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Evaluation of toxic effects of heavy metals on unicellular algae

IV - Effects of inoculum size on inhibition type**

The noxious effects of heavy metals on unicellular algae may manifest themselves according to three different inhibition types, i.e. with three different growth curves, in accordance with what has been stated by BLANKLEY (1973); a study of this subject was carried out on algae *Cyanidium caldarium* and *Chlorella saccharophila* (ALBERTANO & al., 1980a).

Each metal usually gives rise to a peculiar type of inhibition: it is known from the literature that such metals as copper cause an inhibition of type III, which manifests itself by prolonging the lag phase as the metal concentration increases (STEEMAN-NIELSEN & KAMP-NIELSEN, 1970); it is also known that an inhibition of the same type is caused by mercury (KAMP-NIELSEN, 1971).

Later on it was also shown that the metals that give rise to an inhibition of type III, besides being extremely toxic, are those which manifest their toxicity in very short times (ALBERTANO & al., 1980b).

Nevertheless, during some experiments on the two abovementioned algae, we could often notice that, in spite of the accuracy of our procedure, the answer obtained in different tests was not the same: more precisely the same metal gave rise on the same alga sometimes to inhibition of type III, sometimes to inhibition of type II (decreased growth rate).

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Since the only parameter which had varied during such experiments was the inoculum concentration, we have surmised that this could be the cause; this hypothesis was suggested to us also by the observation of the fact that the inoculum parameter sometimes weighs heavily on the limits of toxicity of H. M. on unicellular algae (ALBERTANO & al., 1979).

Our aim was that of ascertaining which relation exists between size of inoculum and type of inhibition in the case of highly toxic metals.

MATERIALS AND METHODS

We have employed the alga *Cyanidium caldarium,* strain 001 from our collection, and the alga *Chlorella saccharophila,* strain 211-9a Gö from the Culture Centre of Algae and Protozoa, Cambridge, i.e. the same two algae that had already been employed for similar experiments (ALBERTANO & al., 1980a).

Growth tests were made on three alga-metal pairs that have shown inhibition curves of type III in the above-mentioned study, namely *C. caldarium* with mercury and *C. saccharophila* with copper and with mercury. The fourth pair of this type, i.e. *C. caldarium* with tungsten, was excluded because of the heavy interference in the readings due to substances produced by the alteration of pigments by tungsten.

On these three alga-metal pairs tests were made at various and suitably chosen concentrations of H. M. ($HgCl_2$ and $CuCl_2.2H_2O$), and employing two different inocula (corrected values in the figs. 1-3). For each alga the larger inoculum gave a reading of 0.24 a.u. (uncorrected value) on the colorimeter, the smaller was obtained by a dilution 1:3.

The whole experiment was prepared and carried out exactly in the same way as the one previously described (ALBERTANO & al., 1980a); all the tests were repeated thrice.

RESULTS

The results of our experiment are summarized in fig. 1 *a, b* for the *C. caldarium* - mercury pair, in fig. 2 *a, b* for the *C. saccharophila* - copper pair, and in fig. 3 *a, b* for the *C. saccharophila* - mercury pair. In each figure are shown the growth curves obtained with a small (*a*) and a large (*b*) inoculum.

The results of one of the three probings (the whole experiment was carried out in three probings) are shown in figs. 1-3. The results of the two others showed slight variations in quantity as far as the limits of toxicity are concerned, though they fully confirmed the general development of the phenomenon described here.

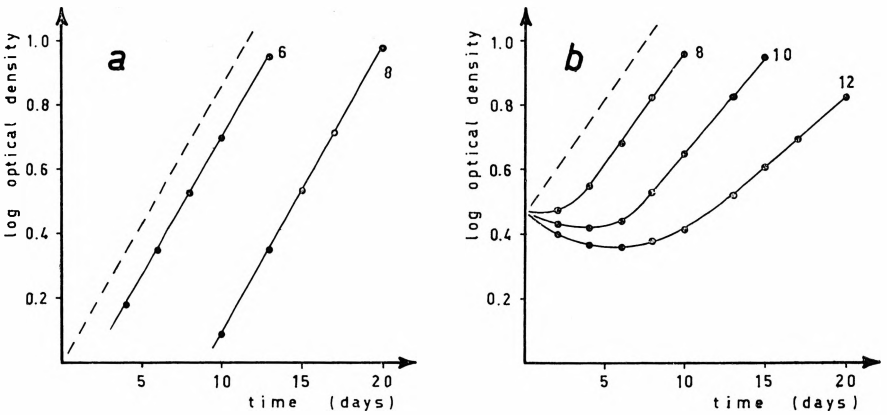

Fig. 1 - Growth curves of alga *Cyanidium caldarium* when in the presence of various mercury concentrations. The tests represented in fig. 1*a* were made with a small algal inoculum, those of fig. 1*b* with an inoculum thrice as large as the former (0.24 a.u.). At the end of each curve the mercury concentration x 10^{-6}M is given. On the ordinates log *absorbance x 10* is indicated (in order to avoid negative values). The dashed line refers to the control (without mercury). NOTA: With a small inoculum (*a*) the metal gives rise to an inhibition of type III (increasing of the lag phase), while with a larger inoculum (*b*) an inhibition of type II (decreasing in the growth rate) appears. One should also take note of a lowering of toxicity as the inoculum increases.

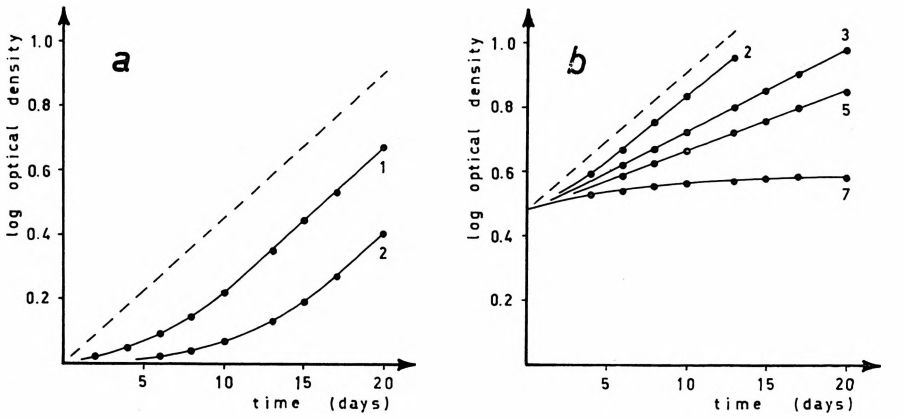

Fig. 2 - Growth curves of alga *Chlorella saccharophila* when in the presence of various copper concentrations. The tests represented in fig 2*a* were made with a small algal inoculum, those of fig. 2*b* with an inoculum thrice as large as the former (0.24 a.u.). At the end of each curve the copper concentration x 10^{-5}M is given. On the ordinates log *absorbance x 10* is indicated (in order to avoid negative values). The dashed line refers to the control (without copper). NOTA: As in fig. 1.

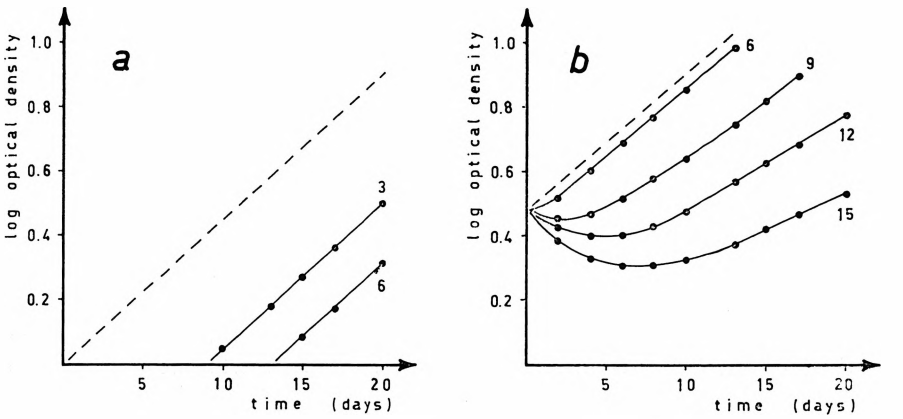

Fig. 3 - Growth curves of alga *Chlorella saccharophila* when in the presence of various mercury concentrations. The tests represented in fig. 3*a* were made with a small algal inoculum, those of fig. 3*b* with an inoculum thrice as large as the former (0.24 a.u.). At the end of each curve the mercury concentration x 10^{-6}M is given. On the ordinates log *absorbance x 10* is indicated (in order to avoid negative values). The dashed line refers to the control (without mercury). NOTA: As in fig. 1.

Discussion and conclusions

In the Introduction we have put forth the hypothesis that the use of algal inocula of various sizes may give rise to different types of the inhibition caused by some heavy metals.

In this connection the results shown in fig. 2, referring to the *Chlorella saccharophila* - copper pair, are particularly significant in our opinion. Actually the use of a small inoculum (fig. 2*a*) gives rise to an inhibition of type III, i.e. the higher the concentration of H. M., the longer the lag phase, followed by an exponential phase with a growth rate equal to that of the control.

When an inoculum thrice as large as that is employed (fig. 3*b*), besides a foreseable decreasing of toxicity (cf. Albertano & al., 1979), one notices that the inhibition caused by the H. M. (copper) becomes more or less clearly of type II, i.e. characterized by a decreasing of the growth rate as the metal concentration is increased.

Similar results, indisputable though not so manifest, were also obtained both with mercury on *Cyanidium caldarium* (fig. 1 *a, b*) and with mercury on *Chlorella saccharophila* (fig. 3 *a, b*).

In any case, it is evident from figs. 1*b* and 3*b* that, especially in the presence of high concentrations of metal, the inhibition becomes a blend of types II and III; i.e. it is characterized, as the concentration of metal increases, by an increasing of the lag phase and at the same time by a decreasing of the growth rate in the following exponential phase.

A confirmation of this type of behaviour is found in a study by Ben-Bassat & Mayer (1975) of the volatilization of mercury by algae — a slight indication of type II inhibition by mercury with large inocula, is visible in fig. 1.

The phenomenon we have pointed out is presumably to be connected with the very causes of type III inhibition. Indeed it was shown that dead algae produce a detoxification of the culture medium (Albertano & al., 1980b), presumably also on account

of the formation of compounds, the toxicity of which is lower than that of metallic ions.

When the concentration of algae increases, the apparent toxicity of the metal decreases, but at the same time (with higher concentrations of metal) the quantity of dead cells increases and, as a consequence, the concentration of the above-mentioned compounds also increases, though some of them (as in the case of Hg) may be eliminated through their volatilization: « It is therefore possible that during the lag period of 8-9 days, the Hg in the medium was transformed chemically to a form which is rather less permeable or less toxic to the cells. The overall process which occurs when Chlorella cells interact with $HgCl_2$ might be as follows: The Hg concentration in the medium decreases rapidly as a result of uptake by the algal cells and of volatilization. The latter occurs as a result of reaction between the Hg and a compound from the cells which gives rise to a new form of Hg. This form is more volatile and less permeable or toxic. » (BEN-BASSAT & MAYER, 1975).

It is therefore evident that whenever certain concentrations of the above-mentioned compounds of the metal are reached, the latter behave like harmful (non toxic) compounds and give rise in surviving algae to a type II inhibition that overlaps the toxic effects (type III) caused at first by metallic ions.

SUMMARY

The authors show that the type of inhibition caused by a heavy metal on unicellular algae may vary depending on the size of the inoculum.

In three alga-metal pairs examined (*Cyanidium caldarium* — mercury; *Chlorella saccharophila* — copper; *C. saccharophila* — mercury), by employing a small inoculum, an inhibition of type III has been noticed (increasing of the lag phase), while a gradual shift to a type II inhibition (decreasing of the growth rate) has been noticed with a larger inoculum.

The authors suggest that this phenomenon is due to the rise of slightly harmful compounds of the metal, the inhibitory action of which (type II) is made stronger by their higher concentration in the presence of larger inocula and higher concentrations of H. M.; such an action may overlap the toxic effects (type III) caused at first by metallic ions.

RIASSUNTO

Gli autori dimostrano che il tipo di inibizione esercitata da un metallo pesante sulle alghe unicellulari può variare al variare della concentrazione dell'inoculum.

In tre coppie alga-metallo esaminate (*Cyanidium caldarium* — mercurio; *Chlorella saccharophila* — rame; *C. saccharophila* — mercurio), usando un basso inoculum si è riscontrata inibizione di III tipo (prolungamento della fase di latenza), mentre con inoculum maggiore si è riscontrato un graduale passaggio all'inibizione di II tipo (diminuzione della velocità di crescita).

Gli autori ipotizzano che questo fenomeno sia dovuto alla formazione di composti del metallo di limitata tossicità, la cui azione inibente (II tipo), resa più intensa da una loro maggiore concentrazione agli inocula più elevati e con più alte concentrazioni di M.P., andrebbe a sovrapporsi alla azione tossica (III tipo) esercitata inizialmente dallo ione metallico.

REFERENCES

ALBERTANO P., PINTO G., TADDEI R., 1979. *Evaluation of toxic effects of heavy metals on unicellular algae. I-The influence of inoculum concentration on the evaluation of toxicity.* Delpinoa, **20**: 75-86.

— —, 1980 a. *Idem. II-Growth curves with different concentrations of heavy metals.* Delpinoa, **21**: 23-34.

— —, 1980 b. *Idem. III-Subtraction of the toxic element from the medium by the cells.* Delpinoa, **21**: 47-61.

BEN-BASSAT D., MAYER A. M., 1975. *Volatilization of mercury by algae.* Physiol. Plant., **33**: 128-132.

BLANKLEY W. F., 1973. *Toxic and inhibitory materials associated with culturing.* In Stein J. R. ed., Handbook of Phycological Methods: 207-229, Cambridge.

KAMP-NIELSEN L., 1971. *The effects of deleterious concentrations of mercury on the photosynthesis and growth of Chlorella pyrenoidosa.* Physiol. Plant., **24**: 556-561.

STEEMANN NIELSEN E., KAMP-NIELSEN L., 1970. *Influence of deleterious concentrations of copper on the growth of Chlorella pyrenoidosa.* Physiol. Plant., **23**: 828-840.