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EFFECT OF Cd^{2+} ON THE METABOLISM OF THE MARINE UNICELLULAR ALGAE

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ABSTRACT:

Some metabolic modifications of Chaetoceros simplex v. calcitrans Pauls., Cyclotella caspia Grun., Skeletonema costatum Grev., Chlamydomonas sp. and Platymonas impellucida McLaughlan & Parke, which occured under the influence of Cd^{2+} are analysed. The specificity of Cd^{2+} on different algae has been demonstrated and the mechanism of their protection against Cd^{2+} effect has been explained.

After WOOD's classification (8) regarding toxicity degree of heavy metals, Cd^{2+} is included in the category of highly toxic elements, which are largely available, to the living organisms.

The presence of Cd^{2+} in the sea water, especially in the coastal zone, is due to the inflow of domestic and industrial waste waters.

Cadmium concentrations varying between tiny traces and 2.3 µg/l have been detected in the coastal zone of the Black Sea waters.

Owing to the large capacity of the marine organisms for accumulation of chemical elements, heavy metals have been detected in vegetal and animal organisms at values generally some tens

times higher than in the water environment all over the Romanian coastal zone investigated.

In the particular case of Cd^{2+}, determinations on tissues of Mytilus galloprovincialis L. (6) demonstrated that this extremely toxic metal was accumulated up to mean values ranging between 1.9 and 5.5 µg/g dry substance.

We have intended here to study the Cd^{2+} action - under conditions of acute contamination on the marine unicellular algae, taking into account several considerations; (1) algae are the most active organisms as regard the accumulation factor; (2) they constitute the basic element in the nutrition of many animal organisms which are part of the human alimentary consume; (3) having a good knowledge of the exact limits of heavy metal accumulation of its effects as well especially in fish organisms, quality standards can be established for the marine environment; (4) as their physiology is known after years of numerous experiments, these organisms constitute a basis for detecting the inner mechanisms for the action of these pollution agents.

MATERIAL AND METHODS

The unicellular algae Chaetoceros simplex var. calcitrans, Cyclotella caspia, Skeletonema costatum, Chlamydomonas sp. and Platymonas impellucida, isolated from the coastal zone of the Romanian sea water, were used for experimentation.

The tested concentrations were 0.04, 0.1, 0.5, 1 and 2 µg/l Cd^{2+} as $CdCl_2$, so ranging within the limits detected in the natural medium. The tests were conducted as compared to a control variant, represented by the algal species considered, the development of which was followed in the MS medium.

Cultures were in the log phase, being developed in the MS medium, the trophic particularities of which were known from previous observations.

The following determinations were performed on the algae:

1.- divizion rate $K = \frac{Nt - No}{T_{t1} - T_{to}}$, in order to express cellular divizion increment per time unit;

2.- fresh weight, dry weight, moisture and ashes;

3.- Cd^{2+} accumulation;

4.- accumulation of other divalent ions present in the nutrient solution at detectable levels in the utilised determination regime.

Dosage of heavy metals was done by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

1. CELLULAR DIVISION RATE

A stimulating effect on the cellular division process was noticed, with a mention that there is much specificity of the algae to Cd^{2+} concentrations inducing this process.

Chaetoceros simplex v.calcitrans is stimulated at concentrations between 0.04 - 0.1 µg/l (Fig.1).

Cyclotella caspia is subject to stimulation of cellular division at 0.04 - 1 µg Cd^{2+}/l values (Fig.2).

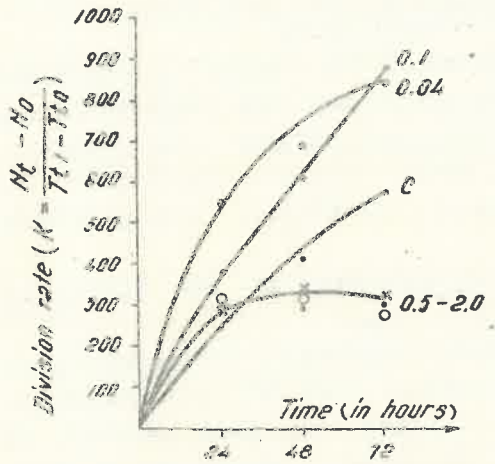

Fig.1 - Cellular division rate of the alga Chaetoceros simplex v. calcitrans under conditions of acute Cd^{2+} contamination

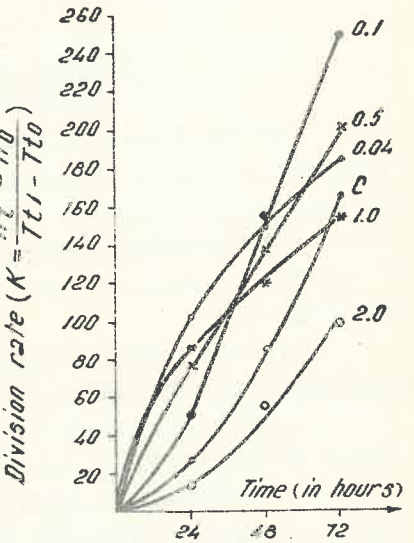

Fig.2 - Cellular division rate of the alga Cyclotella caspia under conditions of acute Cd^{2+} contamination

In Skeletonema costatum, only the concentration of

1 µg/l causes a slight division stimulation and only for the first 24 hours of Cd^{2+} contamination (Fig. 3); concentrations exceeding this value have an inhibitory effect even in the first hours of contamination. The behaviour observed at the concentration of 0.1 µg/l shows that as the Cd^{2+} effect grows chronic, extremely low concentrations may become toxic.

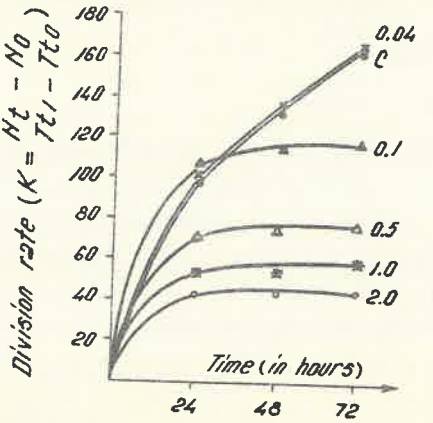

Fig. 3 - Cellular division rate of the alga Skeletonema costatum under conditions of acute Cd^{2+} concentration

Of the three diatoms on which the results have been reported, the strongest stimulating effect has been obtained on the species Cyclotella caspia and Chaetoceros simplex v.calcitrans.

Skeletonema costatum shows higher sensibility to Cd^{2+} concentrations, as well as to chronicized action of this heavy metal.

In the green algae Chlamydomonas sp. and Platymonas impellucida, the following have been found:

- Cd^{2+} contamination causes destruction during the first 24 hours of action, which is proportional with Cd^{2+} concentration;
- a certain adaptive capacity has been noticed, as expressed by the possibility for the algae to resume the cellular division process after 48 hours from the beginning of the contamination;
- after adaption to the Cd^{2+} presence, this agent may induce metabolism stimulation, followed by an increase in the division rate;
- the stimulation effect in the green algae is also

characterized by high specificity; Chlamydomonas sp.is stimulated at all tested concentrations, there being a positive correlation between Cd^{2+} concentration and cellular division increases (Fig.4).

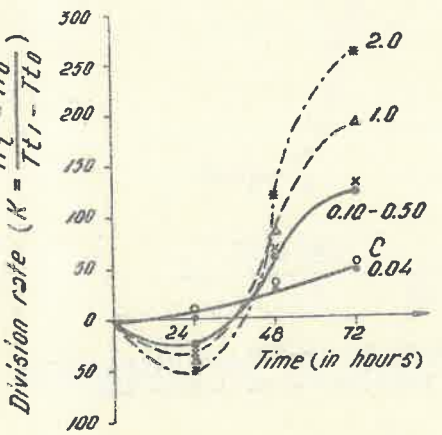

Fig.4 - Cellular division rate of the alga Chlamydomonas sp. under conditions of acute Cd^{2+} contamination

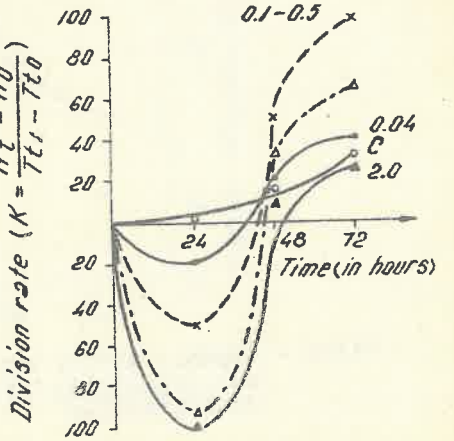

Fig.5 - Cellular division rate of the alga Platymonas impellucida under conditions of acute Cd^{2+} contamination

In the alga Platymonas impellucida, the effect of cellular division stimulation was noticed only at concentrations between 0.04 - 0.5 µg/l; at higher values, Cd^{2+} concentration inhibits cellular division (Fig.5).

2. ORGANIC MATTER ASSIMILATION

One of the mechanisms for protection of the unicellular algae against the toxic action of the heavy metals is the intracellular accumulation of synthetic products. STEEMAN-NIELSEN and WIUM-ANDERSEN (5) noticed an increase of the intracellular products with copper concentration increase in the alga Chlorella sp.

A rise in the activity of organic synthesis was rendered evident only in the diatom algae within the frame of this study. The most significant values as compared to the control were obtained in the species Chaetoceros simplex v.calcitrans and Cyclotella nana (Fig.6)

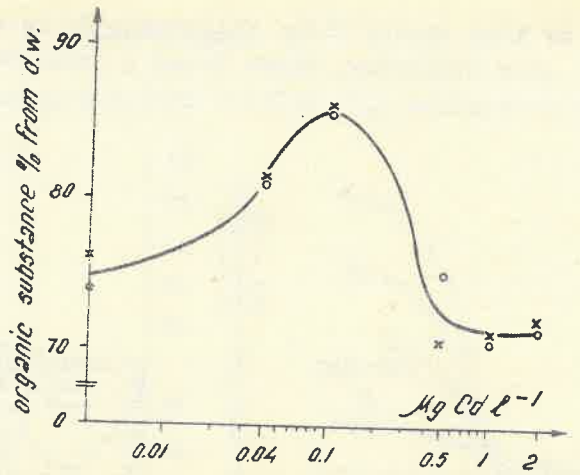

Fig.6 - Organic matter synthesis in the algae Chaetoceros and Cyclotella under conditions of acute Cd^{2+} contamination

3. MODIFICATION OF THE MINERAL METABOLISM

The determination of the mineral content of the algae subjected to the acute action of Cd^{2+} pointed out the following (Fig.7):

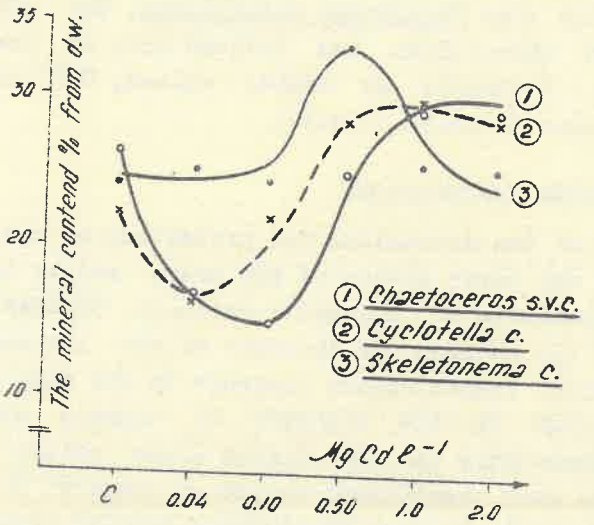

Fig.7 - Variation of mineral content as a function of Cd^{2+} contamination

-a decrease in the mineral content of the algae at Cd^{2+} concentrations at which stimulations of organic synthesis occured;

- in the diatoms Chaetocerog, Cyclotella and Skeletonema, the process occurs at concentrations between 0.04 and 0.1 μg Cd^{2+}/l;

- exacerbation of the mineral metabolism was observed when the concentration of the Cd^{2+} beyond the values which stimulate organic synthesis;

- yet the activation of the mineral metabolism does not occur but within certain limits of Cd^{2+} concentration, beyond which an attenuation of the process is observed, taking values closer to those of the control.

These manifestations of the mineral metabolism as superposed on the aspects of organic synthesis and division rate show that the protection factors are different for different Cd^{2+} concentrations.

Concentrations between 0.04 - 0.1 μg Cd^{2+}/l trigger organic synthesis functions which seem to have a role in fixing Cd^{2+} and making it harmless to the development of the cellular metabolism.

Of the category of organic products with a protection role, the synthesis of metalothioneins has been recently proved in several blue-green algae (3).

Studying the effect of Zn^{2+} on the alga Skeletonema costatum (unpublished data), the role of glycine in the disintoxication mechanism was pointed out. The activation of the glycine synthesis induced a correlative increase in the quantities of aspartic acid and serine. Accordingly, in parallels with the synthesis of the protection factors, synthesis of other organic compounds is stimulated, which is followed by increase in the division potentiality.

An increase in Cd^{2+} concentration has correspondence in a change in the protection mechanism; the synthesis of organic products decreases and stimulation of the mineral metabolism is noticed.

Accordingly, in order to withstand the toxic effect, a protection mechanism based on ionic exchange is started.

4. HEAVY METAL ACCUMULATION FROM THE MEDIUM

In the present study we have followed the accumulation of Cd^{2+} as a toxic factor, as well as the accumulation of the divalent ions Cu^{2+} and Zn^{2+}, the presence of which in the medium is in close connection with the chemical structure of the culturing solution.

In the MS culture medium, Zn^{2+} reached a concentration of 254.4 μg/l and Cu^{2+}, 381.23 μg/l.

In order to study the absorptive capacity of the algae for the above mentioned divalent ions, the algae were calcinated at 480°C. Passing into solution was made with Merck HCl by 2 ml in 2 stages. After this treatment, the material was heat-mineralized. In a first stage, mineralization went up to the moment of total evaporation of the acid; in the second stage, the acid evaporated nearly 80%, then the material was cooled.

Passing into solution was made with redistilled water up to a volume of 25 ml.

The samples prepared in this way were read at an atomic absorption spectrophotometer Pye Unicam Type SP 2900 S.C.

The results of the readings pointed out the following:

- Under conditions of acute contamination, the Cd^{2+} quantities accumulated in the algae after 72 hours of experimentation are below the detection limit of the spectrophotometer utilized for the analyses.

- Presence of Cd^{2+} had no significant effect on Zn^{2+} accumulation.

- Spectrophotometric determinations showed plainly a Cu^{2+} accumulation and indicated it as dependent on the Cd^{2+} presence and concentration.

- Only the Cd^{2+} concentrations between 0.1 and 0.5 determine significant increase of Cu^{2+} accumulation as compared to the control values.

- Absorption and intracellular storage of Cu^{2+} demonstrates a sharp specificity (Figs. 8-9): Chaetoceros, Cyclotella and Chlamydomonas sp. absorb the ion in excess, reaching a concentration factor of 552 - 1252 μg/g dry substance. As compared to the control, the process is higher by 63.76 - 76.52%.

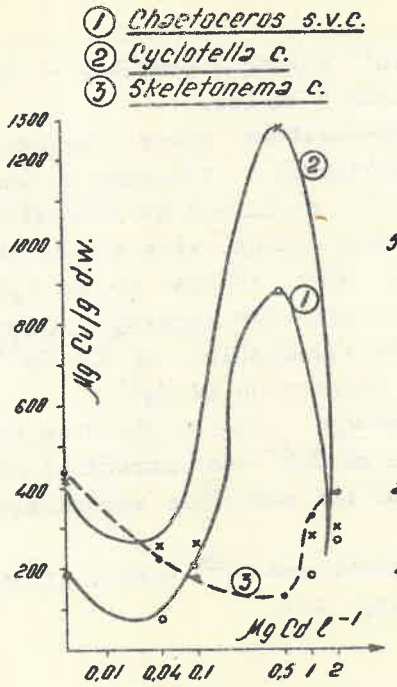

Fig.8 - Cu^{2+} accumulation under conditions of acute Cd^{2+} contamination

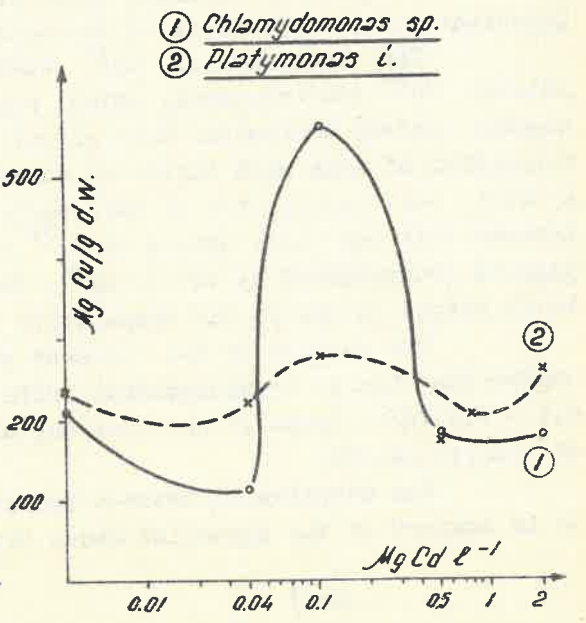

Fig.9 - Cu^{2+} accumulation under conditions of acute Cd^{2+} contamination.

In Platymonas impellucida the accumulation exceeds the control value by only 27.53%.

In Skeletonema costatum, this Cu^{2+} accumulation does not occur, but within the limits of the control.

The results of the study on the accumulation of divalent ions lead to the supposition that, on the basis of the mineral metabolism, another way of protection against the toxic agents is opend.

Taking into account both Cu^{2+} absorption and organic matter synthesis (Figs.6,8,9), the following are to be observed.

For concentrations of 0.04 - 0.1 µg Cd^{2+}/l, at which organic synthesis stimulation takes place, the quantity of Cu^{2+} decreases below that which is dosed in the control. The decrease is explained by activation of synthetic metabolism. It is known that there is correspondence between the high metabolism and the active influx of nutrient elements, among which N and P are the

most important.

An eflux of divalent ions, Cu^{2+} having - probably - an important role, corresponds to the strong P influx.

The increase of Cd^{2+} concentration above 0.1 µg/l induced Cu^{2+} concentration, which superposes a decrease in the organic matter synthesis. This effect is explained by selective absorption of ions with identical electric charge, with a role in braking the penetration of the toxic ions. AGRAWAL et al. (1) noticed that the toxic effect of Co^{2+} in the alga Anacystis nidulans, is counteracted by 40% by Mg^{2+}. The elimination of the Co^{2+} toxic effect is due to the competitive absorption of Mg^{2+}.

The results of the present study demonstrate that the copper ion has a compensational role at Cd^{2+} concentrations of 0.1 - 0.5 µg/l, hence it provides one of the antitoxic mechanisms of mineral nature.

The correlation between hydrature and Cu^{2+} accumulation is in support of the assertion above (Fig. 10).

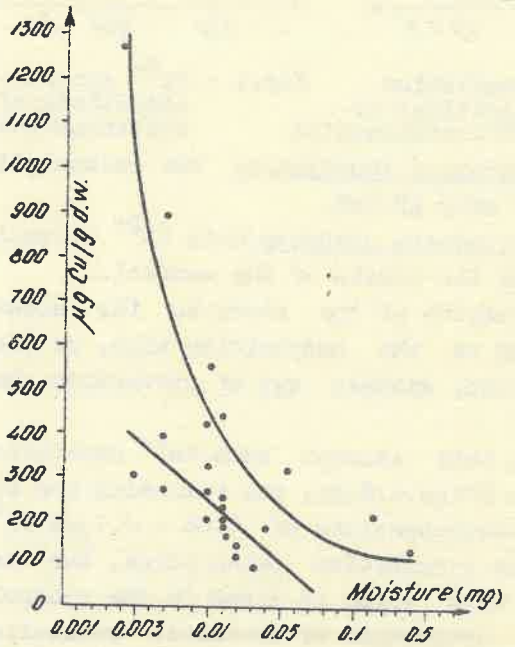

Fig.10 - Correlation between moisture and Cu^{2+} accumulation

The diminution of Cu^{2+} accumulation capacity when Cd^{2+}

exceeds 0.5 µg/l in the species Chaetoceros simplex v.calcitrans, Cyclotella caspia and Chlamydomonas sp.or when there is no super-stimulation of its absorption as compared to the level of the control variant for the algae Skeletonema costatum and Platymonas impellucida, suggests that, beside the above mentioned protection mechanisms, the algae may develop some other such mechanisms.

The cessation of division (ex. Skeletonema costatum at Cd^{2+} concentration of 0.5 - 2 µg/l), the destruction of a certain fraction of a population and the recovery of the population from the cells which have resisted (Chlamydomonas sp. and Platymonas impellucida) prove that the cells lose only temporarilly their division potential; after having been subject to numerous perturbations, they regain their initial structure and recover their integrity and energetic potential.

Latency or destruction of cells can be explained on the basis of the observations of TINGLE et al. (7), who showed that sublethal Hg^{2+} concentrations disorganize the chondriom in the ciliates. Yet this disorganization is temporary,as after 24 hours the cells recover their normal state.

After OLAFSON and THOMPSON (4),for the vertebrates, and McLEAN et al. (2),for the blue-green alga Anacystis nidulans,this adaptation period reflects the disintoxication process induced by a protein susceptible of complexation of the metal ions.

Subsequent to the adaptation, the toxic agent may become stimulative.

Cadmium, in the present case, under conditions of acute contamination, acts upon some algae as a stimulant or as an inhibator from the first hours of contamination. Its not being discernible after 72 hours of action demonstrates that it does not accumulate into cells,or it does in an infinitesimal quantity, during this interval. Stimulation of the metabolic processes occurs indirectly,by triggering the protection mechanisms:a) synthesis of organic products, or b) making the membrane penetrable by other ions, which activate the metabolism or have an intrinsic role in the synthetic processes.

CONCLUSIONS

1.- Under conditions of acute contamination with Cd^{2+}, at concentrations between 0.04 - 2 µg/l (values identified in the natural medium), this heavy metal stimulates neoformation processes in most of the unicellular algae.

2.- The Cd^{2+} concentrations stimulating the division rate in the algae are strictly specific.

3.- The stimulating effect of Cd^{2+} is indirect, by triggering the protection mechanisms.

4.- The protection mechanisms of the algae against the toxic effect of Cd^{2+} consist of either synthesis of some organic compounds, or selective absorption of other ions with identical electric charge.

5.- The antitoxic protection of the unicellular algae, based on the mineral metabolism, is explained by absorbing of some ions with a role in maintaining a membrane equilibrium, which stop the penetration of Cd^{2+} into the cells.

6.- It was demonstrated that, of the ions with a protection function, Cu^{2+} has a most important role.

7.- Under conditions of acute contamination, Cd^{2+} enters the cells in infinitesimal quantities or has not the time necessary to penetrate into the cells.

8.- The spectrophotometrical determinations after 72 hours of contamination demonstrate the absence of Cd^{2+} in the algae.

9.- In the natural medium, Cd^{2+} concentration varies between infinitesimal values expressed as traces and less than 1 µg/l along the 5 m isobath, the values higher than 1 µg/l being found only in front of the waste water drains.

10.- The concentration of Cd^{2+} in the natural medium, beside other factors, explains - at least for the algae we have studied - the actual level of the phytoplanktonic biomasses.

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