

# ALGAL PHYTOCHELATINS AS INDICATOR OF CADMIUM BIOAVAILABILITY IN AQUATIC ENVIRONMENT

Tadeusz Skowroński, Jacek Pirszel, Institute of Ecology P.A.S., Experimental Station, Niecala 18, 20-080 Lublin, Poland; e-mail: [skowron@golem.umcs.lublin.pl](mailto:skowron@golem.umcs.lublin.pl)

## ABSTRACT

Production of phytochelatins (PCs), heavy metal-binding peptides was studied in the green alga *Stichococcus bacillaris* upon exposure to cadmium in the presence of calcium, manganese and chloride ions. PCs were analyzed by reversed phase HPLC. The mentioned ions due to competition or complexation decreased the bioavailability of Cd<sup>2+</sup> in aquatic environment. It resulted in lower production of phytochelatins in algal cells.

## INTRODUCTION

Eukaryotic algal cells in response to heavy metal stress produce metal-binding peptides - phytochelatins (PCs) of general structure ( $\gamma$  Glu-Cys) Gly (n = 2-11) (Gekeler et al., 1988). PCs are considered to play an important role in metal homeostasis and metal detoxification (Zenk, 1996). In addition, it has been suggested that PCs may serve as a bioindicator of metal exposure (Robinson, 1990). Phytochelatins are synthesized by the enzyme phytochelatin synthase, which is activated by free heavy metal ions (Zenk, 1996). Cadmium is the most effective inducer of PCs. Therefore the synthesis of phytochelatins is dependent on heavy metal uptake. The studies on the effects of heavy metals on algae established, that the toxicity of metal is dependent on its free ion concentration rather than its total concentration. In aquatic systems, a number of environmental factors affect metal uptake and toxicity. The most essential factors are other cations and anions, which can influence the metal uptake through various types of interactions. For example, in algal cells Mn<sup>2+</sup> and Ca<sup>2+</sup> ions can compete with Cd<sup>2+</sup> ions for binding sites and intracellular transport system (Skowroński, 1986; Pawlik, Skowroński, 1994), while chloride ions, the mobile and persistent complexing agents of heavy metals, can change metal speciation (Skowroński, 1992). This paper presents investigations on the influence of some ecologically relevant anions and cations on the changes in phytochelatin production in the green microalga *Stichococcus bacillaris* exposed to cadmium.

## METHODOLOGY

*Stichococcus bacillaris* Näg. was cultivated in the modified Pratt's medium and harvested as described (Skowroński, 1984). If not otherwise specified, the alga cells from exponential phase of growth were suspended in 5 mM HEPES buffer (pH 6.8) containing cadmium at the concentration 0.9  $\mu$ M (100  $\mu$ g/l) and other bivalent cations (Ca<sup>2+</sup>, Mn<sup>2+</sup>) or Cl<sup>-</sup> ions, and incubated for 24 h in light (80  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) at 25°C.

After exposure to cadmium algae were filtered on Whatmann GF/C filter, rinsed with 2 mM EDTA and H<sub>2</sub>O and then homogenized in 5% sulfosalicylic acid + 6.3 mM DTPA. The homogenate was filtered (0.1  $\mu$ M) and analyzed by HPLC. Acid soluble thiols were separated on RP-18 column with 0 to 50% gradient of acetonitrile in 0.05% TFA. Sulfhydryl groups were

detected at 412 nm after postcolumn derivatization with 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman's reagent).

Cadmium uptake was studied using  $^{109}\text{Cd}$ . After exposure to cadmium, algal cells were separated by centrifugation, twice rinsed with 5 mM EDTA and then intracellular Cd concentration was measured.

## RESULTS AND DISCUSSION

*Stichococcus bacillaris* upon exposure to cadmium synthesized thiol containing compounds. In algal crude extract, HPLC analysis showed the occurrence of glutathione (GSH) and other thiol-containing peptides which have been identified as phytochelatins: PC<sup>2</sup>, PC<sup>3</sup> and PC<sup>4</sup>, whereas in algae not exposed to cadmium the GSH was stated only. At the studied cadmium concentration (0.9  $\mu\text{M}$ ) the main component of PCs was dimer (PC<sup>2</sup>). Phytochelatin content and cadmium concentration in *Stichococcus bacillaris* cells increased with time parallelly, during 24 h exposure. It has been stated, that  $\text{Mn}^{2+}$  and  $\text{Ca}^{2+}$  ions compete with  $\text{Cd}^{2+}$  ions for transport system and inhibit intracellular cadmium uptake (Skowroński, 1986; Pawlik, Skowroński, 1994). Therefore manganese in *S. bacillaris* can reduce cadmium toxicity. As can be seen in Fig 1, calcium and manganese significantly decreased the amount of intracellular cadmium as well as the PC level. Fig 2 presents HPLC chromatograms of *S. bacillaris* extracts after 24 h incubation of algal cells in solution containing cadmium or cadmium and manganese. The surplus of manganese caused a significant decrease of PC level; other oligomers of phytochelatins has not been synthesized. In general, it is assumed that free metal ions are toxic to phytoplankton and that compounds able to reduce the free ion activity can also reduce metal toxicity (Laegreid et al., 1983). Chemical metal speciation depends on many physico-chemical factors; among others on the presence of inorganic ligands. The important complexing factors of cadmium in the sea and in fresh-water, particularly contaminated with mining waters and sewages of high salinity are chloride ions. As can be seen in Fig 1. in the solution containing chloride, where chloride complexes such as  $\text{CdCl}^+$ ,  $\text{CdCl}_2$ ,  $\text{CdCl}_3^-$  exist, the bioavailability of cadmium was markedly limited. Simultaneously, decrease of PCs<sup>3</sup> production was observed. The obtained results suggest, that the level of phytochelatins in algal cells is connected with bioavailability of cadmium in aquatic environment.

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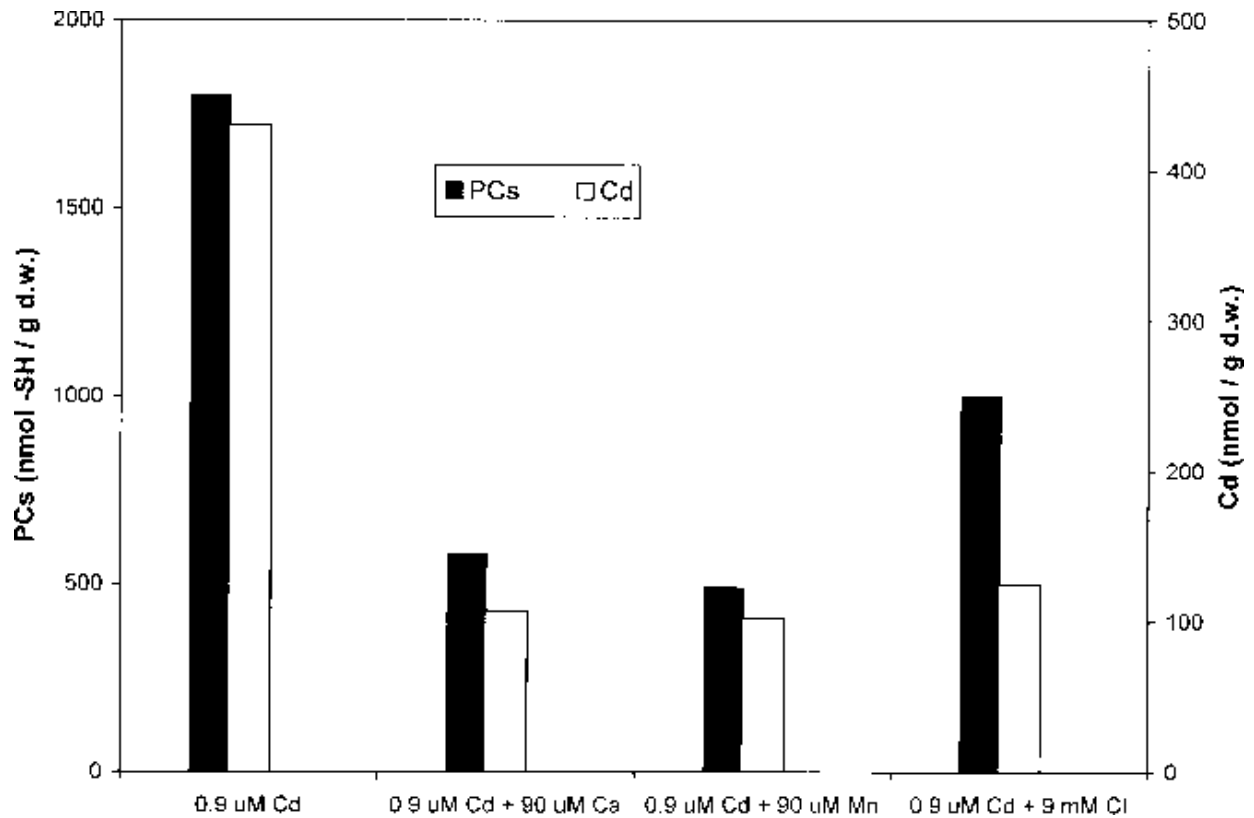


Fig. 1 Phytochelatin and cadmium concentrations in *S. bacillaris* cells exposed to cadmium or cadmium together with other ions.

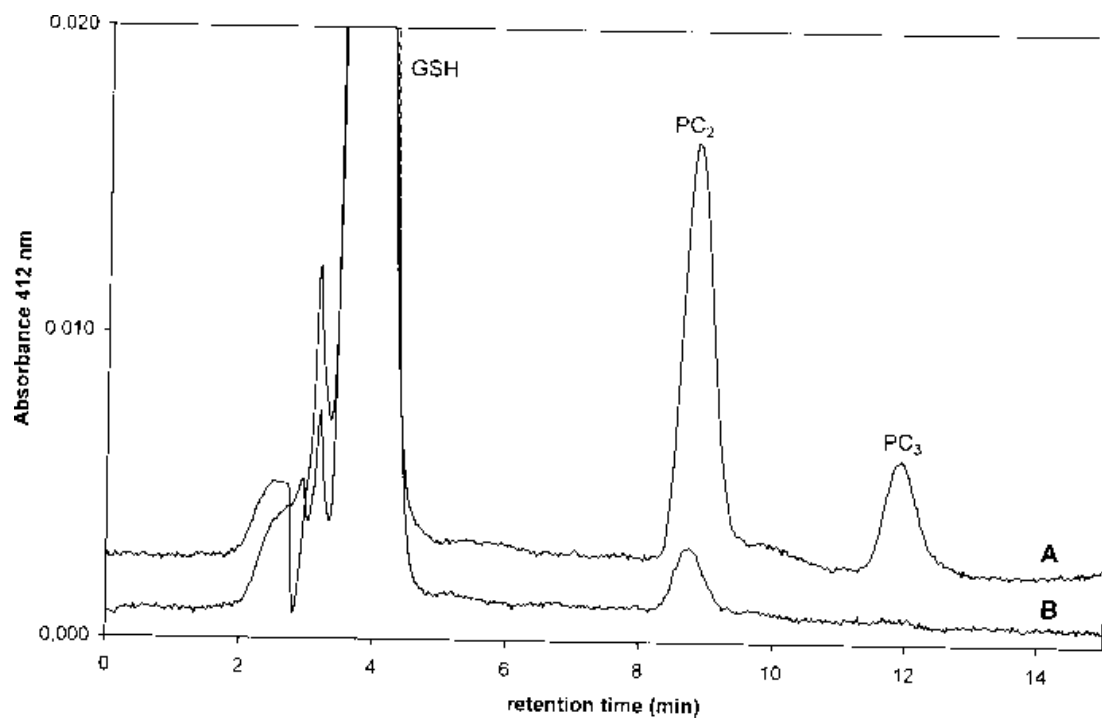


Fig. 2 Chromatograms of extracts from *S. bacillaris* cells exposed to 0.9 μM Cd (A) and 0.9 μM Cd + 90 μM Mn (B)