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Seasonal variation in cyanobacterial toxin production in two Nepalese lakes

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Introduction

Two subtropical lakes in the midhill region of Nepal, Phewa Tal and Begnas Tal, were sampled in 1997–1998 to determine whether cyanobacterial toxins showed a seasonal pattern in response to the summer monsoon and protracted dry period during fall–spring. An empirical relationship was sought between their limnological characteristics and toxin values. These warm monomictic lakes are the most studied lakes in Nepal (HICKEL 1973, LOHMAN et al. 1988, NAKANISHI et al. 1988, JONES et al. 1989, DAVIS et al. 1998) and the present study furthers the understanding of how they respond to the monsoon climate. Based on N:P mass ratios both lakes are seasonally nitrogen limited and support occasional cyanobacterial blooms.

Methods and materials

Water samples were collected from Phewa and Begnas Tals, Pokhara Valley, Nepal during the summer monsoon (August 1997–1998), during winter mixis (January 1997–1998) and during spring shortly after thermal stratification was re-established (March/April 1997–1998). Post-monsoon samples were also collected in November 1998 to better characterize the seasonal pattern. Additional samples were collected in March 2000. Both lakes were sampled at several sites on two to five sampling dates during a given sampling trip. Values from ≤3 m were averaged across sites and dates to arrive at a seasonal mean value used to represent lake characteristics during that particular collection. Subsamples (10 mL) for total phosphorus (TP) and total nitrogen (TN) analyses were transferred to acid-cleaned culture tubes; TN samples were acidified to stop bacterial activity. Digestion and analyses of TP and TN were performed in the original culture tube (Standard Methods 4500-P E, EATON et al. 1995, CRUMPTON et al. 1992, respectively). Chlorophyll (Chl) and net-Chl (>35 µm), corrected for phaeophytin, were extracted in hot ethanol and measured using a fluorometer (KNOWLTON 1984, SATORY & GROBBELAAR 1984).

Algal samples were collected for toxin analysis if algal cells that could be retained on 64-µm netting were present. The net was dragged through the water to concentrate algae, which were then placed into a 125-mL amber HDPE bottle. Samples were frozen and lyophilized, and stored at –80 °C. Algal samples (10 mg) were extracted three times for toxin analysis using deionized water. Samples were sonicated for 5 min at 35 °C before the first extraction, and were centrifuged for 10 min at 13,000 × *g* between extractions. Toxin concentration was determined by a microcystin enzyme-linked immunoassay method from Envirogard®. Plates were read on a Labsystems Multiskan MS 3.0 microplate reader. Toxin concentrations measured by this method include the microcystin variants –LR, –RR, –YR, as well as nodularin (herein referred to as microcystin).

Results

Climate

Annual rainfall at Pokhara averaged 3950 mm during 1984–1999, with 80% of the total during the June–September monsoon (Fig. 1). Monsoon rainfall in 1997 was 80% of the long-term average. Monsoon rainfall in 1998 was 125% of this average, largely because of several >100-mm storms coinciding with the lake collections. Temperatures in this subtropical location were near the long-term average during both years (Fig. 1).

Limnological characteristics

There was temporal variation in the limnological characteristics of Phewa Tal (Table 1) and Begnas Tal (Table 2). In Phewa Tal, TN values in fall and winter were 50% greater than the other seasons and were attributed to internal loading during fall destratification. Algal biomass responded to this nitrogen sufficiency; Chl

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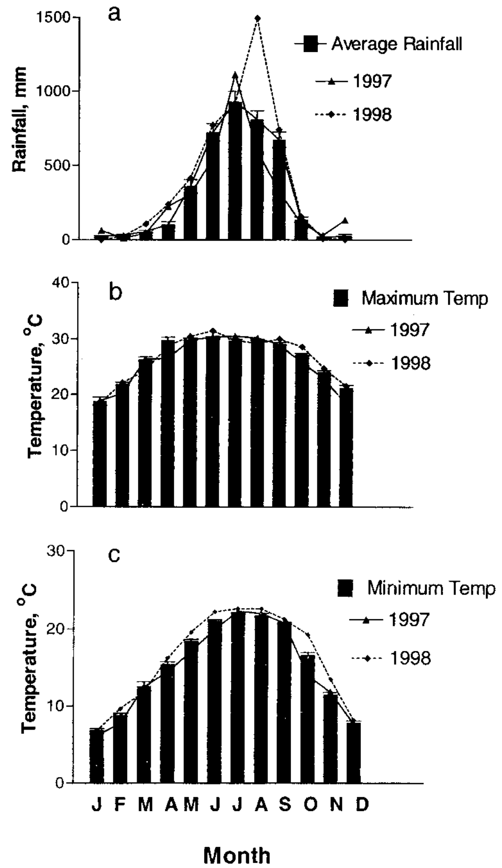

Fig. 1. (a) Rainfall, (b) maximum temperature, and (c) minimum temperature measured in Pokhara, Nepal during 1984–1999. Average monthly rainfall, maximum and minimum temperature from 1984 to 1999 (bars). Rainfall, maximum and minimum temperature for 1997 and 1998.

values during fall and winter were approximately double those of the other seasons, when TN:TP ratios (Table 1) suggest that phytoplankton were nitrogen limited. Little seasonal variation in TP was observed in Phewa Tal. Samples from summer 1998 were collected during peak inflow; 750 mm of rain were recorded in the valley during this period and lake water chemistry was influenced by external inputs and advective mixing. Values of TP declined from 63 µg/L at the start of field sampling to 24 µg/L within 10 days, presumably from sedimentation of particulate materials. During this period, non-volatile suspended solids declined from 30 mg/L to 6 mg/L. Samples from spring 2000 contained <30 µg/L TN, the lowest value reported for this lake.

In Begnas Tal, TN showed a strong seasonal pattern; values reached maxima in winter in conjunction with internal loading during fall destratification, then declined by about 30% in most spring samples and further declined to about 20% of the maximum during the monsoon (Table 2). An increase in TN in the fall 1998 sample relative to the monsoon was consistent with the internal loading, associated with weakening thermal stratification. Values of TP in Begnas Tal were similar to those in Phewa Tal and did not show strong seasonal variation. The smallest TN and TP values in Begnas Tal were during maximum inflows in the 1998 monsoon. Values of Chl did not closely follow nutrient levels in Begnas Tal, maximum Chl was measured in the fall and monsoon values were generally lower than in other seasons (Table 2).

Cyanobacterial toxins and empirical relationships

Microcystin in Phewa Tal was maximal in spring 1997 and 1998, at levels of 2–>100 times greater than measurements during the monsoon and fall/winter (Table 1, Fig. 2a). Values in spring 2000 were about 10% of previous spring measurements. No strong correlation was found between microcystin and nutrient parameters in Phewa Tal, but the correlation between microcystin and pH was significant (P = 0.015, Fig. 3). Phewa Tal has a modest buffering capacity (LOHMAN et al. 1988) and photosynthetic activity may have increased pH values during spring (PAERL 1988, SHAPIRO 1990).

Across seasons, microcystin values in Begnas Tal were over an order of magnitude larger than concurrent collections from Phewa Tal. The minimum microcystin value from Begnas Tal (262 µg/g, summer 1998) matched the maximum in Phewa Tal (spring 1997 and 1998). Microcystin in Begnas Tal was >2400 µg/g except during the monsoon and spring 2000, when values were <590 µg/g (Table 2, Fig. 2b).

Table 1. Nutrient and microcystin concentrations from Phewa Tal, Nepal, collected in winter (W), spring (Sp), and summer (monsoon; Su) 1997 and 1998, fall (F) 1998, and spring 2000. Total nitrogen (TN), total phosphorus (TP), Chl (chlorophyll) units are µg/L. Microcystin is expressed in µg toxin/g algae.

Season	TN	TP	TN/TP	Chl	Microcystin
1997					
W	238 ± 10	16.5 ± 0.7	14.5 ± 0.4	14.3 ± 1.3	22
S	126 ± 11	14.4 ± 1.3	9.2 ± 0.9	5.1 ± 0.3	245
Su	125 ± 16	18 ± 2	6.9 ± 0.3	8.5 ± 2.3	12
1998					
W	197 ± 16	12 ± 0.3	17.7 ± 0.5	15.5 ± 0.4	102
Sp	182 ± 3	13 ± 0.4	14.2 ± 0.4	6.8 ± 0.2	277
Su	208 ± 9	42 ± 3	5.2 ± 0.3	9.6 ± 1.0	2.2
F	226 ± 18	12.7 ± 0.9	17.6 ± 1.0	20.4 ± 2.4	3
2000					
Sp	25.5 ± 3.2	12.3 ± 0.4	2.0 ± 0.2	5.3 ± 0.3	27

Table 2. Nutrient and microcystin concentrations from Begnas Tal, Nepal, collected in winter (W), spring (Sp), and summer (monsoon; Su) 1997 and 1998, fall (F) 1998, and spring 2000. Total nitrogen (TN), total phosphorus (TP), Chl (chlorophyll) units are µg/L. Microcystin is expressed in µg toxin/g algae.

Season	TN	TP	TN/TP	Chl	Microcystins
1997					
W	745 ± 9	16 ± 0.7	48 ± 1	10.3 ± 0.8	2900
Sp	671 ± 9	17 ± 0.4	39 ± 1	11 ± 0.5	2500
Su	179 ± 3	15.4 ± 0.7	11.8 ± 0.6	6.1 ± 0.3	552
1998					
W	699 ± 12	17.2 ± 0.7	41 ± 1	9.4 ± 1.5	3300
Sp	308 ± 7	13.5 ± 0.8	23 ± 1	6.8 ± 0.7	2733
Su	131 ± 12	7.9 ± 0.6	17 ± 1	2.8 ± 0.1	262
F	366 ± 13	15.5 ± 0.9	24 ± 0.7	18.4 ± 1.8	2415
2000					
Sp	453 ± 8	13.4 ± 1.2	35 ± 3	6.01 ± 0.12	590

There was a positive correlation between microcystin and TN ($P = 0.039$; Fig. 4a) but not TP or TN:TP (Figs. 4b, 4c). With the exception of spring 2000 data there was a positive correlation between TN/TP and microcystin ($P = 0.014$). The relation with Chl was not significant, but there was significance with net-Chl (Fig. 4d).

Discussion

Environmental factors controlling the production of cyanobacterial toxins are not well understood (Chorus 1993). Seasonal variations in nutrients, N:P ratios, light, pH, temperature, water column stability and other factors largely determine phytoplankton composition and potential dominance by cyanobacteria (Paerl 1988, Shapiro 1990), but patterns in relation to climate are unclear (Reynolds 1987). Climate in Nepal, dominated by a summer monsoon followed by a protracted dry period during fall–spring and brief pre-monsoon rains in spring and early summer, has a major impact on lake characteristics, including algal biomass and composition (Hickel 1973, Lohman et al. 1988, Davis et al. 1998).

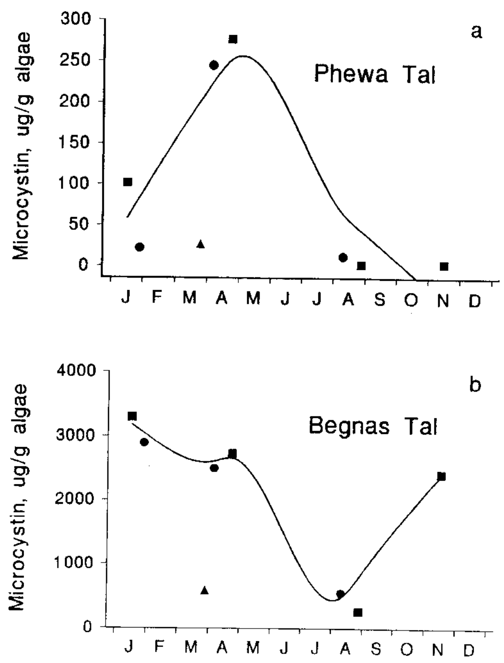

Fig. 2. Algal microcystin values from (a) Phewa Tal and (b) Begnas Tal by season during the project. Lowess plot used data from 1997 (closed circle) and 1998 (closed square). Data from spring 2000 (closed triangle) are shown but were not included in characterizing the seasonal pattern.

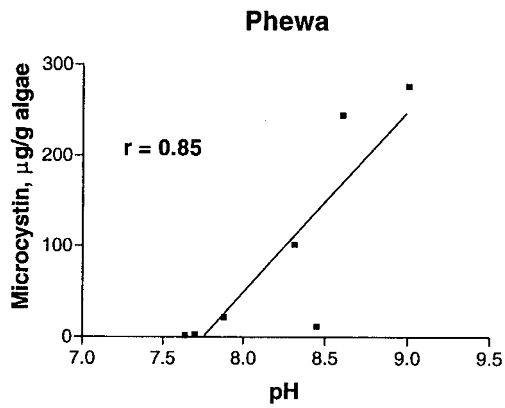

Fig. 3. Relationship between algal microcystin and water pH in Phewa Tal. P = 0.015

Algal toxins showed strong recurring, but different, annual patterns in the two lakes sampled. In Phewa Tal, microcystins reached maxima seasonally in spring 1997 and 1998 (275 µg/g) concurrent with the lake switching from light and phosphorus limitation during winter mixis to nitrogen limitation (unpublished nutrient bioassay experiments) that persists during thermal stratification in spring/summer. Spring conditions in Phewa Tal – including water column stability, warm water temperatures, lower TN and TN:TP ratios, and pH >8.6 – favor cyanobacteria (REYNOLDS 1987, PAERL 1988). Low microcystin measurements in spring 2000 (Table 1) suggest that toxins may not always attain maxima in March/April. The climate, prior to the spring 2000 collection, was drier and cooler than usual and may have been a factor; rainfall during January–March 2000 was about half that of the long-term, and 1997–1998 amounts (Fig. 1), and minimum temperatures during February and March 2000 matched record lows for those months. Low TN in Phewa Tal during spring 2000 (Table 1) suggested that nitrogen fixation and/or external inputs were modest relative to previous spring collections. Microcystin values were lowest during the monsoon (2–12 µg/g) when the water column was unstable – interflows and advective mixing during monsoon storms often disrupt thermal stratification in this lake (DAVIS et al. 1998). Such conditions are unfavorable to cyanobacteria (REYNOLDS 1987, AN & JONES 2000). Microcystin values were intermediate in winter (44 µg/g) when the lake was nitrogen sufficient, and a diatom–desmid association characterized the phytoplankton (HICKLE 1973, LOHMAN et al. 1988).

In Begnas Tal, microcystin averaged 400 µg/g during the monsoon and >2400 µg/g during the remainder of the year. These microcystin measurements (Table 2) matched or exceeded maximum values from lakes in other geographic regions (KOTAK et al. 1995, JACOBY et al. 2000). Begnas Tal has TP levels virtually identical to those in Phewa Tal but averaged about 2.5 times the TN value. Field notes suggest that large cyanobacteria are routinely

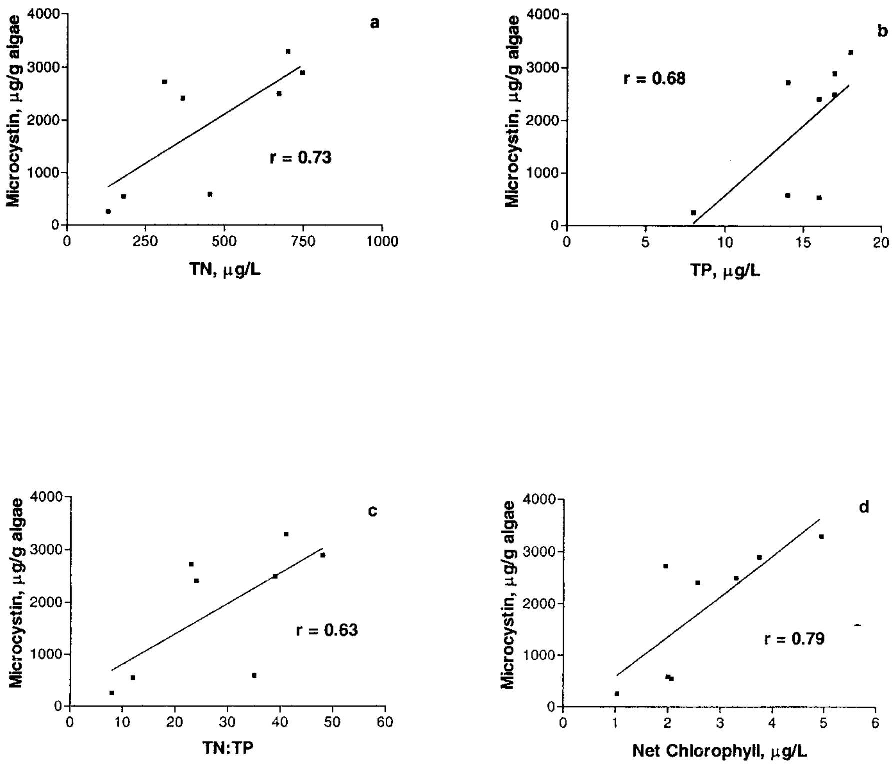

Fig. 4. Relationship between algal microcystin in Begnas Tal and (a) TN, (b) TP, (c) TN:TP and (d) net chlorophyll.

present in Begnas Tal and nitrogen fixation probably contributes to the TN budget. The correlation between microcystin and net-Chl (Fig. 4d) supports the conclusion that toxins are directly related to the abundance of large algae within the lake. The data suggested that water column stability in Begnas Tal was disrupted at the height of the monsoon when toxin levels, TN and TN:TP ratios were at an annual minimum (Table 2). As in the case of Phewa Tal, climate may account for smaller toxin levels in spring 2000 relative to previous years.

Low microcystin values during the monsoon in both study lakes are consistent with observations elsewhere in Asia that intense monsoon conditions are unfavorable for cyanobacteria (Zafar 1986, An & Jones 2000). This seasonal pattern may be characteristic of microcystin production in lakes influenced by the monsoon. Regional weather patterns are known to affect the timing of cyanobacteria blooms (Soranno 1997) and potential toxin levels. In temperate climates, without monsoon influence, peak concentrations of microcystin generally occur during the warm season, between early spring and late fall, but within- and among-year variation in toxin levels in individual lakes is large (Kotak et al. 1995, Jacoby et al. 2000).

Correlations between toxin concentrations and environmental factors such as water column stability, high temperatures, high pH and N:P ratios <5, have been found, but no single factor triggers toxin production (Carmichael 1997). Kotak et al. (1995) found that microcystin was correlated with TP, dissolved P, and Chl whereas Jacoby et al. (2000) found a correlation with soluble reactive phosphorus. Jungmann et al. (1996) found no correlation between microcystin and environmental variables. Low TN:TP ratios did not favor toxin production in either of the study lakes (Tables 1 and 2). The correlation with pH in Phewa Tal was consistent with the belief that cyanobacteria have a competitive advantage in high pH waters (REYNOLDS 1987). The correlation with TN in Begnas Tal may result from favorable conditions for cyanobacteria in this lake and their role in nitrogen fixation. This study advances the understanding of how the monsoon climate influences lakes in Nepal, but suggests that no single environmental cue can be used to predict the presence of algal toxins in these subtropical lakes.

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