CYANOBACTERIAL TOXINS AND TOXICITY IN AQUATIC ECOSYSTEMS AND FISH OF RIVER NILE

By

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B.Sc. Agric. Sci. (Animal Production), Fac. Agric., Alex. Univ., 2002.

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

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In

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Department of Agricultural Microbiology
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Cairo University
EGYPT

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APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: Cyanobacterial Toxins and Toxicity in Aquatic Ecosystems and Fish of River Nile.
Supervisors: Dr. Aziz Mohamed Aziz Higazy
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Department: Agricultural Microbiology. **Approval:** 16 / 6 /2010

ABSTRACT

The aim of the present study was to detect and determine cyanobacterial toxic blooms in two water bodies of river Nile and study the effect of isolated toxic cyanobacterial species on the 10- day old tilapia fish. Water, sediment, algae and fish samples were collected monthly from September 2007 to March 2009 from Port-Said freshwater canal and Rosetta branch.

Twenty one species of Cyanophyta were identified, only three species were capable to form bloom. These species were *Microcystis aeruginosa, Oscillatoria brevis, Oscillatoria princeps,* maximum growth of these species were observed during winter seasons.

Toxicity determination of the collected samples using brine shrimp bioassay and mouse bioassay showed that higher toxicity was recorded during winter 2008 and winter 2009. Also microcystin determinated using High Performance Liquid Chromatogrphy (HPLC) showed a variation of average concentrations in collected samples of water, algal bloom and fish muscles, fish gills and fish intestines during during winter seasons. The average of concentrations ranged between from 0.2 - 5.6 µg l^{-1}, 0.1 - 6.5 µg g^{-1}, 1.8 - 3.2 µg kg^{-1}, 1.6 - 4.3 µg kg^{-1} and 2.0 - 3.8 µg kg^{-1} respectively.

Correlation analysis between physicochemical parameters and blooming cyanobacterial indicates that the bloom formation were positively correlated with TDS, nitrate, ammonium and phosphate.

The mortality percentage of 10-days old tilapia fish *Oreochromis niloticus* was correlated positively with increasing cells dry weight concentrations of *Oscillatoria brevis* and *Microcystis aerugenosa* and also with increasing the time exposure.

Key words: Cyanobacteria, cyanobacterial bloom, brine shrimp, cyanotoxins, river Nile

DEDICATION

I dedicate this work to my FATHER and my MOTHER for all the support through my life, as well as to my dear brothers for all the support and help in all my life. My special and sincere thanks to my brother Dr.Reda, who encourage me througout my graduation study. Also, I would like to thank my wife for her patience and help to complete my work.

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اسم الطالب: ضياء عطية جاب الله مريز **الدرجة:** ماجستير

عنوان الرسالة: سموم وسمية السيانويكتريا فى النظم البيئية المائية والأسماك لنهر النيل

المشرفون : دكتور : عزيز محمد عزيز حجازى
دكتور : زكريا يحيى ضو
دكتور : محمد نصرالدين محمد جمعة

قسم: الميكروبيولوجيا الزراعية **تاريخ منح الدرجة:** ٢٠١٠/٦/١٦

المستخلص العربي

تهدف الدراسة إلى الكشف عن وتقدير سموم السيانوبكتريا فى نهر النيل ودراسة تأثير هذه السموم على نفوق الأسماك من خلال تجميع عينات شهرية من مياه وطمى وطحالب وأسماك فى الفترة من سبتمبر ٢٠٠٧ حتى مارس ٢٠٠٩ بطول ترعة بورسعيد وفرع رشيد.

تم تعريف واحد وعشرون نوعا من من السيانوبكتريا خلال فترة الدراسة منها ثلاث أنواع فقط لها القدرة على تكوين الإزدهار الطحلبى وهذه الأنواع هى *Microcystis aeruginosa, Oscillatoria brevis, Oscillatoria princeps* حيث كان أقصى نمو لهذه الأنواع خلال شتاء ٢٠٠٨ وشتاء ٢٠٠٩.

تم الكشف عن سمية العينات المجمعة بإستخدام التقييم الحيوى ليرقات بيض الجمبرى وفئران التجارب حيث سجلت أعلى سمية للعينات خلال شتاء ٢٠٠٨ وشتاء ٢٠٠٩. أما بالنسبة لتقدير الميكروسيستين بإستخدام جهاز التحليل الكروماتوجرافى ذو الأداء العالى فقد تراوح تركيز الميكروسيستين فى عينات المياه بين ٠,٢ – ٥,٦ ميكروجرام/ لتر خلال فصلى الشتاء. بينما تراوح فى عينات الكتلة الحية للسيانوبكتريا ما بين ٠,١- ٦,٥ ميكروجرام/ جرام فى فصلى الشتاء. تم الكشف عن سمية عينات اللحم والخياشيم والأحشاء لأسماك البلطى المجمعة من فرع رشيد بإستخدام التقييم الحيوى ليرقات بيض الجمبرى وفئران التجارب حيث سجلت أعلى سمية للعينات أيضا خلال شتاء ٢٠٠٨ وشتاء٢٠٠٩. أما التقدير الكمى للميكروسيستين بإستخدام جهاز التحليل الكروماتوجرافى ذو الأداء العالى تراوح التركيز فى عينات اللحم بين ١,٨- ٣,٢ ميكروجرام/ كجم، وتراوح التركيز بين ١,٦ – ٤,٣ ميكروجرام/ كجم فى عينات الخياشيم، أما عينات الأحشاء فتراوح التركيز بين ٢- ٣,٨ ميكروجرام/ كجم وذلك أثناء فترة إزدهار السيانوبكتريا فى فصلى الشتاء.

أظهرت نتائج الدراسة أن هناك علاقة إرتباط بين العوامل البيئية وإزدهار السيانوبكتريا ، حيث كان هناك إرتباط موجب مع تركيز الجوامد الصلبة الكلية وتركيزات النترات والأمونيا والفوسفور.

كما أظهرت الدراسة أيضا تأثير كل من *Oscillatoria brevis* و *Microcystis aeruginosa* على نفوق أسماك البلطى، فبزيادة تركيز السيانوبكتريا زادت نسبة نفوق الأسماك، كما زادت نسبة نفوق الأسماك بزيادة زمن التعرض.

الكلمات الدالة: السيانوبكتريا، إزدهار السيانوبكتريا، يرقات بيض الجمبرى ،سموم السيانوبكتريا، نهر النيل.

سموم وسمية السيانوبكتريا فى النظم البيئية المائية والأسماك لنهر النيل

رسالة الماجستير
في العلوم الزراعية
(الميكربيولوجيا الزراعية)

مقدمة من

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بكالوريوس في العلوم الزراعية (إنتاج حيوانى)- كلية الزراعة- جامعة الأسكندرية، ٢٠٠٢

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التاريخ / /

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للحصول على درجة

الماجستير

في

العلوم الزراعية
(الميكروبيولوجيا الزراعية)

قســـــــم الميكروبيولوجيا الزراعية
كليـــــة الزراعـــة
جـامعـة القـاهرة
مصـــــــر

٢٠١٠

RESULTS AND DISCUSSION

The main objective of the study is the detection and determination of cyanobacterial toxic bloom in the river Nile. In order to fulfill such objective we tried to answer several questions that may be the key for such problem. The first question was do we have regular occurrence of cyanobacterial bloom in the river Nile and do this bloom is toxic bloom or not. The second is there any relation between the physicochemical parameter and the occurrence of such bloom or its toxicity. The third is there any effect of this bloom on the fish mortality.

1. Physicochemical parameters

To answer the question about relation between physicochemical parameters and the occurrence of cyanobacterial blooms or its toxicity, water physicochemical parameters were measured at sampling time. Correlations were made between these parameters and the occurrence of the blooms.

a. Water temperature

Variations in water temperature illustrated in Fig. (5) showed a general seasonal trend at different studied sites. A little difference in temperature could be seen among the sites. The water temperature had a range of 26-31˚C during summer and 16-18˚C during winter.

As shown in Table (3), no significant differences were detected among the temperature at the different sampling sites. However, significant differences ($P<0.01$) among the different seasons were observed (Table 3).

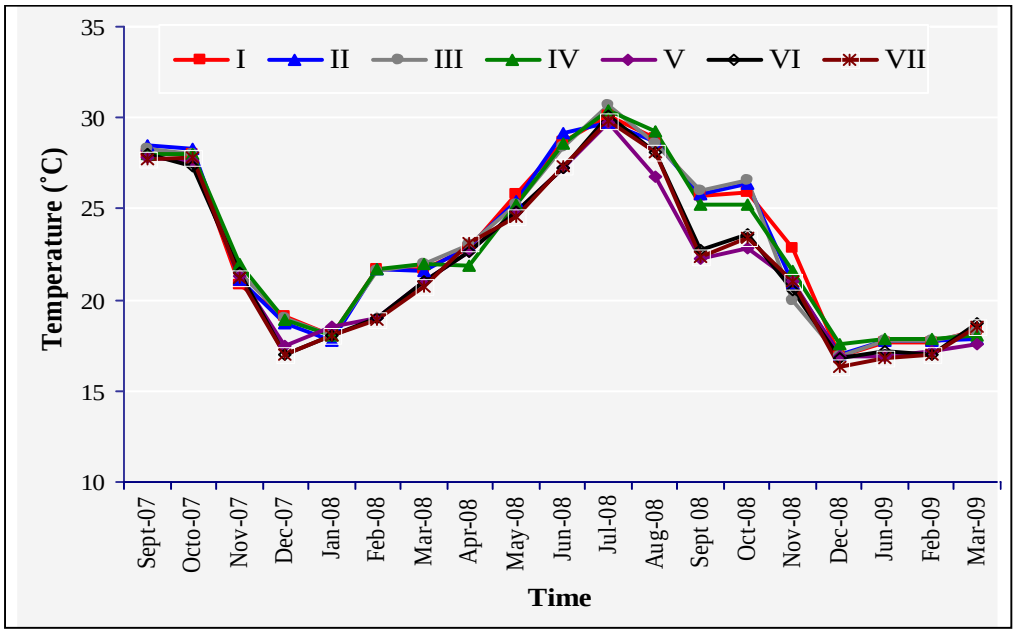

Fig. 5. Variations in water temperature at different sites of Port-Said and Rosetta branch during different months, $LSD_{0.05}$ = 1.52.

b. Water pH

Water pHs at different sampling sites are present in Fig. (6). The pH did not greatly depart of 7.3 - 8.2 throughout the period of study. The minimum pH was recorded as 7.33 at site V during December 2007. No significant differences were detected among the different sampling sites pH in Port-Said sites, as well as among the different seasons. The highest pH of 7.95 was recoreded in winter 2009 followed by autumn 2007. Significant differences were detected among Rosetta branch sites, but no significant difference was detected among the different seasons (Table 3).

c. Turbidity

Determination of turbidity levels throughout the period of study (Fig. 7) showed seasonal variations and ranged between 1.5 and 87 NTU.

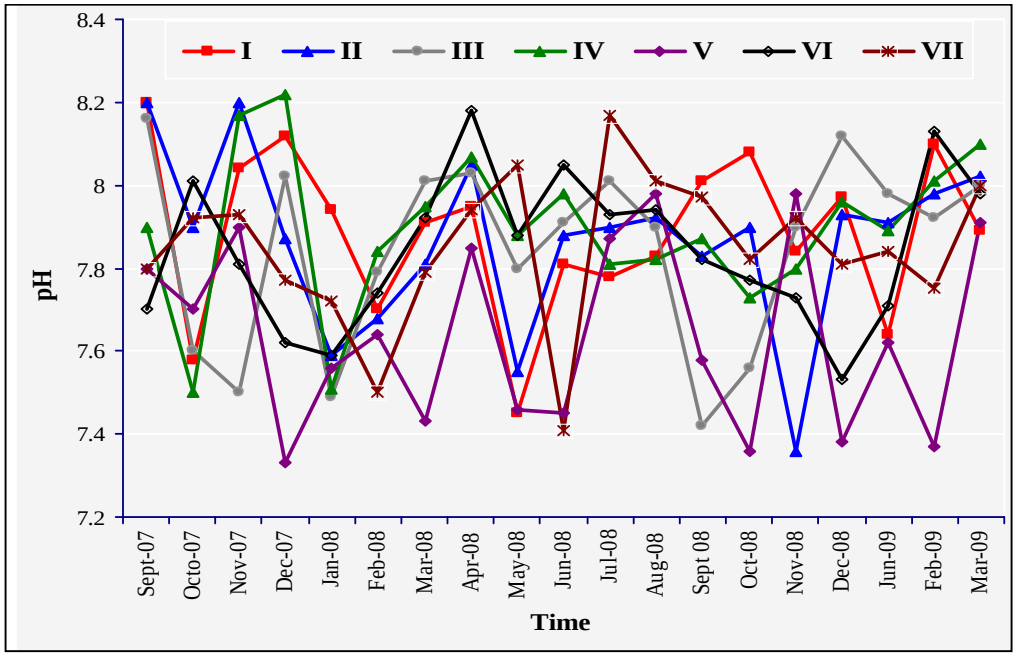

Fig. 6. Levels of water pH of the selected sampling sites during different seasons $LSD_{0.05}$ = 0.34.

Table 3. Measurements of temperature (˚C) and pH in Port-Said and Rosetta branch locations during the study period.

Season	Mean±SE			
	Port-Said freshwater canal		Rosetta Branch	
	Temperature	pH	Temperature	pH
Autumn, 07	$25.9^{B} \pm 0.97$	$7.9^{A} \pm 0.08$	$25.8^{B} \pm 0.97$	$7.8^{A} \pm 0.04$
Winter, 08	$19.5^{D} \pm 0.48$	$7.8^{A} \pm 0.18$	$18.1^{D} \pm 0.27$	$7.6^{B} \pm 0.05$
Spring, 08	$23.3^{C} \pm 0.47$	$7.9^{A} \pm 0.59$	$22.8^{C} \pm 0.56$	$7.7^{AB} \pm 0.10$
Summer, 08	$29.3^{A} \pm 0.22$	$7.9^{A} \pm 0.02$	$28.3^{A} \pm 0.43$	$7.9^{A} \pm 0.09$
Autumn, 08	$24.3^{BC} \pm 0.68$	$7.8^{A} \pm 0.06$	$22.2^{C} \pm 0.68$	$7.8^{A} \pm 0.07$
Winter, 09	$17.6^{E} \pm 0.09$	$7.8^{A} \pm 0.03$	$16.9^{D} \pm 0.09$	$7.7^{AB} \pm 0.08$

(n= 9) $p < 0.05$

No significant differences were detected among the different sampling sites in Port-Said. Data in Table (4) indicated significant differences among seasons ($P<0.01$), the highest turbidity 24.9 NTU was recoreded in autumn 2008 followed by summer 2008. No significant differences were observed between winter 2008 and winter 2009 which recorded the lowest turbidity value. Significant differences were recorded among the different sampling sites in Rosetta branch ($P<0.05$). Also, Significant differences were detected among the different seasons ($P<0.01$), the highest turbidity 22.5 NTU was recoreded in summer 2008 followed by spring 2008.

Significant differences were detected between winter 2008 and winter 2009 which also recorded the lowest turbidity value (Table 4).

d. Alkalinity

Water alkalinity at different sampling sites (Fig. 8), showed a general indpent seasonal trend. It was ranged between 110 and 348 mg l^{-1}, except at site V whereas the alkalinity recorded the highest value and reached 660 mg l^{-1} in June 2008. Significant differences were detected among the different sampling sites ($P<0.05$) and seansons ($P<0.01$) except between winter seasons (Table 4).

e. Electerical conductivity

The measurements of conductivity are shown in Fig. (9). Electerical conductivity values had wide variations between sites of Rosetta branch throughout the period of study. Their values ranged between 362 µmohs cm^{-1} and 7670 µmohs cm^{-1} at site I in Jun 2008 and site V in May 2008, respectively.

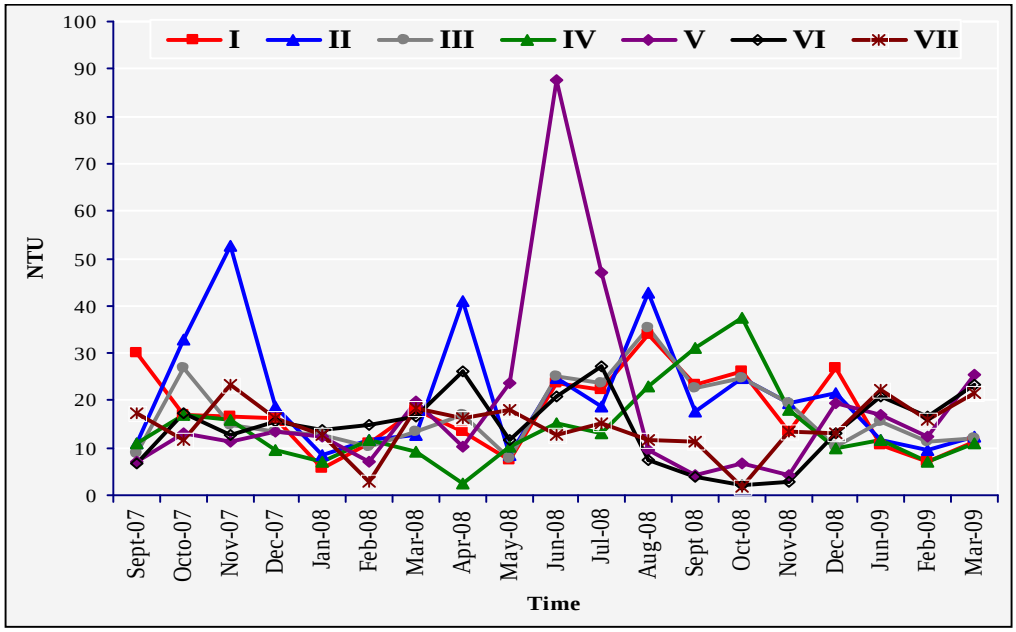

Fig. 7. Variations in turbidity (NTU) measurements at different sites of Port-Said freshwater canal and Rosetta branch, $LSD_{0.05}$ = 3.22.

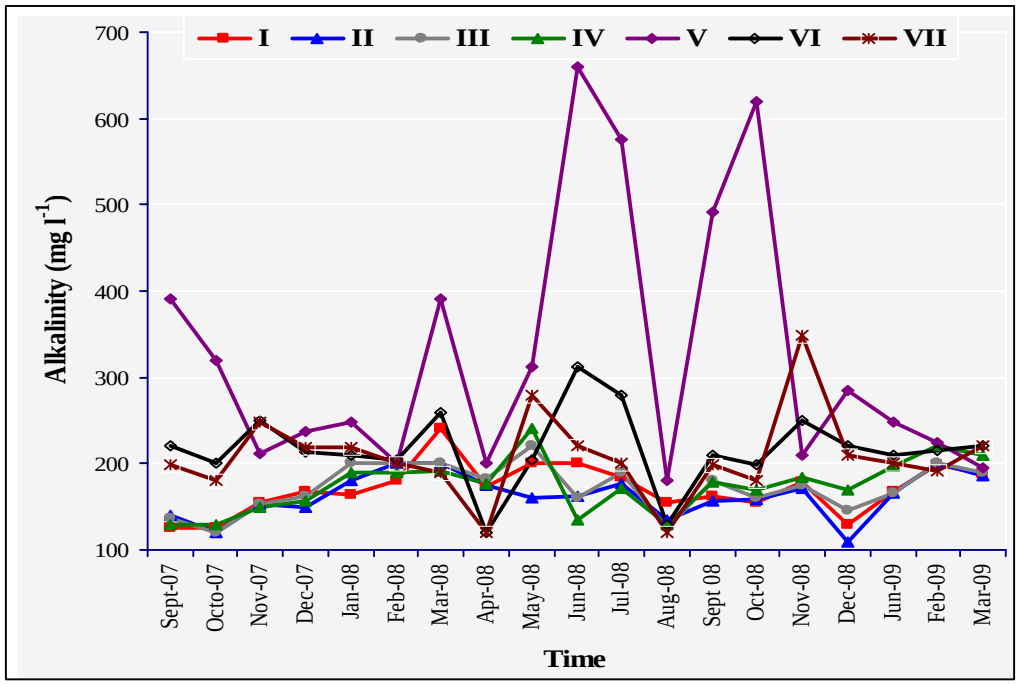

Fig 8. Variations in measurements of alkalinity (mg l^{-1}) at different sites of Port-Said and Rosetta branch, $LSD_{0.05}$ = 16.98.