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Influence of Humic Acid on the Toxicity of Copper, Cadmium and Lead to the Unicellular Alga, *Synechosystis Aquatilis*

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Humic acids are known to play a significant role in phytoplankton productivity by regulating the trace metals required for plant growth (Prakash and Rashid 1968). Although few attempts have been made to evaluate the influence of humic acids on heavy metal toxicity to aquatic organisms (Sunda and Lewis 1978), their interaction in natural waters is well documented (Buffle et al. 1977; Knezevic and Chan 1977; Reuter and Perdue 1977; Schnitzer and Khan 1972; Williams 1969).

The present study was undertaken to evaluate the influence of humic acids (HA) extracted from mangrove sediments of this region (Lat. 14° 15'N & 74°07'E) on Cu, Cd and Pb toxicity to the unicellular alga, Synechosystis aquatilis.

MATERIALS AND METHODS

Humic acid was extracted from sediments by the standard alkali extraction procedure (Kononova 1966) and purified by 95% ethanol several times. The test species, S. aquatilis, was cultured on a large scale (in tanks of 50 L capacity) from a stock culture carefully isolated from natural plankton samples, and suitable media was provided for the growth. The algal culture was used for toxicity bioassays at the exponential growth stage, when the chlorophyll extract measured in a spectrophotometer at the optical density of 663 and 645 nm was above 0.6. A standard algal toxicity bioassay method (APHA 1980) was followed to test the toxicity of Cu, Cd and Pb. The chlorophyll reduction due to exposure to heavy metals for 6 hours was taken as an index of toxicity. The experiment was conducted in triplicate, both under dark and light conditions. Chlorophyll levels in the samples were estimated by the method of Parsons et al. (1984).

Toxicity tests were conducted with three metals in eight logarithmic concentrations ranging from 0.061 to 1.00 ppm with 100-ml algal samples in 125-ml glass vials with screw caps. In another set, 0.01 ppm of HA solution was added to each test container for all three metals and a control was maintained in each case for both light and dark. A light hood (light intensity ≃

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5000 lux; temp. 25 ± 2°C), having both incandescent and day-light lamps, was used for 'light' experiments, and for dark, the containers were fully covered with aluminium foil and black paper. The final volume of each test container was adjusted with water to 110 ml. It should be noted here that the eight test concentrations were the same for all three metals (they were 0.061, 0.255, 0.352, 0.512, 0.653, 0.720, 0.889 and 1.000 ppm). For studies of metal-HA interaction, 0.01 ppm of HA solution was added to each test concentration of metal. The results were subjected to probit analysis as given by Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

The toxicity of metals with or without HA are presented in Table 1. The results suggest that a greater toxic effect on S. aquatilis was felt with Cu, Cd and Pb alone; whereas there was a significant ($r= 0.981$ at EC16; 0.993 at EC50; 0.978 at EC84 levels; $P<0.01$; d.f. 5) reduction in toxicity when 0.01 ppm of HA was added. Further, the toxicity reduction due to HA addition to individual metal was more under light than under dark. This suggests that metals inhibit chlorophyll synthesis more under light than in dark by interfering with Mg ion of chlorophyll (Azeez and Banerjee 1987). This interference is further reduced in the presence of small amount of HA. HA chelates significant amounts of metal ions and reduces the interference with chlorophyll by free metal ions, hence a reduction in toxicity. On the other hand in dark, the light dependent inhibition of metal ions is not observed (Azeez and Banerjee 1987), but chelation alone reduces the toxic effect.

In control tests the addition of HA caused a 10% increase of chlorophyll under light in six hours, but not under dark. This agrees with Prakash and Rashid (1968) who found that HA enhanced chlorophyll synthesis (growth) of marine algae and diatoms.

The toxicity of metals to S. aquatilis were in the order Cu>Cd>Pb both in light and dark. On addition of HA, the order of toxicity remained the same but the percentage reduction in toxicity was more with Cu. A similar observation was made earlier by Sunda and Lewis (1978) on Monochrysis lutheri, not with HA but a natural organic ligand believed to contain HA. Further, the addition of HA to Cu reduced the toxicity to S. aquatilis by 10.09% in light and 9.51% in dark. Similarly Cd toxicity was reduced by 5.55% in light and 5.05% in dark and Pb toxicity reduced by 7.15% in light and 7.14% in dark, compared to individual metals' toxicity.

Table 1. Comparative toxicity values of Cu, Cd and Pb without and with 0.01 ppm of humic acid exposure to *S. aquatilis* for six hours.

Factor	EC16 (ppm)	EC50 (ppm)	EC84 (ppm)	Slope
		Cu		
L	0.24	0.65 (0.62-0.71)	1.07	2.20 (1.61-2.75)
D*	0.25	0.70 (0.65-0.75)	1.14	2.24 (1.65-2.83)
		Cd		
L	0.31	0.70 (0.57-0.88)	1.09	1.92 (1.56-2.28)
D*	0.42	0.79 (0.65-0.95)	1.16	1.67 (1.47-1.87)
		Pb		
L	0.44	0.92 (0.77-1.11)	1.41	1.82 (1.53-2.12)
D*	0.48	0.95 (0.81-1.14)	1.45	1.75 (1.50-2.00)
		Cu + HA		
L	0.34	0.72 (0.66-0.76)	1.06	1.79 (1.47-2.11)
D*	0.35	0.77 (0.71-0.79)	1.15	1.86 (1.50-2.23)
		Cd + HA		
L	0.42	0.74 (0.67-0.92)	1.15	1.65 (1.56-1.74)
D*	0.50	0.83 (0.73-0.98)	1.18	1.53 (1.42-1.65)
		Pb + HA		
L	0.55	0.99 (0.85-1.17)	1.43	1.62 (1.44-1.80)
D*	0.64	1.02 (0.90-1.16)	1.40	1.48 (1.32-1.59)

't' value = 0.749 between light and dark for the above sets at EC50; significant at $P<0.5$; d.f. 5.

L= light; D= dark; * includes chlorophyll reduction in in control also; values in paranthesis indicate 95% confidence limits.

It can be concluded that humic acid reduce the toxic effect of heavy metals to algae significantly, more under light by reducing the free metal ions which are relatively more toxic. Further, HA does not seem to interfere with the light-dependent enzyme action in algae.

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