Toxicity Assessment of Freshwater Blue-green Algae under Different Nitrogen and Phosphorus Ratios

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Abstract: The river Nile and Ismailia Canal are the main sources of potable drinking water supply in Egypt. Under certain environmental circumstances, algae multiply to produce “blooms” and may also synthesize products (biotoxins) that can be harmful to marine and freshwater organisms as well as people. Therefore, the main objectives of this study were: To study the effects of essential nutrients; nitrogen (N) and phosphorus (P) on the blue-green algal growth and its toxicity using animals as test organisms. 2-To evaluate toxins if present is chronic hazard or acute hazard. For these purposes, algal bioassay procedure was used to study the effect of N:P and P:N ratios on three test organisms belonging to fresh water blue-green algae, namely; Anabaena flos-aquae, Oscillatoria limnetica, and Nostochoposis wichmannii. The most important finding of this study are, the short term toxicity effect (Acute) of exocellular and endocellular of the tested species of blue-green algae, revealed no signs of toxins for Daphnia and mice. While, long term toxicity effect (Chronic) by oral administration to mice, only heterocystis cyanobacteria test organism; Nostochoposis wichmannii, extraction affect the mice harmfully.

Key words: Blue-green algae, Nitrogen, Phosphorus, Toxicity, Daphnia, Mice.

INTRODUCTION

Algae play a vital role in productivity, may also synthesize products (biotoxins) which adversely affect other aquatic organisms elsewhere in the food web. An increasing dependency on marine microorganisms as a potential source for drugs and other chemicals, and their modern applications for genetic engineering, so recent concerns about aquatic biotoxins, particularly from microorganisms, was take place[1].

Harmful algal bloom with potential environmental human health impacts are also found in fresh and brackish water. Cyanobacteria produce potent neuro- and hepato-toxins, and extensive blooms occur in some public drinking water supply as well as their occurrence in some dialysis clinic. This outbreak suggests that at least some current treatment technologies may not effectively remove algal toxins from drinking water[2,3,4,5].

Toxin–producing genera of fresh– and brackish water cyanobacteria are now known to include filamentous Anabaena, Aphanizomenon, Nodularia and Oscillatoria, plus coccoid Microcystis. Also, other several cyanobacterial genera are implicated in animal and human water based toxicosis. These freshwater algae can form thick surface accumulations of cells as the water–bloom develops within the water body. They can be found in many temperate–latitude eutrophic lakes and ponds. Some species and strains; not all members, within these five genera are known to contain cyclic hepatotoxic hepta- or pentapeptides and /or neurotoxic alkaloids. So, they are responsible for sporadic but widespread outbreaks of wild and domestic animal illness or death. They are also implicated in human poisonings in certain municipal and recreational water supplies. However, not all members of these genera are toxic and it is not possible to tell by looking at a bloom or by microscopic examination of the water whether it is toxic or not[6,7,8,9,10,11].

Microcystins are now known to be produced by four planktonic cyanobacteria genera; three are filamentous, Anabaena, Oscillatoria, and now Nostoc while one is coccoid, Microcystis. Microcystis produces the greatest variety of toxins differing in the L-amino acids variant[9,10,12]. Also[13,14,15], stated that, strains of certain genera of cyanobacteria, including Anabaena, Aphanocapsa, Hapalosiphon, Nostoc, Pseudanabaena, Planktothrix and Microcystis, can produce cyclic peptide microcystins (MC).

It may be worthy to note, these natural products are more than just academic interest, since they may cause massive animal mortalities as well as environmental, legal, recreational, drinking water, and health–related problems[13]. Therefore, the main objectives of this study were:
1- To study the effects of essential nutrients; nitrogen (N) and phosphorus (P) on the blue-green algal growth and its toxicity using animals as test organisms.

2- To evaluate toxins if present is chronic hazard or acute hazard.

MATERIAL AND METHODS

1- Algal Bioassay Procedure: Algal bioassay procedure was used to study the effect of N:P ratios and P:N ratios on freshwater algal growth and its toxicity via batch system.

For this purpose three test organisms belonging to blue-green algae were isolated from river Nile water in pure culture form. Two species of filamentous nitrogen-fixing cyanobacteria (Anabaena flos-aquae and Nostochopsis wichmannii) (Fig 1 A & B), and one filamentous non-nitrogen-fixing species (Oscillatoria limnetica) (Fig 1 C) were used. Each test organism was cultivated in specific growth algal media; mod-Watanabe medium for (A. flos-aquae), BG-11 medium with fifth of nitrate content for (O. limnetical) and BG-11 medium without nitrate for (N. wichmannii).

Toxicity Bioassay Test: Two animals have been used as test organisms for toxicity bioassay. Daphnia magna was used as a sensitive test organism and male mice as a model for human injury when exposed to harmful blue-green algae.

2-a- Daphnia Magna: For algal toxicity determination, segregate and collect 24-h-old neonate Daphnia released from its mothers. Ten animals were introduced to beakers contain 100 ml of each alga's filtrate growth media (triplicate beakers). A control test was applied at the same time (100 ml Daphnia magna growth media + 10 animals). Both stimulatory and cytotoxic effects were observed after 24, 48 and 96 hrs by counting live D. magna neonates.

2-b – Mouse Bioassay Test: The toxicity of the freeze-dried algal material is determined by using male Swiss mice weighting 18-23 g. Two techniques were applied to examine the algal toxicity which were:

2-b -1- Intraperitoneal Injection "I.P." (Short-Term Toxicity "Acute"): Toxicity is tested by intraperitoneal injection (i.p.) of 0.1-1.0 of lysate algal cells by freeze-thawing. The material dissolved in 0.9% NaCl at a concentration of 50 mg wet weight per ml and suspended by vortexing for 30 min followed by 30 min centrifugation at 4000 rpm. The mice were observed during the following 24 h for any signs of poisoning.

2-b -2-Oral Administration (Long-Term Toxicity "Chronic"): Five mice were housed in polystyrene cage containing wood shavings at room temperature (22-25 °C). They were supplied with drinking water of algal material extraction suspended in tap water. Five animals represented the control group and received tap drinking water. Daily observation of mice took place. Body weight and the liver weight of the tested mice were examined with the observation of any difference in the appearance and texture of mice liver treated with tap water (Control) and the others treated with drinking water contain algal extraction.

Results:
Effects of Some Environmental Factors on Bloom of Cyanophyta: Substantial changes in morphological shape took place in the three test organisms after exposure to changes of some environmental factors (Nitrogen, Phosphorus and Light intensity) (Fig. (1); A, B&C). At maximum growth (Blooming) of the three blue-green algal species; exocellular and endocellular extract were investigated to evaluate toxins if present is acute or chronic hazard.

Toxicity Test:
2-l- Acute Toxicity Test:
2-l.1. Toxicity to Daphnia Magna: Acute toxicity test (Short-Term Toxicity) was carried out by exposure Daphnia magna (neonates 24 hrs old) to filtrate growth media of both Anabaena flos-aquae and Oscillatoria limnetica. Table (1) illustrated that, no change in number of D. Magna was observed during the exposure period. This elicited that, the filtrate growth media of A. flos-aquae, N. wichmannii and O. limnetica did not contain any signs of toxins.

2-l.2. Toxicity to Mice: Three mice for each extract of Anabaena flos-aquae, Oscillatoria limnetica and Nostochopsis wichmannii were used. Mice were injected intraperitoneal (ip) with 1 ml of the test extract solution which containing equivalent final concentrations per mouse of 1 mg of suspended cyanobacterial matter (45.5 mg/Kg mouse). Control mice were injected with 1 ml of the solution used for extraction contain only 0.9% NaCl. The injected animals were observed for different times up to 24 hrs. Behavioral symptoms and survival time were recorded in Table (2). The results showed that, the injection of both A. flos-aquae, N. wichmannii and O. limnetica extracts into mice did not yield any signs of neuro-as well as hepato-toxicity.

From the results of acute toxicity test, it can be concluded that; the exocellular toxins and endocellular toxins were not present in both three tested blue-green algae species.
Fig. 1: Changes in Morphological Shape of Blue-Green Algae Strains Exposed To Different Nitrogen and Phosphorus Ratios.
Table 1: Effect of Blue-Green Algae Exo-Toxin on Daphnia magna

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<tr>
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<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>* Anabaena flos-aquae*</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Nostochoposis wichmannii</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Oscillatoria limnetica</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
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</table>

Table 2: Toxicity of Cyanobacterial Extraction on Mice

<table>
<thead>
<tr>
<th>Organism Name</th>
<th>Neurotoxin (1-30 mins)</th>
<th>Hepatotoxin (45-240 mins)</th>
<th>Hepato+Neuro Toxin (15-30 mins)</th>
<th>(4-24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.9% NaCl only)</td>
<td>NE*</td>
<td>NE</td>
<td>NE</td>
<td>NT**</td>
</tr>
<tr>
<td>* Anabaena flos-aquae*</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>Nostochoposis wichmannii</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td></td>
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<tr>
<td>Oscillatoria limnetica</td>
<td>NE</td>
<td>NE</td>
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* NE = No Effect **NT = No Toxins

2-P. Chronic Toxicity Test: To evaluate the chronic toxicity (Long-Term Toxicity) of cyanobacteria, the mouse has been used as the experimental model, since the liver and kidney function of mouse are very similar to humans. In these experiments oral administration of filtrate growth media of Nostochoposis at maximum growth of three N:P ratios, namely; 0 N:1 P, 1 N:1 P and 16 N:1 P. Dry weight of Nostochoposis at this growth stage were 489, 416 and 361 mg/L, respectively.

2-II.1. Changes in Mice Body Weight and External Features: The results of body weight measurements are recorded in Table (3). The body weight data showed all groups of mice gain weight reaching 33-40 gm after 40 days of oral administration.

Control mice ended the experiment without any visible injury or disability. Visual observation of the mice receiving water of cyanobacterial growth media did not show any signs of symptoms during the first 29 days of tested water uptake. At the end of experiment (30-40 days), symptoms by inspection showed that all the groups became hyperactive and severely agitated and scratched each other (Fig. 2).

It may be worthy to note that, the filtrate of Anabaena flos-aquae, and Oscillatoria limnetica did not affect all mice groups.

2-II.2. Gross Anatomy: Postmortem examination failed to show changes in gross morphology or appearance in any organ (Fig. 3). Particular care was taken in looking of liver. Pale necrotic areas and areas of gross erythrocyte congestion of liver were seen by naked eye (Fig. 4). No liver tumor was observed in any mice livers. There were significant changes in mice liver weight drunk cyanobacterial filtrate. However, liver weight decrease than control by 4.6, 8.2 & 14.2% respectively for 0 N:1 P, 1 N:1 P and 16 N:1 P. This is indicated that, the magnitude of blue-green alga Nostochoposis wichmannii changes according to change in nutrient enrichment (Table 4).

Discussion: Harmful algal blooms (HABs) are frequently reported in many countries around the world. Even though this phenomenon has been known for a long time, the causes of HABs and toxicity releases are still not understood. Effective management of HABs of cyanobacteria requires an understanding of both the environmental factors associated with their formation and the effectiveness of available management alternatives for a given body of water. Toxins produced by cyanobacteria have been reported in marine as well as freshwater environments throughout the world. Blooms formed by cyanobacteria produce not only cells accumulation, but also an increase in toxins concentrations to levels hazardous to humans or livestock. HABs can affect the quality of water bodies, thereby disrupting drinking water supplies, impacting recreational uses, and releasing toxins that can harm ecosystems and humans. HABs present a growing problem to water resource managers.

Our findings revealed that, the short term exposure to extracellular and endo-cellular of blue-green algae namely; Anabaena flos-aquae or Oscillatoria limnetica showing no signs of toxins for both Daphnia magna and Mice. This indicated that, both two tested algal organisms A. flos-aquae or O. limnetica did not contain any toxins. These results confirmed with that obtained from long-term exposure (40 days) of mice by oral administration of extracellular of A. flos-aquae or O. limnetica.
Fig. 2: Effect of *Nostochopsis wichmannii* Extraction on Mice

![Mice Drinks Tap Water](image1) ![Mice Drinks Water Containing Algal Extraction of *Nostochopsis wichmannii*](image2)

**a**- Mice Drinks Tap Water (Control)  
**b**- Mice Drinks Water Containing Algal Extraction of *Nostochopsis wichmannii*

Fig. 3: Gross Anatomy of Mice

![Control (Drinking Tap Water)](image3) ![Treated (Drinking Water containing *Nostochopsis wichmannii* Extract)](image4)

1- Control (Drinking Tap Water)  
2- Treated (Drinking Water containing *Nostochopsis wichmannii* Extract)

Fig. 4: Morphological Changes in Liver of Mice

**Table 3**: Chronic Toxicity of *Nostochopsis wichmannii* on Mice Body Weight

<table>
<thead>
<tr>
<th>Types of Drinking Water</th>
<th>Body Weight (gm)</th>
<th>Average (gm)</th>
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</thead>
<tbody>
<tr>
<td>Tap Water</td>
<td>34, 33, 32.5</td>
<td>33</td>
</tr>
<tr>
<td>0 N : 1 P</td>
<td>39, 36.5, 35.5</td>
<td>37</td>
</tr>
<tr>
<td>1 N : 1 P</td>
<td>37, 37.8, 36.6</td>
<td>37.1</td>
</tr>
<tr>
<td>16 N : 1 P</td>
<td>38, 38.2, 40</td>
<td>38.7</td>
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Ostrofsky and Forsyth et al.\textsuperscript{[21,22]} reported that the cyanobacterium Anabaena releases algal toxins (exotoxin), which reduces thoracic lumb beat frequency, the feeding rate reproduction of Daphnia pulex and D. Carinata. Jungmann\textsuperscript{[23]} found that, a fraction of an extract from Microcystis aeruginosa, which did not contain microcystin, was much more toxic to Daphnia than another fraction containing microcystins.

In contrast,\textsuperscript{[24]} have demonstrated that the toxicity of Microcystis strains to mice does not always coincide with the toxicity to Daphnia. Thorsrup and Christoffersen\textsuperscript{[25]} have shown that Daphnia can accumulate up to 24.5 μg/l of toxin/g dry weight; this amount is not toxic to fish which prey on Daphnia, but they can accumulate the toxins in their body.

The results of oral administration of Nostochopsis bloom extracellular to mice for long term period (40 days) showing no signs of toxins symptoms during the first days of receiving contaminated water by extracellular of Nostochopsis. However, at the end of experiment (30-40 days) the mice became agitated and scratched each other. In addition, the extracellular of Nostochopsis sp. bloom cause decrease in liver weight as well as pale and white patches of liver were seen be naked eyes. Also, data showed that, the body weight of all mice groups increased. These results raise concerns that the long term exposure to even very low levels of cyanotoxin may significant and could ultimately result in liver diseases.

In contrast,\textsuperscript{[26]} stated that the highest dose of Microcystis extract supplies showed evidence of a general effect in body weight reduction, which was not recovered during the trial. Also, they found no visible symptoms of gastroenteritis occurred, only the liver exhibited injury, as demonstrated by analysis of plasma for liver function indicators and by histopathology.

Conclusions and Recommendations:
1. It is greatest important to determine the effects of long-term exposure to low concentration of cyanotoxins in drinking water.
2. Further investigations are necessary to understanding the succeeding development of algal populations and the ability of cyanobacteria to survive under different condition.
3. More precise investigations of potential regulatory mechanisms of cyanotoxins biosynthesis require knowledge of the genes and enzymes involved.

**REFERENCES**