Bull Environ Contam Toxicol (2011) 86:263–267
DOI 10.1007/s00128-011-0208-0

Effects of Nitrite and Toxic *Microcystis Aeruginosa* PCC7806 on the Growth of Freshwater Rotifer *Brachionus Calyciflorus*

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Received: 27 January 2010 / Accepted: 25 January 2011 / Published online: 3 February 2011

Abstract Over the last two centuries, anthropogenic activities have increased the nitrogen amount in aquatic ecosystems, which has resulted in increased occurrences of blooms of cyanobacteria. This study investigated the effects of nitrite and the cyanobacterium *Microcystis aeruginosa* on population growth in the rotifer *Brachionus calyciflorus*. The rotifer was treated for 12 days with nitrite alone (medium containing 0, 3, 6, 10 mg NO_2^-–N L^{-1}), *M. aeruginosa* alone (medium containing 0 mg NO_2^-–N L^{-1} + 5.0 × 10^5 cell ml^{-1} *M. aeruginosa* precultured at 0, 3, 6, 10 mg NO_2^-–N L^{-1}), and nitrite in combination with *M. aeruginosa* (medium containing 3, 10 mg NO_2^-–N L^{-1} + 5.0 × 10^5 cell ml^{-1} *M. aeruginosa* precultured at corresponding nitrite concentrations). We observed that a nitrite concentration of 10 mg NO_2^-–N L^{-1} markedly inhibited the growth of *B. calyciflorus*; however, rotifer growth declined slightly in the presence of *M. aeruginosa* precultured at 6 mg NO_2^-–N L^{-1}. Furthermore, reduced population growth of *B. calyciflorus* was observed when it was treated with both nitrite and *M. aeruginosa* compared to nitrite alone or *M. aeruginosa* alone. These results suggested that a high tolerance of *B. calyciflorus* to nitrite levels may be attributed to the absence of specific respiratory structures and pigments; and that the increased toxicity of nitrite in combination with *M. aeruginosa* may have been due to increased production of microcystin. It is also possible that nitrite and microcystin could act in a synergistic way in causing toxicity.

Keywords Growth · Microcystin · Nitrite · *Brachionus calyciflorus* · *Microcystis aeruginosa*

During the past two centuries, and especially over the last five decades, humans have substantially altered the global nitrogen cycle, increasing both the availability and the mobility of nitrogen over large regions of earth (Galloway and Cowling 2002). Consequently, a large amount of inorganic and organic nitrogen can enter aquatic ecosystems and result in increased concentrations of inorganic nitrogen in surface water (Rouse et al. 1999; Smith et al. 1999; Li et al. 2009). Nutrient concentration plays an important role in the growth of phytoplankton, and generally a combination of high nutrient load with warm, stable condition can stimulate phytoplankton to grow excessively and form blooms (Paerl 1988). *Microcystis aeruginosa* blooms are common and widespread in eutrophied freshwater ecosystems. Some studies have revealed that many strains of this cyanobacterium produce microcystins, which pose a threat to humans by hepatotoxicity and inhibition of protein phosphatase types 1 and 2A (Mackintosh et al. 1990; Dawson 1998). The increase of nitrogen in aquatic ecosystem can also cause significant enhancement of nitrite concentration due to imbalances in nitrification and denitrification processes (Philips et al. 2002), In addition, certain human activities direct increase the amount of nitrite, such as industrial wastewater effluents, municipal sewage effluents, runoff from agriculture

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(Lewis and Morris 1986; Camargo and Alonso 2006; Li et al. 2009). On the basis of acute toxicity data, several authors have suggested nitrite levels over 0.35 mg NO_2^-–N L^{-1} were toxic to sensitive aquatic animals (Camargo and Alonso 2006), causing dysfunctions of the oxygen carrying pigments, as well as reductions in extracellular chloride concentration and muscle potassium content (Lewis and Morris 1986; Philips et al. 2002; Camargo and Alonso 2006).

Zooplankton groups, particularly rotifers, cladocerans and copepods, represent the dominant component in freshwater bodies and are the natural food link between the primary producer (algae) and zooplanktivorous fish (Pennak 1989). As such they are important in the maintenance of an ecological balance in fresh water ecosystems. Although numerous studies have examined the effects of microcystin produced by *M. aeruginosa* on zooplankton, few studies have focused on rotifers (Smith and Gilbert 1995; Nandini 2000; Geng et al. 2006). Moreover, there is little information on the influence of nitrite on rotifers and microcystin toxicity. Thus, in the present study, we examined the effects of nitrite and *M. aeruginosa* alone and in combination, upon the growth in a laboratory culture of the rotifer *Brachionus calyciflorus*.

Materials and Methods

B. calyciflorus was collected from Lake Taihu (China) and maintained in the laboratory for 6 months prior to initiation of this experiment. A clone of this species was derived from a single female and cultured in EPA medium (Teresa et al. 2004) at 25°C, 300 lux and a 12 h light: 12 h dark cycle, on a diet of 5.0×10^5 cell ml^{-1} *Scenedesmus obliquus*. *M. aeruginosa* PCC7806 and *S. obliquus* were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). *M. aeruginosa* PCC7806, which produces microcystin-LR (Downing et al. 2005), was grown in the sterilized modified BG-11 medium, where nitrate concentration was 50 mg NO_3^-–N L^{-1} (Chen et al. 2009), at 25°C, 2500 lux and a 12 h light: 12 h dark cycle. *S. obliquus* was cultured in sterilized HB-4 medium (Ma et al. 2004) under similar condition. Cell densities were monitored by enumeration with microscope and hemacytometer.

The experiment followed a factorial design with two factors, nitrite and *M. aeruginosa*. It investigated the effects of nitrite alone, *M. aeruginosa* alone and *M. aeruginosa* in combination with nitrite on *B. calyciflorus*. The effect of nitrite on *B. calyciflorus* experiment was carried out in four nitrite treatments (0, 3, 6, 10 mg NO_2^-–N L^{-1}) achieved by adding $NaNO_2$ to the EPA medium, based on the range of nitrite-N concentration reported by Masser et al. (1999), Spieles and Mitsch (2000) and Li et al. (2009).

To investigate the impact of *M. aeruginosa* on *B. calyciflorus*, *M. aeruginosa* was precultured in modified BG-11 medium with 0, 3, 6, 10 mg NO_2^-–N L^{-1}. The nitrite concentration of medium was held constant by adding nitrite daily. The initial cell density was about 5×10^4 cell ml^{-1}. After 9 days of precultivation, the algae were harvested, centrifuged at 3,000*g* for 10 min, and transferred to EPA medium to form four different treatments: 0 mg NO_2^-–N L^{-1} + 5.0×10^5 cell ml^{-1} *M. aeruginosa* precultured at 0, 3, 6, 10 mg NO_2^-–N L^{-1}; the experiment of effect of nitrite in combination with *M. aeruginosa* on *B. calyciflorus* had two nitrite levels of 3 and 10 mg NO_2^-–N L^{-1}. After preculturing at these two nitrite levels for 9 days, *M. aeruginosa* was transferred to EPA medium with 3 and 10 mg NO_2^-–N L^{-1}, respectively to achieve two treatments: 3 mg NO_2^-–N L^{-1} + 5.0×10^5 cell ml^{-1} *M. aeruginosa* precultured at 3 mg NO_2^-–N L^{-1}, 10 mg NO_2^-–N L^{-1} + 5.0×10^5 cell ml^{-1} *M. aeruginosa* precultured at 10 mg NO_2^-–N L^{-1}.

All experiments were conducted on a diet of 5.0×10^5 cell ml^{-1} *S. obliquus* at 25°C, 300 lux and a 12 h light: 12 h dark cycle. Five neonates (<6 h old) were introduced in 20 ml glass beakers containing 10 ml designated treatment medium. The animals were transferred daily to fresh treatment medium, and the numbers of all live individuals were recorded. The experiments were terminated after 12 days. Every treatment had three replicates. Based on population abundance data, the growth rate (r) was calculated using the equation $r = \ln (N_t/N_0)/t$, where N_0 is the initial population density and N_t is the population density after t days. Data of the maximum population density and r were treated statistically using one-way analysis of variance (ANOVA). Post hoc pairwise comparisons were performed (Tukey test) to compare a given treatment to all other treatments in every experiment. A probability of $p < 0.05$ was considered statistically significant. All analyses were performed with SPSS.

Results and Discussion

The population density and growth rate of *B. calyciflorus* at different nitrite concentrations are presented in Fig. 1 and Table 1. There were no significant impacts at nitrite concentrations of 0, 3 and 6 mg NO_2^-–N L^{-1}on *B. calyciflorus* ($p > 0.05$), but a statistically significant inhibition on growth of *B. calyciflorus* was observed at nitrite concentration of 10 mg NO_2^-–N L^{-1} ($p < 0.05$), where the growth rate and maximum population density decreased by 2.9 and 19%, respectively compared to 0 mg NO_2^-–N L^{-1}.

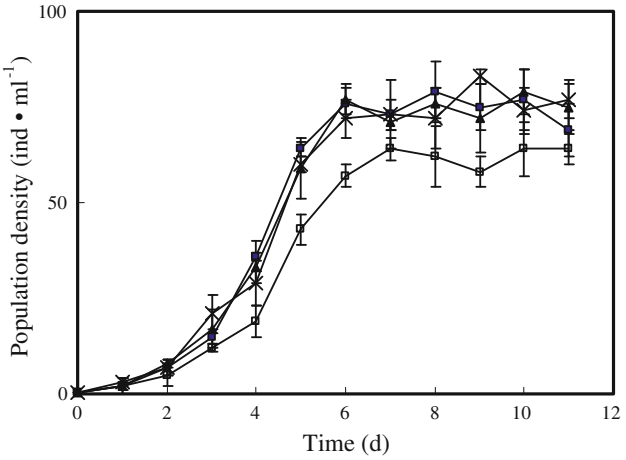

Fig. 1 The population density of *Branchionus calyciflorus* in nitrite treatments. 0 mg NO_2^-–N L^{-1} *filled square*, 3 mg NO_2^-–N L^{-1} *filled triangle*, 6 mg NO_2^-–N L^{-1} *times symbol*, 10 mg NO_2^-–N L^{-1} *square*. *Error bars* denote the standard deviation of triplicate incubations

The study of population growth is commonly used to evaluate the effects of many substances such as pesticide and heavy metals to zooplankton. In general, a reduction in the growth rate indicates that a substance is toxic to zooplankton (Forbes and Calow 1999). Therefore, the present study suggested the nitrite concentration of 10 mg NO_2^-–N L^{-1} caused toxicity to *B. calyciflorus*. Many laboratory studies have examined nitrite concentration inducing toxicity to freshwater invertebrates and fishes. These studies revealed *Cherax quadricarinatus* (decapod; adult), *Oncorhynchus mykiss* (salmonid; fry), *Pimephales promelas* (cyprinid; fry), *Hexagenia sp* (ephemeropteran; larvae) and *Gammarus fasciatus* (amphipod; adult) exhibited acute toxicity (96-hour LC_{50}) lower than 3 mg NO_2^-–N L^{-1} (Russo et al. 1981; Ewell et al. 1986; Rouse et al. 1995; Camargo and Alonso 2006). Compared to *B. calyciflorus*, these species seemed to be more sensitive to nitrite, possibly resulting from a difference in respiratory structures between *B. calyciflorus* and these species. *C. quadricarinatus*, *O. mykiss*, *P. promelas*, *H. sp* and *G. fasciatus* have gills and singular oxygen-carrying pigments. In contrast, *B. calyciflorus* has no specific respiratory structures and pigments, and the gas exchange is performed by simple diffusion through the body wall. The gills are the organs of active nitrite uptake, there is strong evidence that nitrite toxicity to aquatic animal is mitigated when inhibition of active diffusion of nitrite through the gill occurs (Russo et al. 1981; Lewis and Morris 1986; Camargo and Alonso 2006; Alonso and Camargo 2008). In addition, nitrite could cause the conversion of oxygen-carrying pigments to a form that is incapable of carrying oxygen (Lewis and Morris 1986; Philips et al. 2002; Camargo and Alonso 2006). Hence, the absence of these respiratory structures could be the reason for the high tolerance of *B. calyciflorus* to nitrite toxicity (Alonso and Camargo 2008).

Figure 2 and Table 1 show the effects of *M. aeruginosa* precultured at different nitrite concentrations on *B. calyciflorus*. The growth of animals in medium with *M. aeruginosa* precultured at 0 and 3 mg NO_2^-–N L^{-1} did not exhibit significant change ($p > 0.05$). However, the population growth of *B. calyciflorus* was statistically significantly inhibited in the presence of *M. aeruginosa* precultured at 6 and 10 mg NO_2^-–N L^{-1} ($p < 0.05$).

As mentioned in the introduction, microcystin has been found to be toxic to humans, animals and plants. Some laboratory investigations have demonstrated that though *B. calyciflorus* was tolerant to microcystin to some extent,

Table 1 Maximum population density and growth rate of *Branchionus calyciflorus* in different treatments. Data are means ± SD (n = 3)

Nitrite (mg NO_2^-–N L^{-1})		Growth rate ($days^{-1}$)	Maximum population density(ind. mL^{-1})
Nitrite treatments	0	0.454 ± 0.006	79 ± 5
	3	0.456 ± 0.010	79 ± 6
	6	0.458 ± 0.005	83 ± 9
	10	0.441 ± 0.003[a]	64 ± 7[a]
M. aeruginosa treatments	0	0.456 ± 0.008	80 ± 11
	3	0.460 ± 0.006	80 ± 7
	6	0.443 ± 0.006[b]	69 ± 8[b]
	10	0.413 ± 0.011[b]	50 ± 5[b]
Nitrite in combination with *M. aeruginosa* treatments	3	0.427 ± 0.003[c]	65 ± 6[c]
	10	0.121 ± 0.006[d]	28 ± 4[d]

[a] significant difference with the 0, 3, 6 mg NO_2^-–N L^{-1} in nitrite treatments

[b] significant difference with the 0, 3 mg NO_2^-–N L^{-1} in *M. aeruginosa* treatments

[c] significant difference with the 3 mg NO_2^-–N L^{-1} in *M. aeruginosa* treatments and in nitrite treatments

[d] significant difference with the 10 mg NO_2^-–N L^{-1} in *M. aeruginosa* treatments and in nitrite treatments (^{a,b,c,d} $p < 0.05$)

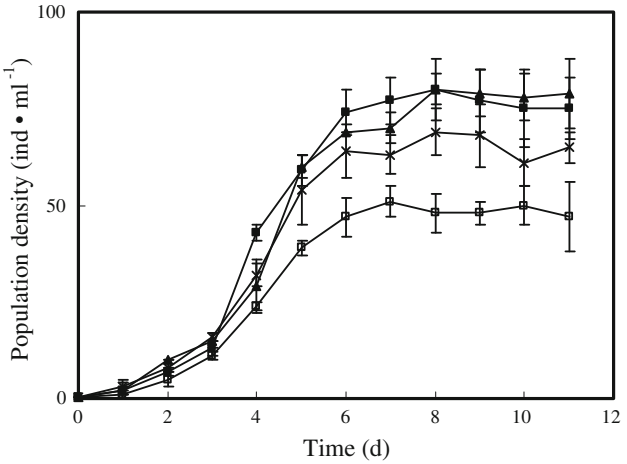

Fig. 2 The population density of *Branchionus calyciflorus* in *M. aeruginosa* treatments. 0 mg NO_2^-–N L^{-1} + 5.0 × 10^5 cell ml^{-1} *M. aeruginosa* precultured at 0 *filled square*, 3 *filled triangle*, 6 *times symbol* and 10 *square* mg NO_2^-–N L^{-1}. *Error bars* denote the standard deviation of triplicate incubations

its growth was adversely inhibited by microcystin at *M. aeruginosa* concentration of 10^6 cells ml^{-1}(Smith and Gilbert 1995; Nandini 2000; Geng et al. 2006). Therefore, we propose that the decreased population growth of rotifers in the presence of *M. aeruginosa* precultured at 6 and 10 mg NO_2^-–N L^{-1} indicates a higher amount of microcystin. This would indicate an increase in toxin production per cell, given the equal cell density of all *M. aeruginosa* treatments. The production of toxin by algae has been suggested to have special biological functions, and can be governed by biotic and abiotic factors, such as temperature, pH, light intensity, nutrient concentration, zooplankton and fish (Westhuizen and Eloff 1985; Downing et al. 2005; MarÍa and Daniel 2005). Recently, Jang et al. (2004, 2006) and Gong et al. (2009) reported some chemicals that could stimulate microcystin production of *M. aeruginosa*. Moreover, our previous work found that high nitrite concentration caused a significant increase in MC-LR of *M. aeruginosa* (Chen et al. 2010). Thus, it is reasonable to hypothesize that nitrite stimulated microcystin production in the algae, and the increasing toxin concentration resulted in the suppression of population growth in *B. calyciflorus*.

In comparing the effect of 3 mg NO_2^-–N L^{-1} in combination with *M. aeruginosa* on *B. calyciflorus* to the medium with 3 mg NO_2^-–N L^{-1} or *M. aeruginosa* precultured at 3 mg NO_2^-–N L^{-1} alone, a significant decrease in population growth of *B. calyciflorus* was observed in the combination treatment of 3 mg NO_2^-–N L^{-1} and *M. aeruginosa* precultured and tested in 3 mg NO_2^-–N L^{-1} (Table 1; Fig. 3; $p < 0.05$). Treatments of 3 mg NO_2^-–N L^{-1} alone or *M. aeruginosa* alone precultured at 3 mg NO_2^-–N L^{-1} did not result in significant population growth differences ($p > 0.0.05$). Similarly, the growth of animals

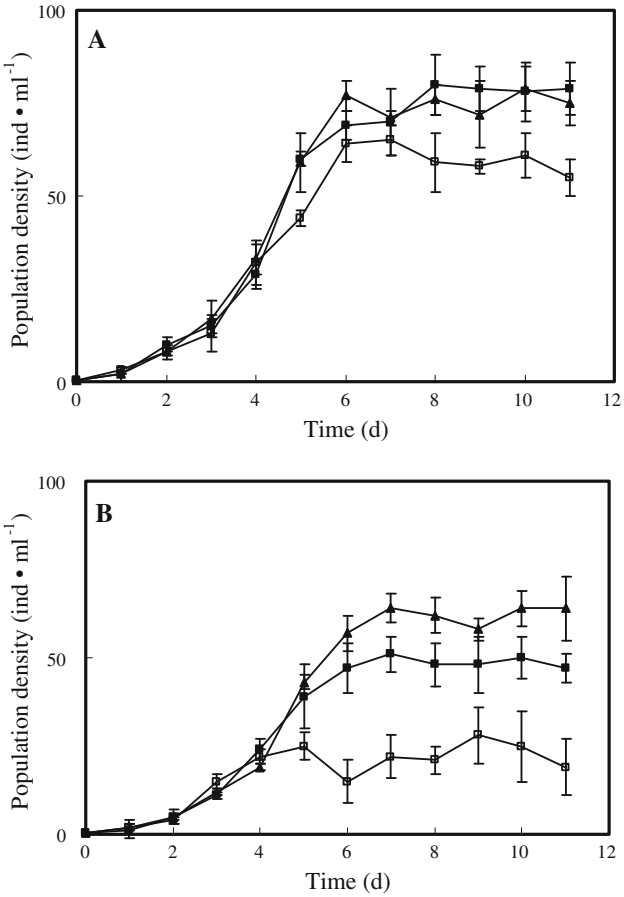

Fig. 3 The population density of *Branchionus calyciflorus* in nitrite in combination with *M. aeruginosa* treatments and corresponding nitrite treatments, *M. aeruginosa* treatments. **a** 3 mg NO_2^-–N L^{-1} *filled triangle*, 0 mg NO_2^-–N L^{-1} + 5.0 × 10^5 cell ml^{-1} *M. aeruginosa* precultured at 3 mg NO_2^-–N L^{-1} *filled square*, 3 mg NO_2^-–N L^{-1} + 5.0 × 10^5 cell ml^{-1} *M. aeruginosa* precultured at 3 mg NO_2^-–N L^{-1} *square*; **b** 10 mg NO_2^-–N L^{-1} *filled triangle*, 0 mg NO_2^-–N L^{-1} + 5.0 × 10^5 cell ml^{-1} *M. aeruginosa* precultured at 10 mg NO_2^-–N L^{-1} *filled square*, 10 mg NO_2^-–N L^{-1} + 5.0 × 10^5 cell ml^{-1} *M. aeruginosa* precultured at 10 mg NO_2^-–N L^{-1} *square*. *Error bars* denote the standard deviation of triplicate incubations

in the medium with both 10 mg NO_2^-–N L^{-1} and *M. aeruginosa* precultured at 10 mg NO_2^-–N L^{-1} showed a greater inhibition than the medium with 10 mg NO_2^-–N L^{-1} alone, or *M. aeruginosa* precultured at 10 mg NO_2^-–N L^{-1} alone(Table 1; Fig. 3; $p < 0.05$).

The influence of different abiotic and biotic factors on the toxicity of nitrite or microcystin is a subject of continued interest from different investigators. For example, the presence of chloride, calcium and selenium in the ambient water may significantly reduce nitrite toxicity to aquatic animals (Russo et al. 1981; Lewis and Morris 1986; Wang et al. 2009). Dithioerythrito and silymarinand protected against microcystin-induced toxicity toward cultured rat hepatocytes (Mereish and Solow 1990). The present study examining the mutual influence of nitrite and

microcystin showed that nitrite in combination with *M. aeruginosa* caused a greater reduction in population growth than either nitrite treatment alone or *M. aeruginosa* treatment alone.

In summary, *B. calyciflorus* showed a high tolerance to nitrite toxicity, possibly owing to the absence of respiratory structures and pigments. *M. aeruginosa* precultured at higher nitrite levels (6 and 10 mg NO_2^-–N L^{-1}) showed inhibition of population growth in *B. calyciflorus*, likey caused by promotion of algal growth and increased production of microcystin. In addition, the possibility exists that nitrite and microcystin could act synergistically in causing the observed toxicity. This suggests that the influence of *M. aeruginosa* on the toxicity of nitrite should be taken into account when the ecotoxicological risk assessment of nitrite is performed in aquatic ecosystems, and vice versa.

Acknowledgments This work was supported by a cooperative project between Nankai University and Tianjin University funded by Educational Ministry of China.

References

Alonso A, Camargo JA (2008) Ameliorating effect of chloride on nitrite toxicity to freshwater invertebrates with different physiology: a comparative study between amphipods and planarians. Arch Environ Contam Toxicol 54:259–265

Camargo JA, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int 32:831–849

Chen WM, Zhang QM, Dai SG (2009) Effects of nitrate on intracellular nitrite and growth of *Microcystis aeruginosa*. J Appl Phycol 21:701–706

Chen WM, Liu H, Zhang QM et al. (2010) Effect of nitrite on growth and microcystins production of *Microcystis aeruginosa* PCC7806. J Appl Phycol doi:10.1007/s10811-010-9558-y

Dawson RM (1998) Toxicology of microcystins. Toxicon 7:953–962

Downing TG, Sember CS, Gehringer MM et al (2005) Medium N:P ratios and specific growth rate comodulate microcystin and protein content in *Microcystis aeruginosa* PCC7806 and *M. aeruginosa* UV027. Microb Ecol 49:468–473

Ewell WS, Gorsuch JW, Kringle RO et al (1986) Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. Environ Toxicol Chem 5:831–840

Forbes VE, Calow P (1999) Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? Environ Toxicol Chem 18:1544–1556

Galloway JN, Cowling EB (2002) Reactive nitrogen and the world: 200 years of change. Ambio 31:64–71

Geng H, Xie P, Xu J (2006) Effect of toxic *Microcystis aeruginosa* PCC7820 in combination with a green alga on the experimental population of *Brachionus calyciflorus* and *B. rubens*. Bull Environ Contam Toxicol 76:963–969

Gong Y, Chou HN, Tu CD et al (2009) Effects of arsenate on the growth and microcystin production of *Microcystis aeruginosa* isolated from Taiwan as influenced by extracellular phosphate. J Appl Phycol 21:225–231

Jang MH, Ha K, Joo GJ et al (2004) Changes in microcystin production by *Microcystis aeruginosa* exposed to phytoplanktivorous and omnivorous fish. Aquat Toxicol 68:51–58

Jang MH, Ha K, Jung JM et al (2006) Increased microcystin production of *Microcystis aeruginosa* by indirect exposure to nontoxic cyanobacteria: potential role in the development of *Microcystis* bloom. Bull Environ Contam Toxicol 76:957–962

Lewis WM, Morris DP (1986) Toxicity of nitrite to fish: a review. Trans Am Fish Soc 115:183–195

Li YF, Wan WF, Song J et al (2009) Classification of groundwater contamination in Yuxi river valley, Shaanxi Province, China. Bull Environ Contam Toxicol 82:234–238

Ma JY, Lin FC, Zhang RZ et al (2004) Differential sensitivity of two green algae, *Scenedesmus quadricauda* and *Chlorella vulgaris*, to 14 pesticide adjuvants. Ecotoxicol Environl Saf 58:61–67

Mackintosh C, Beattie KA, Klump S et al (1990) Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett 264:187–192

MarÍa VA, Daniel AW (2005) Effects of iron, ammonium and temperature on microcystin content by a natural concentrated *Microcystis aeruginosa* population. Water Air Soil Pollut 168: 235–248

Masser MP, Rakocy J, Losordo TM (1999) Recirulating aquaculture tank production system: management of recirculating systems. SRAC Publ 452 (Aubum, 11 pp)

Mereish KA, Solow R (1990) Effect of antihepatotoxic agents against microcystin-LR toxicity in cultured rat hepatocytes. Pharm Res 7(3):256–259

Nandini S (2000) Responses of rotifers and cladocerans to *Microcystis aeruginosa* (cyanophyceae): a demographic study. Aquat Ecol 34:227–242

Paerl HW (1988) Nuisance phytoplankton blooms in coastal estuarine inland waters. Limnol Oceanogr 33:823–847

Pennak RW (1989) Fresh-water invertebrates of the United States. The Ronald-Press Co, New York

Philips S, Laanbroek JH, Verstraete W (2002) Origin, causes and effects of increased nitrite concentrations in aquatic environments. Rev Environ Sci Biotechnol 1:115–141

Rouse DB, Kastner RJ, Reddy KS (1995) Toxicity of ammonia and nitrite to hatching redclaw crayfish, *Cherax quadricarinatus*. Freshw Crayfish 10:298–303

Rouse JD, Bishop CA, Struger J (1999) Nitrogen pollution: an assessment of its threat to amphibian survival. Environ Health Perspect 107:799–803

Russo RC, Thurston RV, Emerson K (1981) Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*): effects of pH, nitrite species, and anion species. Can J Fish Aquat Sci 38:387–393

Smith AD, Gilbert JJ (1995) Relative susceptibilities of rotifers and cladocerans to *Microcystis aeruginosa*. Arch Hydrobiol 132: 309–336

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ Pollut 100:179–196

Spieles DJ, Mitsch WJ (2000) The effects of season and hydrologic and chemical loading on nitrate retention in constructed wetlands: a comparison of low-and high-nutrient riverine systems. Ecol Eng 14:77–91

Teresa RP, Sarma SSS, Nandini S (2004) Effects of mercury on the life table demography of the rotifer *Brachionus calyciflorus* Pallas (rotifera). Ecotoxicology 13:535–544

Wang HW, Xu HM, Xiao GH et al (2009) Effects of selenium on the antioxidant enzymes response of *Neocaridins heteropoda* exposed to ambient nitrite. Bull Environ Contam Toxicol 84(1): 112–117

Westhuizen AJV, Eloff JN (1985) Effect of temperature and light on the toxicity and growth of the blue-green alga *Microcystis aeruginosa* (UV-006). Planta 163:55–59