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# THE EFFECT OF HEAVY METAL IONS ($Pb^{2+}$ and $Cu^{2+}$) ON THE CHLOROPHYLL FLUORESCENCE IN VIVO*

The effect of $Cu^{2+}$ and $Pb^{2+}$ on Chlorella pyrenoidosa cells was studied. It was found that $Cu^{2+}$ ions are probably actively transported into the cells opposite to $Pb^{2+}$ ions and that $Cu^{2+}$ ions disorganize the energy coupling between PSII and PSI while $Pb^{2+}$ ions do not affect the excitation energy transfer between PSII and PSI.

Heavy metals have been defined as those with specific gravity greater than 4 or 5, located from atomic numbers 22 to 34 and 40 to 52 on the periodic table, and having a specific biological response [1].

The interaction of heavy metal ions with microorganisms is of great concern. Some microalgae can cummulate selectively large amounts of heavy metals and are seriously considered as means for the recovery of useful metals from nature [2]. The application of sewage from waste treatment plants, as manure, on agricultural land is most often limited by heavy metal concentration [3].

It is clear from the above that depending on the problem the biocummualtion of heavy metals may be considered as an advantageous or disadvantageous process. Simply, it would be nice to have a way by which one could regulate the process of metal cum-

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mulation by microorganisms. To reach this goal still many of experiments have to be done.

In this paper the interaction between some common heavy metal ions ($Cu^{2+}$ and $Pb^{2+}$) and viable and heat-killed Chlorella pyrenoidosa cells have been investigated. Especial focus has been made on the following problems:

i) the effect of $Cu^{2+}$ and $Pb^{2+}$ ions on the chlorophyll fluorescence in vivo;

ii) the adsorption mechanisms;

iii) the time dependence of the cummulation of $Cu^{2+}$ and $Pb^{2+}$ ions.

## The effect of $Cu^{2+}$ and $Pb^{2+}$ on the chlorophyll fluorescence in vivo

Heavy metal ions: $Cu^{2+}$ and $Pb^{2+}$, differ remarcably in their effects on the chlorophyll (chl) fluorescence in vivo - see Fig. 1. Fluorescence spectrum of Chlorella cells, with $Pb^{2+}$ added, reveals two maxima: at 695 and 735 nm; whereas cells with $Cu^{2+}$

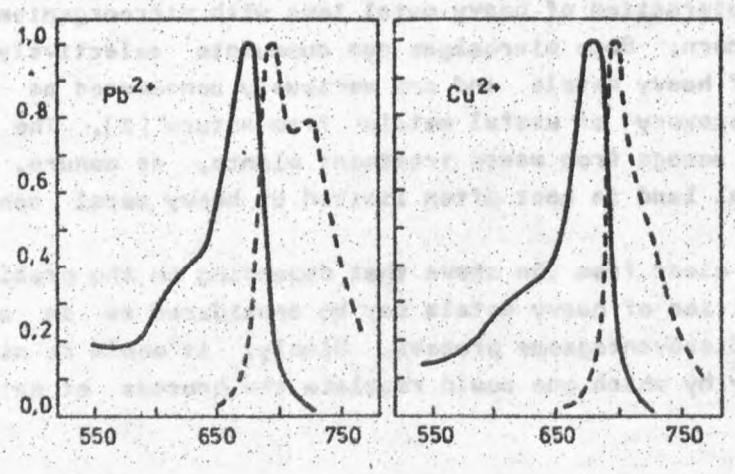


Fig. 1. Absorption (——) and fluorescence (---) (arbitrary units) spectra of Chlorella suspensions with heavy metal ions ($\sim 10^{-4}$ M) added

Widma absorpcji (——) i fluorescencji (---) (jednostki względne) zawiesiny Chlorella po dodaniu jonów metalu ciężkiego ($10^{-4}$ mol/l)

added have the spectrum with one pronounced maximum (at 695 nm) and a shoulder (at 735 nm).

It is known from the literature that, in vivo, chl fluoresces, depending on its location, (Fig. 2) at:

i) 686 nm (chl from antenna);

ii) 695 nm (chl from PSII);

iii) 715-735 nm (chl from PSI).

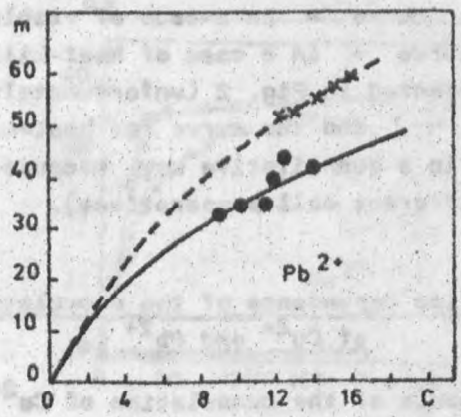


Fig. 2. $Pb^{2+}$ absorption isoterms for viable (xxx) and heat-killed (...) Chlorella suspensions. Dashed and continuous curves are plots of Langmuir's ($m = \frac{\alpha C}{I + \beta C}$) and Freundlich's ($m = k \cdot C^n$) relations, appropriately. The plotted curves correspond to the measured values of $Pb^{2+}$ cummulation by cells

Izoterma absorpcji $Pb^{2+}$ dla żywych (xxx) i martwych po ogrzaniu (...) zawiesin Chlorella. Przerywane i ciągłe krzywe wytyczone odpowiednio wg równań Langmuira ($m = \frac{\alpha C}{I + \beta C}$) i Freundlicha ($m = k \cdot C^n$). Przedstawione krzywe odpowiadają wartościom nagromadzania jonów Pb przez komórki

With these data in mind, the disappearance of the maximum at 735 nm, in a case of $Cu^{2+}$ presence, may be interpreted as an effect of some damage to the "energetic link" between PSII and PSI. In a result the excitation energy transfer from PSII to PSI becomes less probable and the maximum that characterizes fluorescence of chl from PSI less pronounced.

Both, $Cu^{2+}$ and $Pb^{2+}$ ions, seem to have no effect on the excitation energy tunneling from the antenna chlorophyll to PSII or PSI.

## $Cu^{2+}$ and $Pb^{2+}$ adsorption mechanisms

Two kinds, Langmuir's and Freundlich's adsorption isoterms have been examined in a case of $Pb^{2+}$ ions and viable and heat-killed cells of Chlorella pyrenoidosa. For this purpose both, Langmuir's and Freundlich's relations have been transformed from their original forms to the linearized formulae.

It has been found that the experimental points fitted quite well the Langmuir's curve - in a case of viable cells, and the Freundlich's curve - in a case of heat-killed cells only. The results are presented in Fig. 2 (unfortunately, the curve for viable cells (---) and the curve for heat-killed cells (——) cannot be compared in a quantitative way because they have been obtained for two different cell preparations).

## The time dependence of the cummulation of $Cu^{2+}$ and $Pb^{2+}$

The time dependence of the cummulation of $Cu^{2+}$ and $Pb^{2+}$ ions by viable and heat-killed cells of Chlorella pyrenoidosa is presented in Fig. 3.

In a case of heat-killed cells the character of curves is similar for both kinds of the investigated metal ions. An another interesting feature that is worth of notice is that there is no essential difference in cummulation of Pb by viable and heat-killed cells.

Quite different situation is seen in a case of $Cu^{2+}$ ions. Heat-killed cells absorb $Cu^{2+}$ ions immediately (what resembles the case with $Pb^{2+}$); whereas in a case of viable cells some time (of the order of 2 hrs) is needed for the reaching the saturation in the cummulation curve.

The presented results are very similar to those observed by R e b o t n o v a and P o z m o g o v a [4] with $Cu^{2+}$ and $Ag^{2+}$ ions and suspension of Candida utilis.

On the basis of these data it seems that some metal ions are absorbed immediately, and the adsorption does not depend on the physico-chemical conditions (temperature, viability, and so on) - one can put $Pb^{2+}$ and $Ag^{2+}$ ions to this group; whereas other

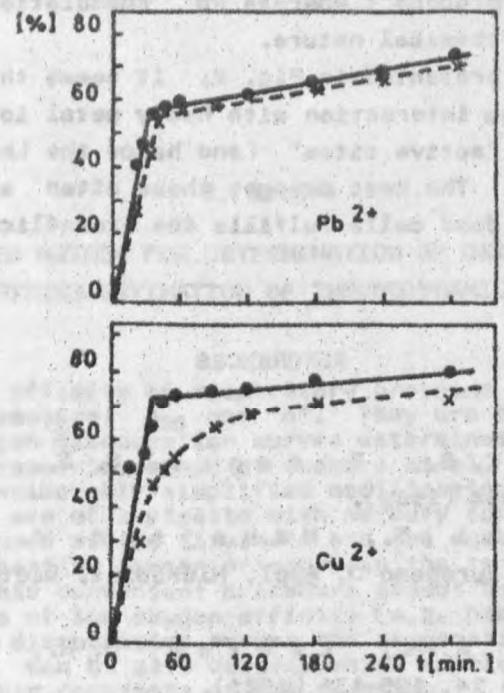


**Fig. 3. Time dependences of the cummulation of the heavy metal ions by viable (---) and heat-killed (——) cells of Chlorella pyrenoidosa**

**Zależność nagromadzenia jonów metali ciężkich od czasu przez żywe (- - -) i martwe (——) komórki Chlorella pyrenoidosa**

metals, here $Cu^{2+}$ may serve as an example, get into cells via the "active transport process" (which surely depends on the environmental conditions and, of course, on the viability).

## Conclusions

On the ground of the obtained results it can be said that the interaction of $Cu^{2+}$ and $Pb^{2+}$ ions with Chlorella pyrenoidosa cells differ in several respects:

- $Cu^{2+}$ ions damage the "energetic link" between PSII and PSI, whereas $Pb^{2+}$ ions seem to have no effect on the excitation energy transfer from PSII to PSI,
- $Cu^{2+}$ ions are most probably provided to cells by the cell's

"active transport process"; whereas $Pb^{2+}$ cummulation seem to be of "pure" physico-chemical nature.

From the data presented in Fig. 2, it seems that in a case of viable cells the interaction with heavy metal ions is mostly via, so called, "active sites" (and hence the Langmuir's relation must hold). The heat damages these sites and the adsorption process for dead cells fulfills the Freundlich's mechanism.

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## WPŁYW JONÓW METALI CIĘŻKICH ($Pb^{2+}$ I $Cu^{2+}$) NA FLUORESCENCJĘ CHLOROFILU IN VIVO

Badano wpływ $Cu^{2+}$ i $Pb^{2+}$ na komórki Chlorella pyrenoidosa. Stwierdzono, że jony $Cu^{2+}$ są prawdopodobnie transportowane aktywnie do wnętrza komórek w przeciwieństwie do jonów $Pb^{2+}$ i że jony $Cu^{2+}$ dezorganizują sprzężenie energetyczne pomiędzy PSII i PSI, podczas gdy jony $Pb^{2+}$ nie mają wpływu na przenoszenie energii pobudzenia pomiędzy PSII i PSI.