Dynamics of cyanobacteria and cyanobacterial toxins and their correlation with environmental parameters in Tri An Reservoir, Vietnam

Thanh-Son Dao, Jorge Nimptsch and Claudia Wiegand

ABSTRACT

This study evaluates the water quality from Tri An Reservoir, a drinking water supply for several million people in southern Vietnam, in terms of cyanobacterial biomass and their potent toxins, microcystins (MCs). Cyanobacteria, their toxins and environmental parameters were monitored monthly for 1 year (April 2008–March 2009) at six stations covering a transect through the reservoir. Dynamics of cyanobacterial abundance in relation to cyanobacterial biomass, toxins and environmental factors were investigated. Environmental variables from Tri An Reservoir favored algal and cyanobacterial development. However, cyanobacterial biomass and proportion varied widely, influenced by physical conditions, available nutrients and nutrient competition among the phytoplankton groups. Cyanobacterial biomass correlated slightly positively to temperature, pH and biochemical oxygen demand (BOD_5), but negatively to total inorganic nitrogen concentrations. During most of the sampling times, MC concentrations in the reservoir were quite low ($\leq 0.07\ \mu g\ L^{-1}$ MC-LR equivalent), and presented a slight positive correlation to BOD_5, total nitrogen:total phosphorus ratio and cyanobacterial biomass. However, in cyanobacterial scum samples, which now and then occurred in the reservoir, MC concentrations reached up to $640\ \mu g\ g^{-1}\ DW^{-1}$. The occurrence of MC in the reservoir poses a risk to local residents who use the water daily for domestic purposes.

Key words | cyanobacterial biomass, environmental factors, microcystins, phytoplankton

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INTRODUCTION

As primary producers, algae and cyanobacteria play a key role in aquatic ecosystems. Their occurrence is defined by aquatic environmental factors, but their mass proliferation also reacts to shifts in these factors. Light intensity and temperature in freshwater lakes and reservoirs regulate the photosynthesis of phytoplankton (Wetzel 2001) so that these two factors could shape the distribution as well as abundance of phytoplankton temporally and spatially (Zhang & Prepas 1996; Marinho & de Moraes Huszar 2002). Other physical factors such as turbulence, pH, and water current also have an influence on phytoplankton communities (Wetzel 2001; Marinho & de Moraes Huszar 2002). Many dissolved chemicals, including nitrogen and phosphorus compounds, closely relate to the development of algae and cyanobacteria, as different phytoplankton species have different chemicals and nutrient requirements for their optimal growth (Sivonen 1990; Wetzel 2001; Sabour *et al.* 2009). For diatoms' abundance, silica is essential (Tilman *et al.* 1986). The ratio of total nitrogen to total phosphorus by weight (TN:TP) influences the phytoplankton community. Cyanobacterial species composition is reduced when this ratio exceeds 29:1 (Smith 1983), while low ratios potentially favor blooms

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of heterocystous cyanobacteria (Havens *et al.* 2003), which are able to fix atmospheric dinitrogen (Bothe 1982) to enhance their competition over other phytoplankton in case of inorganic nitrogen depletion in the water.

Freshwater quality is decreasing due to intensification of adjacent agriculture and pollution by anthropogenic activities throughout the world. In many reservoirs and lakes these phenomena induced eutrophication leading to mass proliferation of cyanobacteria (Carmichael 1992), of which 25–75% was estimated to be toxic (Sivonen & Jones 1999; Zurawell *et al.* 2005). In freshwater bodies, toxic cyanobacteria are of concern owing to their detrimental effects on aquatic organisms and notorious incidents of human illness in relation to the toxins (Zurawell *et al.* 2005). Cyanobacterial toxins (e.g. microcystins (MCs)) have been recorded throughout the world and cause both acute and chronic toxicities to animals and human (Metcalf & Codd 2012). Chronic toxicity is of concern to the public due to the association with cancer (Hernandez *et al.* 2009). To reduce the risk of human fatalities, the World Health Organization established a guideline value of maximum 1 μg microcystin-LR (MC-LR) (or equivalents of other MC forms) per liter of drinking water (WHO 2003).

In Vietnam, toxic cyanobacteria, cyanobacterial blooms and their toxins were only recently reported in some lakes and reservoirs (Hummert *et al.* 2001; Nguyen *et al.* 2007; Duong *et al.* 2013). Tri An Reservoir in southern Vietnam, where toxic cyanobacteria scum has been observed (Dao *et al.* 2010), is directly and indirectly supplying drinking water for millions of local residents. Nevertheless, cyanobacterial abundance dynamics and cyanobacterial toxins have not been monitored in the reservoir. Despite the WHO guideline of 1 μg L^{-1} for drinking water, cyanobacterial toxins are not yet considered as important factors for water quality in Vietnam. Processes for complete removal of cyanobacterial toxins are not included in drinking water purification processes in Vietnam. Hence, local people may be facing chronic health risks or hazards caused by the toxins via daily domestic use. Therefore, in this study, monitoring of parameters such as temperature, pH, turbidity, biochemical oxygen demand (BOD_5), conductivity, total dissolved solids (TDS), nutrients, phytoplankton, and in particular cyanobacterial abundance and toxin concentration in the waters of Tri An Reservoir was implemented.

MATERIALS AND METHODS

Study area and sample collection

Tri An Reservoir is about 70 km northeast from Ho Chi Minh City. It has a surface area of 323 km^2, is around 50 km long, 2–15 km wide, with mean and maximum depths of 8.4 m and 27 m, respectively (Figure 1). It has a total volume of 2.7 billion m^3 and an elevation approximately 62 m above sea level at its highest capacity. The annual rainfall and average temperature in the study area are 2,400 mm and 25.4 °C, respectively (Vietnam Ministry of Science Technology & Environment 2001). Receiving water from Dong Nai and La Nga Rivers, Tri An is a reservoir used for multiple purposes such as hydroelectric power, flood control, domestic and industrial water supply, fisheries and irrigation of agricultural fields. In addition to the agriculture upstream, both fish caging and wastewater from the sugar factory (located at the inflow of La Nga River) have led to nutrient enrichment supporting algal growth and cyanobacterial development in the reservoir.

In Tri An Reservoir, samples of algae, cyanobacteria and MC were taken monthly at six sites (TA1–TA6) at the water surface from April 2008 to March 2009, with the exception of January 2009 (Figure 1). Physical and chemical factors were also measured at the same six sites and times, except in April 2008. Qualitative samples of algae and cyanobacteria were taken with a conical net (25 μm), and quantitative samples were taken at the surface and fixed with neutral Lugol solution (Sournia 1978) in the field. Surface water samples for nutrients (inorganic nitrogen, phosphorus), BOD_5 and MC analyses were collected, kept on ice in the field until analyzed in the laboratory the same day, or filtered and stored at –70 °C until analysis.

Physical and chemical analysis

Physical and chemical factors of surface water were measured *in situ* including pH (Metrohm 744), turbidity (Hach DR/2010), conductivity and TDS (WTW LF197 multi-detector), temperature and dissolved oxygen (DO) (WTW Oxi197i multi-detector). Nutrients in surface water were analyzed colorimetrically with a spectrophotometer (Hach DR/2010) and BOD_5 was determined by the

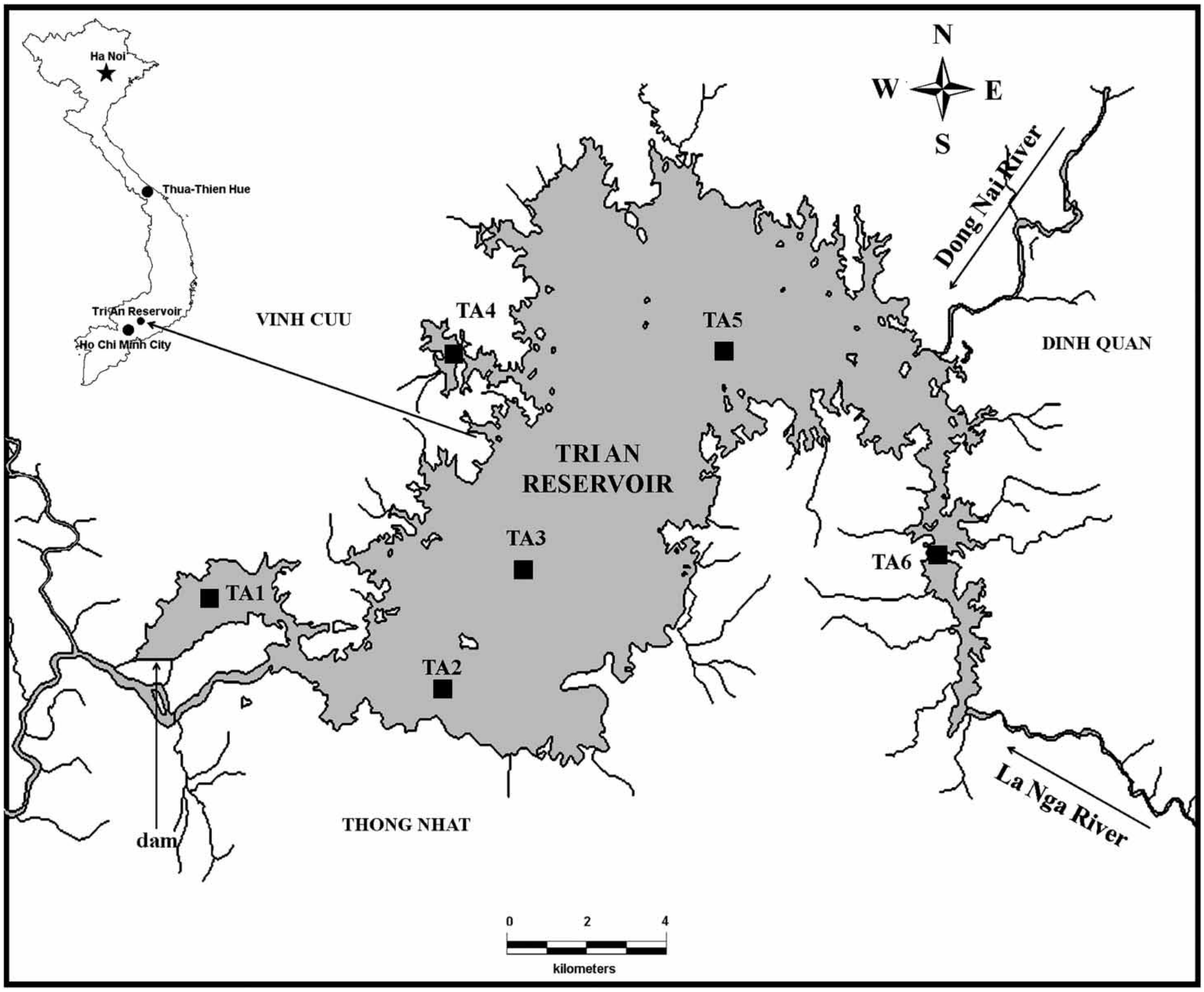

Figure 1 | Map of Tri An Reservoir with sampling sites (TA1–TA6) for the monitoring of environmental factors, phytoplankton and cyanobacterial toxins.

difference of DO concentrations in samples after five days according to *Standard Methods* (2005). The detection limits of nutrient parameters were 0.02 (nitrate), 0.002 (nitrite), 0.04 (ammonium), 0.06 (total Kjeldahl nitrogen) and 0.05 mg L^{-1} (for both orthophosphate and TP).

Algal and cyanobacterial identification, counting and biomass estimation

Phytoplankton was observed at 400–800 × magnification (Olympus BX51 microscope). Identification was based on morphology following the system of Komárek & Anagnostidis (1989, 1999, 2005) for cyanobacteria, Krammer & Lange-Bertalot (2004) for diatoms, and other taxonomy books for green, golden and yellow algae, dinoflagellates and euglenoids. For counting, 10 mL of sample was settled overnight in a tubular counting chamber (Utermöhl-chamber; KC Denmark A/S). Algae and cyanobacteria were counted in an inverted microscope. The biomass of cells and/or trichomes was calculated based on geometrical formulae, and the algal biomass was estimated according to Olrik *et al.* (1998).

MC determination

One liter of water was filtered on GF/C filters (Whatman). The filters were dried at 50 °C overnight and kept at –70 °C prior to

MC determination. Extraction of samples was prepared according to Fastner *et al.* (1998) with minor modification. Briefly, the field samples on GF/C filters were homogenized and firstly extracted overnight in 70% MeOH (Carl Roth) containing 5% acetic acid (Merck) and 0.1% trifluoroacetic acid (TFA; Merck) followed by 3×60 minutes in 90% MeOH containing 5% acetic acid and 0.1% TFA with 30 seconds sonication during the last extraction. After centrifugation (4,500 rpm, 10 min, 4 °C), the supernatants of all extraction steps from each sample were pooled, dried at 35 °C, re-dissolved in 0.5 mL MeOH (100%) and centrifuged at 14,000 rpm, 1 °C for 10 min. MC in the supernatant was analyzed according to Pflugmacher *et al.* (2001) by high performance liquid chromatography (HPLC; Waters Alliance, Eschborn) on a reverse phase column (RP18; 5 μM LiChrospher 100) by UV and photodiode array detection between 200 and 300 nm. Separation of 80 μL injection volume was achieved at 40 °C by a gradient of Milli-Q water and acetonitrile (ACN; Rathburn, Walkerburn, UK), both enriched with 0.1% (v/v) TFA at a flow rate of 1 mL min^{-1}, starting at 35% ACN, increasing to 55% ACN within 15 min, cleaning at 100% ACN and 10 min equilibration to start conditions. MC standard, MC-LR, was purchased from Axxora (Germany).

Statistical analysis

Principal component analysis (PCA; Statistica 7.0, StatSoft) and the Pearson correlation test (SPSS, version 16) were implemented for examination of relationships between cyanobacterial biomass or MC concentration and environmental parameters. The relationship between chlorophyll and phosphorus concentration in Tri An Reservoir was equated according to the equation reported by Reynolds (2006): $\log[\text{chlorophyll}] = 0.91 \times \log[\text{TP}] - 0.435$.

RESULTS

Chemical and physical parameters of water samples

Temperature of surface water ranged from 25 to 35 °C, higher in September 2008 and lower in December 2008, with little changes among the sampling sites and times of monitoring. The pH of water in Tri An Reservoir ranged between 6.0 and 7.6 (Table 1), being lower in October–December 2008. Conductivity values were 31–66 μS cm^{-1}, quite similar at each sampling time at most sites, except site TA6. Water turbidity ranged from 2 to 305 NTU (nephelometric turbidity unit), varying only slightly among sites TA1–TA4 during September 2008–March 2009, but more variable in May–August 2008 and higher at sites TA5 and TA6. TDS values ranged from 17 to 35 mg L^{-1}. DO values were in the range 6–8.2 mg L^{-1} with little change among the sites during the monitoring. The BOD_5 in the water commonly ranged from 0.5 to 3 mg oxygen L^{-1} but increased to 7 mg oxygen L^{-1} at site TA4 in May 2008.

In the reservoir, maximum concentrations of ammonium, nitrite and nitrate were 0.12 mg L^{-1}, 0.108 mg L^{-1} and 0.78 mg L^{-1}, respectively (Table 1). The nitrate concentrations were higher from June to August 2008 and lowest in March 2009 and, correspondingly, nitrite increased from June to August 2008 and decreased afterwards. Concentrations of nitrate and nitrite did not vary much between the sites, except site TA6. Concentrations of total Kjeldahl nitrogen (TKN) ranged from 0.06 to 1.637 mg L^{-1}, except those at sites TA2 (May 2008) and TA3 (August 2008) which were higher, 2.15 mg L^{-1} and 2.17 mg L^{-1}, respectively. The TKN concentration varied among the sampling sites; TKN was higher in August 2008 and lower in March 2009. TN (defined as the sum of TKN, nitrite and nitrate) in Tri An Reservoir ranged from 0.25 to 2.63 mg L^{-1} varying among the sites and sampling times (Table 1), higher in July, August and November 2008 and lower in May 2008 and March 2009. Soluble phosphorus concentration of up to 0.1 mg L^{-1} was detected only at site TA6, in July, September and October 2008; otherwise it was below detection level. Concentrations of TP ranged from 0.05 to 0.33 mg L^{-1}. Those at the sites TA1–TA4 were similar, a little higher at site TA5 but more varied at site TA6 with the incoming river (Table 1). The concentrations of TP were higher from June to August 2008, but decreased from October to December 2008. The TN:TP ratio values varied from 4.5:1 to 30:1 except one value of 49:1 at site TA2, in May 2008. The ratio values varied among the sampling sites and during the monitoring (Table 1).

Phytoplankton composition and biomass

During the monitoring period in Tri An Reservoir, 197 species of phytoplankton were recorded belonging to seven classes,

Table 1 | Physical and chemical parameters in Tri An Reservoir from May 2008 to March 2009

	Sampling site					
Parameter	**TA1 min–max mean**	**TA2 min–max mean**	**TA3 min–max mean**	**TA4 min–max mean**	**TA5 min–max mean**	**TA6 min–max mean**
Temperature (°C)	28.3–33.6 30.6	25.3–33.9 29.2	25.7–35.3 28.9	26.6–33.9 30.1	26.4–31 28.8	27.4–31.1 29.5
pH	6.5–7.4 6.8	6.0–7.5 6.9	6.2–7.5 6.9	6.4–7.4 6.9	6.4–7.4 6.9	6.4–7.6 6.9
Conductivity (µS/cm)	32–49 41	31–48 41	34–50 41	34–47 40	34–50 41	34–66 53
Turbidity (NTU)	4–78 25	2–79 28	2–134 44	6–122 39	8–182 81	11–305 114
TDS (mg/L)	18–25 21.7	17–26 21.8	19–27 22	19–25 21.6	18–27 22	18–35 28
DO (mg/L)	6.5–7.4 6.9	6–8.2 7.2	6.4–7.9 7	6.3–7.7 7.1	6.3–7.6 7	6.4–7.5 6.9
BOD_5 (mg oxygen/L)	0.5–3 1.2	0.5–3 1.5	0.5–3 1.2	0.5–7 1.8	1–2 1.3	1–2 1.8
Ammonium (mg/L)	0.04–0.06 0.046	0.04–0.08 0.045	0.04–0.08 0.047	0.04–0.06 0.044	0.04–0.1 0.058	0.04–0.12 0.069
Nitrate (mg/L)	0.02–0.4 0.24	0.02–0.33 0.21	0.02–0.43 0.24	0.02–0.39 0.22	0.1–0.42 0.28	0.32–0.78 0.5
Nitrite (mg/L)	0.002–0.047 0.014	0.002–0.04 0.013	0.002–0.035 0.015	0.002–0.04 0.015	0.002–0.052 0.017	0.007–0.108 0.039
TKN (mg/L)	0.06–1.18 0.669	0.06–2.15 0.735	0.47–2.17 0.846	0.34–1.40 0.705	0.06–1.5 0.674	0.1–1.637 0.807
TN (mg/L)	0.28–1.12 0.865	0.25–2.24 0.913	0.47–2.63 1.099	0.44–1.63 0.896	0.46–1.5 0.893	0.61–1.93 1.299
PO_4^{3-} (mg/L)	BDL	BDL	BDL	BDL	BDL	0.05–0.1 0.56
TP (mg/L)	0.05–0.09 0.069	0.05–0.1 0.07	0.05–0.13 0.076	0.05–0.14 0.08	0.05–0.2 0.104	0.05–0.33 0.142
TN:TP ratio	6–22:1 13:1	5–22(49):1 14:1	6–25:1 15:1	5–20:1 12:1	5–25:1 10:1	5–30:1 12:1

Minima (min), maxima (max) and mean values. TDS, total dissolved solids; DO, dissolved oxygen; BOD_5, biochemical oxygen demand (after 5 days); TKN, total Kjeldahl nitrogen; TN, total nitrogen; PO_4^{3-}, orthophosphate; TP, total phosphorus; TN:TP, total nitrogen to total phosphorus ratio by weight; BDL, below detection limit (0.05 mg P/L).

Cyanophyceae (cyanobacteria), Chlorophyceae (green algae), Bacillariophyceae (diatoms), Chrysophyceae (golden algae), Xanthophyceae (yellow algae), Euglenophyceae (euglenoids) and Dinophyceae (dinoflagellates). Species number of phytoplankton assemblages ranged from 28 to 75, of which cyanobacteria comprised 9–30% of the total (Figure 2).

During the monitoring period, the total biomass of phytoplankton in the reservoir strongly varied, from 0.013 to 7.717 mg L^{-1}. Its maximal values were recorded in April–May 2008 and February–March 2009. The biomass was highest at site TA4 followed by TA1, TA2, TA3, TA5 and minimal at site TA6 (Figure 3(a)–3(f)). Diatoms biomass consisted mainly of the genera *Aulacoseira* and *Synedra*, cyanobacteria consisted of *Microcystis* and *Anabaena*, green algae were mainly from the orders Chlorococcales and desmids, dinoflagellates consisted mainly of the genera *Ceratium* and *Peridinium*, and euglenoids were from the *Trachelomonas* and *Euglena* genera. The proportion of cyanobacterial biomass over total phytoplankton biomass ranged from 1 to 98%. The proportion was higher at sites TA1, TA2, TA3 and

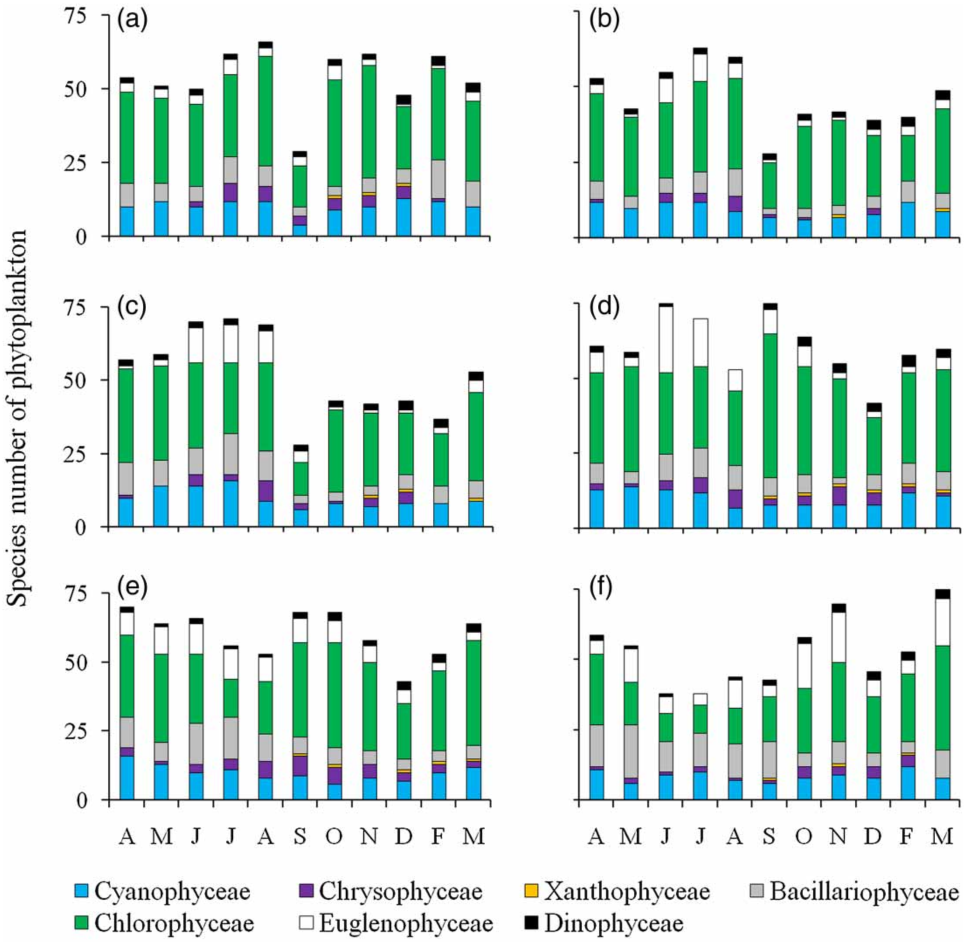

Figure 2 | Phytoplankton diversity as number of species in Tri An Reservoir at sites: (a) TA1, (b) TA2, (c) TA3, (d) TA4, (e) TA5 and (f) TA6 throughout the sampling period.

TA5, and lower at sites TA4 and TA6 (Figure 3(g)–3(l)). Generally, the proportion of cyanobacteria increased from May to September 2008. Absolute biomass was lowest at most sites from August till December 2008 with the exception of site TA6, where the opposite phytoplankton development occurred with maxima in December 2008 (Figure 3(a)–3(f)). Cyanobacterial biomass during the monitoring period ranged from 0.009 to 0.834 mg L^{-1}. The biomass was higher at the sites TA1–TA4 and minimal at site TA6. After a peak development in April–May at four out of six stations, phytoplankton biomass was reduced (Figure 3). Generally, biomass of cyanobacteria was mostly attained from Chroococcales and Nostocales (Figure 4(a)–4(d)).

In Tri An Reservoir, the annual mean concentrations of TP and chlorophyll were 0.09017 mg L^{-1} and 2.356 μg L^{-1}, respectively. The relationship between TP and chlorophyll in the case of Tri An Reservoir was then equated as log [chlorophyll] = 0.91 × log[TP]–0.499.

MC concentrations

The cell-bound MC concentration from the reservoir reached up to 0.072 μg L^{-1}. MC was detected at low concentration at some sites in the reservoir (Figure 4(a)–4(f)). However, in most of the samples the concentration was below the detection limit of HPLC-UV.

Correlation between cyanobacterial biomass, toxins and environmental factors

The PCA indicated that cyanobacteria biomass seemed to be positively correlated with temperature, pH and MC

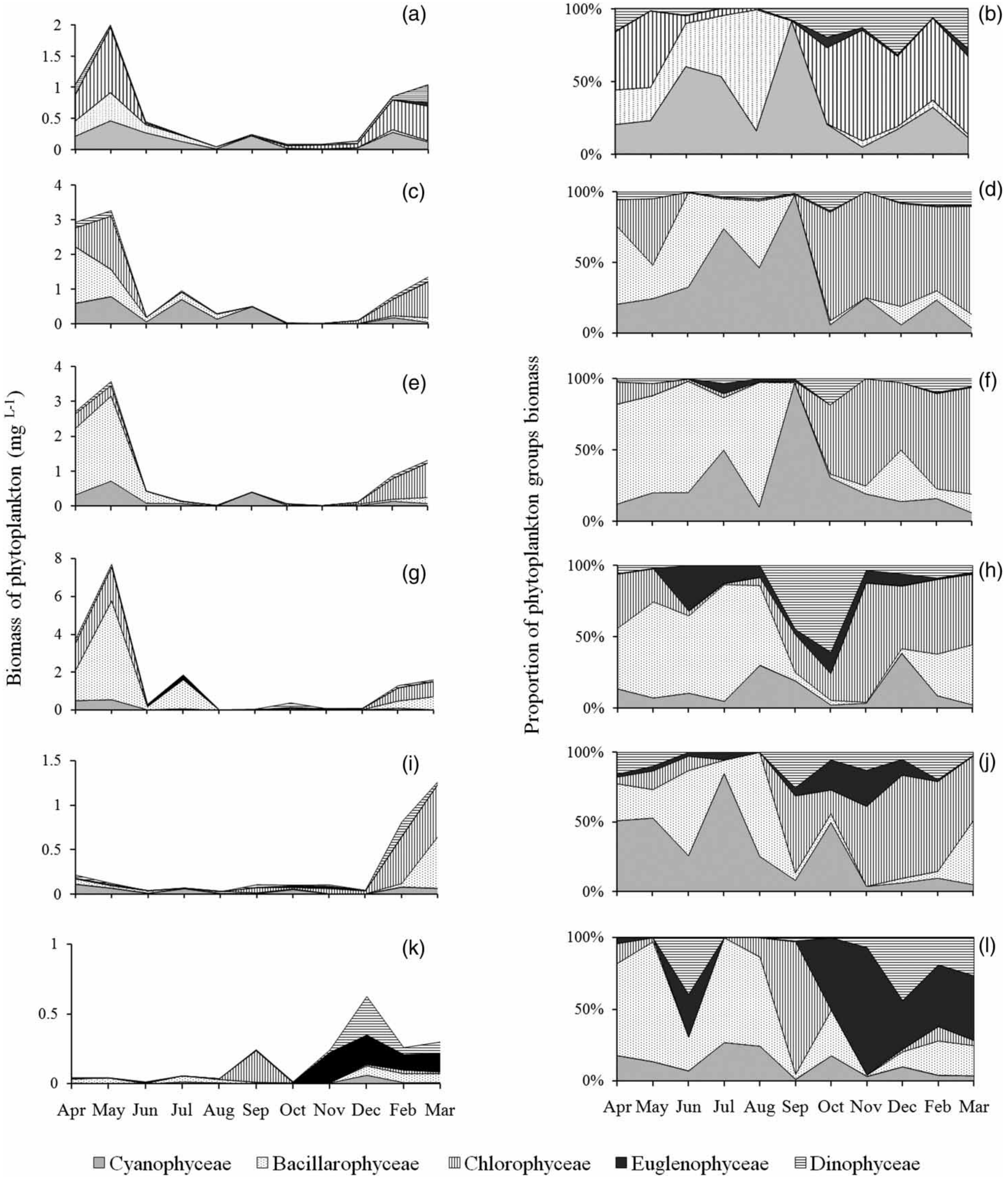

Figure 3 | Spatial and temporal variation of phytoplankton biomass and proportion in Tri An Reservoir: (a)–(f) phytoplankton biomass at sites TA1–TA6, respectively (note: different scales of phytoplankton biomass); (g)–(l) biomass proportion of phytoplankton at sites TA1–TA6, respectively.

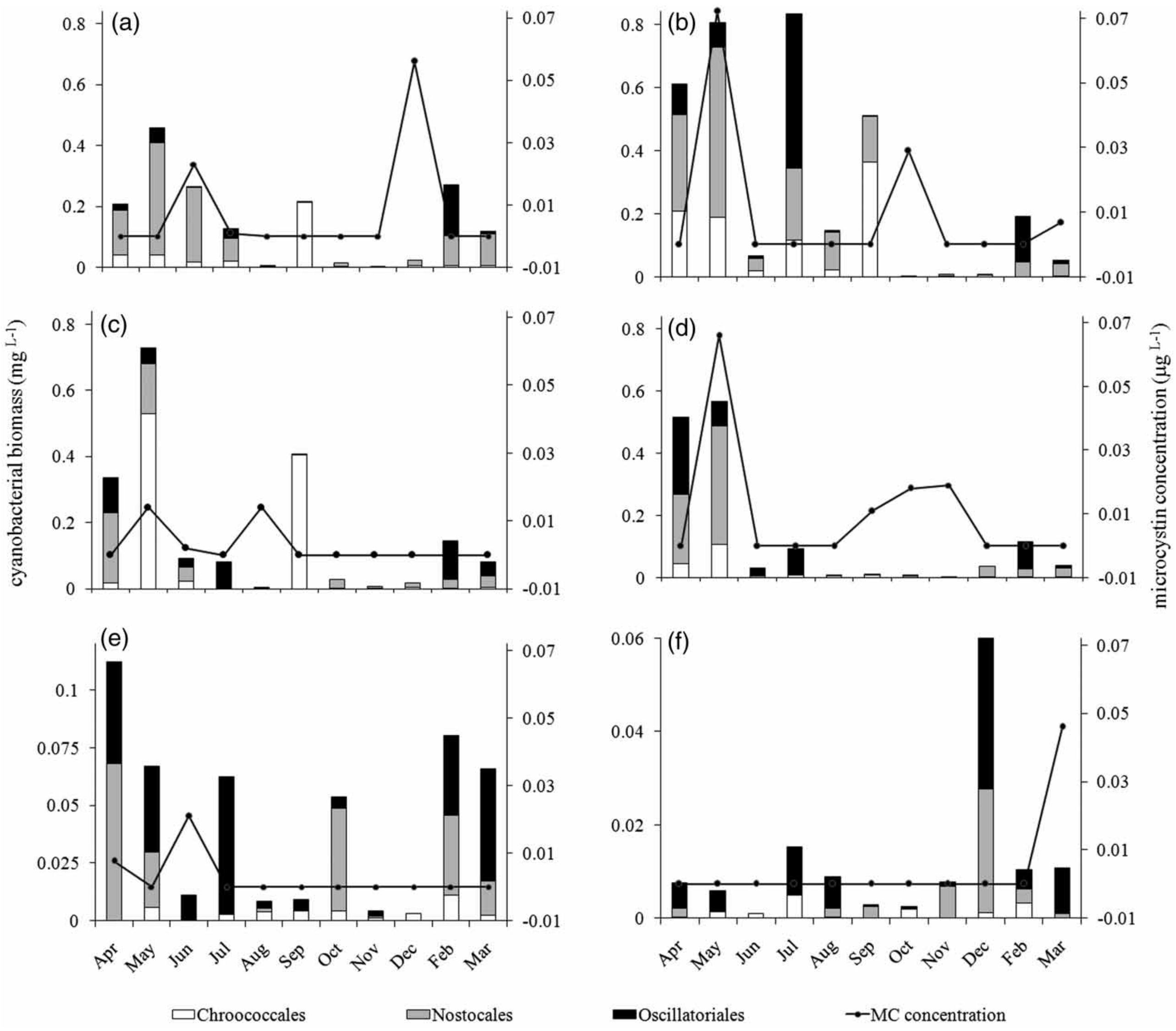

Figure 4 | Spatial and temporal variation of cyanobacterial biomass and MC concentration in Tri An Reservoir: (a)–(f) phytoplankton biomass and MC concentration at sites TA1–TA6, respectively (note: different scales of cyanobacterial biomass).

concentration, and negatively correlated with total inorganic nitrogen (Figure 5). The Pearson correlation test showed that cyanobacterial biomass correlated positively with temperature ($r = 0.305$; $p < 0.05$), pH ($r = 0.364$; $p < 0.01$), DO ($r = 0.290$; $p < 0.05$), BOD_5 ($r = 0.494$; $p < 0.01$), but negatively with total inorganic nitrogen ($r = -0.358$; $p < 0.01$) among the studied environmental parameters. In contrast, MC concentration only had a positive correlation with BOD_5 ($r = 0.408$; $p < 0.01$), TN:TP ratio ($r = 0.324$; $p < 0.01$) and cyanobacterial biomass ($r = 0.372$; $p < 0.01$) (Table 2).

DISCUSSION

Chemical and physical parameters

Tri An Reservoir is a tropical water body, hence the temperature does not vary much diurnally and over the seasons. Temperature ranged within the algal and cyanobacterial optimum; hence conditions were favorable for phytoplankton development (Wetzel 2001). Neutral and slightly acidic pH values during October–December 2008 (Table 1) were within the range of those in Nui Coc Reservoir in Vietnam

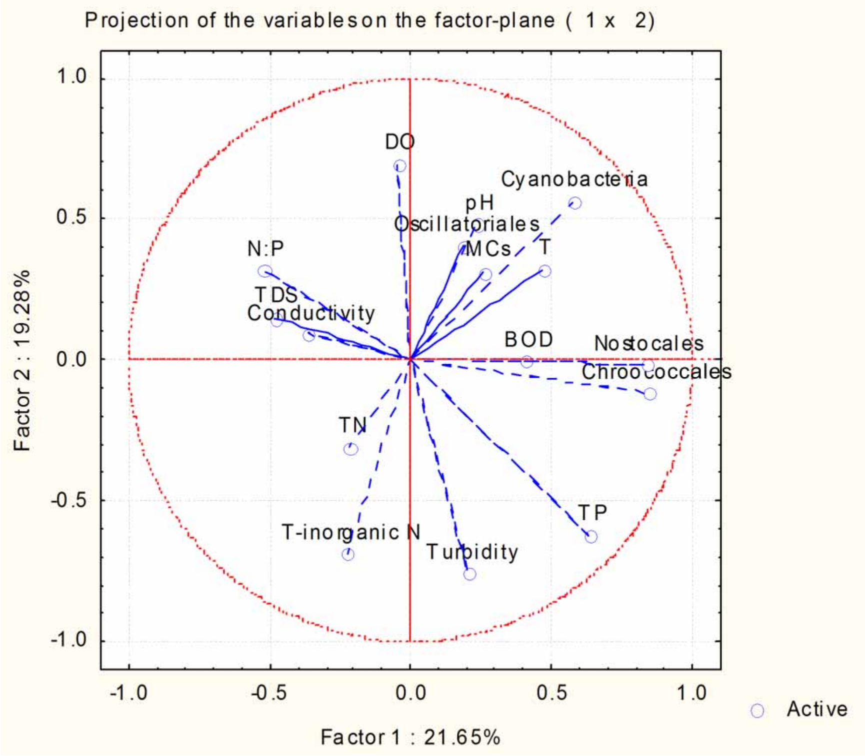

Figure 5 | Principal component analysis based on cyanobacterial biomass, toxin concentration and environmental parameters from Tri An Reservoir; T, temperature; DO, dissolved oxygen, MCs, microcystin concentrations; other abbreviations, please see Table 1.

Table 2 | Correlations between cyanobacterial biomass and/or MC concentration and environmental variables based on Pearson correlation test

	Cyanobacterial biomass			**MC concentration**		
Variables	**r**	***p***	**df**	**r**	***p***	**df**
Temperature	0.305	*	58	0.053	ns	58
pH	0.364	**	58	0.111	ns	58
Turbidity	−0.179	ns	58	−0.145	ns	58
TDS	−0.078	ns	58	−0.102	ns	58
Conductivity	0.027	ns	58	−0.047	ns	58
DO	0.290	*	58	0.134	ns	58
BOD_5	0.494	**	58	0.408	**	58
Total inorganic nitrogen	−0.358	**	58	−0.174	ns	58
TN	−0.017	ns	58	0.148	ns	58
TP	−0.118	ns	58	−0.110	ns	58
TN:TP ratio	0.104	ns	58	0.324	*	58
Cyanobacterial biomass				0.372	**	58

r, correlation coefficient; *, $p < 0.05$; **, $p < 0.01$; ns, not significant ($p > 0.05$); df, degree of freedom (n–2).

(Duong *et al.* 2013) and in Juturnaiba and Botafogo Reservoirs, Brazil (Marinho & de Moraes Huszar 2002; Lira *et al.* 2009).

During the rainy season (May–November in southern Vietnam), the two incoming rivers bring organic and inorganic matter into the reservoir. Consequently, the water turbidity strongly increased at sites TA5 and TA6, close to their entry. The other sites (TA1–TA4) were less influenced, and turbidity at these four sites was lower (Table 1). Conductivity and TDS in the reservoir revealed a low trophic state (Wetzel 2001). DO concentrations in the waters of the reservoir were high but not saturated. High BOD_5 values demonstrated a richness of organic compounds for heterotrophic bacterial development. Despite the input of organic matter during the rainy season at the reservoir entrance it was diluted out in the volume of the reservoir, and may also settle. However, during the dry season, the input via the La Nga River was evident.

Variations of total inorganic nitrogen concentrations (and nitrate as the biggest part of them) were possibly due to the seasonal changes of input via the two rivers. The nitrogen concentrations in the reservoir fall into the range of mesotrophic to eutrophic water characteristics (Wetzel 2001). Additionally, fish caging activities and wastewater from a sugar factory located at the inflow of La Nga River have contributed to the nutrient enrichment. Although the concentration of orthophosphate in the reservoir was below detection limit (0.05 mg L^{-1}) for most samples, the TP concentrations (0.05–0.33 mg L^{-1}, Table 1) characterized eutrophic conditions according to Padisak (2003) and Reynolds (2006). Nitrogen-fixing cyanobacteria are inferior competitors for phosphorus in comparison to other phytoplankton such as diatoms and green algae (Huisman & Hulot 2005). The values of the TN:TP ratio in the reservoir (4.5:1–30:1 (45:1)) were mostly below the threshold of 29:1, where cyanobacteria co-exist with microalgae and start to dominate phytoplankton communities (Smith 1983). Besides, the structure of other trophic levels (e.g. Cladocera, planktivorous fish) in a water body could alter the response of phytoplankton to nutrients (Cronberg 1982), and at the ratio values of TN:TP $< 29:1$ many environmental factors (e.g. light availability, temperature, CO_2, TN, TP) should be involved in the development and dominance of cyanobacteria (Smith 1986).

Phytoplankton structure and biomass

The phytoplankton assemblage in Tri An Reservoir consisted of most major taxonomic groups of freshwater algae and cyanobacteria. This record was similar to that in a previous investigation of phytoplankton in tropical water bodies in northern Vietnam (Duong *et al.* 2013) and in Malaysia (Yusoff & McNabb 1997). According to the hypothesis of Smith (1983), physical and chemical conditions, especially the TN:TP values in Tri An Reservoir (Table 1) were suitable for a diverse community of phytoplankton. During the monitoring period, cyanobacteria gained around 15% of the species number of the phytoplankton assemblage. However, this percentage varied at each sampling site, from 9 to 30% (Figure 2), with the changing TN:TP ratio and temporal and spatial alteration of environmental conditions.

Total phytoplankton biomass in the reservoir was quite variable, from 0.013 to 7.717 mg L^{-1} (mean value of 0.623 mg L^{-1}), decreased in the rainy season and increased in the dry season. In the total biomass, biomass of green algae and diatoms was dominant, followed by that of cyanobacteria. This could be explained as: (1) green algae have a higher competition capacity for phosphorus, nitrate uptake and light intensity than cyanobacteria; (2) diatoms have an advantage on energy safety for their frustule development over other algal groups; and (3) many cyanobacteria are capable of buoyancy and nitrogen fixation, and capturing a broader light spectrum (Huisman & Hulot 2005; Visser *et al.* 2005). Besides, cyanobacterial dominance is related to water stability (Wicks & Thiel 1990); hence the lowest density was found at the inflowing rivers (TA5, TA6).

Phytoplankton biovolume or biomass and phytoplankton chlorophyll concentration are correlated in natural water bodies (Felip & Catalan 2000). Besides, chlorophyll content in phytoplankton biomass was strongly altered by light intensity, cell size, phytoplankton structure and phosphorus concentration (Desortova 1981; Kasprzak *et al.* 2008). As Tri An Reservoir is located in a tropical region and phytoplankton samples were collected from the surface water, the light intensity in the reservoir can be assumed not to be the limiting factor for the chlorophyll alteration during the monitoring period. In this study we did not measure phytoplankton chlorophyll concentration. However, assuming that phytoplankton chlorophyll concentration gains around 0.505% of phytoplankton biomass (Kasprzak *et al.* 2008) or about $4.036 \times (\text{biovolume})^{0.66}$ (Felip & Catalan 2000), the annual average concentration of phytoplankton chlorophyll in Tri An Reservoir could range from 2.356 μg L^{-1} (calculated according to Felip & Catalan 2000) to 3.145 μg L^{-1} (calculated according to Kasprzak *et al.* 2008).

Kasprzak *et al.* (2008) reported that the proportion of chlorophyll over total phytoplankton biomass decreased when the cyanobacterial biomass increased. However, chlorophyll concentration and biomass of green algae are positively correlated. Varying proportions of golden algae did not influence the chlorophyll to biovolume ratio (Felip & Catalan 2000). Although Felip & Catalan (2000) and Kasprzak *et al.* (2008) built up relationships between phytoplankton biovolume and chlorophyll concentration, Felip & Catalan (2000) did not include diatoms and cyanobacterial groups in their calculation, whereas all main phytoplankton groups (green algae, diatoms, cyanobacteria, Dinophyceae, Cryptophyceae, etc.) were brought into the equation by Kasprzak *et al.* (2008). Phytoplankton abundance in Tri An Reservoir was mainly contributed by three groups, green algae, diatoms and cyanobacteria (Figure 3). Hence, the chlorophyll concentration from Tri An Reservoir extrapolated from the phytoplankton biomass would be calculated more accurately with the equation of Kasprzak *et al.* (2008) than that of Felip & Catalan (2000).

Reynolds (2006) formulated the relationship between TP and chlorophyll concentration (mostly) based on data from temperate lakes and reservoirs, $\log[\text{chlorophyll}] = 0.91 \times \log[\text{TP}]–0.435$. From Tri An Reservoir, this relation was equated as $\log[\text{chlorophyll}] = 0.91 \times \log[\text{TP}]–0.499$. Hence, our study confirmed the formulation with the data from a tropical water body.

Cyanobacterial biomass in Tri An Reservoir was positively correlated with water temperature (Table 2), a cyanobacterial characteristic reviewed by Paerl & Huisman (2008). The positive correlation between pH and DO concentration and cyanobacterial biomass is a result of photosynthetic activity, especially at favorable temperature. A high biomass releases organic compounds, which in turn could be decomposed by bacteria, resulting in elevated BOD_5 values. However, correlations between cyanobacterial biomass and temperature, pH and DO, BOD_5 remained low in our study ($r \leq 0.49$).

Phosphorus concentration and in particular the TN:TP ratio support cyanobacterial abundance, growth and bloom development (Tilman *et al.* 1986; Zhang & Prepas 1996). However, Kotak *et al.* (2000) showed that biomass of the cyanobacterium *Microcystis aeruginosa* was negatively correlated with TN:TP ratio. Unexpectedly, in Tri An Reservoir, cyanobacterial biomass did not correlate with the TN:TP ratio and TP during the monitoring period (Table 2). Jensen *et al.* (1994) found that in shallow temperate lakes TP was closely related to dominance of cyanobacteria up to TP < 0.8 mg L^{-1} and green algae from TP > 1 mg L^{-1}. During our monitoring, the maximum TP was 0.33 mg L^{-1} (Table 1) and cyanobacterial biomass was higher than green algal biomass from April until September (Figure 3). At TA5, cyanobacteria benefited from the introduction of nutrients from the rivers, whereas at TA6, the river characteristics dominated. Further into the reservoir, with increasing lacustrine characteristics, biomass increases and the phytoplankton community becomes lake typical. Total inorganic nitrogen concentrations were weakly negatively correlated with cyanobacterial biomass ($r = –0.358$), which was in agreement with the results of Kotak *et al.* (2000). This weak and negative correlation could be explained by heterocystous (nitrogen-fixing, e.g. *Anabaena* spp.) and gas-vacuole containing cyanobacterial species (able to shift vertically, e.g. *Microcystis* spp.) mainly contributing to cyanobacterial biomass in Tri An Reservoir (Figure 4). Heterocystous cyanobacteria are stronger competitors than algae in depleted nitrogen concentrations in the reservoir as manifested elsewhere (Huisman & Hulot 2005).

Cyanobacterial biomass ranged from 0.0009 to 0.834 mg L^{-1} (Figure 4) which was comparable or higher than that reported from a reservoir in central Vietnam (Nguyen *et al.* 2007), but similar or lower than that recorded at many tropical and temperate lakes throughout the world (Trimbee & Prepas 1987; Zhang & Prepas 1996; Marinho & de Moraes Huszar 2002). More than 10 genera of cyanobacteria were recorded and several of them commonly occurred in Tri An Reservoir (Dao *et al.* 2010). However, only *Microcystis* and *Anabaena* were dominant, while the proportion of other genera (e.g. *Cylindrospermopsis, Aphanizomenon*) was minor in cyanobacterial biomass. This could be because not all cyanobacteria have the capacity for buoyancy regulation and nitrogen-fixing or a similar phosphorus uptake rate resulting in different competitive abilities for occurrence and development. Although *Cylindrospermopsis* and *Aphanizomenon* demonstrated a lower demand for nitrogen for development than *Anabaena* (Dao *et al.* 2010), their biomass in the reservoir was lower. This might be related to the preference of *Cylindrospermopsis* for shallow and turbid water bodies (Stuken *et al.* 2006), while Tri An Reservoir is on average 8.4 m and at its deepest point 27 m deep. Together with low orthophosphate concentration (Table 1), the phosphorus uptake competition among phytoplankton might have limited the mass development of cyanobacteria in Tri An Reservoir. Furthermore, algal and cyanobacterial assemblages and abundance are strongly connected to phytoplankton grazers, which were excluded in our study, and need further investigation.

MC concentrations and their correlation with environmental parameters and cyanobacterial biomass

In Tri An Reservoir, both toxic and non-toxic strains of *Microcystis* sp. were co-existing (Dao *et al.* 2010). The low MC concentrations correspond to low abundance of cyanobacteria and the co-existence of toxin-producing and non-producing strains, as also found by Nguyen *et al.* (2007) in another reservoir in central Vietnam where no MC was detected at very low cyanobacterial biomass concentrations (<0.006 mg L^{-1}).

Effects of environmental factors on toxin production of cyanobacteria have been reported with controversial results: in the field, temperature, pH, and DO were strongly and positively correlated to total MC concentration of *Microcystis* sp. while orthophosphate concentration was weakly and negatively correlated (Wicks & Thiel 1990). However, Jacoby *et al.* (2000) showed a positive correlation between phosphorus concentrations and MC concentrations from *Microcystis* and *Anabaena*. In *Planktothrix*, Jann-Para *et al.* (2004) suggested a negative relationship between MC and light intensity, temperature, pH and phosphorus content but a positive correlation between MC and TN:TP ratio. MC production by *Oscillatoria* isolates correlated with high nitrogen concentrations, and also depended on phosphorus concentrations if those were below 0.4 mg L^{-1} (Sivonen 1990). TP strongly correlated with MC concentration in the field (Kotak *et al.* 2000). In the mentioned investigations, different environmental factors and cyanobacterial species/strains were used for correlative assessment, hence resulting in variable outcomes.

The proportion of toxic cyanobacterial strains to non-toxic ones can have a wide range: from 6 to 86% (Shirai *et al.* 1991). Consequently, several cyanobacterial biomass samples were dominated by toxin-producing strains, whereas others were entirely non-toxic (Orr & Jones 1998; Wiedner *et al.* 2002). In case the proportion does not change, toxin production would more or less be correlated with cyanobacterial biomass, which requires further investigations in Tri An Reservoir.

Although MC concentrations in Tri An Reservoir were far below the WHO guideline (WHO 2003), toxic cyanobacteria and their toxins were evidenced (Dao *et al.* 2010). The reservoir is also used for fisheries with an annual fish yield of more than 350 tonnes (personal communication with the director of the reservoir management board), which are consumed daily by local people. Cyanobacterial toxins accumulate in fish muscle and in particular in the liver at concentrations beyond the recommended WHO (2003) consumption guideline value (Magalhaes *et al.* 2003). Dangerously, the viscera of fish such as the liver and heart are a favorite food for many Vietnamese consumers. Hence, via drinking water and fish consumption, local residents might be at risk from chronic low exposure to cyanobacterial toxins. Moreover, as the reservoir is continuously being enriched with nutrients and other anthropogenic pollutants (from fish caging, wastewater from the sugar factory, and agricultural activities upstream of the reservoir), the dynamics of cyanobacterial communities, abundance and toxins could change to a dominance of toxic strains. Hence regular monitoring of cyanobacteria and their toxins and further studies related to toxin accumulation in fish from the reservoir is strongly recommended.

CONCLUSIONS

During the monitoring in Tri An Reservoir, temperature and pH were stable while the water turbidity changed seasonally. Nitrogen, phosphorus and TN:TP ratio were favorable for phytoplankton development. Phytoplankton assemblage in the reservoir consisted of the major taxonomic groups of freshwater algae and cyanobacteria. Phytoplankton biomass and proportion varied temporarily and spatially, influenced by physical conditions, available nutrients and nutrient competition among the phytoplankton groups. Phytoplankton chlorophyll and TP concentrations were closely related as previously formulated by Reynolds (2006). Cyanobacterial biomass correlated positively with temperature, pH, BOD_5 but negatively with total inorganic nitrogen concentrations. MC concentrations in the reservoir were low and weakly positively correlated with BOD_5, TN:TP ratio and cyanobacterial biomass. Additionally, co-existence and competition for nutrients of both toxic and non-toxic strains in the reservoir influenced the toxin concentration. Further studies on zooplankton and planktivorous fish should be implemented for evaluation of phytoplankton communities and biomass dynamics in the reservoir. Regular monitoring of cyanobacteria and their toxins is recommended for implementation in future.

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