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Reduction of Cadmium Toxicity to Green Microalga *Stichococcus bacillaris* by Manganese

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Investigations of cadmium toxicity to microorganisms are now more concerned with the interactions of cadmium with different environmental factors and other metals. The interactions are complex and have not been thoroughly studied yet. Metal interactions may assume the form of synergism characterized by increase in toxicity, but also of antagonism in which one metal reduces the toxicity of another.

Apart from cadmium interactions with such toxic metals as mercury and lead (Stratton and Corke 1979 ; Wong et al. 1980; Devi Prasad and Devi Prasad 1982), interactions of cadmium with the essential trace elements seem to be very interesting because it has been assumed that algal cells take up cadmium by the system transporting these elements (Broda 1973).

The previous paper (Skowroński 1986a) showed that cadmium transport into Stichococcus bacillaris cells was inhibited by Mn^{2+} ions. Thus, it can be supposed that there exist some possibilities of using those ions antagonistic to cadmium as counterpoison. Showing those possibilities was the aim of the present paper.

MATERIALS AND METHODS

Stichococcus bacillaris Näg. was obtained from the Institute of Microbiology, Warsaw University. The alga was cultivated as described previously (Skowroński 1984). The effect of manganese on cadmium toxicity was studied as follows. The medium of the composition: urea– 0.3 g, KH_2PO_4 – 0.135 g, $MgSO_4 \times 7H_2O$ – 0.5 g, $FeSO_4 \times 7H_2O$ – 0.003 g, $C_6H_5O_7Na_3 \times 2H_2O$ – 0.0057 g, H_3BO_3 –2.137 mg, $MnCl_2 \times 4H_2O$ – 1.81 mg, $ZnSO_4 \times 7H_2O$ –0.22 mg, $(NH_4)_2MoO_4$ – 0.002 mg, $CuSO_4 \times 5H_2O$– 0.07 mg, $Co(NO_3)_2 \times 6H_2O$ – 0.08 mg, NH_4VO_3– 0.01 mg and 1 L deionized water, was inoculated with the algae (100 mg dry weight/L) taken from a 48-hr old culture. Different doses of $CdCl_2 x 2H_2O$ and/or $MnCl_2 x 4H_2O$ were added to the algae suspension at the beginning of the experiment. Restoration of the cadmium treated cultures was studied by the addition of

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$MnCl_2x4H_2O$ to the alga culture after 48 - hr growth in the cadmium containing medium. The levels of both metals were selected from the previous studies of cadmium uptake and toxicity to Stichococcus bacillaris (Skowroński et al. 1985; Skowroński 1986a). pH of the medium was adjusted to 6.5 and corrected every 24 hr. Algae were cultivated in glass tubes (medium volume of 300 mL) at the temperature of 28°C in the fluorescent light (6,000 lux). Air containing 1% CO_2 was constantly bubbled through the medium. Estimation of the toxic effect of cadmium was based on measurements of dry weight and chlorophyll a content and on the number of living cells in cultures. Dry weight was determined by filtering algae on Whatman GF/C filter followed by a wash with distilled water, pH 5.5 and drying at 105°C. The chlorophyll a content in the cultures was determined according to Golterman and Clymo (1971). The number of living cells in cultures was determined by fluorescent microscopy. The effect of both metals on the growth rate of algae was calculated according to Wong et al. (1983):

$$\% \text{ inhibition} = \left(1 - \frac{k_1}{k_2} \right) \times 100$$

where k_1 = growth rate in the presence of both metals
k_2 = growth rate in the control
The growth rate (k) was calculated according to Guillard (1973) on the base of alga dry weight. Statistical data are expressed as mean $\pm$ standard error of the mean. Analysis of variance was carried out according to Ahrens (1970).

RESULTS AND DISCUSSION

Table 1 shows that cadmium in the absence of manganese inhibits the growth rate of S. bacillaris and the percentage of living cells. The addition of manganese had an antagonistic effect on the toxicity of cadmium. This was shown by an increase in growth rate and the percentage of living cells when manganese was added to medium containing cadmium. In the culture with 0.09 mM of Cd the living cells constituted only 28 % whereas in that with a 10 - fold higher concentration of manganese 80 %. These observations are confirmed by the factorial experiment of the type 4 x 3 in which the range of manganese concentration was widened (Table 2). As can be seen manganese alone at the concentration to 1.8 mM caused slightly inhibiting effect on biomass level in the cultures. Cadmium alone caused algal growth inhibition as in the earlier experiments (Skowroński et al. 1985) and 0.09 mM of Cd inhibited algal growth almost completely. In the cultures to which cadmium and manganese were added, dry weight and chlorophyll a content were greater than in the cultures with cadmium only. The experimental combination with 0.045 mM of cadmium and 1.8 mM of manganese is especially spectacular. In this case a 40 - fold higher dose of manganese completely neutralized (comparing with the combination: Cd - 0, Mn - 1.8 mM)toxic effect of cadmium. In the studied range of concentrations of both metals, cadmium toxicity decreased with the increase of manganese concentration in the medium. Comparing dry weight and chlorophyll a content in the cultures it can be seen that addition of manganese resulted also in the increase of chlorophyll a

Table 1. The effect of Cd and Mn on the growth rate of S.bacillaris (% inhibition) and content of living cells after 4 days of cultivation (%)

Cd and Mn added (mM)		% inhibition	% living cells
Cd	Mn		
–	–	–	100
–	0.450	0.06	100
–	0.900	0.02	100
0.045	–	27.70	80
0.045	0.225	21.30	93
0.045	0.450	13.80	98
0.090	–	44.70	28
0.090	0.450	38.30	66
0.090	0.900	34.00	80

content in biomass. The higher concentration of manganese in the culture medium, the higher chlorophyll a content in algal cells. For example, chlorophyll a content in the algal biomass in the presence of 0.045 mM Cd was 0.53 %, while in the experimental combination: 0.045 mM Cd and 1.8 mM Mn - 1.51 %, reaching the level close to the control. The results included in Table 2 were subjected to the analysis of variance (Ahrens 1970), and showed that dry weight and chlorophyll a content in the cultures signifi - cantly depended on Cd and Mn concentrations (statistical proba - bility - 99.9 %).

Upitis et al. (1973) stated that growth of Chlorella in culture medium containing cadmium was greater when the medium had been enriched with Mn, Zn or Fe. Experiments carried out with S. bacil - laris showed that toxicity of even very high doses of cadmium (e.g. 0.09 mM) might be considerably reduced after adding appropria- te doses of manganese.

S. bacillaris takes up Cd by adsorption and energy-dependent trans- port. Mn^{2+} ions evidently affect both processes. They reduce the amount of Cd adsorbed due to the competition for cadmium bin - ding sites on the cell surface (Skowroński 1986b). However they reduce the amount of Cd taken up by means of energy-dependent transport as well, which may result from Mn^{2+} and Cd^{2+} ion com - petition for their common transport system (Skowroński 1986a). Cadmium may be toxic after entering a cell. Thus, inhibition of Cd transport to cells by Mn^{2+} ions may account for lower Cd toxicity in the media containing higher Mn level as shown in the paper.

The above results concern the experimental combination in which both elements were simultaneously added into the growth medium. It also seemed interesting to study the effect of manganese on algal cultures treated with cadmium earlier. Fig.1 presents the experi - mental results when manganese was added just after 2 day incuba -

Table 2. Simultaneous influence of Cd and Mn on dry weight and chlorophyll a content in algal cultures after 4 day cultivation

Cd \ Mn	0	0.045 mM	0.9 mM	1.8 mM
	Dry weight (mg/L)			
0	1813±87	1633±52	1867±18	1753±13
0.045 mM	723±22	1220±75	1680±10	1707±53
0.090 mM	220±15	300±12	1080±47	1517±71
	Chlorophyll a content (µg/L)			
0	29360±78	23280±590	24960±788	26350±237
0.045 mM	3800±266	12695±331	21050±803	25790±681
0.090 mM	970±135	1180± 62	10970±672	16790±866

Data are expressed as a mean ± standard error of the mean, N = 3

tion of algae with cadmium. During the first 48 hours cadmium toxic action caused biomass and chlorophyll decrease in S.bacillaris cultures. However, after addition of manganese whose concentration was 20 - fold higher than cadmium molar concentration, significant increase both in dry weight and chlorophyll a content in the cultures took place in comparison with the manganese free cultures. After five day incubation, algal dry weight content in the cultures with manganese was three times as large as that in the manganese free cultures. Thus manganese acted as a detoxicant and restored S. bacillaris culture. This effect can be explained by manganese competitive inhibition of cadmium transport into algal daughter cells. It should also be taken into consideration that after penetration into the cells, manganese could protect photosynthetic apparatus against harmful action of cadmium as in the case of higher plants. Baszyński et al. (1980) studying photosynthetic activities of cadmium-treated tomato plants stated that addition of manganese into the growth medium caused not only chlorophyll synthesis increase and chloroplast structure restoration, but also restoration of photosystem II activity.

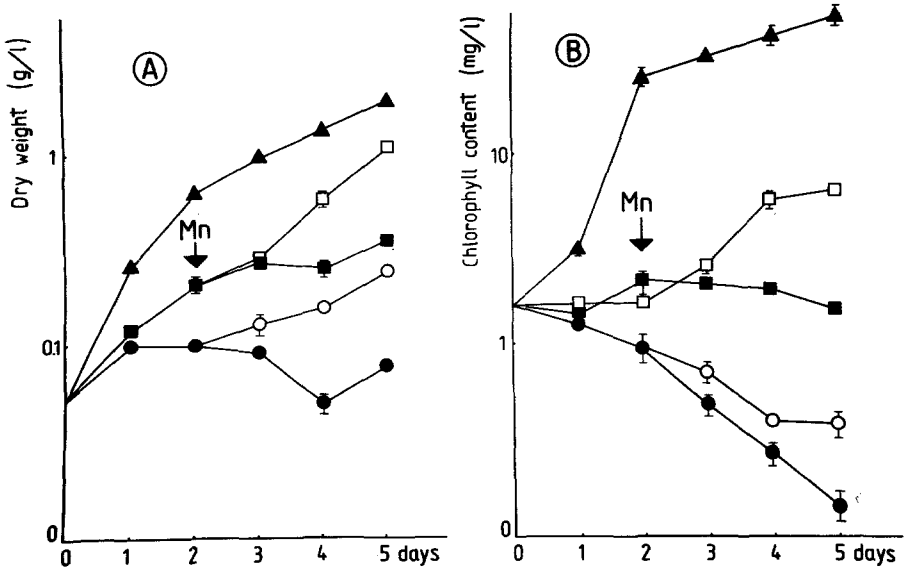

Figure 1. Detoxic effect of manganese to cadmium-treated cultures of Stichococcus bacillaris. A – algal dry weight in the cultures, B – chlorophyll content in the cultures (▲) – without Cd and Mn; (□) – 0.045 mM Cd + 0.9 mM Mn; (■) – 0.045 mM Cd; (○) – 0.09 mM Cd + 1.8 mM Mn; (●) – 0.09 mM Cd. The arrow indicates the addition of manganese. Data are expressed as a mean + standard error of the mean, N = 3. If SE is not denoted, it is smaller than the symbols in the Figure.

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REFERENCES

Ahrens H (1970) Analysis of variance. PWN Warszawa, Poland

Baszyński T, Wajda L, Król M, Wolińska D, Krupa Z, Tukendorf A (1980) Photosynthetic activities of cadmium-treated tomato plants. Physiol Plant 48:365 – 370

Broda E (1973) Wie treten die esentielle und schädige Spurelementen in die Nahrungskette ein? Naturw Rdsch 9:381 – 389

Devi Prasad PV, Devi Prasad PS (1982) Effect of cadmium, lead and nickel on three freshwater green algae. Water Air and Soil Pollut 17:263 – 268

Golterman HL, Clymo RS (1971) Methods for chemical analysis of fresh waters. Blackwell Sci Publ, Oxford London-Edinburgh-Melbourne

Guillard RRL (1973) Division rates. In: Stein JR (ed) Handbook of phycological methods. Culture methods and growth measurements. Cambridge University Press, Cambridge, p 289

Skowroński T (1984) Energy-dependent transport of cadmium by Stichococcus bacillaris. Chemosphere 13: 1379–1384
Skowroński T (1986a) Influence of some physico - chemical factors on cadmium uptake by the green alga Stichococcus bacillaris. Appl Microbiol Biotechnol 24: 423 - 425
Skowroński T (1986b) Adsorption of cadmium on green microalga Stichococcus bacillaris. Chemosphere 15: 69 -76
Skowroński T, Jakubowski M, Pawlik B (1985) Cadmium toxicity to green alga Stichococcus bacillaris. Acta Microbiol Polon 34: 309 - 312
Stratton GW, Corke CT (1979) The effect of mercuric, cadmium and nickel ion combinations on a blue -green alga. Chemosphere 10:731-740
Upitis VV, Pakalne DS, Nollendorf AF (1973) Dosage of microelements in nutrient medium as a factor for increasing resistance of Chlorella to unfavourable cultivation conditions. Mikrobiologia 42: 854 - 858
Wong PTS, Chau YK, Patel D (1983) The use of algal batch and continuous culture techniques in metal toxicity study. In: Nriagu JO (ed) Aquatic toxicology, J Wiley and Sons Inc, New York, p 449
Wong PTS, Mayfield CI, Chau YK (1980) Cadmium toxicity to phytoplankton and microorganisms. In: Nriagu JO (ed) Cadmium in the environment, part I, J Wiley and Sons Inc, New York, p 571

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