



Biochemical and Histopathological Effects of Copper Exposure on the Liver of *Oreochromis niloticus* Treated with *Spirulina platensis*

Dina T. Meshhal¹, Medhat H. Hashem², Omaila A. Khamiss²,
Fatma A. S. Mohamed¹

¹ National Institute Of Oceanography and Fisheries, (NIOF), Egypt

² Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat City, Egypt

* Corresponding Author: d.talaat@yahoo.com

ARTICLE INFO

Article History:

Received: Nov. 9, 2022

Accepted: Dec. 29, 2022

Online: June 27, 2023

Keywords:

Oreochromis niloticus,
liver,
copper,
Spirulina platensis

ABSTRACT

Copper (Cu) is an essential trace metal in aquatic environments. However, excess Cu can induce toxic effects at levels of biochemical and histopathological aspects. Previous studies have reviewed the importance of spirulina supplementation in neutralizing the harmful influences of heavy metals in various fish species. In the present work, the biochemical and histopathological effects of 0.4 and 0.8mg/ l of Cu were addressed on *Oreochromis niloticus* fed spirulina-free diet (SFD) or spirulina-supplemented diet (SSD). Hepatic protein content showed a highly significant increase in fish exposed to 0.8mg/ l of Cu and fed SSD for 7 days. On the 14th day, a significant increase in lipid content was noticed in the liver of fish exposed to 0.8mg/ l of Cu and fed SFD. Both protein and lipid content showed a significant increase in the liver of fish fed SSD without Cu exposure. On the 7th day, a significant increase was found in the liver glycogen of fish exposed to 0.4mg/ l of Cu and fed SFD. While, on day 21, a highly significant increase was found in the liver glycogen concentration in fish exposed to 0.4mg/ l of Cu and fed SSD. Cu induced many pathological lesions including vacuolar degeneration, pyknosis and degenerative and necrotic changes till the loss of architecture and hemorrhagic lesions. The liver histopathological changes observed were more evident with high Cu concentrations and time. The impact of sublethal concentrations of copper on *O. niloticus* is intense. The role of 1% *spirulina platensis* supplementation was limited in neutralizing the harmful influences of Cu on *O. niloticus*

INTRODUCTION

Copper (Cu) is an essential trace metal within aquatic environments and an important micronutrient for many aquatic species (Zitoun, 2019). The toxic effects of metals may begin after excretory, metabolic, storage and detoxification mechanisms are no longer able to match the uptake (Jorgensen, 2012; Kaoud, 2015). Fish can exhibit serious cellular alteration due to the toxicity impact of Cu (Al-Bairuty *et al.*, 2013). Numerous studies have reviewed the importance of spirulina supplementation in neutralizing the deleterious influences of heavy metals in various fish species (Bangeppagari *et al.*, 2014; Dar *et al.*, 2014; Vetrivel *et al.*, 2014; Sayed *et al.*, 2017; Mohanty & Samanta, 2018; Priya *et al.*, 2018). Moreover, spirulina

supplementation attenuated the toxic effect of some pesticides (Abdelkhalek *et al.*, 2015; Abdelkhalek *et al.*, 2017; Fadl *et al.*, 2022). In the present work, the biochemical and the histopathological effects of 10% and 20% of maximum tolerable concentration (MTC) of copper were examined on *Oreochromis niloticus* fish fed spirulina free diet (SFD) or spirulina supplemented diet (SSD).

MATERIALS AND METHODS

Fish sampling

The present study was carried out on *Oreochromis niloticus* coinciding with the studies of Trewavas (1981, 1982). Fish were reared in NIOF aquarium under healthy conditions. Fish stockage, weighing 9.99 ± 2.6 g was done without any feeding during the 24 hour post-transporting. Aeration of aquaria's water was carried out by using air pumps and air stones. The excess food and the fecal matter at the bottom of aquarium was removed and replaced by overnight kept water with the help of plastic tubing using siphon technique. Fish were fed with artificial pellets (30% protein). Two forms of diet were used, spirulina free diet (SFD) and 1% spirulina supplemented diet (SSD). *Spirulina platensis* dried powder was obtained from Hydrobiology lab, NIOF. Cupric sulfate pentahydrate (Lobal Chemei for Laboratory Reagents and Fine Chemicals, India) was used in the present study. Pure compound of cupric sulfate pentahydrate was dissolved in distilled water.

Toxicity screening test

Concentrations of Cu were conducted for toxicity screening in 80-L glass aquaria. Groups of *O. niloticus*, with ten fish for each, were used for toxicity test. One control group was maintained in clean water (control). Other groups were exposed to 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0 and 8.0mg of Cu/ l, respectively. Fish in each aquarium were continuously observed during the period of the experiment (96 hrs). The dead fish were immediately removed upon detection. Mortalities and survival time were recorded.

Chronic exposure to sublethal concentrations of copper in fish fed SFD or SSD

Sublethal concentrations of copper (0.4 and 0.8mg/ l) were used. Two forms of diet were used (SFD and SSD), supplemented with 1% *spirulina platensis* dried powder. The effects of sublethal concentrations of copper were studied after 7, 14, and 21 days via:

Biochemical studies

Samples from the liver of the studied fish were stored in freezer (at -20°C) till used. Samples were subjected for colorimetric analysis total protein content according to Gornall *et al.* (1949) and total lipid content according to Frings and Dunn (1970), using Spectrum Diagnostic kit, Egypt. Meanwhile, glycogen content was measured in fish liver following the anthrone method (Seifter *et al.*, 1950).

Data were collected, coded and entered as spread sheet using Microsoft Excell. Data were analyzed using IBM Statistical package for social science software (SPSS). One way ANOVA test was employed to compare each group with control group.

Histopathological studies

The collected samples were fixed in 10% formalin for 24 hours, washed by running water, dehydrated in alcohol, cleared in xylene, and sections of 4-6 μ were prepared according to Saad *et al* (2012).

RESULTS

Toxicity test

The percentage of mortality for *O. niloticus* at different concentrations of copper was recorded. The studied fish tolerated the exposure to 4mg/ l of Cu for 96hrs since no mortality was recorded. Increasing the concentrations of Cu induced the increment of mortality percentage, reaching 100% at 6mg/ l or more of Cu (Table 1). The maximum tolerable concentration (MTC) of Cu for the studied fish was 4mg/ l.

Chronic exposure to sub-lethal concentrations of copper in fish fed SFD or SSD

The effects of Cu (0.4 and 0.8 mg/l) were studied in the liver of the studied fish fed SFD or SSD via:

Biochemical studies

Protein content. On day 7, the liver of fish exposed to 0.8mg/l of Cu and fed SSD induced highly significant increase in the protein content ($P \leq 0.001$) (Table 2). On the 14th and 21st days, no significant differences were detected between control group and other groups (Table 2). Protein content showed significant increase ($P \leq 0.05$) in the liver of fish fed SSD without Cu exposure.

Lipid content. On the 7th and 21st days, no significant difference was observed in the lipid content of the liver of fish exposed to different concentrations of Cu compared to the control. On day 14, a significant increase was detected in the lipid content of the liver of fish exposed to 0.8mg/l Cu and fed SFD ($P \leq 0.05$) (Table 3). Lipid content showed a significant increase ($P \leq 0.05$) in the liver of fish fed SSD without Cu exposure.

Glycogen content. On the 7th day, a significant increase ($P \leq 0.05$) was recorded in the liver glycogen of fish exposed to 0.4mg/ l of Cu and fed SFD.

There were non-significant differences between control and fish exposed to different concentrations of copper on the 14th day. Whereas, on day 21, a highly significant increase ($P \leq 0.001$) was registered in the liver glycogen concentration in fish exposed to 0.4mg/ l of Cu and fed SSD (Table 4).

Table 1. Mortality percentage (%) of *O. niloticus* exposed to different concentrations of copper (Cu)

Copper (mg/l) Exposure time (hrs)	0 (control)	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0
24 hrs	0	0	0	0	0	0	0	0	2	6	10	10	10
48 hrs	0	0	0	0	0	0	0	0	1	1	0	0	0
72 hrs	0	0	0	0	0	0	0	0	0	2	0	0	0
96 hrs	0	0	0	0	0	0	0	0	0	0	0	0	0

n= 10 fish individuals for each group.

Table 2. Comparison between the studied groups as per liver protein concentration (g/100g) of *O. niloticus* at different follow-up periods.

Cu (mg/l)	Diet	7 days				14 days				21 days			
		Mean	±	SD	P-value	Mean	±	SD	P-value	Mean	±	SD	P-value
0	SFD	2.46	±	0.38	-	2.46	±	0.38	-	2.46	±	0.38	-
0	SSD	4.40	±	0.59	0.018*	4.40	±	0.59	0.018*	4.40	±	0.59	0.018*
0.4	SFD	1.59	±	0.56	0.360	2.48	±	0.84	1.000	3.92	±	1.79	0.911
0.4	SSD	1.30	±	0.54	0.105	3.16	±	1.24	0.794	4.60	±	1.56	0.636
0.8	SFD	2.54	±	1.38	1.000	3.17	±	0.86	0.785	3.06	±	1.28	0.091
0.8	SSD	4.60	±	1.56	0.001**	6.30	±	4.90	0.875	2.33	±	1.66	1.000

*:p≤0.05 is considered statistically significant

**:p≤0.001 is considered statistically highly significant.

SFD: Spirulina Free Diet

SSD: Spirulina Supplemented Diet.

Table (3): Comparison between the studied groups as per liver lipids concentration (g/100g) of *O. niloticus* at different follow-up periods.

Cu (mg/l)	Diet	7 days				14 days				21 days			
		Mean	±	SD	P-value	Mean	±	SD	P-value	Mean	±	SD	P-value
0	SFD	0.41	±	0.32	-	0.41	±	0.32	-	0.41	±	0.32	-
0	SSD	0.92	±	0.24	0.036*	0.92	±	0.24	0.036*	0.92	±	0.24	0.036*
0.4	SFD	0.85	±	0.52	0.271	0.62	±	0.46	0.761	0.42	±	0.07	1.000
0.4	SSD	0.86	±	0.43	0.236	0.15	±	0.11	0.593	0.24	±	0.13	0.681
0.8	SFD	0.49	±	0.22	0.999	0.98	±	0.25	0.021*	0.44	±	0.18	1.000
0.8	SSD	0.36	±	0.23	1.000	0.13	±	0.12	0.508	0.47	±	0.16	0.993

*: $p \leq 0.05$ is considered statistically significant **: $p \leq 0.001$ is considered statistically highly significant

SFD: Spirulina Free Diet

SSD: Spirulina Supplemented Diet

Table (4): Comparison between the studied groups as per liver glycogen concentration (g/100g) of *O. niloticus* at different follow-up periods.

Cu (mg/l)	Diet	7 days			14 days			21 days		
		Mean	± SD	P-value	Mean	± SD	P-value	Mean	± SD	P-value
0	SFD	2.28	± 0.67	-	2.28	± 0.67	-	2.28	± 0.67	0.99
0	SSD	1.82	± 1.25	0.99	1.82	± 1.25	0.99	1.82	± 1.25	0.69
0.4	SFD	5.04	± 2.19	0.01*	1.69	± 1.31	0.94	3.24	± 0.97	0.59
0.4	SSD	1.79	± 0.79	0.99	1.86	± 0.96	0.99	3.34	± 0.57	<0.001**
0.8	SFD	2.67	± 1.34	1.00	2.07	± 0.72	1.00	9.72	± 1.81	0.99
0.8	SSD	2.34	± 0.66	1.00	2.53	± 1.39	1.00	1.87	± 0.83	0.99

* :p≤0.05 is considered statistically significant

** :p≤0.001 is considered statistically highly significant.

SFD: Spirulina Free Diet. SSD: Spirulina Supplemented Diet.

1.1. Histopathological studies:

As shown in (Fig. 1 A), in the control *O. niloticus*, the liver of fish showed hepatocytes that are arranged in a definite chord-like pattern around sinusoids. The liver of fish fed with SSD without exposure to Cu exhibited pyknosis in the nucleus of some hepatocytes with slight vacuolation in the hepatocytes (Fig. 1 B).

On day 7, *O. niloticus* exposed to 0.4 mg/l Cu and fed SFD showed some pathological lesions in the liver. Vacuolar degeneration of hepatocytes, pyknosis of the nucleus and focal areas of necrosis and, hemorrhage between hepatocytes (Fig. 1 C) was observed. *O. niloticus* exposed to 0.4 mg/l Cu and fed SSD showed moderate recovery of hepatocytes (Fig. 1 D). Meanwhile, infiltration of inflammatory cells and hemorrhage (Fig. 1 D) were noticed.

O. niloticus exposed to 0.4 mg/l copper and fed SFD for 14 days showed increased vacuolar degeneration, increased dilation and congestion of blood sinusoids (Fig. 1 E). In addition, coagulative necrosis (Fig. 1 F) and degenerative and necrotic changes (Fig. 1 G) were presented. *O. niloticus* exposed to 0.4 mg/l copper and fed SSD for 14 days showed a recovery and improvement in many pathologic lesions (Fig. 1 H).

O. niloticus exposed to 0.4 mg/l copper and fed SFD for 21 days showed increased severity of histopathological lesions. The liver showed severe vacuolar degeneration in the hepatocytes and focal areas of necrosis (Fig. 1 I). Coagulative necrosis of hepatocytes (Fig. 1 J) was noticed. Hemorrhage between hepatocytes (Fig. 1 K) was observed. Meanwhile, liver of fish fed SSD and exposed to copper showed less severe lesions. The liver showed vacuolar degeneration in the hepatocytes and thrombosis of hepatic vessel (Fig. 1 L).

On the other hand, the exposure to the higher copper concentration (0.8 mg/l) for 7 days induced more severe vacuolar degeneration of the hepatocytes and pyknosis of the nucleus (Fig. 2 A). However, supplementation of spirulina reduced vacuolar degeneration of the hepatocytes (Fig. 2 B). Moreover, hemorrhagic lesions were observed (Fig. 2 C).

On day 14 after exposure to 0.8 mg/l of copper, the lesions increase in the intensity. The liver showed severe vacuolar degeneration in the hepatocytes (Fig. 2 D), degenerative and necrotic changes and finally loss of the liver architecture (Fig. 2 E). Focal areas of necrosis were observed between the hepatocytes (Fig. 2 F). Hemorrhagic lesions were noticed between the hepatocytes (Fig. 2 G). However, SSD improved hepatocytes appearance with persistent focal areas of necrosis between the hepatocytes (Fig. 2 H) and intravascular hemolysis within hepatoportal blood vessels (Fig. 2 I) were observed.

On day 21 after exposure to 0.8 mg/l of copper, intensive hepatic damage and loss of the liver architecture were found. Severe vacuolar degeneration with hemorrhage between hepatocytes was noticed (Fig. 2 J). Meanwhile, on exposure to 0.8 mg/l of copper and fed SSD, the liver showed severe vacuolar degeneration in the hepatocytes (Fig. 2 K). Also, hemorrhage between hepatocytes (Fig. 2 L) was noticed.

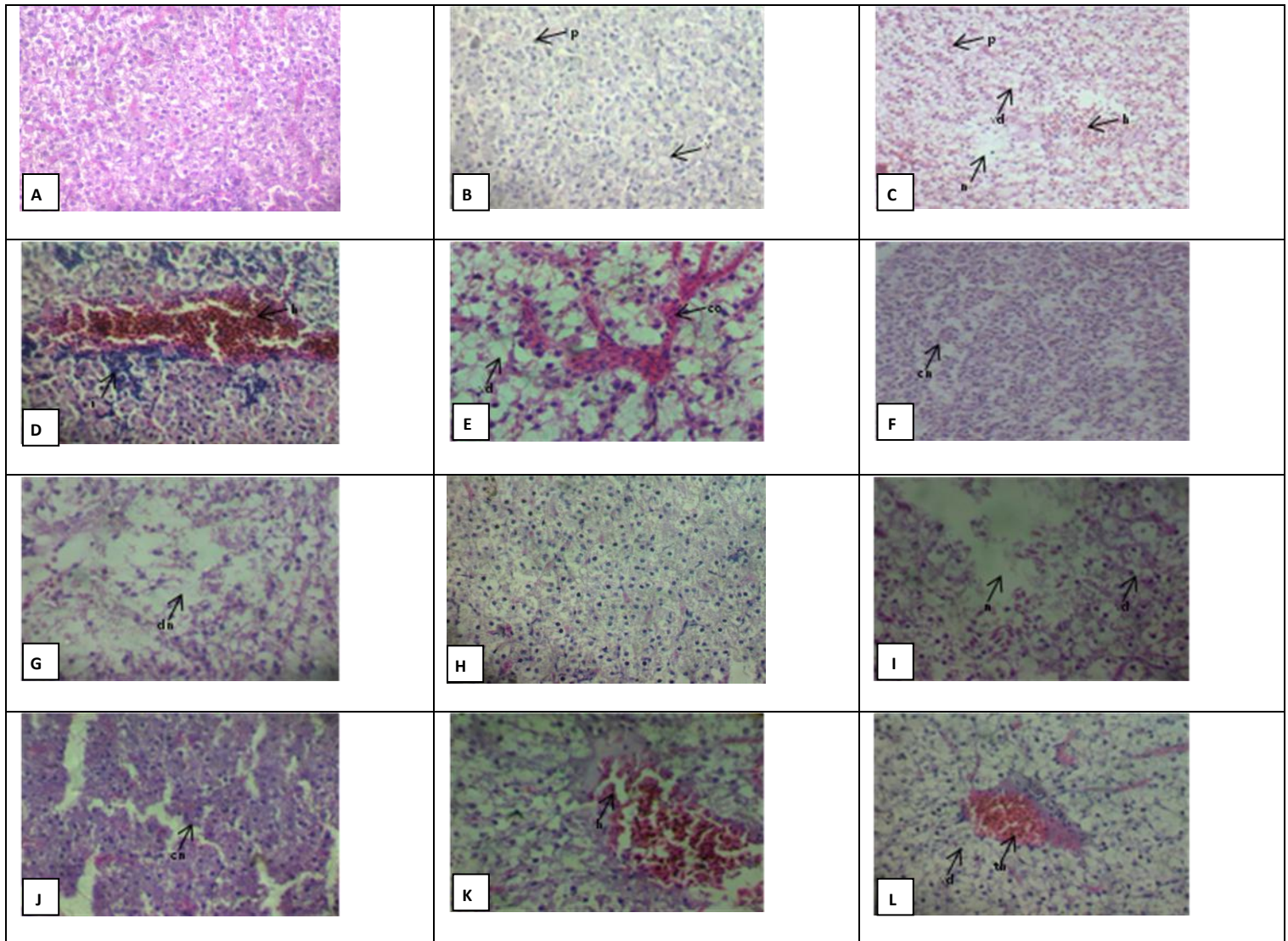


Figure (1): Liver of *o. niloticus* control group fed SFD (A), the liver of fish from control group fed SSD showing pyknosis(p) in the nucleus of some hepatocytes with slight vacuolation(v) in the hepatocytes (B), liver of fish exposed to 0.4 mg/l of Cu and fed SFD for 7 days showing vacuolar degeneration (vd) of hepatocytes, pyknosis(p) of the nucleus and focal area of necrosis(n), hemorrhage(h) between hepatocytes (C), fish exposed to 0.4 mg/l of Cu and fed SSD for 7 days showing moderate recovery of hepatocytes, infiltrations of inflammatory cells(i) and hemorrhage(h) between hepatocytes (D), fish exposed to 0.4 mg/l of Cu and fed SFD for 14 days showing increased vacuolar degeneration(vd), dilation and congestion(co) of blood sinusoids (E), coagulative necrosis(cn) (F) and degenerative and necrotic changes(dn) (G), fish exposed to 0.4 mg/l of Cu and fed SSD for 14 days showing slight recovery of hepatocytes (H), fish exposed to 0.4 mg/l of Cu and fed SFD for 21 days showing severe vacuolar degeneration (vd) in the hepatocytes and focal area of necrosis(n) (I), coagulative necrosis(cn) of hepatocytes (J) and hemorrhage (h) between hepatocytes(K), fish exposed to 0.4 mg/l of Cu and fed SSD for 21 days showing vacuolar degeneration (vd) in the hepatocytes and thrombosis (th) formation in hepatic blood vessel(L) (X400).

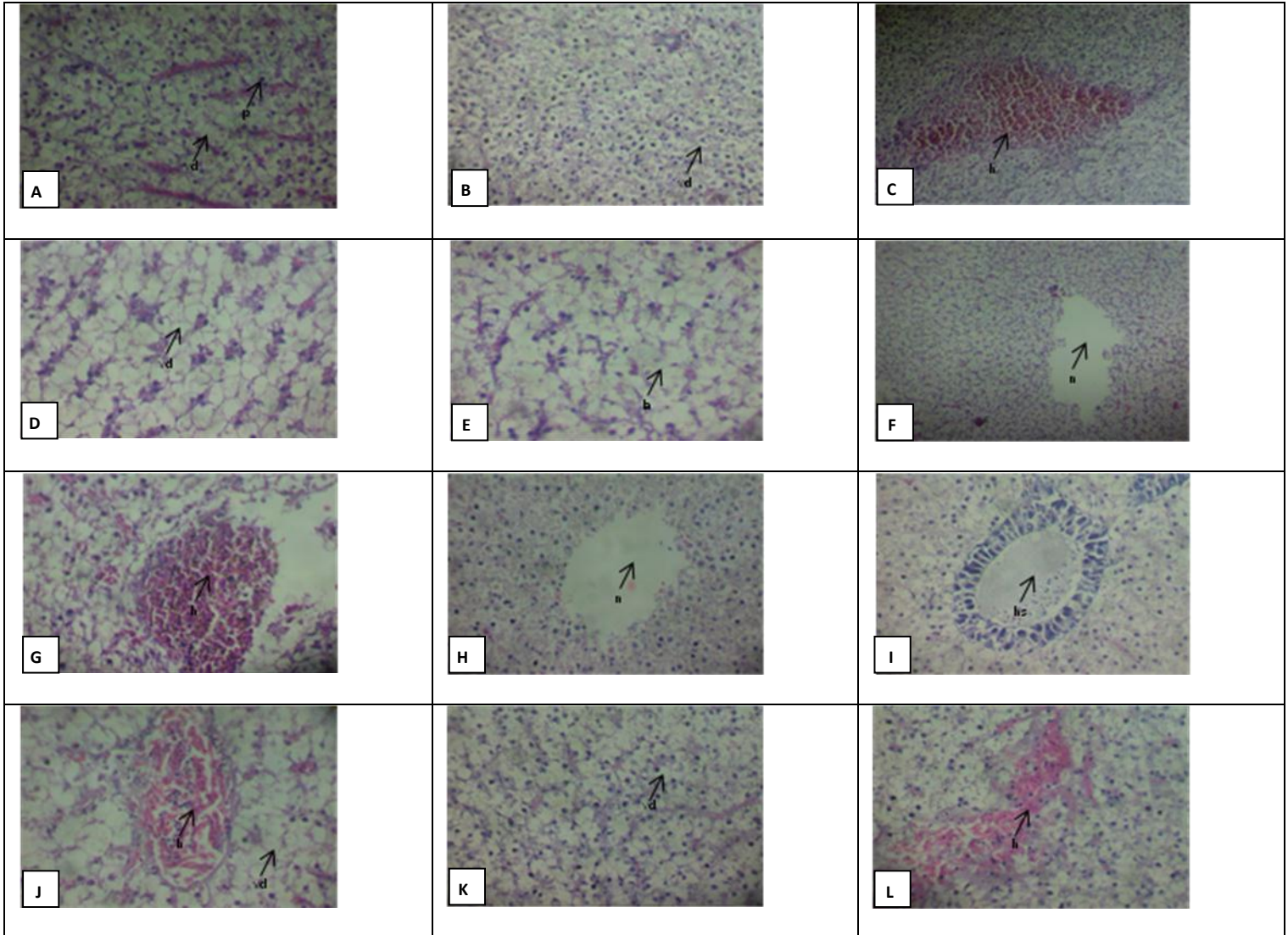


Figure (2):Liver of fish exposed to 0.8 mg/l of Cu and fed SFD for 7 days showing severe vacuolar degeneration (vd) of the hepatocytes, pyknosis (p) of the nucleus (A), fish exposed to 0.8 mg/l of Cu and fed SSD for 7 days showing moderate vacuolar degeneration (vd) of the hepatocytes (B) and hemorrhage(h) between the hepatocytes (C), fish exposed to 0.8 mg/l of Cu and fed SFD for 14 days showing severe vacuolar degeneration (vd) of the hepatocytes (D), loss of the liver architecture(1a) (E), focal area of necrosis(n) between the hepatocytes (F) and hemorrhage(h) between the hepatocytes (G), Cu-exposed fish and fed SSD for 14 days showing slight recovery in the hepatic architecture with presence of focal area of necrosis(n) (H) and hemolysis(hs) within hepatoportal blood vessel (I), fish exposed to 0.8 mg/l of Cu and fed SFD for 21 days showing Severe vacuolar degeneration(vd) with hemorrhage(h) between hepatocytes (J), fish exposed to 0.8 mg/l of Cu and fed SSD for 21 days showing severe vacuolar degeneration(vd) (K) and extra hemorrhage (h) between the hepatocytes (L) (X400).

DISCUSSION

There are extensive regulatory requirements for fish acute toxicity data in support of both risk assessment and also hazard classification (**Braunbeck and Lammer, 2006**). This ensures the value of studying sublethal concentrations of different toxins. The maximum tolerable concentration (MTC) is the highest concentration of a substance in an environmental medium that does not cause the death of the test organisms or species. MTC of Cu for *O. niloticus* was 4 mg/l in the present study. Disparate safe, tolerable and toxic levels have been established for Cu on fish. **National Academy of Sciences (NAS)** (1977) reported acute toxicity of Cu at 0.01 – 0.02 mg/L. **Eisler**, (1998) observed that a concentration of Cu between 0.004-0.01 mg/l had sublethal effects to fish and aquatic food chain. Meanwhile, **Bradl**, (2005) reported that Cu concentration is toxic to fish when exceeds 0.02 mg/L. **Mustapha and Agunloye (2016)** found that Cu concentrations ranged from 0.01 to 0.10 mg/l within different aquacultures with the highest concentration recorded in the natural pond and the lowest found in the collapsible pond. The levels of Cu in the ponds were slightly elevated above the references (**Mustapha and Agunloye, 2016**).

In present work, sublethal concentrations of Cu were selected regarding to MTC. Fish was exposed to 10% and 20% of MTC of Cu (0.4 and 0.8 mg/l, respectively) for 7, 14 and 21 days in presence or absence of spirulina supplemented diet. Complex process of physiological and biochemical changes is involved in fish to cope with stress and nutrient metabolism (**Polymeropoulos et al., 2017**). In present study, significant increase in protein content in liver of fish fed exposed to 0.8mg/l Cu and fed SSD for 7 days.. The significant increase in hepatic protein content in fish exposed to Cu may be required for fish to meet the increased demand for tissue repair response during Cu exposure (**Javed et al., 2017; Soliman et al., 2021**). and enhanced by presence of spirulina. Many previous literatures showed the hepatoprotective effects of spirulina against drugs, chemicals and xenobiotics induced hepatic injury (**Abdelkhalek et al., 2015 and 2017**).

The significant raise in the protein content in fish fed SSD without Cu exposure come in accord with previous studies (**Velasquez et al., 2016; Cao et al., 2018; Roohani et al., 2019; Mohammadiazarm, et al., 2021**). This may explained by Spirulina contain high amounts of protein between 55 and 70% by dry weigh (**Zhang et al., 2020**).

Lipids are necessary to maintain cellular energy homeostasis and exert a vast array of functions (**O'Brien et al., 2015**). Pollutants include heavy metals can cause an imbalance between the free radical species production and reduction in fish (**Kamollerd et al., 2019**). The free radicals can attack lipid, protein and DNA molecules to induce oxidative stress products and DNA damage (**Vilela et al., 2018; Kamollerd et al., 2019; Hashem et al., 2020**). In present study, significant increase in lipid content in liver of fish exposed to Cu 0.8mg/l and fed SFD for 14 days. Copper exposure induced significant increase in

lipid content in liver of many fishes (**Liu *et al.*, 2010; Chen *et al.*, 2013; Wang *et al.*, 2019**).

Meanwhile, increase of lipid content in liver of studied fish fed SSD without Cu exposure may be as a result of the role of spirulina in increasing concentration of some beneficial long-chain PUFAs and decreased concentration of unwanted SFAs (**Jafari *et al.*, 2014**).

The glycogen metabolism is the main pathway of energy acquisition, especially in the stressful environment (**Oliveira *et al.*, 2004; Bacca *et al.*, 2005**). In the present study, the raise of hepatic glycogen content was exhibited in fish exposed to 0.4 mg/l Cu and fed SFD for 7 days and fish exposed to 0.4 mg/l Cu and fed SSD for 21 days. In general, glycogen content reduced during stress because of increased glycogenolysis and decreased glycogen synthesis (**Van Cromphaut, 2009**). However, metabolic reprogramming may take place to adapt to available energy reserve during stress. Metabolic reprogramming was reported during environmental hypoxia (**Gracey *et al.*, 2011**) for replenishment of glycogen that was over-consumed (**Javed and Usmani, 2015**). **Li *et al.* (2018)** reported increased glycogen content in chronic hypoxia and decrease in acute hypoxia. Also, the significant increase in glycogen content may be due to enhancement of gluconeogenesis probably due to lactate oxidation through the change of metabolic fuel preference to the use of lactate as a gluconeogenic substrate as occur in chronic hypoxia (**Omlin and Weber, 2010**).

Histopathological study was performed in liver of *O. niloticus* exposed to different concentrations of Cu and/or fed spirulina supplemented diet. The histopathological lesions are highly sensitive and accurate for evaluation of hepatic damages in toxicological studies (**Dabrowska *et al.*, 2012**). In present study, Cu induced many pathological lesions including vacuolar degeneration, pyknosis, degenerative and necrotic changes till loss of architecture. Hemorrhagic, hemolytic and vascular damages were also observed in liver of fish exposed to Cu. Many literatures agreed with results of present study regarding the alternations induced by Cu such as increase of cytoplasmic vacuolation (**Maharajan *et al.*, 2016; Alkobaby and Abd El-Wahed, 2017; Padriilah *et al.*, 2018**) and nuclear pyknosis (**AL-Bairuty *et al.*, 2013; Maharajan *et al.*, 2016; Sabullah *et al.*, 2017; Alkobaby and Abd El-Wahed, 2017**). In accordance with literature, Cu induced degenerative-necrotic changes (**Maharajan *et al.*, 2016; Abdel-Tawwab, 2016**). Disorganization and loss of hepatic architecture were also reported after Cu exposure (**Padriilah *et al.*, 2017**). Increased dilation and congestion of sinusoids were reported after Cu exposure (**Padriilah *et al.*, 2017**).

Braunbeck *et al.* (1990) referred that nuclear alterations are signs of increased metabolic activity and pathological action. Pyknosis is one of hepatocellular pleomorphism thought to be exhibited in sub lethally injured cells resulting from exposure to several types of toxicant (**Agamy, 2012**). Cu toxicity increase ROS formation and decrease antioxidant protection causing oxidative stress. This may result in structural

changes in biomembranes, loss of liver integrity, decreased metabolic activity, and finally cell death (**Daggulli et al., 2014**). Increased cytoplasmic vacuolation might indicate imbalance between the synthesis rate of different molecules in parenchymal cells and the rate of releasing them into systemic circulation (**Hadi and Ahwan, 2012**). Meanwhile, increased vacuolation of the hepatocytes is a degenerative process suggestive of metabolic damage (**Pacheco and Santos, 2002**). Degenerative necrotic conditions could be explained by Cu-induced oxidative stress in the tissue of the liver (**Holye et al., 2007**). Necrosis of some portions of the liver tissue is attributed to presence of chemicals disturbing the physiological process within cells (**Mela et al., 2007**). Also, hepatic degeneration and necrosis may be resulted from stasis of the blood and impaired blood flow due to the vascular engorgement and congestion of the blood vessels and sinusoids (**Mohamed, 2001**). Loss of the hepatic architecture is probably accompanied with cell necrosis and degeneration of structural proteins in the membrane of the hepatocytes (**Pacheco and Santos, 2002**).

The degenerative alterations and necrosis are given the highest importance factor because they are considered a direct effect of toxicants, they are generally irreversible, and their persistence or progression may lead to partial or total loss of organ function (**Agamy, 2012; Biuki et al., 2013; Mela et al., 2013; Abdel-Moneim, 2014; Afifi et al., 2014**).

Hemolysis and hemorrhage are a result of blood channel disruption, and indication of severe physical damage. The hemolysis, dilation, and congestion may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals.

Infiltration of inflammatory cells was seen in liver of fish exposed to Cu and fed SSD. **Kevat et al. (1988)** owed cellular infiltration to inflammation and reaction of microcirculation and movement of fluids and leukocytes from the blood into the extra vascular tissue. This may be line of immunological response. Also, that was classified by other researchers as a moderate injury and it is often associated with neutralization and destroying the source aggressor, cleaning the tissue by removing dead cells, as well as inducing the recovery of damaged tissue (**Bernet et al., 1999**).

The liver histological changes observed were more evident in fish exposed to the higher Cu concentrations in agreement with **Al-Tamimi et al. (2015)** and **Padrilah et al. (2017)**. Also, the degree of liver histopathological lesions induced by Cu seemed to be more pronounced with time. These histological lesions were considered indicative of the impact of Cu on the health of *O.niloticus* fish.

In present study, spirulina supplementation induced moderate recovery in hepatocyte morphology. **Soliman et al. (2021)** found that exposed fish pre-fed the basal diet supplemented with spirulina maintained a more normal histological structure than those that were exposed to CuSO₄ without supplementation. Meanwhile, many pathological lesions were still well presented in Cu exposed fish liver during feeding SSD. This may

be owed to contamination with cyanotoxins or toxic metals (**Kerna *et al.*, 2022**). Meanwhile, spirulina reported to produce toxins (microcystins, β -methylamino-L-alanine (BMAA) which might contribute to liver damage (**Simran *et al.*, 2022**).

CONCLUSION

In current study, the impact of sublethal concentrations of copper on *o. niloticus* was intense. The role of 1% spirulina supplementation in neutralizing the harmful influences of Cu on *o. niloticus* was limited.

REFERENCES

- Abdelkhalek, N. K.; Eissa, I. A.; Ahmed, E.; Kilany, O. E.; El-Adl, M.; Dawood, M. A. and Abdel-Daim, M. M.** (2017). Protective role of dietary *Spirulina platensis* against diazinon-induced oxidative damage in Nile tilapia; *Oreochromis niloticus*. Environmental Toxicology and Pharmacology, 54: 99-104.
- Abdelkhalek, N. K.; Ghazy, E. W. and Abdel-Daim, M. M.** (2015). Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, *Oreochromis niloticus*: impact on lipid peroxidation and oxidative stress. Environmental Science and Pollution Research, 22(4): 3023-3031.
- Abdel-Moneim, A. M.** (2014). Histopathological and ultrastructural perturbations in tilapia liver as potential indicators of pollution in Lake Al-Asfar, Saudi Arabia. Environmental Science and Pollution Research, 21(6): 4387-4396.
- Affi, M.; Alkaladi A.; Abu-Zinada, O. and Ali, N. Z. Z.** (2014): Histopathological and ultrastructural alterations in skin, gills, liver and muscle of *Siganus canaliculatus* and *Epinephelus morio* caught from Jeddah and Yanbou coast as bio-indicators of oil hydrocarbons pollution. Life Science Journal, 1(8): 880-888.
- Agamy, E.** (2012). Histopathological changes in the livers of rabbit fish (*Siganus canaliculatus*) following exposure to crude oil and dispersed oil. Toxicologic pathology, 40(8): 1128-1140.
- Al-Bairuty, G. A.; Shaw, B. J.; Handy, R. D. and Henry, T. B.** (2013). Histopathological effects of waterborne copper nanoparticles and copper sulfate on the organs of rainbow trout (*Oncorhynchus mykiss*). Aquatic toxicology, 126: 104-115.
- Alkobaby, A. I. and Abd El-Wahed, R. K.** (2017). The acute toxicity of copper to Nile tilapia (*Oreochromis niloticus*) fingerlings and its effects on gill and liver histology. Journal of Aquaculture Research and Development, 8(1): 1-6.
- Al-Tamimi, A. H.; Al-Azzawi, A. J. and Al-A'dhmi, M. A.** (2015). Chronic toxicity assessment of histological changes and micronuclei in fish *Cyprinus carpio* L. after exposed to copper. American Academic Scientific Research Journal for Engineering, Technology, and Sciences, 13(1): 194-210.

- Bacca, H.; Huvet, A.; Fabioux, C.; Daniel, J. Y.; Delaporte, M.; Pouvreau, S. and Moal, J.** (2005). Molecular cloning and seasonal expression of oyster glycogen phosphorylase and glycogen synthase genes. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 140(4): 635-646.
- Bangeppagari, M.; Gooty, J. M.; Tirado, J. O.; Mariadoss, S.; Thangaswamy, S.; Maddela, N. R. and Ortiz, D. R.** (2014). Therapeutic efficiency of spirulina against lead acetate toxicity on the fresh water fish *Labeo rohita*. *American Journal of Life Sciences*, 2(6): 389-394.
- Bernet, D.; Schmidt, H.; Meier, W.; Burkhardt-Holm, P. and Wahli, T.** (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of fish diseases*, 22(1): 25-34.
- Biuki, N. A.; Savari, A.; Mortazavi, M. S.; Zolgharnein, H. and Salamat, N.** (2012): Liver histopathological changes in Milkfish (*Chanos chanos*) exposed to Petroleum Hydrocarbon Exposure. *World Applied Sciences Journal*, 18(9): 1315-1320.
- Biuki, N. A.; Savari, A.; Mortazavi, M. S.; Zolgharnein, H. and Salamat, N.** (2013): Liver histopathological changes in Milkfish (*Chanos chanos*) exposed to acute toxicity of diesel oil. *World Applied Sciences Journal*, 14 (10): 1487-1492.
- Bradl, H. (Ed.)**. (2005). *Heavy metals in the environment: origin, interaction and remediation*. Elsevier.
- Braunbeck, T. and Lammer, E.** (2006). Background paper on fish embryo toxicity assays. German Federal Environment Agency, Dessau.
- Braunbeck, T.; Storch, V. and Bresch, H.** (1990). Species-specific reaction of liver ultrastructure in zebrafish (*Brachydanio rerio*) and trout (*Salmo gairdneri*) after prolonged exposure to 4-chloroaniline. *Archives of Environmental Contamination and Toxicology*, 19(3): 405-418.
- Cao, S. P.; Zou, T.; Zhang, P. Y.; Han, D.; Jin, J. Y.; Liu, H. K. and Xie, S. Q.** (2018). Effects of dietary fishmeal replacement with *Spirulina platensis* on the growth, feed utilization, digestion and physiological parameters in juvenile gibel carp (*Carassis auratus gibelio* var. CAS III). *Aquaculture Research*, 49(3): 1320-1328.
- Chen, Q. L.; Luo, Z.; Pan, Y. X.; Zheng, J. L.; Zhu, Q. L.; Sun, L. D. and Hu, W.** (2013). Differential induction of enzymes and genes involved in lipid metabolism in liver and visceral adipose tissue of juvenile yellow catfish *Pelteobagrus fulvidraco* exposed to copper. *Aquatic toxicology*, 136: 72-78.
- Dabrowska, H.; Ostaszewska, T.; Kamaszewski, M.; Antoniak, A.; Kopko, O.; Lang, T. and Lehtonen, K. K.** (2012). Histopathological, histomorphometrical, and immunohistochemical biomarkers in flounder (*Platichthys flesus*) from the southern Baltic Sea. *Ecotoxicology and Environmental Safety*, 78: 14-21.
- Daggulli, M.; Dede, O.; Utangac, M. M.; Bodakci, M. N.; Hatipoglu, N. K.; Penbegul, N. and Yüksel, H.** (2014). Protective effects of carvacrol against

methotrexate-induced testicular toxicity in rats. *International Journal of Clinical and Experimental Medicine*, 7(12): 5511-5516.

Dar, B. A.; Khaliq, R.; Jha, G. N.; Kour, P. and Qureshi, T. A. (2014). Protective effects of dietary spirulina against cadmium chloride exposed histoarchitectural changes in the liver of freshwater catfish *Clarias batrachus* (Linnaeus, 1758). *Indian J Fish*, 61(3): 83-87.

Eisler, R. (1998). Copper hazards to fish, wildlife, and invertebrates: a synoptic review (No. 33). US Department of the Interior, US Geological Survey.

Fadl, S. E.; Elbialy, Z. I.; Abdo, W.; Saad, A. H.; Aboubakr, M.; Abdeen, A. and Abdelhiee, E. Y. (2022). Ameliorative effect of spirulina and *Saccharomyces cerevisiae* against fipronil toxicity in *Oreochromis niloticus*. *Ecotoxicology and Environmental Safety*, 242: 113899.

Frings, C. S. and Dunn, R. T. (1970). A colorimetric method for determination of total serum lipids based on the sulfo-phospho-vanillin reaction. *American Journal of Clinical Pathology*, 53(1): 89-91.

Gornall, A. G.; Bardawill, C. J. and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. biol. Chem*, 177(2): 751-766.

Gracey, A. Y.; Lee, T. H.; Higashi, R. M. and Fan, T. (2011). Hypoxia-induced mobilization of stored triglycerides in the euryoxic goby *Gillichthys mirabilis*. *Journal of Experimental Biology*, 214(18): 3005-3012.

Hashem, M. H.; Tayel, S. I.; Sabra, E. A.; Yacoub, A. M. and Heiba, A. A (2020). Impact of the water quality of El-Rahawy Drain on some genetic and histopathological aspects of *Oreochromis niloticus*. *Egyptian Journal of Aquatic Biology and Fisheries*, 24(2): 19-38.

Hadi A. and Ahwan S. (2012). Histopathological changes in gills, liver and kidney of fresh water fish, *Tilapia zillii*, exposed to aluminum. *International Journal of Pharmacy and Life Sciences*, 3(11): 2071–2081

Hoyle, I.; Shaw, B. J. and Handy, R. D. (2007). Dietary copper exposure in the African walking catfish, *Clarias gariepinus*: Transient osmoregulatory disturbances and oxidative stress. *Aquatic toxicology*, 83(1): 62-72.

Jafari, S. M. A.; Rabbani, M.; Emtiazjoo, M. and Piryaee, F. (2014). Effect of dietary *Spirulina platensis* on fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) fillet. *Aquaculture international*, 22(4): 1307-1315.

Javed, M.; Ahmad, M.; Usmani, N. and Ahmad, M. (2017). Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity and histopathology) in *Channa punctatus* exposed to heavy metal loaded waste water. *Scientific reports*, 7(1): 1-11.

Javed, M. and Usmani, N. (2015). Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish *Channa punctatus* inhabiting river polluted by Thermal Power Plant effluent. *Saudi Journal of Biological Sciences*, 22(2): 237-242.

- Jorgensen, S.W.** (2012). Ecotoxicology: a derivative of encyclopedia of ecology". Academic Press, London, 390.
- Kamollerd, C.; Senaphan, K.; Tengjaroenkul, B.; Monkheang, P. and Neeratanaphan, L.** (2019). Oxidative stress and genetic differentiation in experimental tilapia fish exposed to heavy metals in a reservoir near a municipal landfill. *Applied Ecology and Environmental Research* 17(6):12893-12907.
- Kaoud Hussein, A.** (2015). Article review: heavy metals and pesticides in aquaculture: health problems. *European Journal of Academic Essays* 2(9): 15-22.
- Kaoud, H. A.; Mahran, K. M.; Rezk, A. and Khalf, M. A.** (2012). Bioremediation the toxic effect of mercury on liver histopathology, some hematological parameters and enzymatic activity in Nile tilapia, *Oreochromis niloticus*. *Researcher*, 4(1): 60-70
- Kerna, N. A.; Nworie, U.; Ortigas, M. A. C.; Chawla, S.; Pruitt, K. D.; Flores, J. V. and Anderson, I. I.** (2022). Spirulina miscellany: medicinal benefits and adverse effects of spirulina. *EC Nutrition*, 17: 25-36.
- Kevat, S.; Ahern, M. and Hall, P.** (1988). Hepatotoxicity of methotrexate in rheumatic diseases. *Medical toxicology and adverse drug experience*, 3(3): 197-208.
- Li, M.; Wang, X.; Qi, C.; Li, E.; Du, Z.; Qin, J. G. and Chen, L.** (2018). Metabolic response of Nile tilapia (*Oreochromis niloticus*) to acute and chronic hypoxia stress. *Aquaculture*, 495: 187-195.
- Liu, X. J.; Luo, Z.; Xiong, B. X.; Liu, X.; Zhao, Y. H.; Hu, G. F. and Lv, G. J.** (2010). Effect of waterborne copper exposure on growth, hepatic enzymatic activities and histology in *Synechogobius hasta*. *Ecotoxicology and environmental safety*, 73(6): 1286-1291.
- Lushchak, V. I.** (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic toxicology*, 101(1): 13-30.
- Maharajan, A.; Kitto, M. R.; Paruruckumani, P. S. and Ganapiriya, V.** (2016). Histopathology biomarker responses in Asian sea bass, *Lates calcarifer* (Bloch) exposed to copper. *The Journal of Basic and Applied Zoology*, 77: 21-30.
- Mela, M.; Guiloski, I. C.; Doria, H. B.; Randi, M. A. F.; de Oliveira Ribeiro, C. A.; Pereira, L. and De Assis, H. S.** (2013). Effects of the herbicide atrazine in neotropical catfish (*Rhamdia quelen*). *Ecotoxicology and environmental safety*, 93: 13-21.
- Mela, M.; Randi, M. A. F.; Ventura, D. F.; Carvalho, C. E. V.; Pelletier, E. and Ribeiro, C. O.** (2007). Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. *Ecotoxicology and Environmental Safety*, 68(3): 426-435.
- Mohamed, F. A. S.** (2001). Effects of phenol on the histological structures of the intestine and gonads of the freshwater teleost *Tilapia zillii* (Gervais, 1848). *Egyptian Journal of Aquatic Biology and Fisheries*, 5(1); 195-223.
- Mohammadiazarm, H.; Maniat, M.; Ghorbanijeze, K. and Ghotbeddin, N.** (2021). Effects of spirulina powder (*Spirulina platensis*) as a dietary additive on Oscar fish,

Astronotus ocellatus: Assessing growth performance, body composition, digestive enzyme activity, immune-biochemical parameters, blood indices and total pigmentation. *Aquaculture nutrition*, 27(1); 252-260.

Mohanty, D. and Samanta, L. (2018). Dietary supplementation of spirulina ameliorates iron-induced oxidative stress in Indian knife fish *Notopterus*. *Environmental Toxicology and Pharmacology*, 61, 71-78.

Mustapha, M. and Agunloye, J. T. (2016). Copper toxicity of four different aquaculture ponds. *Journal of Tropical Life Science*, 6(3): 95583.

National Academy of Sciences (NAS), (1977). Copper. National Research Council, National Academy of Sciences, Washington DC.

O'Brien, D. M. (2015). Stable isotope ratios as biomarkers of diet for health research. *Annual review of nutrition*, 35: 565-594.

Oliveira, G. T.; Rossi, I. C.; Kucharski, L. C. and Da Silva, R. S. (2004). Hepatopancreas gluconeogenesis and glycogen content during fasting in crabs previously maintained on a high-protein or carbohydrate-rich diet. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 137(2): 383-390.

Omlin, T. and Weber, J. M. (2010). Hypoxia stimulates lactate disposal in rainbow trout. *Journal of Experimental Biology*, 213(22): 3802-3809.

Öner, M.; Atli, G. and Canli, M. (2009). Effects of metal (Ag, Cd, Cr, Cu, Zn) exposures on some enzymatic and non-enzymatic indicators in the liver of *Oreochromis niloticus*. *Bulletin of environmental contamination and toxicology*, 82(3): 317-321.

Pacheco, M. and Santos, M. A. (2002): Naphthalene and β -naphthoflavone effects on *Anguilla anguilla* L. hepatic metabolism and erythrocytic nuclear abnormalities. *Environment International*, 28(4): 285–293.

Padrihah, S. N.; Sabullah, M. K.; Shukor, M. Y. A.; Yasid, N. A.; Shamaan, N. A. and Ahmad, S. A. (2018). Toxicity effects of fish histopathology on copper accumulation. *Pertanika Journal of Tropical Agricultural Science*, 41 (2): 519 – 540.

Patnaik, B. B.; Howrelia, H.; Mathews, T. and Selvanayagam, M. (2011). Histopathology of gill, liver, muscle and brain of *Cyprinus carpio communis* L. exposed to sublethal concentration of lead and cadmium. *African Journal of Biotechnology*, 10(57), 12218-12223.

Polymeropoulos, E. T.; Elliott, N. G. and Frappell, P. B. (2017). Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 213: 28-35.

Priya, N. P.; Remya, V. K. and Krishnaveni, M. S. (2018). Phycoremediation potential of *Spirulina platensis* on cadmium induced toxicity in *Oreochromis mossambicus*. *International Journal of Fisheries and Aquatic Studies*, 6(4): 271-276.

Roohani, A. M.; Abedian Kenari, A.; Fallahi Kapoorchali, M.; Borani, M. S.; Zoriezahra, S. J.; Smiley, A. H. and Rombenso, A. N. (2019). Effect of *Spirulina*

platensis as a complementary ingredient to reduce dietary fish meal on the growth performance, whole-body composition, fatty acid and amino acid profiles, and pigmentation of Caspian brown trout (*Salmo trutta caspius*) juveniles. *Aquaculture Nutrition*, 25(3): 633-645.

Saad, S. M. M.; El-Deeb, A. E.; Tayel, S.I.; Al-Shehri, E. and Ahmed, N. A. M. (2012). Effect of heavy metals pollution on histopathological alterations in muscles of *Clarias gariepinus* inhabiting the Rosetta branch, River Nile, Egypt. 1st International Conference Biotechnology Applications in Agric., Benha Univer., (1): 79-87, Moshtohor and Hurghada, 18-22, February 2012, Egypt.

Sabullah, M. K.; Ahmad, S. A.; Shukor, M. Y.; Gansau, A. J., Shamaan, N. A., Khalid, A. and Sulaiman, M. R. (2017). Alteration in morphological features of *Puntius javanicus* liver exposed to copper sulfate. *International Food Research Journal*, 24(3): 1314-1320

Sayed, A. E. D. H.; El-Sayed, Y. S. and Ali, H. (2017). Hepatoprotective efficacy of *spirulina platensis* against lead-induced oxidative stress and genotoxicity in catfish; *Clarias gariepinus*. *Ecotoxicology and environmental safety*, 143: 344-350.

Seifter, S.; Dayton, S.; Novic, B. and Muntwyler, E. (1950). The estimation of glycogen with the anthrone reagent. *Arch. Biochem.*, 25: 191-200.

Simran, G.; Jaspreet, K.; Kartik, S.; Rasane, P.; Jyoti, S.; Vishesh, B.; Prashant, K. and Sapna J. (2022). Spirulina- An Edible Cyanobacterium with Potential Therapeutic Health Benefits and Toxicological Consequences. *Journal of the American Nutrition Association*, 1-14.

Soliman, H. A.; Hamed, M. and Sayed, A. E. D. H. (2021). Investigating the effects of copper sulfate and copper oxide nanoparticles in Nile tilapia (*Oreochromis niloticus*) using multiple biomarkers: the prophylactic role of Spirulina. *Environmental Science and Pollution Research*, 28(23): 30046-30057.

Trewavas, E. (1981): Addendum to Tilapia and Sarotherodon. *Buntharscha Bull.*, 87:12.

Trewavas, E. (1982). Genetic groupings of Tilapiini used in aquaculture. *Aquaculture*, 27(1): 79-81.

Van Cromphaut, S. J. (2009). Hyperglycaemia as part of the stress response: the underlying mechanisms. *Best Practice and Research Clinical Anaesthesiology*, 23(4): 375-386.

Velasquez, S. F.; Chan, M. A.; Abisado, R. G.; Traifalgar, R. F. M.; Tayamen, M. M.; Maliwat, G. C. F. and Ragaza, J. A. (2016). Dietary spirulina (*Arthrospira platensis*) replacement enhances performance of juvenile Nile tilapia (*Oreochromis niloticus*). *Journal of applied phycology*, 28(2): 1023-1030.

Vetrivel, C.; Pugazhendy, K. and Prabakaran, S. (2014). Protective effect of spirulina against the lead acetate induced ALP and ACP activity in the liver tissue of fresh water fish, *Labeo rohita*. *Int J Modn Res Revs*, 2: 226-228.

Vilela, C.L.S.; Bassin, J. P. and Peixoto, R. S. (2018). Water contamination by endocrine disruptors: Impacts, microbiological aspects and trends for environmental protection. *Environmental Pollution* 235: 546-559.

Wang, T.; Wen, X.; Hu, Y.; Zhang, X.; Wang, D. and Yin, S. (2019). Copper nanoparticles induced oxidation stress, cell apoptosis and immune response in the liver of juvenile *Takifugu fasciatus*. *Fish and Shellfish Immunology*, 84: 648-655.

Zhang, F.; Man, Y. B.; Mo, W. Y. and Wong, M. H. (2020). Application of Spirulina in aquaculture: a review on wastewater treatment and fish growth. *Reviews in Aquaculture*, 12(2): 582-599.

Zitoun, R. (2019). Copper speciation in different marine ecosystems around New Zealand (Doctoral dissertation, University of Otago).