

THE EFFECT OF CHROMIUM ON THE GROWTH OF *CHLORELLA PYRENOIDOSA* ALGAL CULTURES

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Abstract

Different forms of chromium can be found in soil, water and even in the air. Around 60% of the chromium in the air is a result of human activities, the rest occurs naturally (by way of volcanic eruptions, fires). Gaseous chromium cannot be found in the atmosphere, chromium binds to the solid particles or to the atmospheric aerosol drops.

Most of the forms of Cr (VI) occur in the environment as a result of industrial activity, having chromite as main source, also known as iron and chromium chromate. The ideal composition is FeCr₂O₄.

The chromium in the water originates from dust from the air and cliff erosion. The chromium in freshwater varies in the range 0.1-800 mg/l. In polluted areas the values can be even higher. In natural waters Cr(III) and Cr(VI) is stable.

Key words: toxicity, green algae, heavy metals, phytotoxicity.

INTRODUCTION

This paper studies the effects of a toxic heavy metal ion, Cr(VI), in addition to Cr(III), on certain growing processes of *Chlorella pyrenoidosa* green algae. *Chlorella pyrenoidosa* is commonly used in biochemical and biological stress experiments due to its photosynthetic apparatus resembling that of higher plants, and its fast reproduction. For that reason, the experiments with *Chlorella pyrenoidosa* can be carried out in large numbers and with multiple repetitions; they are easily reproducible and controlled.

Living beings assimilate from the environment the materials, implicitly various metal ions, necessary for the organism and metabolism development. When the concentration of toxic (or essential) metal ions in the environment, that can be assimilated, a certain threshold is reached leading to the inhibition of a series of metabolism processes of living beings, which in this case may cause the death of the living being. Subjected to the stress of heavy metals, these organisms try to decrease the degree of toxicity as much as possible.

Interest in chromium comes from its large scale usage, given that the metallurgical, paints,

electrochemical and leather industries release chromium in high quantities in the liquid, solid and vapour form, thus causing major biological and ecological problems. Chromium can be found in countless states of oxidation, although only Cr(III) and Cr(VI) is fairly stable in nature, Cr(IV) and Cr(V) are unstable intermediaries in the redox transition of Cr(III) and Cr(VI).

The ratio of the two forms of chromium is determined by the physical and chemical parameters of the environment. In a well oxygenated, pH \geq 7 environment, the presence of CrO₄²⁻ ions is expected, while Cr(III) ions are expected in a poorly oxygenated, pH \leq 6 environment. This is greatly influenced by the oxidation and reduction agents and by molecules capable to produce complexes. Except for the aerated surface waters with a higher level of oxygen, Cr(VI) occurs in the form of H₂CrO₄ only at a low pH level. Within the normal pH values of natural waters, Cr(III) occurs in the form of hydro-complexes, organic complexes and less mobile Cr(OH)₃ precipitations, but in this case the production of complexes lowers the precipitation production possibility. Depending on environmental conditions, Cr(VI) knows many forms and their

solubility and agility is significantly higher than that of Cr(III).

According to current knowledge, Cr(III) is essential to human and other living beings, it has an important role in the normal functioning of the lipids and glucose metabolism. In the case of Cr (VI), however, toxic and teratogenic effects are known to exist.

Plants, primarily due to solubility aspects, cannot assimilate Cr(III), and Cr(VI) has a toxic effect, especially in higher concentrations.

THE INTERACTION OF CHROMIUM AND MICRO-ORGANISMS

Research on the interaction between algae and metals has a significant past.

Experiments regarding Chromium began in the 70s, followed by a long pause; however, interest has grown in recent years.

Meisch and Schmitt-Beckmann researched the effect of Cr(III) and Cr(VI) compounds of various compositions on a strain of wild and laboratory *Chlorella*.

In the case of low concentrations, both a growth and a photosynthesis stimulating effect was observed, while in the case of higher concentrations the decrease of the cell reproduction and size was observed, as well as the inhibition of chlorophyll synthesis (Meisch and Schmitt-Beckmann, 1979).

In the case of *Glaucocystis nostochinearum* the EC₅₀ value was observed to be 1 mg dm⁻³ and in atomic absorption research a notable Chromium accumulation was observed (Rai et al., 1992).

Metal accumulation and toxicity symptoms - the chlorophyll concentration, protein content, and the nitrate and nitrite reductase - have been shown to be dependent on concentration (Rai et al. 1992).

In the study of individual and combined effects of several toxic heavy metals (Cu, Cr, Ni) synergistic effects in growth, photosynthetic activity and the chlorophyll synthesis in the case of *Chlorella pyrenoidosa* 251 were observed.

In the case of heavy metals used individually in the study of the algal species, toxicity decreased towards Cu, Cr, Ni (Wong and Chang, 1990).

MATERIALS AND METHODS

In the preparation of the culture medium the purest compounds available on the market were used. The Bacto Agar used in the solid culture medium.

The growth medium used for incubating the algae is a standard medium containing a wide range of nutrients to ensure the natural growing conditions of the organism.

All vessels used for the test are made of glass, glass is known as an inert material.

In our studies we used the green algae *Chlorella pyrenoidosa* (IAM-128) in the collection at the Department of Microbiology at the University of Szeged, Hungary. The algal cells were kept in a solid culture medium and sterility was maintained. The algal cells were cultivated in special 500 ml algae growing vessels, in a liquid C-30 modified culture medium, in a sterile medium at 25°C. Table 1 features the composition of the culture medium. Trace elements were added to the medium substrate from the basic solution prepared beforehand. For the inoculation, I have calculated and used an amount necessary to obtain a 10⁴ cells/ml initial cellular density which proved to be optimal for growing experimental cultures. During the 72 hours test period, the daily density of the algae suspensions in each test recipient was tested by using a Thoma chamber and a microscope.

The results were then subjected to statistical analysis.

Table 1. Trace elements

KNO ₃	2.02 g
KH ₂ PO ₄	0.113 g
K ₂ HPO ₄	0.087g
MgSO ₄ x7H ₂ O	0.24 g
CaCl ₂ x6H ₂ O	0.037 g
NaHCO ₃	1.0 g
Fe (Fe ²⁺ -EDTA)	5 mg
H ₃ BO ₃	1.43 mg
MnCl ₂ x4H ₂ O	0.905 mg
ZnSO ₄ x7H ₂ O	0.111 mg
CuSO ₄ x5H ₂ O	0.0395 mg
Na ₂ MoO ₄ x2H ₂ O	0.0126 mg
NH ₄ VO ₃	0.0115 mg
Co (NO ₃) ₂ x6H ₂ O	0.0245 mg

RESULTS AND DISCUSSIONS

As Cr (III) $\text{Cr}_2(\text{SO}_4)_3$ was used, and as the compound Cr (VI) ($\text{Na}_2\text{Cr}_2\text{O}_7$). When cultures of algae reached 10^4 cell/ml in the culture medium, the algae were treated with 1-10 mg ions of Cr(III) and Cr(VI) with the help of basic solutions prepared in advance. Untreated cultures in identical conditions were used for each experiment. After applying the treatments, the algae were cultivated for 48-72 hours.

I studied the algae cell growth using several methods:

- a./by counting cells using a cytometer
- b./in the case of a low cell number I performed orientation measures using photometry on 600 and 660 nm (Rodrigues and Lopez, 1964).

The effect of Chromium on the growth of *Chlorella pyrenoidosa*.

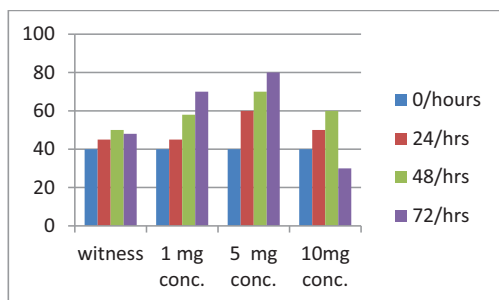


Figure 1. The evolution of cell growth in the presence of Cr III

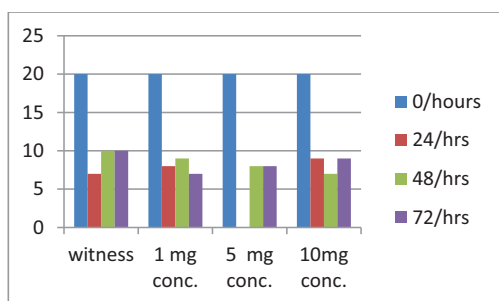


Figure 2. The evolution of cell growth in the presence of Cr VI

I studied the effect of Cr(III) and Cr(VI) with increasing concentration on various growth parameters of *Chlorella pyrenoidosa*. Although the published literature points out that Cr(VI) is significantly more toxic than Cr(III), I

performed measurements with $\text{Cr}_2(\text{SO}_4)_3$ besides $\text{K}_2\text{Cr}_2\text{O}_7$.

The results show that not even in a 1-5 mg concentration did Cr(III) significantly affect the green algae growth parameters; it did not produce any significant differences in relation to the control medium. At a 5 mg concentration a slight increase in the algae cells can be observed. This happens due to the chemical properties of Cr(III) because it is found in nature in the form of various precipitations depending on the pH conditions. Depending on the soil conditions and the medium of the roots, in the case of land plants, the electrochemical features and the pH of the soil allow for the Cr(III) \rightarrow Cr(VI) transformation, if the Cr(VI) form is possible (Kotás and Stasicka, 2000).

The measurement data are similar to data from the published literature, where, except a few cases, Cr(III) is not considered toxic for plants (Samantaray et al. 1998).

In the case of treatment with 1 mg concentration Chromium, there are no significant differences between the control and the treated samples. As for the content of solid material, the cell and chlorophyll number, there was no difference observed at this concentration.

However, the increase in the concentration of Cr(VI), has produced a significant decline both in terms of the density of cells and cell number. At a concentration of 5 mg, significant modifications in relation to the control sample took place.

Crops treated with 1-5 mg concentration Cr(VI) showed chlorotic symptoms.

The algae cells removed from the culture recipient containing 5 mg concentration Cr(VI) did not reproduce anymore when they were placed in a fresh, Cr(VI)free culture medium. The data suggest that the lethal concentration of Cr(VI) in the case of *Chlorella pyrenoidosa* is 10 mg.

CONCLUSIONS

The ratio of the two forms of chromium is determined by the physical and chemical parameters of the environment.

In a well oxygenated medium with a $\text{pH} \geq 7$ the presence of CrI_4^{2-} ions is expected and in a medium poor in oxygen and with a $\text{pH} \leq 6$ the

presence of Cr(III) ions is expected, this being influenced greatly by the potential oxidation and reduction agents and the molecules capable of producing complexes.

According to current knowledge Cr(III) is essential to humans and other living things and plays an important role in the normal functioning of the metabolism of lipids and glucose.

However, in the case of Cr(VI) we do know that it has toxic and teratogenic effects.

Plants - primarily because of solvency issues - cannot assimilate Cr(III), and - especially in larger concentrations - Cr(VI) has toxic effects. The study of plant tolerance to Chromium is just the beginning, physiological and biochemical aspects are mostly unexplored.

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