Histopathological Changes in the Liver of the Nile Fish *Oreochromis niloticus* Fed on the Blue-Green Algae *Microcystis aeruginosa* under Laboratory Conditions

Samia M. Sanad, Mostafa A. Al Gamaal* and Dalia K. Hemmaid*

Abstract**—**The present study was planned to investigate the histopathological changes occurring in the liver of an edible and econimacally important tilapian fish in Egypt, *Oreochromis niloticus* after feeding with Microcystis aeruginosa cells mixed with their food under laboratory condition. Fish used in the present work were obtained from a fish hatchery in the central laboratory of fish research in Abassa, Abo Hamaad, Sharkia Governerate, Egypt.

Five groups each of three replica were established, three aquaria for the control group and three aquaria for each of the experimental groups. All fish (300) received diet twice daily at a daily feeding rate of 3% of the actual body weight, six days weekly. All aquaria were provided with continuous aeration and water was changed partially daily (siphoning) and totally three times weekly (every other day). During experimentation, fish of the control group were fed with fish food twice a day for 30 days. Fish of 3 experimental groups were fed for 30 days with fish food plus toxic cells of *Microcystis aeruginosa* (0.623, 1.246 and 1.869 gm Microcystis cells/kg food pellets). Fish of the fifth group were fed twice daily for 30 days with fish food plus toxic cells of Microcystis aeruginosa (1.869 gm Microcystis cells/kg food pellets) and were fed twice daily for another 30 days with food pellets free of cyanobacteria.

Samples of the liver were taken from both control and treated fish at the end of the exposure regimen. Various histopathological changes were observed in the liver tissue which were represented by degeneration and vaculation of the cytoplasm, pyknosis of the nuclei, fibrotic connective tissue patches, dilation of the central veins and blood vessels which had thickened walls and were congested with blood. Infiltration of the plasma cells and monocytes were noticed. The pancreatic cells appeared hypertrophied and were loaded with eosinophilic zymogen grnaules. There was a dose response relationship; the higher the exposure dose, the more drastic were the changes. Most of the changes were reversible and after 30 days of with drawal of *Microcystis aeruginosa* cells from the food of fish, recovery of the liver was observed and the picture appeared more or less normal.

Keywords**—**Histopathology, Liver, Nile fish *Oreochromisniloticus*, Blue Green Algae *Microcystsis aeruginosa*, Laboratory Conditions..

Prof. Samia M. Sanad, Zoology Department, Faculty of Science, Zagazig University, Egypt. Email Egypt-sbes@hotmail.com.

Mostafa A. Al Gamaal, and Dalia K. Hemmaid are with Limnology Department, Abbasa Central Laboratory for Auaculture Research, Egypt

I. Introduction

MASS growth of blue-green algae in eutrophic water bodies leads to a large amount of toxins, e.g. microcystins (Jing *et al*., 2014). In another recent study, Davis *et al.* (2014) pointed out that, many harmful cyanobacterial genera have strains that can produce potent toxins and other biologically active compounds that present a risk to the health of humans and other animals that consume or contact contaminated water. The occurrence of heavy cyanobacterial blooms has become a worldwide problem, as a consequence of eutrophication of the aquatic ecosystems; furthermore, 60% to 75% of these blooms have been found to be toxic (Mitsoura *et al*. 2013). Moreover, Steffen *et al*. (2014) reported that, the pervasive, persistent and highly toxic cyanobacterial bloom in Grand Lake St. Marys (Ohio, USA) led to the collapse of the local tourism industry, with microcystin concentrations exceeding 2000 $\mu g\ mL^{-1}$ at some locations during the peak of the bloom. Massive accumulations of cyanobacteria (blue-green algae), known as freshwater harmful algal blooms , are a common global occurrence in water bodies used for recreational purposes and drinking water purification (Weirich and Miller, 2014). Hence, Smutná *et al*. (2014) considered the toxic cyanobacterial blooms as a global threat to human health and aquatic biota. Buratti *et al.* (2013) reported that many cyanobacterial species can produce cyanotoxins, among which microcystins (MC) are a group of ≈100 congeners of hepatotoxic cyclic heptapeptides. In addition, He *et al.* (2013) pointed out that, the cyanobacterial toxin, MC-LR, is predominantly presented during toxic cyanobacterial blooms and is consumed by phytoplanktivorous and zooplanktivorous fish directly. Moreover, Acuna *et al.* (2012) pointed out that, the toxic cyanobacterium *Microcystis* sp. is a potential and unquantified threat to the health and survival of aquatic organisms such as fish. The authors added that the microcystins (MCs) produced by *Microcystis sp.* are hepatotoxic and represent a potential threat to the fishery. Fish can be exposed to MCs either during feeding (cyanobacterial blooms) or passively when the toxins pass through the gills during breathing (Malbrouck and Kestemont, 2006). Microcystins (MCs) are toxic to fish at concentrations as low as a few micrograms per liter (μg/L) or even fractioal μg/L (Ibelings and Havens, 2007).

http://dx.doi.org/10.15242/IICBE.C0215063

Fish occupy the top of the aquatic food chain and mostly are affected by exposure to microcystins. Therefore, consumption of the affected fish may pose a great risk to humans (Maglhaes *et al*., 2001). Moreover, Bruno *et al.* (2012) considered cyanobacterial toxins as a worldwide cause of human poisoning and an important health hazard for human beings. According to Djediat *et al.* (2010), microcystins constitute a public health threat via drinking water and food chains. The risk of toxicity by cyanobacterial microcystins came from the fact that the latter have been detected in drinking water and in tissues of aquatic organisms used for human consumption (Verspagen *et al*., 2006; Bruno *et al*., 2009; Kim *et al*., 2010). Consumption of the toxicated fish by humans and other animals may lead to several health complications (Mehra *et al*., 2009). Fish consumption was considered by Dyble *et al*. (2011) as a potential route of human exposure to the hepatotoxic microcystins, especially in lakes and reservoirs that routinely experience significant *Microcystis* sp. blooms predicting potential human health impacts. The powerful microcystin toxins produced by *Microcystis* can initiate cancer and promote tumor formation in the liver of humans, fish and wildlife animals (Zegura *et al*., 2003; De Figueiredo *et al*. 2004; Herfindal and Selheim, 2006; Ibelings and Havens, 2008 and Lehman *et al*., 2010). The International Agency for Research on Cancer (IARC, 2006&2010) classified microcystins as 2B in its cancerogenic scale. This level means that, they are very effective tumor promoters. Microcystins were linked to liver and colon cancer (Fleming *et al*., 2002 and Zhou *et al*., 2002). The potential consequences of low-level chronic exposure to *Microcystis* blooms and cyanobacterial toxins could result in liver disease, and promotion of carcinogenic tumors in animal models (Falconer, 2008). Chronic exposure of humans to microcystins through drinking water and fish consumption has been correlated with increased liver damage in local residents (Fleming *et al*., 2002) and recreational contact in water experiencing *Microcystis sp.* blooms (Backer *et al*., 2010).

Wolf and Wolfe (2005) pointed out that, there is a solid evidence showing that, fish should be acknowledged as a worthy models for assessing hepatotoxicity in terms of both field and laboratory-based research. However, Ibelings and Havens (2008) pointed out that, although the potential impact of *Microcystis sp.* blooms on human health is known, its potential impact on the structure and function of aquatic food webs is poorly understood. Although a great deal of research has been dedicated to the effects of cyanotoxins (cyanobacterial toxins are of the cyanotoxins) on warm-blooded terrestrial animals, the ecological role of these toxins in the aquatic environment remains under debate (Wiegand & Pflugmacher,2005 and Leflaive &Ten-Hage, 2007).

Little informations are available on the toxic effects of microcystins (MCs) produced by cyanobacteria on phytoplanktivorous fishes which are more frequently exposed to cyanobacterial toxins under natural conditions because of the habitat and feeding mode. There have been very few studies concerned with the subchronic oral toxicity of microcystis to fish under laboratory conditions. Therefore, the present study was designed to investigate the histopathological changes occurring in the liver of an edible and econimacally important tilapian fish in Egypt, *Oreochromis niloticus* fed on cells of *Microcystis aeruginosa*. The toxic effects of chronic exposure to these cyanobacteria and their MCs need to be evaluated in order to elucidate a better understanding of mechanism underlying hepatic intoxication in fish. The toxicological histopathological data which could be obtained would greatly reduce the degree of uncertainty in this concern and determine the risk assessment of such cyanobacteria cells exposure.

II. Material And Methods

A. The cyanobacteria (Microcystis aeruginosa):

In the present study, The used cyanobacteria (blue green algae) were *Microcystis aeruginosa* isolated from the fish farm of Abbasa Central Laboratory for Aqaculture Research belonging to the Agriculture Research Center located in Sharkia Governorate, Egypt.

B. Microcystis aeruginosa Culture:

Culture of algae was obtained by cultivation of *Microcystis aeruginosa* in 250 ml Erlenmeyer flasks containing 150 ml sterilized medium (Allen). The Erlenmeyer flasks were stoppered by cotton plugs and sterilized in an autoclave at 121.5ºC and 1.5 atm. for 20 minutes. Sterilized flasks were cooled and then inoculated with 30 ml of the pure algal culture and aerated by air pumps. The flasks were incubated at 30±2ºC under continuous illumination by white light fluorescent tubes providing 3000-3500 lux light intensity.

C. Mass culture of Microcystis aerigunosa:

The *Microcystis aerigunosa* culture was gradually amplified by cultivation using 2 liters flasks. In all the cultivation and mass production steps the flasks were maintained at the same above conditions. Full mass culture production was achieved when 1500 ml from the pre-culture were obtained and this was enough to inoculate the 20 liter carboys. The inoculated carboys were aerated and kept at the same above conditions of illumination and temperature. Providing carboys with compressed air was necessary to keep the *Microcystis aeruginosa* cells in suspension and supply them with CO_2 needed for the process of photosynthesis.

D. Harvesting the Microcystis aeruginosa Biomass:

The cultures of the cyanobacteria were harvested at the stationary phase (16-18 days). When the culture growth reached its maximum growth at the stationary phase, the cells were harvested after stoppage of the pumping system.

Feed Preparation:

The growing *Microcystis* growth was left to precipitate in a measuring cylinder and the superficial somewhat clear layers of water were discarded by siphoning. The remaining part of water suspension (950 ml) containing cells of *Microcystis aeruginosa* was left in the measure to determine the volume of the basal precipitated part loaded with heavy cellular suspension.The volume of such green suspension was estimated to be 300 ml.After that , the whole original suspension (950 ml) was manually shaked in the cylinder to

distribute homogeneously the cyanobacterial cells. Then, the solution was divided into 3 quantities; the first part consisted of 158.33 ml which contained 50 ml of heavily precipitated cells (out of 300 ml). The second part consisted of 316.66 ml which contained 100 ml of the heavily precipitated cells (i.e, 100 out of 300ml). The Third part consisted of 457 ml which contained 150 ml of the heavily precipitated cells (i.e, 150 out of 300 ml). Thus, this arrangement could be summarized as follows:

The total solution contained suspended *Microcystis aeruginosa* cells = 950 ml Volume of the heavily precipitated suspension in the above solution = 300 ml. The weight of *Microcystis aeruginosa* cells added to food stock in each treatment group was calculated and included:

Treatment I food stock: The weight of the dry *Microcystis aeruginosa* cells was o.623 gm/kg food pellet.

Treatment II food stock: The weight of the dry *Microcystis aeruginosa* cells was 1.246 gm/kg food pellet.

Treatment III food stock: The weight of the dry *Microcystis aeruginosa* cells was 1.869 gm/kg food pellet.

Table (1) shows the weight of *Microcystis aeruginosa* cells per 1 kg food pellet used in the three treated groups.

TABLE I
WEIGHT OF THE SUSPENDED *MICROCYSTIS AERUGINOSA* CELLS IN THE 3 TREATMENTS

Treatment	Volume of homogeneous solution (out of 950 ml)	Volume of heavily suspended solution (0ut of 300 ml)	Weight of *Microcystis aeruginosa* cells per 1 kg food pellet
First Treatment (T I)	185.33 ml	50 ml	0.623 gm
Second Treatment (T II)	316.66 ml	100 ml	1.246 gm
Third Treatment (T III)	457 ml	150 ml	1.869 gm

Fish *(Oreochromis niloticus)*:

Studies were conducted using *Oreochromis niloticus* (Nile tilapia, Perciformes: Cichlidae) with a mean weight of 30 ± 7 g (4–5 weeks old, length ~10± 2cm). Fish were obtained from a fish hatchery in the central laboratory of fish research in Abassa, Abo Hamaad, Sharkia Governerate, Egypt. The fish were acclimated to laboratory conditions for fifteen days in aquaria (20 individuals/aquarium) with 96 L of fresh water before beginning of experimentation. Filling the tanks at least three days before the fish were introduced minimized exposure to chlorine (aged dechlorinated tap water). Fish were kept at a water temperature of 22 ± 2°C in water under continuous filtration and aeration.

The water parameters remained constant throughout the experiment at dissolved oxygen (6–8 mg/l), pH (7.6 ± 0.2), CO_2 (10 mg/l), NH_3 (0.02 mg/l), alkalinity (150 mg/l), hardness (180 mg/l), NO_2 (0.01 mg/l) and NO_3 (0.4 mg/l). Mean values for additional parameters of water quality were: conductivity 292 ¼S/cm, Ca_2± 0.60 mM/L, and Mg_2± 0.3 mM/L. Fish were fed standard laboratory floating pellets (32% protein content) twice a day (at 8 AM and 1 PM).Faecal matter was removed before each morning feeding.

Exposure:

The experiment was carried out using fifteen glass aquaria (70X40X30 cm) with twenty fish in each. Twenty fish (approximately of the same size, 30g average) were stocked into each of the 15 aquaria which contained 50 liter of water, three aquaria for the control group and three aquaria for each treatment (treatments I, II, III and recovery group). The aquaria were provided with continuous aeration and their water was changed partially daily (siphoning) and totally three times weekly (every other day). All fish (300) received diet twice daily at a daily feeding rate of 3% of the actual body weight, six days weekly for four weeks (30 days).

The first group of fish (control group) received only the food pellets free of cyanobacteria. The tilapias of the treated groups were divided into 3 groups each group of 3 replicas (three aquaria for each group containing 20 fish/aquarium). During experimentation, fish were fed with fish food plus toxic cells of *Microcystis aeruginosa* according to the following groups:

1- Treatment group I (T1): In this group, each fish were fed twice daily for 30 days with fish food plus toxic cells of *Microcystis aeruginosa* (0.623 gm *Microcystis* cells/kg food pellets).
2- Treatment group II (T2): In this group, each fish were fed twice daily for 30 days with fish food plus toxic cells of *Microcystis aeruginosa* (1.246 gm *Microcystis* cells/kg food pellets).
3- Treatment group III (T3): In this group, each fish were fed twice daily for 30 days with fish food plus toxic cells of *Microcystis aeruginosa* (1.869 gm *Microcystis* cells/kg food pellets).
4- Treatment group IV (Recovery group): In this group, each fish were fed twice daily for 30 days with fish food plus toxic cells of *Microcystis aeruginosa* (1.869 gm *Microcystis* cells/kg food pellets). Fish of this group were fed twice daily for another 30 days with food pellets free of cyanobacteria.

E. Histological and histopathological studies:

For histological and histopathological studies, small pieces of the liver were taken from control and treated fish at the end of exposure regimen (30 days). For the recovery group, fish were dissected at the end of the second month (60 days).

Samples were fixed in 10% buffered formalin for twenty-four hours at 4ºC, dehydrated in ascending grades of ethanol, immersed in xylol, and embedded in paraffin wax. Sections of 4-5 µm thick were mounted on clean glass slides, deparaffinized, rehydrated, stained with hematoxylin and eosin and mounted with DPX . Sections were examined using a light microscope.

III. RESULTS

Liver of Control Fish:

The liver of the normal *Oreochromis niloticus* is composed of a parenchyma covered by a thin capsule of connective tissue

is divided into irregular lobules by the hepatopancreas. The weakly organized lobules are built up of cords of hepatocytes parenchyma covered by a thin capsule of connective tissue. It is radiated out from a centrally located central vein or large central sinusoid (Figs. 1&2).

In *Oreochromis niloticus*, the liver and pancreas are combined into a single organ, the hepatopancreas. The liver portion of the hepatopancreas lacks the distinct lobulation known in mammals. The combined hepatic and pancreatic tissue are collectively called the hepatopancreas (Figs. 2, 3 &4).

The hepatocytes comprise the main bulk of the liver forming parenchyma and they are arranged in cords radially surround large blood sinusoids or central vein. Hepatocytes are similar in appearance and vary from polyhedral to round shape. Each hepatocyte contains a large, spherical central nucleus with a prominent dark nucleolus. Within the parenchyma, the hepatocytes are radially arranged in cords around a central sinusoid. Large cells resting on the luminal surface of the sinusoid endothelium are present; these cells are known as Kupffer cells (Figs. 1, 3 & 4).

The hepatocyte-sinusoidal structure of the fish liver is of the solid form in which the major part of the hepatocyte lining is multi-layered. The hepatic sinusoids are narrow and short and have tortuous capillaries. Some of the hepatocyte surfaces line the sinusoidal channel (sinusoidal surface), while other surfaces are in contact with the adjacent hepatocyte (intercellular surfaces). Some of the adjacent hepatocyte surfaces have bile canaliculi running between them. The lumen of the sinusoids contains mainly erythrocytes and macrophages. Sinusoids are covered by typical endothelial cells with flattened nuclei.

The Pancreatic tissue proliferates along branches of the hepatic artery and eventually appears as randomly distributed regions surrounded by a liver portion and are seen as pancreatic "islands" scattered throughout the organ. There is a duct system into which the pancreatic cells empty their secretion. Blood vessels may run through the pancreatic "island." The erythrocytes demonstrated inside such blood vessels are nucleated.

The exocrine pancreas or hepatopancreas has an acinar arrangement. Cells of the pancreatic patches are separated from the remaining hepatic parenchyma by a thin layer of connective tissue. The pancreatic cells usually proliferate around branches of the hepatic portal artery. They are separated from the artery by a basal membrane and reticular fibers The portal vessels are clearly distinguishable due to the pancreatic acini which surround it. The basal region of the inner row of the hepatopancreatic cells is adjacent to the basal membrane of the portal vein. The basal region of the outer hepatopancreatic row of cells is in contact with the surrounding thin layer of the connective tissue.

The exocrine acinar cells are tall and columnar possessing euchromatic basally located spherical nucleus with a prominent dark nucleolus. Acinar cells have basophilic cytoplasm at their lower pole but many large eosinophilic zymogen granules are located in the apical ends of these cells

Liver of Treated Fish

Liver of fish fed with 0.623 gm *Microcystis aeruginosa* cells/kg food pellets for 30 days(T I):

Following feeding *Oreochromis niloticus* fish with the lowest dose of Microcystis aeruginosa algae for 30 days, histopathological lesions were demonstrated in the liver (Figs. 5-8). These pathological perturbations were represented by cytoplasmic degenerations in addition to the presence of pyknotic nuclei. The central vein was mostly congested with blood and monocytes .At some regions of the hepatic parenchymal portion, fibrotic connective tissue was demonstrated. Such regions were crowded with infiltration of plasma cells, monocytes and pyknotic nuclei.

Concerning the hepatopancreas, the portal vessel was clearly congested with coagulative eosinophilic secretion. Such aggregations were nearly homogeneous and inturrpted by spherical vacuoles of variable diameters . Cells of the pancreatic acini revealed lowered eosinophilia. There was patchy fibrotic regions scattered in the hepatic portion. These fibrotic parts were densly basophilic, branched and characterized by the presence of pyknotic nuclei .

Liver of fish fed with 1.246 gm *Microcystis aeruginosa* cells/kg food pellets for 30 days (T II):

Administration of tilapia fish with the medium dose of *Microcystis aeruginosa* cells exhibited more pathological deteriorations in the liver (Figs. 9-12). Cytoplasmic degenerations of hepatocytes were prominent. Numerous pyknotic nuclei were seen especially in the cells scattered in the fibrotic patches. Dilation and enlargement of the central veins or large blood vessels were obvious. Such dilated vessels have thickened walls crowded with basophilic cells. Infiltrated monocytes and microphages could be demonstrated. In some sections, the extensively dilated central vein was obviously congested with nucleated blood cells and monocytes.

The pancreatic cells were enlarged and loaded with eosinophilic zymogen granules. The portal artery in the center of the pancreatic tissue was congested and filled with homogeneous purple secreation . Cells of the pancreatic patches sometimes showed hypertrophy. Degenerative portions were demonstrated in the hepatic tissue surrounding these pancreatic patches.

Liver of fish fed with 1.869 gm *Microcystis aeruginosa* cells/kg food pellets for 30 days(T III):

Inspection of hepatopancreatic sections from livers of fish fed on the highest applied dose of *Microcystis aeruginosa* cells for 30 days displayed numerous drastic pathological changes (Figs. 13-19).The vascular pathological deteriorations were prominent as exemplified by the sections seen in figs 13, 14, 15, & 18. The blood vessels were completely occluded with congested blood which was stained deep buffy or bricky in addition to the presence of inflammatory cells. The endothelial lining of these vessels were thickened and harboring necrotic cells.

The cytoplasmic degeneration in hepatocytes was prominent and involved the entire hepatic portion. Hepatocytes were vacuolated and it was hard to recognize the nuclei of these cells. Parts in the hepatic portion showed necrosis and

conspicuous degenerations Fibrotic connective tissue harboring pyknotic nuclei and cellular infiltration were commonly detected in the hepatic portion of the liver (Fig. 18). These fibrotic parts together with the associated pyknotic cells exhibited a bunch-like appearance. The central veins were generally congested with blood and infiltration of immune cells. The central vein seen in Fig. (17) was obviously enlarged and conspicuously dilated while the lumen was filled with a huge number of nucleated blood cells and monocytes. Sometimes the congestion in the large blood vessels was stained deep buffy or purplish and completely occludes the whole cavity of the vein (Fig. 15). Some vessels were markedly elongated and congested with blood. In such vessels, subsidiary branches were seen opening in the large elongated ones. The large blood vessel shown in Fig. (18) acquired a bizarre appearance. Its elongated rectangular lumen was filled with a huge number of nucleated blood cells and cells from the immune system. At one of its corners coagulative clumps were aggregated and stained deep purplish to violet or buffy. Many thin vascular branches were extending to various directions from such large vessels (Fig. 15).

Pancreatic cells were enlarged and the portal vein which was circumscribed by such cells was congested with blood and monocytes (Figs. 14, 16 & 19). Cells of the pancreatic portion seen in Fig. (16) were loaded with eosinophilic zymogen granules and cellular infiltration was demonstrated in the vicinity. Purplish to buffy secretory material was occasionally demonstrated in some pancreatic patches.

Liver of fish fed with 1.869 gm *Microcystis aeruginosa* cells/kg food pellets for 30 days and left for another 30 days for recovery (T IV):

Investigation of liver sections from Oreochromis niloticus fish fed with the highest dose of *Microcystis aeruginosa* cells for 30 days and left for another 30 days on food free from the toxic cyanobacteria exhibited a picture more or less similar to those of the controls (Figs. 20& 21).

In the hepatic portion of the liver, no cytoplasmic degenerations were observed. Hepatocytes displayed a normal radial arrangement similar to the control architecture. The cells were organized in nearly solid cords around the central vein. No fibrotic tissues or pyknotic nuclei were demonstrated in the liver. The nuclei of hepatocytes were intact and exhibited normal appearance. No congestion was observed in the central veins or large blood vessels.

The pancreatic portion of the hepatopanceas showed to a large extent a control-like architecture. Cells of the pancreas were tall with the normal staining affinity. Their bases were basophilic while the apex contained eosinophilic zymogen granules

IV. Discussion

Microcystis and other freshwater cyanobacterial blooms represent a worldwide concern because their frequency and distribution are increasingly prevalent in water reservoirs throughout the world (van Apeldoorn *et al*., 2007; Fristachi *et al*., 2008; and Paerl and Huisman, 2009). This concern came from the fact that they produce a surface scum on the surface of the water column (Lehman *et al*., 2008). This superficial scum impedes recreation, reduces aesthetics, lowers the dissolved oxygen concentration and causes taste and odor problems in drinking water (Paerl *et al*., 2001). Recently, Sotton *et al.* (2014) pointed out that, cyanobacterial cells constitute a part of food resource for herbivorous zooplanktonic taxa during bloom periods. Therefore, the present investigation was planned to explore the risk of feeding an economically important fish worldwide and in Egypt namely the tilapia fish (*Oreochromis niloticus*) with the famous cyanobacteria (*Microcystis aeruginosa*) forming such blooms.

The cyano-toxicological effects on the tilapia fish (*Oreochromis niloticus*) are thought to be quite important taken in consideration its commercial importance as a product of inland fisheries and aquaculture. Although acute toxicity experiments are useful in the study of toxicokinetics and histopathological changes, such experiments do not represent the mode of exposure in a natural environment (Ferrão-Filho and Kozlowsky-Suzuki, 2011). Because the chronic and subchronic effects in aquatic organisms might be more relevant than the acute lethal effects, in the present laboratory investigation fish were fed with the cyanobacteria for one month to mimic the real situation occurring during blooms.

Multiple studies and reports pointed out that , cyanobacteria threat human and ecosystem health (Babica *et al*., 2006; Backer *et al*., 2008; Paerl and Huisman, 2009 and Pathak & Singh, 2010). Toxins of *Microcystis sp*. have serious risks to humans including hepatic or gastrointestinal (colorectal) cancer due to chronic exposure to subacute doses in drinking water (Humpage *et al*., 2000; Zegura *et al*., 2003; Herfindal and Selheim, 2006 and Chen *et al*., 2009). The latter authors detected microcystins, for the first time, in the serum of a chemically exposed human population (fishermen) together with indication of hepatocellular damage.

In the present laboratory study, the route of *Microcystis aeruginosa* administration was the oral route. This route was chosen because it simulates the real case occurring by fish in water during their nutrition. Bury *et al*. (1998) considered the ingestion as the most probable route for microcystin uptake in fish. This is because the gills and skin epithelia of freshwater fish form a barrier to microcystin transport (Tencalla *et al*., 1994 and Bury *et al*., 1995). In this concern, Butler *et al*. (2009) reported that, several studies have observed severe liver damage in fish following oral administration of microcystins, usually in the form of freeze-dried cyanobacterial cells. In nature, usually fish are subjected to sublethal impacts resulting from exposure to microcystins over days or weeks. The World Health Organization (WHO) in 1999 reported that, microcystin concentrations in cyanobacterial blooms commonly reach 20,000 µg MC/kg algae and have been reported as high as 129,000 µg MC/kg algae.

The liver is of utmost physiological importance as it is the chief site for storage of high energy foods (glycogen, lipids) and it also plays a vital role in detoxification, digestion and other physiological processes (Channa and Mir, 2009). In the studies concerned with toxicity of fish by microcystins the

liver was the most affected organ among various fish organs. This finding was true in *Cyprinus carpio* (Fischer and Dietrich, 2000) and *Oreochromis sp*. (Jos *et al*., 2005). The present investigation is concerned with exploring the effects of feeding fish with the cyanobacteria *Microcystis aeruginosa* for one month under Laboratory conditions.

The presently applied feeding regimen in which fish were fed with the low, intermediate or the highest doses for 30 days, the liver exhibited an obvious cytoplasmic degenerations in all cases. Degenerative cytoplasmic regions demonstrated in hepatic cells of treated fish may reflect rupture of lysosomal enzymes and high activity of lysosomes. According to Marie et al. (2012), cyanobacterial toxic blooms constitute a potential health risk to human populations as well as to fish and other aquatic organisms. During the blooming period in lakes, bioaccumulation in the intracellular and extracellular microcystins in the fish (Silver and bighead carp) was reported (Guo *et al*., 2014). Hence, studies of Malbrouck and Kestemont (2006) concluded that, *Microcystis sp*. blooms affect fish health through impacts on growth rate, histopathology and behavior.

In the Nile tilapia (*Oreochromis niloticus*), Palikova *et al*. (2011) demonstrated accumulation of microcystins (toxins of *Microcystis sp*.) in organs of the fish. In a recent study (Trinchet *et al*., 2013), microcystin concentrations in the organs revealed that accumulation was particularly high in the digestive tract and the liver, which are known to be classical targets of MCs. Mohamed *et al*. (2003) found that, the liver of fish is the target organ for microcystins. They attributed such accumulation in the liver to process of presystematic hepatic elimination which prevents or at least minimizes the distribution of foreign chemicals to other parts of the body. It has been earliearly suggested (Klassen and Watkins, 1984) that, when the presystematic hepatic elimination is overwhelmed or bypassed by exposure to toxins, these may circulate to other organs. Field studies have confirmed the uptake of microcystins into the liver and muscles of planktivorous (Xie *et al*., 2004 and Ibelings *et al*., 2005), omnivorous (Cazenave *et al*., 2005) and piscivorous fish (Xie *et al*., 2005). In the liver of the Nile tilapia (*Oreochromis niloticus*) the accumulated concentrations of microcystins were higher than in the muscles (Zhao *et al*., 2006 and Deblois *et al*., 2011). According to Dyble *et al*. (2011), there is an initial uptake of microcystin into the liver and then partially redistributed into the muscle. Moreover, Runnegar *et al*. (1993) suggested selective uptake of microcystins into hepatocytes occurring via active transport resulting in organ specificity of accumulation. Recently, Amrani *et al*. (2014) determined the presence of microcystins in different tissues including the hepatopancreas and liver of the common carp and the European eel captured from an Algerian lake. Moreover, Best *et al*. (2003) found that, exposure of the freshwater rainbow trout to intact cells of a microcystin-producing cyanobacterium led to an increase in the liver mass (hepatosomatic index) by approximately 18%. Also, in fish obtained from 4 Swedish lakes, Larson *et al*. (2014) recorded bioaccumulation of microcystins in their livers due to exposure to *Microcystis aeruginosa*.

Liver histopathological lesions demonstrated in the present investigation reflect toxicity effects of the applied species of cyanobacteria (*Microcystis aeruginosa*). In this concern, Lehman *et al*. (2010) scored seven characteristics of the fish liver lesions following exposure to *Microcystis aeruginosa* in estuarine food web production in San Francisco Estuary. These characteristics included: glycogen depletion, eosinophilic protein droplets, cytoplasmic inclusions depletion, single cell necrosis, fatty vacuolation or lipidosis, macrophage aggregates and focal/multifocal parenchymal leucocytes or lymphocytes.

Liver alterations in the present histopathological investigation were evident mainly in the form of loss of the hepatic architecture and dose dependent vacuolation as well as cytoplasmic degenerations which corroborates with earlier studies (Falconer and Yeung, 1992; Snyder *et al*., 2002 and Li *et al*., 2003). In general, the gross histopathological changes in the liver of *Oreochromis niloticus* following administration of *M. aeruginosa* are similar in many respects to those seen in mammals (Zurawell *et al*. 2005). Nevertheless, several dissimilarities do exist because species-specific differences in MC tolerance are indicated by the degree of liver damage and liver enzyme activities in the blood of exposed fish (Fischer and Dietrich 2000 and Best *et al*. 2001).

The cytoplasmic degeneration in hepatic cells was one of the noticeable histopathological lesions demonstrated in the present study. Such degeneration was recorded in the livers of fish in the three treated groups . Many investigators studied the histopathological findings and the mechanism of microcystins influence. Similar studies have emphasized on the histopathological findings of cyanobacteria in *Cyprinus carpio* (Li *et al*., 2003&2004), in rainbow trout (Kotak *et al*., 1996), in *Tilapia rendalli* (Soares *et al*.,2004) and in *Oreochromis niloticus* (Oruc and Uner, 2000; Mohamed *et al*., 2003 and Jos *et al*., 2005). It is well established that, one cyano-toxigenic mechanism is the potent inhibition of the protein phosphatases 1 and 2A. This inhibition results in the abrogation of the protein phosphorylation status, which is directly associated with the cytotoxic effects and tumour-promoting activity of cyanotoxins (Carmichael, 1994 and Hooser, 2000).

The histopathological observations noticed in the present study seem to be in agreement with previous reports concerning cyanobacterial toxicity (Malbrouck and Kestemont, 2006; Qui *et al*., 2007 and Jiang *et al*., 2011). Hydropic degeneration and necrosis observed in the present study are in agreement with the findings reported by Snyder *et al*. (2002) and Liu *et al*. (2002). The latter authors detected histological findings in the common carp exposed to microcystin represented by loss of the parenchymal architecture, hepatocyte necrosis with vacuolization of the cytoplasm and the presence of lipid droplets. Vacuolization has been reported in isolated hepatocytes from the common carp (Li *et al*., 2001) after oral exposure to microcystins. Vacuolar dystrophy of hepatocytes of carp accompanied with nuclear damage was observed in fish exposed to cyanobacterial extract (Palikova *et al*., 2004). According to Molina *et al*. (2005) and Atencio *et al*. (2008), vacuolization might indicate an imbalance between the rate of synthesis of

substances in the parenchymal cells and the rate of release of these substances into the systemic circulation.

In the present investigation, administration of the applied food regimen containing *Microcystis aeruginosa* cyanobacteria produced characteristic histopathological changes in the fish liver. These were exemplified by fatty infiltration, liver degeneration, inflammatory cell infiltration, and focal necrosis. Liver necrosis was described in other studies by various authors (Fischer and Dietrich, 2000). According to the latter authors, necrosis of hepatocytes represents primary events in microcystins induced hepatotoxicity and apoptotic cell death is a secondary event. Djediat *et al*. (2010) reported that, subacute and acute exposure to microcystin contamination can exacerbate physiological stress, induce pathological damage and affect the immune response of the fish. Focal necrosis and hepatic cells vacuolization were detected by Palikova *et al*. (2004) in *Cyprinus carpio* larvae exposed to cyanobacterial extract containing 85% of *Microcystis aeruginosa*.

Results of the present study displayed a dose-dependent effects following *Microcystis aeruginosa* administration. The highest dose exhibited the most drastic effect of such treatment. In this concern, Zhang *et al*. (2008) documented a dose-dependent effect of microcystins on cat fish. Moreover, Mehra *et al*. (2009) showed dose-dependent effects on the fish *Heteropeustus fossilis* after oral ingestion of cyanobacterial blooms containing *Microcystis sp*. under laboratory conditions. Other investigators (Prieto *et al*., 2007) focused on time-dependent response of *Oreochromis niloticus* after acute exposure to microcystins in the lab. These findings suggest accumulation of microcystins in the fish liver which can detoxify them after a certain time period. Dyble *et al*. (2011) found that, the concentration of microcystin in the fish liver was elevated by 4-6 hours and peaked at 8-10 hours after oral dosing. The findings of Mohamed *et al*. (2003) proved that, *Oreochromis niloticus* can accumulate high levels of microcystins that, although not toxic to the fish, may lead to levels above the recommended levels for drinking water to humans. The investigators detected the presence of high numbers of toxic *Microcystis* cells in the gut of fishes graze on cyanobacteria in a fish farm containing blooms of them. According to Harada *et al*. (1996), the high risk on humans from toxicated fish came from the fact that, microcystins are heat stable and they are not broken down by cooking

Soares *et al*. (2004), found that, under laboratory conditions , liver of *Tilapia rendalli* accumulated microcystins in the liver when they were fed daily with fish food plus toxic cells of *Microcystis aeruginosa* for 15, 28 and 42 days . Djedial *et al*. (2010) using immunohistochemical methods, recorded accumulated microcystins in contaminated fish tissues. Several studies concluded that, cyanobacterial toxins are known to bioaccumulate in common aquatic vertebrates and invertebrates, including fishes (Amrani *et al*., 2014). Such bioaccumulation poses a potential risk to both animal and human health if such aquatic animals are consumed (Ibelings and Chorus, 2007).

Lang *et al*. (2006) suggested a categorization system for the diagnosis of liver histological alterations in terms of the toxicological relevance of these alterations based on the findings in flat fish species. van Dyk *et al*. (2011) have applied this system to evaluate pollution-related histopathological changes in the catfish. These categories include non-specific lesions, early toxicopathic non-neoplastic lesions, pre-neoplastic lesions and neoplasms. The alterations listed within each of these categories are widely accepted as useful indicators for monitoring the biological effects of contaminants on fish (Feist *et al*., 2004). Using the proposed categories as guidelines, many of these alterations were also observed in *Oreochromis niloticus* during the present study, such as early toxicopathic, non-neoplastic lesions, including hepatocellular necrosis, nuclear pyknosis, and vacuolization or cytoplasmic degeneration.

Concerning the interpretation of the mechanisms and causes of pathological lesions occurring under the effect of cyanobacterial intoxication, many studies suggested certain explanations. Falconer *et al*. (1983) interpreted the mechanism of liver damage by toxicity from *Microcystis aeruginosa.* Falconer and Yeung In (1992), attributed the microcystin toxicity in fish hepatocytes to cytoskeletal damage leading to loss of the cell morphology, cell to cell adhesion and finally cellular necrosis. The disruption of the hepatocyte cytoskeleton in animals (Ding *et al*., 2000 & Ding and Ong, 2003) and fish (Li *et al*., 2003) in addition to hyperphosphorylation of cytosolic and cytoskeletal proteins are the agents responsible for damage of hepatocytes. It is well established that, one of the toxic mechanisms of microcystins is their ability to inhibit protein phosphatases 1 and 2A leading to such disruption of hepatocyte cytoskeleton and finally disintegration of these cells. The inhibition of cellular protein phosphatases by microcystins induces oxidative DNA damage (Zegura et al., 2003) and activation of some proto-oncogens (Li *et al*., 2009). Earlier, Eriksson *et al.* (1989) pointed out that, the histopathological deformation produced by toxic microcystins induces degenerative changes of the tissue. The widely accepted mechanism of such toxicity is the irreversible inhibition of proteinphosphatses 1 and 2A, which are key regulatory enzymes in catalyzing dephosphorylation of serine/threonine residues in various phosphoproteins (structural proteins, enzymes, regulators). Fujiki and Suganuma (2009) interpreted toxicity by microcystins on the basis that they inhibit the activity of serine/theronine protein phosphatases 1 and 2A. This inhibition resulted from covalent binding of microcystins and these protein phosphatases enzymes. Inhibition of protein phosphatases is followed by loss of cytoskeletal integrity and subsequent cytolysis or apoptosis, primarily of hepatocytes (Dietrich & Hoeger, 2005). At acutely toxic doses, rounding of hepatocytes occurred concurrently with the loss of normal hepatic architecture. The latter pathological changes are considered to result from the interaction of MC with serine/threonine protein phosphatases-1 and -2A (PP), essential for maintaining the monomerization (phosphorylation)/ polymerization (dephosphorylation) equilibrium of the cytoskeletal intermediate filaments (Eriksson *et al*., 1989). Earlier studies (Dawson, 1998) concluded that, the inhibition of serine/theronine protein by microcystins can cause disintegration of the liver structure,

hepatocytes damage, necrosis and internal hemorrhage in the liver. Inhibition of these protein phosphatases enzymes in fish can ultimately result in widespread cellular death and loss of liver structure (Malbrouck and Kestemont, 2006). Another important biochemical mechanism of microcystin toxicity is the oxidative stress. Butler *et al*. (2009) reported that, such inhibition is believed to be a mechanism by which microcystins destroy livers. Hooser (2000) showed that, hepatocytes from animals treated with microcystins appear to die by programmed cell death or suicide (apoptosis). Thus, it could be suggested that, the presently demonstrated disarrayed liver architecture and disintegrated hepatocytes following *Microcystis aeruginosa* administration mostly attributed to the mentioned enzymatic inhibition. Also, other studies (Boaru *et al*., 2006 and Butler *et al*., 2009) documented that, microcystins are actively taken up by the liver in fish where they disrupt normal cellular activity by inhibiting protein phosphatases. Microcystins have been shown to induce formation of reactive oxygen species that might cause serious cellular damage such as peroxidation of lipid membranes, genotoxicity, or modulation of apoptosis (Ding & Ong, 2003). The formation of reactive oxygen species is the most likely mechanism responsible for oxidative damage of DNA, genotoxic and clastogenic effects of microcystins (Humpage and Falconer, 1999; Humpage *et al*., 2000 and Bouaicha *et al*., 2005). However, the exact mechanism of oxidative stress promoted by microcystins is still not known. In the liver, glutathione s-transferase (GST) is an important enzyme in detoxifying microcystin and mitigating damage from the oxidative stress (Pflugmacher *et al*., 1998). In this concern, Amado and Monserrat (2010) pointed out that, exposure to significant levels of microcystins reduced the available glutathione S-transferase. Such reduction results in increased oxidative toxicity ultimately leading to increased necrosis (Prieto *et al*., 2006 and Amado & Monserrat, 2010). The above discussed interpretations for mechanism of cyanobacterial effects at the cellular level seem to be acceptable and are considered scientifically a logic issue.

In three cyprinid fish species Gavrilovic *et al.* (2014) recorded a decrease in liver catalase after exposure to cyanobacterial blooms. The authors considered this decrease in catalase activity in addition to decrease in glutathione peroxidase activity that was detected in all the three species may be directly responsible for an enhanced susceptibility of fish to potential oxidative stress caused by the cyanobacterial bloom.

van Dyk *et al.* (2011) applied a categorisation system for the diagnosis of liver histological alterations suggested by Lang *et al.* (2006) to evaluate pollution-related histopathological changes in the catfish. These categories include non-specific lesions, early toxicopathic non-neoplastic lesions, pre-neoplastic lesions and neoplasms. Most of these alterations were also observed in *C. carpio* by Mitsoura *et al.* (2013), in addition to hepatocellular necrosis, nuclei pyknosis, vacuolisation and steatosis.

The present results revealed that, the pathological alterations occurred in the fish liver following feeding with *Microcystis aeruginosa* were reversible. This was evident by the findings observed in the recovery group (feeding fish with the highest dose for 30 days and leaving them to feed for another 30 days on food without the toxic *Microcystis aeruginosa* cells). The liver of these fish group exhibited to a large extent a control-like architecture and the hepatocytes appeared more or less normal. Fischer *et al*. (2000) pointed out that, the question remains whether the pathological changes observed in hepatocytes result from the rapid (but reversible) interaction of microcystins with protein phosphatases (PP 1 and PP 2A) or other covalent binding to these enzymes leading to their inhibition is necessary for the initiation of subsequent cascade of changes. Studies of kaya (1996) presented evidence supporting the hypothesis that, the reversible binding of microcystins to the protein phosphatases is sufficient for their inhibition and development of liver pathology. However, Mackintosh *et al*. (1995) documented that, the cyanobacterial toxin microcystin binds covalently to cysteine-273 on protein phosphatase 1. Also, other studies reported that, in hepatocytes, microcystins bind covalently and irreversibly to the catalytic subunit of protein phosphatase 1 and 2A (Bischoff, 2001).

In conclusion, the present laboratory investigation confirms the risk of fish exposure to the cyanobacterial blooms especially those containing *Microcystis aeruginosa* known by its highly toxic microcystins. Risks on the most edible and economically important fish in Egypt and Worldwide, *Oreochromis niloticus*, were proved by detecting the histopathological alterations occurring in the liver of fish fed with the cyanobacteria. Such pathological lesions were represented by cytoplasmic degeneration of hepatocytes, pyknosis, of the nuclei, vacuolar changes, disintegration, fibrotic changes, blood vessels congestion and occlusion of vessels.

The significance of the findings achived in the present work came from the fact that, the pattern of effects on fish liver could be comparable to human liver. Wolf and Wolf (2005) reported that, it may be reassuring to note that, there are similarities than differences between fish and mammals in terms of their macro- and microanatomy, physiological and biochemical characteristics and pathologic responses to hepatotoxic substances. In this concern, Mohamed *et al*. (2003) recommended monitoring of microcystin-producing water blooms in fish farms. In their opinion, fish tissues monitoring as well as blooms monitoring are important issues should be followed to protect human from possible toxicity associated with *Microcystis* blooms.

Djediat *et al.* (2010) pointed out that, microcystins are potent cause of environmental stress and pose a potential health hazard in aquatic ecosystems when heavy blooms of cyanobacteria appear. The authors also reported that, these blooms constitute a public health threat to people via drinking water and food chains. It should be emphasized that, biomarkers measured at the molecular level in fish have been proposed as sensitive "early warning' tools for biological effect measurements in environmental quality assessments (Pathiratne *et al*., 2010). Results of the present work confirms that, there should be appropriate risk management decisions for human consumption of fish harvested from freshwater

bonds characterized with mass occurrence of cyanobacterial blooms. Also, monitoring should be extended to fish farms in which flourished cyanobacterial blooms are prominent. The levels of microcystin accumulation will depend on the level of human consumption of fish and the severity of the toxic blooms in the area where fish are caught (Al-Kahtani and Fathi, 2008). Therefore, it could be recommended that, as a general trend, the viscera of fish should not be eaten. In addition, caution should be taken when fish are caught from farms or natural waters where major or large toxic blooms occur

REFERENCES

[1] Acuña, S.; Deng, D.; Lehman, P. and The, S. (2012): Sublethal dietary effects of Microcystis on Sacramento splittail, Pogonichthys macrolepidotus. Aquatic Toxicology, 110–111: 1–8. http://dx.doi.org/10.1016/j.aquatox.2011.12.004

[2] Amado, L. L. and Monserrat, J. M. (2010): Oxidative stress generation by microcystins in aquatic animals: why and how. Environ Int, 36:226–235. http://dx.doi.org/10.1016/j.envint.2009.10.010

[3] Amrani, A.; Nasri, H.; Azzouz, A.; Kadi, Y. and Bouaïcha, N. (2014): Variation in Cyanobacterial Hepatotoxin (Microcystin) Content of Water Samples and Two Species of Fishes Collected from a Shallow Lake in Algeria. Arch Environ Contam Toxicol, DOI 10. 1007/s 00244-013-9993-2.

[4] Atencio, L.; Moreno, I.; Jos, A.; Pichardo, S.; Moyano, R.; Blanco, A. and Camean A (2008): Dose-dependent antioxidant responses and pathological changes in tenca (Tinca tinca) after acute oral exposure to Microcystis under laboratory conditions. Toxicon. 52:1–12. http://dx.doi.org/10.1016/j.toxicon.2008.05.009

[5] Babica, P.; Bláha, L. and Maršálek, B.(2006): Exploring the natural role of microcystins – a review of effects on photoautotrophic organisms. J Phycol. 42:9–20. http://dx.doi.org/10.1111/j.1529-8817.2006.00176.x

[6] Backer, L.C.; Carmichael, W.; Kirkpatrick, B.; Williams, C.; Irvin, M.; Zhou, Y.; Johnson, T.B.; Nierenberg, k.; Hill, V.; kieszak, S. and Cheng, Y. (2008): Recreational exposure to microcystins during a Microcystis aeruginosa bloom in a small lake. Marine Drugs, 6:369-406. http://dx.doi.org/10.3390/md6020389

[7] Backer, L.; McNeel, S.; Barber, T.; Kirkpatrick, B.; Williams, C.; Irvin, M.; Zhou, Y.; Johnson, T.; Nirenberg, K.; aubel, M.; et al. (2010): Recreational exposure to microcystins during algal blooms in two California lakes. Toxicon, 55:909-921. http://dx.doi.org/10.1016/j.toxicon.2009.07.006

[8] Best, J. H.; Eddy, F. B. and Codd, G. A. (2001): Effects of purified microcystin-LR and cell extracts of Microcystis strains PCC 7813 and CYA 43 on cardiac function in brown trout (Salmo trutta) alevine. Fish Physiol Biochem, 24: 171–78. http://dx.doi.org/10.1023/A:1014081827372

[9] Best, J. H.; Eddy, F. B. and Codd, G. A. (2003): Effects of purified Microcystis cells, cell extracts and lipopolysaccharide on drinking and liver function in rainbow trout Onchorhynchus mykiss Walbaum. Aqu Toxicol, 64: 419-426. http://dx.doi.org/10.1016/S0166-445X(03)00105-X

[10] Bischoff, D. P. (2001): The toxicology of MCYST-LR: Occurrence, toxicokinetics, toxicodynamics, diagnosis and treatment. Vet Hum Toxicol, 34: 294-297.

[11] Boaru, D.; Dragos, N. and Schirmer, K. (2006): Microcystin-LR induced cellular effects in mammalian and fish primary hepatocyte cultures and cell lines: a comparative study. Toxicol, 218(2/3):134-148. http://dx.doi.org/10.1016/j.tox.2005.10.005

[12] Bouaicha, N.; Maatouk, I.; Plessis, M. J. and Perin, F. (2005): Genotoxic potential of microcystin–LR and nodularin in vitro in primary cultured rat hepatocytes and in vivo in rat liver. Environ Toxicol. , 20:341–347. http://dx.doi.org/10.1002/tox.20110

[13] Bruno, M.; Gallo, p.; Messineo, V. and Melchiorre, S. (2012): Health risk associated with microcystin presence in the environment: the case of an Italian lake (Lake Vioco, central Italy).Int. J. Environ. Prot., 2(4):34-41.

[14] Bruno, M.; Melchiorre, S.; Messineo, V.; Volpi, F.; Di Corcia, A.; Aragona, I.; Guglielmone, G.; Di Paolo, C.; Cenni, m.; Ferranti, P. and Gallo, P. (2009): "Microcystin detection in contaminated fish from Italian lakes using ELISA immunoassays and LC-MS/MS analysis," in P. M. Gault and H. J. Marler, Eds., Handbook on Cyanobacteria. Nova Science Publishers, Inc.pp.191-210.

[15] Buratti , F. ;Scardala, S.; Funari, E. and Testai, E. (2013): The conjugation of microcystin-RR by human recombinant GSTs and hepatic cytosol. Toxicology Letters, 219(3): 231–238. http://dx.doi.org/10.1016/j.toxlet.2013.03.015

[16] Bury, N.R.; Eddy, F. B. and Codd, G. A. (1995): The effects of the cyanobacterium Microcystis aeruginosa, the cyanobacterial toxin microcystin-LR and ammonia on growth rate and ionic regulation of brown trout (Salmo trutta L.). J. fish Biol., 46:1042-1054. http://dx.doi.org/10.1111/j.1095-8649.1995.tb01408.x http://dx.doi.org/10.1006/jfbi.1995.0099

[17] Bury, N.R.; Newlands, A.D.; Eddy, F. B. and Codd, G. A. (1998): In vivo and in vitro intestinal transport of H-microcystin- LR , a cyanobacterial toxin, in rainbow trout (Oncorhynchus mykiss). Aqat. Toxicol., 42:139-148. http://dx.doi.org/10.1016/S0166-445X(98)00041-1

[18] Butler, N.; Carlisle, J. C.; Linville, R. and Washburn, B. (2009): Microcystins a brief overview of their toxicity and effects, with special reference to fish, wildlife, and livestock, Ecotoxicology Program Integrated Risk Assessment Branch,Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

[19] Carmichael, W. W. (1994): The toxins of cyanobacteria. Sci. Am., 270:78–86 http://dx.doi.org/10.1038/scientificamerican0194-78

[20] Channa, A. and Mir, I. H. (2009): Distribution of neutral lipids in the intestinal tract and liver of Schizothorax curvifrons Heckel: A histochemical study. Indian J. Applied Pure Biol., 24: 285-288.

[21] Chen, J.; Zhang, D.; Xie, P.; Wang, Q. and Ma, Z. (2009): Simultaneous determination of microcystin contaminations in various vertebrates (fish, turtle, duck and water bird) from a large eutrophic Chinese lake, Lake Taihu, with toxic Microcystis blooms. Sci Total Environ, 407(10):3317–3322. http://dx.doi.org/10.1016/j.scitotenv.2009.02.005

[22] Davis, T., Orr, P.; Boyer, G. and Burford, M. (2014): Investigating the production and release of cylindrospermopsin and deoxy-cylindrospermopsin by Cylindrospermopsis raciborskii over a natural growth cycle. Harmful Algae, 31: 8–25. http://dx.doi.org/10.1016/j.hal.2013.09.007

[23] Dawson, R.M. (1998): The toxicology of microcystins. Toxicon, 36(7):953–962. http://dx.doi.org/10.1016/S0041-0101(97)00102-5

[24] Deblois, C. P.; Giani, A. and Bird, D. F. (2011): Experimenta model of microcystin accumulation in the liver of Oreochromis niloticus exposed subchronically to a toxic bloom of Microcystis sp. Aquat Toxicol.,103(1-2):63-70. http://dx.doi.org/10.1016/j.aquatox.2011.02.006

[25] De Figueiredo, D. R.; Azeiteiro, U. M.; Esteves, S. M.; Goncalves, F. J. M. and Pereira, J. M. (2004): Microcystin-producing blooms—a serious global public health issue. Ecotox Environ Safety, 59:151–63. http://dx.doi.org/10.1016/j.ecoenv.2004.04.006

[26] Dietrich, D. and Hoeger, S. (2005): Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): a reasonable or misguided approach? Toxicology and Applied Pharmacology, 203:273–289. http://dx.doi.org/10.1016/j.taap.2004.09.005

[27] Ding, W.X. and Ong, C.N. (2003): Role of oxidative stress and mitochondrial changes in cyanobacteria–induced apoptosis and hepatotoxicity – Mini Review. FEMS Microbiology Letters, 220:1–7. http://dx.doi.org/10.1016/S0378-1097(03)00100-9

[28] Ding, W. X.; Shen, H. M. and Ong, C. N. (2000): Microcystic cyanobacteria extract induces cytoskeletal disruption and intracellular glutathione alteration in hepatocytes. Environ. Health Perspect., 108: 605-609.

http://dx.doi.org/10.1289/ehp.00108605
[29] Djediat, C.; Malécot, M.; de Luze, A.; Bernard, C.; Puiseux-Dao, S. and Edery, M. (2010): Localization of microcystin-LR in medaka fish tissues after cyanotoxin gavage. Toxicon, 55(2-3) : 531-535. http://dx.doi.org/10.1016/j.toxicon.2009.10.005
[30] Dyble, J.; Gossiaux, D.; Landrum, P.; Kashian, D. and Pothoven, S. (2011): a kinetic study of accumulation and elimination of microcystin-LR in yellow perch (Perca flavescens) tissue and implications for human fish consumption. Mar. Drugs, 9:2553-2571. http://dx.doi.org/10.3390/md9122553
[31] Eriksson, J. E.; Paatero, G. I. L.; Meriluoto, J. A.; Codd, G. A.; Kass, G. E. N.; Nicotera, P. and Orrenius, S. (1989): Rapid microfilament reorganization induced in isolated rat hepatocytes by microcystin-LR, a cyclic peptide toxin. Exp. Cell Res., 185: 86-100. http://dx.doi.org/10.1016/0014-4827(89)90039-6
[32] Falconer, I. R. (2008): Health effects associated with controlled exposures to cyanobacterial toxins. Adv. Exp. Med. Biol.,619:607-612. http://dx.doi.org/10.1007/978-0-387-75865-7_27
[33] Falconer, I. R.; Beresford, A. M. and Runnegar, M. T. (1983): Evidence of liver damage by toxin from a bloom of the blue-green alga, Microcystis aeruginosa. Med. J. Aust. 1(11):511-514.
[34] Falconer, I. R. and Yeung, D. S. K. (1992): Cytoskeletal changes in hepatocytes induced by Microcystis toxins and their relation to hyperphosphorylation of cell proteins. Chem. Biol. Interact., 81: 181–196. http://dx.doi.org/10.1016/0009-2797(92)90033-H
[35] Feist, S. W.; Lang, T.; Stentiford, G. D. and Kohler, A. (2004): Biological effects of contaminants: use of liver pathology of the European flatfish dab (Limanda limanda L.) and flounder (Platichthys flesus L.) for monitoring. ICES Techniques in Marine Environmental Sciences, 38: 42 pp.
[36] Ferrão-Filho, A. A. and Kozlowsky-Suzuki, B. (2011): Cyanotoxins: Bioaccumulation and Effects on Aquatic Animals. Mar. Drugs, 9(12), 2729-2772. http://dx.doi.org/10.3390/md9122729
[37] Fischer, W. J. and Dietrich, D. R. (2000): Pathological and biochemical characterization of Microcystin-induced hepatopancreas and kidney damage in carp (Cyprinus carpio). Toxicol Appl Pharmacol, 164: 73–81. http://dx.doi.org/10.1006/taap.1999.8861
[38] Fleming L. E.; Rivero, C.; Burns, J.; William, C.; Bean, J. A.; Shea, K. A. and Stinn, J. (2002): Blue green algae (cyanobacterial) toxins, surface drinking water, and liver cancer in Florida. Harmful Algae, 1: 57-168. http://dx.doi.org/10.1016/S1568-9883(02)00026-4
[39] Fristachi, A., Sinclair, J.; Hambrook-Berkman, J.; Boyer, G.; Burkholder, J.; Burns, J.; Carmichael , W.; du Four, A.; Frazier, W.; Morton, S. O'Brien, E. and walker, S. (2008): Occurrence of Cyanobacterial Harmful Algal blooms. In Hudnell, HK. (ed.). Proceedings of the Interagency International Symposium on Cyanobacterial Medical Biology. Springer, New York, Vol., 619:37-97.
[40] Fu, W. Y.; Chen, J.P.; Wang, X. M. and Xu, L. H. (2005): Altered expression of p53, Bcl-2 and Bax induced by microcystin-LR in vivo and in vitro. Toxicon, 46:171–177. http://dx.doi.org/10.1016/j.toxicon.2005.03.021
[41] Fujiki, H. and Suganuma, M. (2009): Carcinogenic aspects of protein phosphatases 1 and 2A inhibitors. Progr Mol Subcell Biol, 46:221-254. http://dx.doi.org/10.1007/978-3-540-87895-7_8
[42] Gavrilović, B., Despotović, S.; Gavrić, J.; Borković-Mitić, S.; Ognjanović, B.; Slađan Z. Pavlović, S. and Saičić, Z. (2014): Changes in antioxidant enzyme activities in the livers and gills of three cyprinids after exposure to a cyanobacterial bloom in the Gruža Reservoir, Serbia. Ecological Indicators, 38: 141–148. http://dx.doi.org/10.1016/j.ecolind.2013.10.016
[43] Guo, L.; Wang, Q.; Xie, P.; Tao, M.; Zhang, J.; Niu, Y. and Ma, Z. (2014): A non-classical biomanipulation experiment in Gonghu Bay of Lake Taihu: control of Microcystis blooms using silver and bighead carp. Aqua Cult Res, DOI: 10.1111/are.12375. PubDate: 2014-01-17T23:11:07.75331-05:0
[44] Harada, K. I.; Tsuji, K.; Watanabe, M. F. and Kondo, F. (1996): Stability of microcystins from cyanobacteria—III. Effect of pH and temperature. Phycologia: 35 (6S): 83-88. http://dx.doi.org/10.2216/i0031-8884-35-6S-83.1
[45] He, S.; Liang , X.; Sun, J. and Shen, D. (2013): Induction of liver GST transcriptions by tert-butylhydroquinone reduced microcystin-LR accumulation in Nile tilapia (Oreochromis niloticus). Ecotoxicol. Environm. Safety, 90:128–135. http://dx.doi.org/10.1016/j.ecoenv.2012.12.021
[46] Herfindal, L. and Selheim, F. (2006): Microcystin produces disparate effects on liver cells in a dose dependent manner-Mini-reviews. Med. Chem., 6: 279-285.
[47] Hooser, S. B. (2000): Fulminant hepatocyte apoptosis in vivo following microcystin-LR administration to rats. Toxicol Pathol, 38 (5):726-733. http://dx.doi.org/10.1177/019262330002800513
[48] Humpage, A. R. and Falconer, I. R. (1999): Microcystin-LR and liver tumor promotion: Effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes. Environmental Toxicology,14:61–75. http://dx.doi.org/10.1002/(SICI)1522-7278(199902)14:1<61::AID-TOX10>3.0.CO;2-R http://dx.doi.org/10.1002/(SICI)1522-7278(199902)14:1<61::AID-TOX10>3.3.CO;2-I
[49] Humpage, A. R.; Fenech, M.; Thomas, P. and Falconer, I. R.(2000): Micronucleus induction and chromosome loss in transformed human white cells indicate clastogenic and aneugenic action of the cyanobacterial toxin, cylindrospermopsin. Mutat Res/Genet Toxicol Environ Mutage, 472:155–161.
[50] IARC (2006): Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. The Lancet Oncology, 7;628-629. http://dx.doi.org/10.1016/S1470-2045(06)70789-6
[51] IARC (2010): "Ingested nitrate and nitrite and cyanobacterial peptide toxins," WHO – IARC Eds., Lion, France, IARC Monographs (2006) on the Evaluation of Carcinogenic Risks to Humans, Vol., 94.
[52] Ibelings, B. W.; Bruning, K.; de Jonge, J.; Wolfstein, K.; Pires, L.M.; Postma, J. and Burger, T. (2005): Distribution of microcystins in a lake foodweb: no evidence for biomagnification. Microb Ecol., 49(4):487-500. http://dx.doi.org/10.1007/s00248-004-0014-x
[53] Ibelings, B. W. and Chorus, I. (2007): Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: a review.
[54] Environmental pollution, 150 (1), 177-192. http://dx.doi.org/10.1016/j.envpol.2007.04.012
[55] Ibelings, B. W. and Havens, K. E. (2007): Cyanobacterial toxins: a qualitative meta-analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota. In "International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB)," H.H. Kenneth, Editor, Springer Press, pp.:685-744.
[56] Ibelings, B. W. and Havens, K. E. (2008): Cyanobacterial toxins: a qualitative meta-analysis of concentrations, dosage and effects in freshwater estuarine and marine biota. In: Hudnell, H.K. (ed.). "Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Adv Exp Med Biol., 619:675–732.
[57] Jiang, J. L.; Gu, X. Y.; Song, R.; Zhang, Q., Geng, J. J.; Wang, X. Y. and Yang, L. Y. (2011): Time- dependent oxidative stress and histopathological alterations in Cyprinus carpio L. exposed to microcystin-LR. Ecotoxicology, 20:1000–1009. http://dx.doi.org/10.1007/s10646-011-0646-9
[58] Jing, W.; Sui, G. And Liu, S. (2014): Characteristics of a Microcystin-LR Biodegrading Bacterial Isolate: Ochrobactrum sp. FDT5. Bull Environ Cont Toxic, 92 (1): 119-122. http://dx.doi.org/10.1007/s00128-013-1170-9
[59] Jos, A.; Pichardo, S.; Prieto, A. I.;Repetto, G.; Vazquez, C. M.; Moreno, I. and Camean, A. M. (2005): Toxic cyanobacterial cells containing microcystins induce oxidative stress in exposed tilapia fish (Oreochromis sp.) under laboratory conditions. Aquat Toxicol, 72: 261–71. http://dx.doi.org/10.1016/j.aquatox.2005.01.003
[60] Kaya, K. (1996): Toxicology of microcystins. In Toxic Microcystis (M. Watanabe; K. Harada; W. Carmichael and H. Fujiki, Eds.) pp. 175–202. CRC Press, Boca Raton.
[61] Kim, B. H.; Hwang, S. J.; Park, M.H. and Kim, Y. J. (2010): Relationship between cyanobacterial biomass and total microcystin-LR levels in drinking and recreational water. Bull. Environm. Contamin. Toxicol., 85:457-462.

http://dx.doi.org/10.1007/s00128-010-0121-y

[62] Klaassen, C. D. and Watkins, J. B. (1984): Mechanisms of bile formation, hepatic uptake, and biliary excretion. Pharmacol. Rev., 36(1):1-67.

[63] Kotak, B. J.; Semalulu, S.; Friytz, D. L.; Prepas, E. E.; Hrudey, S. E. and Coppock, R. W. (1996): Hepatic and renal pathology of intraperitoneally administered microcystin-LR in rainbow trout (Oncorhynchus mykiss). Toxicon, 34: 517–25. http://dx.doi.org/10.1016/0041-0101(96)00009-8

[64] Lang, T.; Wosniok, W.; Barsiene, J.; Broeg, K.; Kopecka, J. and Parkoonen, J. (2006): Liver histopathology in Baltic flounder (Platichthys flesus) as indicator of biological effects of contaminants. Mar Pollut Bull, 53:488–496. http://dx.doi.org/10.1016/j.marpolbul.2005.11.008

[65] Larson, D.; Ahlgren, G. and Willén, E. (2014): Bioaccumulation of microcystins in the food web: a field study of four Swedish lakes. Inland Waters, Vol 4(1): 91-104 http://dx.doi.org/10.5268/IW-4.1.627.

[66] Leflaive, J. and Ten-Hage, L. (2007): Algal and cyanobacterial secondary metabolites in fresh waters: A comparison of allelopathic compounds and toxins. Freshwater Biol.; 52:199–214. http://dx.doi.org/10.1111/j.1365-2427.2006.01689.x

[67] Lehman, P. W.; Boyer, G. L.; Satchwell, M. and Waller, S. (2008): The influence of environmental conditions on the seasonal variation of Microcystis cell density and microcystins concentration in San Francisco Estuary. Hydrobiologia, 600:187–204. http://dx.doi.org/10.1007/s10750-007-9231-x

[68] Lehman, P. W.; The, S. J.; Boyer, G. L.; Nobriga, M. L.; Bass, E. and Hogle, C. (2010): Initial impacts of Microcystis aeruginosa blooms on the aquatic food web in the San Francisco Estuary. Hydrobiologia, 637:229-248. http://dx.doi.org/10.1007/s10750-009-9999-y

[69] Li, X. Y.; Chung, I.; Kim, J.; and Lee, J. (2004): Subchronic oral toxicity of microcystin in common carp (Cyprinus carpio L.) exposed to Microcystis under laboratory conditions. Toxicon, 44: 821–27. http://dx.doi.org/10.1016/j.toxicon.2004.06.010

[70] Li, X. Y.; Liu, Y. D. and Song, L. R. (2001): Cytological alterations in isolated hepatocytes from common carp (Cyprinus carpio L.) exposed to microcystin-LR. Environ Toxicol, 16: 517–22. http://dx.doi.org/10.1002/tox.10012.abs http://dx.doi.org/10.1002/tox.10012

[71] Li, X. Y.; Liu, Y. D.; Song, L. R. and Liu, J.(2003): Responses of antioxidant systems in the hepatocytes of common carp (Cyprinus carpio L.) to the toxicity of microcystin-LR. Toxicon, 42: 85-89. http://dx.doi.org/10.1016/S0041-0101(03)00104-1

[72] Li, X. Y.; Xie, P.; Li, G.; Hao, L. and Xiong, Q.(2009): In vivo study on the effects of microcystin extracts on the expression profiles of proto-oncogens(c-fos, c-jun and c-myc) in liver, kidney and testis of male wistar rats injected i.v. with toxins. Toxicon, 53: 169-175. http://dx.doi.org/10.1016/j.toxicon.2008.10.027

[73] Liu, Y.; Song, L.; Li, X. and Liu, T. (2002): The toxic effects of microcystin LR on embryo-larval and juvenile development of Loach, Misgurnus mizolopis. Toxicon. 40(4):395–399. http://dx.doi.org/10.1016/S0041-0101(01)00173-8

[74] MacKintosh, R. W.; Dalby, K. N.; Campbell, D. G.; Cohen, P. T.; Cohen, P. and MacKintosh, C. (1995): The cyanobacterial toxin microcystin binds covalently to cysteine-273 on protein phosphatase 1. FEBS Lett, 371:236–240. http://dx.doi.org/10.1016/0014-5793(95)00888-G

[75] Malbrouck, C. and Kestemont, P. (2006): Effects of Microcystins on fish. Environ Toxicol Chem 25: 72–86. http://dx.doi.org/10.1897/05-029R.1

[76] Marie, B.; Huet, H.; Djediat, C.; Puiseux-Dao, S.; Catherine, A.; Trinchet, I. and Edery, M. (2012): Effects of a toxic cyanobacterial bloom (Planktothrix agardhii) on fish: Insights from histopathological and quantitative proteomic assessments following the oral exposure of medaka fish (Oryzias latipes). Aquatic Toxicology, 114–115: 39–48. http://dx.doi.org/10.1016/j.aquatox.2012.02.008

[77] Mehra, S.; Dubey, J. and Bhowmik, D. (2009): hepatotoxic responses in Heteropneustus fossilis (Bloch) after oral exposure to Microcystis under laboratory conditions. Amer-Euras. J. Toxicol. Sci., 1(2): 50-56.

[78] Mitsoura, A; Kagalou, I.; Papaioannou, N.; Berillis, P.; Mente, E. and Papadimitriou, T. (2013): The presence of microcystins in fish Cyprinus carpio tissues: a histopathological study. Internat Aqua Res, 5:8 doi: 10.1186 /2008-6970-5-8.

[79] Mohamed, Z. A.; Carmichael, W. W. and Hussein, A. A. (2003): Estimation of microcystins in the freshwater fish Oreochromis niloticus in an Egyptian fish farm containing a Microcystis bloom. Environ Toxicol., 18(2):137-41. http://dx.doi.org/10.1002/tox.10111

[80] Mohamed, Z. A.. and Hussein, A. A. (2006): Depuration of microcystins in tilapia fish exposed to natural populations of toxic cyanobacteria: a laboratory study. Ecotoxicol Environ Saf., 63(3): 424-9. http://dx.doi.org/10.1016/j.ecoenv.2005.02.006

[81] Molina, R.; Moreno, I.; Pichardo, S.; Jos, A.; Moyano, R.; Monterde, J. G. and Camean, A. (2005): Acid and alkaline phosphathase activities and pathological changes induced in Tilapia fish (Oreochromis sp.) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. Toxicon, 46: 725–35. http://dx.doi.org/10.1016/j.toxicon.2005.07.012

[82] Oruc, E. and Uner, N. (2000): combined effects of 2,4-D and azinphosmethyl on antioxidant enzyumes and lipid peroxidation in liver of Oreochromis niloticus. Comp. Biochem. Phys., C 127:291-296.

[83] Paerl, H. W.; Fulton, R.S.; Moisander, P.H. and Dyble, J. (2001): Harmful freshwater algal blooms, with an emphasis on cyanobacteria. The Scientifi world, 1;76-113.

[84] Paerl, H. W. and Huisman, J. (2009): Blooms like it hot. Sci., 320: 57-58. http://dx.doi.org/10.1126/science.1155398

[85] Pathak, R. and Singh, J. (2010): The toxicology of microcystins and its implication on human health. J. Ecophysiol. Occup. Health, 10:35-41.

[86] Palikova, M.; Mares, J.; Kopp, R.; Hlavkova, J., Navratil, S.; Adamovsky, O.; Chmelar, L. and Blaha, L. (2011): Accumulation of microcystins in Nile Tilapia, Orechromis niloticus L., and effects of a complex cyanobacterial bloom on the dietetic quality of muscles. Bull Environ Contamin Toxicol. 87:26–30. http://dx.doi.org/10.1007/s00128-011-0279-y

[87] Palikova, M.; Navratil, S.; Tichy, F.; Sterba, F.; Marsalek, B. and Blaha, L. (2004): Histopathology of carp (Cyprinus carpio L.) larvae exposed to cyanobacteria extract. Acta Vetr. Brno, 73:253-257. http://dx.doi.org/10.2754/avb200473020253 http://dx.doi.org/10.2754/avb200473020259

[88] Pathiratne, A.; Pathiratne, K. A. and De Seram, P. K. (2010): Assessment of biological effects of pollutants in a hyper eutrophic tropical water body, Lake Beira, Sri Lanka using multiple biomarker responses of resident fish, Nile tilapia (Oreochromis niloticus). Ecotoxicology,19(6):1019-26. http://dx.doi.org/10.1007/s10646-010-0483-2

[89] Pflugmacher, S.; Wiegand, C.; Oberemm, A.; Beattie, K. A.; Krause, E.; Codd, G. A. and Steinberg, C. E. (1998): Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: the first step of detoxication. Biochim Biophys Acta, 1425:527–533. http://dx.doi.org/10.1016/S0304-4165(98)00107-X

[90] Phillips, M. J.; Roberts, R. J.; Stewart, J. A. and Codd, G. A. (1985): The toxicity of the cyanobacterium Microcystis aeruginosa to rainbow trout, Salmo gairdneri Richardson. J Fish Dis., 8:339–344. http://dx.doi.org/10.1111/j.1365-2761.1985.tb00953.x

[91] Prieto, A. I.; Jos, A.; Pichardo, S.; Moreno, I.; and Cameán, A. M. (2006): Differential oxidative stress responses to microcystins LR and RR in intraperitoneally exposed tilapia fish (Oreochromis sp.). Aquat Toxicol, 77: 314–21. http://dx.doi.org/10.1016/j.aquatox.2005.12.012

[92] Prieto, A. I.; Pichardo, S.; Jos, A.; Moreno, I. and Camean, A.M. (2007): Time dependent oxidative stress response after acute exposure to toxic cyanobacterial cells containing microcystins in tilapia fish (Oreochrmis niloticus) under laboratory conditions. Aquat. Toxicol., 84:337-345. http://dx.doi.org/10.1016/j.aquatox.2007.06.012

[93] Qui, T.; Xie, P.; Ke, Z.; Li, L. and Guo, L. (2007): In situ studies on physiological and biochemical responses of four fishes with different

trophic levels to toxic cyanobacterial blooms in a large Chinese lake. Toxicon. 50:365–376.
http://dx.doi.org/10.1016/j.toxicon.2007.04.006

[94] Runnegar, M.T.; Kong, S. and Berndt, N., (1993): Protein phosphatase inhibition and in vivo hepatotoxicity of microcystins. Am. J. Physiol., 265, 224–230.

[95] Smutná, M.; Babica, P.; Jarque, S.; Hilscherova, K.; Marsalek, B.; Haeba, M. and Blaha, L. (2014): Acute, chronic and reproductive toxicity of complex cyanobacterial blooms in Daphnia magna and the role of microcystins. Toxicon, 79 : 11–18.
http://dx.doi.org/10.1016/j.toxicon.2013.12.009

[96] Snyder, G. S.; Goodwin, A. E. and Freeman, D. W. (2002): Evidence that channel catfish, Ictalurus punctatus (Rafinesque), mortality is not linked to ingestion of the hepatotoxin microcystin-LR. J Fish Dis. 25:275–285.
http://dx.doi.org/10.1046/j.1365-2761.2002.00374.x

[97] Soares, R. M;, Magalhães, V. F. and Azevedo, S. M. (2004): Accumulation and depuration of microcystins (cyanobacteria hepatotoxins) in Tilapia rendalli (Cichlidae) under laboratory conditions. Aquat Toxicol., 70(1):1-10.
http://dx.doi.org/10.1016/j.aquatox.2004.06.013

[98] Sotton, B.; Guillard, J.; Anneville, O.; Maréchal, M.; Savichtcheva, O. and Domaizon, I. (2014): Trophic transfer of microcystins through the lake pelagic food web: Evidence for the role of zooplankton as a vector in fish contamination. Sci Total Environ, 466–467 : 152–163.
http://dx.doi.org/10.1016/j.scitotenv.2013.07.020

[99] Steffen, M.; Zhu, Z.; Mckay, R.; Wilhelm, S. and Bullerjahn, G. (2014): Taxonomic assessment of a toxic cyanobacteria shift in hypereutrophic Grand Lake St. Marys (Ohio, USA). Harmful Algae, 33: 12–18.
http://dx.doi.org/10.1016/j.hal.2013.12.008

[100] Tencalla, F. G. and Dietrich, D. R. (1997): Biochemical characterization of microcystin toxicity in rainbow trout (Oncora Infotrievehynchus mykiss). Toxicon, 35:583–95.
http://dx.doi.org/10.1016/S0041-0101(96)00153-5

[101] Tencalla F.G., Dietrich D.R., Schlatter C. (1994): Toxicity of Microcystis aeruginosa peptide toxin to yearling rainbow trout (Oncorhyncus myskiss). Aquat. Toxicol.;30:215–224.
http://dx.doi.org/10.1016/0166-445X(94)90059-0

[102] Trinchet, I.; Cadel-Six, S.; Djediat, C.; Marie, B.; Bernard, C.; Puiseux-Dao, S.; Krys, S.; Edery, M. (2013): Toxicity of harmful cyanobacterial blooms to bream and roach. Toxicon , 71, : 121–127.
http://dx.doi.org/10.1016/j.toxicon.2013.05.019

[103] Trinchet, I.; Djediat, C.; Huet, H. Dao, S. and Edery, M. (2011): Pathological modifications following sub-chronic exposure of medaka fish (Oryzias latipes) to microcystin-LR. Reprod Toxicol., 32:329–340.
http://dx.doi.org/10.1016/j.reprotox.2011.07.006

[104] Van Apeldoorn, M.; Van Egmond, H.; Speijers, G. and Bakker, G. (2007): Review - Toxins of cyanobacteria. Mol. Nutr. Food. Res., 51:1-60.
http://dx.doi.org/10.1002/mnfr.200600185

[105] Van Dyk, J.; Cochrane, M. and Wagenaar, G. (2011): Liver histopathology of the sharptooth catfish Clarias gariepinus as a biomarker of aquatic pollution. Chemosphere, 87(4):304–311.

[106] Verspagen, J.; Passarge, J.; Johnk, K. Visser, P.; Peperzak, L.; Boers, P.; Laanbroek, H. and Huisman, J. (2006): Water management strategies against toxic Microcystis blooms in the Dutch delta. Ecolog. Applic., 16:313-327.
http://dx.doi.org/10.1890/04-1953

[107] Weirich, C. A. and Miller, T. R. (2014): Freshwater Harmful Algal Blooms: Toxins and Children's Health. Curr Probl Pediat Aldol Heal Care, 44(1): 2-24.
http://dx.doi.org/10.1016/j.cppeds.2013.10.007

[108] WHO (1999): Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. Routledge: London and New York.

[109] Wiegand, C. and Pflugmacher, S. (2005): Ecotoxicological effects of selected cyanobacterial secondary metabolites: A short review. Toxicol. Appl. Pharmacol; 203:201–218.
http://dx.doi.org/10.1016/j.taap.2004.11.002

[110] Williams, D. E.; Craig, M.; Dawe, S. C.; Kent, M. L.; Andersen, R. J. and Holmes, C. F. (1997): C14-labeled microcystin-LR administered to atlantic salmon via intraperitoneal injection provides in vivo evidence for covalent binding of microcystin-LR in salmon livers. Toxicon 35, 985–89.
http://dx.doi.org/10.1016/S0041-0101(96)00196-1

[111] Wolf, J. and Wolfe, M. J. (2005): A brief overview of nonneoplastic hepatic toxicity in fish. Toxicol Pathol, 33:75–85.
http://dx.doi.org/10.1080/01926230590890187

[112] Xie, L.; Xie, P.; Guo, L.; Li, L., Miyabara, Y. and Park, H. D. (2005): Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. Environ Toxicol., 20(3):293-300.
http://dx.doi.org/10.1002/tox.20120

[113] Xie, L.Q.; Xie, P.; Ozawa, K.; Honma, T.; Yokoyama, A. and Park, H.D. (2004): Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment. Environ Pollut., 127(3): 431 – 439.
http://dx.doi.org/10.1016/j.envpol.2003.08.011

[114] Zegura, B.; Sedmark, B. and Filipic, M. (2003): Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2. Toxicon, 41:41-48.
http://dx.doi.org/10.1016/S0041-0101(02)00207-6

[115] Zhang, X.; Xie, P.; Wang, W.; Li, D.; Li, L.; Tang, L.; Lei, H. and Shi, Z. (2008): Dose-dependent effects of extracted microcystins on embryonic development, larval growth and histopathological changes of Southern cat fish (Silurus meridionalis). Toxicon, 51(3):449-456.
http://dx.doi.org/10.1016/j.toxicon.2007.11.005

[116] Zhao, M.; Xie, S.; Zhu, X.; Yang, Y.; Gan, N. and Song, L. (2006): Effect of dietary cyanobacteria on growth and accumulation of microcystins in Nile tilapia (Oreochromis niloticus). Aquaculture, 261:960-966.
http://dx.doi.org/10.1016/j.aquaculture.2006.08.019

[117] Zhou, L.; Yu, H. and Chen, K. (2002): Relationship between microcystin in drinking water and colorectal cancer. Biomed Environm Sci, 15:166–171.

[118] Zurawell, R. W., Chen, H., Burke, J. M., and Prepas, B. R. (2005): Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environment. J. Toxicol. Environ. Health Part B - Critical Reviews, 8:1-37.
http://dx.doi.org/10.1080/10937400590889412

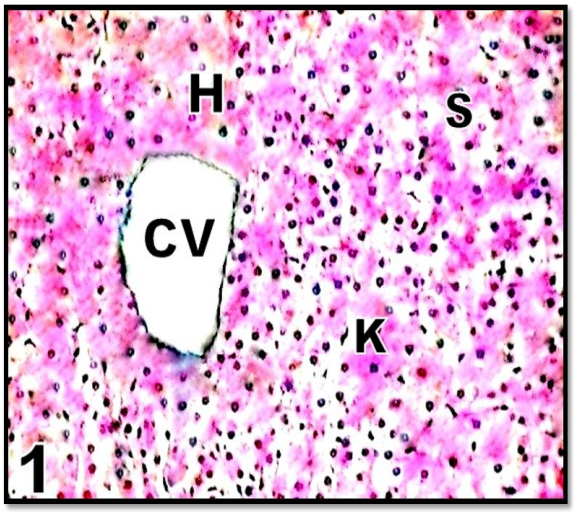

Fig. 1: Section in the liver of a control *Oreochromis niloticus* showing the hepatic portion (H) crowded with polygonal hepatocytes possessing spherical nuclei and arranged radially around the central vein (CV). Sinusoids (S) are narrow and some spindle-shaped Kupffer cells (K) are seen at one side of the endothelial lining of the sinusoid. (H & E) X400.

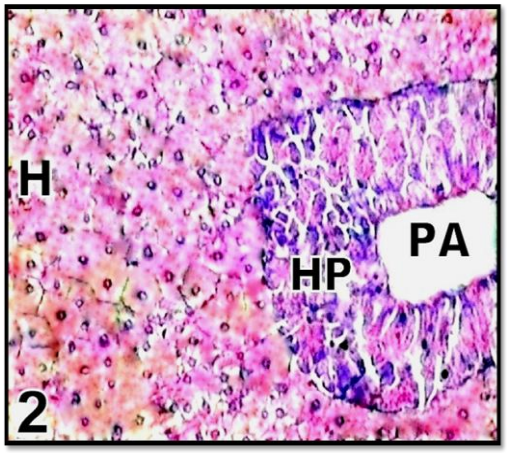

Fig. 2: Section in the hepatopancreas of a control *Oreochromis niloticus* showing the hepatic part (H) harbors normally organized hepatocytes with intact nuclei. The pancreatic portion (HP) possesses long elongated pancreatic cells poliferating around a portal artery (PA). The basal region of the pancreatic cells is basophilic whereas the apex is loaded with eosinophilic zymogen granules. (H & E) X400.

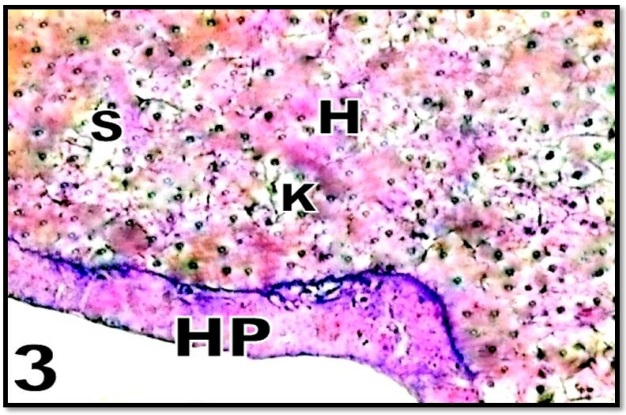

Fig. 3: Section in the hepatopancreas of a control *Oreochromis niloticus* liver. The hepatic part (H) is occupied by the usual polygonal hepatocytes with spherical nuclei. The solid architexture of hepatic cords is interrupted by a few narrow blood sinusoids (S). Kupffer cells (K) could be demonstrated at sides of the sinusoidal lumen. Part of the pancreas (HP) is indicated with its characteristic long elongated cells. These cells have basophilic basal regions and eosinophilic zymogen granules. (H & E) X400.

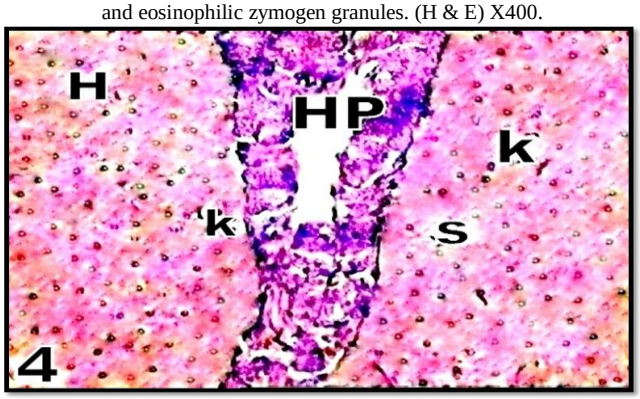

Fig. 4: Section in the hepatopancreas of a control *Oreochromis niloticus* liver. The hepatic part (H) contains polygonal hepatocytes with spherical nuclei. Little number of narrow sinusoids (S) and Kupffer cells (K) are associated with their endothelium. The pancreatic portion (HP) is endwed with long elongated pancreatic cells circumscribing a portal artery. The basal region of the pancreatic cells is basophilic whereas their apex is densely eosinophilic. (H & E) X400.

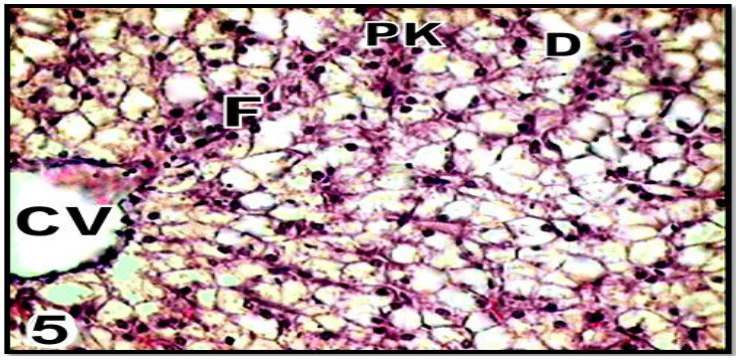

Fig. 5: Section in the liver of *Oreochromis niloticus* fish fed with 0.623 gm Microcystis aeruginosa cells/kg food pellets for 30 days. Hepatocytes are vacuolated and show cytoplasmic degenerations (D) while many nuclei are pyknotic (PK). The central vein (CV) is congested with coagulative blood with infiltration of monocytes. Fibrosis (F) is seen in the liver tiussue. (H & E), X400.

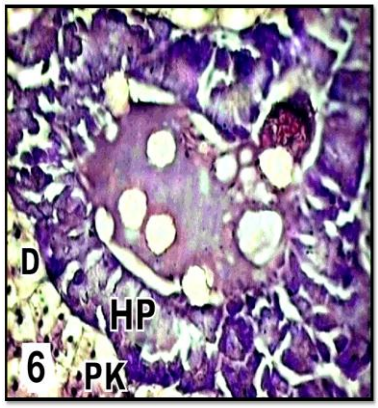

Fig. 6: Section in the hepatopancreas of *Oreochromis niloticus* fish fed with 0.623 gm *Microcystis aeruginosa* cells/kg food pellets for 30 days. Hepatocytes show cytoplasmic degenerations (D) and the nuclei are pyknotic (PK). Part of the pancreas (HP) is indicated with its characteristic long elongated cells. portal vessel is clearly congested with coagulative homogeneous eosinophilic secretion interrupted by spherical vacuoles of variable diameters. (H & E) X400.

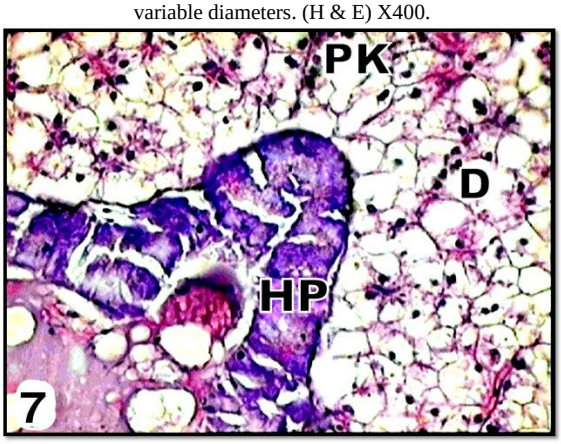

Fig. 7: Another field from the previous section showing the hepatic part containing degenerated hepatocytes with pyknotic nuclei (PK). The long elongated pancreatic cells of the pancreatic portion (HP) surround a portal artery. The central lumen harbors densely eosinophilic secretion and a large buffy necrotic clump. (H & E) X400.

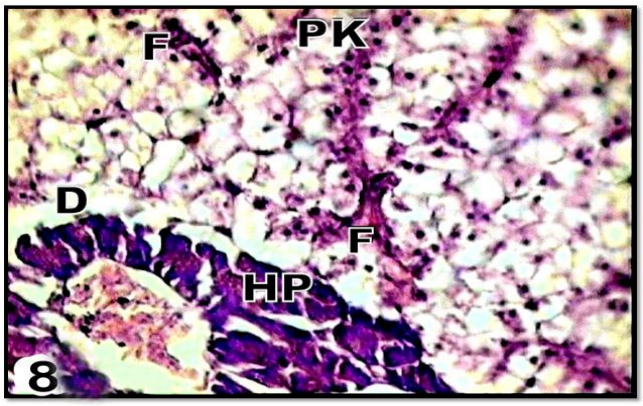

Fig. 8: Section in the hepatopancreas of *Oreochromis niloticus* fish fed with 0.623 gm Microcystis aeruginosa cells/kg food pellets for 30 days. Hepatocytes show cytoplasmic degenerations (D) and the nuclei are pyknotic (PK). Fibrotic patches (F) are scattered in the hepatic portion. pancreatic portion (HP) has long elongated cells surrounding a portal artery congested with eosinophilic clumps. (H & E) X400.

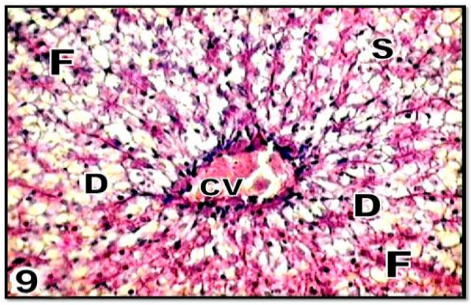

Fig. 9: Section in the liver of *Oreochromis niloticus* fish fed with 1.246 gm Microcystis aeruginosa cells/kg food pellets for 30 days. Hepatocytes display cytoplasmic degeneration (D) while many nuclei are pyknotic. Blood sinusoids (S) are expanded and fibrotic patches (F) are seen. The central vein (CV) is congested with blood and infilamatory cells.(H & E) X400.

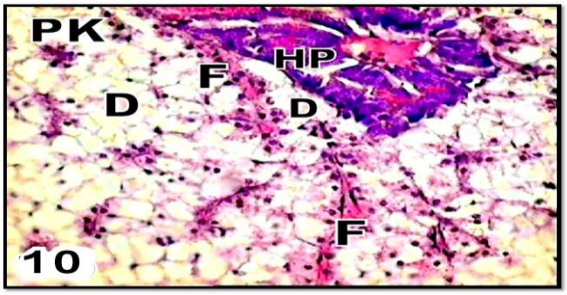

Fig. 10: Section in the liver of *Oreochromis niloticus* fish fed with 1.246 gm Microcystis aeruginosa cells/kg food pellets for 30 days. Hepatocytes display cytoplasmic degenerations (D) while many nuclei are pyknotic. Blood sinusoids are expanded and fibrotic patches (F) are seen. The pancreatic portion (HP) is characterized by conspicuous tall elongated cells surrounding a portal artery which is congested with homogeneous eosinophilic material and inflammatory cells. (H & E) X400.

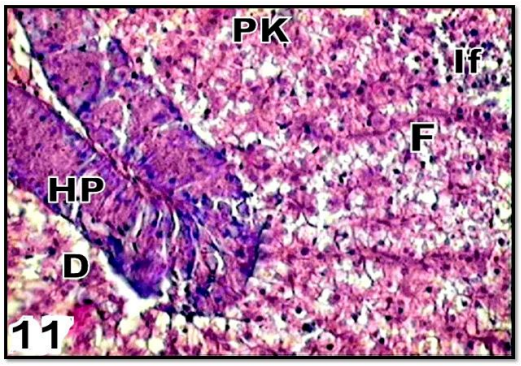

Fig. 11: Section in the liver of *Oreochromis niloticus* fish fed with 1.246 gm Microcystis aeruginosa cells/kg food pellets for 30 days. The hepatic portion shows cytoplasmic degeneration (D) and pyknotic nuclei (PK). Fibrotic patches (F) together with inflammatory cell infiltration (IF) are demonstrated. The pancreatic portion (HP) harbors tall elongated cells exhibiting eosinophilic zymogen granules. These cells surround a portal artery which is congested with homogeneous eosinophilic material and inflammatory cells. (H & E) X400.

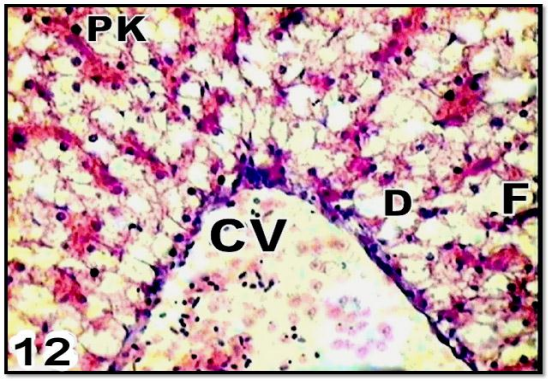

Fig. 12: Section from the liver of the previous case showing part of a highly dilated central vein (CV) congested with blood and monocytes. The wall of the vein is thick and its endothelium has strongly basophilic cells. Hepatocytes suffer from cytoplasmic degeneration (D) while many nuclei are pyknotic (PK). Fibrotic patches (F) are seen distributed throughout the hepatic tissue. (H & E) X400.

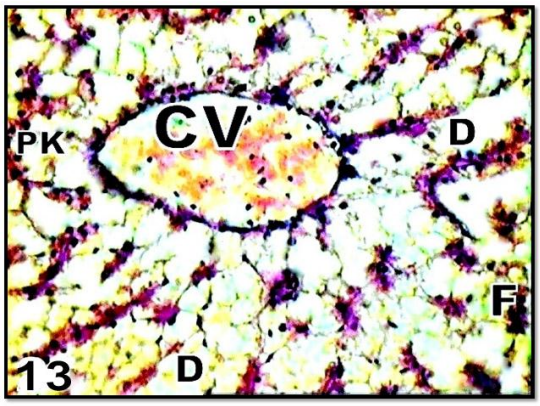

Fig. 13: Section in the liver of *Oreochromis niloticus* fish fed with 1.869 gm Microcystis aeruginosa cells/kg food pellets for 30 days. The hepatocytes show cytoplasmic degenerations (D) and the nuclei are pyknotic (PK). Fibrotic patches (F) are seen distributed throughout the hepatic tissue. The central vein (CV) is congested with blood and monocytes. The wall of the vein is thick with clumped endothelial cells having strongly basophilic nuclei. (H & E) X400.

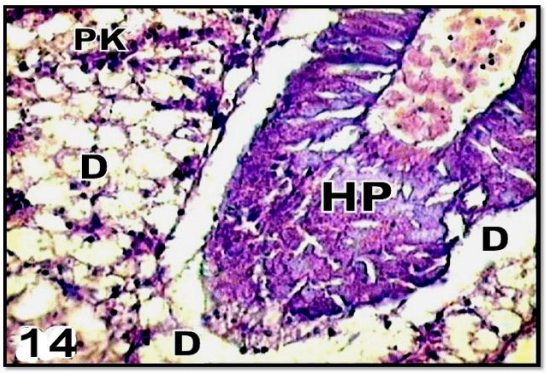

Fig. 14: Section in the liver of *Oreochromis niloticus* fish fed with 1.869 gm Microcystis aeruginosa cells/kg food pellets for 30 days. In the hepatic portion, cytoplasmic degenerations (D) and vacuolar changes are demonstrated in the hepatocytes in addition to pyknotic nuclei (PK). The portal lumen of the pancreas is congested with blood and monocytes. (H & E) X400.

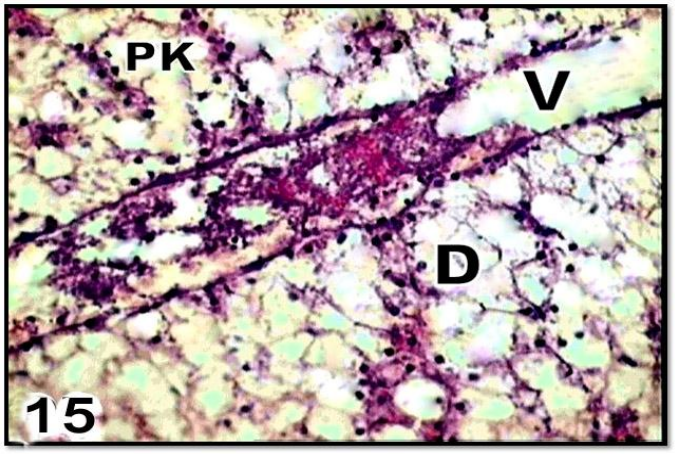

Fig. 15: Section from the liver of the previous case showing degenerated cytoplasm (D) of hepatocytes and pyknotic nuclei (PK). A large elongated and widely dilated vessel (V) is seen congested with buffy to bricky aggregations containing necrotic nuclei. (H & E) X400.

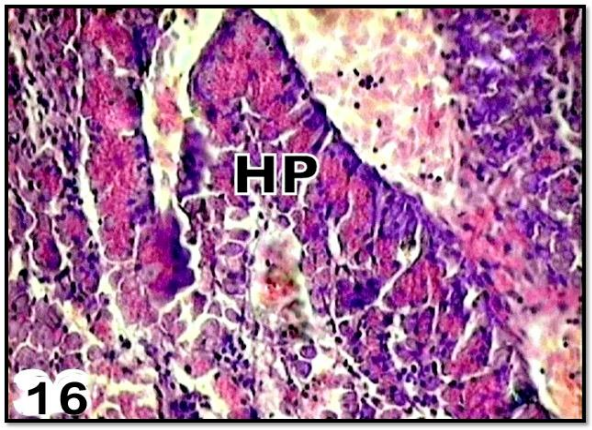

Fig. 16: Section in the liver of *Oreochromis niloticus* fish fed with 1.869 gm Microcystis aeruginosa cells/kg food pellets for 30 days. Hypertrophied portion of the pancreatic part (HP) of the hepatopancreas is seen. Cells of the pancreas are obviously enlarged and possess densly eosinophilic granules. The pancreatic cells surround a portal vessel filled and congested with blood cells in addition to darkly stained monocytes. Inflammatory deeply stained cells are scattered mostly at the left part of the micrograph. (H & E) X400.

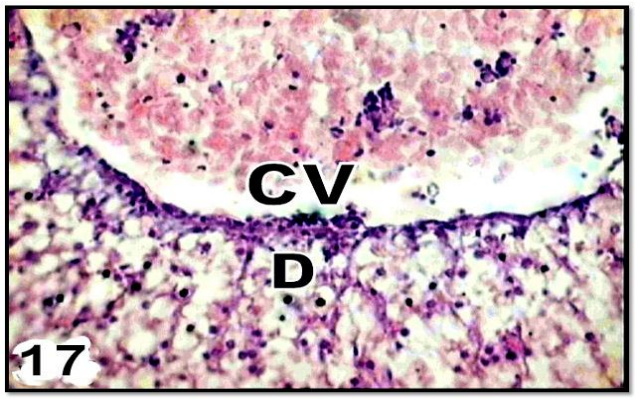

Fig. 17: Section in the liver of *Oreochromis niloticus* fish fed with 1.869 gm Microcystis aeruginosa cells/kg food pellets for 30 days. Hepatocytes show cytoplasmic degenerations (D). Part from a highly dilated central vein (CV) is seen congested with buffy blood aggregations and inflammatory cells. The wall of the vessel is thick. (H & E) X400.

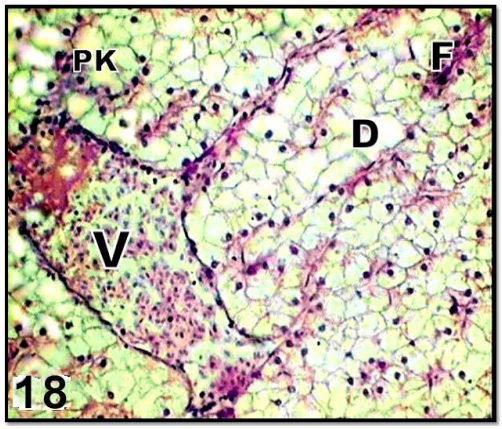

Fig. 18: Section from the liver of the previous case. Degenerative cytoplasm (D) of hepatocytes and pyknotic nuclei (PK) are detected as well as fibrotic patches (F). The large blood vessel has a bizarre appearance. Its elongated rectangular lumen is filled with a huge number of nucleated blood cells and cells from the immune system. At the left corner, coagulative clumps are aggregated and are stained deep purplish to violet or buffy. (H & E) X400.

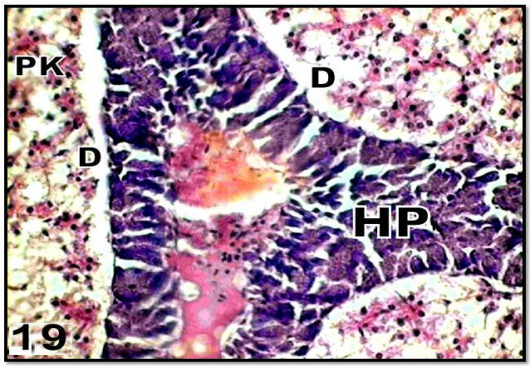

Fig. 19: Section in the liver of *Oreochromis niloticus* fish fed with 1.869 gm Microcystis aeruginosa cells/kg food pellets for 30 days. The hepatic part exhibit degeneration (D) and pyknosis (PK). The pancreatic portion (HP) harbors long elongated cells. In the portal lumen, there is a purplish to buffy secretory material interrupted with spherical vacuoles. (H & E) X400.

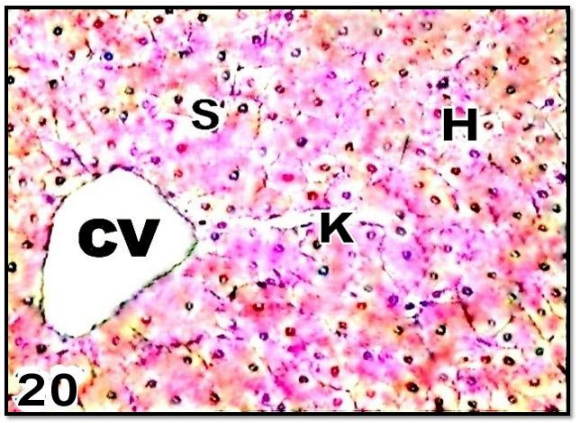

Fig. 20: Section in the liver of *Oreochromis niloticus* fish fed with 1.869 gm Microcystis aeruginosa cells/kg food pellets for 30 days and left for one month for recovery. Nearly, a normal architecture of the liver is detected. The hepatic portion (H) is harboring polygonal hepatocytes arranged radially around the central vein (CV). Sinusoids (S) are narrow and a spindle-shaped Kupffer cell (K) are seen. (H & E) X400.

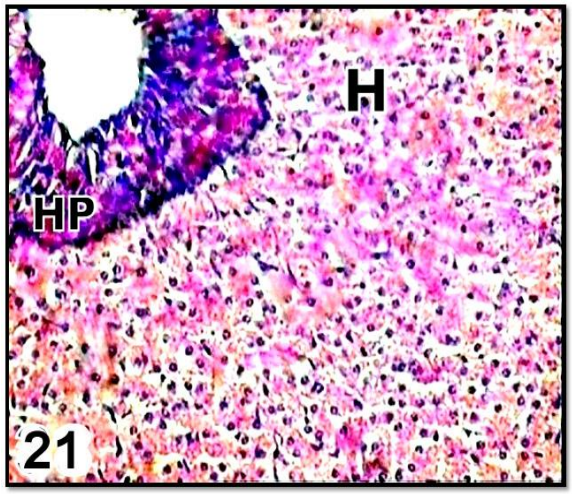

Fig. 21: Section in the liver of *Oreochromis niloticus* fish from the previous recovery group. The hepatic part (H) exhibits a rather control-like appearance. The pancreatic portion (HP) harbors long elongated cells containing eosinophilic granules. These cells circumscribe a portal vessel. (H & E) X400.