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Harmful cyanobacterial blooms in Slovenia – Bloom types and microcystin producers

Škodljiva cianobakterijska cvetenja v Sloveniji – Tipi cvetenj in proizvajalci mikrocistinov

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Abstract. Up to now, research on cyanobacteria and their biologically active substances has been directed principally towards their harmful effects on humans, and little has been done to elucidate their ecological role. In order to understand better the biological success of cyanobacterial blooms, and in order to be able to compare the results of different scientific investigations, we must find and agree on a definition of the phenomenon. We propose a definition of harmful cyanobacterial blooms based on the OECD boundary system of eutrophication with the addition of phycocyanin values. We have found a direct linkage between the trophic conditions in the water-bodies and the frequency of formation of cyanobacterial blooms.

Specific toxic species and their strains have been studied intensively. However, in order to elucidate the mechanisms that enable cyanobacteria to overtake eutrophic water bodies we must change our approach. Cyanobacterial blooms should not be treated merely as different species or strains but as superorganisms. It is their intraspecific diversity that permits cyanobacteria to be successful in a variable water environment. We here focus attention on microcystin producers and microcystins as an adaptation to the limited light conditions, which arise in cyanobacterial blooms. The conclusions are illustrated with some data from surface water-bodies in Slovenia.

Key words: cyanobacteria, blue-green algae, harmful bloom, microcystins, eutrophication.

Izvleček. Raziskave cianobakterij in biološko aktivnih snovi, ki jih proizvajajo so bile do sedaj usmerjene predvsem na proučevanje škodljivih učinkov na človeka. Zelo malo je bilo storjenega v smeri preverjanja njihove ekološke vloge. Za boljše razumevanje razvojnega uspeha cianobakterijskih cvetenj in da bi bili sploh sposobni primerjati rezultate različnih znanstvenih raziskav moramo najti najprej definicijo tega pojava. Predlagamo opredelitev škodljivih cianobakterijskih cvetov, ki temelji na OECD razmejitvenem sistemu evtrofikacije z dodanimi vrednostmi za fikocianine. Ugotovili smo neposredno zvezo med trofičnim stanjem vodnih teles in pogostostjo pojavljanja cianobakterijskih cvetov.

Številne toksične cianobakterijske vrste in soje so že podrobno proučevali. Vendar za boljše razumevanje mehanizmov, ki jim omogočajo prevlado v evtrofnih vodnih

telesih, moramo spremeniti naš pristop. Cianobakterijske cvetove ne moremo obravnavati le kot zmesi različnih vrst in sojev, temveč kot superorganizme. Prav njihova intraspecifična raznovrstnost jim omogoča uspeh v spremenljivem vodnem okolju. Našo pozornost bomo osredotočili na tiste cianobakterije, ki so sposobne proizvajati mikrocistine in na mikrocistine kot možno prilagoditev na omejene svetlobne razmere, kakršne nastopajo ob cvetenjih. Zaključki smo podkreplili s podatki o vodnih telesih v Sloveniji.

Ključne besede: cianonbakterije, modrozelene-alge, škodljivo cvetenje, mikrocistini, evtrofikacija.

Introduction

The deterioration of surface water quality is becoming one of the main problems facing humanity in the near future. Some of the most evident consequences are the dense cyanobacterial blooms that further contribute to poor quality and the need for expensive treatment of affected waters (*e.g.* FERGUSSON et al. 1996). Cyanobacteria are also able to produce a wide range of toxic metabolites – microcystins (MC) being the most abundant – that additionally reduce water utilization (CARMICHAEL 1992).

Contemporary scientific concern is based primarily on an anthropocentric point of view, and this is the main reason why cyanobacteria and microcystins have been given serious attention. They are frequently the cause of health problems in humans and livestock (CARMICHAEL 1992, LAHTI 1997). As a consequence, microcystins have been treated only as toxins, and little attention has been paid to their ecological role.

The universal view of microcystins as hepatotoxins does not take into account the fact that cyanobacteria were present more than two billion years ago, long before the appearance of higher organisms (SCHOPF 1993, SCHOPF 2000). The assumption that cyanobacteria in their early stage of evolution already produced microcystins is, of course, speculative but on the other hand it is unlikely that they developed these complex substances just as a defence mechanism against potential predators. The overall logic of evolution is based principally on egoism rather than aggressiveness, and we can therefore expect organisms to be stimulated to produce substances that favour better adaptation, rather than cause harm to rivals. The implication of this statement will be explained later, once we have defined the harmful cyanobacterial bloom where the mass production of microcystins frequently takes place.

Material and methods

Field sampling

Eighty-four surface water bodies were regularly inspected for cyanobacterial blooms over an eight years period (1994-2001). Three different samples were taken at each location where the blooms occurred.

1) Water samples. Samples for chlorophyll *a* determination were taken beneath the water surface in order to avoid the surface bloom and from the bloom itself. The extraction was performed with hot methanol according to VOLLENWEIDER (1974).
2) Net samples. Qualitative 25 mesh net samples were taken as a vertical profile, preserved in 5% formaldehyde and analysed for phytoplankton species composition and their abundance rated using three categories: present, subdominant and dominant based on their relative biomass (ORLIK 1981)

3) Bloom samples. Cyanobacterial bloom samples were collected by skimming across water surface with a 25 µm plankton net for toxin analysis. We separated larger particles and zooplankton by using different sieves. The samples were then concentrated by placing the material in glass cylinders under natural light. In this way, cell buoyancy was increased and the cyanobacteria floated to the surface.

Cyanobacterial species analysis

The species were identified with the use of an inverted microscope according to Komarek (1958, 1991), Starmach (1966) and Hindak (1978).

Microcystin analysis

The lyophilised bloom material processed according to HARADA et al. (1988), as described elsewhere (SEDMAK & KOSI 1997a). The toxic fractions separated using HPLC were estimated by comparison of the retention times, spectra and peak areas of the standard microcystins.

Results and discussion

Bloom definitions

A classic bloom definition is that proposed by LINDHOLM (1994), who describes blooms as »remarkable phytoplankton maxima, in which organisms are highly concentrated and often almost monospecific«.

The expression "bloom" is still poorly defined. Usually it describes a phytoplankton biomass that is significantly higher than the average found in a water body. As the blooms are usually composed of one or two plankton species they are accordingly named after the dominant phytoplankton species. Additionally there is another problem derived from the varying cell volume of the phytoplanktonts. Which organism is actually blooming, the minute but very frequent one, or the bigger although seldom appearing? The representation only by cell concentration cannot reflect the real situation, since cells of voluminous species never occur as frequently as the minute ones even if they constitute the bulk of biomass. In freshwaters with high productivity we are confronted with different blooms such as green algal blooms, diatom blooms, cyanobacterial blooms, etc.

The fact that bloom forming cyanobacteria are able to produce a broad range of biologically active substances (CARMICHAEL 1994) that are harmful to humans as well as to other organisms, has created the need for a better definition of this phenomenon.

Waters rich in nutrients - eutrophic waters - are favourable to the mass development of phytoplankton. There is an indisputable linkage between water eutrophication and cyanobacterial bloom formation. Since in oligotrophic waters the cyanobacterial bloom forming species are extremely rare or even absent, we have based our definition of harmful cyanobacterial blooms on the OECD boundary system of eutrophication (ANON. 1982).

Table 1: OECD boundary values for trophic categories supplemented with proposed phycocyanin values.
Tabela 1: Mejne OECD vrednosti za trofične kategorije voda z dodanimi predlaganimi vrednostmi za fikocijanine.

Trophic category	**P** (mg/m^3)	***Chl.*** (mg/m^3)	Max. ***chl.*** (mg/m^3)	***Phycocy.*** (mg/m^3)	*Max.* ***Phycocy.*** (mg/m^3)	***Secchi*** (m)	Min. ***Secchi*** (m)
Ultra-oligotrophic	≤ 4	≤ 1	≤ 2.5	≤ 1	≤ 2.5	≥ 12	≥ 6
Oligotrophic	≤ 10	≤ 2.5	≤ 8	≤ 2.5	≤ 8	≥ 6	≤ 3
Mesotrophic	10 – 30	2.5 - 8	8 - 25	2.5 - 8	8 - 25	6 - 3	3 – 1.5
Eutrophic	35 - 100	8 - 25	25 - 75	8 - 25	25 - 75	3 – 1.5	1.5 – 0.7
Hypertrophic	≥ 100	≥ 25	≥ 75	≥ 25	≥ 75	≤ 1.5	≤ 0.7

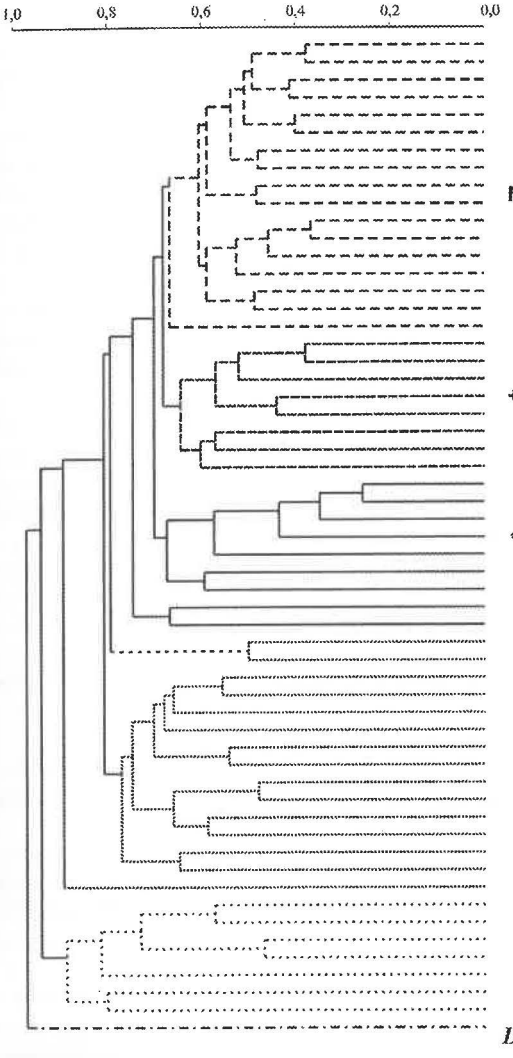
Keeping in mind the potentially harmful effects of cyanobacteria we propose a definition for harmful cyanobacterial blooms as follows:"A harmful cyanobacterial bloom is a seasonal dense phytoplankton growth, where more than 50% of the biomass comprises cyanobacteria, where the total chlorophyll value and the total phycocyanin value are each higher than 8 mg/m^3. (Tab. 1).

From this statement derives that harmful cyanobacterial blooms are mostly surface blooms and scums. Nevertheless they can present themselves in a less visible forms as metalimnetic blooms in stratified lakes or as dispersed dense blooms in eutrophic and hypertrophic water-bodies. In such blooms the concentration of cyanobacteria and their biologically active products is very high.

The accessory pigment phycocyanin is present in only few phytoplankton groups like *Cyanobacteria* and *Rhodophyta*. Additionally in our fresh waters the *Rhodophyta* species are exclusively benthic organisms. Phycocyanin is therefore in our opinion a better indicator for the presence of cyanobacterial blooms than chlorophyll. Since the contents of phycocyanin and chlorophyll are similar and, at the same time, extremely variable, we propose the same boundary values for the two photosynthetic pigments.

Our definition contains all the basic technical information necessary for easy identification, and is in conformity with the limits usually applied to potable and recreational waters. In Europe these limits are set at 10 mg m^{-3} of chlorophyll ***a***, which corresponds to ca. 10^7 cyanobacterial cells / l.

Field data on cyanobacterial blooms in Slovenia indicate that mesotrophic water bodies are potential and random sites of harmful cyanobacterial blooms, while the eutrophic and hypertrophic water bodies are sites with regular blooms with high microcystin production (Fig. 1). The metalimnetic blooms in natural lakes clearly show a different type of association demonstrating that the term "seemingly oligotrophic" is appropriate.



Legend:	OECD Value	(Chlorophyll mg/m³)	Cyanobacterial blooms
———	Hypertrophic	≥ 25	Frequent and regular
- - - - - -	Eutrophic	8 - 25	Frequent and regular
- - - - - -	Mesotrophic	2.5 - 8	Occasional
- · - · - · - L	Seemingly oligotrophic	≤ 2.5	Frequent and regular metalimnetic
·····	Oligotrophic	≤ 2.5	No
·····	Ultraoligotrophic	≤ 1	No

Figure1: Multivariate cluster analysis using a modified Bray-Curtis method (CLARKE & WARWICK 1990). Comparison of phytoplankton associations in surface water bodies of different trophic categories in Slovenia, based on chlorophyll contents of the water.

Slika 1: Multivariantna klasterska analiza po prilagojeni Bray-Curtisovi metodi (CLARKE & WARWICK 1990). Primerjava fitoplanktonskih združb v slovenskih površinskih vodnih telesih različnih trofičnih kategorij uvrščenih na podlagi vsebnosti klorofila.

Cyanobacterial bloom types in Slovenia

There are three basic types of cyanobacterial blooms in Slovenia which differ in the origin of nutrients:

*a.) **Planktonic blooms*** build up in eutrophic and hypereutrophic water bodies with nutrients evenly dispersed in the water, and where nutrient availability is influenced by diurnal stratification (all bloom forming species involved).

*b.) **Metalimnetic blooms*** build up in deeper mesotrophic and eutrophic reservoirs and in "seemingly" oligotrophic lakes where the nutrients become available as a consequence of seasonal stratification (predominantly filamentous species).

*c.) **Benthic blooms*** (cyanobacterial mats) build up in eutrophic and mezotrophic shallow water bodies, where the benthic cyanobacteria utilize nutrients from the sediment (*Oscillatoria princeps*).

In a temperate climate with seasonal changes, as in Slovenia, we are faced with stratification in all water bodies when the vertical mixing is weak. Therefore regard must also be paid to nutrient availability as a factor that triggers the beginning of cyanobacterial blooms (Reynolds 1984a). However, once the cyanobacterial bloom has started to form, light takes over as the major limiting factor in phytoplankton growth.

Basic cyanobacterial freshwater bloom configurations

Cyanobacterial blooms appear in different forms, depending on cyanobacterial abundance and on climatic and meteorological conditions.

– ***Dispersed blooms*** occur at the beginning of bloom formation and can appear secondarily as the consequence of vertical mixing due to high wind velocities (George & Edwards 1976). In such an environment the lower light conditions are mainly due to mutual shading of the plankton.

– ***Metalimnetic blooms*** occur in clear stratified lakes, where light penetrates beyond the depth of the epilimnion. Such blooms arise where opposing gradients of irradiance and nutrients are established due to the mobilisation of nutrients from the lake bottom (Ganf & Oliver 1982, Konopka 1989). In upper layers the light conditions are good and the position of cyanobacteria is due to their regulation of buoyancy (Reynolds & Walsby 1975),

– ***Surface blooms*** occur in calm weather and good insolation, when the speed of the wind is less than 2 – 3 m s^{-1}, resulting in low mixing rates (Webster & Hutchinson 1994). The light conditions below the bloom are bad, but are rescued by buoyancy regulation within the cyanobacterial population. Cyanobacteria alternatively migrate towards the surface in a constant exchange of cells and colonies at the water surface. Under favourable conditions such surface blooms may give the mistaken impression of a persistent bloom (Kromkamp & Walsby 1990).

– ***Scums*** originate from persistent blooms where there is a physical restraint on vertical movement (Ibelings & Mur 1992, Walsby 1994). Cyanobacterial scums prevent the penetration of light to deeper layers. The cells at the surface are often severly damaged by the high light intensities that can induce dehydration and cell senescence.

The most evident common characteristic of cyanobacterial blooms is the light environment, which ranges from low to very low and in extreme circumstances to even almost complete darkness. In several publications Mur emphasises the importance of light for cyanobacterial dominance. Results from competition experiments on the growth of *Scenedesmus* and *Oscillatoria* have shown that

cyanobacteria can reach higher growth rates than green algae only under extreme light limitation (*e.g.* MUR 1983).

Light limitation can be provoked by dispersed particles of different origin, or by mutual shading of phytoplankton. In shallow eutrophic water bodies there are different periodic progressions from one dominant phytoplankton assemblage to another (REYNOLDS 1980). These environments reduce the light to such an extent that only cyanobacteria remain competitive, giving rise in time to a massive population and out-competing other autotrophs.

With the appearance of cyanobacteria the light availability rapidly decreases. Gas vesicles present in buoyant cyanobacterial species induce additional horizontal light scattering that diminishes further light availability in deeper layers (WALSBY 1994).

Cyanobacterial blooms and microcystins in Slovenia

Our interpretations are based on results obtained from natural populations in Slovene water bodies, and on laboratory experiments with isolated cyanobacterial species and strains grown *in vitro* under controlled conditions. Our most frequent bloom forming cyanobacterial genera are *Microcystis*, *Anabaena*, *Aphanizomenon* and *Oscillatoria*.

Dispersed blooms are planktonic blooms, occurring in all eutrophic water bodies at the beginning of bloom formation or as a product of vertical mixing. They can be either toxic or non-toxic. The strains of cyanobacteria that are present at the beginning of bloom formation are in the majority of cases of non microcystin producing types.

Isolates of strains from different natural blooms have led to the identification of several non-producing and diverse producing strains of cyanobacteria. The isolation of a relatively high percentage of non-producing strains from toxic natural blooms can be explained by the fact that, for a successful isolation, single cells or filaments or small colonies are used. It has been namely demonstrated that larger colonies in most cases belong to producing strains (JUNGMANN & al. 1996). This observation leads to the assumption that producing strains are better adapted to bloom conditions, since they proliferate with faster dividing rates than non-producing strains, resulting in bigger colonies. Of course there are also several producing and non-producing strains that do not aggregate in colonies and proliferate in single cell configuration.

The evolution of a cyanobacterial bloom is a highly dynamic process in which a broad variety of strains are involved. The constant changes in the light environment in the bloom, due to the growth of cyanobacteria on the one hand and to meteorological and hydrological changes on the other, give different strains the opportunity to proliferate. With the aggravation of light conditions, strains that are better adapted predominate. It has long been known that *Microcystis* is non-toxic at the beginning of the growing season, but develops high toxicity during the first strong biomass increase (BENNDORF & HENNING 1989). Already in the sixties it was established that the possibility of a cyanobacterial bloom being toxic is over 50% (OLSON 1964). In our investigations this rises to over 80% (SEDMAK & al. 1994, SEDMAK & KOSI 1997a). The main difficulty in comparing such results from the literature lies in the poor definition of the bloom. Adopting our definition for cyanobacterial blooms and taking into consideration only the principal cosmopolite microcystin-producing genus *Microcystis*, the statement that cyanobacterial blooms evolve to toxic becomes a rule. We conclude that producing strains that prevail overwhelmingly in the bloom are better adapted to a low light environment. When we concentrate such dispersed blooms we can detect diverse microcystins that could originate also from different strains.

All filamentous and non-filamentous bloom-forming genera appear occasionally in the form of dispersed blooms.

Metalimnetic blooms are common in deeper stratified lakes and reservoirs. The main bloom forming species in Slovenia are filamentous *Aphanizomenon flos-aquae, Anabaena flos-aquae and Oscillatoria rubescens* (SEDMAK & KOSI 1997a, SEDMAK & KOSI 1991). They may be either producing or non-producing. We are concerned with Lake Bled, since it is the main centre of tourism in the region. The term "seemingly" oligotrophic is used because the productivity of the lake is normally low and the inflows are rich and permanent. The nutrients in the phase of summer stratification diffuse from the lake bottom and support metalimnetic blooms. *O. rubescens* grows almost every year when water stratification is established. In favourable meteorological and climatic conditions *Oscillatoria* migrates to the surface forming a surface bloom or even scum frequently covering almost the entire lake surface. Such blooms can persist on the surface even in January and can grow under the ice cover. In such cases we can normally detect microcystin-YR in bloom samples. It appears that the strain capable of MC-YR production is the best adapted to counter the lake environment. Lake Bled has two marked depressions which function as two independent sites of cyanobacterial growth. For this reason, unusual surface blooms can be observed as separate blooms of *O. rubescens* and *An. flos-aquae,* which subsequently merge in a unique mixed surface bloom. Blooms composed of equal parts of *O. rubescens* and *M. aeruginosa* have also been observed.

Surface blooms and scums prevail in summer and autumn in smaller eutrophic and hypertrophic water bodies such as reservoirs, fishponds and abandoned gravel pits. The main species are *Microcystis aeruginosa, Microcystis wesenbergii* and *An. flos-aquae* (Sedmak & Kosi 1997b). *Microcystis* species are almost always toxic. Occasionally there are also blooms of *Aphanizomenon flos-aquae, Oscillatoria limnetica* and *Oscillatoria agardhii,* which till now were all identified as non-producing. Mixed blooms are also common. *M. aeruginosa* appears frequently together with *M. wesenbergii* or *An. flos-aquae*.

Benthic cyanobacterial species like *O. princeps* can also rise to the surface and form surface blooms in the case of high proliferation rates. So far we have not been able to detect productive strains of this species. However there are some cases when microcystins have been produced by benthic cyanobacteria (MEZ et al. 1997).

All blooms have at least two things in common, high fluctuations in oxygen content and high light limitation. Additionally, the *Microcystis* blooms and scums are, to a large extent, associated with microcystin production. These blooms in a similar environment in North-eastern Slovenia all end with an almost identical pattern of production of microcystins-RR and –LR (SEDMAK & KOSI 1977a). Again we can assume that these productive strains are the best adapted to take over the highly eutrophic water bodies in the specific environment of the region.

A concise survey of microcystin production and their possible role

Today it is perfectly clear that the ability of a strain to produce microcystins depends on its possession of the necessary genes (MEIßNER et al.1996; DITTMANN et al. 1997). Thus there are strains able to produce microcystins to different extents and others that are not able to produce them at all. Meanwhile, the amount of production is dependent principally on ecological conditions (*e.g.* WATANABE & OISHI 1985). So it is obvious that the success of a particular strain depends on its adequacy under given conditions. There is great intraspecific biodiversity in the production of those biologically active substances that benefit the producing organisms, which is also the case in microcystin synthesis (NEILAN et al. 1999). So in the space of time from the origin of a bloom to its senescence, we have a

series of physiologically diverse cyanobacteria that can be successful to different degrees in various conditions, even in the framework of the same species. The possibility of natural genetic transformation in bloom conditions is very low due to the short time span of the bloom and relatively low dividing rates of cyanobacteria.

So far it seems that there is no single factor responsible for the variation in toxicity of cyanobacteria. From various data *in vitro* as well as from data obtained from natural blooms, it is evident that the differences in microcystin production between strains are very large, microcystin contents ranging from zero to 1.5% of cyanobacterial biomass according to JUNGMANN and co-workers (1996) and 1.84% microcystins /dry weight according to UTKILEN and GJØLME (1992). In our analyses total microcystin content can reach 2% of cyanobacterial dry weight (calculated value 0.64 pg/cell) in natural populations (SEDMAK & KOSI 1997a). On the other hand, the chlorophyll ***a*** content of *M. aeruginosa* is on average 1.5% of cell dry weight (0.26 – 0.43 pg/cell) (REYNOLDS 1984b).

Such huge microcystin production, comparable to the content of the indispensable chlorophyll ***a***, emphasizes the ecological importance of these substances for the producing cyanobacteria. The most recent findings indicate that microcystin synthesis proceeds via a multienzyme complex consisting of both peptide synthetase and polyketide modules (KAEBERNICK & NEILAN 2001). In such a synthesis significant cellular energy is required. The role of microcystin should be correspondingly great.

As already mentioned, cyanobacteria were among the first organisms to inhabit our planet. It is more probable that their evolution involved biologically active substances that would aid them in their adaptive capabilities rather than harm rivals that did not even exist. From a quick survey of scientific data we can summarize that there is no evolutionarily adaptive value for cyanobacteria to kill land animals and fish (JUNGMANN et al. 1996). It can be expected that cyanobacteria and their products interact primarily with organisms in the same environment. Microcystins are not likely defence substances against grazers, since experiments have demonstrated that they are toxic to zooplankton only at very high concentrations (DE MOTT et al. 1991) and that there are other, more effective substances against them isolated from cyanobacteria (*e.g.* NIZAN et al. 1986). Therefore according to *Occam's razor* (WILLIAM OCKHAM 1285 – 1349) whereby »unnecessary assumptions should be abandoned«, the assumption that microcystins are defence substances is, in our opinion, unnecessary.

We have proposed that there is no environmental factor capable of converting a non-producing strain into a producing strain (SEDMAK & KOSI 1998a, SEDMAK & KOSI 1998b), but that there must be an ecological factor that augments the growth of microcystin producing cyanobacteria in order to prevail over other species and strains. In the last decade, research has been focused on environmental factors that could trigger microcystin production, rather than looking at cyanobacterial species as complex mixtures of different strains with diverse adaptive values.

Light availability and microcystin production

Work on microcystin production has been concentrated on the study of specific producing strains of cyanobacteria, in order to find optimal conditions where they proliferate and produce and with the aim of understanding how they would behave in bloom conditions. However, in specific bloom conditions, they may represent changeable proportion of the cyanobacterial biomass. In nature it is the environment that gives the opportunity to the fittest. During the evolution of the bloom there is a constant change of conditions. Specific strains are favoured and, with their proliferation, there is, in turn, an additional change in environmental conditions that offers the opportunity to another better adapted strain to propagate. With the growth of the bloom, light conditions become worse and only strains capable of proliferation under such extreme environments can prevail. In our opinion, microcystin producing strains are better adapted to low light conditions than non-producing strains (SEDMAK

2001). This explains why the blooms become more toxic with the increase in cyanobacterial biomass. It also explains the poor permeability of cells to microcystins, which are primarily designed to influence their own physiology.

Orr and Jones (1998) have shown that the highest microcystin concentrations are produced under conditions optimal for cell growth. There is a high probability therefore that under optimal conditions there will be an optimal production of all the microcystin variants that a strain is capable of producing. These optimal conditions for producing cyanobacteria coincide with relatively low light conditions. It is necessary therefore to be able to estimate the availability of light in eutrophic water bodies with abundant phytoplankton growth. The optimal light intensities for producing *M. aeruginosa* strains have been estimated as being less than 40 microeinsteins m^{-2} s^{-1} (Utkilen & Gjølme 1992). Similar values have been reported for other microcystin producing cyanobacteria (Rapala et al. 1997). Such intensities are normally found in cyanobacterial blooms at a depth of about 1 m (Utkilen & Gjølme 1992). From these data it is evident that producing cyanobacteria are adapted to the low light conditions characteristic of an already existing bloom. Cyanobacteria that start such an environment usually belong to the non-producing or poorly producing strains. Microcystin production is energy consuming and becomes an advantage only in an adequate environment. We have found a strong positive correlation between microcystin production and cyanobacterial cell concentration in the bloom, suggesting that producing strains are capable of more dense bloom and scum formation and of survival in consequent low light environment (Fig 2).

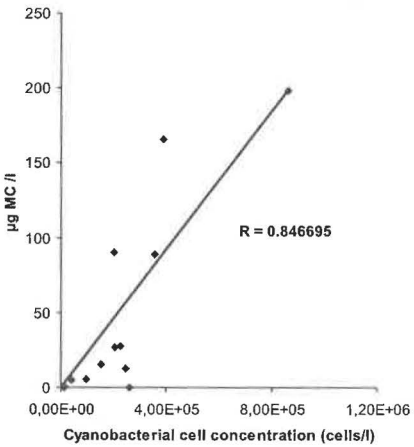


Figure 2: The relationship the cumulative values of produced microcystins (ΣMC) in the bloom and the cyanobacterial cell concentration.
Slika 2: Razmerje med vsebnostjo vseh mikrocistinov (ΣMC) in koncentracijo cijanobakterijskih celic v cvetu.

There have been a few attempts to find a linkage between photosynthesis and microcystin production. Microcystins have been found primarily in the thylakoid and nucleoid regions (Shi et al. 1995) and it is believed that the ADDA moiety of the toxin may bind to the thylakoid. This association with the photosynthetic apparatus of cyanobacterial cells may indicate a function in the light harvesting and chromatic adaptation mechanisms exhibited by these organisms (Orr & Jones 1998) Light dependent processes are essential to both prokaryotic cyanobacteria and eukaryotic algae, which compete in the same habitat. Thus the production of biologically active substances that gives an advantage in this crucial area is clearly supported in the process of evolution.

Can microcystins influence other phytoplankton species in the environment?

Despite the established opinion that microcystins are generally not cell-permeable, except the hepatocytes, which have a specific uptake system, we have demonstrated that they can influence the growth of different phytoplankton species in culture even at low concentrations (10^{-7} M) (SEDMAK & KOSI 1998a, SEDMAK & KOSI 1998b). Recent investigations have confirmed the possibility of non-specific translocation of oligopeptides (to undecamer) across plasma membranes (OELHKE et al. 1997). Microcystins are heptapeptides and as such they can pass the cell membrane. Whether the release of microcystins is due to cell death or to cell leakiness, it remains a fact that microcystins can be detected in the environment during cell proliferation (RAPALA et al. 1997). In such a way, non-producing strains also may persist longer in the bloom, preserving a bigger biodiversity of strains. *In vitro* experiments have namely confirmed that non-producing strains exposed to microcystins achieve higher proliferation rates under low light conditions than otherwise (SEDMAK & KOSI 1998a, SEDMAK & KOSI 1998b). Such diverse cyanobacterial association can dominate for a longer period in the continuously changing water environment. In such an unpredictable situation, the role of microcystins is also to preserve the variability of strains. The cyanobacterial bloom is functioning as a superorganism where different strains take over in conformity with temporary conditions. This assumption is supported by the fact that we can isolate non-producing strains, irrespective of how dense and toxic the monospecific cyanobacterial bloom or scum may be.

The presence of microcystins can also influence the growth of other phytoplanktonts in the bloom. The diversity in the blooms is namely low. We have found a correlation between microcystin production and the presence of phytoplankton species in toxic cyanobacterial blooms (Fig.3). We suggest that there is a combined effect of light limitation together with microcystin influence on susceptible phytoplankton species.

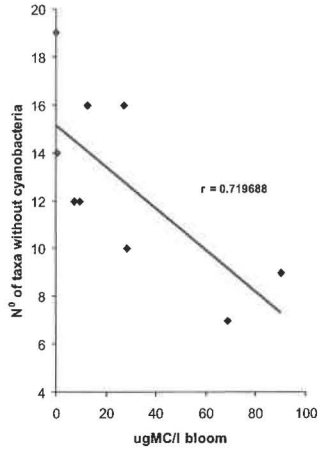


Figure 3. The relationship between microcystin production in the bloom and phytoplankton diversity.
Slika 3: Razmerje med koncentracijo mikrocistinov v cvetu in biodiverziteto fitoplanktona.

Cyanobacteria can control their buoyancy, but in old and very dense blooms the competition for carbon dioxide can depress photosynthesis to such a degree, that the turgor pressure in the cell can no longer rise and the gas vesicles are not able to collapse. Such a bloom is thus trapped at the water surface (*e.g.* WALSBY 1994). Exposure to direct sunlight, with photooxidation of the photosynthetic pigments, leads to the death and disintegration of cyanobacteria. In this situation, the moribund and lysed cells release massive amounts of microcystins into the water. They can stimulate the growth of other phytoplankters (*i.e. Scenedesmus* spp.), that tolerate the presence of microcystins, giving rise to another, this time green algae, bloom. Frequently, after the collapse of a cyanobacterial bloom, green algae become dominant (LIN 1972). In our opinion the disintegration of a hepatotoxic bloom accelerates the proliferation of defined tolerant genera (SEDMAK & KOSI 1998a, SEDMAK & KOSI 1998b), while several other susceptible taxa can be excluded from the environment.

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