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MECHANISM UPTAKE METALS OF POLLUTED LAKES-SEA WATER FOR BIOLOGICAL TREATMENT BY SOME ALGAE AND PROTECTIVE EFFECT

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Abstract

This study aimed to investigate the use of two algal species *Dunaliella spp.* and *Sargassum spp.* as biological treatment and protective effect on some heavy metals . Two elements were chosen mercury Hg and lead Pb to study biosorption and desorption mechanisms by algae and influence on some biochemical parameters in Nile tilapia (*Oreochromis niloticus*). Fingerlings were investigated with an average weight 28.5±6.5g\fish were applied in glass aquaria each was stocked randomly with 20 fingerlings each reared in aquarium (60x70x50 cm) for six treatments polluted water in addition to control treatment non- polluted water. Active transport was the mechanism for Hg and Pb uptake by two algal species. *Sargassum spp.* showed higher accumulation ratios than living algal cells, the reverse was true for *Dunaliella spp.* Mercury Hg and lead Pb residues in the muscles and gills of experimented fish were measured together with some biochemical parameters. All investigated biochemical parameters increased significantly ($P< 0.05$) during accumulation period and returned back to the normal values after elimination period, it was significantly higher in fish group T_5 and T_6 compared to that of fish group T_3 and T_4 ($P< 0.05$). There were significant differences ($P< 0.05$) in AWG, ADG and SGR among treatments, The higher values of heavy metals mercury Hg and lead Pb were accumulated in fish group T_1 and T_2 ($P< 0.05$), while the lowest value was obtained at fish group T_5 and T_6 ($P< 0.05$). Accumulation rate increased with the increasing of exposure concentration or period and elimination significantly decreased the levels of both mercury and lead. Body dry matter (%) was not significantly ($P< 0.05$) affected by the experimental treatments, while CP%, EE% and ash% significantly differed among treatments ($P< 0.05$). Tilapia fingerlings in T_5 and T_6 produced lower value of Creatinine, Cholesterol, Glucose and Uric acid while gave

higher value of total protein, follicle stimulating hormone (FSH) and gave best value of AST, ALT in plasma. Dried. *Sargassum spp.* can be used successfully for bioremediation of mercury Hg and lead Pb from the contaminated water, while living *Dunaliella spp.* could be used for the removal of these elements from lakes and sea water and metals bioremediation from aquatic environment. The results also demonstrated the efficiency of algae in reducing the pollutant effect of some heavy metals in water, and decreasing their negative impact, where it gave better water quality and fish safety adverse health human effects.

Key words: *Dunaliella spp.**, *Sargassum spp.**, protective effect, mercury and lead, polluted, aquatic environment, bioremediation, Nile tilapia, growth performance, accumulation and elimination, biochemical parameters.

INTRODUCTION

Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Accumulation levels vary considerably among metals, toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer able to counter uptake (Parker, 2002).

The increase in aquatic environment during the last years has been causing the increase of risk factors concerning fish health. One of these risks is heavy metal pollution. Mercury (Hg) is one of the high toxic heavy metals and it grouped under black list pollutants because of its high toxicity, persistence and bioaccumulation in the ecosystem (Canli and Furness, 2011). The estimated global mercury discharge is 1300 metric tons each year to water from natural resources (US National Academy of Science, 1977).

Most of the increases in mercury discharge into the environment occur in the less developed regions of the world. Mercury has no known biological function in the animal body and is described as an ultra trace element, fish can absorb Hg from water mainly through gills and from food through digestive tract (Verma and Tonk 2015; Zillious *et al.,* 1993).Most cases of mercury poisoning arise from contaminated fish consumption. Lead (Pb) the most toxic metal, is detectable in practically all phases of the inert environment and all biological system, because it is toxic to most living things at high exposure

levels. Lead is non-essential element and it is a bone-seeking element, it is processed along with calcium, however, tissues other than bone are considered to be storage sites for lead in fish (Abbas, 1994; Aboul Ezz and Abdel-Razek, 1991). Studies performed on blood usually reflect the toxicity of pollution.

In natural waters, the uptake of a given isotope in relation to other fission products may be quite different from that on land, for example plankton algae were found to accumulate Pb to a much greater extent than Co, whereas for land plants the reverse is true (Gazso, 2014; Shfik, 1993). Among the aquatic food chains, algae may absorb radioisotopes from the aquatic environment, both ionic and particulate forms of radionuclide, and both passive and active mechanisms of uptake may be involved.

This activity requires high-quality feeds, which should contain not only necessary nutrients but also complementary feed additives to keep organisms healthy, favor growth and environment-friendly aquaculture. Tartiel, (2009) reported that *Dunaliella salina*, a unicellular green alga that can be induced to accumulate massive amounts of ß-carotene (pro-vitamin A), is the most important factor responsible, and found that the highest accumulation of antioxidant vitamins in *Dunaliella salina, and* content of folic acid 4.8, B_1 29, B_2 19.6, B_6 2.2, B_{12} 0.7, Biotin 0.9, Nicotine 79.3, ß-carotene 82.5, Vitamin E 116.3, Vitamin C 163.2 (µg/g dry matter). In this investigation two of the hazards nuclide found in the aquatic environment was chosen, mercury (Hg) and lead (Pb), biosorption and desorption of these radionuclide, by the marine macroalga *Sargassum spp.* as representative for marine environment and *Dunaliella spp.* the unicellular alga as representative for lakes environment in relation to their surface area, were investigated (Allen and Nelson, 1974; Davis *et al.*, 2015; Shalaby, 2000). Also, the effect of these radionuclide on cell activities, in addition to side rophores production were also investigated, aiming to find safe, practical mechanism for radionuclide removal with the recycling of biosorbant either from marine environment or from lakes (Sorensen, 2008). Small amount of algae is sufficient to prevent and cure scurvy, however, larger amount may be essential to maintain good health during environmental

adversities, situation of physiological stress and conditions of infections and parasitic diseases Tartiel *et al.,* 2009; Watson, 1997).

Therefore, the present study was carried out to explore the effect of algae on intoxication, protection against of inorganic lead-mercury toxicity and its impact on some biochemical parameters, growth performance, blood indices and liver function of Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Experimental design and fish culture technique:

The present work was carried out in Fish Research Center, Abbassa, Abo-Hammad, Sharkia, Egypt, the present study aims to investigate the effect of both *Dunaliella spp.* and *Sargassum spp.* on intoxication of mercury (Hg) and lead (Pb), the residues of both mercury and lead in *Oreochromis niloticus* fingerlings muscles and gills after accumulation and elimination periods, also the effect on some biochemical parameters, growth performance and survival rate of Nile tilapia (*Oreochromis niloticus*). The lethal concentration (LC_{50}) was obtained by exposing Nile tilapia to different concentration of mercury (Hg) and lead (Pb) in static system for 96 hours. The concentration LC_{50} was determined according to the method of Behreus and Karber (1953). Four hundred and twenty fish fingerlings were stocked in 21 glass aquaria representing the seven treatments in triplicates, each aquarium (50X70X60 cm) supplied with tap water through a closed recycling system at constant level of 195 liters were used. Each aquarium was supplied with compressed air through a central air compressor. Water samples for chemical analysis were monitored biweekly. Temperature and dissolved oxygen were measured on site using a YSI model 58 oxygen meter and recorded daily, where the average of dissolved oxygen was above 6.8 mg/l. Other water quality parameters including pH and ammonia were measured every two days, where the average range of total ammonia was 0.12 – 0.23 mg/l and pH was in range of 7.2 ± 0.5 during the experiment according to the method of (APHA)(1985) (USEPA, 1991) . All treatments were applied with diets received commercial pelleted fish feed

(artificial diet) containing 25% crude protein and fed at a daily rate of 3 % of fresh fish body weight from two times daily; the experiment lasted for 5 weeks, two weeks of accumulation period and three weeks of elimination period. Fish were daily fed at a rate 3% of their biomass and weekly weighed for adjusting food amounts according to the new weights. The calculated amount of food was daily offered by hand to fish, in equal portions at 8.00 am and 2.00 pm and fed three times daily. Chemical proximate analyses of feed ingredients used in the presents study are presented in Table (1). The experimental diets were formulated from available ingredients in the local market, Essential amino acid analysis of the bases diet Table (2). Chemical analysis of the ingredients used in the experimental diets on DM basis Table (3), to contain according to recommended requirements needed for this stage of feeding (Jauncey,2000). The required amount of the diet was prepared every two weeks, and stored in a refrigerator. The fish were distributed randomly at a rate of 20 fish per glass aquarium, 20 fish each treatment, three replicate/glass aquaria representing seven nutritional groups. Lead Pb-polluted water was prepared by using $Pb(NO_3)_2$ while Hg- polluted water was prepared by using $HgCl_2$ mercuric chloride, produced by El-Nasr chemical company (Egypt) and prepared in aquatic solution to provide the required concentration. One group served as control (fish raised in non polluted tap water) and six groups for each as follow:

1. fish raised in 6mg Pb/l polluted water.
2. fish raised in 0.25mg Hg/l polluted water.
3. fish raised in 6mg Pb/l polluted water + *Dunaliella*
4. fish raised in 0.25mg Hg/l polluted water + *Dunaliella*
5. fish raised in 6mg Pb/l polluted water + *Sargassum*
6. fish raised in 0.25mg Hg/l polluted water + *Sargassum*

Faeces and feed residues were removed by siphoning from each aquarium, and a half of aquariums water was replaced with de-chlorinated tap

water and dead fish were removed. At the end of the experiment, fish were collected, counted, weighed and kept in a deep freezer at -18°C until the chemical analysis at the end of the experiment.

Measuring residues in fish tissues:

Fish were dissected to get muscles, liver and gills for residual analysis according to the method described by Official Methods of Analysis (1990) where fish samples were burned in muffle furnace for 16 hours at 550 °C to be ached, to the ached sample, add 2ml HNO3 were added, evaporated carefully just to dryness on hot plate, transferred to cooled furnace, temp., slowly raised to 450°C and holed at this temp. for 1 hour, 10ml of IN HCL were added and ash were dissolved by heating cautiously on a hot plate, transferred to 25 ml volumetric flask, cooled and diluted to volume. Hg and Pb residues were measured using atomic absorption spectrophotometer (model: Perkin Elmer, 2280).

Algae materials:

The unicellular alga, *Dunaliella spp.* (Chlorophyta), It was obtained from Lake Bardawil. The lake is situated on the North of Sinai-Egypt, isolates, purified and identified according to Guillard, R.R.L.(1973). The microalgae were subculture in a solid BBM (Bischoff and Bold, 1963). The cultures were allowed to grow in the algae culture room at 25°C and 14/10 light-dark cycle (5000lux). Stock culture were transferred from Dokki lab to the wet lab of CLAR in two liters capacity flasks for 5-6 days, then inoculated in carboy cultures at a density of 6×10^5 cells/ml. The carboy cultures were used as inoculums for two different phases of production in indoor and outdoor in glass aquarium. The transfer of the algal cells to fish aquarium was achieved at a density of $5x10^6$ cells / ml.The aquaria were used to inoculate the fiberglass tanks ($5m^3$), the following formula was used to compute for the required volume of stock green algae to be added into the aquaria (Tendencia *et al.*, 2005 & Becker, 1987).

Volume to be added = (desired density-existing density)x volume of water in pond

Density of stock culture

The quality of the algal biomass harvesting by the two methods were compared by determining their moisture and crude protein contents. Since cells of microalgae at the stationary phase of growth tended to settle to the bottom of the cultivation tank, primary separation of the algal cells from the liquid phase was achieved by gravity separation. After the algal culture reached maximum growth, the circulation provided by the pumping system was stopped and the algal cells settled and formed thick sediment at the bottom of the cultivation tank within 24h. *Sargassum* (Phaeophyta) was the second biosorbant alga used in this investigation, was collected from Abu Qir locality in Alexandria. Ten grams fresh weight samples were washed thoroughly with sterilized distilled water several times, Fresh samples of *Sargassum spp.* used as biosorbant were transferred to flasks containing 50 ml medium prepared. Dried samples of ***Sargassum*** were prepared (in parallel with the fresh samples) by drying in an oven at 60°C, then ground to a size in range 500-700µm and stored in a desiccators (Allen and Nelson, 1974).

Sampling and analytical methods:

Representative samples of fish were randomly taken at the beginning and at the end of the experiments. Fish samples were killed and kept frozen (-18°C) until performing the body chemical analysis. Samples of the experimental fish feed were taken, ground and stored in a deep freezer at - 18°C until proximate analysis. All of chemical analyses of fish and fish feed were analyzed for crude protein (CP%), ether extract (EE%), crude fiber (CF%), ash (%) and moisture while whole body composition of fish samples were determined according to (BOE, 1991; Caraway, 1955; Behreus and Karber, 1953; Boyd, 1991), the procedures described by standard A.O.A.C. (1995).The nitrogen free-extract (NFE%) was calculated by differences. For determination of protein digestibility the diets and faeces were collected during the last 15 days of the experimental period. Any uneaten feed or faeces from each aquarium was carefully removed by siphoning about 30 min. after the last feeding. Faeces were collected by siphoning separately from each replicate

aquarium before feeding in the morning. Collected faeces were then filtered, dried in an oven at 60°C and kept in airtight containers for subsequent chemical analysis, (Becker, 1987).

Biochemical analysis and Analytical methods:

The basal diet and fish samples from each treatment were analyzed using the methods of A.O.A.C. (1995) for determination of moisture, crude protein, total lipids and ash. Blood samples were collected from the caudal veins and blood was allowed to set for 30 min. at 4°C to clot, and then centrifuged for 5 min. at 1000 rpm. The serum samples were stored at -20°C until later used to analyze total protein, creatinine, uric acid, glucose and enzymes activity aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to the method described by Henry (1974). Blood samples were preserved in sodium fluoride for estimating blood glucose was determined according to Trinder (1969). For measuring blood minerals, 0.5 ml plasma was digested with a mixture of concentration. The studied elements were determined using atomic absorption spectrophotometer (Perkin Elmer model 2280). Sodium and potassium were determined by flame photometry (AOAC,1995; Reitman and Frankel 1957).

Growth performance parameters:

The growth performance parameters were calculated according to the following equations:

Average weight gain (AWG) = Average final weight (g)- Average initial weight (g).

Daily weight gain (DWG) = Gain / experimental period.

Relative weight gain (RWG %) = Gain / initial weight X 100

Specific growth rate (SGR%) = (In W_1 – In W_0) / T) X 100

Where W_1 is the fish weight at the end (final weight), W_0 is the weight at the start (initial weight), In is the natural log. As described by Bagenal and Tesch (1978) and T is the number of days in the feeding period.

Feed and protein utilization parameters:

The feed utilization parameters were calculated according to the following equations:

Feed conversion ratio (FCR) =Total feed fed (g/fish) / total wet weight gain (g/fish).

Protein efficiency ratio (PER) = Wet weight gain (g/fish) / protein intake (g/fish).

Energy utilization (EU%) = Retained energy (Kcal)/ energy intake (Kcal) X 100

Statistical analysis of data:

Statistical analysis was performed using the Analysis of variance (ANOVA) and Duncan's multiple Range Test, to determine differences between treatments means at significance rate of $P < 0.05$. The standard errors of treatment means were also estimated. All statistics were carried out using Statistical Analysis program (SAS, 2000).

RESULTS AND DISCUSSION

Active transport was the mechanism of mercury Hg and lead Pb uptake by both *Dunaliella spp.* and *Sargassum spp,* since the accumulation ratios of these nuclides under the concentration used exceeded. Both *Dunaliella* and *Sargassum* used exhibited a number of metabolic-dependent and metabolic-independent processes of uptake and accumulation of radionuclide and heavy metals (Canli and Furness, 2011). All biochemical parameters were significantly affected by algae *Dunaliella* and *Sargassum*, heavy metals, and their interaction ($P<0.05$) Table 4 and 5. There was a significant ($P<0.05$) increase in glucose and lipids levels in treatments are shown in Table (4) after rising in Hg-polluted water (250µg/l) or Pb-polluted water (6mg/l) where the recorded values after accumulation period (2 weeks) were 1.52±0.52, 1.69±0.8, 1.36±0.2, 1.72±0.66, 1.77±0.44 and 2.03±0.2 respectively, while the control value was 0.847±0.33g/l, were total lipids recorded values 24.10±0.05,

25.30±0.04, 24.70±0.08, 24.50±0.04, 23.20±0.03 and 23.80±0.88 respectively, while the control value was 18.02±0.077 g/l. Elimination period (3weeks) caused both treatments values to be decreased significantly ($P<0.05$) in comparison to their values at the end of accumulation period, but these values still significantly ($P<0.05$) higher than control in most cases, values recorded after 3 weeks of elimination period were illustrated in Table (6) 1.64±0.02, 1.95±0.41, 0.921±0.21, 0.995±0.34, 0.831±0.65 and 0.850±0.32 respectively, while the control value was 0.847±3.41g/l. Total lipids values recorded 23.0±0.31, 24.0±0.62, 18.9±0.25,19.5±0.54, 16.7±0.52 and 17.2±0.63 respectively, while the control value was 17.8±0.22 g/l. In the present study, glucose and serum lipids in control, T_5 and T_6 were significantly ($P<0.05$) lower than those of T_1 and T_2, lowest values were obtained in T_5 followed by T_6 then control treatment non-polluted water and without algae. These results are in agreement with those of Moore and Rmamoorth (1984) who reported that the intensity of hyper lip media may reflect the degree of stress imposed on the fish under the influence of toxic agents and environmental pollutants. The tendency of *Sargassum* (dried) to accumulate Hg and Pb was due to the large surface area of dried algal, the adsorptive power was high and accordingly the accumulation ratio was greater than in living algal. Larsson *et al.*,(2007) found that living *Scenedesmus* removes large amounts of phosphorous from water by an active transport system. In the contrary, the accumulation ratio in living cells of *Dunaliella* exceeded that of the dried cells. In the unicellular algae, *Chlorella* and *Scenedesmus*, dried algal cells had high adsorption power for metals at very low concentration, and to accumulate them with large quantities, via active transport (Verma and Tonk, 2015).

Serum total protein, albumin and globulin decreased significantly ($P<0.05$; Table 4) with the increase of mercury and lead toxicity, water treatment with algae exhibited high levels compared to water that were not algae at the end of accumulation period (2weeks). Serum total protein fingerlings after rising in Hg-polluted water (250µg/l) or Pb-polluted water (6mg/l) where the recorded values of serum total protein were 12.5±0.24,

11.9±0.41, 12.2±0.68, 12.4±0.36, 13.1±0.74 and 12.6±0.91 respectively, while the control value was 23.6±0.25g/l. Elimination period (3weeks) caused both treatments values to be increased significantly ($P<0.05$) in comparison to their values at the end of accumulation period, but these values still significantly ($P<0.05$; Table 6) were 12.4±0.45, 12.2±0.67, 22.1±0.41, 22.8±0.67, 23.6±0.31 and 23.0±0 respectively, while the control value was 24.3±0.33 g/l. Serum protein, albumin and globulin were significantly ($P<0.05$) lower in T_1 and T_2. Higher values were obtained in control followed by T_5 and T_6 then T_3 and T_4 compared to those without algae (T_1 and T_2). Elevated serum total protein may be attributed to the damage of kidney and gills as a result of exposure to mercury Hg and lead Pb, which in turn leads to disturbance in smog regulation (Ghazaly *et al.,* 1994 & Gill and Pant 1981). These results may be due to the disturbances in the liver protein metabolism due to Hg and Pb toxicity, as was found to be the case with other contaminants (Halim, 1987). On the other hand Lin *et al.,* (2008) reported that a low albumin may result from impaired synthesis, loss through urine or feces or increased catabolism. Its suggestible that exposure to mercury or lead may causing partial irreversible in some organs such as liver, the case in which the increase in enzymes levels hardly return to normal values. Racicot *et al.,* (2010) reported that liver damage due to accumulation of lead elevate the levels of GPT activities. Gatlin (2012) reported that cell injury of certain organs lead to the release of tissue-specific enzymes into the blood stream. Performing blood chemistry analyses often provide vital information aiding the diagnosis for health assessment and management of cultured fish (Saleh, 1987& Shalaby, 2000& Shakoori *et al.,* 2007).

Table 5 is showing the data of some blood constituent (Creatinine, Cholesterol, uric acid, AST and ALT) in plasma of Nile tilapia (*Oreochromis niloticus*) as affected by both algae, these parameters increased significantly ($P<0.05$) with exhibited lower values of creatinine, uric acid, AST and ALT compared to those without algae (T_1 and T_2). Serum ALT values recorded in the

mentioned treatments after 2weeks (accumulation period) of exposure were 34.2±0.42, 33.7±0.52, 30.1±0.63, 32.2±0.93, 30.2±0.38 and 29.7±0.71 respectively. Elimination period after 3weeks caused both enzymes values to be decreased significantly ($P<0.05$; Table 7) in comparison to their values at the end of accumulation period, in spite of its values recorded at the end of the elimination period were significantly lower than its values at the end of accumulation period, but its values recorded in fishes raised in 0.25mg Hg/l and 6 mg Pb/l (18.2±0.52, 19.4±0.56, 15.9±0.53 and 15.7±0.44 respectively). Creatinine and uric acid levels are indicators of kidney function. In the present study, creatinine and uric acid showed a significant increase in fish exposed to heavy metals. These results may be due to the action of heavy metal on glomerull filtration and heavy metals may cause pathological changes to the kidney resulting in dysfunction (Davis *et al.,* 2015& Tartiel *et al.,* 2009). Slight variations were noticed among treatment in regard to the content of different blood parameters. However, treatments 5 and 6 where *Sargassum* were used showed the lowest content of creatinine, cholesterol, uric acid and alanine aminotransferase and aspartate aminotransferase (ALT and AST), but the highest content FSH. Blood parameters content in fish of both T_5 and T_6 had moderate vacillating values and fall between the values of T_1 and T_2. Hematological tests and analyses of serum constituents have proved useful in the detection and diagnosis of metabolic disturbances, such tests should be supplemented with clinical and biochemical analysis for diagnostic purposes. In *Sargassum*, the cell wall played the main role in mercury accumulation. The release of metals by this alga has been frequently associated with exudation of metal chelated to poly-phenolic compounds known to be present in this genus (Alam and Maughan, 1995& Chaisukant, 2003). In this investigation, *Sargassum* showed also a great sorption capacity for mercury Hg and lead Pb suggesting that they are suitable biosorbant for the treatment of water containing these radio nuclides, sorption activity was shown to be dependent on the initial ion concentration. Allen, (1995) concluded that the initial rapid uptake of an element would be corresponding to extracellular adsorption and to passive intracellular uptake (metabolism-independent) involving cell surface

adsorption and simple diffusion into cells or intercellular spaces. Rice and Willis, (1959) & Kalay and Canli,(2000) reported that living microalgae have an advantage to be used as biosorbant, due to metabolic uptake and continuous growth. In dried *Sargassum* material, there was an increase in accumulation ratios with all the treatments used. This again spots the light on the free ion activity hypothesis of algal surface. The above results indicated that: dried cells of *Sargassum* had high adsorption power for lead Pb with less efficiency for mercury Hg. A slower uptake will be corresponding to metabolism-dependent incorporation in the cell body, Shakoori *et al.*,(2007) also supported this postulation Valdmar and Leite (2008).

As shown in Table (8) the highest mercury Hg were recorded after 2 weeks in the muscles of tilapia fingerlings raised in 250µg Hg/l polluted water. However three weeks of elimination resulted in a significant ($P<0.05$) decrease of heavy metals residues in the muscles of fingerlings, values of 0.972±0.47 and 0.021±133 respectively. Higher mercury quantities were accumulated in the liver than muscles. Hg levels in the liver of fingerlings were 2.55±0.41, 3.70±0.35, 3.36±0.22, 2.90±0.81, 2.77±0.19 and 2.89±0.14 respectively, after accumulation period. These values significantly decreased ($P<0.05$) to values of 3.62±0.52, 3.81±0.61 T_1 and T_2 without algae treatment of water, while T_3 to T_6 with algae treatment, values were 0.842±0.34, 0.853±0.92, 0.921±0.84 and 0.872±0.63 respectively. Mercury Hg levels higher in liver than gills and muscles. Hg levels in the gills of fingerlings were 1.82±0.32, 1.94±0.21, 1.45±0.25, 1.67±0.24, 1.48±0.48 and 1.77±0.71 respectively, after accumulation period. These levels significantly decreased ($P<0.05$) after three weeks (Elimination period) to levels of 2.50±0.18, 2.90±0.62, 0.123±0.36, 0.154±0.33, 0.121±0.45 and 0.118±0.21 respectively. The lowest accumulation rate off all studied metals in muscles, gills and liver were detected in fish organs from T_3 followed by T_5 and T_6 while T_4 ranked in the last in regard to the accumulation rate. This may be attributed to the complex formation between both metal ions and the protein structure in gills which contain nitrogen, oxygen and sulfur as previously reported by Cotton and Wilkinson (1980). Lead Pb

residues in the muscles, liver and gills as shown in Table (9), recorded a significant increase than control in fingerlings exposed to lead concentration (6mg/l water) during accumulation period. Highest lead concentration after 2 weeks in the muscles was recorded 0.510±0.53. An elimination period was sufficient for Pb residues in muscles to be significantly decreased ($P<0.05$) to a value of 0.015±0.74. Higher quantities of lead were accumulated in the liver than muscles. Hg levels in the liver of fingerlings were 3.23±0.42, 2.67±0.91, 4.83±0.18, 3.91±0.55, 3.87±0.44 and 4.65±0.24 respectively, after accumulation period. These levels significantly decreased ($P<0.05$) after three weeks (Elimination period) to levels of 3.41±0.62, 3.35±0.83, 1.056±0.94, 1.035±0.42, 0.904±0.61 and 0.962±0.37 respectively. Higher lead in the liver than gills was recorded 1.27±0.94 in gills of fingerlings after accumulation period, while after elimination period was recorded 0.116±0.47, but this value still significantly higher than control. The reason for the high accumulation of Pb and Cu in the liver could be related to the specific metabolism process and enzyme catalyzed reaction involving Pb and Cu taking place in the liver, the high metal concentrations in liver reflect its multifunctional role in detoxification and storage, (Centinkaya *et al.*,1999& Phillips, 1990). These findings reflect the effect of the treatment with *Scenedesmus dimorphus* in reducing the accumulation rate of heavy metals in the organs of fish. However, all metals concentrations on average below the maximum tolerance levels for human consumption established by the FAO/WHO, (1999). It is well documented that pollutants, such as metals and organic compounds can be accumulated by aquatic biota (USEPA, 2007). Bioaccumulation measurements refer to studies or methods monitoring the uptake and retention of pollutants like metals or biocides in organs and tissues of organisms, such as fish (Tartiel *et al.,* 2009). Therefore the ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal accumulated in that specific tissue, in the fish can also influence the bioaccumulation of particular metal (Jones, 1969). This can only take place if the rate of uptake by the organism exceeds the rate of elimination (Kotze, 1997& Maita *et al.,* 2012).

The growth performance parameters and feed conversion ratio (FCR), average weight gain (AWG), daily weight gain (DWG), relative weight gain (RWG), specific growth rate (SGR) and survival rate (SR%) of Nile tilapia (*Oreochromis niloticus*) fingerlings were significantly ($P<0.05$). Data in Table (10) illustrated the effect of both algae *Dunaliella* and *Sargassum*, on treatment of water enhanced fish growth; meanwhile the exposure to heavy metals reduced them. However, fish groups fed both *Dunaliella spp.* and *Sargassum spp.* Average of initial body weight of Nile tilapia (*Oreochromis niloticus*) fingerlings fed the algae T_3, T_4, T_5 and T_6 at the start did not differ, indicating that groups were homogenous. Whereas, the highest values of final body weight was found to be 37.80 ±0.12 control and T_5 T_6 (36.30±0.52 & 35.60±0.18) respectively, compared with the other treatments, SGR% of control value 0.80±0.01 and (0.74±0.32 &0.68±0.44) respectively and SR% of control value 96.6% and (85.6% & 84.20%) respectively. The results agreed with those of Pena et al., (2004). The main outcome of the present study was that the variations in dietary additives had only a minor effect on fish growth performance. These results are in disagreement with Santiago *et al.,* (2006) who found that the addition of algae, aquatic plant and yeast in fish diets did not differ significantly ($P<0.05$) less than 20, 25 and 10% respectively, while, Abbas, (1994) stated that the daily body weight gain did not differ significantly ($P<0.05$) affecting by different addition of microalgae *Spirulina* in fish diets for *Tilapia mossambicus* less than 30 and 50% respectively. Similarly Allen (1995), showed that the addition of water hyacinth, water primrose and algae in fish diets improved growth performance of Nile tilapia and common carp. The use of microalgae as fish feed inputs has been studied with encouraging results. (Badr, 1997), reported positive growth performance in fish feed diets containing algae cells.

Protein and feed utilization: Data in Table (11) representing means for protein efficiency ratio (PER) and feed efficiency ratio (FER) were significantly ($P<0.05$) higher with fish compared with the control treatment. The best FER, highest PER and the highest of T_5 and T_6 were significantly ($P<0.05$) improved

in comparison with the other groups and better than the control treatment. The PER was found to be 1.82±0.31 (control), 2.20±0.41 and 1.37±0.35 respectively. The same trend was observed in PER where the fish groups T_5 and T_6 showed better ($P<0.05$) FER values compared with the other groups. The FER was found to be 0.54± 0.61 (control diet), 0.82±0.25 and 0.71±0.02 respectively. In the present study, the commercial feed additives used significantly ($P<0.05$) enhanced feed efficiency. These results are in agreements with the findings of Bomba *et al.*(2002). In practical terms, this means that the use of additives can decrease the amount of feed necessary for animal growth which could result in reductions of production cost. Similarly Abu Zead (2001), showed that the addition of water hyacinth, water primrose and algae in fish diets improved growth performance of Nile tilapia and common carp. The use of microalgae as fish feed inputs has been studied with encouraging results. (Broun, 1980), reported positive growth performance in fish feed diets containing algae cells. The differences among the above studies may be related to the difference in algae sources and algae species in the rearing water. The fish growth was improved in T_3 , T_4 and T_5 , T_6 suggesting that algae treatment could play a role in reducing Hg and Pb toxicity. *Dunaliella spp.* and *Sargassum spp* are known to act against metal toxicity by forming protein and metals complex (Badr and Abou-Waly, 1997& Shfik, 1993& Kotze, 1997). In the present study, algae played a role in enhancing feed intake with a subsequent enhancement of the fish body composition. The low feed utilization in heavy metals challenged groups (T_1 and T_2) may have been due to the fact that the heavy metals may led to reduction in fish appetite or complete fish fasting resulting in reduced growth. These results indicated the necessity of additives for fish growth and feed utilization. Meanwhile, additives deficiency in the diet impaired cellular immune response in rainbow trout, and was associated with poor growth, depressed feed intake. Algae have attention as a possible alternative protein source for cultured fish, particularly in tropical and subtropical regions where algae production high and their good protein, vitamins and essential fatty acids contents (Tartiel, 2008& Moore and Ramamoorth, 1984).

All fish body constituents except moisture content were significantly ($P<0.05$) affected by addition algae in pollution water and their interaction. Table 12 is showing the data of fish body chemical composition; moisture, crude protein, total lipids and ash content. No significant ($P<0.05$) variations were noticed among treatments in regard to body moisture content which ranged between 72.7 and 76.51%. Crude protein content was higher in fish body of T_5 was recorded 58.3%, higher than control was recorded 57.4% and T_6 was recorded 55.6% respectively. T_6 showed the lowest lipids content in fish body while T_3 and control treatment showed the highest one. No significant differences were found in ash content of T_1, T_2 and T_4. It is obvious that T_5, where *Sargassum,* were used, showed the highest protein content and the lowest lipids content in fish body. Crude protein and total lipids decreased significantly ($P<0.05$) with heavy metals (T_1 and T_2). Ash content and metals residues in T_1 and T_2 were significantly ($P<0.05$) lower than those of T_5 and T_6. These results are indicating the healthy status of Nile tilapia in this study irrespective to additives levels in the diet. These results are in agreement with the results of Yang and Volesky, (1999) who found the fish diet containing algae up to 45% did not cause a significant variation in carcass CP, ash and dry matter comparing with control diet for Nile tilapia (*O. niloticus*).

Table 1. Composition and proximate chemical analyses (on DM bases) of the experimental diet used.

Diet 25 % CP	Ingredients (%)
10	Fish meal (60% CP)
12	Corn gluten (60%CP)
22	Soybean meal (44% CP)
20	Wheat bran
30	Yellow corn
3.5	Sunflower oil
0.5	Di-calcium phosphate
1.5	Vitamin & Mineral [1]
-	*Dunaliella spp.**
-	*Sargassum spp.**
0.5	Cr_2O_3 [2]
100	Total
Proximate analysis	Chemical composition (%)
90.78	Dry matter DM
25.20	Crude protein CP
6.70	Ether extract EE
6.90	Crude fiber CF
7.69	Ash
53.70	Nitrogen free extract NFE[3]
426.09	Gross Energy Kcal/100g[4]

1- Each Kg vitamin & mineral mixture premix contained Vitamin A, 4.8 million IU, D_3, 0.8 million IU; E, 4 g; K, 0.8 g; B_1, 0.4g; Riboflavin, 1.6 g; B_6, 0.6 g, B_{12}, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4g; Biotin, 20 mg; Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu. 4g; I, 0.4 g; Selenium, 0.4 g and Co, 4.8 mg.

2- Cr_2O_3 : Chromic Oxide

3- NFE (nitrogen free extract) = 100 – (protein + lipid + ash + crude fiber).

4- Gross Energy (GE) : Calculated according to NRC (1993) as 5.64, 9.44 and 4.11 kcal / g for protein, lipid and NFE, respectively.

Table 2. Essential amino acid analysis of the bases diet.

Amino acid	Basal diet	Requirements*
Therionine	3.76	3.75
Cysteine	1.25	1.18
Methionine	2.72	2.68
Isoleucine	3.92	3.11
Leucine	7.68	3.39
Phenylalanine	5.21	3.75
Valine	4.24	2.80
Lysine	5.12	5.12
Histdine	2.23	1.72
Arginine	4.22	4.20

* Santiago and Lovell (1988).

Table 3. Chemical analysis of the ingredients used in the experimental diets on DM basis.

Proximate analysis (%)	Fish meal	Soybean meal	Yellow corn	Corn gluten	Rice bran	*Dunaliella spp.**	*Sargassum spp.**
Dry matter	92.21	90.57	87.30	91.26	91.18	96.10	95.2
Crude protein	72.00	44.00	7.7	60.00	12.8	45.8	43.6
Ether Extract	8.8	2.1	4.1	2.9	14.00	12.3	13.5
Crude fiber	0.6	7.4	2.5	1.6	11.0	5.90	8.36
Ash	10.2	6.5	1.3	2.0	11.3	8.70	12.90
NFE*	8.4	40	84.4	33.5	50.9	27.30	21.64
GE**	525.9	458.0	429.8	506.8	452.2	486.62	462.2

NFE* (nitrogen free extract) = 100 – (protein + lipid + ash + crude fiber).

GE** (Gross Energy Kcal/100g): Calculated according to (NRC, 2004)

Table 4. Some blood constituent (in plasma) of Nile tilapia fingerlings (*Oreochromis niloticus*) after Accumulation period.

Treatment	Total protein(g/l)	Total lipids(g/l)	Albumin(g/l)	Globulin(g/l)	Glucose(g/l)
Control	23.6±0.25^{a}	18.02±0.077^{c}	15.74±0.34^{a}	7.93±0.54^{a}	0.847±0.33^{d}
T1	12.5±0.24^{c}	24.10±0.05^{ab}	8.10±0.61^{c}	3.22±0.85^{d}	1.52±0.52^{b}
T2	11.9±0.41^{d}	25.30±0.04^{a}	7.27±0.94^{cd}	3.68±0.28^{cd}	1.69±0.81^{ab}
T3	12.2±0.68^{c}	24.70±0.08^{a}	8.31±0.97^{c}	3.74±0.61^{cd}	1.36±0.21^{bc}
T4	12.4±0.36^{c}	24.50±0.04^{ab}	8.12±0.79^{c}	4.12±0.34^{c}	1.72±0.66^{bc}
T5	13.1±0.74^{bc}	23.20±0.03^{b}	9.41±0.37^{b}	4.33±0.41^{c}	1.77±0.44^{ab}
T6	12.6±0.91^{c}	23.80±0.88^{b}	7.55±0.27^{cd}	3.81±0.22^{cd}	2.03±0.21^{a}

Means and SE were calculated from n = 3aquaria. 0.31

Means having the same letter in the same column are n0.62ot significantly different at (P>0.05).

Table (5): Some blood constituent (in plasma) of Nile tilapia fingerlings (*Oreochromis niloticus*) after Accumulation period.

Treatment	Creatinine(mg/l)	Cholesterol (mg/l)	Uric acid (mg/l)	AST	ALT	FSH
Control	5.88±0.32^{d}	12.78±0.38^{d}	16.50±0.02^{d}	17.34±0.02^{d}	14.8±0.34^{d}	0.17±0.04^{a}
T1	14.92±0.44^{a}	22.4±0.34b^{c}	27.02±0.09^{a}	40.32±0.09^{a}	34.2±0.42^{a}	0.13±0.55^{b}
T2	13.98±0.71^{b}	23.8±0.25^{b}	26.04±0.07^{ab}	37.51±0.07^{ab}	33.7±0.52^{a}	0.12±0.64^{bc}
T3	14.66±0.62^{a}	23.6±0.62^{b}	25.07±0.06^{b}	36.22±0.05^{b}	30.1±0.63^{b}	0.14±0.33^{ab}
T4	13.97±0.92^{b}	25.7±0.41^{ab}	27.12±0.04^{a}	32.47±0.04^{bc}	32.2±0.93^{ab}	0.11±0.08^{c}
T5	13.88±0.83^{b}	28.9±0.23^{a}	24.08±0.07^{b}	31.81±0.02^{bc}	30.2±0.38^{b}	0.12±0.07^{bc}
T6	15.0±0.90^{a}	22.4±0.64^{bc}	25.32±0.08^{ab}	29.52±0.03^{c}	29.7±0.71^{bc}	0.14±0.82^{ab}

**ALT alanine aminotransferase enzyme **AST aspartate aminotransferase enzyme

**F.S.H.: Follicle stimulating hormone Means having the same letter in the same column are not significantly different at (P>0.05).

Table 6. Some blood constituent (in plasma) of Nile tilapia fingerlings (*Oreochromis niloticus*) after Elimination period .

Treatment	Total protein(g/l)	Total lipids(g/l)	Albumin(g/l)	Globulin(g/l)	Glucose(g/l)
Control	24.3±0.33^{a}	17.8±0.22^{bc}	15.28±0.12^{a}	7.30±1.32^{b}	0.872±3.41^{c}
T1	12.4±0.45^{c}	23.0±0.31^{ab}	8.33±0.45^{d}	3.25±2.41^{c}	1.64±0.02^{a}
T2	12.2±0.67^{c}	24.0±0.62^{a}	7.87±0.47^{d}	3.93±1.52^{c}	1.95±0.41^{a}
T3	22.1±0.41^{b}	18.9±0.25^{bc}	12.74±0.35^{c}	7.96±1.61^{ab}	0.921±0.21^{b}
T4	22.8±0.67^{b}	19.5±0.54^{b}	12.81±0.66^{c}	8.34±0.51^{a}	0.995±0.34^{b}
T5	23.6±0.31^{a}	16.7±0.52^{c}	14.40±0.37^{ab}	8.62±1.22^{a}	0.831±0.65^{c}
T6	23.0±0.41^{ab}	17.2±0.63^{bc}	13.22±0.47^{b}	8.21±1.48^{a}	0.850±0.32^{c}

Means and SE were calculated from n = 3aquaria.

Means having the same letter in the same column are not significantly different at (P>0.05).

Table 7. Some blood constituent (in plasma) of Nile tilapia fingerlings (*Oreochromis niloticus*) after Elimination period .

Treatment	Creatinine (mg/l)	Cholesterol (mg/l)	Uric acid (mg/l)	AST	ALT	FSH
Control	5.91±0.15^{d}	11.60±2.1^{bc}	14.52±0.55^{c}	18.6±0.33^{d}	15.6±0.23^{d}	0.18±0.45^{b}
T1	14.52±0.48^{a}	21.83±2.3^{a}	23.38±0.21^{a}	42.0±0.41^{a}	32.3±0.24^{a}	0.12±0.62^{c}
T2	14.31±0.12^{a}	22.77±3.12^{a}	23.44±0.14^{a}	39.6±0.61^{ab}	31.8±0.42^{a}	0.14±0.33^{c}
T3	6.34±0.23^{cd}	12.20±0.90^{bc}	15.20±0.32^{bc}	20.91±0.55^{c}	18.2±0.52^{bc}	0.22±0.45^{a}
T4	6.20±0.26^{cd}	11.96±0.29^{bc}	15.70±0.64^{bc}	21.80±0.22^{c}	19.4±0.56^{bc}	0.20±0.58^{b}
T5	5.87±0.35^{d}	10.70±0.19^{c}	14.84±0.81^{c}	18.70±0.14^{d}	15.9±0.53^{d}	0.19±0.81^{b}
T6	5.98±0.41^{d}	11.20±0.93^{bc}	14.95±0.61^{c}	19.40±0.71^{d}	15.7±0.44^{d}	0.20±0.47^{ab}

**ALT alanine aminotransferase enzyme **AST aspartate aminotransferase enzyme

**F.S.H. : Follicle stimulating hormone Means having the same letter in the same column are not significantly different at (P>0.05).

Table 8. Average concentrations of mercury Hg (µg/g dry wt.) in tissue/organs of Nile tilapia (*Oreochromis niloticus*) after accumulation period and after elimination period.

	Accumulation period (without algae) 2weeks			Elimination period (supplemented algae) 3weeks		
Organs Treatments	**Muscle**	**Liver**	**Gills**	**Muscle**	**Liver**	**Gills**
Control	0.001 ± 0.27^{d}	0.002 ± 0.22^{d}	0.001 ± 0.22^{d}	0.001 ± 1.54^{d}	0.002 ± 0.48^{d}	0.001 ± 0.71^{d}
T1	0.883 ± 0.29^{bc}	2.55 ± 0.41^{bc}	1.82 ± 0.32^{ab}	1.14 ± 1.22^{ab}	3.62 ± 0.52^{a}	2.50 ± 0.18^{b}
T2	0.942 ± 0.45^{b}	3.70 ± 0.35^{a}	1.94 ± 0.21^{a}	1.66 ± 1.81^{a}	3.81 ± 0.61^{a}	2.90 ± 0.62^{a}
T3	0.972 ± 0.47^{a}	3.36 ± 0.22^{a}	1.45 ± 0.25^{c}	0.021 ± 133^{c}	0.842 ± 0.34^{b}	0.123 ± 0.36^{c}
T4	0.960 ± 0.21^{a}	2.90 ± 0.81^{ab}	1.67 ± 0.24^{bc}	0.033 ± 1.42^{c}	0.853 ± 0.92^{b}	0.154 ± 0.33^{c}
T5	0.971 ± 0.33^{a}	2.77 ± 0.19^{b}	1.48 ± 0.48^{c}	0.027 ± 1.66^{c}	0.921 ± 0.84^{ab}	0.121 ± 0.45^{c}
T6	0.952 ± 0.64^{ab}	2.89 ± 0.14^{ab}	1.77 ± 0.71^{b}	0.023 ± 0.85^{c}	0.872 ± 0.63^{b}	0.118 ± 0.21^{c}

Means having the same letter in the same column are not significantly different at (P>0.05).

Table 9. Average concentrations of lead Pb (µg/g dry wt.) in tissue/organs of Nile tilapia (*Oreochromis niloticus*) after accumulation period and after elimination period.

	Accumulation period (without algae) 2 weeks			Elimination period (supplemented algae) 3 weeks		
Organs Treatments	**Muscle**	**Liver**	**Gills**	**Muscle**	**Liver**	**Gills**
Control	0.001 ± 1.33^{d}	0.004 ± 1.25^{d}	0.003 ± 1.38^{d}	0.002 ± 0.078^{d}	0.004 ± 0.51^{d}	0.003 ± 0.82^{d}
T1	1.36 ± 2.50^{b}	3.23 ± 0.42^{bc}	1.36 ± 0.81^{cb}	1.44 ± 0.02^{a}	3.41 ± 0.62^{a}	1.90 ± 0.37^{a}
T2	1.48 ± 1.80^{a}	2.67 ± 0.91^{c}	1.48 ± 0.66^{cb}	1.52 ± 0.44^{a}	3.35 ± 0.83^{a}	1.70 ± 0.25^{a}
T3	0.462 ± 0.55^{bc}	4.83 ± 0.18^{a}	1.70 ± 0.54^{ab}	0.017 ± 0.66^{c}	1.056 ± 0.94^{bc}	0.251 ± 0.14^{bc}
T4	0.434 ± 0.36^{c}	3.91 ± 0.55^{ab}	1.95 ± 0.42^{a}	0.019 ± 0.07^{c}	1.035 ± 0.42^{bc}	0.142 ± 0.69^{c}
T5	0.510 ± 0.53^{bc}	3.87 ± 0.44^{ab}	1.56 ± 0.36^{b}	0.015 ± 0.74^{c}	0.904 ± 0.61^{c}	0.170 ± 0.58^{bc}
T6	0.482 ± 0.57^{bc}	4.65 ± 0.24^{a}	1.27 ± 0.94^{c}	0.014 ± 0.22^{c}	0.962 ± 0.37^{c}	0.116 ± 0.47^{c}

Means having the same letter in the same column are not significantly different at (P>0.05).

Table 10. Growth performance of Nille tilapia fingerlings raised in either (Pb and Hg) at the end of the Study after 5 weeks.

Items	Treatments						
	Control	(1) Pb	(2) Hg	(3) Pb+*Du.**	(4) Hg+*Du.**	(5) Pb+*Sar.**	(6) Hg+*Sar.**
Initial weight (g/fish)	28.5^{a} ±0.06	28.02^{a} ±0.04	28.30^{a} ±0.02	27.94^{a} ±0.01	28.32^{a} ±0.02	28.10^{a} ±0.05	28.04^{a} ±0.04
Final weight (g/fish)	37.80^{a} ±0.12	30.81^{c} ±0.17	30.01^{c} ±0.22	34.50^{ab} ±0.16	32.9^{ab} ±0.33	36.30^{b} ±0.52	35.60^{ab} ±0.18
Weight gain (g/fish) AWG	9.30^{a} ±0.23	2.78^{cd} ±0.44	1.71^{d} ±0.27	6.56^{ab} ±0.38	4.58^{c} ±0.17	8.20^{a} ±0.26	7.56^{b} ±0.39
Weight gain (%)	24.6^{a} ±0.09	9.02^{c} ±0.41	5.7^{d} ±0.75	19.01^{b} ±0.64	13.92^{ab} ±0.53	22.6^{a} ±0.28	21.24^{a} ±0.41
Daily weight gain (g/day/fish) DWG	0.27^{a} ±0.01	0.08^{d} ±0.04	0.05^{d} ±0.02	0.19^{bc} ±0.07	0.13^{c} ±0.01	0.23^{b} ±0.06	0.22^{b} ±0.05
Relative weight gain (RWG)	32.6^{a} ±0.46	9.92^{d} ±2.25	6.04^{c} ±3.42	23.5^{b} ±5.22	16.2^{cd} ±4.60	29.18^{a} ±5.57	26.96^{b} ±8.04
Feed conversion ratio (FCR)	1.84^{a} ±0.55	0.99^{c} ±0.04	0.83^{c} ±0.08	1.73^{c} ±0.41	1.61^{ab} ±0.03	1.22^{bc} ±0.22	1.40^{ab} ±0.06
Specific growth rate (SGR %)	0.80^{a} ±0.01	0.26^{cd} ±0.09	0.17^{d} ±0.06	0.60^{b} ±0.70	0.43^{c} ±0.03	0.74^{ab} ±0.32	0.68^{b} ±0.44
Survival rate (SR %)	96.6^{a} ±0.04	56.5^{c} ±0.03	51.3^{c} ±0.00	83.7^{ab} ±0.00	82.4^{ab} ±0.9	85.6^{b} ±0.00	84.20^{b} ±0.6

Data are represented as mean of three samples replicates ± standard error

Means in the same row with the same letter are not significant difference (P>0.05).

Table 11. Feed utilization parameters of Nille tilapia fingerlings raised in either (Pb and Hg) at the end of the Study after 5 weeks.

Items	Treatments						
	Control	**(1) Pb**	**(2) Hg**	**(3) Pb+*Du.****	**(4) Hg+*Du.****	**(5) Pb+*Sar.****	**(6) Hg+*Sar.****
Feed intake (g feed/fish)	17.12^{a} ±0.01	2.75^{cd} ±0.02	1.42^{d} ±0.09	11.35^{b} ±0.01	7.37^{c} ±0.33	10.0^{ab} ±0.60	10.58^{ab} ±0.03
Protein intake in feed (PI)	5.12^{a} ±0.069	3.08^{b} ±0.55	2.25^{d} ±0.011	4.46^{ab} ±0.08	2.97^{c} ±0.04	3.72^{b} ±0.18	4.37^{ab} ±0.06
Feed efficiency ratio[1] (FER	0.54^{c} ±0.61	1.01^{a} ±0.32	1.20^{a} ±0.66	0.58^{c} ±0.07	0.62^{bc} ±0.28	0.82^{ab} ±0.25	0.71^{b} ±0.02
Protein gain (PG)	533.8^{a} ±0.22	123.7^{cd} ±0.91	71.3^{d} ±0.47	333.2^{c} ±0.11	239.5^{bc} ±0.37	478.0^{ab} ±0.34	420.3^{b} ±0.41
Protein efficiency ratio (PER)	1.82^{ab} ±0.31	0.90^{d} ±0.04	0.76^{cd} ±0.62	1.47^{c} ±0.71	1.54^{c} ±0.24	2.20^{a} ±0.41	1.37^{b} ±0.35
Retained protein (RP)	16.36^{a} ±0.81	12.46^{c} ±0.54	6.79^{d} ±0.01	14.17^{b} ±0.62	14.8^{b} ±0.26	16.42^{a} ±0.61	15.6^{a} ±0.61
Apparent protein utilization[2] (APU%)	31.18^{c} ±0.63	44.98^{b} ±0.07	50.21^{a} ±0.08	29.35^{d} ±0.41	32.51^{c} ±0.09	47.8^{ab} ±0.62	39.7^{b} ±0.67

Data are represented as mean of three samples replicates ± standard error

Means in the same row with the same letter are not significant difference (P>0.05)

1- Feed efficiency ratio (FER) = body weight gain (g)/ feed intake (g)

2- Apparent protein utilization (APU%) = Protein gain in fish (g) / protein intake x 100

Table 12. Proximate chemical analysis (% on dry matter basis) of whole body of Nile tilapia (*Oreochromis niloticus*) at the end of the study after experimental period .

Items	Treatments						
	Control	(1) Pb	(2) Hg	(3) Pb+*Du.**	(4) Hg+*Du.**	(5) Pb+*Sar.**	(6) Hg+*Sar.**
Moisture	74.30[a] ±0.01	72.70[a] ±0.62	73.20[a] ±0.34	74.60[a] ±0.22	72.80[a] ±0.07	75.45[a] ±0.33	76.51[a] ±0.12
Crude protein (CP %)	57.4[a] ±0.02	44.5[cd] ±0.04	41.7[d] ±0.11	50.8[c] ±0.51	52.3[b] ±0.61	58.3[a] ±0.04	55.6[ab] ±0.18
Total lipids (%)	21.61[a] ±0.07	18.5[b] ±0.41	19.3[ab] ±0.14	21.5[a] ±0.06	20.6[ab] ±0.51	18.4[b] ±0.09	16.52[c] ±0.63
Ash (%)	18.20[ab] ±0.03	16.8[bc] ±0.58	16.3[bc] ±0.03	17.43[b] ±0.17	15.65[c] ±0.05	19.50[a] ±0.02	17.73[b] ±0.40
Gross energy (Kcal)	539.1[a] ±0.08	507.6[c] ±0.04	510.7[c] ±0.80	531.6[b] ±0.11	536.6[ab] ±0.33	518.1[bc] ±0.01	511.2[bc] ±0.02

Data are represented as mean of three samples replicates ± standard error.
Means in the same row with the same letter are not significant difference (P>0.05).

NFE (nitrogen free extract) = 100 – (protein + lipid + ash + crude fiber).

Gross Energy (GE): Calculated according to NRC (1993) as 5.64, 9.44 and 4.11 kcal / g for protein, lipid and NFE, respectively.

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آلية امتصاص ملوثات مياة البحار والبحيرات والمعالجة البيولوجية بواسطة بعض الطحالب والتأثير الوقائي لها

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الملخص العربى

أجريت هذه الدراسة بهدف ايجاد حلا بيولوجيا للسيطرة على مخاطر التلوث البيئى ومايترتب عليه من تراكم العناصر السامة فى الكائنات البحرية وأسماك البحيرات بما يمثل خطورة على صحة الانسان من تناولها حيث تصب مياه المصارف فى بعض البحيرات الهامة والتى تعتبر من أهم مصادر الثروة السمكية وكذلك ظهور بقعة زيتية (بترول) في المياة الاقليمية المصرية تسببت في مشكلة بيئية كبيرة وتوضح هذه الدراسة آلية التخلص من الملوثات البيئية بواسطة بعض الطحالب ، تم اختيار طحلب الديونيلا وهو من طحالب البحيرات وطحلب سارجاسم وهو من الطحالب البنية الشائعة على سواحل البحار، وقد اجريت تجربة على الطحالب حية وجافة ووجد ان معدلات التراكم للعناصر المستخدمة تزيد في الخلايا الحية لطحلب الديونيلا عنها في الخلايا المجففة بينما كان العكس صحيحا بالنسبة للطحلب البحري سارجاسم حيث فاقت كمية العناصر الممتصة علي سطح الخلايا المجففة عدة مرات الكميات الممتصة على الخلايا الحية . أختصت هذه الدراسة بعنصري الزئبق Hg، الرصاص Pb حيث انها عناصر شديدة السمية. تم تطبيق ستة معاملات: (T_6 ، T_5، T_4 ، T_3 ، T_2 ، (T_1بالاضافة الى معاملة المجموعة الضابطة (الكنترول) فى ثلاثة مكررات لكل معاملة ووزعت أصبعيات البلطي النيلي بمتوسط وزن 28.5 ±6.5 جم بمعدل 20 سمكة/حوض سعة 100لتر وأستمرت التجربة لمدة 5 أسابيع واستغرقت المرحلة الاولى أسبوعين حيث وضعت الاسماك فى ماء ملوث (مرحلة التراكم) بعد ذلك المرحلة الثانية تم استخدام الطحالب لمدة ثلاثة أسابيع (مرحلة الازالة)، تم قياس العناصر السامة فى كل من المياة والاسماك بصفة دورية على مدار التجربة خلال مرحلتى التراكم والازالة وكذلك تم قياس بعض العوامل البيوكيميائية لسيرم الدم للاسماك واوضحت النتائج مايلى:

- ان لمساحة سطح الطحلب ومدة الاستخدام دور رئيسى فى الكميات الممتصة والمدمصة من العناصر السامة حيث ان كمية العناصر التى ازيلت بواسطة طحلب السارجاسم فاقت بكثير كمية العناصر بواسطة طحلب الديونيلا.

- زيادة التراكم فى الخياشيم عن العضلات وكذلك زيادة المتبقيات من العناصر بشكل ملحوظ فى جميع المعاملات ولكن أدت مرحلة الازالة والتنقية بواسطة الطحالب الى تقليل المتبقيات من العناصر السامة خاصة فى العضلات بشكل ملحوظ الى تركيز أقل من الحد المسموح به كما أدت الى تحسن قيم العوامل البيوكيميائية الى حد قريب من قيم المجموعة الضابطة (كنترول) .
- تأثرت معنويا قيم كل من الزيادة فى الوزن ((AWG ومعدل النمو اليومى (ADG) ومعدل النمو النوعى((SGR حيث سجلت المعاملة الخامسة والسادسة اعلى قيم لهذه المقاييس.
- لم يتأثر معنويا محتوى الاسماك من المادة الجافة باختلاف المعاملات بينما تأثر محتوى الجسم من البروتين والمستخلص الاثيرى والرماد باختلاف المعاملات وكان هناك فروق معنوية.
- أظهرت النتائج أن أسماك المعاملة الخامسة والسادسة أعطت أقل قيم للكرياتين، والكوليسترول والجلوكوز وحمض اليوريك فى بلازما الاسماك بينما أظهرت نتائج تلك المعاملات أعلى قيم لانزيمات نشاط الكبد (AST، ALT) الانزيمات الناقلة للاحماض الامينية وكذلك الهرمون المشجع لنمو الحويصلات FSH فى بلازما الدم.

لذلك توصى هذه الدراسة الى استخدام طحلب السارجاسم المجفف وخلايا طحلب الديونيلا الحية للتخلص بكفاءة من ملوثات مياة البحار والبحيرات والي تحسين جودة ونوعية المياة وكذلك الى التوازن البيئى المحيط بالاسماك بما يضمن تواجد اسماك نظيفة وآمنة صحيا.