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Influence of Light on Chlorophyll, a Content of Blue-Green Algae Treated with Heavy Metals

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The toxicity of heavy metals is manifested in multifarious forms. The metals cause alterations in various metabolic activities like protein synthesis (Hart and Scaife 1977), carbon assimilation and chlorophyll content (Rebhum and Ben-Amotz 1984; Azeez and Banerjee 1986) and so on. Factors like illumination influence the inhibitory effect of heavy metals on chlorophyll metabolism and photosynthetic activities (Cedano-Maldonado and Swader 1972; Colbert et al. 1983; Baker 1984; Wu and Lorenzen 1984).

The present study was undertaken to explore the effect of light on the chlorophyll A (Chl A) content of blue green algae. This is in continuation of heavy metal toxicity and accumulation studies on cyanobacteria reported earlier by the present authors.

MATERIALS AND METHODS

Blue-green algae *Anacystis nidulans* and *Spirulina platensis* were grown in an aquous medium containing per liter NaHCO3 (18.0 gm); K2H PO4 (0.5 gm); NaNO3 (2.5 gm); K2SO4 (1.0 gm); NaCl (1.0 gm); MgSO4 (0.2 gm); CaCl2 (0.04 gm); FeSO4 (0.01 gm) and 1 mL A5 solution (containing per liter H3BO3, 2.9 gm; MnCl2, 1.8 gm; ZnCl2, 0.11 gm; CuSO4, 0.08 gm and (NH4)MoO4, 0.18 gm) (Azeez 1986). They were treated with Cu, Cd, Ni and Cr. These metals were added as aquous solutions of CuSO4.5H2O, 3CdSO4.8H2O, NiSO4.5H2O and K2Cr2O7, for Cu, Cd, Ni and Cr respectively in concentrations of 0.01; 0.1; 1.0 and

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10.0 ppm in each case. 10 ml cultures of the experimental species were taken in screw cap tubes and the metals were added singly as well as in combinations of twos (CuxCd; CuxNi; CuxCr; CdxNi; CdxCr and CrxNi) in all the above mentioned concentrations. Half of the tubes were covered with black paper and aluminium foil completely so as to prevent exposure to light and kept along with the others under a light hood (light intensity ∽ 2500 lux; room temperature 27 $\pm$ 2° C). For each concentration of metal 4 replicates were run. Controls were also run for both dark and light conditions. After the algae had been exposed to the metals for 6 hrs under light or dark conditions, they were harvested by filtration. Chl A content in the algal samples was extracted with 80% acetone and optical densities of the extract were determined (at 663 nm and 645 nm) by a Bausch & Lomb Spectronic 1001 spectrophotometer. The optical densities were converted to Chl A contents following the relation given by Venkataraman (1983). This measurement provides data on the net balance of the synthesis and degradation of Chl A.

RESULTS AND DISCUSSION

The unicellular species *Anacystis* contains more Chl A than the filamentous *Spirulina*. It was observed that the Chl A contents of these two species were 0.74 $\pm$ 0.02% and 0.42 $\pm$ 0.01% of the total dry weight respectively. Exposure of the algae to the metals for 6 hrs reduced the Chl A content. This reduction in Chl A content varied with the metal and also with the experimental species. The decrease in Chl A content when *Anacystis* and *Spirulina* were treated with Cu and Cd has been reported earlier by the present workers (Azeez and Banerjee 1986). When *Anacystis* was treated with the metals, under illuminated conditions, 10.0 ppm Cu resulted in the highest reduction in Chl A content. In terms of percent reduction it was 40.5%. The same treatment under dark conditions resulted in the reduction of Chl A content by 24.2%. In case of *Spirulina* the highest reduction in Chl A content was observed when treated with 10.0 ppm Ni. In this case, Chl A content was reduced by 57.4% and 52.7% under light and dark conditions respectively. The decrease in Chl A content in the presence of Cu, Cd, Ni and Cr followed the general relation, Chl A = K + nln C (C = concentration of metal in ppm; K and n = intercept and slope respectively). The slopes of this equation provide good indices for comparing the reduction of Chl A content by the metals. Based on

Table 1. Constants of the equations denoting the reduction of Chl a content of the cyanobacteria, when treated with Cu, Cd, Ni and Cr singly and in combinations of twos in different concentrations. (L and D denote light and dark, respectively)

Metals	Anacystis				Spirulina			
	Intercept		Slope*		Intercept		Slope*	
	L	D	L	D	L	D	L	D
Cu	5.385	5.650	-0.4473 ±0.020	-0.3040 ±0.020	3.070	3.160	-0.2215 ±0.009	-0.1867 ±0.006
Cd	5.649	5.820	-0.2897 ±0.006	-0.1781 ±0.009	2.930	3.010	-0.2562 ±0.009	-0.2389 ±0.008
Ni	5.840	5.934	-0.2910 ±0.006	-0.2354 ±0.005	2.427	2.492	-0.2762 ±0.005	-0.2567 ±0.003
Cr	6.040	6.017	-0.2041 ±0.011	-0.1837 ±0.006	3.330	3.390	-0.1471 ±0.015	-0.1173 ±0.006
Cu x 0.01 ppm Cd	5.055	5.177	-0.4517 ±0.007	-0.4260 ±0.020	3.668	3.656	-0.2797 ±0.016	-0.2163 ±0.012
x 0.1 ppm Cd	4.517	4.707	-0.3718 ±0.011	-0.3696 ±0.009	3.023	3.162	-0.2862 ±0.007	-0.2784 ±0.003
x 1.0 ppm Cd	3.778	4.030	-0.3774 ±0.013	-0.3822 ±0.002.	2.392	2.525	-0.3305 ±0.004	-0.3192 ±0.005
x 10.0 ppm Cd	3.213	3.282	-0.3466 ±0.011	-0.3718 ±0.010	1.986	2.029	-0.3183 ±0.010	-0.3266 ±0.015
Cd x 0.01 ppm Cu	5.573	5.720	-0.3144 ±0.007	-0.2866 ±0.006	3.878	3.922	-0.2145 ±0.009	-0.1655 ±0.007
x 0.1 ppm Cu	4.736	4.904	-0.3010 ±0.002	-0.3092 ±0.001	3.221	3.284	-0.2315 ±0.009	-0.2137 ±0.011

Table 1 continued..

x 1.0 ppm Cu	3.746	3.915	-0.2923 ±0.010	-0.2997 ±0.003	2.566	2.598	-0.2445 ±0.009	-0.2297 ±0.008
x 10.0 ppm Cu	2.998	3.134	-0.2319 ±0.014	-0.2397 ±0.005	1.704	1.828	-0.2636 ±0.011	-0.2840 ±0.018
Cu x 0.01 ppm Ni	5.305	5.377	-0.5385 ±0.021	-0.4239 ±0.010	3.500	3.730	-0.2606 ±0.013	-0.2128 ±0.020
x 0.1 ppm Ni	4.492	4.644	-0.5064 ±0.013	-0.4504 ±0.022	3.182	3.363	-0.2176 ±0.003	-0.2124 ±0.004
x 1.0 ppm Ni	3.823	3.979	-0.4599 ±0.006	-0.4721 ±0.006	2.864	3.014	-0.1811 ±0.011	-0.2202 ±0.020
x 10.0 ppm Ni	3.006	3.120	-0.4399 ±0.005	-0.4560 ±0.010	2.489	2.527	-0.1637 ±0.005	-0.1764 ±0.007
Ni x 0.01 ppm Cu	5.943	6.004	-0.4078 ±0.021	-0.2962 ±0.022	3.695	3.843	-0.2323 ±0.011	-0.1950 ±0.009
x 0.1 ppm Cu	4.872	4.992	-0.3500 ±0.010	-0.3110 ±0.011	3.287	3.459	-0.1633 ±0.004	-0.1941 ±0.008
x 1.0 ppm Cu	3.737	3.858	-0.3218 ±0.009	-0.3340 ±0.006	2.877	2.905	-0.1112 ±0.011	-0.1368 ±0.006
x 10.0 ppm Cu	2.721	2.877	-0.3031 ±0.010	-0.3283 ±0.009	2.378	2.664	-0.1407 ±0.017	-0.1811 ±0.020
Cu x 0.01 ppm Cr	6.497	6.538	-0.2892 ±0.021	-0.2189 ±0.008	3.700	3.730	-0.2606 ±0.005	-0.2491 ±0.004
x 0.1 ppm Cr	5.748	5.891	-0.2667 ±0.006	-0.2489 ±0.009	3.600	3.490	-0.1954 ±0.019	-0.2332 ±0.010
x 1.0 ppm Cr	5.218	5.365	-0.2341 ±0.001	-0.2323 ±0.002	3.560	3.646	-0.1433 ±0.006	-0.1294 ±0.007
x 10.0 ppm Cr	4.659	4.771	-0.2006 ±0.003	-0.2119 ±0.005	3.400	3.490	-0.0869 ±0.009	-0.1068 ±0.010

Table 1 continued.

Cr x 0.01 ppm Cu	6.317	6.392	-0.3175 ± 0.011	-0.2545 ± 0.012	4.170	4.270	-0.1563 ± 0.010	-0.1129 ± 0.009
x 0.1 ppm Cu	5.728	5.871	-0.2949 ± 0.008	-0.2619 ± 0.007	3.830	3.920	-0.0825 ± 0.008	-0.1081 ± 0.006
x 1.0 ppm Cu	5.250	5.347	-0.2714 ± 0.005	-0.2610 ± 0.003	3.570	3.550	-0.0695 ± 0.017	-0.0356 ± 0.004
x 10.0 ppm Cu	4.690	4.831	-0.2258 ± 0.006	-0.2423 ± 0.009	3.160	3.205	+0.0304 ± 0.011	+0.0586 ± 0.013
Cd x 0.01 ppm Ni	5.532	5.576	-0.3110 ± 0.009	-0.2879 ± 0.010	3.540	3.680	-0.2475 ± 0.006	-0.1477 ± 0.022
x 0.1 ppm Ni	5.142	5.186	-0.2979 ± 0.008	-0.2727 ± 0.010	3.217	3.432	-0.2284 ± 0.010	-0.1902 ± 0.009
x 1.0 ppm Ni	4.816	2.905	-0.2721 ± 0.004	-0.2649 ± 0.005	2.957	3.145	-0.1894 ± 0.005	-0.1867 ± 0.003
x 10.0 ppm Ni	4.327	4.386	-0.2762 ± 0.002	-0.2836 ± 0.001	2.688	2.693	-0.1364 ± 0.009	-0.1603 ± 0.016
Ni x 0.01 ppm Cd	6.059	6.089	-0.2028 ± 0.005	-0.1724 ± 0.011	3.790	3.850	-0.2041 ± 0.020	-0.1303 ± 0.009
x 0.1 ppm Cd	5.425	5.469	-0.1737 ± 0.017	-0.1681 ± 0.009	3.385	3.510	-0.1542 ± 0.005	-0.1433 ± 0.002
x 1.0 ppm Cd	4.753	4.808	-0.1711 ± 0.002	-0.1668 ± 0.002	2.967	3.071	-0.1112 ± 0.011	-0.1424 ± 0.003
x 10.0 ppm Cd	4.117	4.189	-0.1568 ± 0.003	-0.1659 ± 0.002	2.534	2.669	-0.0942 ± 0.006	-0.1420 ± 0.013
Cd x 0.01 ppm Cr	6.360	6.388	-0.2779 ± 0.013	-0.2080 ± 0.007	4.089	4.111	-0.1377 ± 0.010	-0.1034 ± 0.005
x 0.1 ppm Cr	5.666	5.723	-0.2749 ± 0.009	-0.2384 ± 0.009	3.852	3.985	-0.1459 ± 0.006	-0.1346 ± 0.002
x 1.0 ppm Cr	4.997	5.127	-0.2719 ± 0.004	-0.2545 ± 0.004	3.685	3.800	-0.1325 ± 0.01	-0.1346 ± 0.009

Table 1 continued.

	x 10.0 ppm Cr	4.350	4.435	-0.2519 ±0.008	-0.2693 ±0.007	3.453	3.586	-0.1147 ±0.006	-0.1294 ±0.007
Cr	x 0.01 ppm Cd	6.228	6.258	-0.3101 ±0.021	-0.2341 ±0.013	4.260	4.375	-0.0499 ±0.002	-0.0478 ±0.002
	x 0.1 ppm Cd	5.623	5.659	-0.2927 ±0.006	-0.2723 ±0.007	3.970	4.107	-0.1021 ±0.006	-0.0808 ±0.005
	x 1.0 ppm Cd	5.003	5.103	-0.2949 ±0.007	-0.2797 ±0.002	3.632	3.740	-0.0873 ±0.002	-0.0913 ±0.002
	x 10.0 ppm Cd	4.400	4.510	-0.2823	-0.2975	3.382	3.504	-0.0721 ±0.007	-0.0704 ±0.006
Cr	x 0.01 ppm Ni	6.060	6.048	-0.3409 ±0.014	-0.2645 ±0.016	3.900	3.920	-0.0990 ±0.009	-0.0695 ±0.004
	x 0.1 ppm Ni	5.272	5.414	-0.3522 ±0.010	-0.3027 ±0.013	3.563	3.749	-0.1277 ±0.011	-0.0790 ±0.010
	x 1.0 ppm Ni	4.610	4.679	-0.3214 ±0.002	-0.3114 ±0.003	2.234	3.530	-0.0942 ±0.009	-0.0508 ±0.011
	x 10.0 ppm Ni	3.953	4.015	-0.3209 ±0.005	-0.3322 ±0.006	3.030	3.220	-0.0912 ±0.012	-0.0912 ±0.013
Ni	x 0.01 ppm Cr	6.114	6.114	-0.3244 ±0.021	-0.2571 ±0.011	3.744	3.884	-0.1529 ±0.011	-0.1064 ±0.009
	x 0.1 ppm Cr	5.402	5.436	-0.3088 ±0.031	-0.2086 ±0.011	3.539	3.688	-0.1312 ±0.010	-0.0734 ±0.006
	x 1.0 ppm Cr	4.668	4.741	-0.3014 ±0.002	-0.2988 ±0.003	3.285	3.464	-0.1433 ±0.010	-0.1008 ±0.006
	x 10.0 ppm Cr	3.831	3.941	-0.2966 ±0.007	-0.3183 ±0.011	3.050	3.320	-0.1303 ±0.008	-0.1129 ±0.006

*± 95% confidence level.

the slopes (n) of the above equation the order of toxicity was found to be Cu > Ni > Cd > Cr in case of <u>Anacystis</u> and Ni > Cd > Cu > Cr in case of <u>Spirulina</u> (Table 1). The slopes were more for experiments under illumination than under darkness. This held true for both sets of observations, one with the single metals and the other with the combination of two metals. Exceptions to this general observation were noted in the presence of any one metal along with 10.0 ppm of any other metal. In these cases the slopes of metal concentration vs. Chl A content plots, of the illuminated samples were equal to or lesser than those under dark conditions. Results of Chi-square test conducted on all the observations (Table 1) are $X2 = 10\ 45$; $df = 1$; $p < 0.01$. Steeman-Nielsen and Wium-Andersen (1971), Cedano-Maldonado and Swader (1972), Tripathy et al. (1981) and Baker et al. (1982) have discussed about the light dependent inhibition of metals on photosynthetic processes. Some processes involved in the synthesis as well as degradation of chlorophyll molecule are to a great extent light dependent (Baker 1984). The possible reason for lesser reduction of Chl A by heavy metals under dark may be the light dependent inhibition of enzymes and other factors which get activated by illumination. The displacement of Mg from chlorophyll molecules by metal atoms (Wu and Lorenzen 1984) leading to a change in the functional characteristics may also be a possible reason. These processes result in alteration of the metabolic turnover of chlorophyll in the presence of toxic metals.

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