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DYNAMICS OF THE CYANOBACTERIAL WATER BLOOM WITH FOCUS TO *MICROCYSTIS* AND ITS RELATIONSHIP WITH ENVIRONMENTAL FACTORS IN BRNO RESERVOIR

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Abstract

STRAKOVÁ LUCIE, KOPP RADOVAN, MARŠÁLKOVÁ ELIŠKA, MARŠÁLEK BLAHOSLAV: *Dynamics of the cyanobacterial water bloom with focus to Microcystis and its relationship with environmental factors in Brno reservoir.* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 5, pp. 1383–1390

Our paper brings new information about long-term changes of the phytoplankton communities in the Brno reservoir with the focus on the *Microcystis* abundance using the semi-monthly monitoring data covering the period 2006–2012. The main aim is to extract from this long-term data set differences in number of *Microcystis* cells depending on environmental factors. The development of cyanobacteria in Brno reservoir is caused by excessive phosphate loading from wastewater treatment facilities upstream and from non–point sources along the Svratka river. It focuses management effort on upstream controls of reservoir condition. High abundance in millions of cyanobacteria cells in 1ml observed in Brno reservoir before was reduced to values in the order of thousands cells in 1ml in last two years through a combination of measures (liming, precipitation of phosphorus on inflow, aeration and destratification). Phytoplankton composition was also changed and at the expense of cyanobacteria promoted the development of green algae and diatoms.

cyanobacteria, blue-green algae, destratification, phosphorus

Eutrophication is one of the greatest challenges for management of water resources, since its effects have affected the water quality of many ecosystems. The spread and magnification of the eutrophication process in freshwater systems are now often linked to the increased frequency and extent of cyanobacteria blooms, including harmfull species (Kosten *et al.*, 2012). Cyanobacterial blooms have become a worldwide environmental problem and the mechanisms and processes involved in the initiation of cyanobacterial blooms are of great concern. In freshwater ecosystems, algal blooms caused by several cyanobacteria genera, including *Aphanizomenon*, *Cylindrospermopsis*, *Dolichospermum* (*Anabaena*), *Microcystis* and *Planktothrix* (*Oscillatoria*) have been extensively documented (Chorus and Bartham, 1999). Among these genera, *Microcystis* is well known to be the most ecologically damaging group due to its widespread prevalence in water bodies and its toxicity to aquatic and terrestrial organism (Carmichael *et al.*, 2001). The overwhelming dominance of *Microcystis* in various lakes and reservoirs of the world has been explained by water temperature, underwater light climate, nutrients, buoyancy regulation and zooplankton or fish grazing (Reynolds, 2006).

High nutrient levels in eutrophic/hypertrophic waters are regarded as the main influencing factors for the growth of cyanobacteria water blooms. Smith (1983) concluded that bloom-forming cyanobacteria tended to dominate in lakes and reservoirs where the N_T:P_T mass ratio was less than 29. On the other hand, Xie *et al.* (2003) described results indicating that low N_T:P_T ratio is not the cause of *Microcystis* blooms, but a result of the blooms. The different incorporation rates of P and N from sediments by *Microcystis* blooms resulted in low N_T:P_T ratio. Reactive phosphorus is a key regulatory factor for the dominance of the non-nitrogen-fixing cyanobacteria like *Microcystis aeruginosa* (Trimbee *et al*., 1987; Xie *et al*., 2003).

Several studies indicate that *Microcystis* species rapidly grew in the water column at the end of spring and then dominated during summer period (Preston *et al*., 1980). However, *Microcystis* occurrence at unfavourable environmental conditions in winter and early spring was also considered to play an important role in the formation of subsequent water blooms. Many studies have paid attention to cyanobacterial recruitment as determining the contribution of *Microcystis* in sediments to subsequent water blooms in the water column (Brunberg and Blomqvist, 2002). *Microcystis* numbers in the water column, especially in the lowest layers, play an important role in forming the next water bloom (Xu *et al*., 2010).

This paper concentrates on long-term changes of *Microcystis* cells counts using the semi-monthly monitoring data covering the period 2006–2012. The main aim is to extract from this long-term data set differences in numbers of *Microcystis* cells depending on environmental factors.

MATERIALS AND METHODS

Study site

The Brno reservoir (49°N, 16°30´E) is an artificial lake dammed up in 1940 at an altitude of 231 m. The reservoir stretches for 10 km in the valley of the Svratka River with a maximum width of 0.8 km in the main basin next to the dam. The maximum and average depths are 19 m and 7.7 m, respectively, and the total volume is some 17.7.10^6 m^3. The lake area is 2.59 km^2, and the annual average water discharge is 7.68 m^3.s^{-1}. The reservoir is used mainly for recreational and generation of electricity but also serves as a backup drinking water reservoir.

Water of Brno reservoir is classified as highly eutrophic with regular occurrence of cyanobacterial water bloom. Approximately 32.3 tons of phosphorus flow into this reservoir yearly (Gardavská *et al*., 2012). Summer stratification, oxygen saturation in euphotic layer and anaerobic conditions in hypolimnion are typical during the growing season. Project "Clean Svratka basin" which has to improve poor water quality in Brno reservoir and deals especially with reduction of phosphorus load started in 2003. Some measures and interventions for better water quality were in progress during our monitoring.

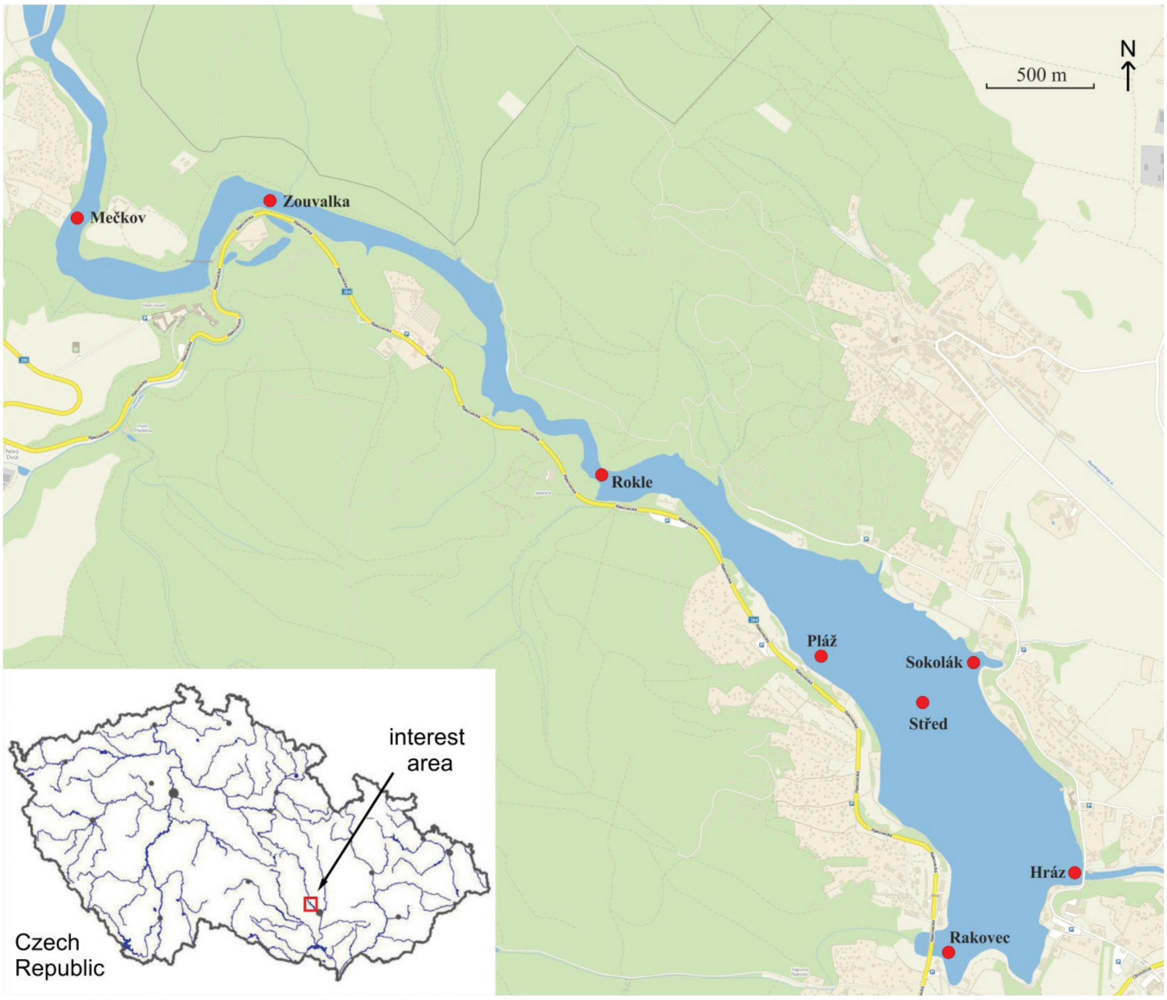
Aerial liming of exposed banks and bottom was made to accelerate the mineralization of sediment, 200 kg.ha^{-1} of limestone hydrate was applied in autumn 2007 and 400 kg.ha^{-1} in spring and autumn 2008 and in spring 2009 as well. Decrease of total phosphorus from 2710 mg.kg^{-1} to 965 mg.kg^{-1} was observed in sediment upper layer (Maršálek *et al*., 2012). Afterwards, the water level begun to decrease at about 30 cm per day and final lowering was of 10 m in 2009. Due to the summering was significantly reduced amount of cyanobacteria inoculum in sediment, from 34.6–42.3 mil. cells.ml^{-1} in 2006–2008 to 0.5–5.4 mil. cells.ml^{-1} in 2009–2012 (Moronga *et al*., 2012).

As a next step was removed part of the fish stock (over the years 2008–2012). Involved was overgrowth population of small cyprinids as a bream (*Abramis brama*) or silver bream (*Abramis bjoerkna*). In addition, some fish predators were delivered after refiling of reservoir in 2010–2011, exactly 4.400 of pike (*Esox lucius*) and 12.500 of zander (*Sander lucioperca*) (Moronga *et al*., 2012). Another arrangement is a dispenser of PIX113 (41% ferric sulphate) on the inflow, installed 2010, which is automatically depending on the volume of water flow and content of phosphorus in inflow water. Dose of 20–60 mg.l^{-1} ferric sulphate precipitated 0.25–0.85 mg.l^{-1} of phosphate on the inflow to values around 0.01 mg.l^{-1} (Maršálek *et al*., 2012; Moronga *et al*., 2012). Water destratification is provided by twenty aeration towers located in deeper part of the reservoir since 2011(approximately near the sampling sites Hráz and Střed). Five towers are equipped with aerators and other 15 have got mixing pumps. Control of the system is performed by in-situ oxygen sensors with the main purpose to prevent oxygen decline at the bottom of the reservoir bellow 2 mg.l^{-1}(Maršálek *et al*., 2012).

Sampling and analytical methods

Water sampling of Brno reservoir was realized from a boat in 8 sites from May to October 2006–2012 (Fig. 1). Water samples for microscopic determination and quantification of phytoplankton (*Microcystis*) were collected from the motor boat by diversion tube call "Angels tube", which shows the profile from the surface to a depth 30 cm. The samples in plastic test tubes (capacity 50–100 ml) were stored in the cooling box until analysis. Determination and quantification was performed in native samples and remainder fixed in formaldehyde (4%) for later check-up. If necessary, samples were concentrated by filtration equipment by Marvan (Marvan, 1957). Colonies of cyanobacteria were disintegrated according to the methodology ČSN 757717. *Microcystis* cells were quantified with counting chamber Bürker and microscope Olympus BX51 and after identified to the species level and expresses as cells per millilitre.

Basic physico–chemical parameters (temperature, pH, dissolved oxygen, transparency, chlorophyll a) were measured immediately on the locality at a depth of 0–50 cm. The transparency of the water was assessed using a Secchi disc. Temperature and pH were measured by device Hanna Combo HI 98129 (Hanna instruments, USA), dissolved oxygen by LDO sensor (Hach–Lange, Colorado, USA). From



1: *Map of the Brno reservoir with the study sites indicated*

the year 2009, water temperature, oxygen and pH were measured in–situ using the data probe YSI 6600 V2–4 (YSI Incorporated, Ohio, USA). The chlorophyll a was determined by Fluoroprobe bbe Moldaenke (bbe Moldaenke GmbH, Germany) during the years 2006–2008, from the year 2009 the data were measured using the probe YSI 6600 V2–4.

RESULTS

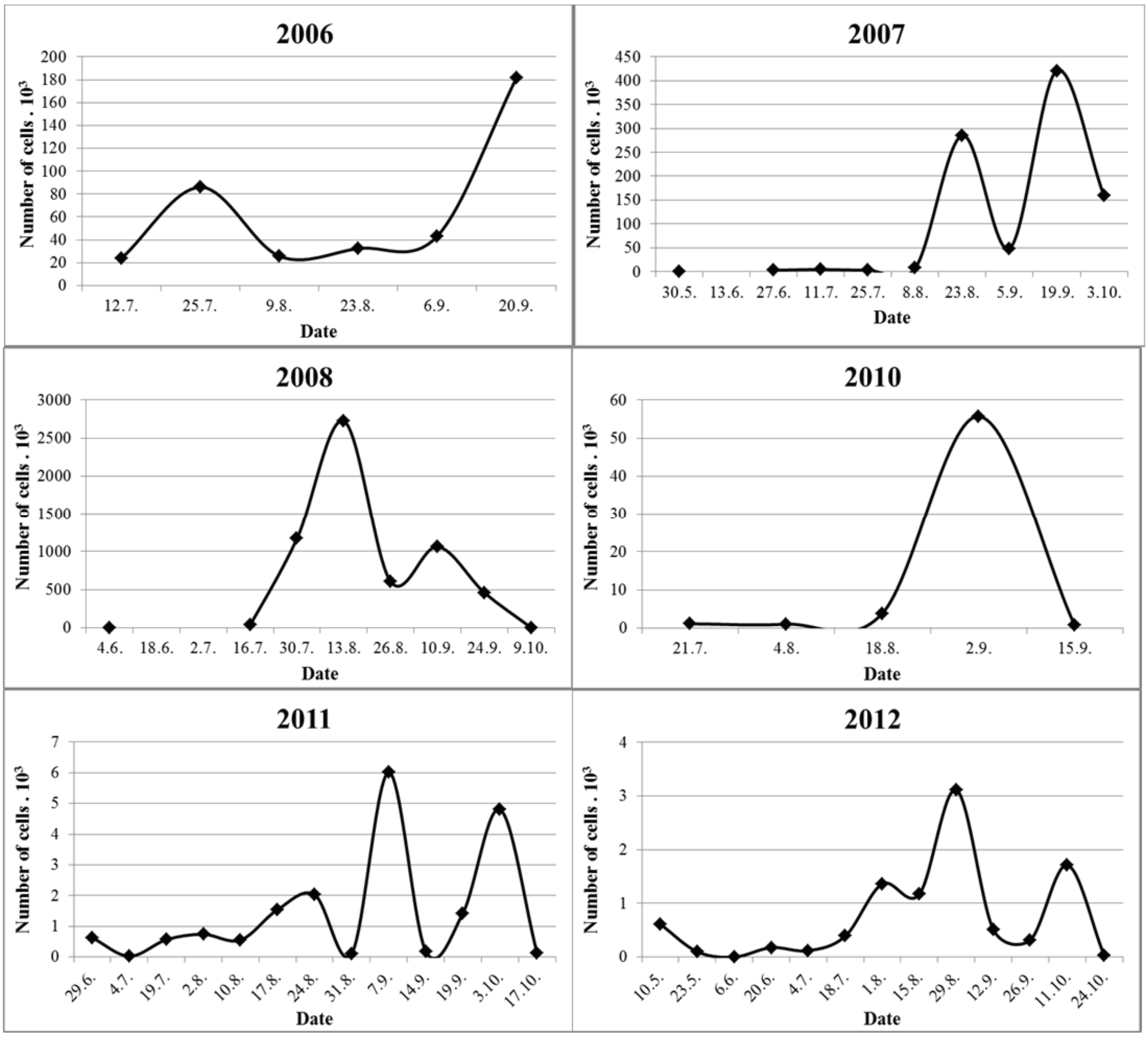
Basic physical and chemical parameters of Brno reservoir are listed in Tab. I. Detected values respond to this type of water reservoir and observed changes of environment are the result of human activity in recent years. Different values of water temperature and transparency were monitored after water lowering about 10 m in 2009. Due to destratification and precipitation of phosphorus on inflow are observed higher water transparency and lower values of chlorophyll a in last two years.

Fig. 2 show average abundance of the genus *Microcystis* in Brno reservoir. Cyanobacteria *Microcystis* entered in this reservoir at the beginning of July of 2006. *Microcystis*, especially *M. aeruginosa* and *M. flos-aquae*, were dominant group of primary producers (91%) during the sampling on 12. 7. 2006. The number of cells gradually increased up to 487 000 cells of *Microcystis* on study site Zouvalka at the end of July. Dominant became mainly *M. aeruginosa* and *M. ichthyoblabe* and species occurring in minority were *M. flos-aquae*, *M. wesenbergii* and *M. viridis*. *Microcystis* remained the dominant genus of primary producers though the number of cyanobacteria and algae cells decreased in August. This situation endured until the beginning of September when abundance started to significantly increase and number of cells extended 1 milion on site Hráz. Dominant species were still *M. aeruginosa* and *M. ichthyoblabe*. Genus *Microcystis* formed 71–96% of the total abundance of cyanobacteria and algae during the July–September of 2006.

Cyanobacteria *Microcystis aeruginosa* was observed in lower abundance in the upper part of the water reservoir (Mečkov and Zouvalka) on the end of May of 2007. The presence of these cyanobacteria was also recorded on research site Rokle on the end of June. While the species *M. ichthyoblabe* and *M. viridis* occurred sporadically, *Microcystis aeruginosa* was found in lower abundance at all locations during July and August. Basically genus *Microcystis*

I: *Physicochemical characteristics (mean ± SD / min. – max.) of the Brno reservoir from May to September in years 2006–2012, N = 30*

Year	temperature °C	pH	dissolved oxygen $mg.l^{-1}$	transparency cm	chlorophyll a $\mu g.l^{-1}$
2006	19.9 ± 3.9 13.8–26.0	8.67 ± 0.58 7.49–9.37	10.36 ± 2.59 6.69–16.62	111 ± 55 40–230	20.4 ± 18.8 1.5–101.6
2007	19.8 ± 2.4 15.3–22.8	8.13 ± 0.63 6.41–9.05	9.48 ± 1.19 7.69–13.34	139 ± 94 60–400	22.8 ± 14.9 2.3–62.6
2008	19.0 ± 3.5 11.2–23.8	9.06 ± 0.51 7.78–9.83	13.66 ± 3.09 9.28–19.48	102 ± 39 40–200	49.5 ± 37.6 10.5–143.1
2009	17.6 ± 3.0 10.9–22.1	8.47 ± 0.65 7.46–9.78	12.31 ± 1.76 9.55–16.33	47 ± 28 20–120	25.2 ± 17.4 4.9–79.0
2010	18.9 ± 3.3 14.3–26.8	8.51 ± 0.40 7.60–9.30	11.18 ± 2.47 7.18–15.06	113 ± 62 40–300	18.3 ± 11.2 2.7–39.5
2011	19.6 ± 2.5 13.6–23.3	8.18 ± 0.42 7.38–8.90	10.25 ± 1.63 8.14–13.70	182 ± 94 40–400	4.5 ± 2.7 1.1–13.1
2012	19.4 ± 2.8 11.6–24.5	7.47 ± 0.39 6.49–8.93	7.42 ± 2.03 2.05–12.62	165 ± 49 100–280	6.1 ± 4.1 1.3–24.5



2: *The number of cells of genus Microcystis during the growing seasons 2006–2012 (Average values from 8 sites)*

formed minority of phytoplankton in this period. Since mid-August, there was a significant increase of total abundance of cyanobacteria. Aside already mentioned species were observed *M. flos-aquae* and *M. wesenbergii*. Dominant genus *Microcystis* occupied 75–93% of total abundance of cyanobacteria and algae in this period. In higher abundance were observed species *M. wesenbergii* and *M. viridis* as well. Maximum abundance of genus *Microcystis* was observed on site Hráz in September 19, 2007 (870 000 cells in 1 ml).

In 2008, first cells of *Microcystis* in low abundance were found in upper part of water reservoir (site Mečkov) in early June. In this case was observed otherwise sporadically seen species *M. flos-aquae.* Genus *Microcystis* became dominant throughout the Brno reservoir since mid-July. The most frequent species was *M. aeruginosa*, in lower abundance occurred also *M. ichthyoblabe* and *M. viridis*. The number of cells in 1ml was approximately in tens of thousands, the highest abundance was observed on site Rokle, 65 000 of cells. Cyanobacteria became absolutely dominant part of phytoplankton in Brno reservoir at the end of July. Number of *Microcystis* cells exceeded 1 million in most of the monitored sites, the highest abundance was in site Rakovec, 3.6 mil. cells per 1 ml. Complete dominance and high abundance of *Microcystis* was observed until the end of September 2008. The highest abundance, 5.7 million of cells per 1ml, was calculated on site Střed. Even at the end of September (24. 9. 2008) were 2.3 million of *Microcystis* cells per 1 ml on site Hráz. Main species was again *M. aeruginosa*, in lower abundance were found *M. viridis*, *M. flos-aquae* and *M. wesenbergii*. The rapid death of cyanobacteria population occurred on all monitored sites in early October, when the number of cells basically did not exceed 2 000 in 1 ml.

The year 2009 was very atypical because the reservoir was partly drained (water level dropped about 10 m below normal). Significant reduction of water area allowed only three sampling sites. Cyanobacteria *Microcystis aeruginosa* were found just in sample Hráz from 20th May 2009 (4 300 cells in 1 ml). In further were cyanobacteria found only in samples collected by phytoplankton net.

Redevelopment of cyanobacteria *Microcystis* was again observed after re-filling of the reservoir in 2010. Genus *Microcystis* was in abundance 15 000 cells per 1 ml only in the upper part of the reservoir at the end of July. Throughout the season occurred mainly *M. aeruginosa*, the presence of other species *M. ichthyoblabe* and *M. wesenbergii* was observed rarely. There was a short-term increase of abundance in early September, cyanobacteria occurred mainly in the body of the reservoir. The maximum number of cells was 200 000 per 1 ml on research site Rokle. A significant reduction in the development of cyanobacteria came in mid-September, when was counted only 2 500 of cells per 1 ml. During October was not found genus *Microcystis* on any of monitored sites.

The growing season 2011 was characterized by a lower abundance of cyanobacteria and algae. Genus *Microcystis*, especially *M. aeruginosa*, *M. viridis*, *M. ichthyoblabe* and *M. wesenbergii* appeared at the end of June and the abundance was in hundreds and thousands until the end of August. The number of cells increased at the end of August and the first half of September, maximum value (63 000 of cells per 1 ml) was observed on site Hráz. Slow decline in phytoplankton biomass started in mid-September and finally the abundance of *Microcystis* was in hundreds in 1 ml.

During the season 2012 was genus *Microcystis* observed already in May. The main species was *M. aeruginosa,* but the abundance was low until early August, maximum measured concentration was 5 500 cells per 1 ml. Some other species, *M. viridis*, *M. ichthyoblabe* and *M. wesenbergii* were found in low abundance in August. Total abundance of *Microcystis* was constantly in thousands cells with the exception of site Zouvalka, where was counted 24 000 of cells per 1 ml. The abundance of cyanobacteria decreased again to hundreds of cells in 1ml during September and October. Finally, there was no significant development of cyanobacteria *Microcystis* for growing season 2012 and this genus remained in minority representation.

DISCUSSION

The monitoring of nutrient sources in the watershed was performed in the Brno reservoir in nineties. The study continued in surveys carried out in previous years, which documented the permanent nutrient inflow by the Svratka river and strong occurrence of cyanobacteria (mainly *Microcystis aeruginosa*) in late summer (Beránková *et al.*, 1993). In last twenty years was number of cyanobacteria cells in order of tens millions cells per millilitre.

Temperature plays an important role in the phytoplankton composition in Brno reservoir. It is commonly observed that warm temperatures favour cyanobacteria. Robarts and Zohary (1987) showed that the temperature optima for the growth rate of *Microcystis* was 25 °C or greater, and growth of *Microcystis* will be severely limited by temperatures below 15 °C. The development of cyanobacterial blooms formed by *Microcystis* in Brno reservoir is mainly during the warmest period of the year.

The start of the cyanobacteria season *(Microcystis)* in Brno reservoir affects the intensity of inflow and higher flows during the growing season. Cyanobacteria of genus *Microcystis* have developed later in July 2006 due to the extreme water inflow (190 $m^3.s^{-1}$) during the April 2006. Higher inflow affected the development of cyanobacteria also in 2009 and 2010. The year 2009 was characterized with almost no cyanobacteria development due to water lowering about 10m. Discharge, flow velocity and turbulence have been identified as important factors influencing the development of cyanobacterial blooms (Mitrovic *et al.*, 2011; Roelke and Pierce, 2011).

Grazing pressure is also thought to be an important factor to regulate the phytoplankton structure. Inedible algal species growth can be promoted by the high herbivore grazing pressure for the edible algae in the early summer (Sommer *et al.*, 1986). Ortega-Mayagoitia *et al.* (2003) indicated that the presence of planktivorous fish and the different composition of zooplankton have minor importance in phytoplankton composition under hypertrophic conditions. Large cladocerans often cannot grow and reproduce fast enough to prevent bloom formation, and they always disappear when the bloom becomes dense (Gliwicz 1990). Biomanipulation with fish stock had probably an insignificant effect on the cyanobacteria bloom development.

On the other hand a big impact had an application of limestone in Brno reservoir in 2007 and 2009. This application accelerates mineralization of organic matter and increased biomass of cyanobacteria and algae in year after application (2008). However, liming causes a reduction of cyanobacteria biomass in the long term. Multiple moderate doses of lime on eutrophic lakes and reservoirs were effective at reducing chlorophyll a and phosphorus concentrations over longer periods (Prepas *et al.*, 2001).

Reactive phosphorus is a key regulatory factor for the dominance of the non–nitrogen–fixing cyanobacteria like *Microcystis aeruginosa* (Xie *et al.*, 2003; Wang *et al.*, 2012). It is striking that the abundance of *Microcystis* was significantly positively correlated with amount of total phosphorus (Xu *et al.*, 2010). Xie *et al.* (2003) indicated that cyanobacteria may indirectly enhance the release of phosphorus from sediments. Cyanobacteria biomass developments in the reservoir depend on allochthonous inputs or to internal loading of phosphorus from anoxic sediments (Lehman, 2011). The development of cyanobacteria in Brno reservoir is caused by excessive phosphate released from wastewater treatment facilities upstream and from non–point sources along the Svratka river. It focuses management effort on upstream controls of reservoir condition. Alternative sources of phosphorus can be from internal loading after episodic mixing events, which inject nutrient–rich hypolimnetic water into the epilimnion. On inflow to reservoir during vegetation period (since 2010) is dosing PIX 113 (41% ferrous sulphate) for decrease of phosphorus, automatically in dependence on body of inflow water and content of phosphorus (Maršálek *et al.*, 2012; Moronga *et al.*, 2012). The influence of iron-phosphate precipitation on the trophic level of water and decrease of chlorophyll-a concentration described Jaeger (1994). Amount of phytoplankton significantly decreased due to the lower concentration of available phosphorus in 2011 and 2012. Species composition was also changed and at the expense of cyanobacteria promoted the development of green algae and diatoms.

Artificial mixing in the eutrophic Brno reservoir was successful in preventing blooms of the cyanobacterium *Microcystis*. During the two years of artificial (2011 and 2012) deep mixing, the number of cells of *Microcystis* per millilitre was lower than in the several preceding years. Artificial mixing has been widely adopted as a method to control algal blooms. In most cases, air bubble plumes have been installed to induce destratification, leading to efficient mixing and oxygenation (Visser *et al.*, 1996; Lindenschmidt and Chorus, 1997; Becker *et al.*, 2006).

The chlorophyll a concentrations were lower in the mixed reservoir. Some studies showed that destratification decreased algal biomass (Simmons 1998; Imteaz *et al.*, 2009). The phytoplankton shifted from cyanobacteria dominated community in summer to a mixed community of green algae and diatoms. Reduced sedimentation losses in the mixed reservoir, probably in combination with lower pH and oxygen distribution with depth, favoured non-buoyant algae, while the entrainment of cyanobacteria in the turbulent flow nullified their advantage of buoyancy.

Be based on long term monitoring of *Microcystis* abundance at the Brno reservoir we can state a significant reduction in the number of cells during the growing season in recent years. High abundance in millions of cyanobacteria cells in 1ml observed in 2008 was reduced to values in the order of thousands cells in 1ml in last two years through a combination of measures (liming, precipitation of phosphorus on inflow, aeration and destratification). The question is, what would be the development of phytoplankton in Brno reservoir without all these measures and leaving the natural succession of planktonic communities.

SUMMARY

The Brno reservoir is an artificial lake dammed up in 1940 at an altitude of 231 m. Water of Brno reservoir is classified as highly eutrophic with regular occurrence of cyanobacterial water bloom. Our paper brings information about long-term changes of the phytoplankton communities in the Brno reservoir with the focus on the *Microcystis* abundance using the semi-monthly monitoring data covering the period 2006–2012. Dominant species from genus *Microcystis* in the Brno reservoir used mainly *M. aeruginosa* and *M. ichthyoblabe* and species occurring in minority were *M. flos-aquae*, *M. wesenbergii* and *M. viridis*. The main aim is to extract from this long-term data set differences in number of *Microcystis* cells depending on environmental factors. Reactive phosphorus is a key regulatory factor for the dominance of the non–nitrogen–fixing cyanobacteria genus *Microcystis* in the Brno reservoir. The development of cyanobacteria in Brno reservoir is caused by excessive phosphate released from wastewater treatment facilities upstream and from non–point sources along the Svratka

river. It focuses management effort on upstream controls of reservoir condition. Be based on long term monitoring of *Microcystis* abundance at the Brno reservoir we can state a significant reduction in the number of cells during the growing season in recent years. High abundance in millions of cyanobacteria cells in 1 ml observed in 2008 was reduced to values in the order of thousands cells in 1ml in last two years through a combination of measures (liming, precipitation of phosphorus on inflow, aeration and destratification). The phytoplankton shifted from cyanobacteria dominated community in summer to a mixed community of green algae and diatoms.

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