation (H & E 400 X).

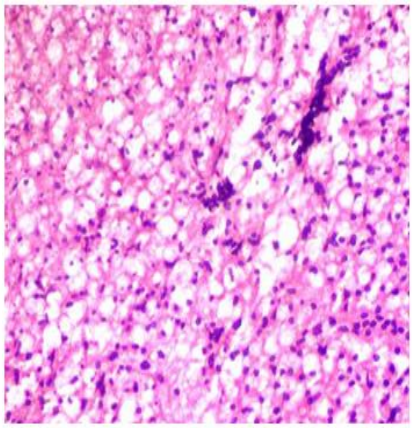

Fig.2c Liver showing vacuolation of hepatocytes and lymphocytic infilteration (H & E 400 X).

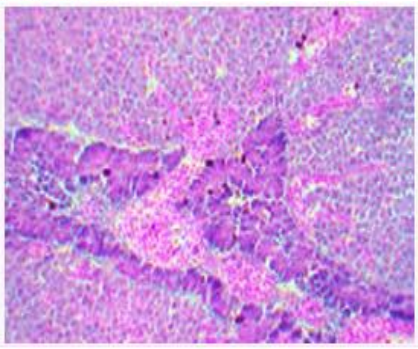

Fig.2$_d$ Hepatic parenchyma of Hg intoxicated fish showing hemorrhages (H&E 400X)

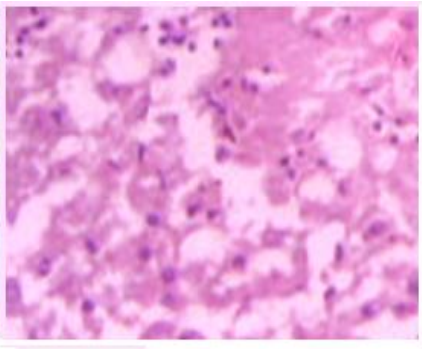

Fig.2$_e$ Hepatic parenchyma of Hg intoxicated fish showing degeneration (H&E 400X)

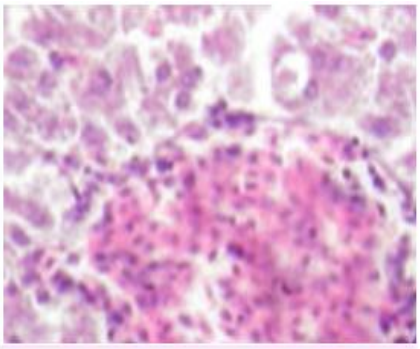

Fig.2$_f$ Hepatic parenchyma of Hg intoxicated fish showing congestion (H&E 400X)

Fig.2: Histopathology of liver tissue in Nile tilapia, *Oreochromis niloticus*,exposed to mercury toxicity.

4. Discussion

The 96 h LC_{50} value for Hg in Nile tilapia, *Oreochromis niloticus*, was calculated by the simple graphic method to be 0.30 mg $HgCl_2$ L^{-1}. Ishikawa et al. (2007) and Kaoud & Mekawy (2011) found that the value of LC_{50} for 96 h exposure to Hg in Nile tilapia, Oreochromis *niloticus* were 0.22 and 0.24 mg·L^{-1}, respectively, which are relatively lower than that obtained in the present study. Higher value (0.739 mg·L^{-1}) was obtained by Ramamurthi et al. (1982) for *Tilapia mossambicus*.

Variations of LC_{50} may be attributed to some differences in standard techniques that were adopted in the experiments such as the larger size of the test-organisms (Ishikawa et al., 2007; Buhl, 1997 and Boening, 2000). The acute toxicity of waterborne heavy metals on aquatic organisms is highly variable even among phylogenetically closely related species and depends on metal speciation, being the free ions (WHO, 1992).

The present study reveals that the fish exposed to Hg alone showed significant reduction in RBCs, Hb and HCT than those exposed to Hg with *Spirulina platensis*. The reduction of these parameters in *O. niloticus* at sub-lethal levels of Hg might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemsynthesis that 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 severe anemia (James and Sampath, 1999). Also Abdel-Tawwab et al., (2004) found a significant reduction in the RBCs, Hb and HCT in Nile tilapia, *Oreochromis niloticus*, after exposure to sublethal doses of inorganic Hg (0.125 and 0.250 mg Hg L^{-1}).

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 Hg 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 and Kaoud *et al*., 2011). Hepatic AST and ALT activities were decreased at high dose of Hg *Oreochromis niloticus* exposed to inorganic Hg (Abdel-Tawwab et al., 2004)

Gill *et al*. (1990) who found a marked reduction in hepatic, branchial and renal AST and ALT in rosy barb (*Puntius conchonius*) after toxication with mercuric chloride. They mentioned that, the reduced levels of aminotransferase in various organs may result from tissue damage and consequently the reduction of enzyme turnover causally related to the presence of toxic mercury. Also, Abu El-Ella (1996) ,Shalaby (2000) and Kaoud *et al*., 2011) found similar results with grass carp, *Ctenopharyngodon idella*; common carp, *Cyprinus carpio* and *Oreochromis niloticus* when exposed to Cd, respectively. These results may be attributed to liver necrosis (because of toxicant) that led to leakage from liver into the blood and/or tactual inhibition of liver enzymes.

The decrease of ALP activity in plasma due to Hg toxicity was similar to that obtained by Abdel-Tawwab et al. (2004) who recorded that a significant reduction in ALP in liver and kidney of catfish, *Oreochromis niloticus* after toxication with Hg. This decrease may be due to the damage and dysfunction of the liver.

The hepatic parenchyma of fish exposed to mercury showed lymphocytic infiltration, increase of cytoplasmatic vacuolation, hepatocellular necrosis, increased HSI and decreased in the number of hepatocytes nucleus per mm^2.

Biological parameters are sometimes indicative of toxicant effects (Mayer et al. 1992). Our results reveal that the HSI increased with mercury toxicity. Figueiredo- Fernandes *et al.* (2006b) found an increase of HSI in male and female tilapia, *O. niloticus*, exposed to paraquat. Also Figueiredo- Fernandes *et al.* (2007) found an increase of HSI in tilapia, *O. niloticus*, exposed to copper. Huuskonen & Lindström-Seppa (1995) and Stephensen et al. (2000) found that the high HSI observed in the perch (*Perca fluviatilis*) and sculpin (*Myoxocephalus scorpius*) can be indicative of increased activity of xenobiotic

biotransformation enzymes. Figueiredo-Fernandes et al. (2006b) also suggested a positive relationship between the relative liver weight and the xenobiotic-metabolizing enzymes of tilapia exposed to paraquat, additionally Figueiredo-Fernandes *et al.* (2007) found an increase of HSI In contrast in tilapia, *O. niloticus*, exposed to copper. .

Spirulina platensis reduced the Hg toxicity in water and fish which in turn enhanced the ALP activity. On other hand, The ACP activity increased significantly in fish exposed to Hg alone more than control. The obtained results showed that all the tested biochemical parameters were improved in due to *Spirulina platensis* 10 mg L^{-1} which is considered as an optimum dose could improve the health status of fish.

The alkaline phosphatase (ALP) in plasma was significantly decreased in fish exposed to Hg **(**1.213 ±0.110IU/L, P<0.05). On the other hand, addition of *Spirulina platensis* Hg –polluted media enhanced ALP activity in fish and became similar to that of control fish. The acid phosphatase (ACP) increased significantly in fish exposed to Hg alone at 15 days (32.50 ± 8.60 IU/L, P<0.05, respectively). Contrarily, the ACP activities of fish exposed to Hg with high dose Spirulina *platensis* 10 mg L^{-1} became similar to that of control fish group at 15 days.

Addition of *Spirulina platensis* to the Hg polluted media reduced significantly (*P<0.05*) the Hg level in aquarium's water as compared to that of Hg alone. Hg concentration in water exposed Hg alone was 69.880±0.156µg Hg L^{-1} and declined significantly (*P<0.05*) to 22.92 ±0.426 and 4.95 ±0.085 with 5 mg, and 10 mg L^{-1} of *Spirulina platensis*, respectively.

These results suggest that *Spirulina platensis* could chelate Hg ions producing a stable complex, thus reducing the chance for metal uptake. The formation of Hg- chelate complex in water evidently reduced the metal burden in tissues and thereby improved the hematological and biochemical parameters of fish exposed to Hg. Kaoud and Mekawy (2011) found that, *Lemna gibba L* (weed and extract) were effective in removing Hg from water and reducing Hg bioaccumulation in liver and muscular tissues of *O.niloticus* fish .

Cd-binding complex was isolated from Chlorella fusca and has shown to be composed of phytochelating peptides $(\gamma\text{-Glu-Cys})_n$ – Gly,n=2-5.Members of six of the ten classes of Phycophyta revealed phytochelation synthesis after exposure to cadmium ions . Phytochelation was also induced by ions of lead,zinc,silver,copper and mercury (Gekeler et al, 1988).

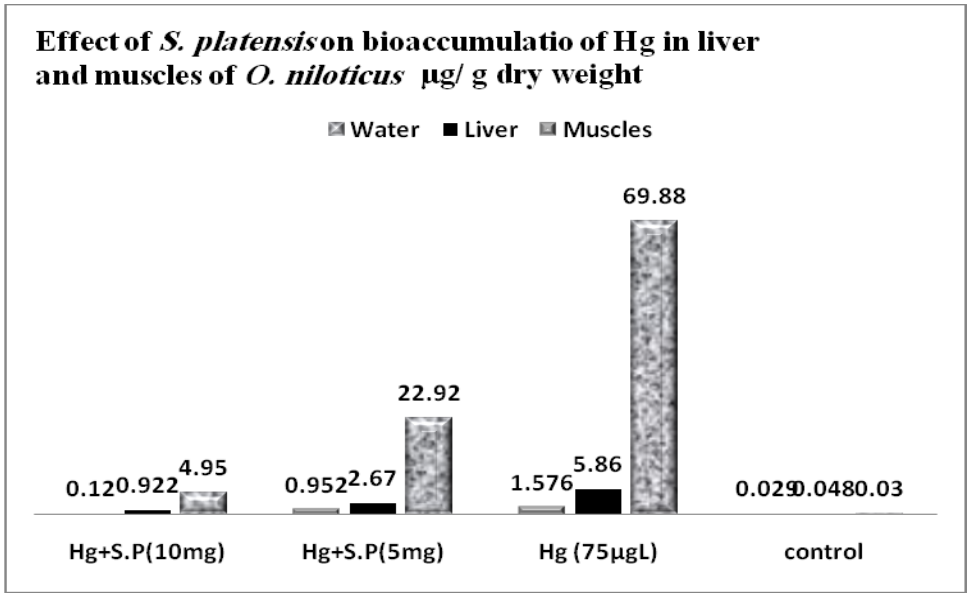

Fig.3: *Spirulina platensis* reduced the Hg in water and its bioaccumulation in liver & muscles of *O.niloticus*.

Spirulina (*Arthrospira*) has gained a high economic value (Cohen et al., 1995) particularly because it contains some fine compounds such as essential fatty acids and amino acids, antioxidant vitamins and minerals *etc*. at relatively high concentrations (Roughan,

1989). Various species of cyanobacteria and algae have been known to adsorb and take up heavy metal ions (Kuyucak and Volesky, 1999). Microbial biomass-related technologies have also been tested for heavy metal removal from polluted water bodies (Volesky and Holan, 1995) as the conventional methods are expensive (Eccles, 1999; Volesky and Holan, 1995; Chang *et al*., 1997). Components found in the cell wall of *Spirulina*, such as peptydoglycan, teichuronic acid, teichoic acid, polysaccharides and proteins (Schiewer and Wong, 2000) which display mainly carboxylic, hydroxyl and phosphate groups (Aksu, 2002; Markai *et al*., 2003) may give algal wall binding properties. Cell wall of *S.platensis* having lots of negative carboxyl and phosphate groups, which are the dominant binding sites of toxic and heavy metals cations (Vonshak, 1997; Ari *et al*., 1999). Furthermore, it has been found that microalgae to be very effective biosorbents, as they possess a large surface area and high binding affinity (Roy *et al*., 1993).

Finally, we could conclude that *Spirulina platensis* provided protection against the toxic action of Hg and increased the chance of blood and enzymes regeneration.

Corresponding Author:
Dr Hussein .A. Kaoud
Department of Veterinary Hygiene, Environmental Pollution and Management, Faculty of Veterinary Medicine, Cairo University, Giza 11221, Egypt.
Email: ka-oud@link.net

References

Abdel-Tawwab M.,. Shalaby A. M.E, Ahmad M. H. and Khattab Y. A.E.2004. Effect of Supplementary Dietary L-Ascorbic Acid (Vitamin C) on Mercury Detoxification, Physiological Aspects and Growth Perfomance of Nile Tilapia (*Oreochromis niloticus* L.) Proceedings 6th International Symposium on Tilapia in Aquaculture,12-16 Sept.2004. International Convention Center Roxas Boulevard Manilla,Phillipines. 159-171,

Adami G. M., Barbieri P., Fabiani M., Piselli S., Predonzani, S., Reisenhofer E., (2002). Levels of cadmium and zinc in hepatopancreas of reared *Mytilus galloprovincialis* from the Gulf of Trieste (Italy). Chemosphere, 48 (7), 671 – 677.

Aksu, Z. (2002) Determination of the equilibrium, kinetic and thermodynamic parame-ters of the batch biosorption of nickel(II) ions onto *Chlorella vulgaris*. Process Bio-chem. 38: 89-99.

Ari, A.B., Mel, M., Hasan, M.A. and Karim, M.I.A. (1999) The kinetics and mechanism of lead(II) biosorption by powderized *Rhizopus oligosporus*. World J. Microbiol. Biotechnol. 15: 291-298.

American Public Health Association (APHA) (1998). Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, Washington, DC.

American Public Health Association (APHA) (1992). Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association, Washington, DC.

Bancroft D, Stevens A, Turner R. 1996. Theory and practice of histological techniques. Fourth edn., Churchill Livingstone, Edinburgh, London, Melbourne.

Barbosa A.C., Jardim W., Dorea J.G., Fosberg B., Souza J., (2001). Hair mercury speciation as a function of gender, age and body mass index in inhabitants of the Negro River basin, Amazon, Brazil. Arch Environ Contam Toxicol 2001; 40: 439–44.

Boussiba and Richmond, 1976 cited in The growth and functioning of leaves Edited

Boening, D.W. 2000 Ecological effects, transport, and fate of mercury: A General Review. *Chemosphere, 40:* 1335-1351.

Britton, C.J. (1963): "Disorders of the Blood" 9th ed. I. A. Churchill, Ld. London.

Buhl, K.J. 1997 Relative sensitivity of three endangered Þ shes, Colorado SquawÞ sh, Bonytail, and Razorback Sucker, to selected metal pollutants. *Ecotoxicol. Environ. Safety*, *37*: 186-192.

Cohen, Z., Cristina, M. and Tomaselli, L. (1995) Chemotaxonomy of cyanobacteria. Phytochem. 4: 1155-1158.

Chang, J.S., Law, R. and Chang, C.C. (1997) Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU 21. Water Res. 31: 1651-1658.

Dacie, J. V. and Lewis, S. M. (1984): Practical Haematology. Churchill Living Stone. London .

Doshi ¹H., Ray A. and Kothari I.L. 2006.Bioremediation potential of live and dead *Spirulina*: Spectroscopic, kinetics and SEM studies. (2007), Bioremediation potential of live and dead *Spirulina*: Spectroscopic, kinetics and SEM studies.

Biotechnology and Bioengineering, 96: 1051–1063.

Eccles, H. (1999) Treatment of metal-contaminated wastes: why select a biological process? TIBTECH. 17: 162-165.

FAO, (1985). Manual of methods in aquatic environment research. Part 4. Bases for selecting biological tests to evaluate marine pollution: FAO fish tech paper 164.

Figueiredo-Fernandes A., Fontaínhas-Fernandes A., Peixoto F., Rocha E. & Reis- Henriques M.A. 2006b. Effect of paraquat on oxidative stress enzymes in tilapia *Oreochromis niloticus* at two levels of temperature. Pest. Biochem. Physiol. 85:97-103.

Figueiredo-Fernandes A., Ferreira-Cardoso J. V. , Garcia-Santos S., Monteiro S. M., Carrola.J, Matos P. and Fontaínhas-Fernandes A.2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper Pesq. Vet. Bras. 27(3):103-109

Gekeler W.,Grill E.,Winnacker E.,and Zenk M.H.1988.Algae sequester heavy metals via synthesis of phytochelation complexes.Arch Microbiol.150:197-202.

Gill, T.S., Tewari, H. and Pande, J. 1990. Use of the fish enzyme system in monitoring water quality: Effects of main tissue enzyme. Comp. Biochem. Physiol., 97C: 287-292.

Hamilton, M.A.; Russo, R.C.; Thurston, R.V. 1977 Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ Sci. Technol., 11:* 714-719.

Huuskonen S. & Lindström-Seppa P. 1995. Hepatic cytochrome P4501A and other biotransformation activities in perch (*Perca fluviatilis*): the effects of unbleached pulp mill effluents. Aquat. Toxicol 31:27-41.

Ishikawa N M 1; Maria JosÈ Tavares Ranzani-Paiva M J T, Julio Vicente Lombardi J V (2007): Acute toxicity of mercury ($HgCl_2$) to Nile Tilapia, *Oreochromis niloticus*, *B. Inst. Pesca*, São Paulo, *33*(1): 99 – 104.

James, R.; Sampath, K.; Jancy Pattu, V and Devakiamma, G. (1992): Utilization of *Eichhorina crassipes* for the reduction mercury toxicity on food transformation in *Heteropneustes fossilis*. J. Aqua. Trop., 7 :189- 196.

Khangarot, B. S and Tripathi, D. M (1991): Changes in humoral and cell- mediated immune responses and in skin and respiratory surfaces of cat fish *Saccobranchus fossillis*, following copper exposure. Ecot. Envir. Safety., 22 (3): 291-308.

Kaoud H. A. and Mekawy M. M. 2011**.** Bioremediation the Toxic Effect of Mercury-Exposure in Nile Tilapia (*Oreochromis Niloticus*) by using *Lemna gibba L*. Journal of American Science;7(3):336-343.

Kaoud H. A., Zaki M. M., El-Dahshan A. R., Saeid S. and El Zorba H.Y.2011. Amelioration the Toxic Effects of Cadmium-Exposure in Nile Tilapia (*Oreochromis Niloticus*) by using *Lemna gibba L.* Life Science Journal. 2011;8(1):185-195.

King, P. R. N. and King, J. (1954): Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine. J. Clin. Path., 7:322-326.

Kuyucak, N. and Volesky, B. (1989) The mechanism of cobalt biosorption. Biotech. Bioeng. 33: 823-831.

Lee, Y.K. (1997) Commercial production of microalgae in the Asia-Pacific rim. J. Appl. Phycol. 9: 403-411.

Manahan, S.E. (1979). Environmental Chemistry, 3rd (ed.). Willand grant press, Boston, Massachusetts. pp.453-455.

Myers M.S., Rhodes L.D. & McCain B.B. 1987. Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions, and other iodiopathic hepatic conditions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. J. Natl Cancer. Inst. 78:333-363.

Moussa, M. A. (1999): Biological and physiological studies on the effect of the gramoxon and stomp herbicides on Nile tilapia *(Oreochromis niloticus*). Fact. Sci. Zool. Dep. Cairo. Univ.,200p (Ph.D. Thesis).

Markai, S., Andres, Y., Montavon, G. and Gram bow, B. (2003) Study of the interac-tion between europium(III) and *Bacillus subtilis*: fixation sites, biosorption modelling and reversibility. J. Colloid Interface Sci. 262: 351-361.

Michael, A.B. (1999) Commercial production of microalgae: ponds, tanks, tubes and fer-menters. J. Biotechnol. 70: 313-321.

Mathis, B.J. and T.F. Cummings, 1973. Selected metals in sediments, water and biota of the Illinois River. J. Water Poll. Cont. Trop., 45: 1573-1583. PMID: 4720140.

Parada, J.L., de Caire, G.Z., de Mule, M.C.Z. and de Cano, M.M.S. (1998) Lactic acid bacteria

growth promoters from *Spirulina platensis*. Int. J. Food Microbiol. 45: 225-228.

Ramamurthi, R.; Naidu, K.A.; Subbiah, M.B.; Balaji, N.; RAO, M.V.R. 1982 Toxicity of mercury to some freshwater organisms. *Geobios Jodhpur, 9:* 89-90.

Reitman, S. and Frankel, S. (1957): Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol., *28*: 53-56

Rec, G. S. (1972): Determination of alkaline phosphatase. J. Clin. Chem. Clin. Biochem., 10:182.

Roughan, P.G. (1989) *Spiulina*: A source of dietary gama-linolenic acid J. Sc. Food Agric., 47: 85-93.

Roy, D., Greenlaw, P.N. and Shane, B.S. (1993) Adsorption of heavy metals by green algae and ground rice hulls. J. Envi-ron. Sci. Health A. 28: 37-50.

Shalaby, A.M.E. 2000. Sublethal of heavy metals copper, cadmium and zinc alone or in combinations on enzymes activities of common carp (*Cyprinus carpio* L.). Egypt. J. Aquat. Biol. Fish., 4(2): 229-246.

Snedecor, O.W. & Cochran, W.O. (1982). Statistical Methods. 7th Ed,, Iowa University Press, Ames, Iowa.

Svoboda, M (2001): Stress in fish – review. Bul. VURH Vodnany., 37: 69- 191.

Sprague, J.B. 1971 Measurement of pollutant toxicity to fish. III. Sublethal effects and safe concentrations. *Water Res., 3:* 793-821.

Schiewer, S. and Wong, M.H. (2000) Ionic strength effects in biosorption of metals by marine algae. Chemosphere. 41: 271-282.

Van Kampen, E. J. and Zijlstra, N. C. (1961): Determination of haemoglobin. Clin. Chem. Acta, *5*: 719-720.

Verlhac, V. and Gabaudan, J. 1994. Influence of vitamin C on the immune system of

salmonids. Aquaculture and Fisheries Management, 25: 21-36.

Volesky, B. and Holan, Z.R. (1995) Biosorp-tion of heavy metals. Biotechnol. Prog. 11: 235-250.

Vonshak, A. 1997 (Ed.) Spirulina platensis (Arthrospira) Physiology, cell-biology and biotechnology. Taylor & Francis, London.

Waqar A., (2006). Levels of selected heavy metals in Tuna fish. Arab. J. Sci. Eng., 31 (1A), 89–92.

Wintrobe, M. M (1978): In: Clinical hematology. Henry Kimpton, London., pp: 448.

WHO (1992). Environmental Health Criteria, No. 134, Environmental aspects, Geneva WHO.

Yamawaki, K.; Hashimoto, W.; Fujii, K.; Koyama, J.; Ikeda, Y. and Ozaki, H. (1986): Hematological changes in carp exposed to low cadmium concentration . Bull of the Japanese. Soc. Sci. Fish., 59 (3):459- 466.

Zhang, X.C. (1998) Large-scale cultivation of *Spirulina* in China; Today and tomorrow. Biosyst. Studies. 1: 66-73.

11/12/2011