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Interactive Effects of Nitrogen and Copper on Growth of Cyanobacterium *Microcystis*

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Among nutrients, nitrogen and phosphorus are responsible for considerable growth and emergence of blooms of planktonic algae in highly eutrophic lakes, ponds and reservoirs during the warm season. In many cases the scum of cyanobacteria consists almost entirely of cells of Microcystis species. This unicellular alga produces toxic substances which harm and cause even death of domestic animals (Gentile 1971; Watanbe and Oishi 1982).

Copper is an essential trace element needed in small quantities by algae for various metabolic activities. The effects of copper on microbes like algae, for example, have been documented by numerous researchers (Sunda and Lewis 1978; Toledo et al. 1979; Petersen 1982). This essential micronutrient, however, may induce toxic response at submicromolar concentrations by inhibiting nitrate uptake, reduction and nitrite release (Kashyap and Gupta 1982), acid and alkaline phosphatase activity (Gupta 1983), and synthesis of photosynthetic pigments and macromolecules (Gupta 1986). Biochemical significance of copper in photosystem II has also been established recently (Gupta 1988).

Interactions involving micronutrient availability have generated increasing environmental concerns (Kuwabara 1982; Gupta 1987). Results from laboratory experiments for biological response of various concentrations of a single nutrient or toxicant may not be appropriate in field conditions due to lack of sufficient information on nutrient interactions. Herein an attempt has been made to investigate the interactive effects of inorganic nitrogen nutrients and copper on growth of Microcystis sp.

MATERIAL AND METHODS

A dense bloom of natural Microcystis population was collected from one of the lakes of the Indian Botanic Garden, Botanical Survey of India, Howrah, using a plankton net (100 μM aperture). Clonal cultures were raised and maintained in ASM-1 medium (Gorham et al.

1964) modified by Reynolds and Jaworski (1978) employing standard microbiological techniques. The cultures were tested periodically for bacterial contamination by plating on bacteriological media. Cultures were maintained in an incubator at 24 ± 1 C, continuously illuminated with cool fluorescent tubes (irradiance 3.87 Wm^{-2}) for 16:8 light and dark period, respectively. Concentrations of initial nitrate nitrogen and copper were selected in such a way that the stationary growth phase would be attained within the 14 days culturing period so that copper and nitrogen nutrients effects would be discernible over the selected concentration ranges.

Copper was supplemented as $CuSO_4.5\ H_2O$ (British Drug Houses, India) from 1 M copper stock solution to achieve desired concentrations. Graded concentrations of different forms of nitrogen nutrients were added depending on the requirement. Exponentially grown cultures were washed by repeated centrifugation and inoculated in a nitrogen-deficient media for 12 hours before the start of the experiments. Cell counts were made to establish an inoculum of approximately 10^6 cells. Growth was monitored spectrocolorimetrically (Systronics, India) at 650 nm and the specific growth rate (k) was calculated using the following equation given by Myres and Kratz (1955):

$$k = \frac{2.32(\log N_2 - \log N_1)}{T_2 - T_1}$$

where,

N_1 = initial cell density at time T_1, and

N_2 = final cell density at time T_2

Two sets of experiments have been conducted. Of these, one set related to copper toxicity in natural water and basal medium and the other to the interactive effects of various inorganic nitrogen nutrients and copper concentrations.

RESULTS AND DISCUSSION

Growth of Microcystis was observed to be in direct relation to the concentration of copper employed.

Copper concentration as low as 0.1 µM affected the specific growth rate (k) and cell-division whereas higher copper concentration of 0.5 µM and above have proved to be toxic causing the death of the organism. Death of Microcystis in the basal medium was faster (50% survival after 40 h of treatment) than in the natural water (68% survival) (Table 1). This might be due to the absence of organic matter in the basal medium or sufficient physiological differences present in the natural water and basal medium rendering them more sensitive in the latter case. It has been reported that natural waters possess copper-binding capacities which is attributed to humic and fulvic acids in such waters (Geisy et al. 1978; Sunda and Guillard 1976). Detoxification of copper as a result of natural complexation influencing copper bioavilability to organisms is reported to trigger uncontrollable algal blooms in natural water (Nor 1987).

Copper (0.1 µM) serves as an algistatic in the presence of high nitrate concentrations (5-10 mM) but becomes algicidal when present in low concentrations (<5 mM). Thus high nitrate concentrations, which supported better growth of Microcystis, reduced the algicidal effect of copper. Low 'k' values were observed when nitrate was replaced either by nitrite-N or ammonium-N. However, the 'k' values increased as the nitrite-N concentrations increased from 1 to 5 mM. But in the presence of copper and nitrite-N the 'k' values have been significantly lower (Table 2). This clearly indicates that other inorganic nitrogen sources caused more inhibition than nitrate-N in presence of copper. Growth response of Microcystis to various copper concentrations and nitrate-N suggests that in nature growth limitation by an essential nutrient other than nitrate-N or copper (e.g. phosphates) could shift to other limiting conditions as a result of elevated copper concentrations. This study indicates that elevated copper concentrations may limit cyanobacterial biomass by interfering with the nitrogen metabolism (Kashyap and Gupta 1982).

There are various mechanisms by which microbial cells can tolerate high levels of heavy metals:(a) removal of the toxicant from the cell and intracellular detoxication, (b) detoxication by production of extracellular organic material, and (c) prevention of intracellular uptake and accumulation. Microcystis probably falls in the first category. The increased sensitivity of this cyanobacterium suggests a possibility of the production of a toxic substance of proteineous nature (Watanbe and Oishi 1982) involved in

Table 1. Inactivation of Microcystis in natural water and basal medium by copper (0.5 µM)

Treatment (h)	Survival	
	Basal medium	Natural pond water
0	100	100
20	82.5	90
40	50	68
60	32.5	48.5
80	25.8	37
100	12.5	20
120	0.0	5.5

Table 2. Interactive effects of different nitrogen nutrients and copper in Microcystis

Copper (µM)	NO_3-N(mM)			NO_2-N(mM)		NH_4-N(mM)	
	1.0	5.0	10.0	1.0	5.0	1.0	5.0
0.0	0.042 ±0.001	0.055 ±0.003	0.060 ±0.001	0.040 ±0.001	0.045 ±0.004	0.025 ±0.002	0.035 ±0.001
0.1	0.045 ±0.002	0.050 ±0.002	0.060 ±0.001	0.035 ±0.001	0.030 ±0.001	0.020 ±0.003	0.019 ±0.001
0.2	0.020 ±0.004	0.025 ±0.001	0.030 ±0.001	0.030 ±0.001	0.020 ±0.003	0.015 ±0.004	0.010 ±0.005
0.5	0.000	0.000	a	0.000	a	0.000	a

[a] Not observed

transporting copper into the cells. Unicellular cyanobacteria, including Microcystis, are comparatively more sensitive than the filamentous ones (Gupta et al. 1985) and since Microcystis is often found in association with other planktonic filamentous forms like Phormidium and Anabaena, the differential sensitivities have to be borne in mind while treating planktonic algae in polluted ponds, reservoirs and lakes as only sensitive forms might be killed leaving behind other resistant for survival and creating additional blooms.

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REFERENCES

Geisy JP, Briese LA, Leversee GJ (1978) Metal binding capacity of selected Maine surface waters. Environ Geol 2:257-268

Gentile J (1971) Blue-green and green algal toxins.In: Kadis S, Ciegler A, Ajl S (eds) Microbial Toxins, Academic Press, New York, pp 27-66.

Gorham PR, McLachlan J, Hammer UT, Kim WK (1964) Isolation and culture of toxic strains of Anabaena flos-aquae (Lyngb) Breb. Ver Int Verein Theor Angew Limnol 15:796-804

Gupta SL (1983) Acid and alkaline phosphatase activity in the cyanobacterium Anacystis nidulans under copper stress. Folia Microbiol 28: 458-462

Gupta SL (1986) Copper uptake and inhibition of growth, photosynthetic pigments and macromolecules in the cyanobacterium Anacystis nidulans. Photosynthetica 20:447-453

Gupta SL (1987) Influence of inorganic-nitrogen nutrients on copper toxicity in Anacystis nidulans and cyanophage AS-1 resistant mutant. Bull Bot Surv India 27:142-144

Gupta SL (1988) Copper inhibition of photosystem II activity in a cyanophage AS-I-resistant mutant. J Gen Appl Microbiol 34:1-5

Gupta SL, Kashyap AK, Singh AP (1985) Responses of blue-green algae to copper: A comparative study. Ind J Environ Hlth 27:224-229

Kashyap AK, Gupta SL (1982) Effect of lethal copper concentration on nitrate uptake, reduction and nitrite release in Anacystis nidulans. Z Pflanzenphysiol 107:289-294

Kuwabara JS (1982) Micronutrients and kelp cultures: Evidence for cobalt and mangnese deficiency in Southern California deep sea water. Science 216: 1219-1221

Myres J, Kratz WA (1955) Relations between pigment content and photosynthetic characteristics in a blue-green alga. J Gen Physiol 39:11-22

Nor YM (1987) Ecotoxicity of copper to aquatic biota: A review. Environ Res 43:274-282

Peteresen R (1982) Influence of copper and zinc on the growth of a fresh water alga Scenedesmus quadricauda. The significance of chemical speciation. Environ Sci Technol 16:443-447
Reynolds CS, Jaworski GHM (1978) Enumeration of natural Microcystis populations. British Phycol J 13:269-277
Sunda WG, Guillard R (1976) The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J Mar Res 34:511-529
Sunda WG, Lewis JAM (1978) Effect of complexation by natural organic ligands on the toxicity of copper to a unicellular alga Monochrisis lutheri. Limnol Oceanog 23:870-876
Toledo APP, Tundisi JG, D'Aquino VA (1979) Humic acid influence on the growth and copper tolerance of Chlorella sp. Hydrobiologie 71:261-263
Watanbe MF, Oishi S (1982) Toxic substance from a natural bloom of Microcystis aeruginosa. Appl Environ Microbiol 43:819-822

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