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Toxicity and Accumulation of Copper and Cadmium in the Alga *Scenedesmus obliquus* LH

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Reports on the occurrence of copper and cadmium in the environment, especially in the hydrosphere, are appearing at even increasing frequency. In particular, cadmium is an undesirable pollutant because of its highly deleterious effects on health. Both metals effect also the hydrobiology and ecology of the aqueous environment.

In aqueous environments both Cu and Cd affect algal cells. The effect on algal growth and on their biochemical and physiological properties, as well as the accumulation of both elements in algal biomass, have been studied by a number of authors. Effect of copper has recently been studied, e.g., by Arturo and Swader (1974), Nakajima et al. (1979), Sandmann and Boger (1980), Butler et al. (1980), and others. The metals were found to exert inhibitory action on algal growth, photosynthesis, respiration and morphology even though trace concentrations of copper have been reported to stimulate algal growth (Januszko 1976). A marked toxic effect on algae, substantially higher than that of the related zinc, was found with cadmium (Wong et al. 1979; Cain et al. 1980). Accumulation and sorption of cadmium in algae was investigated, e.g., by Sakaguchi et al. (1979), Brauwers (1982) and Geisweid and Urbach (1983).

The techniques of determination of toxic and inhibitory effects, and of measuring the kinetics of metal sorption, used by individual authors differ widely in basic parameters, especially in the experimental concentrations of algal suspensions and in methods of separation of algae. The results are therefore comparable only to a limited extent. Some authors assume that the drop in the concentration of the metal in the solution, or its concentration in the biomass, are a measure of sorption of the metals by the algae. This is not entirely correct; our study led to this conclusion on the basis of measurement of inhibition of growth and sorption of copper and cadmium ions in dense algal suspensions in a photoautotrophic regime of an intensive culture, and comparison

of disappearance of these ions from the solution in the absence of the algae.

MATERIALS AND METHODS

Inorganic medium according to Šetlík (1967) at half concentration was used. The initial concentration of algae in the inoculated culture was about 2 g d.w. L^{-1}. The content of copper and cadmium in the inoculum was determined and was taken into account in calculation of results. Before the experimental cultivation, both metals were supplied from stock solutions to a concentration of 0.5 mg L^{-1} in variant I and 1.0 mg L^{-1} in variant II. Synthetic medium of the same composition and concentration was processed in the same manner as the culture; however, no algae were added to it and spontaneous growth of algae was prevented by coating the cultivation tubes with aluminium foil.

A sample of 70 mL suspension was collected daily. A volume of 10 mL was used for dry weight and extinction determination, the rest was centrifuged and the supernatant was dried by lyophilization. The lyophilizate was used for analyses. The first collections were done immediately after inoculation and further at 24-h intervals. The last was done after 120 or 144 h of cultivation. One measurement is a mean of six parallel assays. The experimental device and culture parameters are given in Véber et al. (1981).

The algal strain Scenedesmus obliquus LH was obtained from the collection of the Department of Autotrophic Microorganisms, Institute of Microbiology, Třeboň.

Algal dry weight was determined daily by a standard method, i.e. filtering and drying to a constant weight at 100°C. The solution containing the nutrient medium with appropriate Cu and Cd additions without the algae was bubbled through by a gas mixture in the same device and for the same time interval as algal cultures. After the experiment the solution was centrifuged analogously to algal suspensions and the supernatant was analyzed to determine the Cu and Cd content.

Cadmium and copper were assayed in the supernatant by atomic absorption spectrometry on a VARIAN AAS-1200 flame photometer using an acetylene-air flame.

RESULTS AND DISCUSSION

The experimental results given in Table 1 showed the following removal efficiency: Cu 83-97 % , Cd 78-99 % . In control parallel experiments (without algae and without light) the removal was 47-56 % Cu and 61-78 % Cd in the

same period and under the same conditions. The role of S. obliquus in the adsorption or absorption of the metals is thus not so big (Cu max. 49 % , Cd max. 39 %) as it does appear without the comparison. The lowering of concentration of copper and cadmium compounds in a solution containing other chemicals such as phosphates and ferrous ions, through carbon dioxide is bubbled at pH 6-7, is not surprising as such. Copper and cadmium salts at indicated concentrations can under these conditions form colloid hydroxides, phosphates and carbonates or coprecipitate with colloid ferric hydroxide. Centrifugation of the solution separates these particles and the supernatant contains then lowered concentrations of the elements. In the presence of algae it is very difficult to distinguish sorption of metals from this precipitation which can, and probably actually does, occur in parallel with the sorption. Centrifugation of cells results in pelleting of these colloid or microscopic particles. This has to be taken into account when evaluating experiments with biosorption of heavy metals. We attempted to appraise this effect in a comparative parallel experiment. However, this appraisal is only approximate since the presence of algae in the system can affect the precipitation and coagulation processes of metal compounds in diverse ways. The kinetics of removal of the metals from the media is shown in Table 1.

TABLE 1. Course of removal of Cu and Cd from the medium.

Variant	Cultivation time	Cu		Cd	
		Final concentration in the supernatant / Removal efficiency			
No.	h	mg L^{-1}	%	mg L^{-1}	%
I.	0.5	0.128	83.0	0.087	82.6
	24	0.023	96.9	0.030	94.0
	48	0.042	94.4	0.018	96.4
	72	0.061	92.0	0.005	99.0
	96	0.061	92.0	0.002	99.6
	+120	0.107	85.8	0.015	97.0
	120	0.280	56.0	0.195	61.0
II.	0.5	0.476	60.0	0.277	77.6
	24	0.071	94.0	0.012	98.8
	48	0.081	93.0	0.021	97.9
	96	0.160	86.5	0.003	99.7
	+144	0.106	91.0	0.005	99.5
	144	0.224	47.6	0.223	78.0

+ Control without the algae

Cultures of the alga S. obliquus with an initial concentration about 2 g L^{-1} were slightly inhibited by the addition of copper plus cadmium (0.5 mg L^{-1} each); even when the concentrations of the two metals were increased to more than 1 mg L^{-1} each, the toxic effect was not as pronounced as anticipated (Table 2). The time shift in the appearance of the maximum concentration of algae along with the relative difference of concentrations reached by the experimental and the control culture are measurable consequences of the toxicity of the metals towards the algae in this experiment.

TABLE 2. Course of growth during cultivation.

Cultivation time	Variant	Biomass concentration with - without metals		Variant	Biomass concentration with - without metals	
h	No.	g d.w. L^{-1}		No.	g d.w. L^{-1}	
0.5	I.	2.4	2.4	II.	1.8	1.8
24		3.5	3.8		2.7	3.3
48		4.5	5.3		3.4	5.4
72		7.0	7.3		4.9	7.0
96		6.0	6.8		5.8	9.7
120		6.1	7.0		7.7	12.9
144		-	-		5.8	11.0

In any case, the inhibition of dry weight increase was higher at 1 mg Cu plus 1 mg Cd per 1 litre suspension than at 0.5 mg L^{-1} of each of the metals.

Microscopic examinations showed no death of the algal cells in any of the variants. The difference between our data and the toxic concentrations determined in other studies can be most probably attributed to differences in initial concentration of the algae and also possibly type of growth medium, light intensity, and temperature employed.

It can be said that the removal of copper and cadmium is accelerated and increased in the presence of the alga Scenedesmus obliquus, even though the actual active biosorption is perhaps much lower than would correspond to the measured changes in the concentrations of the metals in cultivation media.

The results permit us to conclude that the inhibitory effect of Cu and Cd on the growth of the alga S. obliquus at initial biomass concentrations about 2 g dry weight per litre is perceptible and the algae accelerate and increase the removal of copper and cadmium from the medium.

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