

Influence of inorganic nutrients and dissolved organic matter on the growth of cyanobacteria *Microcystis aeruginosa* isolated from the Gulf of Riga

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Abstract

The influence of nutrient additions in N-limiting, P-limiting and in DIN:DIP-balanced conditions with and without dissolved organic matter (DOM) of land origin was studied on the growth and toxin content of *Microcystis aeruginosa* isolated from the Gulf of Riga. The dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) supply in balanced ratio of DIN : DIP = 16 is the main determinative factor for *M. aeruginosa* development, promoting increases in cell biomass, chlorophyll *a* content and particulate organic matter. In medium enriched only by DOM, *M. aeruginosa* used 31 % of the available dissolved organic nitrogen and 53 % of the dissolved organic phosphorus. The experiment conditions did not invoke increment of toxicity. The experiments showed that the development of *M. aeruginosa* is promoted by the raised load of inorganic nutrients and dissolved organic matter to the brackish ecosystems of the Baltic Sea.

Key words: cyanobacteria, dissolved organic matter, *Microcystis aeruginosa*, nutrient limitation, the Gulf of Riga.

Introduction

Nutrients, light and temperature are the most important factors for the development of marine phytoplankton. Human activities contribute to increased nutrient discharge to aquatic ecosystems from agriculture, municipal and industrial waste waters world wide. These anthropogenic nutrient sources have led to increased nutrient fluxes also in the Baltic Sea invoking eutrophication processes, followed by reduced water transparency, increased phytoplankton primary production, changes of planktonic species composition etc. (HELCOM 2007). Significant impact on nutrient input is favored also in terms of climate change. For the Baltic Sea ecosystems important changes are in the timing of the seasonal events: water discharge increment in winter in association with the reduction of spring runoff and shortening of the ice and snow cover period. Estimates indicate that river runoff may increase by up to 15 % averaged over the Baltic Sea catchment region, enlarging the export of dissolved organic matter to the brackish ecosystems (HELCOM 2007). Significant dependence of the river discharge on climate change has been also

found in Latvia (Kļaviņš, Rodionovs 2007).

Two bottom-up mechanisms have been invoked as major contributors in the increase of toxic algal blooms in last decades. From one side, human activities significantly increase the input of algal nitrogenous and phosphorus nutrients to estuarine and coastal waters, while the silicon concentration has remained constant or has even decreased in river loads to brackish estuaries (Rahm et al. 1996). It is assumed that in such conditions the proliferation of organisms having little or no requirement for silicon, such as flagellates and cyanobacteria, is favored (Humborg et al. 2000). On the other side coastal waters close to estuaries receive large quantities of dissolved organic matter (DOM), which may favor the growth of auxotrophic/photo-heterotrophic species versus that of autotrophic species (Paerl 1988).

Cyanobacteria have been responsible for the most harmful phytoplankton events in the Baltic Sea. Over the entire Gulf of Riga the potentially toxic cyanobacteria *Aphanizomenon flos-aquae*, *Nodularia spumigena*, *Snowella lacustris* and *Anabaena lemmermannii* can develop, whereas in the coastal areas common species are *Anabaena spiroides*, *Anabaena flos-aquae* and *Microcystis aeruginosa* (Balode, Purina 1996). The potentially toxic cyanobacteria *Microcystis aeruginosa* is mostly known to develop in freshwater, but it is also frequently observed in marine coastal waters (Kononen, Sellner 1995). Additionally, high concentration of *M. aeruginosa* biomass is transported to estuarine areas via rivers. In the coastal areas of the Gulf of Riga maximum development of *M. aeruginosa* continues from late June till September., the development usually occurs sporadically, but often are initiated blooms in the coastal zones (Balode, Purina 1996; Seppälä, Balode 1999). *M. aeruginosa* abundance is related with high nutrient concentrations (Rantajärvi et al. 1998). Studies on this species have intensified in the last decades mainly due to the discovery of potent hepatotoxic heptapeptides, called microcystins, shown to be deleterious to wild and domestic animals and also humans.

In natural water-bodies *M. aeruginosa* is found as several toxic and non-toxic genotypes. Whether a genotype can produce microcystins depends on the presence of intact genes from the microcystin synthetase gene complex (Kurmayer et.al. 2002). A seasonal succession of different *Microcystis* genotypes might often be a key mechanism determining microcystin concentrations in *Microcystis*-dominated lakes (Kardinaal et.al. 2007a) and other water-bodies. In nature several mechanisms might be involved in seasonal succession of toxic and non-toxic *Microcystis* strains: selective feeding by bivalves (Dionisio-Pires, Van Donk 2002) and zooplankton (Lüring 2003), *Microcystis* strains may differ in their buoyancy (Dunton, Walsby 2005) and competitive ability for light (Kardinaal et al. 2007b). The changes in nutrient concentrations and ratios in the environment affect not only phytoplankton community structure, but also algal physiology. Thus N-rich PSP toxins are synthesized during excess N and P-limitation but not during N-limitation (Granéli et al. 1998).

The estimated microcystin concentrations of *M. aeruginosa* isolated from the Gulf of Riga are low and range between 0.8 to 4.5 ng mg⁻¹ dry mass. The potentially toxic cyanobacteria *Nodularia spumigena* also can produce hepatotoxic toxin nodularin in high concentrations. However the observed toxicity of *Nodularia spumigena* in the Gulf of Riga is lower (nodularin 0.030 - 0.123 mg L⁻¹) than in the Open Baltic Sea (0.03 - 1.35 ng mg⁻¹); the toxin content of the Gulf of Riga *Nodularia spumigena* filaments is about 12 times higher than those from the open Baltic (Balode, unpublished data).

The aim of the present study was to test the effect of dissolved organic matter and raised inorganic nitrogen and phosphorus concentrations in different DIN : DIP ratios on the growth and toxicity of potentially toxic cyanobacteria *M. aeruginosa*, isolated from the coastal area of the Gulf of Riga, the Baltic Sea.

Materials and methods

The clone culture of *Microcystis aeruginosa* Kützing (MAGR-2) was isolated from the Gulf of Riga, Baltic Sea. The stock culture was grown in F medium of Guillard and Ryther (1962), the N : P ratio was adjusted to the Redfield value – 16 (1934). Iron was added in the form of Fe-EDTA.

The *M. aeruginosa* mother culture was obtained after centrifugation in sterile conic flasks. Supernatant containing the growth media was removed and cells were re-suspended in nutrient-free sterile seawater and left for 24 h in the culture room. Then, centrifugation and re-suspension were repeated, resulting in partly nutrient-depleted *M. aeruginosa* cells in a mother suspension free from dissolved nutrients. The culture was inoculated to experimental 5 L bottles, providing an initial concentration of *M. aeruginosa* 18.7×10^6 cells per liter. The cultures were grown at 18 ± 1 °C, with a 16 h / 8 h light/dark period at $53 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance from cool white fluorescent lamps.

The growth of *M. aeruginosa* was tested in natural seawater (control), in phosphorus limitation condition (DIN : DIP = 100), in nutrient balanced (DIN : DIP = 16), as well as in nitrogen limitation (DIN : DIP = 3) conditions. All experimental treatments were incubated in three replicates. To test the effect of dissolved organic matter (DOM) a parallel set of treatments was established with DOM addition, also in three replicates. For the experimental phosphorus limitation, balanced N and P and nitrogen limitation treatments, pre-autoclaved sterile nutrient solutions of NaNO_3 and/or KH_2PO_4 and 0.22 μm filtered DOM were aseptically added (Table 1). Cultures were grown in filter-sterilized (0.22 μm) seawater (4 L) in 5-L polycarbonate pre-autoclaved bottles. Enrichments of Fe-EDTA, metals and vitamins were added. Nutrient additions were made gradually from Day 0 to Day 6. Pure seawater and seawater with DOM addition was used as a control in triplicate.

M. aeruginosa growth was monitored daily by *in vivo* fluorescence with a 10 AU Turner Fluorometer (Brand et al. 1981). Estimation of cell number and nutrient concentration was performed on Day 7 (one day after the end of the nutrient addition), Day 9 (end of exponential growth phase), Day 12 and Day 15 (stationary phase). Cell counting was performed until Day 24.

M. aeruginosa cell samples (5 mL; Neubauer cell) were fixed with acid Lugol's solution and counted under a microscope. Chlorophyll *a* was determined spectrophotometrically after filtration through glass microfibre filters (Whatman GF/F), and extraction with pure methanol, at 4 °C, for 1 h in the dark (Jespersen, Christofersen 1987).

Inorganic nutrient concentrations were measured with a Skalar autoanalyzer, following standard procedures for seawater (Valderrama 1995).

Samples for particulate organic carbon (POC) and particulate organic nitrogen (PON) analysis were collected on precombusted glass microfibre filters Whatman GF/F, then decarbonated (HCl fumes, overnight), and analyzed using a CHN analyzer, model 1500 from Carlo Erba. Samples for particulate organic phosphorus (POP) were collected as for

Table 1. Concentration of dissolved organic matter (DOM), dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) added to control (Control + DOM), nutrient balanced (DIN : DIP = 16; (DIN : DIP = 16) + DOM)), phosphorus limited (DIN : DIP = 100; (DIN : DIP = 100) + DOM) and nitrogen limited treatments (DIN : DIP = 3; (DIN : DIP = 3) + DOM), dissolved organic nitrogen – DON; dissolved organic phosphorus – DOP; nitrate – NO₃; phosphate – PO₄.

Treatment	Nutrient	Concentration (μM)
Control	-	-
+ DOM	DON	23.1
	DOP	0.18
DIN : DIP = 100	NO ₃	66.4
	PO ₄	0.66
(DIN : DIP = 100) + DOM	NO ₃	66.4
	PO ₄	0.66
	DON	23.1
	DOP	0.18
DIN : DIP = 16	NO ₃	66.4
	PO ₄	4.16
(DIN : DIP = 16) + DOM	NO ₃	66.4
	PO ₄	4.16
	DON	23.1
	DOP	0.18
DIN : DIP = 3	NO ₃	12.5
	PO ₄	4.16
(DIN : DIP = 3) + DOM	NO ₃	12.8
	PO ₄	4.16
	DON	23.1
	DOP	0.18

POC and PON. POP concentration was obtained as the PO₄ concentration after persulfate digestion at 120 °C (Pujo-Pay, Raimbault 1994).

DOM extract was obtained in May, June and July 1999 from the River Pärnu water, which flows into the Gulf of Riga. Extraction was made using a tangential flow ultra filter (Benner et al. 1997). Firstly, river water was filtered through 1.2 μm and 0.2 μm Opticap filter units (Millipore); then, DOM was concentrated with a tangential device, the Prep/scale™ TFF 6 ft2 cartridge (Millipore). The fraction of U = 10³ - 10⁶ Daltons [Da] [abridged >1000] was obtained and used. Total dissolved nitrogen and phosphorus concentrations were determined after persulfate digestion at 120 °C (Pujo-Pay, Raimbault 1994); DON was calculated as the difference between total nitrogen concentration and DIN (NO₃ + NO₂ + NH₄), and DOP concentration was obtained as difference between total phosphorus and PO₄. The obtained concentrations of DON and DOP in concentrates of DOM were 639 μM and 5.14 μM, respectively (DON : DOP = 124). The DOM extract contained 49 μM NO₃ and 3.37 μM PO₄.

The microcystin content was estimated by protein phosphatase 1A inhibition assay (Ward et al. 1997).

Statistical analysis of variance (ANOVA) was used to test the significance of differences in *M. aeruginosa* growth, biomass increase and nutrient dynamics between experimental conditions

Results

The growth of *M. aeruginosa* started without any lag phase in the control and the + DOM conditions. The cell concentration increased until Day 6 in the control, and until Day 14 in the + DOM condition (Fig. 1). With inorganic and organic nutrient additions, cyanobacteria growth occurred according to the typical pattern for a batch cultures: a 1 to 2 day lag phase followed by an exponential-growth phase lasting from Day 3 until Day 10 or Day 11. During the last five days of the experiment the cell concentration decreased. Differences in cell concentration due to the addition of DOM were visible mostly during the stationary phase.

Chlorophyll *a* concentrations started to increase exponentially from Day 1. Maximal chlorophyll *a* concentrations in the control and the + DOM condition were observed already on Day 5, in other conditions with inorganic and organic nutrient additions at Days 6 - 8 (Fig. 2).

As nutrient additions ended on Day 6, the cultures evolved as batch cultures after that time. Nutrient supplies decreased continuously until the end of culturing. In some cases nutrients were taken up rapidly. This occurred for nitrates (NO_3) in the DIN : DIP-balanced conditions ($p < 0.05$), where NO_3 concentration already reached $< 1 \mu\text{M}$ on Day 9 and

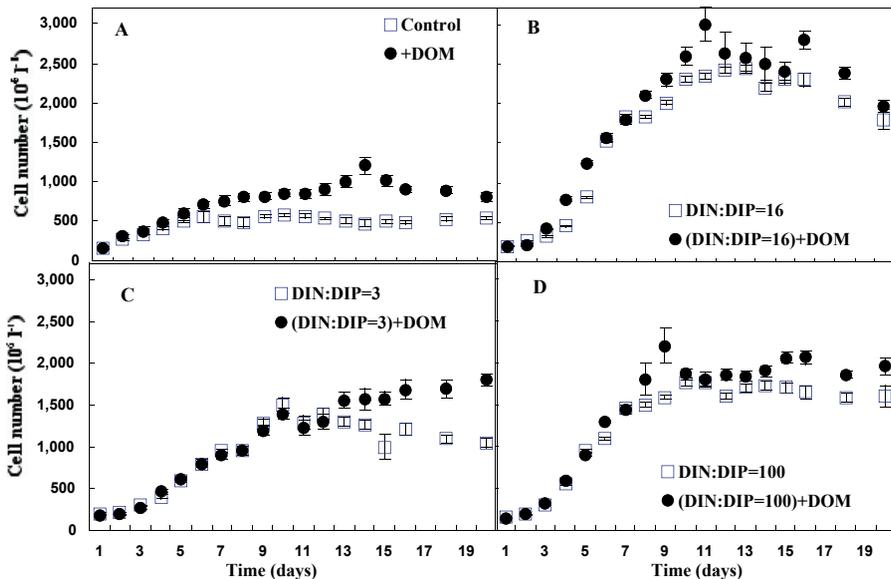


Fig. 1. Cell number of *Microcystis aeruginosa* versus time (days) in the treatment without inorganic nutrient enrichments (A), treatment with nutrient enrichments in balanced ratio (B), N - limited (C) and P - limited treatment (dissolved inorganic nitrogen - DIN; dissolved inorganic phosphorus - DIP; dissolved organic matter - DOM), vertical bars indicates standard deviation between triplicate.

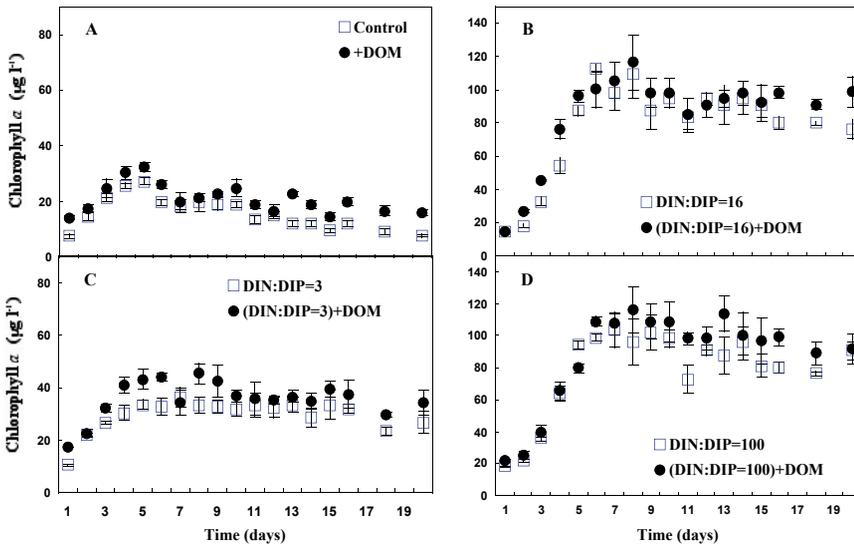


Fig. 2. Chlorophyll *a* concentration of *Microcystis aeruginosa* versus time (days) in the treatment without inorganic nutrient enrichments (A), treatment with nutrient enrichments in balanced ratio (B), N – limited (C) and P – limited treatment (dissolved inorganic nitrogen – DIN; dissolved inorganic phosphorus – DIP; dissolved organic matter – DOM), vertical bars indicates standard deviation between triplicate.

decreased near to detection limit at the end of sampling on Day 15. In contrast, phosphate (PO_4) concentration decreased slightly in the same condition and large amounts of PO_4 were left unused in the cultures on Day 15. In all treatments, NO_3 and PO_4 concentrations were lowest on Day 15.

In all nutrient-enriched cultures cell number together with particulate organic carbon (POC), particulate organic nitrogen (PON) and particulate organic phosphorus (POP) concentrations continued to increase after the last nutrient addition on Day 6, indicating uptake of N and P and biomass production. For all replicates particulate organic carbon, nitrogen and phosphorus concentration on Days 12 and 15 reached the maximum values. Thus, values recorded on Day 15 were used to determine yields and to estimate the relative cellular contents of carbon, nitrogen and phosphorus.

Nitrogen : phosphorus balanced treatment (DIN : DIP = 16 and (DIN : DIP = 16) + DOM)

The highest *M. aeruginosa* numbers and chlorophyll *a* concentrations were observed in the DIN : DIP balanced treatment. Nitrate and phosphate addition in balanced ratio yielded the highest POC, PON and POP increment, while addition of dissolved organic matter had little effect. The biomass increases sustained by DOM were only 7 % for POC, 8 % for PON and 9 % for POP (Table 2).

Phosphorus limited treatment (DIN : DIP = 100 and (DIN : DIP = 100) + DOM)

The second highest cyanobacteria cell and chlorophyll *a* concentrations were obtained under P-limitation. Biomass increases obtained by nitrate and phosphate additions

were lower as in the balanced treatment. *M. aeruginosa* growth in phosphorus-limiting conditions did not benefit so much from the addition of DOM; the corresponding value for POC was 14 %; for PON 13 % and 10 % for POP.

Nitrogen-limiting treatment (DIN : DIP = 3 and (DIN : DIP = 3) + DOM)

Cell number, chlorophyll *a* concentration, POC and PON concentrations were significantly lower ($p < 0.05$) under N limitation than in nitrogen: phosphorus balanced and phosphorus limited conditions (Fig. 1, 2, Table 2). Cells that were nitrogen-limited seemed to benefit from the addition of DOM; POP concentration in the (DIN : DIP = 3) + DOM treatment represented an increase of 20 % compared to the DIN : DIP = 3 treatment ($p < 0.05$).

Unenriched seawater with and without DOM (Control and + DOM)

The *M. aeruginosa* counts in the control and + DOM treatment were lower than in all inorganic nutrient addition treatments ($p < 0.05$, Fig. 1, 2, Table 2). However the addition of DOM to the unenriched seawater (control) significantly ($p < 0.01$) increased cyanobacteria *M. aeruginosa* cell number (Fig. 1) and chlorophyll *a* concentration (increase coefficient: 1.8 ± 0.3 , $n = 24$) in comparison to the control. The increase of cell number was followed by a significant increase of PON and POP concentrations in the +DOM treatment compared to the unenriched control (Table 2). The highest increase by 53 % ($p < 0.05$) occurred for POP concentration, compared to 31 % ($p < 0.05$) for PON concentration. We infer that *M. aeruginosa* acquired nitrogen and phosphorus from DOM in conditions where inorganic nutrients were not added, and that DOP of land origin is to some extent a substitute for PO_4 in P-depleted condition.

Table 2. Mean particulate organic carbon (POC) concentrations, particulate organic nitrogen (PON), particulate organic phosphorus (POP) and PON : POP ratios of *Microcystis aeruginosa* recorded on Day 15 in nutrient balanced and nutrient limited experimental treatments (dissolved inorganic nitrogen – DIN; dissolved inorganic phosphorus – DIP; dissolved organic matter – DOM). Number after (\pm) indicates standard deviation between three replicates. Numbers within parentheses show relative increase of POC, PON and POP concentrations sustained by the addition of dissolved organic matter, in percents.

Treatment	POC (μM)	PON (μM)	POP (μM)	PON : POP
Control	250 \pm 23	12.1 \pm 2.5	0.53 \pm 0.23	22.8 \pm 8.3
+ DOM	340 \pm 37 (26 %)	17.5 \pm 1.4 (31 %)	1.12 \pm 0.09 (53 %)	15.6 \pm 1.7
DIN : DIP = 100	700 \pm 32	54.9 \pm 1.5	1.28 \pm 0.02	42.8 \pm 1.0
(DIN : DIP = 100) + DOM	820 \pm 21 (14 %)	62.8 \pm 1.5 (13 %)	1.43 \pm 0.03 (10 %)	43.9 \pm 1.3
DIN : DIP = 16	790 \pm 39	61.2 \pm 0.2	3.04 \pm 0.11	20.1 \pm 0.9
(DIN : DIP = 16) + DOM	850 \pm 38 (7 %)	66.9 \pm 1.5 (8 %)	3.33 \pm 0.17 (9 %)	20.2 \pm 1.4
DIN : DIP = 3	450 \pm 19	23.7 \pm 2.6	3.02 \pm 0.09	7.8 \pm 0.8
(DIN : DIP = 3) + DOM	610 \pm 12 (26 %)	26.8 \pm 0.9 (12 %)	3.79 \pm 0.25 (20 %)	7.1 \pm 0.7

Discussion

M. aeruginosa reached the highest cell and chlorophyll *a* concentrations in the nitrogen and phosphorus balanced treatment (DIN : DIP = 16 and (DIN : DIP = 16) + DOM). Since the time when Redfield (1934) observed that $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ are taken up by phytoplankton at a constant atomic ratio of 16 : 1 and Fleming (1940) pointed out that this value is also that of the elemental composition of phytoplankton, it has been commonly accepted that values >16 would reflect P-limited and values < 16 would reflect N-limited cell contents. PON : POP values recorded in our two DIN : DIP balanced conditions were somewhat higher (approximately 20) than the expected Redfield's value – 16. It has been reported that chemical contents of cells vary between species. For example Sakshaug and Holm-Hansen (1977) concluded that the point of change for P-deficiency in cells differs between species, for example, for *Skeletonema costatum* PON : POP = 23. We assume that cells of *M. aeruginosa* in DIN : DIP = 16 conditions were neither N- nor P-limited. The DIN and DIP supply in balanced ratio (16) is the main determinative factor for development of *Microcystis aeruginosa*, promoting high cell biomass and chlorophyll *a* content. The addition of DOM did not affect the cell N : P ratio in the presence of NO_3 and PO_4 additions.

In the phosphorus limited treatments (DIN : DIP = 100 and (DIN : DIP = 100) + DOM) the *M. aeruginosa* count was lower than in the DIN : DIP balanced treatment. As the PON : POP ratios of the cells grown in the phosphorus-limiting treatments were 42.8 ± 1.0 and 43.9 ± 1.3 (Table 2) we infer that the cells in both treatments were clearly phosphorus-limited. The DOM addition raised *M. aeruginosa* biomass and cell POP and PON content, while the PON : POP ratio became more P limited, showing that DOP could not compensate the P-deficiency. Similarly Panosso and Graneli (2000) showed that DIP-limited *Nodularia spumigena* was unable to use DOM as a source of phosphorus.

M. aeruginosa cells grown in the nitrogen-limiting treatment (DIN : DIP = 3 and (DIN : DIP = 3) + DOM) clearly showed nitrogen-limitation, as cell PON : POP was 7.8 ± 0.8 and 7.1 ± 0.7 . Hillebrand and Sommer (1999) considered that a N : P < 13 in microalgae indicates nitrogen limitation. In contrast, Panosso and Graneli (2000) observed that DON can provide cyanobacteria cells with nitrogen and increase the yield of *Nodularia spumigena* under conditions of N deficiency. These differing results might have originated from different protocols – extremely severe N and P limitation (DIN : DIP 0.02 and 643) and high amount of DOM added, while in our experiment DOM was added at in-situ like concentrations.

The addition of DOM to unenriched seawater promoted a significant decrease of cell PON : POP ratio by 32 %, changing from a P-limited (PON : POP = 22.8) to a N : P-balanced (PON : POP = 15.6) content (Table 2). As in this treatment the DON : DOP ratio in the medium was 9.2, we infer that cells took up proportionally more organic phosphorus than nitrogen. This idea is supported by the literature, as it is known that several cyanobacteria can acquire phosphorus from organic compounds, and *M. aeruginosa* has large capacity for organic phosphorus mineralization Giraudet et al. (1997). Therefore, we conclude that in the treatment where inorganic phosphorus was not added, *M. aeruginosa* mostly took up dissolved organic phosphorus from river DOM.

The utilization of dissolved organic matter (DOM) in aquatic ecosystems was long thought to be the purview of heterotrophic microorganisms, till Paerl (1991) asked a

fundamental question: how can relatively high numbers of actively growing picoplankton survive and periodically flourish in dissolved inorganic nitrogen-depleted waters? He was the first who showed the light-stimulated incorporation of amino acids by cyanobacteria *Synechococcus* species. Other studies have shown that algal biomass and alkaline phosphatase activity are significantly higher in the presence of riverine humic substances than in nitrate-enriched controls (Carlsson et al. 1993). DOM contributes to a biomass increase of cyanobacteria (Ponosso, Granéli 2000) and iron bound in DOM ensures the iron demands (Stolte et al. 2006). According to Maestrini et al. (1999) the DOM from Daugava River promoted *M. aeruginosa* yield to 38 % of that sustained by optimal nitrate concentration.

Our experiments show that the growth of *M. aeruginosa* was promoted by DOM, but since our cyanobacteria cultures were not bacteria-free, we do not infer that *M. aeruginosa* directly obtained all used N and P from DOM. Bacteria associated with *M. aeruginosa* are involved in ammonification and nitrification (Purvina, unpublished data), but still the mechanisms of DOM and particularly DOP uptake are not unequivocal. DOM enhances cyanobacteria biomass production via algal mixotrophy together with bacterial degradation and photochemical modification of marine humic substances. Altogether, the best growth of *M. aeruginosa* isolated from the Gulf of Riga was achieved by nitrate and phosphate additions in the DIN : DIP balanced (16) treatment. The addition of DOM to the control and the different DIN + DIP conditions increased cell number and raised cellular POC, PON and POP concentrations. In inorganic nutrient depleted conditions, *M. aeruginosa* can acquire nitrogen and phosphorus from riverine dissolved organic matter.

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Neorganisko barības vielu un izšķīdušās organiskās vielas ietekme uz Rīgas liča cianobaktērijas *Microcystis aeruginosa* augšanu

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Kopsavilkums

Pētīja barības vielu ietekmi N-limitējošos, P-limitējošos un DIN : DIP līdzsvarotos apstākļos, ar un bez sauszemes izcelsmes izšķīdušās organiskās vielas (DOM) klātbūtnes, uz cianobaktērijas *Microcystis aeruginosa*, kas izolēta no Rīgas liča, augšanu un toksīna saturu. Izšķīdušā neorganiskā slāpekļa (DIN) un izšķīdušā neorganiskā fosfora (DIP) pievienošana līdzsvarotā attiecībā DIN : DIP = 16 ir galvenais *M. aeruginosa* attīstību noteicošais faktors, kas veicina šūnu biomasas, hlorofila *a* un daļiņveida organiskās vielas pieaugumu. Vidē, kas bija bagātināta tikai ar DOM, *M. aeruginosa* izmantoja 31 % no pieejamā izšķīdušā organiskā slāpekļa un 53 % no izšķīdušā organiskā fosfora. Eksperimenta apstākļi neveicināja cianobaktērijas toksiskuma palielināšanos. Eksperiments parādīja, ka *M. aeruginosa* attīstību veicina paaugstināta neorganisko barības vielu un izšķīdušās organiskās vielas ieplūde iesāļajās Baltijas jūras piekrastes ekosistēmās.