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EFFECTS OF CYANOBACTERIAL TOXINS, MICROCYSTINS ON FRESHWATER INVERTEBRATES

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A b s t r a c t

Cyanobacteria, also known as blue-green algae, are prokaryotic, phototrophic microorganisms that may form massive blooms in eutrophic water reservoirs. Some cyanobacterial strains are able to produce secondary metabolites – cyanotoxins that may be hazardous to aquatic and terrestial animals. These compunds can be grouped into: hepatotoxins, neurotoxins, cytotoxins dermatotoxins and irritant toxins. Microcystins are well-known cyclic heptapeptides acting as inhibitors of protein phosphatases type 1 and 2A. These cyanotoxins induce various adverse effects in freshwater invertebrates including biochemical, physiological and behavioral changes. Moreover, accumulation of microcystins in different tissues occurs, therefore transfer of these cyanotoxins through the food chain to animals being at higher trophic levels may be possible. The purpose of this paper is to review the knowledge on the effects of microcystins on three main groups of freshwater invertebrates: zooplankton, higher crustaceans, mollusks and to indicate possible ecotoxicological consequences of this impact on aquatic environment and invertebrate aquacultures.

DZIAŁANIE TOKSYN SINICOWYCH, MIKROCYSTYN NA SŁODKOWODNE BEZKRĘGOWCE

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S ł o w a k l u c z o w e: sinice, mikrocystyny, małże, ślimaki, widłonogi, wioślarki.

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Abstrakt

Cyjanobakterie (sinice) są prokariotycznymi, fototroficznymi mikroorganizmami, które w eutroficznych zbiornikach wodnych mogą masowo proliferować, tworząc zakwity. Niektóre szczepy sinic zdolne są do produkcji cyjanotoksyn, wtórnych metabolitów, które mogą stanowić zagrożenie dla zwierząt wodnych oraz lądowych. Związki te można podzielić na: hepatotoksyny, neurotoksyny, cytotoksyny, dermatotoksyny oraz toksyny drażniące. Mikrocystyny są dobrze opisanymi cyklicznymi heptapeptydami będącymi inhibitorami białkowych fosfataz typu 1 oraz 2A. Wywołują rozmaite szkodliwe efekty u słodkowodnych bezkręgowców, np. zmiany biochemiczne, fizjologiczne oraz behawioralne. Mikrocystyny dzięki zdolności do akumulacji w różnych tkankach mogą ponadto ulegać transferowi do zwierząt będących na wyższych poziomach łańcucha troficznego. Celem artykułu jest przegląd stanu wiedzy na temat oddziaływania mikrocystyn na trzy główne grupy bezkręgowców słodkowodnych: zooplanktonu, wyższych skorupiaków i mięczaków oraz wskazanie jego możliwych konsekwencji ekotoksykologicznych na środowisko wodne i akwakultury bezkręgowców.

Introduction

Cyanobacteria, (*Cyanophyta, Cyanoprocaryota*) also known as blue-green algae are phototrophic, prokaryotic microorganisms frequently found in many environments, from tropical regions to arctic ice. They play important ecological role as oxygen producers, they also have adaptation to fix atmospheric nitrogen and to tolerate a wide range of temperature (GŁOWACKA et al. 2007, BERMAN-FRANK 2003, WHITTON and POTTS 2000). Some species of cyanobacteria may produce secondary metabolites known as cyanotoxins, which are very harmful to aquatic and terrestial animals (BŁASZCZYK et al. 2010, VALERIO et al. 2010). Some cyanotoxins (such as cylindrospermopsin) may be constantly released during cyanobacterial growth, however most of these compounds pass directly from cyanobacterial cells to surrounding water during the bloom collapse posing a risk of intoxication to various organisms. Toxic effects of various cyanotoxins are well documented in mammals: humans, cattle, dogs and in aquatic organisms such as bacteria, algae, higher plants, invertebrates and vertebrates such as fish. (CARMICHAEL 1997, GRIFFITHS and SAKER 2003, BOWNIK et al. 2012).

Cyanobacterial toxins can be divided on the basis of its toxic action into: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (lipopolysaccharides) (WIEGAND and PFLUGMACHER 2005). Hepatotoxins produced by cyanobacteria include heptapeptide microcystins and pentapeptide nodularin, neurotoxins: anatoxin-a, anatoxin-a(s), homoanatoxin-a, saxitoxins- also known as paralytic shellfish poisons (PSPs). A variety of cytotoxic effects in many organs are induced by cyanobacterial alkaloid cytotoxin – cylindrospermopsin. Irritant cyanotoxins are lipopolysaccharides which are constituents of cyanobaterial cell wall (SIVONEN 2009).

Microcystins are produced by cyanobacterial genera such as *Microcystis, Anabaena, Planktothrix (Oscillatoria), Anabaenopsis, Nostoc, Hapalosiphon, Aphanizomenon* (CARMICHAEL 1992, KAEBERNICK and NEILAN 2001, WIEGAND and PFLUGMACHER 2005) common in many types of freshwater environments such as dam reservoirs, lakes and ponds. These well-known cyanotoxins are cyclic heptapeptides consisting of seven amino acids including two characteristic amino acids: methyldehydroalanine (Mdha) and 3-amino-9methoxy-2,6,8--trimethyl-10-phenyldeca-4,6-dienoic acid (Adda). Currently, more than 85 of their structural variants have been described and microcystin-LR (MC-LR) is one of the most frequently detected in freshwater reservoirs (SIVONEN 2009) (Figure 1). Results from the studies performed on vertebrates indicate that MC-LR targets the liver causing fatal cytoskeletal disruption of hepatocytes induced by specific inhibition of protein phosphatases type 1 (PP1) and 2A (PP2A) (TOIVOLA et al. 1994).

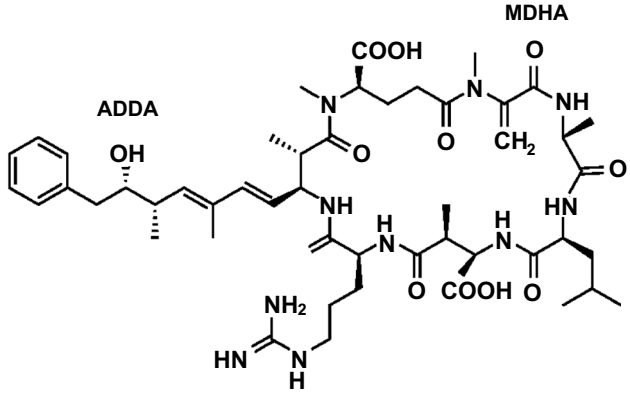

Fig. 1. Structure of microcystin-LR

Effects of microcystins on zooplankton

Zooplanktonic organisms feed on microorganisms such as phytoplankton and bacteria, on the other hand, they are a food source for other predatory species, particularly for fish fry. Scientific data indicate that some cyanobacterial species producing microcystins interact with some zooplanktonic species but the responses are different among closely related species and even individuals (BLANCHTTE and HANEY 2002). Toxic strains of *Microcystis aeruginosa* may increase the production of cyclic heptapeptides in the presence of certain zooplanktonic organisms or their infochemicals (JANG et al. 2008). This type of defensive reaction of phytoplankton usually leads to reduction of grazing

activity of zooplankton. However, there are reports indicating no inhibitory influence on grazing particularly in wild mesoplankton consuming toxic and non-toxic strains of *Microcystis aeruginosa* at similar rates (DAVIS and GOBLER 2011). Consumption of toxic cyanobacterial cells or direct absorbtion of cyanotoxins dissolved in water may induce a variety of toxic changes in susceptible zooplanktonic invertebrates. ROHRLACK et al. (2001, 2005) demonstrated lethal effects in *Daphnia galeata* exposed to cell-bound microcystins. On the other hand, there are reports indicating low impact of microcystins on survival of zooplanktonic species, but showing physiological changes such as disturbances in the heartbeat and the movements of the thoracic limbs, mandibles, second antennae, decreased activity of the foregut, histopathological changes in the midgut and stimulation of its muscles (CHEN et al. 2005).

Microcystin-producing strains of cyanobacteria may induce changes in growth and development of some zooplanktonic species. The growth rate of *Daphnia magna* is reduced after comsumption of *Microcystis aeruginosa* producing microcystins (LÜRLING 2003). Survival of the offsprings is lower when their parents are previously intoxicated with MC-LR. Deformations of the neonates such as incomplete development of the antennae and unrejected tail spin also occur (DAO et al. 2010).

Microcystins induce biochemical changes in zooplanktonic organisms and their mechanism of toxic action seems to be similar to those in mammals. The activity of protein phosphatases P1 and P2A from the extracts of invertebrates *Daphnia pulex*, *Daphnia pulicaria* and *Diaptomus birgei* are inhibited by MC-LR (DEMOTT and DHAWALLE 1995). The cyanotoxins are metabolized by zooplanktonic enzymes which is manifested by stimulation of lactate dehydrogenase and alterations in the levels of glutahione and glutathione-S-transferases in *Daphnia magna*. The changes of acetylcholinesterase activity in *Daphnia pulicaria* may suggest disturbances in neuronal stimulation in daphnids. Microcystins also inhibit the activity of gut proteases, trypsin and chymotrypsin in *Moina macrocopa* and *Daphnia magna* and this may explain their lower feeding activity during cyanobacterial blooms (AGRAWAL et al. 2001, 2005, CHEN et al. 2005).

Although cyanobacteria producing microcystins turned out to be harmful to certain zooplanktonic species, cladocerans developed some mechanims to reduce their toxicity (GUO and XIE 2006, BEDNARSKA 2006). Zooplanktonic organims that are frequently exposed to microcystins seem to be less sensitive in comparison to those crustaceans which had not earlier contact with the cyanotoxins. It was shown that *Daphnia magna* previously exposed to toxic strain of *Microcystis* sp. are more resistant to microcystins than the individuals without prior treatment, which is manifested by their increased survial and larger size (GUSTAFSSON and HANSON 2004).

There are some species-dependent differences in tolerance of zooplankton to microcystins. Toxicological comparative studies indicated that *Daphnia galeata* is more sensitive to MC-LR than *Daphnia magna*. Moreover, pretreatment of these daphnids with purified cyanobacterial lipopolysaccharide (LPS) from *Microcystis CYA 43* increased their resistance to MC-LR (LINDSAY et al. 2006). It was also shown that smaller cladocerans are usually more tolerant than large-sized *Daphnia* in a simultaneous exposure to microcystin-positive strains of *Microcystis aeruginosa* and this could explain the replacement of dominant *Daphnia* by smaller species during summer cyanobacterial blooms (GUO and XIE 2006). Morover, laboratory experiments have shown that blooms of toxic *Microcystis aeruginosa* reduce the numer of large-bodied daphnid cladocerans, such as *Daphnia ambigua* and increase the quantity of smaller copepods such as *Diaptomus reighardi* (FULTON and PAERL 1988). Susceptibility of zooplanktonic organisms to microcystins may also depend on the structural variant and activity of other toxic substances such as microviridins or cyanopeptolins (JUNGMAN and BENNDORF 1994).

Zooplanktonic species possess some adaptations and mechanisms enabling them to reduce the toxic effects of cyanotoxins, however the ability to tolerate toxic cyanobacteria is varied between species. Zooplankton sensitivity to microcystins is different even in closely related species of *Daphnia* that reside in water reservoirs of various trophic profile. The individuals from eutrophic lakes where frequent blooms of toxic strains of cyanobacteria occur may develop mechanisms of resistance which are not present in those organisms from oligotrophic reservoirs (BLANCHETTE and HANEY 2002). Some daphnids are able to select their food and may avoid toxic cyanobacteria. The non-toxic strains were observed to be faster consumed by *Daphnia magna* than the microcystin-positive species (DEGANS and DE MEESTER 2002). On the other hand, *Daphnia pulicaria* was shown to feed unselectively on toxic strains of *Microcsytis aeruginosa*. Physiological sensitivity in addition to a lack of food selectivity make these crustaceans very sensitive to microcystin-producing cyanobacteria (DEMOTT et al. 1991).

Grazing activity and food selectivity of those animals may affect the amount of the ingested cell-bound microcystins and, in a consequence, their toxic impact. For example, it was noted that a crustacean, *Diaptomus birgeri* which is physiologically sensitive to microcystins, inhibits feeding in the presence of toxic *Microcystis aeruginosa*. Reduced grazing activity observed in *Daphnia magna* during the exposure to *Microcystis aeruginosa* may be a type of defensive reaction leading to inhibition of ingestion of cyanobacterial cells containing microcystin (ŁOTOCKA 2001) This effect is possibly induced by reduction of trypsin and chymotrypsin in the gut (AGRAWAL et al. 2005). On the other hand, in some zooplanktonic species such as *Daphnia*

pulicaria, the ingestion rate was not diminished during the exposure to microcystin. As a consequence, these crustaceans may absorb high concentrations of microcystins in addition to their physiological sensitivity (DEMOTT et al. 1991).

Effects of microcystins on decapods and mollusks

Most of freshwater decapods (*Decapoda*) are scavengers and they are vital organisms in maintaining the recycle of organic matter. These animals are senstitive to microcystin-producing strains of cyanobacteria and they may be exposed to microcystins dissolved in water, when grazing on cyanobacterial cells suspended in water or when consuming the detritus containing the decaying material from the bloom with absorbed cyanotoxins. However, they may exhibit symptoms of oxidative stress after the exposure to cyanobacterial cyclic heptapeptides. Production of oxidative stress enzymes was noted in the internal organs such as hepatopancreas and gills of the estuarine crab *Chasmagnathus granulatus* (*Decapoda Brachyura*). Cyanobacterial extracts containing microcystins increased consumption of oxygen, catalase activity, glutathione S transferase and lipid peroxides Microcystins may also induce other toxic effects such as disturbance of the sodium pomp functioning by the reduction of Na^+ and K^+ ATP-ase activity leading to disorders in the metabolism of various cells. (MONTAGNOLLI et al. 2004, PINHO et al. 2005a, 2005b). Decapods are able to metabolize microcystins. The elevated glutathione S-transferase level found in crabs indicated the biotransformation of these heptapeptides (VINAGRE et al. 2002).

Bivalves and gastropods are mollusks including a huge number of species abundant in freshwater environments. Bivalves play important ecological role as filter feeders consuming small organisms and organic particles suspended in water. These organisms may absorb cyanotoxins both in a dissolved form and from ingested cyanobacterial cells. The ability to expell living toxic cyanobacteria into pseudofeces and very efficient depuration of tissues from microcystins are distinct physiological processes enabling these mollusks to tolerate cyanobacterial blooms harmful to other organisms. However, these adaptations are varied among the species.

Although bivalves are resistant to microcystins and do not develop symptoms of acute toxicity, they may show some biochemical changes after a prolonged time of exposure to low concentrations of the heptapeptides. Similarly to decapods, bivalves exposed subchronically to toxic *Microcystis aeruginosa* are prone to oxidative stress by the incereased activity of superoxide dismutase by 50%, catalase by 66% and glutathione-S-transferase by 60% (SABATINI et al.

2011). A zebra mussel, *Dreissena polymorpha* exposed to MC-LR (10 μg/L) for 24 hours exhibited elevated level of glutathione S-transferase in the whole tissue, digestive gland and gills. The activity of antioxidant enzymes, catalase and superoxide dismutase was also increased. However, oxidative stress induced by microcystins is not observed in all bivalves. A mussel, *Unio tumidus* exposed to MC-LR at a concentration of 10 μg/L showed unchanged activity of superoxide dismutase and glutathione S-transferase in the digestive gland and gills (BURMESTER et al. 2012).

Toxic strains of *Microcystis aeruginosa* were noted to induce cytotoxic effects in a freshwater clam, *Corbicula fluminea*. Changes in the expression of proteins involved in the cytoskeleton assembly of cytosolic fraction of gills and digestive tract were noted. (MARTINS et al. 2009). The disruptive effects of microcystins on the cytoskeleton is associated with the inhibitory action of microcystins on protein phosphatases PP1 and P2A

Freshwater gastropods are scavengers and detritus feeders that can assimilate cyanotoxins dissolved in water or they may be intoxicated by consumption of organic matter from the sediments containing these compounds. Another route of intoxication could be grazing on benthic cyanobacteria producing cyanotoxins. The scientific data on the effects of microcystins on gastropods is very scarce. Some results are associated with the accumulation of cyanotoxins other than microcystins in various freshwater snail species. Cyanobacteria producing microcystins induce toxic changes in gastropods found in the pulmonate *Lymnea stagnalis* exposed to the purified MC-LR. The animals exibited reduced egg production and impaired locomotion (GÈRARD et al. 2005).

Accumulation of microcystins in the invertebrates

The results of field and experimental studies suggest that various aquatic animal species tend to accumulate cyanotoxins in the target organs. The increasing concentrations of microcystins observed in the foodchain may be hazardous to predatory species absorbing the heptapeptides with the consumed food.

It is suggested that some invertebrate species may deposit microcystins in their bodies. Microcystins may be accumulated in zooplanktonic organisms that do not feed selectively and may ingest high amounts of toxic cyanobacterial cells. It is estimated that among invertebrates, zooplanktonic crustaceans possess the highest ability to accumulate microcystins reaching the highest values over 1000 μg g^{-1} of DW (Dry Weight) and the avarage values of about 383 μg g^{-1} of DW (FERRÃO-FILHO and KOZLOWSKY-SUZUKI 2011). It was

shown by ELISA (Enzyme Linked Immunosrobent Assay) assay that *Daphnia magna* accumulated microcystins up to 24.5 μg g^{-1} after the exposure to the toxic cells of *Microcystis aeruginosa* CYA228/1 (THORSTRUP and CHRISTOFFERSEN 1999). Microcystins assimilated in zooplankton may be then transferred to predators being at the higher trophic levels such as fish.

Higher crustaceans such as crayfish and freshwater shrimps accumulate microcystins in various organs, mostly in the hepatopancreas and gonads. Mortalities of white shrimps noted in Texas aquaulture ponds with blooms dominated by *Microcystis aeruginosa* and *Anabaena* sp. were associated with assimilation of MC-LR in their tissues. The cyanotoxin was detected in the hepatopancreas at 55 μg g^{-1}, however the toxin concentration in muscles was estimated to be below 0,1 μg g^{-1}. Other crustacean species seem to have a different profile of microcystin distribution. An edible red swamp shrimp (*Procambarus clarkii*) turned out to assimilate the cyanotoxins in the intestine and abdominal muscle (TRICARICO et al. 2008, LIRÅS et al. 1998) and crabs from Brasilian Sepetiba Bay in the muscle tissue (MAGALHÃES et al. 2003).

Bivalves ingest microcystins by filtering the water abundant in toxin-positive cyanobacteria. Results obtained by BAKER et al. (1998) suggested that *Microcystis* cells are selectively consumed by the zebra mussel and filtered out of the water. However, other authors showed that bivalves can reject living toxic *Microcystis* cells with unsuitable food by transporting them to pseudeofeces, a characteristic fluid consisting of mucus and the expelled particles (PIRES et al. 2004, JUHEL et al. 2006). Interestingly, a zebra mussel, *Dreissena polymorpha* is even suspected to be responsible for toxic blooms of *Microcystis aeruginosa* in Lake Erie by expeling pseudofeces containing living cyanobacterial cells (VANDREPLOEG et al. 2001).

Microcystins may be also a hazard to humans in case of their accumulation the edible invertebrates and further comsumption of the contaminated animals. High concentrations of microcystins was noted in an estuarine blue crab (*Callinectes sapidus*) living in a hyper-eutrophic freshwater lake, Lac des Allemands, located in the Barataria estuary system of southeastern Louisiana. The highest tissue concentrations of the cyanotoxins were detected in the hepatopancreas (820 μg kg^{-1}), viscera (65 μg kg^{-1}), muscle (105 μg kg^{-1}) (GARCIA et al. 2010). The amount of the assimilated cyanotoxins during counsumption of these animals would be more than the TDI guideline value 0.04 μg kg^{-1}/day for MC-LR proposed by World Health Organization (WHO) assumed for human body weight (WHO 1999). The most prone organs to microcystins accumulation in invertebrates are hepatopancreas and gonads. If the edible invertebrates are caught for culinary use and are suspected to have previous contact with cyanobacterial blooms, the internal organs should be removed before cooking for health safety.

In summary, freshwater invertebrates should be considered as organisms sensitive to microcystins. Cyanotoxins released during and after cyanobacterial blooms to the aquatic environment may reduce population of invertebrates not only by acute intoxication but also by chronic exposure leading to physiological, biochemical and behavioral changes inducing disturbances in grazing and reproduction. Moreover, the accumulated microcystins observed in invertebrates may be transferred to higher throphic levels in the food web of the aquatic ecosystem (SMITH and HANEY 2006). Higher crustaceans, bivalves and gastropods are also essential for human consumption in some countries. The knowledge on toxic effects and accumulation of microcystins in different organs of invertebrate organisms is very important for human health. Proper periods of animal depuration from microcystins established prior to their culinary use would reduce the risk of microcystin retention in the edible invertebrates (CHEN and XIE 2007). Therefore, constant monitoring of cyanobacteria such as *Microcystis aeruginosa* in ponds and aquacultures where these animals are kept for consumption should be maintained.

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References

AGRAWAL M.K., BAGCHI D., BAGCHI S.N. 2001. *Acute inhibition of protease and suppression of growth in zooplankter, Moina macrocopa, by Microcystis blooms collected in Central India*. Hydrobiologia, 464: 37–44.

AGRAWAL M.K., ZITT A., BAGCHI D., WECKESSER J., BAGHI S.N., VON ELERT E. 2005. *Characterization of proteases in guts of Daphnia magna and their inhibition by Microcystis aeruginosa PCC7806.* Environ. Toxicol., 20: 314–322.

BAKER S.M., LEVINTON J.S., KURDZIEL J.P., SHUMWAY S.E. 1998. *Selective feeding and biodeposition by zebra mussels and their relation to changes in phytoplankton composition and seston load*. J. Shellfish Res., 17: 1207–1213.

BEDNARSKA A. 2006. *Sinice i ich wpływ na roślinożerne zwierzęta planktonowe*. Wiad. Ekol., 52: 59–88.

BERMAN-FRANK I., LUNDGREN P., FALKOWSKI P. 2003. *Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria*. Res. Micobiol., 154: 157–164.

BLANCHETTE M.L., HANEY J.F. 2002. *The effect of toxic Microcystis aeruginosa on four different populations od Daphnia*. UNH Center for Freshwater Biology Research, 4: 1–10.

BŁASZCZYK A., TORUŃSKA A., KOBOS J., BROWARCZYK-MATUSIAK G., MAZUR-MARZEC H. 2010. *Ekologia toksycznych sinic. Zakwity sinic (Cyjanobakterii).* Kosmos, 59: 173–198.

BOWNIK A., RYMUSZKA A., SIEROSŁAWSKA A., SKOWROŃSKI T. 2012. *Anatoxin-a induces apoptosis of leukocytes and decreases the prolifrative ability of lymphocytes of common carp (Cyprinus carpio L.) in vitro.* Pol. J. Vet. Sci., 15: 531–535.

BURMESTER V., NIMPTSCH J., WIEGAND C. 2012. *Adaptation of freshwater mussels to cyanobacterial toxins: Response of the biotransformation and antioxidant enzymes*. Ecotoxicol. Environ. Saf., 78: 296–309.

CARMICHAEL W.W. 1992. *Cyanobacteria secondary metabolites – The cyanotoxins*. J. Appl. Bacteriol., 72: 445– 459.

CARMICHAEL W.W. 1997. *The Cyanotoxins*. Adv. Botan. Res., 27: 211–256.

CHEN W., SONG L., OU D., GAN N. 2005. *Chronic toxicity and responses of several important enzymes in Daphnia magna on exposure to sublethal microcystin-LR*. Environ. Toxicol., 20: 323–330.

CHEN J., XIE P. 2007. *Microcystin accumulation in freshwater bivalves from Lake Taihu, China, and the potential risk to human consumption*. Environ. Toxicol. Chem., 26: 1066–1073.

DAO T.S., DO-HONG L-C., WIEGAND C. 2010. *Chronic effects of cyanobacterial toxins on Daphnia magna and their offspring*. Toxicon, 55: 1244–1254.

DAVIS T.W., GOBLER C.J. 2011. *Grazing by mesozooplankton and microzooplankton on toxic and non-toxic strains of Microcystis in the Transquaking River, a tributary of Chesapeake Bay*. J. Plankton Res., 33: 415–430.

DEGANS H., DE MEESTER L. 2002. *Top-down control of natural phyto- and bacterioplankton prey communities by Daphnia magna and by the natural zooplankton community of the hypertrophic Lake Blankaart*. Hydrobiologia, 479: 39–49.

DEMOTT W.R., DHAWALLE S. 1995. *Inhibition of in vitro protein phosphatase activity in three zooplankton species by microcystin-LR, a toxin from cyanobacteria*. Arch. Hydrobiol., 134: 417–424.

DEMOTT W.R., ZHANG Q.X., CARMICHAEL W.W. 1991. *Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of Daphnia*. Limnol. Oceanogr., 36: 1346–1357.

FERRÃO-FILHO A., KOZLOWSKY-SUZUKI B. 2011. *Cyanotoxins: bioaccumulation and effects on aquatic animals*. Mar. Drugs, 12: 2729–2772.

FULTON R.S., PAERL H.W. 1988. *Effects of the blue-green alga Microcystis aeruginosa on zooplankton competitive relations*. Oecologia, 76: 383–389.

GARCIA A.C., BARGU S., DASH P., RABALAIS N.N., SUTOR M., MORRISON W., WALKER N.D. 2010. *Evaluating the potential risk of microcystins to blue crab (Callinectes sapidus) fisheries and human health in a eutrophic estuary*. Harmful Algae, 9: 134–143.

GÈRARD C., BRIENT L., ROUZIC B.L. 2005. *Variation in the response of juvenile and adult gastropods (Lymnaea stagnalis) to cyanobacterial toxin (microcystin-LR)*. Environ. Toxicol., 20: 592–596.

GŁOWACKA J., WALERON M., SZEFEL-MARKOWSKA M., ŁOJKOWSKA E., WALERON K. 2007. *Cyanobacteria – źródło związków biologicznie czynnych*. Biotechnologia, 79: 95–112.

GRIFFITHS D.J., SAKER M.L. 2003. *The Palm Island mystery disease 20 years on: a review of research on the cyanotoxin cylindrospermopsin*. Environ. Toxicol., 18: 78–93.

GUO N., XIE P. 2006. *Development of tolerance against toxic Microcystis aeruginosa in three cladocerans and the ecological implications*. Environ. Pollut., 143: 513–518.

GUSTAFSSON S., HANSSON L.A. 2004. *Development of tolerance against toxic cyanobacteria in Daphnia*. Aquat. Ecol., 38: 37– 44.

JANG M.H., HA K., TAKAMURA N. 2008. *Microcystin production by Microcystis aeruginosa exposed to different stages of herbivorous zooplankton*. Toxicon, 51: 882–889.

JUHEL G., DAVENPORT J., O'HALLORAN J., CULLOTY S.C., RAMSAY R.M., JAMES K.J., FUREY A., ALLIS O. 2006. *Pseudodiarrhoea in zebra mussels, Dreissena polymorpha (Pallas), exposed to microcystins*. J. Exp. Biol., 209: 810–816.

JUNGMANN D., BENNDORF J. 1994. *Toxicity to Daphnia of a compound extracted from laboratory and natural Microcystis spp, and the role of microcystins*. Freshwater Biol., 32: 13–20.

KAEBERNICK M., NEILAN B.A. 2001. *Ecological and molecular investigations of cyanotoxin production*. FEMS Microbiol. Ecol., 35: 1–9.

LINDSAY J., METCALF J.S., CODD G.A. 2006. *Protection against the toxicity of microcystin-LR and cylindrospermopsin in Artemia salina and Daphnia spp. by pre-treatment with cyanobacterial lipopolysaccharide (LPS)*. Toxicon., 48: 995–1001.

LIRÅS V., LINDBERG M., NYSTRÖM P., ANNADOTTER H., LAWTON L.A., GRAF B. 1998. *Can ingested cyanobacteria be harmful to the signal crayfish (Pacifastacus leniusculus)?* Freshwater Biol., 39: 233–242.

ŁOTOCKA M. 2001. *Toxic effects of cyanobacterial blooms on the grazing activity of Daphnia magna Straus*. Oceanologia, 43: 441–453.

LÜRLING M. 2003. *Effects of microcystin-free and microcystin-containing strains of the cyanobacterium Microcystis aeruginosa on growth of the grazer Daphnia magna*. Environ. Toxicol., 18: 202–210.

MAGALHÃES V.F., MARINHO M.M., DOMINGOS P., OLIVEIRA A.C., COSTA S.M., AZEVEDO L.O., AZEVEDO S.M.F.O. 2003. *Microcystins (cyanobacteria hepatotoxins) bioaccumulation in sh and crustaceans from Sepetiba Bay (Brasil, RJ).* Toxicon., 42: 289–295.

MARTINS J.C., LEÃO P.N., VASCONCELOS V. 2009. *Differential protein expression in Corbicula fluminea upon exposure to a Microcystis aeruginosa toxic strain*. Toxicon., 53: 409–416.

MONTAGNOLLI W., ZAMBONI A., LUVIZOTTO-SANTOS R.J., YUNES J.S. 2004. *Acute effects of Microcystis aeruginosa from the Patos Lagoon estuary, Southern Brazil, on the microcrustacean Kalliapseudes schubartii (Crustacea: Tanaidacea)*. Arch. Environ. Contam. Toxicol., 46: 463–469.

PINHO G.L.L., MOURA DA ROSA C., MACIEL F.E., BIANCHINI A., YUNES J.S., PROENÇA L.A.O., MONSERRAT J.M. 2005a. *Antioxidant responses and oxidative stress after microcystin exposure in the hepatopancreas of an estuarine crab species*. Ecotoxicol. Environ. Saf., 61: 353–360.

PINHO G.L.L., MOURA DA ROSA C., MACIEL F.E., BIANCHINI A., YUNES J.S., PROENÇA L.A.O., MONSERRAT J.M. 2005b. *Antioxidant responses after microcystin exposure in gills of an estuarine crab species pre-treated with vitamin E.* Ecotoxicol. Environ. Saf., 61: 361–365.

PIRES L.M.D., KARLSSON K.M., MERILUOTO J.A.O., KARDINAAL E., VISSER P.M., SIERWERTSEN K., VAN DONK E., IBELINGS B.W. 2004. *Assimilation and depuration of microcystin-LR by the zebra mussel, Dreissena polymorpha.* Aquat. Toxicol., 69: 385–396.

ROHRLACK T., DITTMANN E., BOERNER T., CHRISTOFFERSEN K. 2001. *Effects of cell-bound microcystins on survival and feeding of Daphnia spp*. Appl. Environ. Microbiol., 67: 3523– 3529.

ROHRLACK T., CHRISTOFFERSEN K., DITTMANN E., NOGUEIRA I., VASCONCELOS V., BÖRNER T. 2005. *Ingestion of microcystins by Daphnia: Intestinal uptake and toxic effects.* Limnol. Oceanogr., 50: 440–448.

SABATINI S.E., BRENA B.M., LUQUET C.M., SAN JULIÁN M., PIREZ M., CARMEN RIOS DE MOLINA M.D. 2011. *Microcystin accumulation and antioxidant responses in the freshwater clam Diplodon chilensis patagonicus upon subchronic exposure to toxic Microcystis aeruginosa.* Ecotoxicol. Environ. Saf., 74: 1188–1194.

SIVONEN K. 2009. *Cyanobacterial toxins.* In: *Encyclopedia of Microbiology.* Eds. M. Schaechter, Elsevier Inc., pp. 290–307.

SMITH J.L., HANEY J.F. 2006. *Foodweb transfer, accumulation, and depuration of microcystins, a cyanobacterial toxin, in pumpkinseed sunfish (Lepomis gibbosus)*. Toxicon., 48: 580–589.

THORSTRUP L., CHRISTOFFERSEN K. 1999. *Accumulation of microcystin in Daphnia magna feeding on toxic Microcystis.* Arch. Hydrobiol., 145: 447–467.

TOIVOLA D.M, ERIKSSON J.E, BRAUTIGAN D.L. 1994. *Identification of protein phosphatase 2A as the primary target for microcystin-LR in rat liver homogenates*. FEBS Lett., 344: 175–180.

TRICARICO E., BERTOCCHI S., BRUSCONI S., CASALONE E., GHERARDI F., GIORGI G., MASTROMEI G., PARISI G. 2008. *Depuration of microcystin-LR from the red swamp crayfish Procambarus clarkii with assessment of its food quality.* Aquaculture, 285: 90–95.

VALERIO E., CHAVES S., TENREIRO R. 2010. *Diversity and impact of prokaryotic toxins on aquatic environments: a review*. Toxins, 2: 2359–2410.

VANDERPLOEG H.A, LIEBIG J.R, CARMICHAEL W.W, AGY M.A, JOHEGEN T.H, FAHNENSTIEL G.L, NALEPA T.F. 2001. *Zebra mussel (Dreissena polymorpha) selective filtration promoted toxic Microcystis blooms in Saginaw Bay (Lake Huron) and Lake Erie.* Can. J. Fish Aquat. Sci., 58: 1208–1221.

VINAGRE T.M, ALCIATI J.C, YUNES J.S, RICHARDS J., BIANCHI A., MONSERRAT J.M. 2002. *Effects of extracts from the cyanobacterium Microcystis aeruginosa on ion regulation and gill Na^+, K^+ – ATPase and K^+ – dependent phosphatase activities of the estuarine crab Chasmagnathus granulata (Decapoda, Grapsidae).* Physiol. Biochem. Zool., 75: 600–608.

WIEGAND C, PFLUGMACHER S. 2005. *Ecotoxicological effect s of selected cyanobacterial secondary metabolites, a short review.* Toxicol. Appl. Pharmacol., 203: 201–218.

WHITTON B.A., POTTS M. 2000. *Introduction to the cyanobacteria*. In: *The ecology of cyanobacteria*. Eds. B.A. Whitton, Potts M. Kluwer Academic Publishers, Dordrecht-London-Boston, pp. 1–11.

WHO. 1999. *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management.* Eds. I. Chorus, J. Bartram Routledge: London and New York, pp. 141–142.