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SHORT COMMUNICATION

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EFFECT OF CADMIUM ON PHOTOSYNTHETIC PIGMENTS IN SYNCHRONOUSLY GROWING *CHLORELLA* CELLS

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Cadmium ions introduced at concentration of 30 ppm to the cultivation medium of synchronously growing *Chlorella vulgaris* decreased concentration of chlorophylls a and b, carotenes α and β and lutein at various stages of the cell cycle, while at concentration of 1 ppm synthesis of the photosynthetic pigments was stimulated. The pigment content in the cadmium treated cells was related to the morphometrically determined changes in the size and shape of the cells.

Cadmium in living organisms forms complexes with polyphosphates, proteins and amino acids and is stored in the form of metallothioneins. It links —SH groups of enzymes and inhibits their biological activity [1]. Cadmium, like many other heavy metal ions, leads to pathological changes in living organisms in polluted environment or under laboratory conditions [2, 3]. In unicellular green algae [4] cadmium competes with other ions in membrane transport, decreases synthesis of RNA, DNA, photosynthetic pigments and protein [1]. Consequently, it reduces production of biomass [2, 5].

Chlorella cells are able to grow under conditions of extreme salinity and acidity. Also it has been suggested by Kessler [3] that *Chlorella* cells can be used as a cadmium-bioindicator. A readily measurable criterion of metabolic activity of *Chlorella vulgaris* is the content of chlorophylls and carotenoids [6, 7]. Since both cadmium uptake [5] and protein level in living cells depend on the stage of their cell cycle [4, 8] it seems possible that the effect of cadmium on photosynthetic pigment also depends on the developmental stage of the cell. Therefore, to study the cadmium effect on *Chlorella* cells we have investigated accumulation of photosynthetic pigments at succesive stages of cell cycle and compared these results with morphometric measuremets of the cells.

MATERIALS AND METHODS

Chlorella vulgaris cells Beijerinck 1890, strain A-8 were cultivated at 10 h light : 14 h dark cycle in conditions described earlier [9]. Cell development in such conditions takes place mainly during the light period while the release of aplanosores, their swelling and other non-photosynthetic processes occur in the dark [7, 10]. Since cell development is blocked in the absence of light the content of photosynthetic pigments was measured during or after the light period.

When cadmium as $CdCl_2$ at concentration of 30 ppm was added to the cell suspension at the beginning of cell cycle, the photosynthetic pigments were determined every two hours over the light period. When Cd^{2+} at the concentration of 1 ppm was introduced after 0, 4, 6, 8 and 10 h of cultivation, the amount of photosynthetic pigments was determined at the end (24 h) of each cell cycle. The pigments extracted from 1×10^8 cells were separated by t.l.c. and identified spectrophotometrically according to Goss & Cieplińska [10]. In order to describe morphoquantometric parameters of the cadmium affected cells, Cd^{2+} was added to the cultivation medium at concentrations of 15 and 30 ppm. Our routine morphoquantometric analysis [11], describing [21] parameters of size, shape and intracellular extinction, was carried out in the cadmium exposed cells every two hour during the light period of the cell cycle.

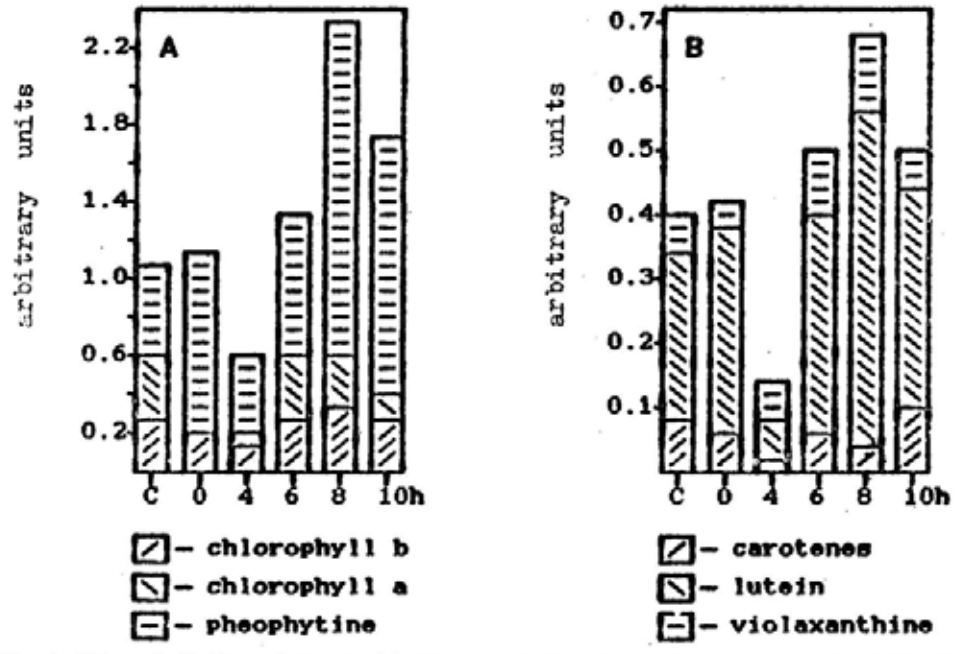

Fig. 1. Chlorophylls (A) and carotenoids (B) concentrations in synchronously growing *Chlorella vulgaris* cells exposed to 1 ppm concentration of cadmium ions introduced at the indicated time of the cell cycle

RESULTS AND DISCUSSION

The concentrations of cadmium used in the experiments are comparable to that in waste waters [2]. Cadmium added at concentration of 1 ppm increased concentration of photosynthetic pigments (Fig. 1) at all stages of cell cycle except at 4 h of cell cycle i.e. at the end of G_1 phase and beginning of S phase.

Table 1

Morphometric changes in the distribution of intracellular extinction (SUEX) expressed in arbitrary units and the total cell surface value (POLC) in μm^2 in Chlorella vulgaris cells exposed to 15 and 30 ppm of cadmium introduced at the beginning of the cell cycle

	Hour of life cycle	0	2	4	6	8	10
SUEX	Control	133	220	313	486	803	1043
	15 ppm Cd^{2+}	133	509	698	565	572	877
	30 ppm Cd^{2+}	133	213	364	269	272	568
POLC	Control	7.2	8.7	11.1	18.2	25.4	33.6
	15 ppm Cd^{2+}	7.2	11.4	15.5	16.1	15.8	22.6
	30 ppm Cd^{2+}	7.2	6.7	11.2	23.5	22.0	15.5

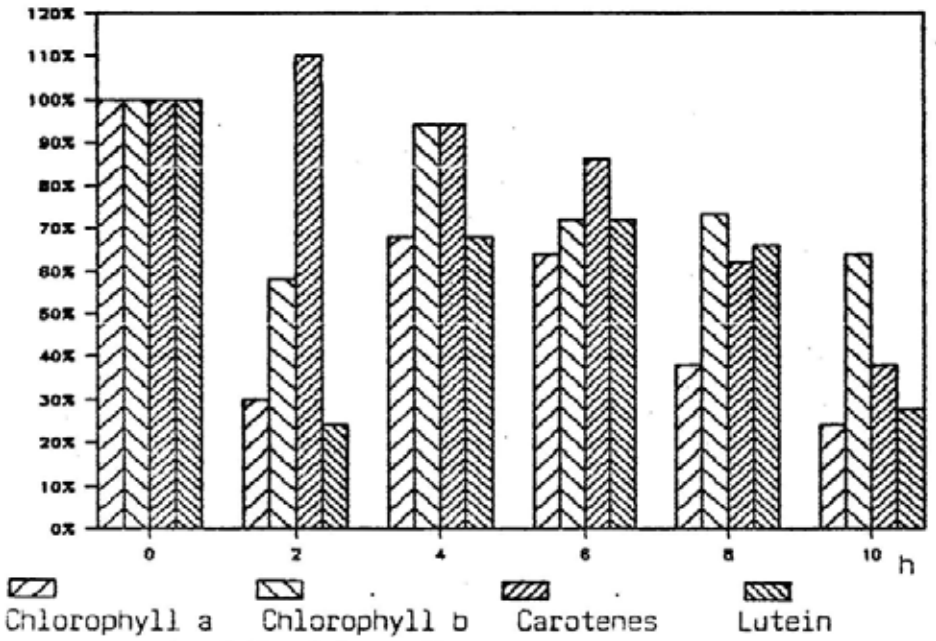

Fig. 2. Concentration of chlorophylles a and b, carotenes α and β and lutein after introduction of Cd^{2+} (30 ppm) at 0 h in synchronously growing *Chlorella vulgaris*, expressed as percentage of pigments content of control cells

Basing on our previous results [10] we measured the effect of cadmium on the content of chlorophylls and carotenoids starting immediately after the end of the dark period (24 h). Cadmium at higher concentration of 15 and 30 ppm was introduced at the beginning of cell cycle and its subsequent effect was measured within 10 h at 2 h intervals. Morphoquantometric analysis of parameters describing cell surface (POLC) (Table 1) suggested adaptation of *Chlorella* cells to the cadmium concentration as high as 15 ppm while 30 ppm concentration seems to be sublethal. However, intracellular extinction (SUEX) demonstrates disturbances of intracellular structure at both cadmium concentrations tested. These disturbances, caused by chloroplast disorganization, must result in changes in the content of photosynthetic pigments. As showing in Fig. 2, the content of chlorophylls a and b, carotenoids and lutein decreased during the cell cycle, however some unexplained oscillations in concentration of the pigments took place at S phase — the period of intensive chlorophyl synthesis in the cells not exposed to cadmium ions. Cadmium affects biosynthesis of the pigments throughout the whole cell cycle of *Chlorella* probably by affecting both the enzymes involved and cell structure.

REFERENCES

1. Viarengo, A. (1985) Biochemical effects of trace metals. *Mar. Pollut. Bull.*, **16**, 153-158.
2. Skowroński, T. (1988) Wpływ kadmu na glony jednokomórkowe. *Post. Mikrobiol.*, **27**, 77-94.
3. Kessler, E. (1985) An extremely cadmium-sensitive strain of *Chlorella*. *Experientia*, **41**, 1621-1622.
4. Thomas, L. (1979) Cadmium in aquatic organisms; in *The Chemistry, Biochemistry and Biology of Cadmium* (Webb, M., ed.) pp. 91-139. Elsevier/North-Holland Biomedical Press. Amsterdam.
5. Geisweid, H. J. & Urbach, W. (1983) Sorption of cadmium by the green microalgae *Chlorella vulgaris*, *Ankistrodesmus braunii* and *Eremosphaera viridis*. *Z. Pflanzenphysiol.* **109**, 127-141.
6. Böhm, H. (1973) Das Chlorella Testsystem. *Wiss. H. d. Pad. H. Köthen*, **1**, 9-17.
7. Chemeris, Y., Grishina, N. A. & Venediktov, P. S. (1979) Differences in the synthesis of chlorophyll a and b in the course of *Chlorella* cell cycle. *Plant Physiol. (Moscow)*, **26**, 378-382.
8. Gipps, J. F. & Coller, B. A. (1980) Effect of physical and culture conditions on uptake of cadmium by *Chlorella* pyrenoidosa. *Aust. J. Mar. Freshwater Res.*, **31**, 747-755.
9. Wilczok, A. & Mazurek, U. (1987) The role of water in size and shape characteristics of newly formed aplanospores of *Chlorella vulgaris*. *Stud. Biophys.*, **122**, 237-248.
10. Goss, M. & Cieplińska, A. (1985) Die Bestimmung der photosynthetischen Pigmente in einer Synchronkultur von Chlorella v. *Wiss. H. d. Pad. H. Köthen*, **1**, 113-116.
11. Wilczok, T., Wacławek, R., Wilczok, A. & Mazurek, U. (1985) Morphometrische Untersuchungen im Chlorella Testsystem. *Wiss. H. d. Pad. H. Köthen*, **1**, 63-65.