

Assessment of Eutrophication and Cyanobacterial Occurrences in a Freshwater Lake, in Turkey

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Abstract

Cyanobacterial dominance in lakes has received much attention in the past because of frequent bloom formation in lakes of higher trophic levels. Study area (Lake Kovada) has been used for recreational purposes; fishing, swimming, garden watering etc. As far to our knowledge, cyanobacterial occurrence, especially *Microcystis aeruginosa* dominance in the lake never has been reported before. Hence intracellular microcystin analyses were performed by immunoassay from filtrate samples where *M aeruginosa* observed high in numbers. In total eighteen species belonging to 10 genera were identified in the lake. Some indicators of water quality in Lake Kovada were also measured in order to characterise the trophic status of the lake. An average of 6 months for soluble reactive phosphate analysis was measured as $109.8 \pm 0.04 \mu\text{g l}^{-1}$. These findings are even exceeding the limits for total phosphate concentration to control cyanobacterial bloom and mean ammonia was detected $668 \pm 58 \mu\text{g l}^{-1}$. Water quality criteria for free NH_3 was suggested to be $20 \mu\text{g l}^{-1}$ by USEPA. According to the results which were obtained from the water quality analyses, the lake water is polluted and under risk of being highly populated with cyanobacteria. The effective monitoring programmes for the reduction of cyanobacterial toxin problems in waters require the identification and use of appropriate guidelines worldwide.

Keywords: Cyanobacteria, Eutrophication, Lake Kovada, *Microcystis aeruginosa*, Water quality analyses.

INTRODUCTION

The production of high persistent concentrations of biomass is closely linked with eutrophication of lakes. It is clear that the increased input of nutrients is the prime cause of the heavy selective pressure on the phytoplankton; it is the system as a whole which determines the final result of this process. When lakes become more eutrophic, the diversity of the phytoplankton assemblage decreases ultimately leading to the dominance of cyanobacteria [1]. The relative abundance of cyanobacteria increases, while first chrysophytes and cryptophytes, and then chlorophytes and diatoms, diminish in importance consequence of eutrophication [2]. Moreover, cyanobacteria are highly successful due to a number of physiological adaptive features and become visible under favourable weather conditions, forming surface scums [3]. Associated with the dominance of cyanobacteria are several negative effects, such as reduced transparency, decreased biodiversity, elevated primary production and the potential occurrence of oxygen depletion, which may result in massive fish kills, odor and taste compounds, as well as production of toxins [4]. One of the most important groups of cyanobacterial toxins, due to their widespread occurrence and high toxicity, are the microcystins (MCs). MCs can be produced by species of *Microcystis*, *Anabaena*, *Nostoc*, *Planktothrix* and several other cyanobacterial genera [5, 6]. Toxic cyanobacterial blooms have

been described in numerous countries [6-9] and several cases of animal and human intoxication have been reported [10,11, 12].

Despite considerable research summarized in Schreurs (1992), the reasons for such outbreaks largely remain unclear [13]. Some changes in environmental conditions, in summer or in autumn may allow the rapid growth of cyanobacteria to dominate population among phototrophic microorganisms. Some studies insist on the importance of temperature [14,15,16], others favour illumination [17] or the concentration of nutrients, such as phosphorus [18,19] and nitrogen [20] (Sivonen, 1990). Others highlighted the importance of the ratio between phosphorus and nitrogen [21, 22]. On the other hand, Ferber et al (2004) expressed the importance of ammonium acquirement [23]. The study has proved that phytoplankton (98% dominated by cyanobacteria) acquired N primarily as ammonium and secondarily as nitrate. Cyanobacteria are commonly observed in eutrophic water bodies and the frequent dominance by cyanobacteria in eutrophic waters troubled water reservoirs used for drinking-, irrigation-, and recreational water constitute a potential risk of public health for many populations.

In the study, physicochemical and biological data were measured to assess the eutrophic level due to dominance of cyanobacteria in the study lake. Here we tested NO_3^- -N; NH_3 -N, and soluble reactive phosphate (PO_4^{3-} -P) instead TP and TN.

MATERIALS AND METHODS

Environmental Sampling And Measurements

Sampling was done between May -June 2005. Six sampling points on Lake Kovada, K1 - K6 were used throughout. (Figure1). Water temperature, pH, conductivity, and dissolved oxygen were measured directly in situ using a multi-parameter probe (WTW model). Water transparency was measured with a Secchi disc (20 cm in diameter). For nutrient analysis, raw water samples were collected from fifty centimetres below the water surface. These samples were shaken vigorously and aliquots were then used for the analysis of nitrate (NO_3^- -N) [24], ammonia (NH_3 -N) [25] and orthophosphate (PO_4^{3-} -P) [24, 25]. Phosphorus was measured by the ascorbic acid method. Concentrations of NH_3 and NO_3^- were determined using the Nessler and sulfosalicylic acid reaction methods, respectively. Qualitative phytoplankton samples were obtained by the filtration of lake water using a plankton net (25 μm mesh size). Some of the samples were separated for cyanobacterial strain isolation and the rest were preserved with formaldehyde (final concentration 4% v/v). For chlorophyll-*a* analysis, 500 ml sub-samples of water stored in the dark until analyses which were carried out according to Richard and Thomson [26] (Eq1).

$$\text{Chlorophyll-}a \text{ (mg/L)} = \frac{v(11,6 \times D_{665} - 0,14 \times D_{630} - 1,31 \times D_{645})}{I \times V} \quad \text{Eq1}$$

v = Volume of acetone extract (ml)

I = Path length of cuvette (cm)

V = Volume of water sample (litres)

Morphological Characterization of Cyanobacteria

Phytoplankton samples preserved in 4% formalin and The strains were identified using taxonomic keys, based on the classification systems of Geitler (1932) [27], Prescott (1973) [28], Anagnostidis and Komárek [29] (1988), Baker (1992) [30], and John et al. (2002) [31]. Cyanobacterial genera and species were identified by microscopic (Zeiss) observation of distinguishing morphological characters cited in the literature.



Figure 1. Lake Kovada and sampling locations on the Lake

Statistical Analysis

Statistical analysis of field data was used in order to reveal relationships of potential significance, using SPSS 10.0 for Windows (Chicago, IL, USA).

RESULTS

Physico-Chemical Data

The Secchi depths never exceeded 68cm and especially, they were recorded below 40cm in July at all sites. The water temperatures were at all sampling dates above 20°C. Mean of monthly temperature measurements was found to be 19.5±4.3. Values of pH between 6.13 and 9.98 were measured and mean pH was 8.6±0.46. The differences in pH values were not significant ($p>0.05$). Conductivity was between 333 and 411 $\mu\text{mhos cm}^{-1}$ and mean conductivity was measured as 357±20.17 $\mu\text{mhos/cm}$. The conductivity results varied significantly in monthly averages. The remaining variables including dissolved oxygen, orthophosphate, chlorophyll-*a*, varied significantly ($p<0.05$). Dissolved oxygen was between 6.8 and 12.6 and monthly average was detected as 9.1±1.5 mg l^{-1} . Dissolved oxygen was low at all sites in May and August. Especially it was recorded below 8 mg l^{-1} at K4 due to high density of water plants at this site. Fig 2. presents physico-chemical conditions of parameters in the Lake, May to November 2005.

Downing et al. (2005) indicated that cellular microcystin quotas were positively correlated with both nitrate uptake cellular nitrogen content[32]. Total nitrate concentrations ranged from 0.81 to 1.42 mg l^{-1} and mean nitrate was 0.951±0.3 mg l^{-1} . The Duncan analysis of variance showed that the nitrate concentration didn't differ monthly ($p>0.05$) (Table 1). Soluble reactive phosphorus (SRP) concentrations were between 0.039

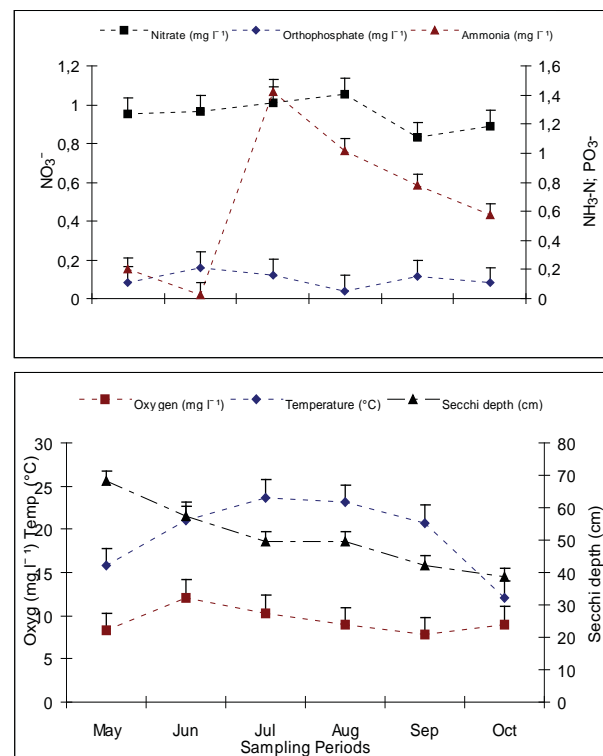


Figure 2. (a) Nitrate, Total Ammonia and SRP (mg l^{-1}) concentrations (b) temperature, Secchi depth and oxygen in Lake Kovada between May and October 2005 ($n=3$)

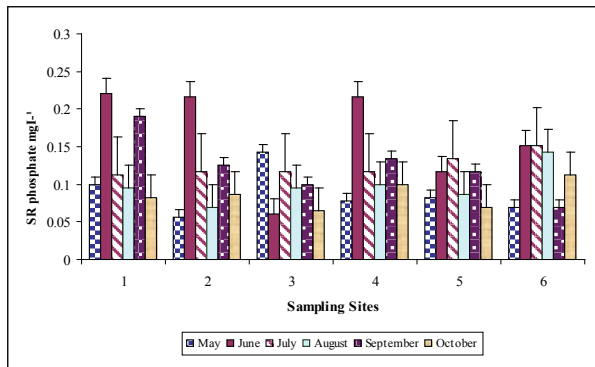


Figure 3. SRP (mg l⁻¹) concentrations at the sampling sites (K1, K2, K3, K4, K5, K6) (n=3)

and 0.160 mg l⁻¹ in monthly averages whereas it reached 0.220±0.015 mg l⁻¹ at site K1, and were over 0.20 at sites K2, K4. In case of study stations, the concentration SRP was slightly higher in June (around 200 µg l⁻¹) (Figure 3). Mean ammonia was detected 0.668±0.058 mg l⁻¹, and the level of NH₃ reached the highest concentration in July (2.23 mg l⁻¹ at K1 sampling location) and decreased down slightly in August due to increase in cyanobacterial biomass.

Chlorophyll-*a* concentration varied with in range of 6.33-60.66µg l⁻¹. The highest chlorophyll-*a* concentration recorded at site K4 in August. Mean chlorophyll-*a* concentration of all

sampling sites in August was 40.44 µg l⁻¹. Duncan analysis of nutrients and biomass were presented at Table 1.

Identification of Cyanobacteria

Cyanobacteria were identified to species level where possible and their abundance at lake study sites during the sampling period is presented in Table 2. Eighteen species belonging to 10 genera were identified in the lake.

Microcystin Assessments

Filtrates were extracted with methanol and analysed with commercial ELISA (Envirogard) kit (R² = 0.9974). Intracellular microcystin concentration of filtrate samples belonging three stations (K1, K4, K3) were given in Figure 4.

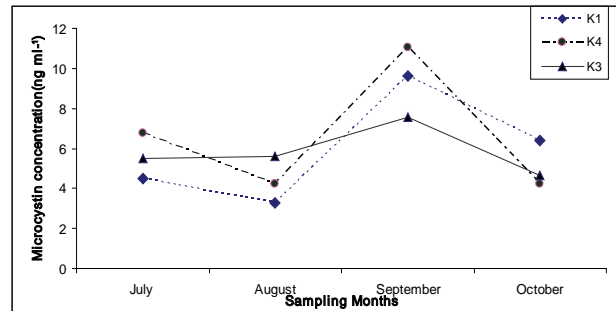


Figure 4. Intracellular microcystin concentration (ng ml⁻¹) of the filtrate samples (K1, K3, K4) (n=6)

Table 1. Mean Concentration of Nutrients and Biomass by Duncan Analysis (n=3)

Sampling duration	Nitrate (mg l ⁻¹)	SRP (mg l ⁻¹)	Ammonia (mg l ⁻¹)	Chlorophyll- <i>a</i> (µg l ⁻¹)
May	0.953a	0.084c	0.201d	8.200d
June	0.968a	0.160a	0.016d	9.888d
July	1.010a	0.121b	1.424a	14.850c
August	1.055a	0.039bc	1.017b	40.440a
September	0.831a	0.117b	0.778bc	22.810b
October	0.889a	0.081c	0.575c	10.961d

* none significancy in mean concentrations at the columns with same letters indicating (p<0,05).

Table 2 . Cyanobacterial Taxonomy of Lake Kovada

Phylum	Cyanobacteria						
Class	Cyanophyceae						
Order	Chroococcales			Nostocales	Oscillatoriales		
Family	Chroococcaceae	Synechococcaceae	Merismopediaceae	Microcystaceae	Nostocaceae	Phormidiaceae	Oscillatoriaceae
	<i>Chorococcus limneticus</i>	<i>Synechococcus elongatus</i>	<i>Synechocystis sp.</i>	<i>Microcystis aeruginosa</i>	<i>Anabaena affinis</i>	<i>Phormidium articulatum</i>	<i>Oscillatoria limnetica</i>
	<i>Chorococcus minimus</i>				<i>Anabaena flos-aquae</i>	<i>Phormidium formosum</i>	<i>Oscillatoria sancta</i>
					<i>Anabaena spiroides</i>	<i>Phormidium nigrum</i>	<i>Oscillatoria subbrevis</i>
					<i>Aphanizomenon flos-aquae</i>	<i>Phormidium limosum</i>	<i>Planktothrix sp</i>
Species							<i>Pseudoanabaena catenata</i>

DISCUSSIONS

Eutrophication has been caused by the enrichment by nutrients such as phosphates and nitrates in the water and hence favoured blooms of cyanobacteria [33]. It is accepted that a combination of factors such as increasing temperatures, decreasing nutrients and increased water column stability may be responsible for the growth of cyanobacteria in large quantities [34]. The quantifying nutrients in water body, is important to acquire information about the Lake conditions. Therefore in the study, some physical conditions (Water temperature, pH, conductivity, dissolved oxygen, conductivity, and transparency), nutrients (nitrate, ammonia, orthophosphate) and chlorophyll *a* were investigated.

Mean Secchi depth was 51.03 ± 17.3 cm and this indicated eutrophic lake conditions [35, 36]. Phytoplankton can take up inorganic dissolved nitrogen in the form of nitrate, nitrite and ammonia. In some arid continental regions, nitrogen is found to be the chief factor limiting phytoplankton growth [37]. Kotak et al. (1995) found that there was a negative correlation between microcystin-LR concentration in water, due to *Microcystis aeruginosa* bloom, and nitrate concentration [38]. Ferber et al. (2004) proved that cyanobacteria acquired N primarily as ammonium (82–98%), and secondarily as nitrate (15–18% in spring and autumn, but <5% in summer) and ammonium and nitrate concentrations were substantial (472.8 mg l^{-1} and 99.2 mg l^{-1}) when the study began in May, but were greatly reduced (to 26.1 mg l^{-1} and 0.6 mg l^{-1}) by the first cyanobacterial bloom in June [23]. In the study, decreased ammonia level was assessed with the increased cyanobacterial growth in August while chlorophyll *a* concentrations at different sampling area were detected to below in July. This outcome has supported by the results of Ferber et al. [23]. From the pollution point of view, the level of free ammonia was detected 15-17 folds in July at some cases higher than the recommended concentration (0.02 mg l^{-1}). McNeely et al. (1979) suggested that natural waters contain concentrations of total ammonia, less than 0.1 mg l^{-1} [39]. Water quality criteria for free NH_3 were suggested to be 0.02 mg l^{-1} or less depending at pH ratio [40].

Phosphate is one of the key nutrients for cyanobacterial increase in lakes. To minimize the risk of cyanobacterial bloom total phosphate concentration was suggested to be kept below $10 \text{ } \mu\text{g P l}^{-1}$. Chorus et al. (2001) reported that if total P concentrations (not only those of soluble P or orthophosphates) are below $10\text{--}20 \text{ } \mu\text{g P l}^{-1}$, mass developments of cyanobacteria are unlikely to happen, and if high turbidity occurs, it may have other causes [41]. In our study SRP concentrations were ranged between 39 and $160 \text{ } \mu\text{g l}^{-1}$ monthly whereas SRP concentration of the sampling stations was slightly higher in June (around $200 \text{ } \mu\text{g l}^{-1}$) (Figure 3). Phosphorus is an indicator of nutrient enrichment and can cause algal blooms or eutrophication in severe situations. Concentrations above 0.1 mg l^{-1} in water may promote slime and algal growths, which affect recreational uses. An average of 6 months for SRP analysis was measured as $109.8 \pm 0.04 \text{ } \mu\text{g l}^{-1}$. These findings are even exceeding the limits for total P concentration to control cyanobacterial bloom. Hence chlorophyll *a* measurements were conducted during the study in monthly. Peak values of chlorophyll *a* for an oligotrophic lake are about $1\text{--}10 \text{ } \mu\text{g l}^{-1}$, while in a eutrophic lake they can reach $300 \text{ } \mu\text{g l}^{-1}$. In cases of hypereutrophy, such as Hartbeespoort Dam in South Africa, chlorophyll *a* can be as high as $3,000 \text{ } \mu\text{g l}^{-1}$ [42]. Most *Microcystis* blooms are found in lakes with an

average summer chlorophyll *a* concentration of $20\text{--}50 \text{ } \mu\text{g l}^{-1}$ and a Secchi transparency of 1-2 m [36]. We detected the highest chlorophyll *a* concentration as $60 \text{ } \mu\text{g l}^{-1}$ at K4 sampling location. In August at all sampling location chlorophyll *a* concentrations were detected over $37 \text{ } \mu\text{g l}^{-1}$. Chlorophyll *a* results also suggested that there was a risk of cyanobacterial bloom. A provisional guideline was suggested with the dominance of cyanobacteria, approximately $50 \text{ } \mu\text{g chlorophyll-a/litre}$, represents a guideline value for a moderate health alert in recreational waters [36, 43].

Overall a positive correlation of chl-*a* with nitrate ($p < 0.05$), ammonium, ($p < 0.01$), temperature ($p < 0.01$) and pH ($p < 0.05$) was found, whereas a negative correlation was detected with Secchi depth ($p < 0.05$) and oxygen ($p < 0.01$). Monthly differences in nitrate concentration found not significant ($p > 0.05$) whereas the mean concentration of ammonia was significant ($p < 0.05$). Ferber et al. (2004) suggested total phosphate and nitrogen regulated algal biomass since they found significant correlations of chlorophyll *a* with these nutrients [23]. Albay et al. (2005) found that the development of an *M. aeruginosa* bloom and the production in the Küçük Cekmece Lagoon resulted from high nutrient availability, high light intensity and stability of the water column together with optimal surface water temperature ($> 24^\circ \text{C}$) [9]. Robarts and Zohary (1987) found that *Microcystis* was severely limited at temperatures below 15°C and were optimal at temperatures around 25°C [34]. Temperature changes were found to induce variations in both the concentration and peptide composition of the toxin [44]. In this study water temperatures were detected 26°C and over at all sampling locations in July. Increase in growth of *Anabaena* spp. was occurred June and July due to higher SRP (please see Figure 3) concentration and *M. aeruginosa* bloom occurred in August due to high ammonia level in July. [45] Forsberg and Ryding (1980) were suggested The lowest N:P ratios, closer to the critical range of 16:1, occur in lakes dominated by nitrogen-fixing cyanobacteria in summer (Müggelsee: 70% cyanobacteria, mainly *Aphanizomenon flos-aquae*.) Later Smith [46] promoted the hypothesis by showing that the relative abundance of cyanobacteria in 17 north temperate lakes correlated negatively with total N : total P (TN : TP) ratio. Dokulil and Teubner [47] were indicated that non nitrogen fixing cyanobacteria *M. aeruginosa* were dominant in lakes with TN:TP ratios much higher than the optimum ratio of N:P=16:1 and suggested that factors causing the dominance of one or the other group are often difficult to reveal because several interacting factors are usually involved. Recently, Davis et al (2010) demonstrated that N enrichment can promote blooms of *Microcystis* more frequently than P and those inorganic nutrients may favor toxic strains over those which cannot produce microcystin [48]. *Microcystis aeruginosa* has the widest distribution, forming blooms all over the world. This species is one of the best studied cyanobacteria in relation to toxins [49].

Many lakes with high trophic level is dominated by large, colony forming species of cyanobacteria such as *Microcystis*, *Plankothrix*, *Limnothrix*, *Anabaena*, or *Aphanizomenon*. If cyanobacterial occurrences become permanent those lakes regarded as critical phase of eutrophication world wide [50]. Excessive abundance or 'blooming' of cyanobacteria generally has detrimental effects on the domestic, industrial and recreational uses of water bodies because of their ability to produce toxins [51, 12]. In this study *M. aeruginosa* was found to be present in the lake from July till November. *M. aeruginosa* occurred in high numbers in August due to high ammonia level

in July. Highest concentrations of intracellular microcystin were recorded at September samples. This may be occurred due to decaying cells because chlorophyll-*a* concentration of water samples were recorded low in September. MC concentrations were found to be 11.2 ng ml⁻¹ at K4 sampling site; 8.60 ng ml⁻¹ at K1 sampling site; 5.55 ng ml⁻¹ at K3 sampling site in September samples (Please see figure 4). *M. aeruginosa* was suspected entering the Lake from Lake Egirdir due to K4 sampling area in front of the canal which brings excess water of Lake Egirdir. Lake Egirdir supports the main water supply of Isparta and Egirdir.

Increase in growth of *Anabaena* spp. was occurred June and mid July. Increase level of nitrogen source and over growth of *Microcystis* were suppressed the further growth of *Anabaena* in late July. The unicellular genus *Synechococcus* is one of the most studied, and geographically most widely distributed, cyanobacteria in the picoplankton. Toxicogenic strains of *Synechococcus* have been reported by [52]. In this study *Synechococcus elongatus* was another common species found all year round in the lake together with *M. aeruginosa*, *P. catenata*. Abundance of cyanobacterial species of Lake Kovada were previously presented by Gurbuz et al. [53]. Ammonia was found key nutrient in summers and triggering source for over growth of non- nitrogen fixing cyanobacteria as *M. aeruginosa* whilst increased SRP concentration triggered the increase of *Anabaena* growth. Therefore these two parameters need be to be controlled in water systems to prevent bloom formation. According to the results we obtained from the water quality analyses, the lake water is polluted and may under risk of being permanently occupied with cyanobacteria.

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