EFFECT OF WATER HYACINTH AND CHLORELLA ON WATER POLLUTED BY HEAVY METALS AND THE BIOCHEMICAL AND PATHOPHYSIOLOGICAL RESPONSE OF EXPOSED FISH

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Abstract

This study aimed to investigate the ability of selected macrophytes to purify some metal pollutants from the water and the effect of such reaction on fish. A total of 720 from each of Nile tilapia (*Oreochromis niloticus*) and African Catfish (*Clarias gariepinus*), with mean initial weight 40±4 g and 50±5 g respectively, were collected from Abbassa Fish Hatchery. They were divided to 13 subgroups, each of 3 replicates. Each replicate was reared in a glass aquarium and fish were given a diet of 25% crude protein. The ranges of water parameters were, pH (7.9-8.2), temperature (25-29 ºC), total ammonia (0.4-0.6 mg/l), salinity (0.2 g/l) and dissolved oxygen (5.2-6.4mg/l). Thirty nine aquaria were used for each experimented fish species and divided to four equal groups. Groups 1-4 survived in water exposed to Pb (0.01±0.001), Cd (0.01±0.001), Hg (0.001±0.001) and a mixture of Pb +Cd + Hg, respectively. The 5th group served as a negative control. The first three aquaria of each treated group were provided by water hyacinth *(Eichhornia crassipes)*, other 3 aquaria were stocked with *Chlorella* sp. and the last three aquaria were left with the metals without treatment. The water and gill, liver as well as muscles of experimented fish were analyzed for metal concentrations. Blood samples and tissue specimens were tested for serum biochemistry and histopathology. The results showed the negative impact of such metals, in a single or mixed form on the serum level of cholesterol, triglycerides, LDH, acid phosphatase and alkaline phosphatase. This negative effect was also recognized through the remarkable circulatory, degenerative, necrotic and inflammatory changes in various organs of experimented fish. On the other hand, the results also demonstrated the efficiency of macrophytes (hyacinth and chlorella) in reducing the pollutant effect of some heavy metals in water, and decreasing their negative impact and residual effect on the exposed fish.

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INTRODUCTION

The heavy metal ions $Cu^{2}+$, $Zn^{2}+$, $Mn^{2}+$, $Fe^{2}+$, $Ni^{2}+$, and Co^{2+} are essential micronutrients for plants, with $Fe^{2}+$ being required by the highest concentrations, while Cd^{2+}, Hg^{2+}, and Pb^{2+} are the non essential metals (Kunze *et al.*, 2001). However, when present in excess, all these metals are toxic. Each plant species has

different tolerance levels to the different contaminants. Tilstone and Macnair (1997) defined heavy metal tolerance as the ability of plants to survive concentrations of metals in their environment that are toxic to other plants.

Several aquatic macrophytes have been used for the removal of heavy metals from the waste water. The use of these plants in biomonitoring of metals (Cardwell *et al.*, 2002) or as biofilters for polluted water (Dunbabin and Bowmer, 1992), and the aspects of removal (Miretzky *et al.*, 2004, Hassan *et al.*, 2007) besides the toxicity of these metals for the plants (Drost *et al.*, 2007) were studied.

In recent years much attention has been given to wastewater treatment using the aquatic plants and recycling of the treated water. After treatment, these aquatic plants can be used for biogas production, as fiber, compost production for solid waste amendments (Haque and Sharma, 1986). Among all, the aquatic macrophytes *Eichhornia crassipes*, *Lemna minor* and *Spirodela polyrhhiza* have a very high growth rate and heavy metal accumulation capacity (Cardwell *et al.*, 2002, Miretzky *et al.*, 2004, Hassan *et al.*, 2007). These plants can survive in extreme conditions and can tolerate very high concentrations of heavy metals which make them an excellent choice for Phytoremediation. Studies on this aspect are restricted to one or a few plants and focus on the removal of 1–3 selected metals.

Aquatic macrophytes have great potential to accumulate heavy metals inside their plant body. These plants can accumulate heavy metals 100,000 times greater than in the associated water (Mishra *et al.*, in press). Therefore, these macrophytes have been used for heavy metal removal from a variety of sources (Miretzky *et al.*, 2004, Hassan *et al.*, 2007, Mishra *et al.*, 2008). The aquatic macrophytes are thought to remove metals through their attachment to the cell wall, accumulation in the root or some parts of the plant.

Analysis of biochemical parameters could help to identify the target organs of toxicity as well as the general health status of animals. It may also provide an early warning signal in stressed organism (Folmar, 1993). The plasma transaminase GOT GPT, as well as acid and alkaline phosphatases (entering the blood after the cell necrosis of certain organs) can be used to establish the tissue damage of the liver and kidney (Nemcsok and Boross 1982). Environmental stress caused marked elevations in plasma glucose levels (Martin and Black 1998). The elevated activities of lactate dehydrogenase in blood reflect damage to the liver, kidney and muscle tissues (Kalender *et al.*, 2005). Cholesterol is an essential structural component of cell membranes, it is the outer layer of plasma lipo proteins and the precursor of all steroid hormones. The primary function of triglycerides is to store and provide cellular energy (Yang and Chen, 2003).

The present study aimed to investigate the capacity of selected aquatic plant (water hyacinth and *Chlorella)* to remove some metal pollution from the water and the effect of such reaction on the serum biochemistry and histopathology of affected fish.

MATERIALS AND METHODS

Fish: A total of 720 from each of Nile tilapia (*Oreochromis niloticus*) and African Catfish (*Clarias gariepinus*), mean initial weight of 40 ±4 g and 50 ±5 g respectively, were collected from Abbassa Fish Hatchery (Central Laboratory for Aquaculture Research (CLAR) Abou Hammad, Sharkia, Egypt. They were divided into four equal groups (each of 180 fish), each of 3 equal subgroups (60 fish each), each subgroup of 3 replicates (20 fish/replicate). Another 60 fish from each species were served as a control negative group and subdivided into 3 equal replicates. Each replicate were reared in a glass aquarium (40×30×40 cm) that was supplied with an aerator and acclimatized for two weeks. Fish were given a diet of 25% crude protein two times per day at feeding levels of 3% from the live body weight, 5 days per week.

Water quality: The average water quality parameters ranges were, pH (7.9-8.2), temperature (25-29 ºC), total ammonia (0.4-0.6 mg/l), salinity (0.2 g/l) and dissolved oxygen (5.2-6.4mg/l).

Chemicals: The analytical grade pure chemicals were used as source of metal ions: cadmium chloride ($CdCl_2 2.5\ H_2O$), Mercury sulphate ($HgSO_4.2H_2O$), and lead chloride ($PbCl_2$). Stock solutions of heavy metals were obtained by dissolving each metal salt in distilled water and the pH of the tested solutions was adjusted to 7.0.

Experiment: Thirty six aquaria were used for each experimented fish species and divided to four equal groups (each of 9 aquaria). Groups 1-4 survived in water exposed to Pb (0.01±0.001), Cd (0.01±0.001), Hg (0.001±0.001) and a mixture of Pb +Cd + Hg, respectively. The 5th group served as a control negative group. The first three aquaria of each group were provided by water hyacinth; the other 3 aquaria were stocked with *Chlorella* sp. and last three aquaria were left with the metal without treatment. In this experiment, 8 plants of water hyacinth *(Eichhornia crassipes)* with an average weight of 45 g± 6.5/ plant and 1 liter *chlorella* sp. ($3.5×10^6$ organism/l) were used. The experiment was extended to 45 days period where fish samples were taken every ten days from all aquaria for analyses.

Metal analysis: The gills, liver and muscles were sampled for the analysis of metal concentration. Eight fish were collected each ten days from all groups including the control. Tissue samples were dried at 65 ºC and kept in desiccators until digestion. Dry tissue was digested with 1:1 HNO_3 (Suprapur1 grade, Merck, Germany) and samples were fumed to near dryness on a hot plate at 120 ºC overnight. After

digestion, the residue was dissolved in 10 ml of 0.2 N HNO_3 and kept in a refrigerator until analyzed for the heavy metals. Cadmium concentrations of tissues were measured using a graphite furnace atomic absorption spectrophotometer (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK). Accumulation factor (AF) is often used to compare the body burden of an organism with the degree of contamination in the water. The following definitions are used here: Accumulation Factor $\overline{o}$AF= Mefw, exp-Me $_{fw, control}$/ Me$_{water}$ where [Me] fw, exp., [Me] fw, control, [Me]water are the metal concentrations in the experimental group, control group and water, respectively, in mg/g (Holwerda, 1991).

Biochemical Estimations: Fish from each experimental and control groups were bled from the dorsal aorta into sterilized glass vials at 4 °C containing the anticoagulant, 1% dipotassium 4ethylenediamine tetra acetate (EDTA). Phosphomonoesterases such as acid phosphatase and alkaline phosphatase activity were assessed according to Hillmann (1971). The lactate dehydrogenase (LDH) activity was measured according to the method reported by Dito (1979). Cholesterol level was determined by the method of Henry (1974). Triglycerides were analyzed by the method of Schettler and Nussel (1975).

Histopathological examination: Tissue specimens of experimented fish were fixed in 10% phosphate buffer formalin. Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin (H & E) (Drury and Wallington, 1980).

Statistical analysis: It was performed using the analysis of variance (ANOVA). Duncan's Multiple Range Test was used to determine the significant differences between means at $P<0.05$. Standard errors of treatment means were also estimated. All statistics were carried out by using Statistical Analysis Systems (SAS) program (SAS, 2000).

RESULTS AND DISCUSSION

Knowledge of heavy metal concentrations in fish is important both with respect to nature management and human consumption of fish. The highest metal concentrations were found in the liver and gills, while the muscle tends to accumulate less metal. The metal concentration in the muscles tissue is important for the edible parts of the fish. The mean concentrations of heavy metals analyzed in the muscles of tilapia and catfish (Table 1) were lower than the maximum permitted concentrations proposed by FAO (1983) in biological treatments. In catfish, heavy metal concentrations in the muscles were higher than those observed in tilapia. Heavy metal levels in different species depend on feeding habits (Mormede and Davies, 2001, Watanabe *et al.*, 2003), age, size and length of the fish (Al- Yousuf *et al.*, 2000) and their habitats (Canli and Atli, 2003). The concentrations of metals in the gills (Table 2)

reflect the concentrations of metals in the waters where the fish species live, whereas the concentrations in the liver represent storage of metals (Rao and Padmaja, 2000). Thus, the liver and gills in fish are more often recommended as environmental indicator organs of water pollution than any other organs. This is possibly attributed to the tendency of liver and also the gills to accumulate pollutants at different levels from their environment as previously reported in the literature (Al-Yousuf *et al.*, 2000, Canli and Atli, 2003). Studies carried out with different fish species have shown that heavy metals accumulate mainly in the metabolic organs such as liver (Table 3) that stores metals to detoxicate by producing metallothioneins (Hogstrand and Haux, 1991). Heavy metals concentrations were lower in the muscles compared to the liver and gills as measured in the two species of this study. Among the metals, Pb had the highest mean value and Hg was lowest in the muscles. Similar results were reported from a number of fish species where the muscles are not an active tissue in accumulating heavy metals (Karadede and Unlu, 2000).

Heavy metal levels were higher in the gills than the muscles tissue of fish with regard to mercury. Metal concentration in the gills could be due to the adhesion of these elements with the mucus that makes it difficult to be completely removed from the lamellae, before tissue is prepared for analysis. The adsorption of metals onto the gills surface, as the first target for pollutants in water, could also be an important influence in the total metal levels of the gills (Heath, 1987). Results presented in this study in table 2 indicated that all metals in the tilapia tissues were lower than those determined in catfish all treatments. Mercury in fish is good indicator for exposure to organic or methyl-mercury contamination. The mercury in fish appears in the form of methyl-mercury (Al-Majeed and Preston, 2000). Therefore, fish diet could be the main source of exposure to methyl-mercury. Therefore, results of this study provide a basis for assessment of human exposure to methyl-mercury. The concentrations of mercury in the fish samples obtained in this study in water hyacinth and Chlorella treatments are not high when compared to other elements. Mercury in the edible portion of various fish species landed at Irish ports during 1993 ranged of 0.1–0.39 with a mean of 0.1 within the values of our study (Nixon *et al.*, 1994). These levels reported to be low and are within the maximum limits of the European Commission for mercury in fisheries products. The accumulation of metals in fish tissues increased with increasing period, the accumulation of Pb in fish tissues higher than other metals. Relatively high concentrations of heavy metals in gills and liver have been found in different fish species (Tables 2 & 3).

From the data presented in table (4), all the tested heavy metals concentrations in water during the experimental period varied significantly from the control groups

(tilapia and catfish) due to the uptake of metals by water hyacinth and chlorella. The highest uptake of metals was recorded in the first ten days followed by second ten days and third. These results indicate that the water hyacinth and chlorella could accumulate all metals during 20 to 30 days by about 4000-10000 times than concentrations in water, this supports the results of Shaker, (2006) and Hassan, *et al.*, (2007). Also, we can conclude that of water hyacinth and chlorella can be used as a biological treatment of polluted water to remove all metals until the permissible limits during about 20 to 30 days.

Floating aquatic macrophyte-based treatment systems also have potential for removing and recovering nutrients and metals in wastewaters from animal-based agricultural operations. In addition to the advantages cited by Hammer (1992) for wetlands, FAMTS have the following positive attributes: (1) high productivity of several large-leaf floating plants, (2) high nutritive value of floating plants relative to many emergent species, and (3) easy in stocking and harvesting. Relatively few studies have been reported on the use of floating plants and phytoplankton-based wastewater treatment. The removal of heavy metals by water hyacinth *(Eichhornia crassipes)* ranged from 80-90% in Pb, 80-90% in Cd and 60-75% in Hg. The removals of heavy metals in all treated groups are lower than non treated metal exposed groups. These results may be due to the complexity of these metals to reduce the accumulation by the aquatic plant and phytoplankton (Tables 4 & 5, graph 1).

The effect of metals (Pb, Cd, Hg and Pb +Cd +Hg) in the blood plasma parameters of *Oreochromis and Clarias* are shown in Tables 6 and 7. Heavy metals pollution stresses the animals and disturbs their metabolism inhibit enzymes, damage and dysfunction the tissues and retard their growth all that associated with biochemical changes. The heavy metals have their own target sites of action, and most of them are metabolic depressors. They generally affect the activity of biologically active molecules such as transaminases, phosphomonoesterases and other enzyme [Vijayavel and Balasubramanian, 2006]. A significant to non significant increase in the acid and alkaline phosphatase activity, in the present work, was observed after chronic exposure to Pb, Cd, Hg and Pb+Cd+Hg with and without water hyacinth and *Chlorella*. Acid phosphatase is a lysosomal enzyme that hydrolyses the phosphorous esters in acidic medium. This enzyme is hydrolytic in nature and acts as one of the acid hydrolyses in the autolysis process of the cell after its death. Alkaline phosphatase splits various phosphorous esters at alkaline pH, its activity is related to the cellular damage. The significant difference in phosphatases activities between the control and experimental groups of fish following the exposure to the heavy metals may be due to the damage of hepatic tissue with disturbed normal liver function as

seen in the histopathological examination. Increased activity of acid phosphatase and alkaline phosphatase in blood plasma could indicate the hepatic damage by the heavy metals. The increase in alkaline phosphatase activity after the exposure of gallium has been implicated due to the direct toxicity of pesticide in fish liver (Yang and Chen, 2003).

LDH is a tetrameric enzyme recognized as a potential marker for assessing the toxicity of a chemical. The elevated levels of LDH in the hemolymph might be due to the release of isozymes from the destroyed tissues. The LDH level in the blood of the chronic exposed fish, in the current study, was decreased in all treated groups. The decrease in the metal exposed group was higher than those treated with water hyacinth and *chlorella*. Several reports have revealed decreased LDH activity in tissues under various pesticide toxicity conditions (Tripathi and Shukla 1990, Mishra and Shukla 2003). This might be due to the higher glycolysis rate, which is the only energy-producing pathway for the animal when it is under stress conditions. LDH is an important glycolytic enzyme in biochemical systems and is inducible by oxygen stress. The significant decline of lactate dehydrogenase activity in *Oreochromis niloticus* and *Clarias gariepinus* blood plasma further suggest the decrease in the glycolytic process due to the lower metabolic rate as a result of heavy metals exposure. Similar findings have been described in plasma of *Oncorhynchus mykiss* acutely exposed to lindane, and *Anguilla anguilla* exposed to insecticide, polychlorinated biphenyls (Strmac and Braunbeck, 2002 and Balint, 1997).

Cholesterol is a steroid lipid found in the cell membranes of all body tissues and transported in the blood plasma. In the present study, the cholesterol content in blood was significantly decreased after chronic of exposure to heavy metals without using the water hyacinth or *Chlorella*. The significant decrease in blood plasma cholesterol level of *Oreochromis niloticus and Clarias gariepinus, after the* exposure to heavy metals without water hyacinth and *Chlorophyll sp.,* was similar those noticed in *Heteropneustes fossilis after* exposure to aldrin and *Oreochromis mossambicus after* exposure to urea (Balasubramanian *et al.*, 1999).

Triglycerides are used to evaluate nutritional status, lipid metabolism, and their high concentrations may occur with nephritic syndrome or glycogen storage disease (Yang and Chen, 2003). In the present study, the activity of triglycerides, after chronic exposure to heavy metals, was significantly decreased than those of control or the groups treated with water hyacinth and *Chlorella sp.* A significant decrease in triglycerides content in the blood plasma of *Cyprinus carpio* by the action of gallium has been shown as an indication of its adverse effects on liver (Yang and Chen 2003).

A similar effect of sublethal concentration of malathion has also been reported in *Clarias batrachus* (Lal and Singh, 1987).

The microscopic examination in the gills and internal organs of the control group revealed no marked pathological changes with normal tissue architecture and cellular details. Some pollutants originate from industrial wastes or agriculture discharges as heavy metals (El-Nabawi *et al.*, 1987). Among those, lead is a highly toxic and cumulative poison to fish. It causes black tails, lordoscoliosis, paralysis, muscular atrophy and degeneration of the caudal fin (Hodson *et al.*, 1982). The histopathology of fish (*Oreochromis niloticus* and *Clarias gariepinus,*) after the chronic exposure to lead in this study, revealed congestion in the gill lamellae and hemorrhage in the gill arch with focal desquamation in the secondary lamellae (Fig. 1). The muscles exhibited hyaline degeneration. Marked degeneration and necrosis in the hepatocytes with nuclear pyknosis were seen. The hepatic vessels contained erythrocytes where some of them were hemolyzed. Proliferation of melanomacrophages was evident in the hepatic parenchyma (Fig. 2). The kidney suffered tubular nephrosis mainly vacuolar degeneration and/or coagulative necrosis and focal necrosis and/or depletion in the hematopoietic and melanomacrophage cells were seen (Fig. 3). Lead also induced alterations in so-me hematological and serological parameters (Gill *et al.*, 1991). The sublethal dose, of lead, acts as a stressor causing immunosupression and increased the susceptibility of fish to infectious diseases (Mazeaud *et al.*, 1977). Our histopathological results are in agreement with Hafez *et al.*, (2003).

Fish exposed to cadmium could show toxicity or unusual responses (Sprague, 1987). The histopathology of fish (*Oreochromis niloticus and Clarias gariepinus),* after the chronic exposure to cadmium, showed congestion in the central venous sinus of the gills and focal epithelial desquamation in the secondary lamellae. The liver showed nuclear pyknosis of hepatocytes with degeneration in the pancreatic acini besides degeneration as well as necrosis of melanomacrophages (Fig. 4) Hyaline degeneration and/or Zenker's necrosis, in the muscles, beside edema and leukocytic infiltration and melanomacrophages proliferation were noticed (Fig. 5). The kidney revealed tubular nephrosis and necrosis in the hematopoietic tissue. It was stated previously that chronic exposure to cadmium produces histopathologicasl changes similar to those reported in the present study (Aly and Nouh, 2004).

The histopathology, of fish (*Oreochromis niloticus and Clarias gariepinus)* after the chronic exposure to mercury, showed congestion and hemorrhages in the gill lamellae. The muscles exhibited hyaline degeneration and/or Zenker's necrosis beside cellular infiltration and some melanomacrophages proliferation. The liver revealed focal congestion and hemorrhage together with marked vacuolar degeneration in the

hepatocytes, nuclear pyknosis and focal necrosis (Fig. 6). The kidney revealed tubular degeneration and focal depletion in the hematopoietic tissue. The histopathological findings of mercury exposed fish were more or less similar to those reported by Galab (1997) but were dose and period dependant.

The microscopic findings of mixed heavy metal pollution were more severe in picture but, after the addition of the aquatic plant (water hyacinth) or the phytoplankton (Chlorella) were decreased in severity in comparison to the metal exposed group only, where the gills showed mild edema in the gill arch and hyperplasia in the gill lamellae (Fig. 7). The muscles suffered hyaline degeneration and melanomacrophage proliferation. The liver revealed congestion and mild degeneration besides aggregation of melanomacrophages. The kidneys showed mild degeneration and proliferation of hematopoietic tissue (Fig. 8). The improvement in these pictures, by using the chelating agents, supports the concept of Hilmy *et al.*, (1986).

It could be concluded that, the removal of heavy metals by aquatic plant (water hyacinth) and phytoplankton (*Chlorella*) improved the water quality and the health status of exposed fish.

Legends

Fig. 1. Gills, of *Oreochromis niloticus* after the chronic exposure to lead, showing mild congestion in the gill lamellae and focal desquamation in the secondary lamellae, H & E stain, x 100.

Fig. 2. Liver, of *Oreochromis niloticus* after the chronic exposure to lead, showing degeneration and necrosis in the hepatocytes and proliferation of melanomacrophages, H & E stain, x 250.

Fig. 3. Kidney, of *Oreochromis niloticus* after the chronic exposure to lead, showing vacuolar degeneration and coagulative necrosis besides focal necrosis in the hematopoietic and melanomacrophage cells, H & E stain, x 250.

Fig. 4. Liver, of *Oreochromis niloticus* after the chronic exposure to cadmium, showing necortic hepatocytes with degeneration and necrosis of melanomacrophages, H & E stain, x 250.

Fig. 5. Muscles, of *Oreochromis niloticus* after the chronic exposure to cadmium, showing hyaline degeneration, Zenker's necrosis, beside edema and leukocytic infiltration, H & E stain, x 250.

Fig. 6. Liver, of *Clarias gariepinus* after the chronic exposure to mercury, showing vacuolar degeneration in the hepatocytes, nuclear pyknosis and focal necrosis, H & E stain, x 400.

Fig. 7. Gills, of *Clarias gariepinus* after the chronic exposure to a mixture of Pb, Cd and Hg, showing mild edema in the gill arch and hyperplasia in the gill lamellae, H & E stain, x 250.

Fig. 8. Kidneys, of *Oreochromis niloticus* after the chronic exposure to a mixture of Pb, Cd and Hg, showing mild degeneration and proliferation of hematopoietic tissue, H & E stain, x 250.

Table 1. Average values of heavy metals in the muscles of Nile tilapia and catfish after experimental period.

Treat Item	Fish	Pb (µg/g)	Cd (µg/g)	Hg (µg/g)	Mixture Pb (µg/g)	Cd (µg/g)	Hg (µg/g)
control	Tilapia	11.6^a ±1.3	10.6^a ±1.1	3.9^a ±0.2	10.1^a ±1.1	8.9 ^{a} ±1	2.8^a ±0.4
	Catfish	11.9^a ±1.4	11.1 ^{a} ±1	3.9 ±0.3a	10.7^a ±1.2	9.3 ^{a} ±1	3.1^a ±0.4
chlorella	Tilapia	1.1 ^{b} ±0.1	0.9^b ±0.1	2.9^b ±0.2	0.8 ^{b} ±0.1	0.8 ^{b} ±0.1	0.7^b ±0.1
	Catfish	1.3 ^{b} ±0.1	1.1^b ±0.1	2.9^b ±0.2	1.1 ^{b} ±0.1	1.0^b ±0.1	0.7^b ±0.1
water hyacinth	Tilapia	1.2 ^{b} ±0.1	1.0^b ±0.1	3.0^b ±0.3	1.0 ^{b} ±0.1	0.9^b ±0.1	0.8^b ±0.1
	Catfish	1.2 ^{b} ±0.1	1.1^b ±0.1	3.1^b ±0.2	1.1 ^{b} ±0.1	1.1^b ±0.2	0.8^b ±0.1

Means in the column followed by different letters are significantly different
(Duncan s Multiple Range Test P<0.05).

Table 2. Average values of heavy metals in the gills of Nile tilapia and catfish after the experimental period.

Treat Item	Fish	Pb (µg/g)	Cd (µg/g)	Hg (µg/g)	Mixture Pb (µg/g)	Cd (µg/g)	Hg (µg/g)
control	Tilapia	45.5^a ±4.2	38.5^a ±3.8	25.1^a ±2.2	38.6^a ±4.1	33.7^a ±3.2	22.4^a ±2.1
	Catfish	48.6^a ±5.1	40.9 ±3.6a	28.3^a ±3.1	41.7^a ±4.2	36.1^a ±3.4	22.9^a ±2.1
chlorella	Tilapia	9.5 ±2.1b	9.2 ^{b} ±1.2	5.1 ±0.8b	8.1^b ±1.1	8.2^b ±1.1	4.3^b ±0.2
	Catfish	11.1^b ±2.1	9.9^b ±1.2	6.7 ^{b} ±0.6	9.2^b ±1.1	8.4^b ±1.2	5.1^b ±0.3
water hyacinth	Tilapia	10.1^b ±1.8	9.2 ^{b} ±1.1	5.5 ^{b} ±0.6	9.3^b ±1.0	8.3^b ±1.1	5.3^b ±0.3
	Catfish	11.2^b ±1.9	10.7^b ±1.4	6.9 ^{b} ±1.0	10.3^b ±1.6	8.7^b ±1.1	5.6^b ±0.3

Means in the column followed by different letters are significantly different
(Duncan s Multiple Range Test P<0.05).

Table 3. Average values of heavy metals in the liver of Nile tilapia and catfish after experimental period.

Treat \ Item		Pb (µg/g)	Cd (µg/g)	Hg (µg/g)	Mixture µg/g		
					Pb (µg/g)	Cd (µg/g)	Hg (µg/g)
control	Tilapia	42.2[a] ±2.4	35.1[a]±3.5	24.8[a]±3.3	39.5[a]±4.2	34.1[a]±3.8	21.8[a]±2.2
	Catfish	48.5[a] ±3.6	40.1[a]±4.2	27.5[a]±3.1	40.9[a]±4.1	35.1[a]±4.1	22.5[a]±3.2
chlorella	Tilapia	8.7[b] ±1.1	8.1[b]±1.7	4.5[b]±0.6	7.7[b]±1.1	7.4[b]±1.2	4.1[b]±0.4
	Catfish	11.1[b] ±1.3	9.4[b]±1.6	6.3[b]±1.0	8.9[b]±1.1	8.1[b]±1.2	4.7[b]±0.5
water hyacinth	Tilapia	9.5[b] ±1.1	8.5[b]±1.4	5.5[b]±1.0	9.2[b]±1.3	8.1[b]±1.3	5.1[b]±0.5
	Catfish	11.2[b] ±1.2	10[b]±1.6	6.6[b]±1.0	10.1[b]+1.6	8.3[b]±1.1	5.4[b] ±0.4

Table 4. Average concentrations of heavy metals in water under different treatments during the experimental period.

Days	Treat.	Pb (µg/g)	Cd (µg/g)	Hg (µg/g)	All mixture		
					Pb (µg/g)	Cd (µg/g)	Hg (µg/g)
initial	Initial	0.01[a] ±0.001	0.01 [a] ±0.001	0.001 [a] ±0.0001	0.01 [a] ±0.001	0.01 [a] ±0.001	0.001 [a] ±0.0001
10	Control	0.009 [a] ±0.001	0.009 [a] ±0.001	0.0009 ±0.0001a	0.009 [a] ±0.001	0.009 [a] ±0.001	0.0008 [a] ±0.0001
	chlorella	0.005 [b] ±0.0004	0.006 [b] ±0.0001	0.0005 [b] ±0.0001	0.005 [b] ±0.0002	0.005 [b] ±0.0003	0.0006 [b] ±0.00003
	water hyacinth	0.006 [b] ±0.0003	0.006 [b] ±0.0002	0.0006 [b] ±0.0001	0.006 [b] ±0.0002	0.006 [b] ±0.0002	0.0006 [b] ±0.00002
20	Control	0.008 ±0.001a	0.008 ±0.001a	0.0008 [a] ±0.0001	0.008 [a] ±0.001	0.008 [a] ±0.001	0.0008 [a] ±0.0001
	chlorella	0.003 [bc] ±0.0002	0.003 [c] ±0.0003	0.0003 [bc] ±0.00004	0.003 [bc] ±0.0002	0.003 [bc] ±0.0002	0.0003 [c] ±0.00002
	water hyacinth	0.004 [bc] ±0.0003	0.004 [bc] ±0.0002	0.0003 [b] ±0.00003	0.004 [bc] ±0.0002	0.004 [bc] ±0.0001	0.0004 [bc] ±0.00001
30	Control	0.008 [a] ±0.001	0.007 [a] ±0.0005	0.0008 [a] ±0.0002	0.007 [a] ±0.0001	0.007 [a] ±0.0001	0.0008 [a] ±0.0001
	chlorella	0.002 [c] ±0.0001	0.002 [c] ±0.0002	0.0001 [c] ±0.00003	0.002 [c] ±0.0001	0.002 [c] ±0.0001	0.0002 [c] ±0.00001
	water hyacinth	0.003 [c] ±0.0003	0.003 [c] ±0.0002	0.0002 [c] ±0.00003	0.002 [c] ±0.0001	0.002 [c] ±0.0001	0.0002 [c] ±0.00001
End	Control	0.008 [a] ±0.001	0.007 [a] ±0.0003	0.0007 [a] ±0.0001	0.007 [a] ±0.0003	0.007 [a] ±0.0002	0.0008 [a] ±0.0001
	chlorella	0.002 [c] ±0.0003	0.001 [c] ±0.0002	0.0001 [c] ±0.00003	0.001 [c] ±0.0001	0.001 [c] ±0.0002	0.0001 [c] ±0.00004
	water hyacinth	0.002 [c] ± 0.0004	0.002 [c] ± 0.0003	0.0002 [c] ±0.00003	0.001 [c] ±0.0002	0.001 [c] ±0.0001	0.0001 [c] ±0.00003

Means in the column followed by different letters are significantly different (Duncan s Multiple Range Test P<0.05).

Table 5. Concentration of pb, Cd and Hg in Cholera sp. and water hyacinth leaves and
roots after experimental period.

Treat.	Fish		Pb (µg/g)	Cd (µg/g)	Hg (µg/g)	Mixture		
						Pb (µg/g)	Cd (µg/g)	Hg
chlorella	Tilapia		74.66 b ±4.5	78.92 b ±6.2	70.42 b ±8.1	71.12 b ±8.2	72.14 b ±6.8	62.78 b ±5.6
	Catfish		75.12 b ±5.8	77.36 b ±8.1	72.18 b ±6.7	76.96 b ±6.9	74.18 b ±8.6	66.06 b ±6.1
water hyacinth	Tilapia	Leaves	13.56 c ±2.1	11.12 c ±1.1	10.72 c ±1.2	13.21 c ±2.3	12.36 c ±1.2	8.78 c ±1.2
		Roots	136.56 a ±13.3	136.60 a ±14.2	124.24 a ±10.9	138.78 a ±8.5	132.22 a ±13.1	106.02 a ±10.3
	Catfish	Leaves	13.48 c ±1.2	12.1 c ±2	10.88 c ±1.2	12.78 c ±1.1	12.46 c ±1.3	8.18 c ±1.3
		Roots	132.12 a ±11.8	130.24 a ±10.8	114.14 a ±12.8	134.04 a ±9.9	126.26 a ±10.2	118.08 a ±11.2

Means in the column followed by different letters are significantly different (Duncan s Multiple Range Test
P<0.05).
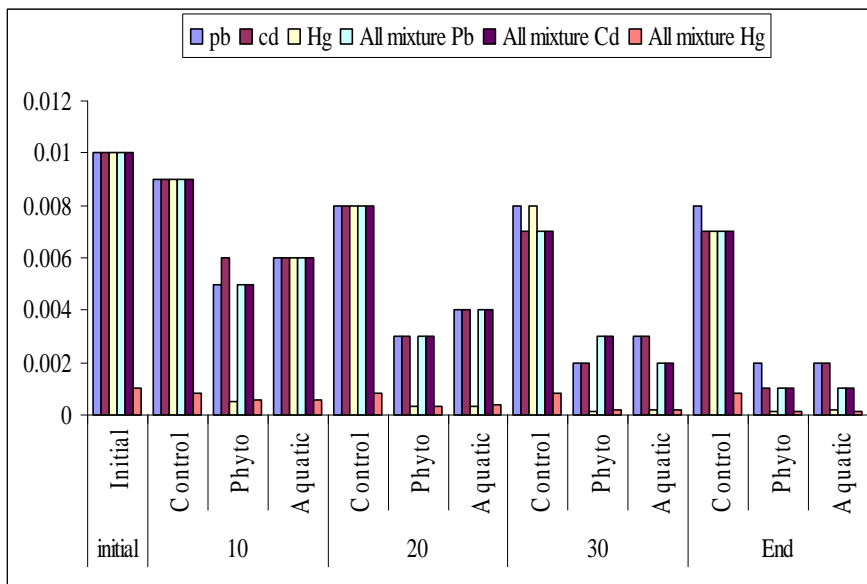
Graph. 1. Effect of aquatic plant and phytoplankton on removal of heavy metals from
water

Table 6. The effect of heavy metals on some biochemical parameters in tilapia nilotica at the end of treatments with Hyacthin and phytoplankton (Chlorella).

Group	Treat.	Chole-sterol	Trigly-cerides	LDH	Acid phosph.	Alk. Phosph.
Lead	Control	80.2 a ± 1.3	50.3 a ±2.2	138.6 a ±3.8	0.322 d ±0.003	0.150 b ± 0.002
	L+H	75.2 b ±1.1	35.8 B ±1.3	120.3 b± 2.9	0.359 c ±0.002	0.210 a ±0.009
	L+C	73.2 b ± 1.2	34.1 b ±1.2	116.5 b± 3.1	0.375 b ±0.005	0.212 a ±0.011
	Lead	69.8 c ±0.9	33.3 b ±1.1	108.3 c± 2.8	0.392 a ± 0.003	0.230 a ± 0.011
Cadmium	Control	80.2 a ±1.3	50.3 a ±2.2	138.6 a ± 3.8	0.322 d ±0.003	0.150 b ± 0.002
	C+H	70.2 b ±1.2	34.2 b ±1.2	106.2 b±2.1	0.448 c ±0.003	0.230 ab ±0.013
	C+C	69.3 b ±2.1	31.1 c ±1.1	96.4 c ± 2.4	0.476 b ±0.008	0.241 a ±0.11
	Cadmium	63.8 c ±1.3	30.5 c ± 1.3	93.5 c± 1.9	0.513 a ±0.009	0.255 a ±0.014
Mercury	Control	80.2 a ± 1.3	50.3 a ±2.2	138.6 a ± 3.8	0.322 d ±0.003	0.150 b ±0.002
	M+ H	57.8 b ± 0.8	26.8 ± b 0.8	96.3 b± 2.2	0.530 c ± 0.009	0.280 b ±0.013
	M+ C	56.4 b ± 0.9	25.6 b ±0.6	86.3 c ±1.8	0.570 b ±0.010	0.310 a ± 0.011
	Mercury	50.3 c ± 0.7	20.2 c ±0.7	82.4 d ± 1.7	0.620 a ±0.009	0.320 a ±0.012
Mixture	Control	80.2 a ±1.3	50.3 a ±2.2	138.6 a ± 3.8	0.322 d ± 0.003	0.150 b ±0.002
	Mx+H	78.6 a ± 1.9	46.2 b ± 1.5	131.2 b± 1.7	0.345 c ± 0.006	0.165 a ± 0.003
	Mx+C	75.0 b ±1.2	43.2 b ± 1.6	126.4 c± 2.3	0.354 b ± 0.011	0.172 a ± 0.008
	Mixture	72.1 c ±0.9	40.2 ±1.8c	121.4 d± 1.9	0.366 a ±0.009	0.185 a ± 0.007

The means have the same latter in the same treatment (Pb, Cd, Hg and their mixture) for the same item are not significant P>0.05

Table 7. The effect of heavy metals on some biochemical parameters in catfish *Clarias gariepinus* at the end of treatments with Hyacthin and phytoplankton.

Group	Treat.	Chole-sterol	Trigly-cerides	LDH	Acid phosph.	Alk. Phosph.
Lead	Control	92.3 [a] ±2.5	56.2 [a] ±1.3	153.2 [a] ±2.6	0.340 [c] ±0.008	0.178 [d] ±0.005
	L+H	76.2 [b] ±1.8	46.3 [b] ±0.8	133.6 [b] ±2.1	0.466 [b] ±0.015	0.230 [c] ±0.008
	L+C	70.3 [c] ±1.8	43.4 [c] ±1.1	130.8 [b] ±1.7	0.478 [a] ±0.017	0.250 [b] ±0.009
	Lead	64.6 [d] ±2.1	40.2 ±0.9d	128.8 ±2.2b	0.489 [a] ±0.019	0.269 [a] ±0.008
Cadmium	Control	92.3 [a] ±2.5	56.2 [a] ±1.3	153.2 [a] ±2.6	0.340 [b] ±0.008	0.178 [c] ±0.005
	C+H	70.3 [b] ±1.6	36.7 [b] ±0.8	128.4 [b] ±3.1	0.538 [a] ±0.017	0.270 [b] ±0.008
	C+C	46.2 [c] ±2.2	33.6 [c] ±0.8	126.2 [b] ±1.8	0.551 [a] ±0.018	0.285 [a] ±0.009
	Cadmium	59.2 [d] ±1.7	32.4 [c] ±0.7	125.3 [b] ±2.1	0.560 [a] ±0.018	0.298 [a] ±0.009
Mercury	Control	92.3 [a] ±2.5	56.2 [a] ±1.3	153.2 [a] ±2.6	0.340 [c] ±0.005	0.178 [d] ±0.005
	M+H	62.3 [b] ±1.4	28.3 [b] ±0.8	170.3 [b] ±1.2	0.602 [b] ±0.021	0.310 [c] ±0.008
	M+C	58.2 [c] ±1.6	22.4 [c] ±0.7	98.2 [c] ±2.3	0.635 [a] ±0.024	0.340 [b] ±0.012
	Mercury	52.3 [d] ±1.5	21.6 [c] ±1.1	96.4 [c] ±1.9	0.670 [a] ±0.018	0.380 [a] ±0.008
Mixture	Control	92.3 [a] ±2.5	56.2 [a] ±1.3	153.2 [a] ±2.6	0.340 [b] ±0.005	0.178 [c] ±0.005
	Mx+H	75.4 [b] ±2.1	48.2 [b] ±1.1	147.2 [b] ±2.8	0.401 [a] ±0.015	0.199 [b] ±0.008
	Mx+C	73.2 [b] ±1.8	45.2 [c] ±0.8	143.2 [b] ± 1.9	0.425 [a] ±0.018	0.205 [b] ±0.009
	Mixture	70.4 [c] ±2.1	40.2 ±1.2d	144.1 [b] ±2.1	0.433 [a] ±0.021	0.225 [a] ±0.008

The means have the same latter in the same treatment (Pb, Cd, Hg and their mixture) for the same item are not significant P>0.05.
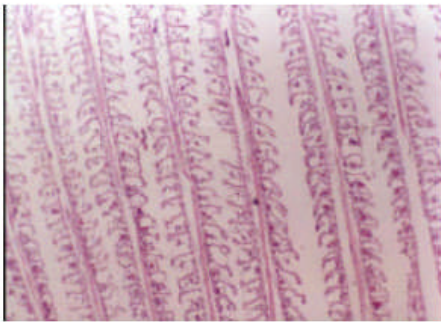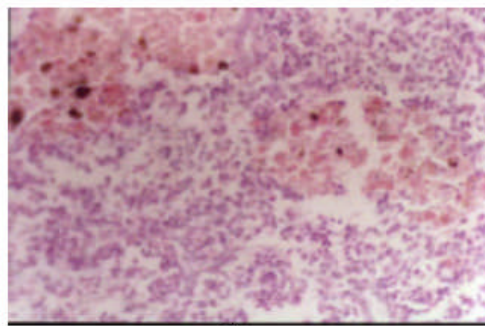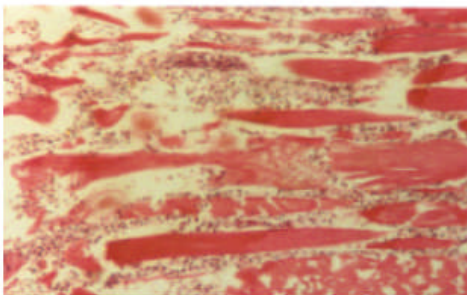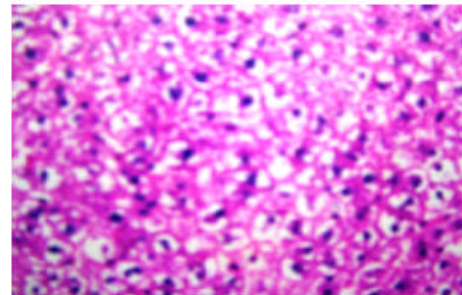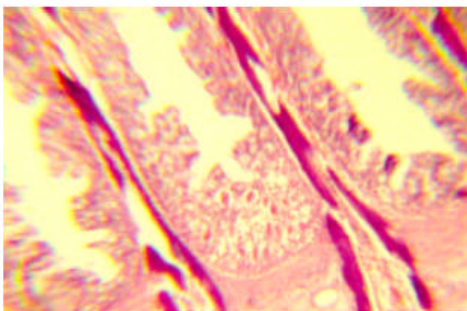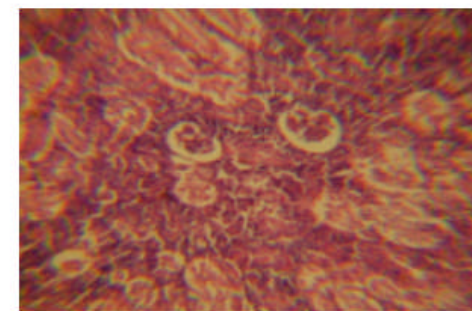
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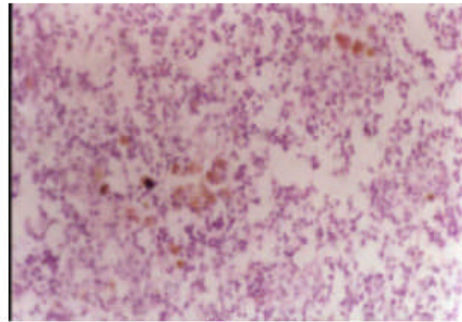
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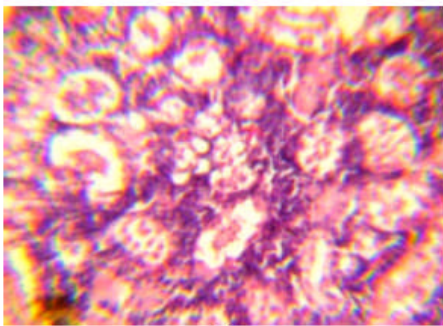
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تأثير ورد النيل وطحلب الكلوريلا على الماء الملوث بالعناصر الصغرى والتأثير البيوكيميائى والباثولوجى على أسماك البلطى النيلى والقرموط الأفريقى

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1- المعمل المركزى لبحوث الثروة السمكية بالعباسة – شرقية
2- المركز الدولى للأسماك (اكلارم) بالعباسة ـ شرقية

فى تجربة معملية لدراسة قدرة نبات ورد البيل وطحلب الكلوريلا على إزالة العناصر الصغرى من المياة الملوثة بعناصر الرصاص- الكادميوم – الزئبق كل على حدة ثم الثلاثة معا وتأثير ذلك على أسماك البلطى والقرموط الأفريقى . تم إختيار 720 سمكة من كل نوع وقسمت الى 12 مجموعة بإجمالى عدد 36 حوض زجاجى لكل حوض تهويته الخاصة. قسمت المجموعة الى عدد 4 تحت مجموعة وهى العناصر بتوزيعها السابق ولكل عنصر تحت مجموعة مقارنة (كنترول) وأخرى للعنصر مع ورد النيل والثالثة مع الكلوريلا وإستمرت التجربة مدة 45 يوم.

وكانت أهم النتائج المتحصل عليها :-

1- تراكم جميع العناصر فى الكبد والخياشيم أكثر من العضلات بإستثناء عنصر الزئبق فلم تكن هناك أى فروق بين الأنسجة الثلاثة.

2- أعلى نسبة تراكم للعناصر داخل أنسجة نبات ورد النيل أو طحلب الكلوريلا كانت فى الأيام العشرة الأولى تليها العشرة الثانية ثم الثالثة وهكذا.

3- قدرة ورد النيل وطحلب الكلوريلا على تجميع من 4000 إلى 10000 ضعف من تركيز العناصر بالمياة .

4- زيادة قدرة ورد النيل وطحلب الكلوريلا على تجميع كميات كبيرة من العنصر فى حالة وجودة بحالة منفردة عنها فى حالة وجودة فى تجمعات مع عناصر أخرى مما يؤدى إلى تكون معقدات يصعب تجميعها.

5- قدرة أسماك القراميط على تجميع العناصر الملوثة للماء أكثر من البلطى.

6- لم تكن هناك فروق واضحة بين ورد النيل أو الكلوريلا فى تجميع العناصر الملوثة للماء.

7- العناصر الملوثة للماء لها تأثير سلبى على الكلوسترول والتراى جلسريد والفوسفات الحامضى وكذلك LDH .

8- معاملات ورد النيل أو الكلوريلا حسنت من خواص الأسماك البيوكيميائية والباثولوجية.

9- تنصح الدراسة بإستخدام ورد النيل أو الكلوريلا فى المعالجة البيولوجية للمياة الملوثة لقدرتها على الوصول الى الحدود المسموح بها عالميا.