ORIGINAL ARTICLE

Use of Phytoplankton Enzyme in the Artificial Lake as Indicator of Pollution

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ABSTRACT: Phytoplankton is important in all environments as it is the primary basis for nutrition. Scientists used it in various fields, including evidence of pollution. Therefore, the current study was conducted to investigate phytoplankton in the tourist lake of Baghdad Island in 2019-2020 to study the distribution of phytoplankton and study the antioxidant enzymes therein and the possibility of using enzymes as evidence of pollution. Monthly samples of surface water were taken from four sampling sites in the lake and results were presented as two dry and wet seasons. A total of 179 algae species have been identified with diatoms dominating. The highest total number of phytoplankton cells in the fourth site was 335 × 10⁴ cells / L in the 2019 dry season, while the lowest was 85 × 10⁴ cells / L in the third site during the 2019 dry season. These results showed the dominance of diatoms among other algal cultivars. They reduced the total number of Chlorophyceae and Cyanophyceace in the lake. The enzymatic study showed an increase in the effectiveness of NADH-nitrate reductase enzymes in the lake during the wet seasons and reduce in enzyme effectiveness during dry seasons, but the changes are not significant, which means more nitrates and less ammonia in the lake. This indicates the stability of environmental conditions, the low level of pollution in them, and the good management of the lake.

INTRODUCTION

Phytoplankton have an important role in the nutrient cycle in aquatic systems. It plays an essential role in maintaining the balance between living organisms and abiotic factors [1, 2]. Phytoplankton have the ability to adapt to various environmental conditions and have a short life cycle and reproduce at high rates. Therefore, it has been used as a warning and monitoring agent for different environmental systems [3-5]. Several researchers mentioned the importance of phytoplankton as a vital indicator in various aquatic ecosystems [4, 5]. Phytoplankton is used in many studies to indicate an environmental condition. [6] He studied the comparison between two lakes (Lakes Fort and Wadral) in Nigeria. The species composition of plankton and the environmental factor found that Lake Fort changes to an eutrophic state. In contrast, another lake was less polluted. [7] It was found that Uzuncayir Dam Lake (Turkey) moderately indicates organic pollution according to their study of phytoplankton. [8] He emphasized the importance of studying the ecological parameter with biological components of aquatic systems (eg. phytoplankton). Despite the important link between large halocarbon emissions from phytoplankton and the state of aquatic communities, only a few studies have been conducted to determine the nature of operational outsourcing.

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involved. Experiments on the formation of bromoform by various diatoms from temperate and subtropical (warm water) and polar regions. Based on finding (that diatom Porosira glacialis releases HOBr and HOI into surrounding seawater, it was concluded that the enzyme is extracellular and most likely present in the apoplast, which is the distance between the cell membrane and freestified. The attempt to determine the nature of the prosthetic group in BPO was inconclusive. However, 0.5 mM H2O2 actually impeded activity. This again strongly indicates that this enzyme is heme peroxidase. So, it is also assumed that other diatoms that have been shown to produce bromoform contain heme peroxide instead of VBPO). In a study conducted by [9] clarifying the relationship between phytoplankton enzymes and evidence of pollution, nitrate reduction is a useful tool where a positive result indicates the use of nitrates and a negative result indicates either growth in ammonium or nitrogen depletion. Growth with other N sources such as nitrites, urea, or amino acids. The enzyme assay appears to be particularly useful for studying the phytoplankton reproduction timeline because it provides a sensitive measure of the initiation and stopping of nitrate uptake.

Therefore, the current study was conducted to investigate phytoplankton in the tourist lake of Baghdad Island in 2019-2020 to study the distribution of phytoplankton and study the antioxidant enzymes therein and the possibility of using enzymes as evidence of pollution.

MATERIALS AND METHODS

Study area

There are many artificial lakes in Iraq, but the tourist lake of Baghdad Island is one of the most attractive lakes for tourists, which contains a water surface, Figure 1. Supplied with water from the Tigris River. As a result of the large number of visitors to the lake and its tourist importance, continuous monitoring of the lake's waters is required. Four sampling sites were taken. The first is at the entrance to the lake, and the second site is located on the northern side of the lake, and the third site is located in the Burj area in the center of the lake, and the fourth site is on the southern side of the lake Table 1.

![Site of sampling](image)

**Figure 1.** Sampling sites on Baghdad Touristic Island Lake, Baghdad-Iraq

<table>
<thead>
<tr>
<th>Site</th>
<th>Longitude (eastwards)</th>
<th>Latitude (northwards)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>44° 19'47.4</td>
<td>33° 22'36.6</td>
</tr>
<tr>
<td>S2</td>
<td>44° 20'32.4</td>
<td>33° 46'39.6</td>
</tr>
<tr>
<td>S3</td>
<td>44° 09'25.2</td>
<td>33° 12'247</td>
</tr>
<tr>
<td>S4</td>
<td>44° 19'47.4</td>
<td>33° 22'366</td>
</tr>
</tbody>
</table>
Sample collection

Phytoplankton samples were collected monthly from four sites in the study area (Table 1) from July 2019 to February 2020 and represented as dry and wet seasons. The quality of phytoplankton was estimated depending on APHA [10], taken a 1L of each sample and preserved by a Lugol’s solution (1:100) ml in a Duran cylinder of a 1L then left to settle for (10-15) days and concentrated the sample to 100 ml finally repeated the process to reach (10 ml). A light microscope was used to identify phytoplankton species (with a power of magnification 400×1000 x). The permanent slides were prepared using a concentrated nitric acid, for diatoms clarification, then classified by utilizing permanent slides [11]. The method of micro- transect was utilized for counting of diatoms(100x) [12-15]. Algal species were identified according to [16], while non-diatom algae were diagnosed by preparing temporary slides and examined at (40x), according to Furet and Benson-Evans [17].

Enzyme assay

The efficacy of the nitrite reductase enzyme was measured by taking a sample filter using glass fiber filters measuring 4.25 cm Whatman GF / C. Then taking a filter pad and adding to it 3.3 ml of a 0.2 M phosphate solution, the pH was 7.9, 1.0 mM dithiothritol with 5-20 mg poly Phenylpyrrolidone is dry and milled in an ice bath. This was then centrifuged for 5-10 minutes at approximately 2000 × g. The supernatant was immediately used as the crude enzyme source. One ml of the enzyme extract was incubated for 30 minutes with NRDH (final concentration, 100-150 pm), MgSO (30-100 mm>), and KNOR (5-10 mm) in a total volume of 1.8 ml at 20-24 °C. The reaction was stopped by adding 5 mL 95% cold ethanol and 0.2 mL of 1 M zinc acetate. The reaction mixtures were then centrifuged. The protein in the ethanol deposits was determined, Cultivation enzyme activity based on protein in extract.

RESULTS AND DISCUSSION

The results of Table 2 show the phytoplankton species that were detected in the lake during the study period. This is necessary to study their community structure for monitoring aquatic ecosystems [7, 18]. A total of 179 algae species have been identified in the lake (Table 2). The algae composition consists of Bacillariophyceae (three classes), Chlorophyceae, Cyanophyceae, Zygmatophyceae, Pyrrhophyceae, Cryptophyceae Euglenophyae, and Chrysophyceae. The results of the present study showed that the total number of phytoplankton in the lake recorded the highest value (335 × 104 cells / L) of phytoplankton observed in the dry season at the Tigris River (S4). In contrast, its value was 225 x 104 cells /Lin th the studied lake (S1). This increase responds to the influence of environmental factors such as length of daylight, intensity of illumination temperature values and decomposition activities [17, 19]. The lowest value at river and lake was 137 x 104 cells /L, 85 x 104 cells / L, respectively, in wet seasons and dry season. Ismail [20] noted these results, while other studies [21] observed an increase during the rainy season. The dominance of diatoms in Iraq’s inland water ecosystems are considered a common phenomenon [22]. This diatoms ability comes from its live and resistance to the different environmental conditions, besides to the availability of silica in the Iraqi water, which the diatoms use in their frustule structure [23], for that the diatoms existed throughout the study period and formed the majority of the total number in all stations.

Cosinodiscophyceae group followed by Bacillariophyceae and was recorded 11×104 cell/L (12%) in the Lake and 25×104 cell/L (11%) in the River. This study showed that the pinnate diatoms (Bacillariophyceae) were dominated on the central diatom (Cosinodiscophyceae). The species Aulacoeira italica that related to Coscinodiscophyceae recorded in the River 14.11×104 cell/L (6.23%), While the species A. granulata recorded 14.95×104 cell/L (6.57%) and these results were agreed with other previous studies (17-20). When the pinnate diatoms (Bacillariophyceae) dominate on the central diatoms (Cosinodiscophyceae),
that indicated water healthy, while the dominance of centric diatoms evidence disturbances in the surface of the water [24, 25]. Fragillariophyceae was represented 8% (12×10^4 cell/L) at River and 7% (11×10^4 cell/L) in the Lake.

Table 2. Showed the Total number and percentage of phytoplankton in the present study

<table>
<thead>
<tr>
<th>Classes</th>
<th>Dry 2019</th>
<th>%</th>
<th>Wet 2020</th>
<th>%</th>
<th>Dry 2019</th>
<th>%</th>
<th>Wet 2020</th>
<th>%</th>
<th>Dry 2019</th>
<th>%</th>
<th>Wet 2020</th>
<th>%</th>
<th>Dry 2019</th>
<th>%</th>
<th>Wet 2020</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACILLARIOPHYCEAE</td>
<td>178</td>
<td>80</td>
<td>99</td>
<td>75</td>
<td>76</td>
<td>75</td>
<td>106</td>
<td>73</td>
<td>60</td>
<td>71</td>
<td>117</td>
<td>74</td>
<td>237</td>
<td>78</td>
<td>113</td>
<td>71</td>
</tr>
<tr>
<td>FRAGILARIOPHYCEAE</td>
<td>13</td>
<td>6</td>
<td>12</td>
<td>8</td>
<td>8</td>
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<td>5</td>
<td>5</td>
<td>10</td>
<td>6</td>
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<td>7</td>
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<td>7</td>
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<td>11</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>7</td>
<td>10</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>45</td>
<td>11</td>
<td>9</td>
<td>6</td>
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<tr>
<td>Cyanophyceae</td>
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<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
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<td>Dinophyceae</td>
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<td>Cryptophyceae</td>
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<tr>
<td>Zygmematophyceae</td>
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<tr>
<td>Total</td>
<td>225</td>
<td>100</td>
<td>137</td>
<td>100</td>
<td>103</td>
<td>100</td>
<td>146</td>
<td>100</td>
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<td>161</td>
<td>100</td>
<td>335</td>
<td>100</td>
<td>162</td>
<td>100</td>
</tr>
</tbody>
</table>

Chlorophyceae was the dominant group after Coscinodiscophyceae by recording 17×10^4 cell/L (11%) in the Lake, while its value in the Tigris River was 8×10^4 cell/L (5%) also this results noticed by [20]. While in Ismail Study [20], it presents more than 20%. The percent of Cyanophyceae reduced from 15.2% (20) to 4% in the present study. Cyanophyceae was recorded 5×10^4 cell/L in the River and 4×10^4 cell/L (5%) in the Lake. The reduction in the total number of Chlorophyceae and Cyanophyceae in the Lake is indicated to the alteration in the trophic status of the Lake that is related the lake management.

Another group, such as Pyrrhophyceae, was recorded 1×10^4 cell/L (1%) in the River and 3×10^4 cell/L (2%) in the Lake. Dinophyceae, Cryptophyceae and Zygmematophyceae were noticed in the Lake only. The phytoplanktonic groups Pyrrhophyceae, Cryptophyceae Euglenophyceae, Chrysophyceae found in the Lake and River but with a low value as showed in Table 2.

Some phytoplankton species were characterized by their presence in most dry and wet seasons, such as A. pediculus, Craticula cuspidate, Staurosira construens, Cosinodiscus spp, C. botrytis, Phacus caudatus and Ceratium hirundinella (Figure 2). The dominant group of River’s phytoplankton was the Bacillariophyceae, that represented by 182×10^3 cell/L (80%), also noticed that dominance in the results of the sites in the Lake, which recorded 273×10^4 cell/L (78%), that dominance due to its rapid response to changes in physical, chemical and biological factors in the aquatic ecosystem, as well as a great capacity for growth and reproduction in the presence of various environmental variations such as high temperature, the intensity of light, salinity and plant nutrients [26, 27] and that capacity to resistance changes environmental conditions its possession of a siliceous wall [28-31]. Bacillariophyceae dominance is identical to previous studies conducted on Tigris River and Lake [19, 20].
The results of Table 3 indicate phytoplankton and starch metabolism activity. Which increases in the wet season, where the highest value was recorded 3.8 *10^-D moles NO-2 formed per µg in S1. While the lowest value was recorded in the dry season 0.3 *10^-D moles NO-2 formed per µg in S4.

A variation in the activity of the enzyme was also observed during the two years of the study, as in 2020 it was higher than in 2019, and this may be due to the increase in the water supply of the Tigris River.

The general use of the enzyme assay is based on the ability of phytoplankton to assimilate both NIL + and N03-, induction of enzyme synthesis with nitrates, and to suppress it during ammonium uptake. The ability to use amino acids or urea as a source of nitrogen, and the ability to suppress NADH-nitrate reductase during this absorption.

Table 3. Activity of NADH-nitrate reductase from Baghdad Touristic Island Lake activity as 10^-D moles NO-2 formed per µg

<table>
<thead>
<tr>
<th>sites</th>
<th>Dry 2019</th>
<th>Wet 2019</th>
<th>Dry 2020</th>
<th>Wet 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>s1</td>
<td>1.1</td>
<td>1.3</td>
<td>0.9</td>
<td>3.8</td>
</tr>
<tr>
<td>s2</td>
<td>2</td>
<td>1.6</td>
<td>1.6</td>
<td>3.5</td>
</tr>
<tr>
<td>s3</td>
<td>1.9</td>
<td>1.2</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>s4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The results indicate the dominance of feathery diatoms. And the lack of numbers of Chlorophyceae and Cyanophyceae. The balance of Bacillariophyceae was observed among other algal groups. The activity and activity of the enzyme NADH-nitrate reductase was high and positive. This indicates the activity of the lake, that it is good in environmental terms, and that the lake has not been directed to eutrophication.

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Conflict of interest

The author declares no conflict of interest.
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