Effect of freshwater toxic and non toxic cyanobacteria, (*Microcystis aeruginosa*) strains on some biochemical parameters of *Oreochromis niloticus*

Mohamed S. Marzouk[1], Mohamed Mostafa[1], Nabil A. Ibrahim[2], Frances R. Pick[3], and Mahmoud S. Sharaf[1]

1- Fish diseases and management Faculty of Veterinary Medicine, Cairo University, Egypt.
2- WorldFish Center, Regional Center for Africa and West Asia, Abbassa, Abou-Hammad,Sharkia, Egypt.
3- Biology Dept. Faculty of Science, University of Ottawa, Canada

ABSTRACT

Thirty six all male *Oreochromis niloticus* were divided into two groups, using six glass aquaria. The treated group was fed on a toxic strain of *M. aeruginosa*, while the control group was fed on a non toxic strain of *M. aeruginosa,* in order to measure their grazing rates from both strains of *M. aeruginosa* and consequently its effect on general fish health represented by, clinical signs, hematological changes and biochemical parameters. Grazing rates and daily intake of *M. aeruginosa* showed close grazing rates between the treated and the control groups with slightly higher intake of non toxic strain than the toxic one were the individual intake was 63,050 and 62,567 cell/ml/fish respectively. Clinical signs in *O. niloticus* associated with exposure to toxic cyanobacteria were investigated. The observed clinical signs were sluggish movement and reduced reflexes during the late stages of exposure period. Plasma glucose, cortisol, T4, creatinine and AST showed higher concentrations in the treated group than that in the control, indicating on increased fish stress that was reflected in its health state and consequently its performance. On the opposite side, plasma total protein concentration was higher in the control than that in treated group. The hematocrit, plasma albumin, LDH and Alkaline phosphatase concentrations did not show any significant difference in both groups. The study concluded that the uptake of toxic strain of *M. aeruginosa* via oral route had deleterious effects on some clinical and biochemical parameters of the blood of Nile tilapia which led finally affected the fish health. The study recommendations include prevention and monitoring of organic and inorganic pollution that promote the harmful algal blooms, and take the early preventive measures to avoid toxic effects of cyanobacteria on fish and consequently on humans.

Keywords: *Microcystis aeruginosa,* cyanobacteria, Nile tilapia, fish health, biochemical parameters

INTRODUCTION

Eutrophication of freshwater lakes and reservoirs leads to water blooms of cyanobacteria in many countries of the world as cyanobacteria constitute up to 70% of the total phytoplankton biomass (Araoz *et al.*, 2010).

The ingestion of intact cells as well as the cyclic peptide toxins (microcystins) can cause illness and death in wild, domestic animals and humans. It is well known that species within certain genera of cyanobacteria, namely, *Anabaena*, *Hapalosiphon*, *Microcystis*, *Nostoc*, and *Oscillatoria* produce cyclic peptide hepatotoxins called microcystins (MCs) that cause liver damage in both mammals and fish through inhibition of protein phosphatase types 1 and 2A. MCs also can be accumulated in the

bodies of these organisms thus constitutes a risk for human being (Carmichael and Falconer, 1993).

In aquatic systems, fish stand at the top of the aquatic food chain, and are possibly affected by exposure to toxic cyanobacteria. Many fish species are imperiled due to eutrophication (Dodds *et al.*, 2009).

M. aeruginosa is the most common toxic cyanobacterium found worldwide, producers of MCs. MCs derive their toxicity from inhibition of protein phosphatases, especially types 1 and 2A, in a manner similar to that of the toxin causing diarrheic shellfish poisoning and okadaic acid (Krishnamurthy *et al.*, 1986).

Barros *et al.* (2010) reported that 10 aquaculture production systems were sampled and found to have MCs-LR concentrations that were highly correlated with *M. aeruginosa* biomass so that most of MCs-LR was produced by this species. The MCs-LR concentrations and *M. aeruginosa* counting were positively correlated with nitrogen-to-phosphorus ratios and suggest that water quality parameters may affect both *M. aeruginosa* biomass and MCs-LR concentrations.

Grosse *et al.* (2006) stated that MC is an endotoxin released when the MCs producing organism dies or is destroyed (In the stomach or after water treatment).

Nyakairu *et al.* (2010) mentioned that both *Oreochromis niloticus* and *Lates niloticus* harvested from L. Mburo and Murchison Bay of L. Victoria and sold for local human consumption and for export were analyzed for MCs in their flesh. The results revealed that the presence of MCs in fish muscle indicated their contamination, which could be a risk of transferring such toxins to humans who are at the end of the food chain. Since the MCs are heat stable, there is a high possibility of toxicity even after heat treatment of fish tissue.

This study was planned to determine the grazing rate of *O. niloticus* from both toxic and non toxic strains of *Microcystis aeruginosa* with its effect on fish health through study of some clinical signs, hematological and biochemical parameters.

MATERIALS AND METHODS

The present study was conducted in the Department of Fish diseases and Management, Faculty of Veterinary Medicine, Cairo University, and the Center of Advanced Research in Environmental Genomics, Faculty of Science, University of Ottawa, Canada, with the collaboration of the WorldFish center Abbassa, Abou Hammad – Sharkia - Egypt

Thirty six all male apparently healthy *Oreochromis niloticus* with an average body weight of 150 $\pm$ 20 g were acclimatized in glass aquaria for two weeks, prior to the experiments. Fish were fed twice daily on a commercial balanced diet and fasted 2 days before start of the experiment according to Dong *et al.* (2009).

Six Glass aquaria (25x50x30 cm) containing dechlorinated tap water were used where six fish were kept in each glass aquarium. The aquaria were cleaned and water was changed every other day for the treated and control groups. Fresh *M. aeruginosa* culture was kept to ensure a constant concentration in the water. Each aquarium was supplied with an electric air pump, where dissolved oxygen was maintained at not less than 8 mg/l, pH ranged from 7.5 to 8 by regular water change. Water temperature was adjusted at 25 ± 2 ºC.

Glass aquaria were kept in incubator to ensure constant water temperature as well as light intensity of 2000 lux (12 h/ 12 h light dark cycle) and humidity of 60-80%.

***Microcystis areuginosa* culture**:

Two strains of *M. aeruginosa* (toxic strain UTCC300 and non-toxic strain (UTCC632) were grown on Sterile-filtered Cyanobacteria BG-11 Freshwater Solution medium (Table 1).

Table 1: Composition of BG-11 cyanobacterium medium.

Salt name	Concentration
calcium chloride dihydrate (mg/l)	36.7
citric acid (mg/l)	5.6
Dipotassium hydrogen phosphate (mg/l)	31.4
Disodium magnesium EDTA (mg/l)	1
Ferric ammonium citrate (mg/l)	6
Magnesium sulfate (mg/l)	36
Sodium carbonate (mg/l)	20
Sodium nitrate (g/l)	1.5

Instruments and chemicals for *M. aeruginosa* counting:

Inverted microscope model Zeiss AXIO A1 equipped with an Empix camera and an Eclipse image analysis system, with the use of Paraformaldehyde Solution (10%) for Picoplankton and Cyanobacteria for fixation of *M. aeruginosa*. (Bitterlich, 1985).

Haematological examinations and biochemical analysis:

Haematological parameters were measured according to Caldwell *et al.* (2006), while plasma glucose was measured according to Young *et al.* (1975), plasma Cortisol according to Kaye and Crapo (1990), thyroxine hormone (T4) according to Schuurs and Van Weeman (1977), plasma AST, plasma alkaline phosphatase, plasma lactate dehydrogenase (LDH-L) and plasma Creatinine according to Tietz (1976), plasma total protein according to Doumas *et al.* (1981) and plasma albumin was measured according to Jacobs (1984).

Experimental design:

Thirty six *O. niloticus* were stocked in 6 aerated aquaria and divided into two groups in order to investigate the effect of *M. aeruginosa* toxic and non-toxic strains on such fish throughout the experimental period by observation of grazing rates, hematological changes and biochemical parameters.

1- The first group (Treated group): included three aquaria each containing six fish with three rips (total of 18 fish) which were exposed to *M. aeruginosa* UTCC300 (toxic strain) with a concentration of 200,000 cell/ml which is considered moderate risk according to Chorus *et al.*, (2000).
2- The Second group (Control group): included three aquaria each containing six fish with three rips (total of 18 fish) which were exposed to *M. aeruginosa* UTCC632 (non-toxic strain) with a concentration of 200,000 cell/ml.

Fresh *M. aeruginosa* cells were added daily to the aquaria to ensure constant concentration of 200,000 cell/ml.

Live Fish were sampled on days 3, 15, 25 and 35 during the exposure period (sampled fish were excluded and not returned back to the aquarium) where on the 3rd day three fish were sampled from each group, on 15th day five fish where sampled, on 25th day four Fish were sampled and on 35th day the rest of five fish (where one fish from each of the treated and control groups was died) were sampled.

Mass cultivation of toxic and non- toxic *M. aeruginosa*:

Pure stocks of both strains of *M. aeruginosa* (toxic and non-toxic) were separately cultured on BG-11 cyanobacteria medium (Table 1). The BG11 medium was previously autoclaved after the addition of about 20 g/l of agar then poured in

petri dishes. The two algae types were individually subcultured on a solid BG11 medium and incubated for 5 weeks in Incubator Conviron® CMP3244 at light intensity of 85 lux, Humidity 100% and temperature 25^{o}C. Incubated agar cultures were transferred to the lamina flow and complete aseptic conditions to carry out mass cultivation process according to Sharaf (2010).

Statistical analysis:

Statistical analysis was performed using Analysis Of Variance (ANOVA) and Duncan's multiple Range test to determine differences between treatments, means at significance level of 0.05. Standard errors of treatment means were also estimated. Pearson correlation test was performed between parameters to calculate correlation coefficient (r). All statistics were carried out using Statistical Analysis Systems (SAS) program (SAS, 2009).

RESULTS

Grazing rate of *M. aeruginosa* by *O. niloticus*:

Daily intake of *M. aeruginosa* UT300 and UT632 by *O. niloticus* (Table 2) showed nearly similar grazing rates for the treated and control groups.

Clinical signs

During the experimental period, both treated and control group showed normal clinical signs but during the last 10 days of the experiment, the treated group showed sluggish movement and reduced reflexes.

Table 2: Number of fish per aquarium and average cell number of *M. aeruginosa* toxic (UTCC300) and nontoxic (UT632) strains after grazing by fish throughout the experimental period*, and average number of consumed cells for both strains per individual fish.

Day	Number of fish per tank	Number of toxic cells after grazing (cell/ml)	Number of non-toxic cells after grazing (cell/ml)	Number of eaten toxic cells per fish (cell/ml)	Number of eaten non-toxic cells per fish (cell/ml)
1	6	431	575	33,262	33,238
3	6	333	614	33,278	33,231
5	5	498	916	39,900	39,817
7	5	206	327	39,959	39,935
9	5	304	196	39,939	39,961
11	5	137	90	39,973	39,982
13	5	614	248	39,877	39,950
15	5	176	92	39,965	39,982
17	3	2,782	209	65,739	66,597
19	3	1,567	1,436	66,144	66,188
21	3	705	1,464	66,432	66,179
23	3	8,478	2,508	63,841	65,831
25	3	2,401	1,215	65,866	66,262
27	2	609	877	99,696	99,562
29	2	2,267	757	98,866	99,622
31	2	3,241	1,338	98,380	99,331
33	2	1,655	847	99,173	99,577
35	2	8,179	699	95,911	99,651
Average per fish				62,567[a]	63,050[a]

*Original concentration of both strains in each aquarium was 200,000 cell/ml.

As shown in Table (3) there was no significant difference (P>0.05) in the Packed cell volume (PCV) between the treatment group exposed to toxic *Microcystis*

and the control group exposed to non-toxic *Microcystis.* Meanwhile the analyses of glucose, Cortisol and Thyroxine hormone (T4) showed significantly higher (P<0.05) concentrations in the treated group than that in the control group.

Table 3: Mean percentage of PCV with mean concentrations of glucose, cortisol and thyroxine (T4) in plasma of Nile Tilapia exposed to toxic (treated) and non toxic (control) *M. aeruginosa*, throughout the experimental period. (Mean ± SE).

Day	Treatment	PCV	Glucose (g/dl)	Cortisol (µg/dl)	T4 (µg/dl)
3	Control	$22.33^{a} \pm 2.60$	$62.87^{a} \pm 10.30$	$10684.22^{b} \pm 9.40$	$4.23^{a} \pm 0.95$
	Treated	$29.67^{a} \pm 1.86$	$61.60^{a} \pm 6.38$	$10769.51^{a} \pm 12.56$	$2.50^{a} \pm 0.62$
15	Control	$22.80^{a} \pm 0.97$	$42.52^{a} \pm 5.01$	$10627.19^{a} \pm 47.45$	$2.17^{b} \pm 0.27$
	Treated	$21.80^{a} \pm 2.08$	$58.82^{a} \pm 13.30$	$10665.47^{a} \pm 25.51$	$3.20^{a} \pm 0.32$
25	Control	$22.00^{a} \pm 1.08$	$30.17^{b} \pm 8.00$	$10310.89^{a} \pm 85.87$	$2.67^{b} \pm 0.48$
	Treated	$22.25^{a} \pm 1.03$	$52.07^{a} \pm 8.90$	$10459.71^{a} \pm 56.61$	$5.55^{a} \pm 0.81$
35	Control	$23.67^{a} \pm 2.33$	$30.14^{b} \pm 1.37$	$10423.73^{b} \pm 51.30$	$1.90^{b} \pm 0.01$
	Treated	$22.40^{a} \pm 0.81$	$46.23^{a} \pm 3.39$	$10648.03^{a} \pm 24.29$	$7.57^{a} \pm 1.07$
General mean	Control	$22.67^{a} \pm 0.72$	$30.14^{b} \pm 1.37$	$10502.99^{b} \pm 37.34$	$2.65^{b} \pm 0.28$
	Treated	$23.47^{a} \pm 1.00$	$46.23^{a} \pm 3.39$	$10627.92^{a} \pm 26.77$	$4.91^{a} \pm 0.51$

Means with different superscripts are significantly different (P≤0.05).

The results of plasma total protein analysis showed significantly (P<0.05) decreased levels (Hypoproteinemia) in the treated group than that in the control. Albumin concentrations showed no significant difference (P>0.05) between the two groups, while creatinine levels were significantly (P<0.05) higher in the treated group than that in the control, (Table 4).

Table 4: Mean concentrations of total protein, albumin and creatinine in plasma of Nile Tilapia exposed to toxic (treated) and non toxic (control) *M. aeruginosa*, throughout the experimental period. (Mean ± SE).

Day	Treatment	Total Protein (g/dl)	Albumin (g/dl)	Creatinine (mg/dl)
3	Control	$2.43^{a} \pm 0.05$	$1.22^{a} \pm 0.01$	$0.68^{a} \pm 0.10$
	Treated	$2.20^{b} \pm 0.01$	$1.26^{a} \pm 0.02$	$0.82^{a} \pm 0.07$
15	Control	$1.95^{a} \pm 0.06$	$1.13^{a} \pm 0.03$	$0.72^{a} \pm 0.05$
	Treated	$2.01^{a} \pm 0.07$	$1.13^{a} \pm 0.03$	$0.78^{a} \pm 0.04$
25	Control	$2.07^{a} \pm 0.02$	$1.04^{a} \pm 0.03$	$0.74^{a} \pm 0.13$
	Treated	$1.74^{b} \pm 0.07$	$1.09^{a} \pm 0.02$	$0.73^{a} \pm 0.05$
35	Control	$2.05^{a} \pm 0.07$	$1.05^{a} \pm 0.03$	$0.71^{b} \pm 0.03$
	Treated	$1.80^{b} \pm 0.08$	$1.09^{a} \pm 0.07$	$1.04^{a} \pm 0.02$
General mean	Control	$2.09^{a} \pm 0.04$	$1.11^{a} \pm 0.02$	$0.71^{b} \pm 0.04$
	Treated	$1.93^{b} \pm 0.05$	$1.13^{a} \pm 0.02$	$0.85^{a} \pm 0.03$

Means with different superscripts are significantly different (P≤0.05).

As shown in Table (5), AST analyses showed significantly higher (P<0.05) concentrations in plasma of *O. niloticus* in the treated group than that in the control,

while LDH and alkaline phosphatase concentrations were not significantly different (P>0.05) in both groups.

Table 5: Mean concentrations of Aspartate amino transferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase in plasma of Nile Tilapia *O .niloticus* exposed to toxic (treated) and non toxic (control) *M. aeruginosa*, throughout the experimental period. (Mean ± SE).

Day	Treatment	AST (IU/L)	LDH (U/L)	Alkaline P. (IU/L)
3	Control	$34.58^{b} \pm 2.55$	$756.83^{a} \pm 96.22$	$104.00^{a} \pm 14.74$
	Treated	$51.98^{a} \pm 1.93$	$614.33^{a} \pm 188.16$	$111.41^{a} \pm 12.97$
15	Control	$55.87^{a} \pm 5.24$	$936.70^{a} \pm 148.27$	$97.83^{a} \pm 38.86$
	Treated	$63.88^{a} \pm 9.86$	$1261.60^{a} \pm 156.85$	$78.53^{a} \pm 14.83$
25	Control	$98.67^{a} \pm 11.85$	$1707.63^{a} \pm 399.59$	$47.73^{a} \pm 9.46$
	Treated	$130.34^{a} \pm 18.33$	$1275.38^{a} \pm 283.61$	$41.83^{a} \pm 2.66$
35	Control	$59.42^{b} \pm 4.99$	$1608.67^{a} \pm 302.12$	$64.50^{a} \pm 9.04$
	Treated	$106.22^{a} \pm 10.78$	$1852.50^{a} \pm 278.11$	$98.51^{a} \pm 15.04$
General mean	Control	$63.74^{b} \pm 5.56$	$1240.70^{a} \pm 147.09$	$81.28^{a} \pm 14.63$
	Treated	$89.87^{a} \pm 7.8$	$1324.41^{a} \pm 135.55$	$81.57^{a} \pm 7.70$

Means with different superscripts are significantly different (P≤0.05).

DISCUSSION

Fish were reared during the experimental period on a sole diet of either *M. aeruginosa* UTCC300 (Toxic strain) for treated group or *M. aeruginosa* UTCC632 (non-toxic strain) for control group.

For investigation of different harmful effects of *Microcystis*, it was important to measure the grazing rate of *M. aeruginosa* by *O. niloticus*, so *M. aeruginosa* from toxic and non toxic strains were added to the water after which samples from the aquaria were taken on the next day for counting of the remaining cells in water. Measuring of the grazing rate was carried out by calculation of the difference between the original concentration ($200X10^{3}$cells/ml) and the counted samples one-day after the addition of algae.

Results of measuring the grazing rates of *O. niloticus* on *Microcystis aeruginosa* showed slightly higher grazing rate (63,050 cell/ml/fish) on non-toxic than toxic strain (62,567 cell/ml/fish) which was not significantly different. This may be due to the higher nutritive value for both strains (more than 65% of protein) in their vegetative stage (de la Fuente *et al.*, 1977; Aliger *et al.*, 2010 & Sharma *et al.*, 2011).

The present results disagreed with those of Beveridge *et al.* (1993) and Keshavanath *et al.* (1994) who noticed that the grazing rates on toxic *M. aeruginosa* were significantly lower in comparison with that non-toxic strain.

Clinical signs in *O. niloticus* associated with exposure to toxic cyanobacteria were investigated, and the only observed clinical signs were sluggish movement and reduced reflexes during late stages of exposure period.

Some biochemical parameters were measured and the following investigations were observed:

Blood glucose is considered an indicator of stress response in fish (Abdel-Baky, 2001). Glucose analyses showed elevated glucose levels in treated group than in control (Table 3). These results agreed with that of Zikova *et al.* (2010) who found a mild to moderate increase in glucose levels indicating mild to moderate stress, while it disagreed with Carbis *et al.* (1996) who found no changes in glucose levels in the common carp *Cyprinus carpio* exposed to toxic *Microcystis* and Kopp *et al.* (2010) who stated that the glucose levels were decreased in carp exposed to toxic *Microcystis*.

The increase in glucose levels in the present experiment indicated that fish in the treated group were stressed by the exposure to microcystin producing *Microcystis aeruginosa*. In this aspect, hyperglaecemia can be viewed as a physiological response of the fish to meet the critical needs for energy under toxic stress. This need may be met by increased break down of liver and muscle glycogen as described by Ferrando & Andreu-Moliner (1991).

Blood cortisol is the major corticosteroid hormone in fish and may have a significant effect on its dynamics (Wendelaar Bonga, 1997 & Mommsen *et al.*, 1999). The results of Cortisol analysis (Table 3) shows that the treated group had higher Cortisol levels than control group. The present result agrees with Zikova *et al.* (2010) who found mild to moderate increase in Cortisol levels indicating mild to moderate stress. The above data may be explained by the activation of hypothalamo–pituitary - inter renal axis with their release of steroid cortisol in blood stream due to stress (Ibrahim 1992, and Reddy & Leatherland, 1998).

As illustrated in Figure (1) there is a strong relationship (r = 0.82) between glucose and cortisol levels in plasma of *O. niloticus,* since their levels are influenced by fish stress.

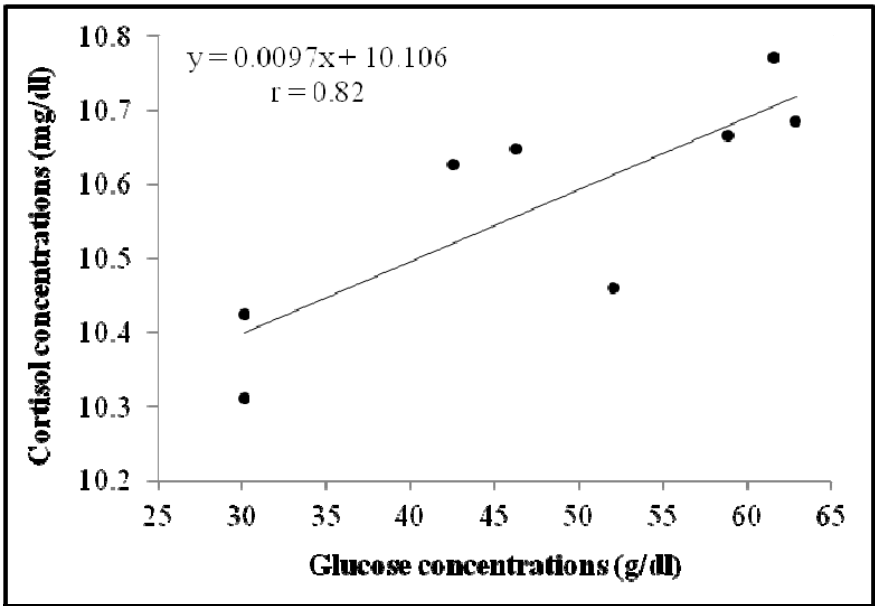

Fig. 1: Regression analysis between glucose and cortisol levels in plasma of *O. niloticus*.

Treated group exposed to toxic *Microcystis* showed higher concentrations of Thyroxin hormone levels than control group, indicating increased metabolic activity causing decreased fish performance. This result disagreed with Li *et al.* (2008) and

Brar *et al.* (2010) who found that injection of microcystin decreases the thyroid hormones levels in plasma and. Such differences in T3 and T3/T4 ratio may point to environment-related alterations in the peripheral conversion of T4 into T3. The disagreement in results may be due to fish species difference.

Proteins are the most important and abundant macromolecules in living beings, which play a vital role in architecture and physiology of the cell and in cellular metabolism (Mommsen & Walsh, 1992), in addition to the regulation of water balance (Heath, 1995).

As shown in Table (4), total protein levels were decreased in the treated group (Hypoproteinemia) compared to control group, as was found by Kopp *et al.* (2010) who treated carp with *Microcystis*. The decreased levels of total protein in the present study could be due to the increase of cortisol in the blood of stressed fish (Figs. 2&3) which suppresses the immunoglobulin function (Wedemeyer 1996; Reddy & Leatherland 1998). Furthermore, under stress conditions, the protein consumed by fishes is not stored in the body tissue (Baskaran & Palanichamy, 1990) and hence, the stressed fish meet their extra energy requirements from body proteins, which are mobilized to produce glucose, that is made available for fishes by the process of gluconeogenesis (Vasanthi *et al.*, 1990) .So, this depletion of the protein levels may have been due to its utilization for metabolic purposes.

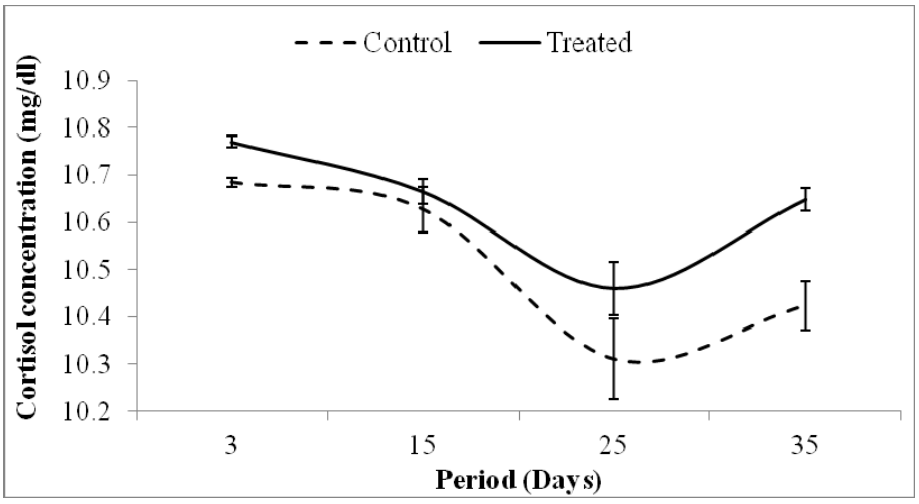

Fig. 2: Cortisol concentration throughout the experimental period.

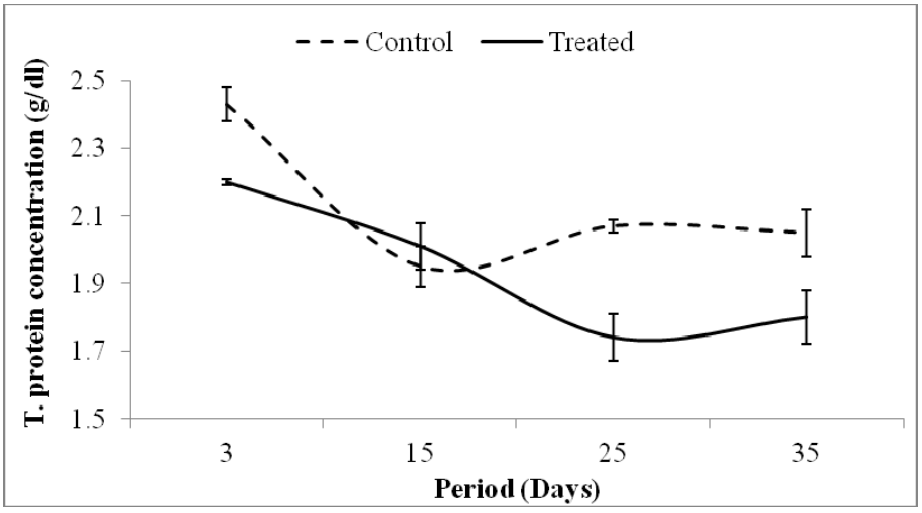

Fig. 3: total protein concentration throughout the experimental period

No significant difference between the treated and control groups in the concentrations of albumin (Table 4). Similar findings were obtained by Carbis *et al.* (1996). However this result disagrees with Kopp *et al.* (2010) who noticed decreased albumin levels in silver carp *hypophthalmichthys molitrix* exposed to toxic *Microcystis* bloom.

Creatinine analyses indicated elevated levels in the treated group than that in the control (Table 4), in contrary with the results of Kopp *et al.* (2010) who stated that the Creatinine levels of the common carp *Cyprinus carpio* exposed to toxic *Microcystis* were decreased, while Carbis *et al.* (1996) found no changes in Creatinine levels in that exposed carp. The increase in creatinine level in the present experiment may indicate kidney damage or malfunction.

Average results of Alanine Aspartate-Transferase (AST) analysis (Table 5) showed higher concentrations in the treated group than that in the control. Liver of vertebrates generally and fish particularly is the principal organ of detoxification (Freeman *et al.*, 1983). So, the increase in AST might be attributed to tissue damage, particularly liver (Gupta & Paul, 1978 and Palanivelu *et al.*, 2005). The elevation of these enzymes in the extracellular fluid or plasma is a sensitive indicator of cellular damage (Van der Oost *et al.*, 2003 and Palanivelu *et al.*, 2005). Thus, the measurement of transaminase and phosphatases activities in blood plasma of fish can be used as indicator for pesticide toxicity (Agrahari *et al*., 2007).

Lactate Dehydrogenase (LDH) concentrations were slightly higher in the treated group compared to the control though not significant (Table 5). This means that the toxic strain of *Microcystis* has almost the same effect of non toxic strain on the LDH enzyme in blood plasma of Nile tilapia.

The results of Alkaline phosphatase analysis (Table 5) show no significant difference between the treated and control groups. Similar results were obtained by Carbis *et al.* (1996) who mentioned that there was no change in Alkaline Phosphatase levels in toxic *Microcystis* exposed carp fish but disagree with Kopp *et al.* (2010) who reported that the Alkaline Phosphatase levels were decreased in toxic *Microcystis* exposed carp fish.

The difference in fish response between the current study and other studies in some blood parameters may due be to different fish species susceptibility, strain of algae, toxins produced and their congeners, dose and duration of exposure.

As shown in (Tables 6&7) the consumption of toxic algae by treated fish changed the linear relationship between some parameters. The positive correlation of glucose with the eaten algae was increased from r = 0.597 for non toxic algae to 0.922 for toxic algae, while the most affected parameter was the thyroxin hormone (T4), since it was markedly changed in a reverse order for all parameters (e.g. positive correlations of all parameters with toxic algae became negative with non toxic algae and vice versa).

Table 6 Pearson correlation matrix for non toxic eaten algae and some blood parameters of Nile tilapia in the control group.

Variables	Non toxic algae eaten	Gluc. (g/dl)	Cor. (µg/dl)	T4 (µg/dl)	Total Prot. (g/dl)	Albu. (g/dl)	Creat. (mg/dl)	AST (IU/L)	LDH (U/L)	Alk. P. (IU/L)
Non toxic algae eaten	**1**									
Glucose (g/dl)	0.597	**1**								
Cortisol (µg/dl)	0.909	0.877	**1**							
T4 (µg/dl)	0.109	0.862	0.512	**1**						
Total Protein (g/dl)	0.057	0.803	0.456	0.946	**1**					
Albumin (g/dl)	0.690	**0.992**	0.928	0.795	0.729	**1**				
Creatinine (mg/dl)	-0.541	-0.838	-0.768	-0.672	-0.797	-0.829	**1**			
AST (IU/L)	-0.805	-0.791	-0.900	-0.452	-0.542	-0.828	0.928	**1**		
LDH (U/L)	-0.854	-0.913	**-0.983**	-0.598	-0.493	**-0.955**	0.711	0.821	**1**	
Alkaline P. (IU/L)	0.921	0.861	**0.999**	0.487	0.422	0.917	-0.742	-0.887	**-0.983**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Table 7: Pearson correlation matrix for toxic eaten algae and some blood parameters of Nile tilapia in the treated group.

Variables	Toxic algae eaten	Gluc. (g/dl)	Cor. (µg/dl)	T4 (µg/dl)	Total Prot. (g/dl)	Albu. (g/dl)	Creat. (mg/dl)	AST (IU/L)	LDH (U/L)	Alk. P. (IU/L)
Toxic algae eaten	**1**									
Glucose (g/dl)	0.922	**1**								
Cortisol (µg/dl)	0.726	0.526	**1**							
T4 (µg/dl)	-0.924	**-0.999**	-0.507	**1**						
Total Protein (g/dl)	0.916	0.872	0.867	-0.858	**1**					
Albumin (g/dl)	0.743	0.803	0.770	-0.778	0.937	**1**				
Creatinine (mg/dl)	-0.361	-0.631	0.328	0.648	-0.179	-0.191	**1**			
AST (IU/L)	**-0.951**	-0.810	-0.903	0.804	**-0.960**	-0.805	0.077	**1**		
LDH (U/L)	-0.707	-0.907	-0.419	0.892	-0.797	-0.882	0.625	0.625	**1**	
Alkaline P. (IU/L)	0.465	0.246	0.946	-0.220	0.688	0.646	0.589	-0.714	-0.213	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Based on the present results, the study concluded that the uptake of toxic strain of *M. aeruginosa* via oral route has deleterious effects on some clinical and biochemical parameters of blood of the Nile tilapia which led to stress that finally affected the fish health.

The general recommendations accrued from this study are the prevention and monitoring of organic and inorganic pollution which have a significant flourishing effect on harmful algal blooms, and periodical monitoring of phytoplankton population in water to avoid the toxic effects of such algae on fish and consequently humans.

REFERENCES

Abdel-Baky, T. E. (2001). Heavy metals concentrations in the catfish, *Clarias gariepinus* (Burchell, 1822) from River Nile, El-Salam canal and lake Manzala and their impacts on cortisol and thyroid hormones. Egypt. J. Aquat. Biol. & Fish., 5: 79-98.

Agrahari, S.; Pandey, K.C. and Gopal, K. (2007). Biochemical alterations induced by monocrotophos in the blood plasma of fish *Channa punctatus* (Bloch) .Pest. Biochem. & Physiol., 88: 268- 272.

Aliger, K.; Arneson P.; Goldman, C. R. and Jooteh S. (2010). Species specific differences in the ingestion of *Microcystis* cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Journal of plankton research. 32(10): 479-1484.

Araoz, R.; Molgo, J. and Tandeau de Marsac, N. (2010). Review: Neurotoxic cyanobacterial toxins. Toxicon., 56: 813–828.

Barros, L. S. S.; Correia de Souza, F.; Tavares, L. H. S. and Amaral, L. (2010). Microcystin-LR in Brazilian Aquaculture Production Systems. Water Environ. Res., 82: 240-248.

Baskaran, P. and Palanichamy, S. (1990). Impact of agricultural (ammonium chloride) fertilizer on physiology and biochemistry of freshwater teleost fish, *Oreochromis mossambicus*. J. Ecobiol., 2: 97-106.

Beveridge, M. C. M.; Baird, D. J.; Rahmatullah S. M.; Lawton, L. A.; Beattie, K. A. and Codd, G. A. (1993). Grazing rates on toxic and non- toxic strains of cyanobacteria by *Hypophthalmichthys molitrix* and *Oreochromis niloticus*. J. of Fish Biol., 43: 901-907.

Bitterlich, G. (1985). The nutrition of stomachless phytoplanktivorous fish in comparison with Tilapia. Hydrobiologia, 121: 173-179.

Brar, N. K.; Waggoner, C.; Reyes, J. A.; Fairey, R. and Kelley, K. M. 2010. Evidence for thyroid endocrine disruption in wild fish in San Francisco Bay, California, USA. Relationships to contaminant exposures. Aquatic Toxicology., 96: 203-215.

Caldwell, S.; Rummer, J. L. and Brauner, C. J. (2006). Blood sampling techniques and storage duration: Effects on the presence and magnitude of the red blood cell β-adrenergic response in rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology, Part A., 144: 188-195.

Carbis, C. R.; Mitchell, G. F.; Anderson, J. W. and McCauley I. (1996). The effects of microcystins on the serum biochemistry of carp, *Cyprinus carpio L.*, when the toxins are administered by gavage, immersion and intraperitoneai routes. Journal of Fish Diseases., 19: 151-159.

Carmichael, W. W. and Falconer, I. R. (1993). Diseases related to freshwater blue green algal toxins, and control measures. In: Falconer IR, editor. Algal toxins in seafood and drinking water. London: Academic Press. 187-209.

Chorus, I.; Falconer I. R.; Salas, H. J. and Bartram, J. (2000). Health risks caused by freshwater cyanobacteria in recreational waters. Journal of Toxicology and Environmental Health 2000, Part B., 3:323-347.

De la Fuente, G.; Flores, A.; Molina, M. R.; Almengor L. and Ricardo Bressani (1977). Some Nutritional Characteristics of a Naturally Occurring Alga (*Microcystis* sp.) in a Guatemalan Lake. Appl. Environ. Microbiol., 33(1):6.

Dodds, W. K .; Bouska, W. W.; Eitzmann, J. L.; Pilger, T. J.; Pitts, K. L.; Riley, A. J.; Schloesser, J. T. and Thornbrugh, D. J. (2009). Eutrophication of U.S. Freshwaters: Analysis of Potential Economic Damages. Environmental Science & Technology., 43: 12-19.

Dong, G.; Zhu X.; Han, D.; Yang, Y.; Song, L. and Xie, S. (2009). Effects of dietary cyanobacteria of two different sources on growth and recovery of hybrid tilapia (*Oreochromis niloticus* X *O. aureus*). Toxicon., 54:208–216.

Doumas, B. T.; Bayse, D. D.; Borner, K.; Carter, R. J.; Eievitch, F.; Garber, C. C.; Graby, R. A.; Hause, L. L.; Mather, A.; Peters, T.; Jr.; Rand, R. N.; Reeder, D. J.; Russell, S. M.; Schaffer, R. and Westgard, J. O. (1981). A Candidate Reference Method for Determination of Total Protein in Serum II. Test for Transferability. Clinical chemistry., 27: 1651-1654.

Ferrando, M.D. and Andreu – Moliner, E. (1991). Effect of lindane on the blood of a freshwater fish. Bull. Environ. Contam. Toxicol., 47: 465-470.

Freeman, H.C.; Sangalang, G.B.; Uthe, J.F.; Garside, E.T. and Daye, P.G. (1983). "A histopathological examination and analysis for polychlorinated hydrocarbons in inshore Atlantic cod (*Gadus morhae*)." Arch. Environ. Contam. Toxicol., J. 12: 627-632.

Grosse, Y.; Baan, R.; Straif, K.; Secretan B.; El Ghissassi F. and Cogliano V. (2006). Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. WHO International Agency for Research on Cancer Monograph Working Group. Lancet Oncology, 7: 628-629.

Gupta, R. G. and Paul, B. S (1978). Influence of malathion (O,O–dimethydithiophosphate of diethyl mercapto succinate) on body enzymes in dermal sub acute toxicity studies in *Bubalus bubalis* species, Bull. Environ. Contam. Toxicol., 20: 811-818.

Heath, A.G. (1995). Water pollution and fish physiology. CRC Press Inc. Boca Raton, Florida.

Ibrahim, A. T. (1992). Effects of some industrial pollutants on *Tilapia nilotica*-Proc. 2nd conf. Fac. Vet. Med., Cairo univ., 47-51.

Jacobs, D. S. (1984). Laboratory test handbook with DRG index Lexi-Comp, Ohio. pp.332.

Kaye, T. B. and Crapo, L. (1990). The cushing syndrome: an update on diagnostic test. ANN. Intern. Med., 112:434.

Keshavanath, P.; Beveridge, M. C. M.; Baird, D. J.; Lawton, L. A.; Nimmo, A. and Codd, G. A. (1994). The functional grazing response of a phytoplanktivorous fish *Oreochromis niloticus* to mixtures of toxic and non-toxic strains of the cyanobacterium *Microcystis aeruginosa*. Journal of Fish Biology., 45: 123-129.

Kopp, R.; Palikova, M.; Navratil, S.; Kubicek, Z.; Zikova, A. and Mares, J. (2010). Modulation of Biochemical and Haematological Indices of Silver Carp (*Hypophthalmichthys molitrix* Val.) Exposed to Toxic Cyanobacterial Water Bloom. Acta Vet. Brno., 79: 135–146.

Krishnamurthy, T.; Carmichael, W. W. and Sarver, E. W. (1986). ToxicPeptides from Freshwater Cyanobacteria (Blue-Green Algae). I. Isolation, Purification and Characterization of Peptides From *Microcystis Aeruginosa* And *Anabaena Flosaquae*. Toxicon., 24: 865-873.

Li, D.; Xie, P. and Zhang, X. (2008). Changes in plasma thyroid hormones and cortisol levels in crucian carp (*Carassius auratus*) exposed to the extracted microcystins. Chemosphere., 74: 13-18.

Mommsen, T. P.; Vijayan, M. M. and Moon, T. W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish. Biol. Fish., 9: 211-268.

Mommsen, T.P. and Walsh, P.J. (1992). Biochemical and environmental perspectives on nitrogen metabolism in fishes. Experientia, 48: 583-593.

Nyakairu, G. W. A.; Nagawa, C. B. and Mbabazi, J. (2010). Assessment of cyanobacteria toxins in freshwater fish: A case study of Murchison Bay (Lake Victoria) and Lake Mburo, Uganda. Toxicon., 55: 939-946.

Palanivelu, V.; Vijayavel, K.; zhilarasibalasubramanian, S. and Balasubramanian, M. P. (2005). Influence of insecticide (Cartap Hydrochloride) from the marine polychaete on certain enzyme systems of the freshwater fish *Oreochromis mossambicus*. J. Environ. Biol., 26: 191-196.

Reddy, P. K. and Leatherland, J. F. (1998). Stress Physiology, In: Fish disease and disorders, Vol.2 Non-infections Disorders. (Eds. Leatherland, J.F. & Woo, P.T.K.), Cabi Pub., 279-301.

Sharaf, M. S. (2010). Green blue algae and their effects on fish health. M. Sc. Thesis, Fish Diseases and Management Dept., Faculty of Veterinary Medicine, Cairo University.

Schuurs, A. H. W. M. and Van Weeman, B. K. (1977). Review, Enzyme-immunoassay. Clinical Chemistry Acta., 81:1.

Sharma, N. K.; Tiwari, S. P.; Tripathi, K. and Rai, A. K. (2011). Sustainability and cyanobacteria (blue-green algae): facts and challenges. J Appl Phycol, 23: 1059-1081.

Statistical Analyses Systems (SAS) (2009). SAS Program ver. 9.1, SAS institute incorporation, Cary, NC 27513 USA.

Tietz, N. (1976). Fundamentals of Clinical Chemistry. W.B. Saunders, Philadelphia, PA., 602-1211.

Van der Oost, R.; Beyer, J. and Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol.,13: 57-149.

Vasanthi, R.; Baskaran, P. and Palanichamy, S. (1990). Influence of carbofuran on growth and protein conversion efficiency in some freshwater fishes. J. Ecobiol., 2: 85-88.

Wedemeyer, G.A. 1996. Physiology of Fish in Intensive Culture Systems. 232, Chapman and Hall, 115 Fifth Avenue New York. pp. 232.

Wendelaar Bonga, S.E. (1997). The stress response in fish .Physiol. Rev. 77: 591-625. W.H.O. (1986). Formations of pesticides in developing countries. United Nation industrial Development Organization, Vienna.

Young, D. S.; Pestaner. L. C. and Gibberman. V. (1975). Effects of Drugs on Clinical Laboratory Tests. Clinical Chemistry., 21: 5.

Zikova, A.; Trubiroha, A.;Wiegand, C.; Wuertz, S.;Rennert, B.;Pflugmacher, S.; Kopp, R.;Mares, J. and Kloas, W. (2010). Impact of microcystin containing diets on physiological performance of nile tilapia (*Oreochromis niloticus*) concerning stress and growth. Environ. Toxicol. and Chemis., 29: 561–568.

ARABIC SUMMARY

تأثير السيانوبكتريا (طحلب ميكروسيستس إريجينوزا) السلالة السامة وغير السامة على بعض المقاييس البيوكيميائية للبلطى النيلى

محمد سيد مرزوق[1] محمد مصطفى محمد[1] نبيل أحمد إبراهيم[2] فرانسيز بيك[3-] محمود سعد شرف[1]

1- قسم أمراض الأسماك ورعايتها كلية الطب البيطري - جامعة القاهرة - مصر

2- المركز الدولى للأسماك المركز الإقليمى لأفريقيا وغرب أسيا - العباسة - أبو حماد- شرقية – مصر

3- قسم البيولوجى – كلية العلوم – جامعة أوتاوا – كندا

لإجراء هذه الدراسة تم تقسيم عدد ستة وثلاثين سمكة من أسماك البلطى وحيد الجنس إلى مجموعتين باستخدام ستة أحواض زجاجية (ثلاثة لكل مجموعة)، المجموعة الأولى (المجموعة المعاملة) تم تغذية أسماكها على السلالة السامة من طحلب ميكروسيستس إريجينوزا والمجموعة الثانية (مجموعة الكنترول) تم تغذية أسماكها على السلالة غير السامة من طحلب ميكروسيستس إريجينوزا لدراسة معدل رعى الأسماك على كلا السلالتين وتأثير ذلك على الصحة العامة للأسماك متمثلة فى العلامات الإكلينيكية و التغيرات الدموية و المقاييس البيوكيميائية. أظهرت النتائج أن معدلات الرعى والمأكول اليومى كانت متقاربة بين المجموعة المعاملة (الطحالب السامة) ومجموعة الكنترول (الطحالب غير السامة) بزيادة طفيفة فى معدلات رعى الطحالب غير السامة عنه للطحالب السامة حيث كان المأكول اليومى الفردى 63050 و62567 خلية/مل/سمكة على التوالى.

أظهرت النتائج أن العلامة الإكلينيكية الوحيدة لأسماك البلطى النيلى المغذاه على الطحالب السامة كانت تباطؤ الحركة و انخفاض رد الفعل خلال المراحل الأخيرة من فترة التعرض.

ارتفع تركيز الجلوكوز و الكورتيزول والثيروكسين و الكرياتنين و AST فى بلازما دم أسماك المجموعة المعاملة عنه فى الكنترول مما رفع من إجهاد الأسماك الأمر الذى يؤثر بالسلب على صحة الأسماك وأدائها ، على النقيض من ذلك فقد ارتفع تركيز البروتين الكلى فى بلازما دم أسماك الكنترول عنه فى الأسماك المعاملة. ولم تتأثر قيم الهيماتوكريت و الألبيومين و LDH و الفوسفاتيز القلوى.

وقد خلصت الدراسة إلى أن تغذى الأسماك عن طريق الفم على السلالة السامة من طحلب ميكروسيستس إريجينوزا كان له أثار ضارة على بعض العلامات الإكلينيكية و المقاييس البيوكيميائية لدم أسماك البلطى النيلى الأمر الذي يؤدى إلى إجهاد الأسماك مما يؤثر في النهاية على صحتها.

توصى هذه الدراسة بمحاولة منع ورصد التلوث العضوى وغير العضوى الذى يؤدى إلى ازدهار الطحالب الضارة، وكذلك المتابعة الدورية لأنواع الطحالب لإجراء تدابير وقائية مبكرة لتجنب الآثار السامة للطحالب على الأسماك وبالتالي على صحة الإنسان.