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Evaluation of toxic effects of heavy metals on unicellular algae

II - Growth curves with different concentrations of heavy metals **

A correct evaluation of the toxic effects of heavy metals on unicellular algae must necessarily be conducted as far as possible under standard conditions. The importance of inoculum concentration for such an evaluation has already been demonstrated in our work (ALBERTANO, PINTO, TADDEI, 1979).

The various evaluation criteria which have been advanced up to the present are based on the inhibition exerted by a heavy metal, the effects of which manifest themselves on algal growth in different ways (BLANKLEY, 1973).

Abundant data are available on this subject in published literature: the effect of chrome on *Nitschia palea* can be seen in a diminution of the growth rate of the latter, the inhibitory action of chrome becoming greater in proportion to an increase in the concentration of the element, while for each concentration the growth-rate remains constant throughout the duration of the experiment (WIUM-ANDERSEN, 1974); the effect of zinc on three species of diatoms is analogous to that already cited, with the additional feature of a progressive slowing-down of the growth rate during the course of the experiment (JENSEN et al., 1974); in other cases, finally, the toxicity of the element reveals itself

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** This study was carried out with support of the Ministero della Pubblica Istruzione.

solely by a prolongation of the lag-phase which is the more pronounced the greater is the concentration of the element (SHRIFT, 1954; BEN-BASSAT, MAYER, 1975).

The scope of the present article is the analysis of various growth-curves of unicellular algae subjected to diverse concentrations of various heavy metals.

MATERIALS AND METHODS

We made use of the alga *Cyanidium caldarium*, strain 001 from our collection and the alga *Chlorella saccharophila*, strain 211.9a Gö from the Cambridge collection.

The enrichment cultures and the experimental tests were conducted in liquid media identical to those employed in our earlier work * (ALBERTANO, PINTO, TADDEI, 1979); the experimental tests, conducted in 14 mm test-tubes, were carried out under conditions identical to those indicated in the above-cited work.

Tests were carried out in the presence of 12 heavy metals in diverse concentrations, at intervals of 0.3 units of log (that is, in a 1/2 relation between two successive concentrations); the metals employed were:

beryllium	as $Be(NO_3)_2 \cdot 4H_2O$	mercury	as $HgCl_2$
cadmium	as $CdCl_2 \cdot H_2O$	molybdenum	as $Na_2MoO_4 \cdot 2H_2O$
chromium **	as $K_2Cr_2O_7$	nickel	as $NiSO_4 \cdot 6H_2O$
cobalt	as $CoCl_2 \cdot 6H_2O$	thallium **	as Tl_2SO_4
copper	as $CuCl_2 \cdot 2H_2O$	tungsten	as $Na_2WO_4 \cdot 2H_2O$
manganese	as $MnCl_2 \cdot 4H_2O$	zinc	as $ZnCl_2$

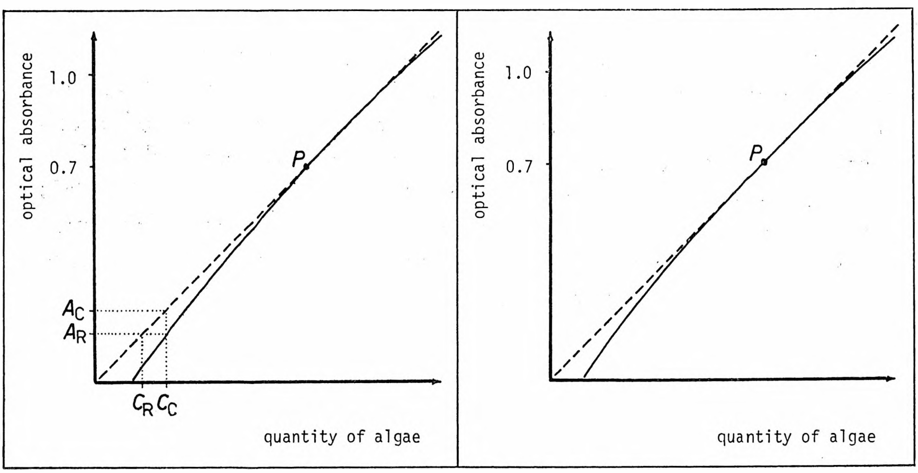
* All the reagents employed in this work were chemically pure and produced by Riedel-De Haën.

** In the case of chromium and thallium the molarity indicated in figs. 2 and 3 refers to the chemical element and not to the salt.

The enrichment cultures were centrifuged and resuspended in a renewed culture medium five days before commencement of the tests: in this way, the inoculum was always effected with algae which were in a fully exponential phase.

The algal growth was followed by means of readings taken from a Bausch & Lomb Spectronic 20 Colorimeter at a wavelength of 550 nm.

Since our investigation turned on growth-curves, we felt the need to verify the reliability of the colorimeter-readings where non-homogeneous samples were concerned like those of algal suspensions. To this end we effected a series of dilutions of algae and carried out the corresponding readings. The results of this preliminary investigation are set out, for the two algae, in fig. 1, a and b respectively.



a *b*

Fig. 1 — Theoretical line (— — —) and experimental curve (———) of correspondance between absorbance and quantity of algae. *a*) relative to *Cyanidium caldarium*; *b*) relative to *Chlorella saccharophila.*

P = arbitrary point of coincidence between the theoretical line and the experimental curve. For any concentration of algae C_C: A_R = colorimeter-reading; C_R = corresponding concentration of algae if there were linearity; C_C = effective concentration of algae; A_C = corrected value of absorbance.

Since the colorimeter-readings were, as we can observe, lacking in linearity, we made the necessary corrections to our readings on the basis of fig. 1; for example, the colorimeter-reading A_R, which would normally correspond to the algal concentration C_R if there were linearity, corresponds in fact to an algal concentration C_C; for this reason the reading A_R will have to be corrected by the value A_C which represents the theoretical absorbance corresponding to the true algal concentration C_C. For both graphs the point P (common to the theoretical line and the experimental curve) was chosen in correspondance to an absorbance of 0.7.

The initial inoculum of algae was such as to allow 0.12 - 0.13 units of absorbance (corrected values) to be registered. The readings were carried out every 2-3 days after addition of H_2O up to the initial level; the experiment lasted 20 days, or rather was interrupted as and when the absorbance overstepped the value of 1.0 units (corrected values).

All the operations relating to the enrichment cultures were carried out under sterile conditions. The experimental tests, on the other hand, were carried out under conditions of semi-sterility; the development of fungi and bacteria proved in fact to be non-existant or absolutely negligible in all the tests. This procedure, quite apart from accelerating the operations, hindered eventual alterations in the substances employed in the course of the sterilisation operations.

Results

The results of the tests are given in fig. 2 for *C. caldarium* and in fig. 3 for *C. saccharophila*. Each graph presents the growth-curves relative to the different concentrations of each of the 12 metals employed.

In the case of certain metals, namely cobalt, copper and nickel, we were obliged to use (for *C. caldarium*) concentrations increased to such an extent that their colouration interfered seriously with the readings: for these elements we have made appropriate corrections throughout the experiment.

In the case of molybdenum, which in lethal/sub-lethal concentrations causes (with regard to *C. caldarium*) a particular effect of alteration in the phycocyanin, in turn causing considerable interference, we substituted, where necessary, the colorimeter-readings by microscopic observation and a count of the number of cells.

Each experiment was repeated 3 times: the growth-curves given in figs. 2 and 3 were drawn on the basis of the mean values attained in the three experiments; the maximum departure from the mean proved throughout inferior to 2.7%.

Discussion and conclusions

Our results demonstrate that inhibition of the growth of algae, due to various concentrations of heavy metal, can manifest itself according to three fundamental types:

I) As a result of an increase in the concentration of the element, the growth-rate of the algae slackens. In the course of the experiment a progressive slowing-down of the growth-rate intervenes, which is the more precocious and violent the greater the concentration of the element. Falling in this type are, for example, the results in figs. 2 b, d, i; 3 i.

II) As a result of an increase in the concentration of the element, the growth-rate of the algae slackens. The growth-rate remains constant throughout the experiment. Belonging to this type are, for example, the results in figs. 2 a; 3 c, d, f, j, l.

III) The toxicity of the element reveals itself solely in a prolongation of the lag-phase, which is the more pronounced, the greater the concentration of the element. From the moment the algae enter the exponential phase of growth, the growth-rate remains constant in time and independent of the concentration of the element. Belonging to this type are, for example, the results in figs. 2 g, k; 3 e, g.

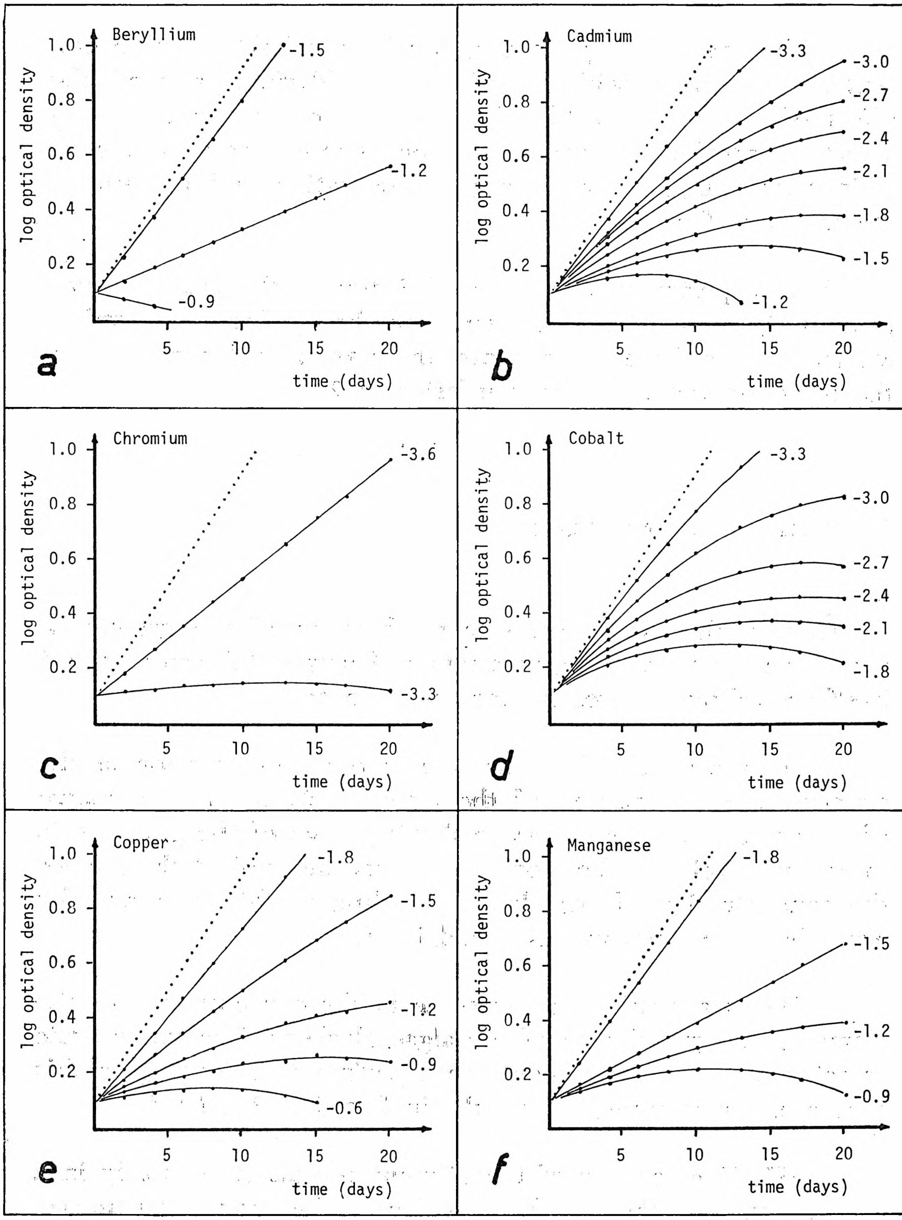
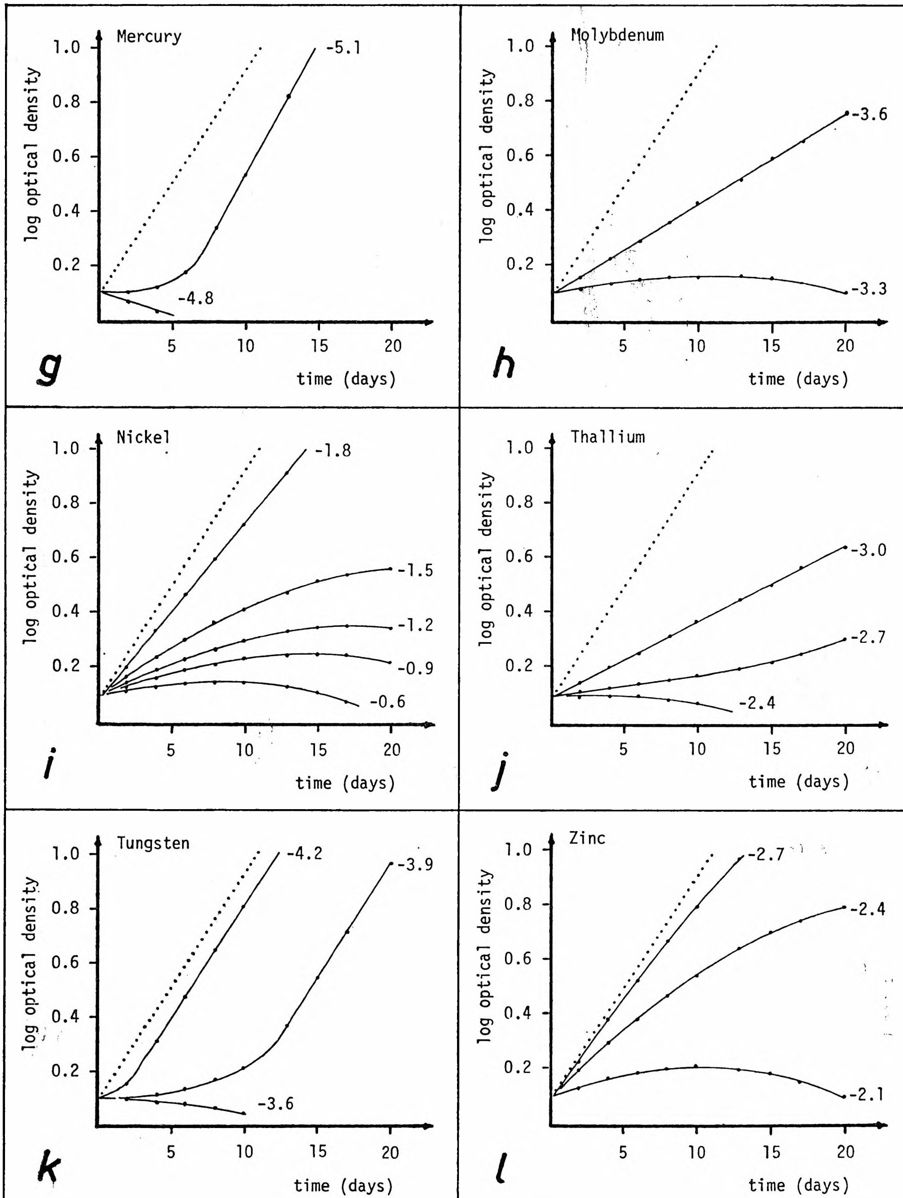


Fig. 2 — Growth-curves of *Cyanidium caldarium* in the presence of diverse concentrations of various heavy metals. For each growth-curve the H.M. concentration is indicated as $\log_{10}$ of the molar concentration of the element. In the ordinates the logs of *absorbance x 10* are given, with the aim of avoiding negative values.



The growth of those cultures to which H.M. was not added is indicated by the dotted line (control). In all the figures, concentrations greater than those given in the graphs proved to be lethal; concentrations weaker than those reported exerted no inhibitory effect in respect to the control culture.

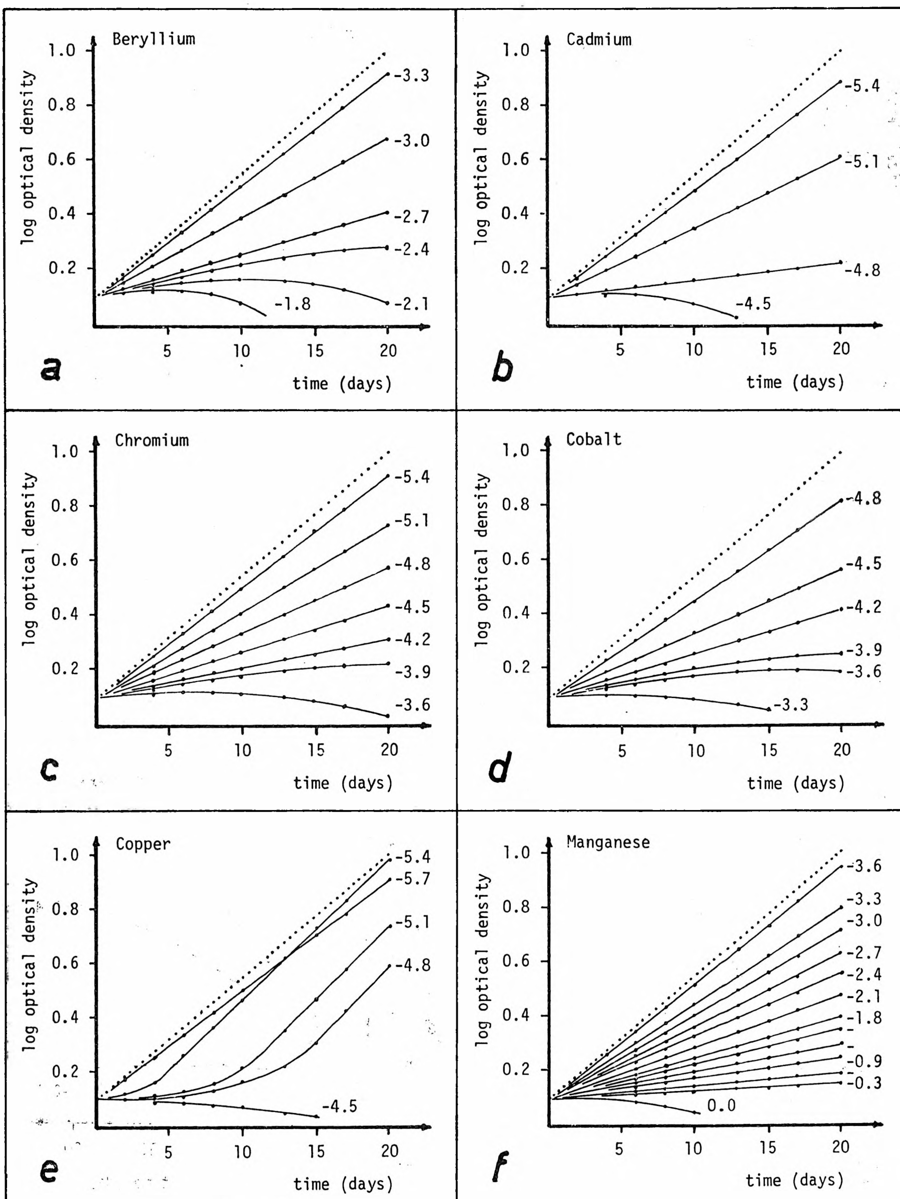
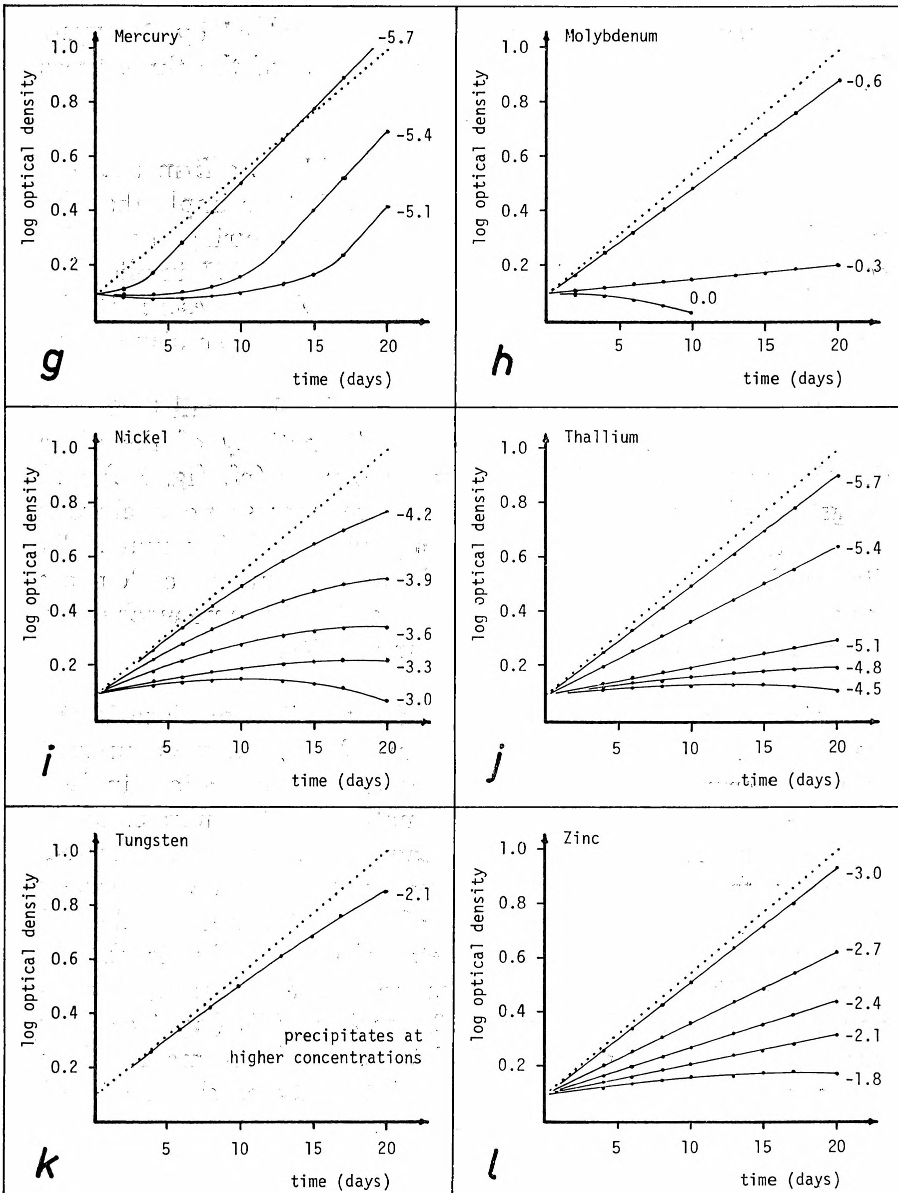


Fig. 3 — Growth-curves of *Chlorella saccharophila* in the presence of diverse concentrations of various heavy metals. For each growth-curve the H.M. concentration is indicated as $\log_{10}$ of the molar concentration of the element. In the ordinates the logs of *absorbance x 10* are given, with the aim of avoiding negative values.



The growth of those cultures to which H.M. was not added is indicated by the dotted line (control). In all the figures, concentrations greater than those given in the graphs proved to be lethal; concentrations weaker than those reported exerted no inhibitory effect in respect to the control culture.

Our results are in accord with those attained by BLANKLEY (1973) also concerning possible combinations of the three types of inhibition.

On the basis of these results we are able to affirm that the reactions of the first (I) and the second (II) type imply that the inhibitory action of the heavy metal persists during the entire course of the experiment; the reaction of type III implies, on the contrary, that the inhibitory action, while present at the outset, disappears completely and definitively after some days.

With regard to this phenomenon we should recall that mercury can be volatilized by the metabolic activity of the algae (BEN-BASSAT, MAYER, 1975). As far as copper (cf. fig. 3 e) and tungsten (cf. fig. 2 k) are concerned, we possess no analogous information; it seems possible that, without being materially displaced by the algae from the culture medium, these elements may, however, be blocked in their toxic action by some mechanism not necessarily linked to any cellular activity.

We observe that the reactions of type III take place in the presence of extremely toxic heavy metals, which were present in our experimental tests in extremely low concentration; in fact, a mechanism inactivating the heavy metal is all the more effective the smaller the quantity of the heavy metal to be inactivated. However, this phenomenon must not necessarily be exclusively dependent on the level of toxicity of the metal, but can depend equally on the quantity of the metal which the alga is able to inactivate. This phenomenon does not manifest itself, for example, in the case of chrome in *C. saccharophila* (fig. 3 c), very probably because the quantity of inactivated chrome is of an order inferior to that of the level of toxicity of chrome itself.

SUMMARY

The authors show that the inhibition of the growth of two unicellular algae, *Cyanidium caldarium* and *Chlorella saccharophila*, due to various concentrations of heavy metals, can manifest itself according to three fundamental types.

In the case of two of these types the inhibition persists throughout the duration of the experiment. In the third type, on the other hand, it manifests itself solely in a prolongation of the lag-phase; from the moment the algae complete this phase, their growth occurs without suffering any apparent inhibition. This third type is characteristic of highly toxic metals.

The authors suggest that this is due to an apparently complete detoxification of the culture medium by the algae themselves.

RIASSUNTO

Gli Autori evidenziano che l'inibizione della crescita di due alghe unicellulari, *Cyanidium caldarium* e *Chlorella saccharophila*, da parte di varie concentrazioni di metalli pesanti, si può manifestare secondo tre tipi fondamentali.

In due di questi tipi l'inibizione è presente per tutta la durata dell'esperimento. Nel terzo tipo invece essa si manifesta unicamente con un prolungamento della lag-fase; dal momento in cui le alghe escono da tale fase, la crescita avviene senza alcuna apparente inibizione. Questo terzo tipo è caratteristico dei metalli altamente tossici.

Gli autori suggeriscono che esso sia dovuto ad una apparentemente completa detossificazione del mezzo di coltura da parte delle alghe stesse.

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