Biological treatment of ammonia using biofloc system for *Oreochromis niloticus* fish

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ABSTRACT

This study aimed to estimate the effect of biofloc technology (BFT) implementation on water quality and production performance of Nile tilapia (*Oreochromis niloticus*) using different concentrations of ammonia and attempt for treatment of excess ammonia by biofloc system with fixed temperature, pH and dissolved oxygen. The improvement and organization of phytoplankton communities in each case was monitored. The hematological and histological alterations of studied fish were also noticeable. Phytoplankton communities developed in this study were dominated by Chlorophyceae followed by Cyanophyceae, Bacillariophyceae and Euglenophyceae.

The collected fish were divided into five groups; the first group was left as the control, the second and third groups were supplemented with ammonia (0.25 LC_{50}, 0.50 LC_{50}) and the fourth and fifth groups were cultured under biofloc system (0.25 LC_{50}, 0.50 LC_{50}). The results revealed that the phytoplankton used in the biofloc system gradually converted ammonia into nitrate. Hematological studies revealed that the studied biochemical parameters of blood obtained from fish cultured under biofloc system were very close to that of the control. Also, histological studies showed that fish cultured under biofloc system appeared close to recovery and similar to that obtained from control. In conclusion, phytoplankton in the biofloc system improves water quality and production performance of cultured fish by enhancing nitrification and transformation, of ammonia (toxic) into nitrate (non-toxic).

INTRODUCTION

Biofloc technology (BFT) is an aquaculture system which focused on a more efficient use of nutrient input with limited or zero water exchange (Widanarni *et al.*, 2012). Phytoplankton is a source of characteristic feed for fish cultivation in the pond. Phytoplankton yield increases by the increasing nutrient content in the pond. Feed and metabolic waste is the wellsprings of nutrient for phytoplankton growth (Arifin *et al.,* 2018). The increasing nutrients entering to the pond affect the phytoplankton composition and its density (Case *et al.,* 2008). Algae need nitrate as a source of nutrients that are absorbed from the water and therefore the concentration of nitrate in the water media was lower (Ebeling *et al.,* 2006). One of the most important groups in biofloc systems is phytoplankton, microalgae use ammonia and compounds as nitrite and nitrate for construction of proteins and they also provide oxygen during day time (Moss, 2002).

Microalgae are able to assimilate a variety of nitrogen sources from an aquatic environment, mainly nitrates (NO_3^-), ammonium (NH_4^+) and urea etc. NH_4^+-N is the most preferred nitrogen source for microalgae growth since it is in a reduced state and low energy is required for its metabolic uptake by microalgae (active transmission at the plasma membrane), which also makes it energetically very efficient source (Perez-Garcia *et al.,* 2011).

BFT systems depend on the living microorganisms in the biofloc (composed of organic matter and microbial biomass) maintained in the water column to aid in ammonia removal via phytoplankton, bacterial uptake and bacterial oxidation of ammonia-N (NH_3-N) to nitrite-N (NO_2-N) and then subsequent oxidation of NO_2-N to nitrate-N (NO_3-N) non-toxic by nitrification (Brune *et al*., 2003; Ebeling *et al.,* 2006 and Hargreaves, 2006). Therefore, these biological processes play a critical part in reducing ammonia and nitrite concentration to levels below from toxic or growth-limiting for cultured fish.

Fish is one of the most important aquatic organisms greatly affected by environmental pollution, this is reflected on function of organs. Tilapia is considered as one the most common genus of fish, widely spread throughout Africa, south America and the middle east. Fish *Oreochromis niloticus* is selected for our investigation because of its economic importance and representing a high percent of the total catch each year along the River Nile and the lakes. It is considered as a well marketable fish and has tolerance for a wide range of environmental condition and shows little susceptibility to diseases (Kadry *et al.,* 2015).

Hematological and histopathological studies are considered as direct display referring to any adverse impact on fish. The most important fish organs affected by water pollution are gills, kidney, liver and spleen (Tayel *et al.,* 2018). Blood components may be used as marker for tissue destruction, because, fish blood is sensitive to pollution-induced stress and certain changes in the blood profile may indicate changes in metabolism and biochemical processes of the organism. The increments in the ALT and AST level activities in serum could consider as indicators for liver damage (Ibrahim and Mahmoud, 2005). However, the increasing of the serum urea and creatinine may be ascribing to kidney disorders (Tayel *et al.,* 2014).

This study was aimed to treat the negative effect of increased ammonia on hematological parameters and histological structures *Oreochromis niloticus* fish using biofloc system. Phytoplankton community was considered as an indicator of toxic ammonia reduction to non-toxic nitrate

MATERIALS AND METHODS

Fish preparation and experiment design

The present study was carried out at the experimental fish farm, El-Serw Station for Fish Research, Inland Water branch, National Institute of Oceanography and Fisheries. A total of 270 fish weighting from 25.0 to 30.0 grams were transported to the laboratory. Fish were acclimatized to the lab conditions for about two weeks.

The experiment was carried out in glass aquaria having volume of 50 L each with water depth of 25 cm. The chemical compound used was ammonium chloride (NH_4Cl).

Determination of 96 hrs-LC_{50} of NH_3-H

A total number of 120 *Oreochromis niloticus* fish with average body weight 27.0 ± 3.0 grams were divided into 12 groups (10 fish/group). First group was left as a control, while, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th groups   were

exposed to 20.0, 30.0, 32.0, 34.0, 36.0, 38.0, 40.0, 42.0, 44.0, 46.0 and 48.0 mg/l of NH_3-H, respectively.

These concentrations were selected as trails for determination of 96 hrs-LC_{50} of NH_3-H concentration. Fish were observed at 12 hrs interval up to 96 hrs. The dead fish was removed immediately up on discovery. Mortalities and survival time were recorded, and then LC_{50} was calculated according to the equation of Behrens and Karper (1953). The calculated 96 hrs-LC_{50} of ammonia for *Oreochromis niloticus* was 38.4 mg/l.

Effect of sub-chronic toxicity of NH_3-H in *Oreochromis niloticus* fish:

A total number of 150 *Oreochromis niloticus* with average body weight 27.0 ± 3.0 grams were divided into 5 groups. Each group has 3 replicates (10 fishes / replicate). Ammonia was prepared to produce the required concentrations, 0.50 (19.2 mg/l NH_3-H) and 0.25 (9.6 mg/l NH_3-H) of 96 hrs-LC_{50} for fish.

The experiment was conducted using 0.50 and 0.25 of 96 hrs-LC_{50} of NH_3-H concentrations and control.

1- The first glass aquarium, control

2- The second glass aquarium, effect of sub-chronic toxicity of NH_3-H on *Oreochromis niloticus* fish, using 0.50 LC_{50} (19.2 mg/l NH_3-H).

3- The third glass aquarium, effect of sub-chronic toxicity of NH_3-H on *Oreochromis niloticus* fish, using 0.25 LC_{50} (9.6 mg/l NH_3-H).

4- The fourth glass aquarium, 0.50 LC_{50} with using biofloc system.

5- The fifth glass aquarium, 0.25 LC_{50} with using biofloc system.

Three replicate units were randomly assigned to each treatment.

Using the biofloc system for the treatment process by adding molasses as a carbon source to encourage the growth of phytoplankton and zooplankton used in treatment.

Using a small compressed air pump, the water in each aquarium was aerated to maintain the oxygen concentration from 7 to 8 mg/l, pH 7.5–8.0 and temperature ranged between 26 to 28°C throughout the experiment.

Phytoplankton identifications and enumerations

Drop technique was applied for counting and identification of phytoplankton species (APHA, 2012), triplicate samples (2 or 5 µl) were taken and examined under inverted microscope ZEISS IM 4738, with magnification power 40 and 100x. The results of phytoplankton density were presented as number of units per liter (units/l).

Phytoplankton identification was performed according to Prescott (1978); Lee (2008) and Bellinger and Sigee (2010).

Canonical Correlation Analysis:

The relationship between the phytoplankton community and environmental factors was analyzed by canonical correlation analysis (CCA) using Canoco for Windows 4.5 soft.

Fish Samples analysis:

Hematological studies:

Blood samples were taken by severance of the caudal peduncle of the studied fish and analyzed at the same day. The samples were collected in small sterilized vials and left to clot and then centrifuged at 3000 rpm for 10 minutes to obtain serum. Supernatant serum was obtained using micropipette (model Labystems K 33071) for biochemical studies. The biochemical analyses were measured using Kits according to the manufacture instructions. Serum glucose level was determined according to GOD-PAP enzymatic colorimetric method (Tietz, 1995), (Spectrum Kit). Serum total proteins level was estimated calorimetrically according to the method of Henry

(1974), (Biorexfars Kit). Serum albumin level was measured calorimetrically according to the procedure of Doumas *et al.* (1971), (Vitro Scient Kit). Serum total cholesterol level was determined according to CHOD-PAP enzymatic colorimetric method (Ellefson and Caraway, 1976), (Spectrum Kit). Serum triglycerides level was estimated according to GPO-PAP enzymatic colorimetric method (Stein, 1987), (Spectrum Kit). The activities of serum ALT and AST were estimated calorimetrically according to the method of Reitman and Frankel (1957), (Spectrum kit). Serum urea level was determined according to urease colorimetric method (Tietz, 1990), (Spectrum Kit). Serum uric acid level was estimated according to uricase-POP enzymatic colorimetric method with 4-amino-antipyrine (Tietz, 1990), (Spectrum Kit). Serum creatinine level was estimated according to a colorimetric method described by Henry (1974).

Histopathological studies:

Immediately after dissection of the studied fish, parts of kidney, liver and spleen were carefully removed and fixed in 10 % formalin at $4°C$, for 48 hours then the samples were dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned into five micrometers thick using Euromex Holland Microtome, and then stained according to Harris Hematoxylin and Eosin method. Finally, the sections were examined microscopically and photographed by a microscopic camera according the methods cited by Tayel *et al.* (2018).

RESULTS AND DISCUSSION

Acute Toxicity (Determination of 96 hrs-LC$_{50}$ of ammonia):

The 96 hrs-LC$_{50}$ of ammonia for *Oreochromis niloticus* fish have been calculated.

Tables (1&2) demonstrated the mortality of *Oreochromis niloticus* at different concentrations of ammonia. The results showed that the 96 hrs-LC$_{50}$ of ammonia was 38.4 mg/l.

Table 1: Preliminary trials for zero and hundred % mortalities in *Oreochromis niloticus* exposed to different concentrations of Ammonia (after 96 hrs).

number	Concentrations of ammonia (mg/l)	Mortality number during 96 hrs				Total mortality Number	Total mortality %
		1st day	2nd day	3rd day	4th day		
1	Control	0	0	0	0	0	0
2	20	0	0	0	0	0	0
3	30	0	0	0	0	0	
4	32	0	0	0	1	1	10
5	34	0	0	1	2	3	30
6	36	0	1	2	2	5	50
7	38	0	1	1	3	5	50
8	40	0	1	2	3	6	60
9	42	0	2	2	3	7	70
10	44	0	2	3	3	8	80
11	46	0	2	2	4	8	80
12	48	0	3	4	3	10	100

Table 2: Actual estimation of 96 hrs-LC$_{50}$ of ammonia for *Oreochromis niloticus*.

Number (N)	Concentrations mg/l	No. of dead fish at 96 hrs	A	b	Axb
2	20	0	0	0	0
3	30	0	10	0	0
4	32	1	2	0.5	1
5	34	3	2	2	4
6	36	5	2	4	8
7	38	5	2	5	10
8	40	6	2	5.5	11
9	42	7	2	6.5	13
10	44	8	2	7.5	15
11	46	8	2	8	16
12	48	10	2	9	18
		Σ axb			96

96 hrs-LC$_{50}$ = Highest dose - Σ axb/n
a= constant factor between two successive doses.
b= the mean of dead fish in each group.
Σ axb= sum of axb.
N= Number of fish in each groups
= 48 – 96/10
= 38.4 mg/l

In the present study ammonia decreased from 9.6 mg/l (0.25 LC$_{50}$) and 19.2 mg/l (0.50 LC$_{50}$) at the first experiment to 0.7 and 4.1 mg/l at the end of the biofloc experiment, this means that the biofloc system removed about 92.7% and 78.7% of ammonia, respectively. As a consequence, nitrite concentration decreased and nitrate concentration increased. Thus, toxic ammonia has been converted into non-toxic nitrate through ammonia oxidation to nitrite then to nitrate by biological factors in the biofloc system such as phytoplankton (Fig. 1). The results of this study revealed that the application of BFT system improved water quality by controlling ammonia level and increasing of phytoplankton. The mode of action of BFT system is claimed to be enhancement of organic matter degradation, nitrification and ammonia removal. Nitrifying bacteria in a pond will always respond to the availability of substrate.

Thus, if a pond has high ammonia levels, nitrifying bacteria will respond by multiplying rapidly and their abundance will decline when ammonia concentration decrease. Phytoplankton in the biofloc system improves water quality and production performance of fish culturing by enhancing nitrification and ammonia removal. This observation agrees with Shalaby (2011); Rapatsa and Moyo (2013) and Sanchez *et al.* (2018).
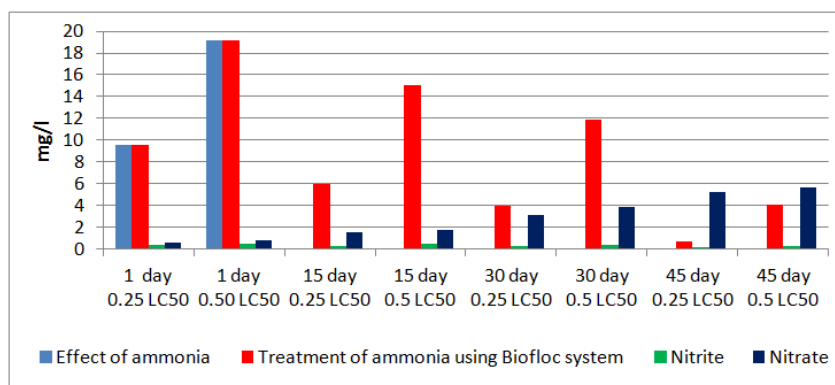

Fig. 1: Concentration of ammonia, nitrite, nitrate under effect of ammonia and biofloc system

Phytoplankton community structure:

Microscopic examination of phytoplankton in experienced BFT system, along the investigated period, revealed that 29 species were identified, and its taxonomical classification was belonging to 4 classes, related to Chlorophyceae, 15 sp., Bacillariophyceae, 6 sp., Cyanophyceae, 5 sp. and Euglenophyceae, 3 sp. (Table 3).

Table 3: Identified phytoplankton in the studied biofloc system

Groups	Species
Chlorophyceae	*Actinastrum hantzschii*
	Ankistrodesmus fractus
	Chlamydomonas angulosa
	Chlorella vulgaris
	Coelastrum microporum
	Elakatothrix gelatinosa
	Kirchneriella aperta
	Kirchneriella obese
	Monactinus simplex
	Mucidosphaerium pulchellum
	Nephrocytium limneticum
	Oocystis pyriformis
	Scenedesmus quadricauda
	Tetraëdron minimum
	Tetraëdron trigonum
Bacillariophyceae	*Aulacoseira granulate*
	Ceratoneis closterium
	Cyclotella meneghiniana
	Fragilaria capucina var. vaucheriae
	Lyrella lyra
	Nitzschia acicularis
Cyanophyceae	*Arthrospira jenneri*
	Chroococcus minor
	Coelosphaerium dubium
	Gloeocapsa punctuate
	Phormidium inundatum
Euglenophyceae	*Lepocinclis acus*
	Phacus chloroplasts
	Trachelomonas hispida

Average of the class composition ratio cleared that Chlorophyceae was the most predominated, forming about 69% and 88% in 0.50 LC_{50} and 0.25 LC_{50} ammonia, respectively from the total phytoplankton density, followed by Bacillariophyceae, representing about 22.1% in 0.50 LC_{50}. While in 0.25 LC_{50} revealed Euglenophyceae in the second order which recorded 4.7% (Fig. 3). Zita and Hermansson (1994) and Arifin *et al.* (2018) showed Chlorophyceae and Bacillariophyceae are the dominant phytoplankton classes in BFT treatments. Schrader *et al.* (2011) mentions that phytoplanktonic composition in an outdoor freshwater pond of catfish, was dominated by green algae, diatoms and cyanobacteria. The population density of phytoplankton in experienced BFT system had examined first day, after 30 days and after 45 days. The highest phytoplankton density of 226×10^5 unit/l and 258×10^5 unit/l in 0.50 LC_{50} and 0.25 LC_{50}, respectively was occurred after 45 days, and the least count was 74×10^5 unit/l and 73×10^5 unit/l in 0.50 LC_{50} and 0.25 LC_{50}, respectively that was recorded in the first day (Fig. 2).
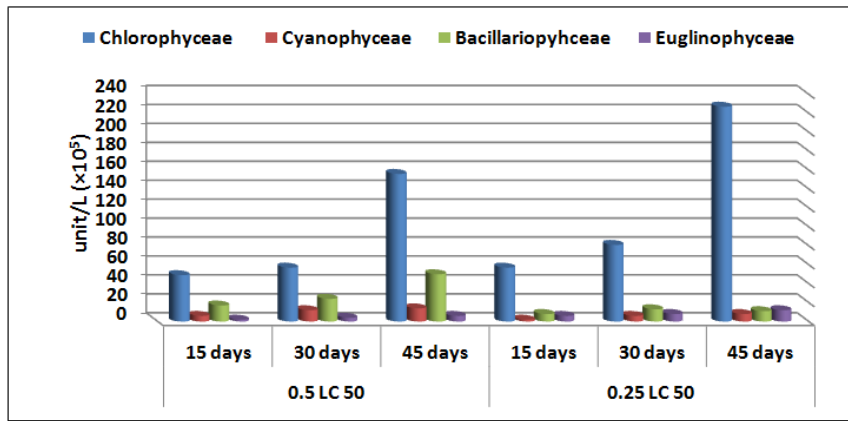
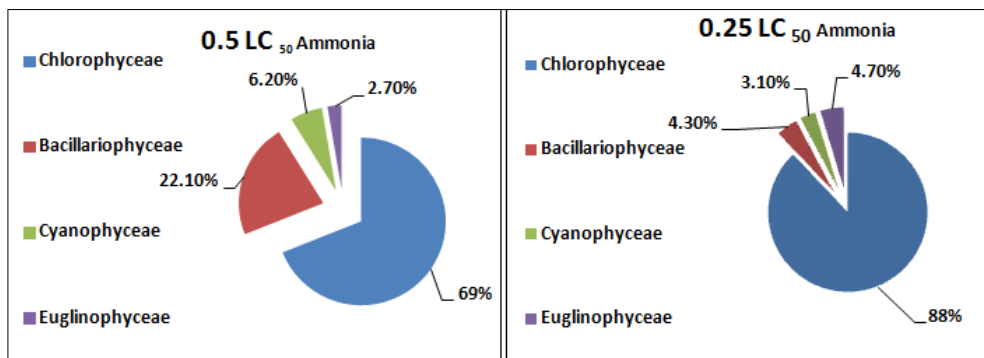Fig. 2: Phytoplankton density in the biofloc system with two concentrations of ammonia.

Fig. 3: phytoplankton class composition ratio in the studied BFT system Canonical Correlation Analysis

Distribution and abundance of the most common species of phytoplankton

Systematic classification of Phytoplankton in BFT system during the investigated period revealed that members of phytoplankton (29 sp.) were manly dominated with *Arthrospira jenneri, Chlorella vulgaris, Chroococcus minor, Coelosphaerium dubium, Gloeocapsa punctata, Phacus chloroplasts, Phormidium inundatum* and *Scenedesmus quadricauda* (Table 4).

Table 4: Densities and abundances of the most leading species of phytoplankton in the studied BFT system

Dominant species	Count (No. of units $\times 10^5$/l)					
	0.5 LC$_{50}$			0.25 LC$_{50}$		
	15 day	30 day	45day	15 day	30 day	45 day
Arthrospira jenneri	2	2	10	2	4	0
Chlorella vulgaris	31	37	89	35	56	180
Chroococcus minor	4	7	2	2	2	2
Coelosphaerium dubium	5	2	13	0	0	2
Gloeocapsa punctata	4	4	8	2	2	0
Phacus chloroplastes	2	4	4	0	0	10
Phormidium inundatum	2	9	17	2	5	7
Scenedesmus quadricauda	10	12	16	14	21	23

Chlorella vulgaris was the most common species of phytoplankton in BFT system which revealed in the final of experiment 39.4% and 69.8% in 0.50 LC$_{50}$ and 0.25 LC$_{50}$ treatments, respectively, followed with *Phormidium inundatum* and *Scenedesmus quadricauda* in 0.50 LC$_{50}$ (7.5% and 7.1%, respectively), while in 0.25 LC$_{50}$, represented *Scenedesmus quadricauda* and *Phacus chloroplasts* as second and third predominant species of phytoplankton (8.9% and 3.9%, respectively).

These results were in agreement with the results of Shawky *et al.* (2015) who revealed that green microalga *Scenedesmus quadricauda* reaching a maximum removal percentage of 1 mg/l ammonia to about 100% after 4 days; while, at 5 mg/l ammonia concentrations, the maximum removal percentage reached 91.8% after 4 days.

Su *et al.* (2012) revealed that removal efficiency of total nitrogen (26.5 mg/l) reached 100% using *Phormidium sp.* and *Chlamydomonas Reinhardtii* after 4 days, respectively. Kim *et al.* (2010, 2013); Choi and Lee (2013) and Zhu *et al.* (2013) studied the removal efficiency of *Chlorella* sp. using different concentrations of ammonia and they revealed that it was able to remove from 64 to 100% ammonia after few days.

Canonical Correspondence Analysis (CCA) is carried out for analyzing 3 environmental variables (NO_2, NO_3 and NH_4) and 8 dominant phytoplankton species (mentioned in table 4) in each of 0.50 LC_{50} and 0.25 LC_{50}, as in Figs. 4 and 5.

Most phytoplankton species showed centroid distribution revealing accumulative affect of different environmental variables such *Chlorella vulgaris, Scenedesmus quadricauda* and *Gloeocapsa punctata*in Figure 4, and *Chlorella vulgaris, Scenedesmus quadricauda, Chroococcus minor* and *Phormidium inundatum* and other species around the center in Figure 5.
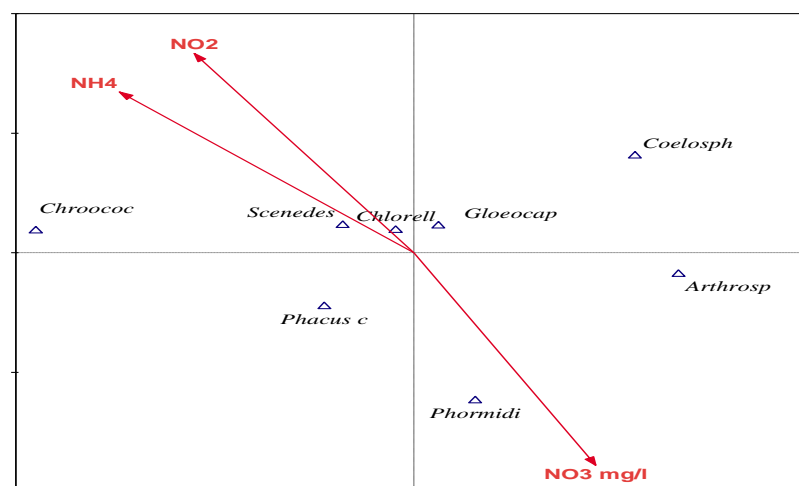

Fig. 4: CCA of phytoplankton species with the environmental parameters at BFT system at 0.50 LC_{50} ammonia concentration .
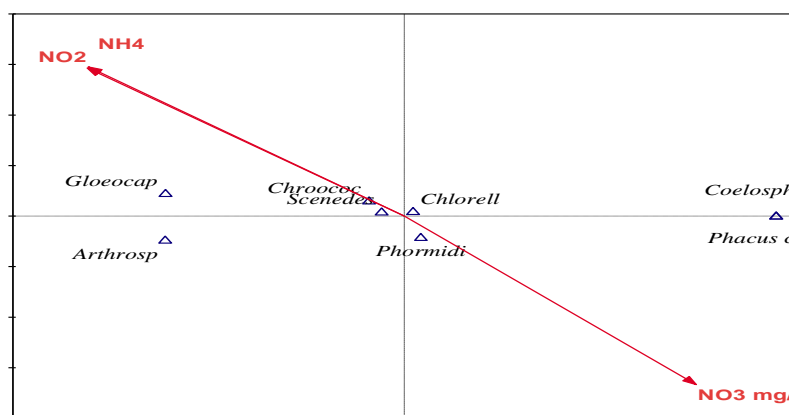

Fig. 5: CCA of phytoplankton species with the environmental parameters at BFT system at 0.25 LC_{50} ammonia concentration

Effect of ammonia and biofloc system on the hematology and histology of *Oreochromis niloticus* fish:

Hematology:

The results of serum glucose, protein, albumin, cholesterol, triglycerides, ALT and AST, urea, uric acid and createnine levels, obtained from experimental fish illustrated in figures (6-15).

The obtained glucose values increased from 125 mg/dI at first experiment affected by 0.25 LC_{50} ammonia to 293 mg/dI at end of experiment affected by 0.50 LC_{50} Ammonia, while the values decreased from 120 to 98 and from 130 to 110 mg/dl affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia and biofloc. In control group glucose value was 88 mg/dI (Fig.6). Total protein values ranged from 3.7 to 4.6 g/dI at the beginning of the experiment to 5.2 and 6.3 g/dI at end of experiment affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia, while value decreased from 3.9 to 3.6 and from 4.3 to 3.8 g/dl in fish affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia and biofloc. In control group, protein level was 3.5 g/dl (Fig. 7). Albumin values decreased from 1.8 g/dI at to 1.1g/dI in fish affected by 0.25 LC_{50} and 0.50 LC_{50} (ammonia), while value increased from 1.6 to 1.8 `g/dl in fish affected by 0.50 LC_{50} ammonia and biofloc. In control group, albumin value was 1.9 g/dl (Fig. 8). Cholesterol values increased from 81mg/dI to 189 mg/dI at in fish affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia, while the value ranged from 85 to 101 g/dI affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia and biofloc respectively. In control group cholesterol value was 78 mg/dl (Fig. 9). Triglycerides values ranged from 120 and 135 mg/dI to 187 and 258 mg/dl in fish affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia, while values decreased from 117 and 130 to 104 and 115 mg/dl in fish affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia and biofloc. In control group, triglycerides value was 100 mg/dl (Fig. 10). AST values ranged from 58.6 to 116 IU/ml at the beginning of the experiment to 183 and 275 IU/ml at end of experiment in fish affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia, while AST values decreased from 55.6 and 113 IU/ml to 40 and 28.7 IU/ml at the end experimental in fish affected by 0.25 LC_{50} and 0.50 LC_{50} ammonia and biofloc. In control group, AST value was 35 IU/ml (Fig. 11). ALT values elevated from 29.3 IU/ml at the beginning of the experiment affected by 0.25 LC_{50} ammonia to 68 IU/ml at end of experiment affected by 0.50 LC_{50} ammonia, while in fish affected by ammonia and biofloc, ALT values ranged from 24.2 to 33.3 IU/ml. In control group ALT value was 20 IU/ml (fig. 12). Urea values increased from 38 mg/dI at the beginning of the experiment affected by 0.25 LC_{50} ammonia to 85 mg/dI at end of experiment affected by 0.50 LC_{50} ammonia, while values ranged from 38 to 26.5 mg/dl in fish affected by ammonia and biofloc. In control group urea value was 25.0 mg/dl (Fig. 13). Uric acid values ranged from 4.8 mg/dI at the beginning of the experiment affected by 0.25 LC_{50} ammonia to 14.5 mg/dI at end of experiment affected by 0.50 LC_{50} (ammonia), while the values ranged from 4.2 to 5.3 mg/dI in fish affected by ammonia and biofloc. In control group uric acid value was 4 mg/dl (Fig. 14). Creatinine values decreased from 0.55 mg/dI at the beginning of the experiment affected by 0.25 LC_{50} ammonia to 0.25 mg/dI at end of experiment affected by 0.50 LC_{50} (ammonia), while values ranged from 0.50 to 0.65 mg/dI in fish affected by ammonia and biofloc. In control group creatinine value was 0.70 mg/dl (Fig. 15). Nowadays cultured Nile Tilapia suffering from several problems such as excess ammonia which is toxic to fish leads to several hematological and histological alterations in cultured fish.

Hematology is important in monitoring physiological and pathological changes in fish. Blood parameters vary in different fish species and this reflects

adaptation to the varied environment conditions (Adeyemo, 2007). A change in the mentioned hematological parameters in present syudy indicates worsening of the organism's health status.It has been claimed by the proponents of BFT system that it enhances the fish immune system. Fish in organically manured ponds are pron to infections by several pathogens. Application of BFT was supposed to boost the fish immunity. In this study, what measured parameters which verified this statement, this noticfication also recorded by Ali *et al*. (2011) and Hanna *et al*. (2014).
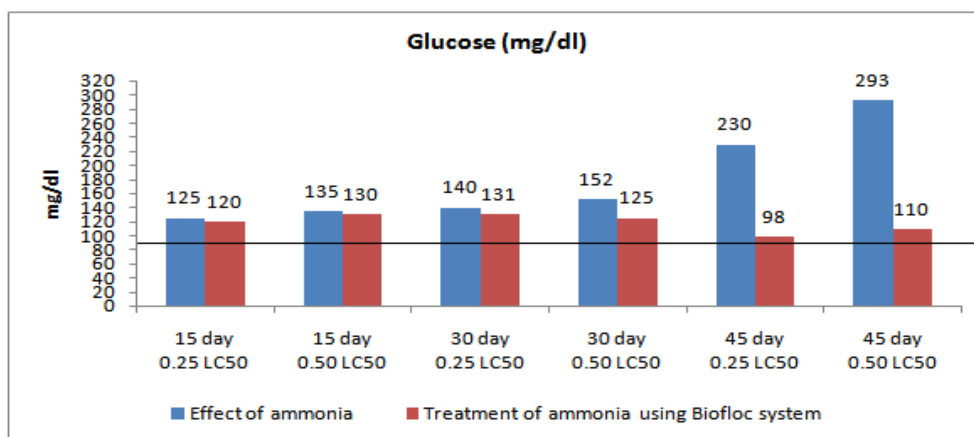
Fig. 6: Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on glucose f *Oreochromis niloticus*, (— Black line) control.
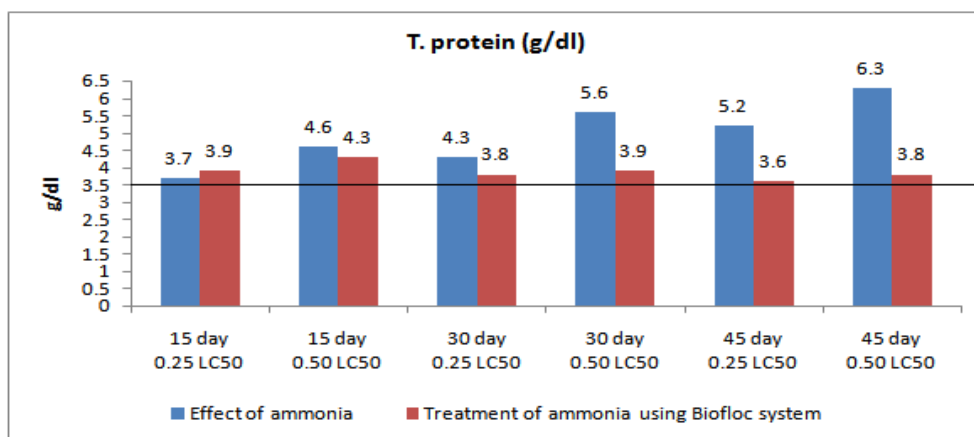

Fig. 7. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on total protein levels of *Oreochromis niloticus*, (——— Black line) control.
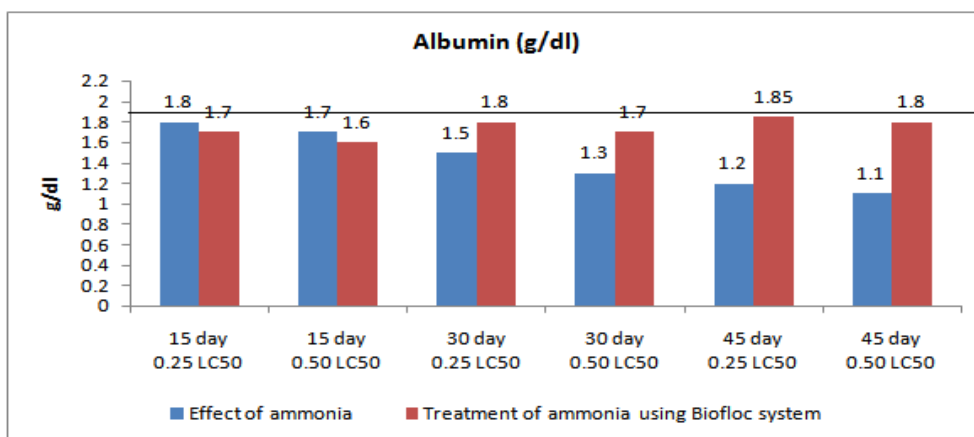

Fig. 8. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on albumin levels of *Oreochromis niloticus*, (——— Black line) control.
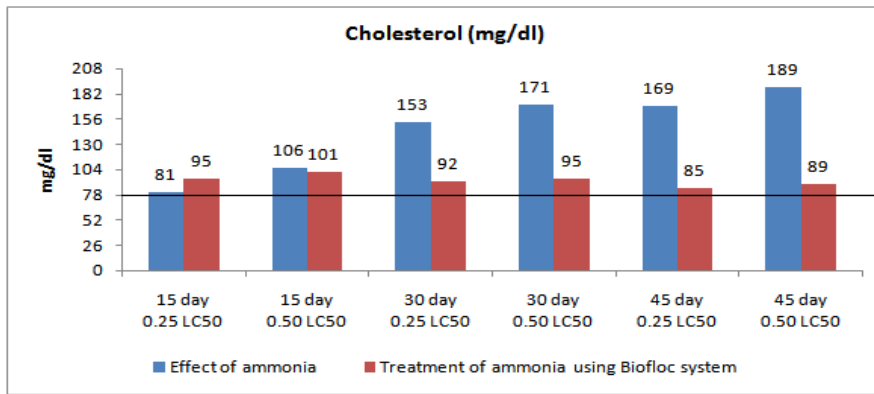
Fig. 9. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on cholesterol levels of *Oreochromis niloticus*, (—— Black line) control.
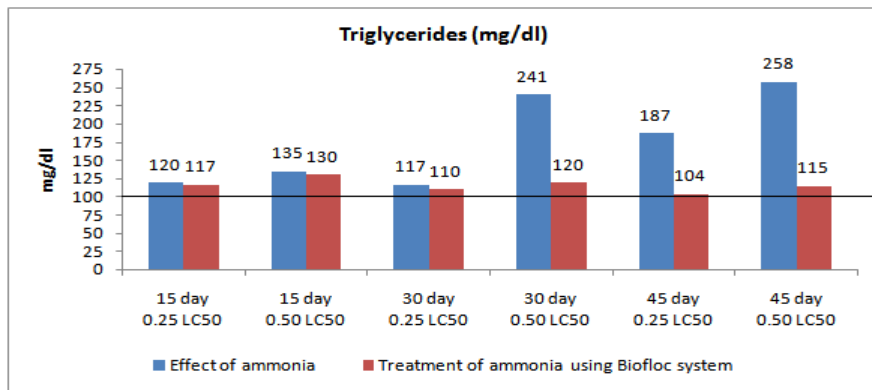

Fig. 10. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia on triglycerides levels of *Oreochromis niloticus*, (—— Black line) control.
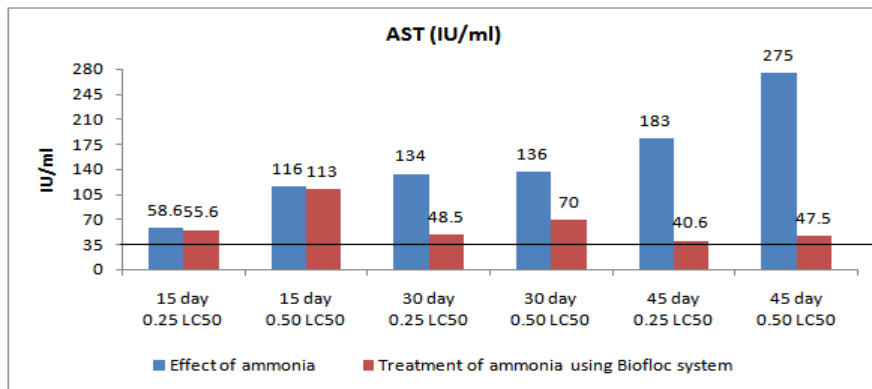

Fig. 11. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on AST activities of *Oreochromis niloticus*, (—— Black line) control.
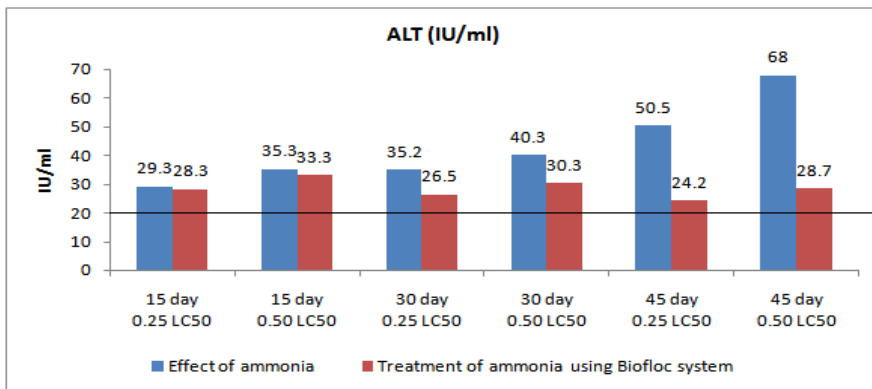

Fig. 12. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on ALT activities of *Oreochromis niloticus*, (—— Black line) control.
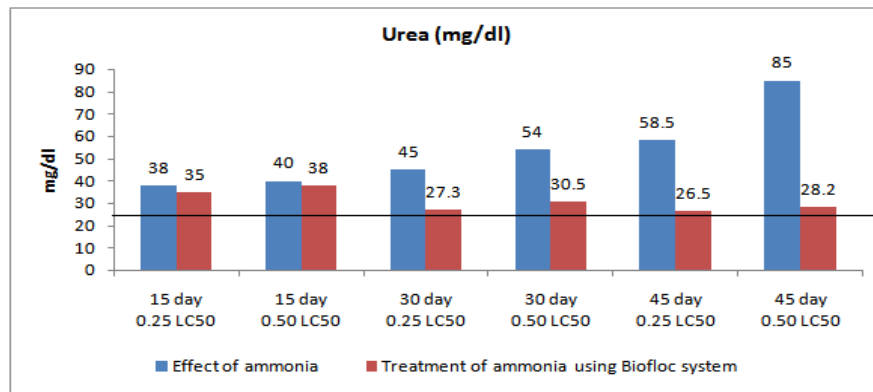
Fig. 13. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on urea levels *Oreochromis niloticus*, (——— Black line) control.
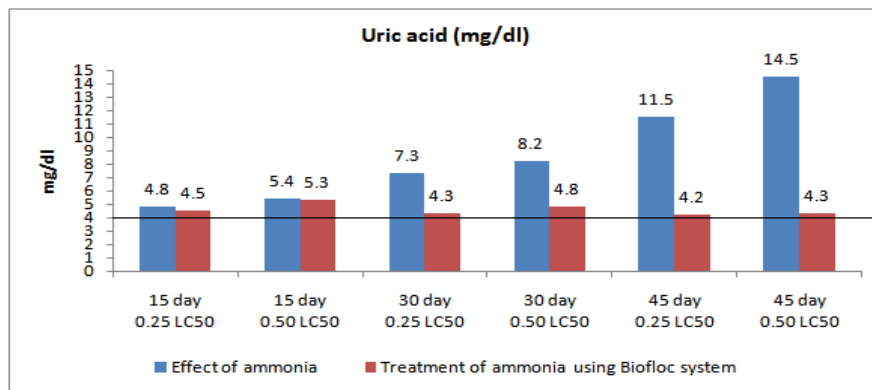

Fig. 14. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on uric acid levels of *Oreochromis niloticus*, (——— Black line) control.
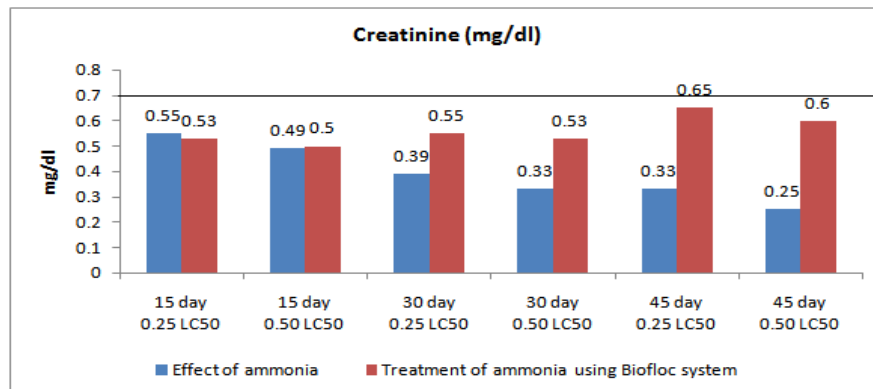

Fig. 15. Effect of ammonia and biofloc system (0.25 and 0.50 LC_{50} of ammonia) on creatinine levels of *Oreochromis niloticus*, (——— Black line) control.

Histology:

Several histopathological alterations were observed in kidneys, liver and spleen of *Oreochromis niloticus* exposed to ammonia, while the control and treated groups appeared nearly normal.

As observed in Figure (16), Kidney tubules and Malpigian corpuscles in control (Fig. 16a) and treated (Figs. 16 d&e) groups showed normal appearance, while the group affected by ammonia (0.25 LC_{50} and 0.50 LC_{50}) (Figs. 16 b&c) showed degeneration and sever degeneration and necrosis, respectivity. In figure (17), the liver section of control group fish showed Hepatic cells are near normal (Fig. 17a). In ammonia affected group (0.25 LC_{50}), the liver showed degeneration, necrosis and condesion in blood sinusoid (Fig. 17b), while the group affected by 0.50 LC_{50} ammonia the liver suffer from severe fatty degeneration and piknosis (Fig. 17c). Fish

group treated by biofloc with 0.25 LC$_{50}$ ammonia, the liver showed improvement in hepatic cells with rare piknosis (Fig. 17d). However, fish that treated by 0.50 LC$_{50}$ and biofloc system cleared slightly improvement in liver cells with piknosis (Fig. 17e).
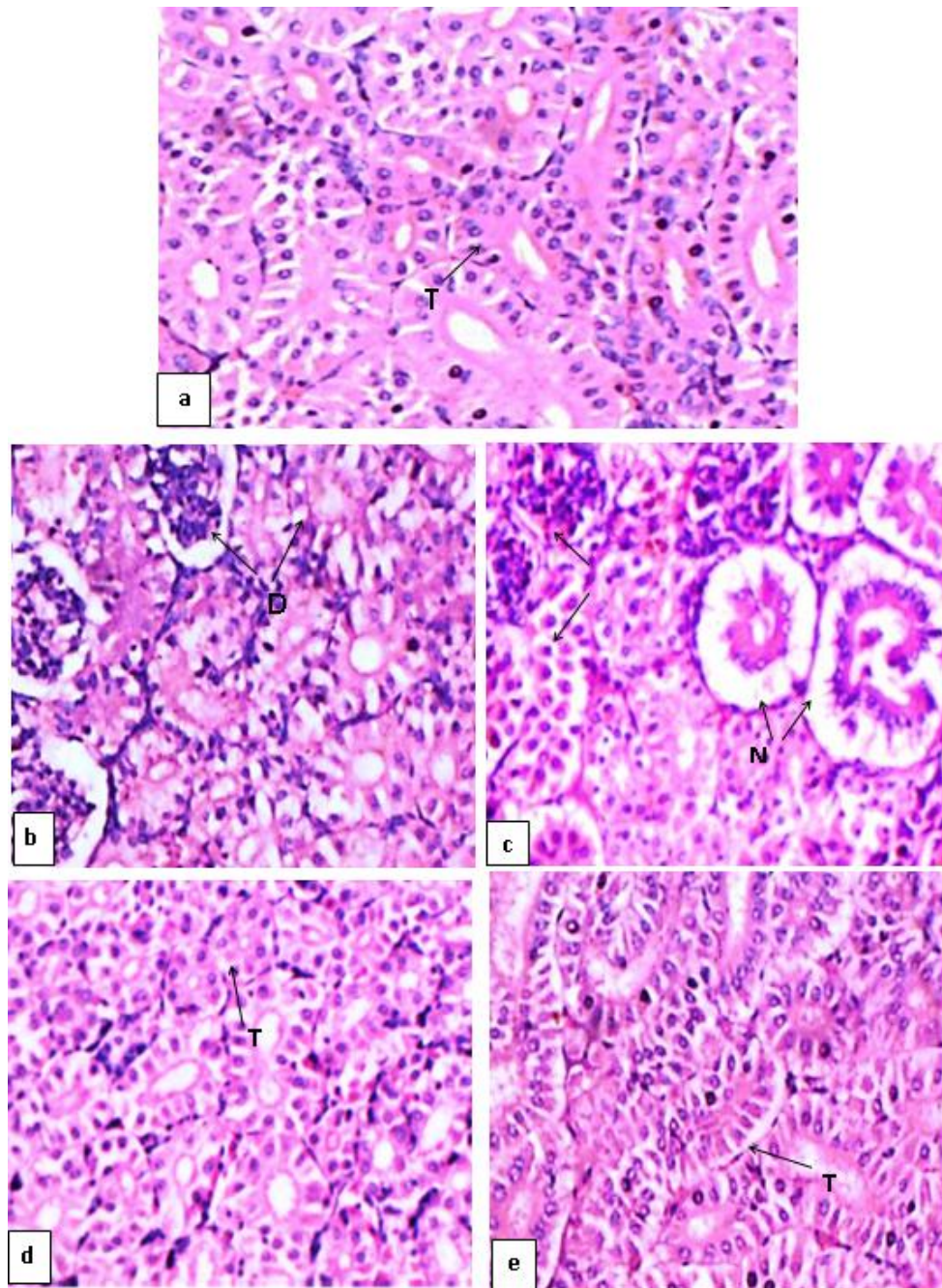

Fig. 16: Showing kidney section; (a): For controled fish showing: Kidney tubules (T) appear nearly as normal structure. (b): For fish affected by 0.25 LC$_{50}$ ammonia showing: Degeneration (D) in Kidney tubules and Malpigian curpacels. (c): For fish affected by 0.50 LC$_{50}$ ammonia showing: Severe degeneration (D) and necrosis (N) in Kidney tubules and Malpigian curpacels. (d): For fish treated by 0.25 LC$_{50}$ Biofloc system showing improvement in Kidney tubules.(e): For fish treated by 0.50 Biofloc system showing slightly improvement in Kidney tubules. HandE.
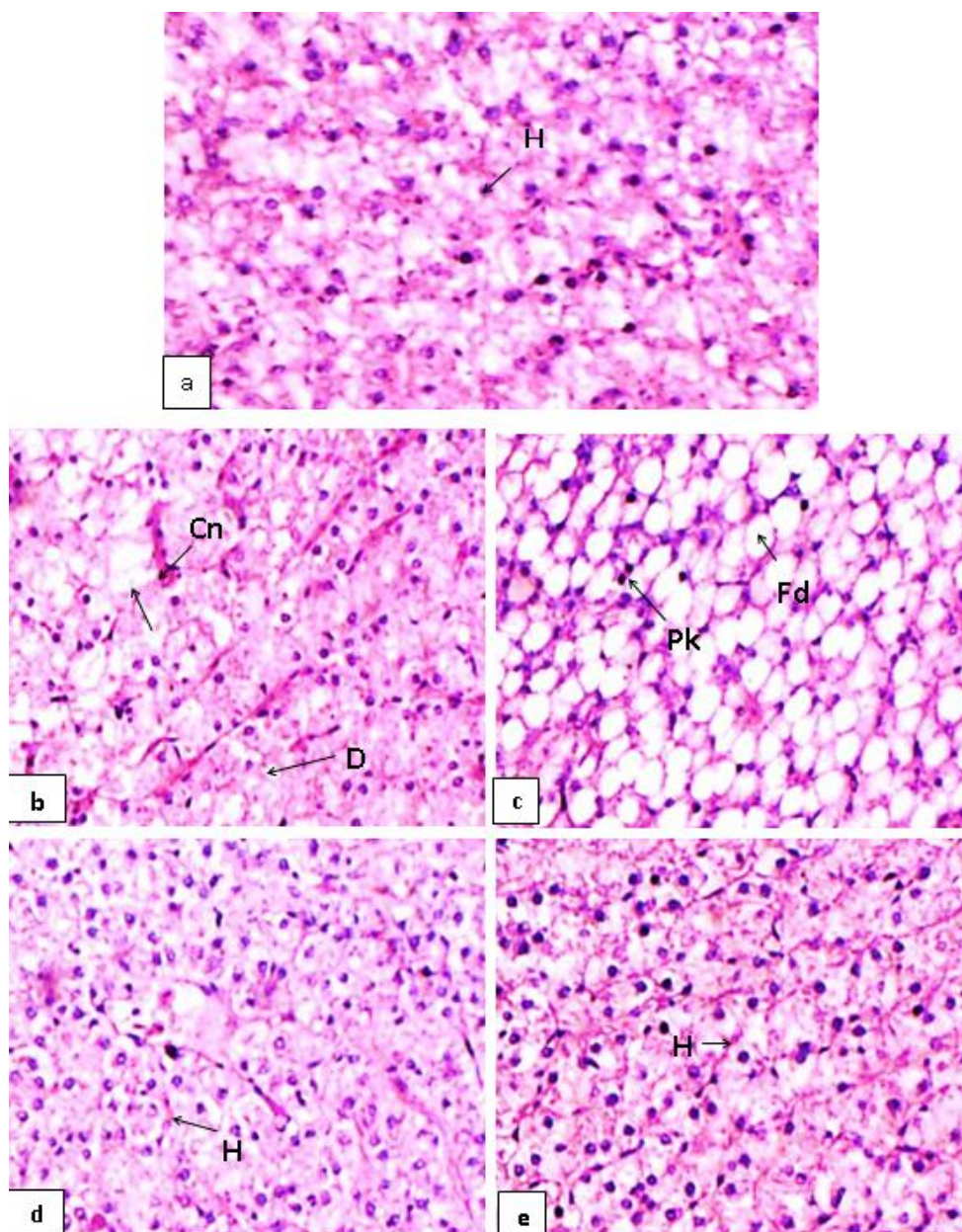
Fig. 17: Showing liver section; (a): For controled fish showing: Hepatic cells are near normal (H). (b): For fish affected by 0.25 LC_{50} ammonia showing degeneration (D), necrosis (N) and Congesion in blood sinusoid (Cn). (c): For fish affected by 0.50 LC_{50} ammonia showing: Severe fatty degeneration (Fd) and Piknosis (Pk) in liver cell nucleus. (d): For fish treated by 0.25 Biofloc system showing improvement in liver cells (H).(e): For fish treated by 0.50 Biofloc system showing slightly improvement in liver cells (H). H&E.

The spleenic tissue in control fish appeared relatively normal (Fig. 18a). There were necrosis, fibrosis, hemorrahge and hemosidrin in the spleen of fish exposed to 0.25 LC_{50} ammonia Fig. 18, while the group affected by 0.50 LC_{50} ammonia showed sever hemosidrin. While, slightly improvement was observed in most of fish treated group (Fig. 18 d&e) as well as red and white bulp were become more improved in 0.25 LC_{50} ammonia and biofloc system.

Kidney is a vital organ in the body and maintains the homeostasis in fish. It is not only involved in removal of wastes from blood but it is also responsible for selective re-absorption, which help in maintaining pH, volume of blood, erythropoiesis and body fluids. Kidney is one of the first organs affected by

contaminants in the water (Tayel *et al.*, 2014 and bayomy *et al.*, 2017).

Liver in fish is responsible of the storage glucose, digestion and filtration. Also, produce many enzymes that are stored in the gall bladder. These enzymes help in the breakdown of food. Generally, it is considered as the principal organ of absorption and detoxification in vertebrates and particularly in fish (Yacoub *et al.*, 2008 and Tayel *et al.*, 2018).
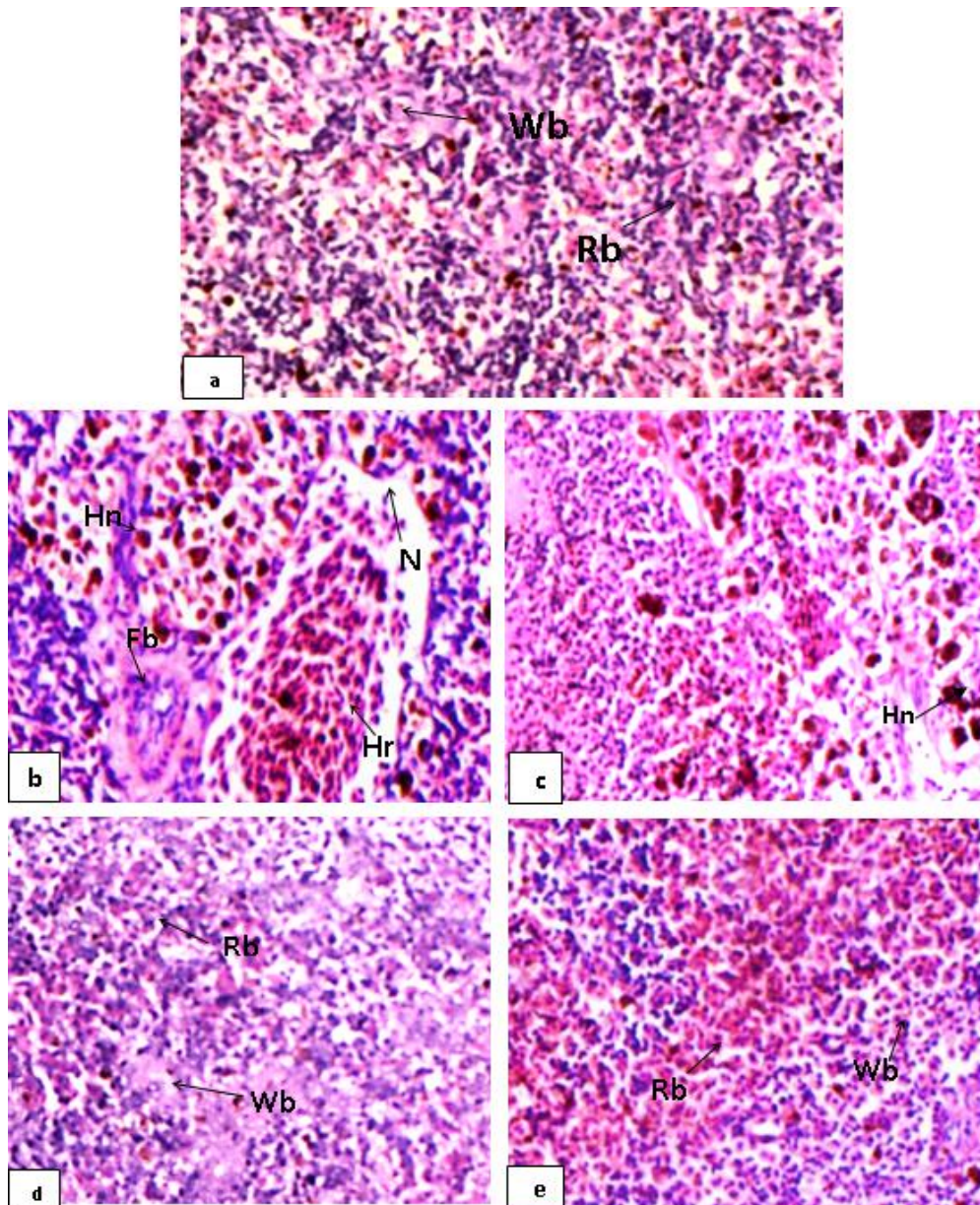

Fig. 18: Showing spleen section; (a): For controled fish showing: Normal in Red (RB) and White (WB) bulb. (b): For fish affected by 0.25 LC$_{50}$ ammonia showing: Necrosis (N), fibrosis (Fb) hemorrahge (Hr) and hemosidrin (Hn) in spleenic tissue. (c): For fish affected by 0.50 LC$_{50}$ ammonia showing: Severe hemosidrin (Hn) in spleenic tissue. (d): For fish treated by 0.25 LC$_{50}$ Biofloc system showing improvement in spleenic tissues (Red (RB) and White (WB) bulb). (e): For fish treated by 0.50 Biofloc system showing slightly improvement in spleenic tissues(Red (RB) and White (WB) bulb). H&E

The Spleen of fish is a hematopoietic tissue which forms the red blood cells and found as a small red mass. The spleen function is the filtration of blood, producing and storage of red blood cells, removing old and abnormal erythrocytes and producing

antibody against blood born antigens (Saad *et al.,* 2011).

The lesions that observed in kidney, liver and spleen of studed fish were degeneration, necrosis, piknosis, congesion hemorrhage, hemosidrin. Although these lesions were not fatal but every of them can be an indicator of a disorder in fish body or farm pond. In intense degeneration, it may lead to fatty degeneration , necrosis and piknosis. Also, hemorrhage and hemosidrin could represent by blood vessel disorders. These observations observation agree with that recorded by Younes *et al*. (2015) and Abu-Elala *et al.* (2016).

In conclusion, the present study indicates that the alterations that observed in the hematological parameters and histological structures in studied fish are mainly related to interactions between ammonia intoxication and biofloc introduced into fish ponds.

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ARABIC SUMMARY

المعالجة البيولوجية للأمونيا بإستخدام نظام البيوفلوك لأسماك البلطى النيلى

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المعهد القومى لعلوم البحار والمصايد (مصر)

تهدف هذه الدراسة إلى تقييم تأثير تطبيق نظام الكتلة الحيوية (البيوفلوك) على جودة المياه وأداء إنتاج أسماك البلطي النيلي باستخدام تركيزات مختلفة من الأمونيا ومحاولة علاج الأمونيا الزائدة بواسطة نظام الكتلة الحيوية فى وجود درجة حموضة والأكسجين المذاب ودرجة حرارة ثابتة. تمت مراقبة تحسين وتنظيم مجتمعات العوالق النباتية في كل حالة. وقد تمت دراسة التغيرات الدموية والنسيجية فى الأسماك محل الدراسة. سيطرت طائفة الطحالب الخضراء على مجتمعات العوالق النباتية التي ظهرت في هذه الدراسة ، تليها طوائف الطحالب الخضراء المزرقة والدياتومات والطحالب اليوجلينية.

تم تقسيم الأسماك التى تمت عليها الدراسة الى خمس مجموعات. المجموعة الأولى كعنصر تحكم (كنترول)، المجموعتين الثانية والثالثة بإضافة تركيزات ربع ونصف الجرعة المميتة من الأمونيا. والمجموعتين الرابعة والخامسة بإضافة تركيزات ربع ونصف الجرعة المميتة من الأمونيا وتحت تأثير نظام الكتلة الحيوية (البيوفلوك). أظهرت النتائج أن العوالق النباتية المستخدمة في نظام الكتلة الحيوية قامت بتحويل الأمونيا تدريجياً إلى نترات. كشفت الدراسات الدموية أن تحاليل الدم التي تم الحصول عليها من الأسماك المستزرعة فى نظام البيوفلوك كانت قريبة جدًا من تلك الموجودة في عنصر التحكم (الكنترول). كما كشفت الدراسات النسيجية أن الأسماك المستزرعة في ظل نظام الكتلة الحيوية (البيوفلوك) تبدو أنها تعافت تقريبًا وتشبه تلك التي تم الحصول عليها من الكنترول. في الختام تعمل العوالق النباتية في نظام الكتلة الحيوية (البيوفلوك) على تحسين جودة المياه وأداء إنتاج الأسماك المستزرعة من خلال عملية النترتة وإزالة وتحويل الأمونيا السامة الى نيترات غير سامة.