Contribuţii Botanice, XXXVI, **2001**
Grădina Botanică "Alexandru Borza"
Cluj-Napoca

STRESS-PHYSIOLOGICAL INVESTIGATION OF ALGAL CELL CULTURES IN POLLUTED MEDIA

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Abstract. Metbolic and developmental parameters of algal cells, on which the primary biomass production of aquatic ecosystems relies, are specifically altered by anthropogenic stress conditions caused by thoughtless use of pesticides and artificial fertilizers, as well as by xenobiotic heavy metals such as the cadmium. The herbicide "diuron" drastically decreases the efficiency of the photochemical conversion of the absorbed light energy. Another herbicide, the paraquat, creates a severe oxidative stress in the illuminated algal cells. Excess amounts of phosphates from artificial fertilizers responsible for the eutrophication of water ponds, induce a very high rate of autospore formation and lead to an early collapse of the algal populations. Water-soluble cadmium ions impair the light-harvesting processes performed by chlorophylls, as well as the inorganic carbon assimilation and cell division. All of these stress factors induce physiological acclimation and tolerance in the algal cells.

Introduction

In a plant physiological sense stress is reflective of the amount of environmental pressure for change that is placed on different biological processes of an organism, inducing an alarm response. These responses may be defensive or adaptive, and stress occurs when the unfavorable environmental factors induce enough physiological change to result in reduced growth, reduced yield, physiological acclimation, species adaptation, or a combination of these. Because of their short life cycle and high contact area of all the cells with the environment, as well as due to their pronounced metabolic plasticity, microalgae are especially suitable organisms to study stress physiology.

Physiological responses to stressors can be divided into two possibilities. In the case of tolerance plants have mechanisms that maintain high metabolic activity under mild stress and reduced activity under severe stress. In contrast, mechanisms of avoidance involve a reduction of metabolic processes, resulting in a dormant state, upon exposure to long-term extreme stress [3,13].

Stress can be regarded as a functional state or as the dynamic response of the whole organism. It represents a significant deviation from the conditions optimal for life, and eliciting changes and responses at all functional levels of the organism which, although at first reversible, may also become permanent. Stress can be regarded as a directional event, induced by highly specific factors, but the physiological response may have common steps for several different stressors. Often, the external factors does not reach the ultimate site of the stress reaction immediately or in its original intensity, because plants possess a variety of protective mechanisms to delay or even prevent disruption of the thermodynamic or chemical equilibrium between environment and cell interior. The stress response is a race between the effort to adapt and the potencially lethal processes in the protoplasm. Thus the dynamics of stress comprises a destabilizing, destructive component, as well as countermeasures promoting restabilization and resistance. Constraint, adaptation and resistance are thus interconnected parts of the whole event [6,8]. Reactions that indicate a state of stress make possible the employment of sensitive plant species as bioindicators of environmental stress, or the use of living plants as biomonitors of specific habitat parameters. Both categories are widely represented among the freshwater microalgae.

Today, as a result of human activities, plants are exposed to far greater amounts of harmful substances than before. These are chiefly xenobiotics to which plants could not become accustomed yet. Land-use practices around the world have often resulted in degraded ecosystems that will not return rapidly to their original state. Commonly, the disturbed habitat has many stressors that impact plant functions, and restoration can be assisted by judicious incorporation of species or ecotypes that can tolerate the stresses of these damaged ecosystems. In this context, stress tolerance of microalgae, as the main primary biomass producers of the aquatic ecosystems, plays a crucial role in the remediation of anthropically polluted water ponds [2,11]. The aim of the present work is to reveal stress-physiological responses of a widespread green microalga against pollution with two different herbicides and a xenobiotic heavy metal, as well as in conditions of eutrophication due to excessive amounts of dissolved phosphates.

Material and Methods

Axenic monoalgal cultures of *Scenedesmus opoliensis* P. Richter, obtained from the culture collection of the Biological Research Institute in Cluj [5], were grown in Kuhl-Lorenzen (KL) nutrient media supplemented, according to the different experimental variants, with 50 micromole diuron (3-(3,4-dichlorophenyl)-1,1-dimethyl-urea), 50 micromole paraquat (1,1'-dimethyl-4,4'-bipyridinium cation), 50 micromole cadmium chloride, or 5 gl^{-1} potassium dihydrogen-phosphate. The pH of all the culture media was adjusted to 6.5 and the cell suspensions were illuminated

14 hours per day with fluorescent lamps at a photon flux density of 80 micromoles $m^{-2}s^{-1}$ on the surface of the cultures.

The dynamics of cell density was determined citometrically, net photosynthetic oxygen evolution was measured oxymetrically with a Clark-type oxygen electrode at saturating light intensity, chlorophyll content was determined spectrophotometrically after extraction with dimethylformamide [7,15] and biomass production was evaluated by dry weight measurements. The values represent the average of five parallel setups for every experimental variant.

Results and Discussion

The capacity for absorption and conversion of light energy, the net photosynthetic yield and the rate of cell divisions are important physiological parameters for characterizing the stress reaction of algae in differently altered environments. The impact of external factors on photosynthetic and developmental processes is of great interest because plant productivity is strongly dependent on the prevailing metabolic rates in a dynamic environment.

The rate of cell divisions in the algal cultures was mostly decreased by paraquat (Fig. 1), probably because the oxidative stress caused by this herbicide impairs the mitotic processes implied in autospore formation. A constantly low cell number was also registered in the medium polluted with cadmium, but the fact that the algal population remained in a stationary phase during the entire period of four weeks reflects that, although they did not multiply intensely, some individuals became tolerant to the applied amount of this heavy metal.

The capacity of this algal strain to cope with xenobiotic stress factors is demonstrated by its responses to diuron. In the presence of this herbicide, after a long lag phase and a moderately performed exponential growth phase, the cell density of the cultures almost reached the one exhibited by the control, probably because of the mutiplication of the few initial cells that succeeded in surviving and accomodating to the selectional pressure. On the other hand, the excess of phosphorus, that is temporarily benefic to the energetic status of the metabolically active cells, causes a valuable blooming of the culture, but in a few weeks the resources get exhausted and the reciprocal shading of the too many individuals, as well as the accumulating by-products, lead to an early decline of the algal population. This dynamics suggests that eutrophication may seem benefic on a short time scale, but finally the excessive amount of a nutrient will cause the collapse of the whole population.

Net oxygen production of algal cells is the difference between the amount of oxygen produced by photosynthetic water-splitting and the amount of oxygen consumed in the same time by photorespiration, mitochondrial respiration and extramitochondrial oxidative procceses. Its dynamics reflects the overall efficiency of

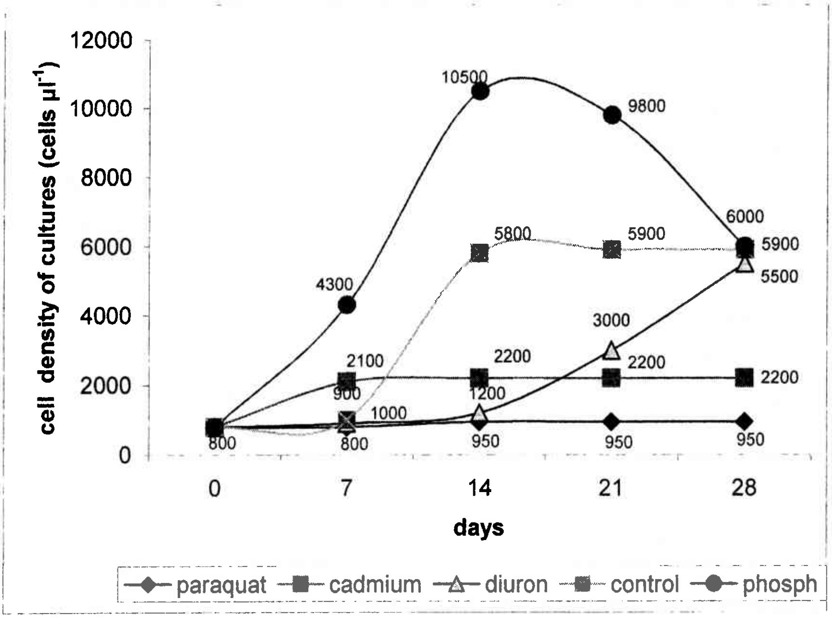

Fig. 1: Influence of different water-polluting agents on the dynamics of cell density in cultures of *Scenedesmus opoliensis*.

the conversion of light into chemical energy stored in the cells. This oxygen evolution, directly related to the intensity of photochemical reactions in the thylakoids and to other aerobic processes of the algal metabolism, is significantly depleted by micromolar concentrations of paraquat, diuron and cadmium (Fig. 2).

In the chloroplasts paraquat is photoreduced by the photosystem I in place of ferredoxin. The reduced paraquat is a free radical which on its turn reduces the molecular oxygen and generates the superoxide anion, which is a very toxic radical. The oxygen consumption of this so-called Mehler-reaction and the subsequent oxidative damages are responsible for the drastical reduction of the net photosynthetic oxygen production registered in the presence of paraquat [1,4].

The other herbicide, the diuron, impairs the water-splitting associated with the function of photosytsem II, due to the fact that in the chloroplasts this substance inhibits the electron transfer from the primary quinone acceptor to the secondary one, because of the competitive binding of the herbicide to the secondary quinone-binding site [4]. This mechanism which occurs in the same functional system with the oxygen-evolving complex, may explain the registered oxygen depletion in the algal cells.

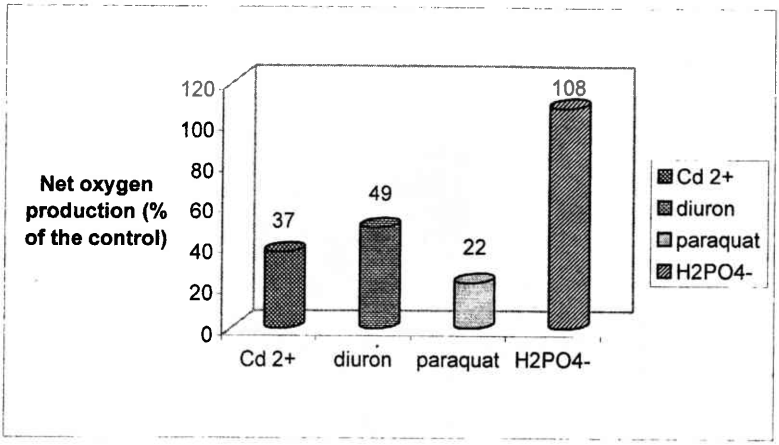

Fig. 2: Influence of cadmium, two herbicides and excess amounts of phosphorus on the net oxygen production of *Scenedesmus opoliensis* cells. For concetrations and culture conditions see the Material and methods section.

Cadmium has specific action sites in both photosystems and also in the photosynthetic carbon assimilation cycle [2,14], and it causes a feed-back inhibition of the oxygen production in the illuminated algal cells.

The main functional molecules for energy absorption and conversion are the light-harvesting pigments, and their optimal quantity in the thylakoid membranes in a prerequisite for a balanced energetic status and a good vitality of the autotrophic algal cells [9,10,12]. Extra amounts of phosphorus in the nutrient medium do not influence significantly the chlorophyll content, and diuron also has a moderate decreasing effect on the quantity of this photosynthetic pigment type (Fig. 3). In contrast, micromolar amounts of cadmium strongly inhibit the accumulation of chlorophylls in the chloroplasts, and the same concentration of paraquat has a similar effect. Although both of these xenobiotics decrease the quantity of light-harvesting pigments, cadmium mainly inhibits the *de novo* biosynthesis of chlorophylls, while paraquat causes an oxidative damage of the preexisting pigment molecules.

Biomass production evaluated on the base of algal dry weight in 4 weeks old cultures, was mostly depleted by micromolar amounts of paraquat and cadmium. After the survival and the multiplication of acclimated tolerant cells, the algal biomass of diuron-polluted aquatic media reaches a moderately higher value. In this context, the most important common feature of cadmium and paraquat pollution, which do not apply in the case of diuron, is the induction of an oxidative stress requiring a considerable energy input in order to survive under such extrem conditions.

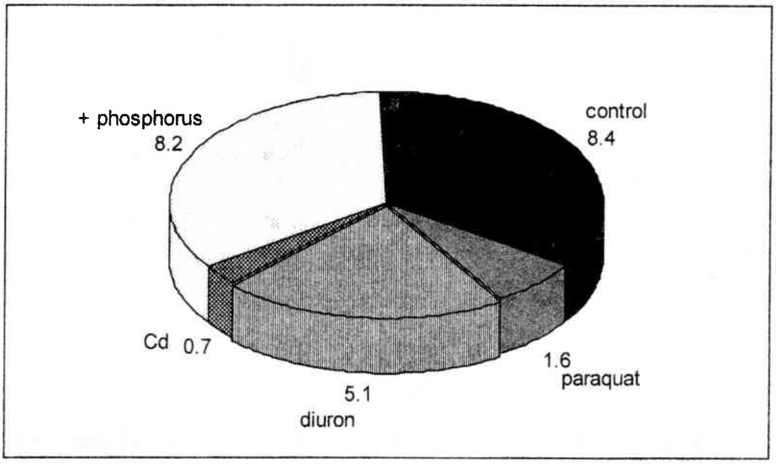

Fig. 3: Influence of water-polluting xenobiotics on the chlorophyll content of *Scenedesmus opoliensis* cell cultures. The numbers represent the chlorophyll content expressed in mg per 100 g dry weight.

Conclusions

Beyond the general physiological mechanisms of self-protection, algal cells develop tolerance and react differently to specific environmental stress factors. Metabolic plasticity and reversible functional acclimation make possible the survival of certain ecotypes in anthropically modified aquatic habitats.

Cadmium affects mainly the light-harvesting pigment molecules and the autospore formation.

Paraquat creates a severe oxidative stress, leading to the depletion of oxygen production and of net biomass accumulation.

Diuron impairs the photochemical reactions connected to the oxygen evolution, but in time the alga is able to develop a certain degree of tolerance to micromolar amounts of this herbicide.

Excess amounts of phosphate that may cause the eutrophication of water ponds, lead to an early collapse of the overpopulated media, but do not influence significantly the chlorophyll content and the net oxygen production of the algal cells.

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INVESTIGAREA STRESULUI FIZIOLOGIC ÎN CULTURI DE CELULE ALGALE AFLATE ÎN MEDII POLUATE

(Rezumat)

Culturi axenice monoalgale de *Scenedesmus opoliensis* au fost tratate separat cu 3 tipuri de substanţe xenobiotice care pot polua mediile acvatice supuse influenţelor antropice: ioni de cadmiu, ierbicidele diuron şi paraquat, precum şi cu exces de fosfat monopotasic, parţial responsabil de eutrofizarea apelor naturale. Formarea de autospori a fost puternic inhibată de paraquat şi de cadmiu, însă în cazul diuronului, pe seama celulelor tolerante, s-a dezvoltat în timp o populaţie cu o densitate celulară relativ ridicată. Atât paraquatul, cât şi cadmiul induc o stare de stres oxidativ şi provoacă scăderea drastică a cantităţii pigmenţilor clorofilieni. Producţia netă de oxigen descreşte semnificativ sub acţiunea paraquatului care în stare fotoredusă transformă oxigenul tripletic în radical superoxidic. Mecanismele funcţionale ale contracarării acţiunii factorilor de stres necesită un

consum energetic ridicat, dar realizează o acomodare fiziologică reversibilă care face posibilă supraviețuirea populațiilor algale în condiții nefavorabile. Cunoașterea mecanismelor antistres este importantă pentru orice acțiune de remediere a ecosistemelor acvatice afectate de poluarea provocată de activitățile umane.