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Mercury toxicity in aquatic environment: histopathology, hematological and enzymatic alterations in *Oreochromis niloticus*; methods of amelioration

H.A.Kaoud[1*], A.R. EL-Dahshan[1] and Quratulan Ahmed[2]

1- Department of Veterinary Hygiene and Environmental Pollution, Faculty of Veterinary Medicine, Cairo University, Egypt. Address: Faculty of Veterinary Medicine, Cairo University, Giza-Egypt

2- Department of Zoology, University of Karachi, Karachi, Pakistan

***Corresponding author*:** H.A.Kaoud

ABSTRACT: The effect of mercury (Hg) toxicity, its impact on liver histopathology, hematological and biochemical changes in Nile tilapia (*Oreochromius niloticus*) were studied. The amelioration effects of *Spirulina platensis* and *Chitosan* were investigated through a 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 96 h LC_{50} value for Hg in *O. niloticus* fingerlings was calculated by the simple graphic method to be 0.30 mg $HgCl_2$ L^{-1}. Fish exposed to Hg resulted in significant reduction *(P< 0.05)* of the erythrocyte count (RBCs), hemoglobin content (Hb) and hematocrit value (Hct). Significant changes in plasma aspartate aminotranseferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) were observed in fingerlings exposed to Hg. Results also, indicated that the addition of *Spirulina platensis* or chitosan to the Hg polluted media reduced significantly *(P<0.05)* Hg level in aquarium's water as compared to that of Hg alone. They improve the hematological parameters (RBCs, Hb, Hct) and ameliorate the toxic effect of Hg in the aquatic environment.

***Keywords*:** Mercury**;** hematological, and biochemical changes; Chitosan; Spirulina platensis; Aquatic environment.

INTRODUCTION

Chemicals derived from agricultural operations (pesticides and herbicides) and industrial effluents, such as metals, ultimately find their way into a variety of different water bodies and can engender a range of toxic effects in aquatic organisms, ranging from alterations to a single cell transmutations in whole populations Bernet . (1), Al-Kahtani (2).

Pollution of aquatic environment with metals is prevalent worldwide and under certain conditions aquatic fauna may concentrate substantial amount of some metals from water in their tissues, Kaoud and El-Dahshan (3). Accumulation of toxic metals of hazardous levels in aquatic biota has become a problem of incrementing concern and could lead to health hazards in man, either through drinking of water and/or consumption of fish, Mathis and Cummings (4).

Mercury (Hg) is one of the most noxious metals in our environment including the lithosphere, hydrosphere, atmosphere and biosphere (Barbosa ., (5). The toxic effects of heavy metals have been reviewed, including bioaccumulation and are circumvented with great care and special paramountcy due to their highly toxic effects on fish as they affect survivability, growth and reproduction (Adami ., (6); Waqar, (7).

A series of intricate chemical transformations allows the three-oxidation states of Hg cycle in the environment (Barbosa ., (5). In the zero oxidation state (Hg0), mercury subsists in its metallic form and vapor is the most abundant shape (98%). The

mercurous and mercuric states are the two higher oxidation states where the mercury atom has lost one (Hg+) or two electrons (Hg2+), respectively. Methyl mercury is the most important form of mercury in terms of toxicity and health effects from environmental exposures ,Jackson, (8); Goyer and Clarsksom, (9); Castro-Gonzalez and Mendez-Armentab, (10).

It has been found that microalgae were very efficscious biosorbents, as they possess a large surface area and high binding affinity ,Roy ., (11). 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, (12).

The aim of this study was to investigate the ability of *dried Spirulina platensis* microalgae and natural *chitosan* on the reduction of mercury from water and tissues of Nile tilapia, exposed to mercury short-term toxicity.

MATERIALS AND METHODS

2. 1 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 from several ponds (belonging to a single population). They were collected and acclimated in the laboratory for seven days before experimentation.

2.2 Mercury chloride

Technical-grade mercury chloride (99% purity) was used.

2.3 Calculation of LC_{50}

Toxicity test was conducted according to the standard procedures described by FAO (13).

2.4 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 2 ppt and hardness 100-150 mg L^{-1} $CaCO_3$ with a photoperiod (10 L: 14 D cycle).

A series of five concentrations of Hg was prepared (0.05, 0.10, 0.20, 0.30, and 0.40 mg Hg $L^{-1)}$, the equivalent on mercury (Hg) plus a control group. No food was supplied during the experiment. Test solutions were superseded by fresh ones of the same respective concentrations every 24 h to maintain the definite concentration of Hg for 96 h APHA, (14). 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.

Air pumps and individual air stone diffusers were used to provide a sufficient aeration. The experiment was carried out at a stocking density of 10 fingerlings /aquarium. Mortalities were recorded at 24, 48, 72 and 96 h of exposure, and dead fish were abstracted regularly from the test solutions.

2.4.1 Microalgae

Spirulina platensis, was used in this study where itt was grown at 25 ± 2 °C in Zarrouk liquid medium Parada ., (15), for 8-10 days under white fluorescent light (90 mmol photon m-2s-1) with 14 hr illumination. At the exponential growth phase, culture was filtered through filters 47 mm (diameter) (Whatman GF/C) and then the filter was put in a glass Petri dish in the oven at 35 °C for 3 days, Boussiba and Richmond, (16).

2.4.2 Chitosan

Chitosan, with a deacetylation degree of approximately 79%, was used in the experimental part. The average particle density was 0.1892 g cm^{-3}. The flake sizes were ranged from 0.22mm to 0.71 mm.

2.4.3 Determination of mercury

APHA (17) recommended procedure were used for the analysis of water samples. At laboratory, the fish samples (liver and muscles) were washed with deionized water and wrapped separately in acid washed polyethylene bag and stored at -20°C until analysis was carried out.

The measurement of the mercury concentration in examined fish sample was carried out at a minimal temperature Diaz-Ravina . (18) and analyzed by using Flame less Atomic Absorption Spectrophotometer equipped with "MHS" mercury hydride system "Cold Vapor Technique."

2.4.4 Hematological and enzymatic investigations

After 15 days of the experiment, blood samples were taken from five fish from each aquarium. The blood samples were taken from the caudal vein of fish by sterile syringe containing EDTA solution as an anticoagulant. These blood samples were

used for determining erythrocyte count, Dacie & Lewis (19) and hemoglobin content, Van Kampen & Zijlstra (20). Hematocrit value (Hct) was calculated according to the formulae mentioned by Britton (21).

Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and non hemolyzed 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 (22). Alkaline phosphatase (ALP) was measured by using Diamond's diagnostics kits according to the method of Rec (23). Furthermore, acid phosphatase (ACP) activity was resolute according to the method of King and King (24).

2.4.5 Histopathological 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 microns thick paraffin sections then stained with Hematoxylin and Eosin Bancroft ., (25) and examined under the light microscope.

2.4.6 Bioremediation

Fishes were distributed randomly into 50 Liters rectangular fiber glass aquaria filled with water at a rate of 25 fish / aquarium. Dissolved oxygen in each tank was maintained at proximate to saturation by aeration. The temperature in each aquarium was maintained by means of thermostats. These aquaria were divided into 4 groups with two replicates each per group. The first group was free from Hg, chitosan and dried *S. platensis* and considered as a control. The second groups were exposed to 75µg of $HgCl_2$ (Equivalent to 1/4 -96 h LC_{50}). The third groups were exposed to 75µg of $HgCl_2$ (Equivalent to 1/4 -96 h LC_{50}) plus chitosan (2 mg L^{-1}).The forth groups were exposed to 75µg of $HgCl_2$ plus dried *S. platensis* (10 mg L^{-1}).
Fish were fed frequently on a diet containing 30% crude protein at a rate of 2% of live body weight.

Siphoning three quarters aquariums were done every day for waste removal and replacing it by an equal volume of new water containing the same concentration of Hg, chitosan and dried *S. platensis*.

2.4.7 Statistical analysis

The data obtained were statistically analyzed using the Trimmed Spearman Karber method Hamilton ., (26) for estimating the median lethal concentration (LC_{50}-96 h) Sprague, (27)

RESULTS AND DISCUSSION

3. Results

3.1 LC_{50}

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

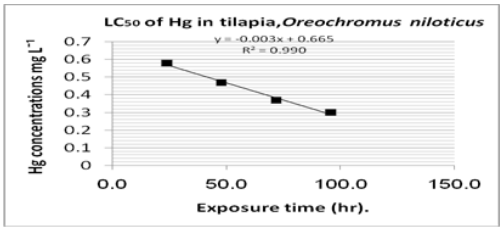

Figure 1. The 96 h LC_{50} value for Hg in O. niloticus

3.2 Hematological Changes

The present study reveals that fish exposed to Hg alone showed significant reduction ($P<0.05$) in RBCs, Hb and HCT than those exposed to Hg with Spirulina platensis or Chitosan.

Table 1. Changes in erythrocyte count (millions/mm3), Hemoglobin (g%) and hematocrite value (%)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)	0.030±0.008[a]	1.390±0.170[a]	4.320±0.354[a]	12.441 ±1.652[a]
Hg alone (75 µg L^{-1})	69.880±0.156[b]	1.090±0.170[b]	3.220±0.554[b]	10.344 ±1.852[b]
Hg + *Chitosan* (2mg)	22.92 ±0.426[c]	1. 55±0.110[a]	4.440±0.534[a]	11.910 ±0.322[a]
Hg + *S.platensis* (10mg)	4.95 ±0.085[c]	1.255±0.110[a]	5.440±0.764[a]	13.210 ±0.322[a]

3.3 Enzymatic Changes

Table 2, shows that AST activity increased significantly in plasma of fish exposed to Hg only. The addition of Spirulina platensis or chitosan 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 or chitosan became proximately kindred to that of control at 15 days.

Table 2: 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 .

Table 2. 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	Water	*Erythrocyte count (RBCs)*	Hemoglobin(HB)	Haematocrit value (Hct)
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 + *Chitosan* (2mg)	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

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

The addition of Spirulina platensis or chitosan to the Hg polluted media reduced significantly (P<0.05) Hg level in aquarium's water as compared to that of Hg alone, Table 3.

3.4 Hg Bioaccumulation

The highest bioaccumulation of mercury was observed in the liver followed by muscles. Mercury hardly traced in the liver of the control fish where the mean residue was 0.029 ± 0.007 µg g^{-1} while those exposed to Hg alone (75 µg L^{-1}), the mean accumulated quantity was 1.756 ± 0.22 µg g^{-1}, Table 3.

Table 3. Changes in mercury (Hg) residue in water (µg Hg L^{-1}), liver and muscles (µg Hg g^{-1} dry weigh) of Nile tilapia (O. niloticus) exposed to Hg with and without Spirulina platensis or chitosan

Group	Water	*Erythrocyte count (RBCs)*	Hemoglobin(HB)
Control (metal free water)	0.030±0.008[a]	0.048±0.013[a]	0.029±0.007[a]
Hg alone (75 µg L^{-1})	69.880±0.156[b]	1.260±0.22[b]	1.576±0.22[b]
Hg + *Chitosan* (2mg)	22.92 ±0.426[c]	1.070±0.038[c]	0.352±0.092[b]
Hg + *S.platensis* (10mg)	4.95 ±0.085[c]	0.922±0.048[d]	0.120±0.089[a]

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

In muscles of control fish, the mean residue was 0.048 ± 0.013 µg g^{-1} while in fish exposed to Hg alone (75 µg L^{-1}), the mean accumulated quantity was 1.260±0.22 µg g^{-1}, Table 3.

3.5 Histopathological alterations

It was noticed that about 40, 60, 30, 15 and 25% of fish exposed to 1\4 LC_{50} of mercury (75 µg L^{-1}) for 15 days showed extensive pathological lesions in their gills, liver, muscle, intestine and kidneys. The observed lesions in the gills were summarized as mucus coagulation and accumulation of cellular debris in the epithelium of lamellae and inter-lamellar regions, hemorrhage, lamellar edema, hyperplasia, epithelial cell necrosis and finally congestion.

The liver of treated tilapia showed degeneration of the hepatocytes with nuclear pyknosis in the majority of the cells, hepatocellular vacuolation as well as the accumulation of the metal binding proteins in their nuclei. Intravascular hemolysis is usually perceived in blood vessels and sinusoids with necrosed hepatocytes.

The muscular tissues showed degeneration in their muscle bundles with aggregations of inflammatory cells (leucocytic infiltration) in between with focal areas of necrosis, atrophy, edema of muscle bundles as well as splitting of muscle fibers were observed.

The intestine of tilapia manifested necrosed mucosa, sub-mucosal hemorrhage, loosely arranged muscle fibers 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 the basement membrane of the villi and blood vessel as well as the lymphocytes. There was a degeneration of the columnar epithelium of the intestine.

The 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.

4. Discussions

The 96 h LC_{50} value for Hg in O. niloticus, was calculated by the simple graphic method to be 0.30 mg $HgCl_2$ L^{-1}. Ishikawa . (28) and Kaoud & Mekawy (29) found that the 96 h LC_{50} values for Hg in Nile tilapia, O. 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 ., (30) 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 ., (28); Buhl, (31) and Boening, (32). The acute toxicity of waterborne heavy metals on aquatic organisms is highly variable even among the closely related species and depends on metal speciation and the amount of free ions WHO, (33).

Histopathological biomarkers have been commonly used in fish to identify and evaluate the toxic effects of pollutants's exposure Rabitto, (34); Oliveira Ribeiro, (35). The presence of necrosis is in fact, one of the most visible damages in tissues affected by a pollutant Rabitto, (34). According to Manahan (36), the occurrence of necrosis is also a consequence of enzymatic inhibition, damages in the cellular membrane integrity, as well as disturbances in the synthesis of proteins and carbohydrate metabolism.

The addition of Spirulina platensis and chitosan 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 with Hg alone was 69.880±0.156µg Hg L^{-1}. The additament of chitosan (2mg L^{-1}) significantly ($P<0.05$) reduced Hg in water to 22.92 ±0.426 and to 4.95 ±0.085 by the addition of Spirulina platensis (10 mg L^{-1}). The highest amount of Hg residue was found in the liver after 15 days of exposure. The uptake of Hg in the liver of fish exposed to Hg alone was 1.576±0.2 µg g^{-1} dry weight. However, it was declined significantly ($P<0.05$) to 0.352±0.092 and 0.120±0.089µg g^{-1} by the addition of 2 mg and 10 mg L^{-1} of chitosan and Spirulina platensis, respectively. Similar trends were observed in fish muscles. Spirulina (Arthrospira) has gained a high economic value Cohen ., (37) particularly because it contains some fine compounds such as essential fatty acids and amino acids, antioxidant vitamins and minerals, etc. at relatively elevated concentrations Roughan, (38),

Several species of cyanobacteria and algae have been kenned to adsorb and take up heavy metal ions Kuyucak and Volesky, (39). Components found in the cell wall of Spirulina, such as peptidoglycan, teichuronic acid, teichoic acid, polysaccharides and proteins Schiewer and Wong, (40) which display mainly carboxylic, hydroxyl and phosphate groups may give algal wall binding properties Aksu, (41), Markai, (42).

The 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, (12); Ari, (43). Furthermore, it has been found that microalgae to be very effective biosorbents, as they possess a large surface area and high binding affinity Roy, (11).

Cd-binding complex was isolated from Chlorella fusca and has shown to be composed of phytochelating peptides $(y\text{-Glu-Cys})_n$ – 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 , (44).

Chitosan is nitrogenous polysaccharides that are made up of acetylglucosamine and glucosamine units. The basic chemical structure: (1,4)-2- amino-2-deoxy-β-D-glucan Sorlier . (45).

Numerous studies have demonstrated that chitosan posses a great sorption capacity and favourable kinetics for most metals. Reviews have been presented by Guibal (46) and Gerente .(47).

CONCLUSION

The present results indicate that Spirulina platensis biomasses and Chitosan are effective in removing Hg from water and reducing Hg bioaccumulation in Tilapia fish. The addition of Spirulina platensis or Chitosan reduced significantly ($P<0.05$) the Hg level in water and the metal uptake as compared to fish exposed to Hg alone.

From the economic point of view, Blue-green alga Spirulina platensis is a promising object for mercury absorption and removing processes of contaminated aquacultures.

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