USEFULNESS OF EUKARYOTIC AND PROKARYOTIC MICROORGANISMS IN THE INVESTIGATION OF WATER QUALITY IN THE MUREŞ RIVER

László Fodorpataki and Judit Papp

Abstract

The development of physiological tolerance to the chemical stress caused by pollution of the aquatic environment with cadmium ions can be detected by different fuctional and biochemical parameters of the algal cells present in the phytoplankton of different river sectors. The investigation of such parameters that indicate early symptoms of heavy metal pollution revealed that the intensity of enzymatic H_2O_2-degradation is a suitable tool to appreciate the tolerance developed by phytoplankters to cope with polluting agents which act as oxidative stress factors. Among the main photosynthetic pigments, only the amount of chlorophyll-b exhibits a regular correlation with the degree of water pollution caused by cadmium. The influence of this heavy metal on the net biomass production of the phytoplankton is also investigated, and the usefulness of prokaryotic decomposers in the estimation of water quality is discussed.

Keywords: phytoplankton, stress tolerance, water pollution, cadmium, oxidative damage

Introduction

Planktonic microalgae are primary biomass producers in the aquatic ecosystems, while heterotrophic bacteria play an important role in decomposing the different organic substances in the water. Both of these two groups of microorganisms exhibit a pronounced metabolic plasticity and a high growth potential. Thus, they may be used to monitor the degree of water pollution with different inorganic and organic chemicals that indicate human impact and may threaten the health of the riverine populations.

The phytoplankton, consisting of microscopic algae that have little or no resistance to currents and live free-floating in open waters, occur as unicellular, colonial or filamentous forms. From a physiological and ecological point of view, the floating cyanobacteria are also included in the phytoplankton. Most of the small organisms are photosynthetic and are grazed upon by zooplankton and other aquatic creatures. Phytoplankton organisms long have been used as indicators of water quality. Some species flourish in highly eutrophic waters while others are very sensitive to different chemical wastes (Hamar 1995). Some species have been associated with noxious blooms, sometimes creating offensive tastes and odors or toxic conditions. Because of their short life cycles, plankters respond quickly to environmental changes, and hence the standing crop and the species composition indicate the quality of the water mass in which they are found. Because of their transient nature and often patchy distribution, information on plankton as indicators is interpreted best in conjunction with concurrently collected, physicochemical and other biological data (Munawar 2000).

The microflora of rivers consists of autotrophic and heterotrophic, aerobe or anaerobe microorganisms that are involved in the natural cycle of chemical elements, in the production, degradation, transformation and mineralization of natural organic compounds, as well as in the bioconversion of xenobiotics originating from different human activities. Many polluting agents that are released in different sectors of rivers seriously endanger the dynamic equilibrium of the aquatic communities. The partial self-cleaning process of water becomes possible only upon the close interaction between autotrophic and heterotrophic microorganisms, that produce and decompose new organic compounds and sustain all of the life forms that are characteristic for an aquatic ecosystem (Fodorpataki and Papp 2000).

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 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 (Schnell 1994). Constraint, adaptation and resistance are thus interconnected parts of the whole event. 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.

The aim of this study is to reveal metabolic properties of microalgal and bacterial populations isolated from different sectors of the Mureş River, and to corelate the physiological parameters with the water quality of the river, in order to achieve a better understanding of how invisible life forms react to the challenge represented by anthropic influences on aquatic communities.

Material and methods

Cell populations of the same algal species (*Scenedesmus opoliensis* P. Richt.) were isolated from 3 different sampling sites along the Mureş River, and cultivated under controlled conditions, in nutrient media without and with supplementation with 0.1 mM cadmium as a polluting agent. The tolerance of the algal populations to this stress condition was investigated by determining the peroxide-scavenging enzyme activities, the chlorophyll *a* and *b* content and the dry biomass production after 10 days of cultivation. The photosynthetic pigment content was determined spectrophotometrically after extraction with methanol and acetone, performed in dim light at 4°C. The activity of H_2O_2 decomposing enzymes was assayed titrimetrically, by measuring the remaining hydrogen peroxide after 1 hour of incubation in the presence of a known amount of H_2O_2. Biomass production was evaluated by dry weight measurement (Fodorpataki *et al.* 2001).

The bacteriological investigations of the water samples include the determination of the total number of heterotrophic bacteria (on nutrient agar, 48h incubation at 37°C), as well as the abundance of some indicator species, such as the coliforms (in Durham tubes with lauryl-sulphate broth) and the streptococci (in inoculation tubes with bromocresol-purple-azide medium). For these determinations water dilutions were prepared from 10^{-1} to 10^{-7}, and these were inoculated in selective nutrient media that allow the growth of specific bacterial groups. The most probable number of the different bacteria was established using the De Man table, based on the number of positive tubes (Fodorpataki and Papp 2000, Greenberg *et al.* 1995, Rompré et al. 2002).

The sampling sites were:

1. Gălăoaia, with unpolluted water on the upper section of the river
2. Gura Arieş, situated downstream to the confluence of the heavily polluted Arieş River with the Mureş
3. Pecica, with partially self-purified water, on the lowest section of the Mureş River.

Results and discussion

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 (Jackson and Black 1993, Kullberg 1995). 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 (Fodorpataki and Trifu 1995).

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 functional 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 the influence of environmental changes on aquatic organisms. 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 (Pugnaire and Valladares 1999).

Many pollutants induce oxidative stress conditions in the living organisms (Ray and Gaur 2001). Algal cells are able to protect themselves against harmful active oxygen compounds, e.g. by decomposing H_2O_2. In the presence of Cd^{2+}, the H_2O_2-scavenging enzyme activity is much higher in the algae selected from polluted water samples (Fig. 1). This indicates that phytoplankters are able to acclimate to variations of the chemical composition of the aquatic environment, and in a relatively short period of time those individuals become dominant which have the ability to develop a more efficient defensive mechanism with the contribution of their inducible enzyme systems. In other words, the microalgal populations originating from polluted sectors of the river are better prepared to cope with the oxidative stress induced by the presence of cadmium ions dissolved in the water. This may be a suitable explanation for the fact that the algal populations isolated from Gura Aries (the sampling site with the most heavily polluted water) exhibit a much higher activity of the peoxide-scavenging enzymes, even in the absence of the chemical stress factor represented by cadmium.

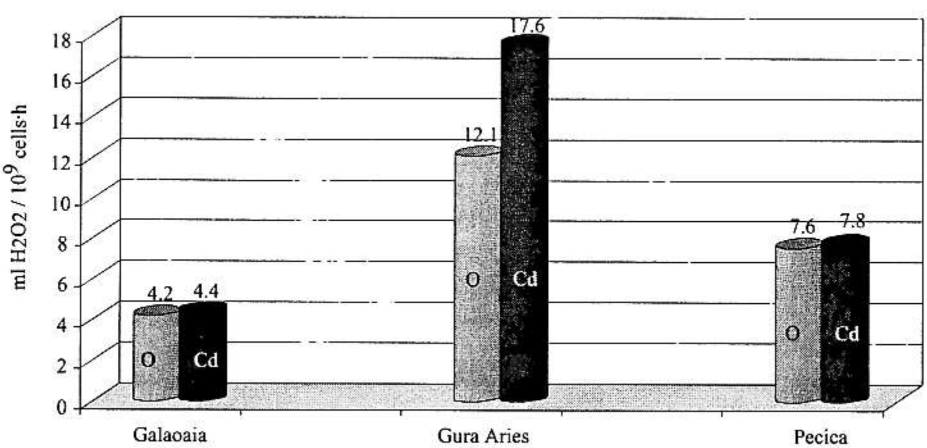

Fig. 1. Activity of hydrogen peroxide-scavenging enzymes in cells of *Scenedesmus opoliensis*, originating in 3 different sectors of the Mureş River, cultivated in unpolluted (0) and Cd-polluted (Cd) media

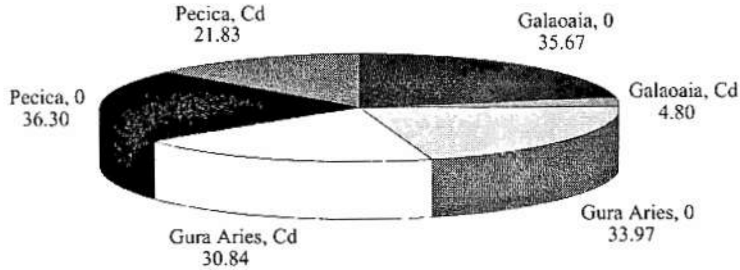

Fig. 2. Changes in the chlorophyll-b content (microgram per gram d.w.) of cells of *Scenedesmus opoliensis* originating in 3 different sectors of the Mureş River, cultivated in unpolluted (O) and cadmium-polluted (Cd) media

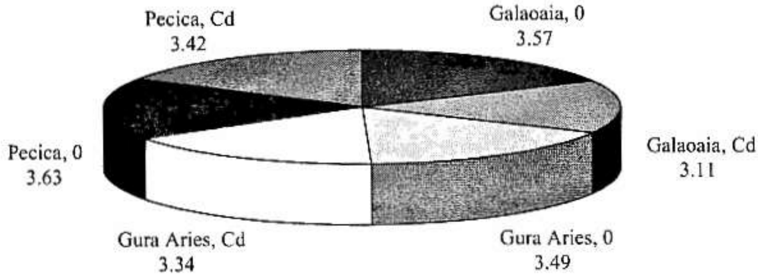

Fig. 3. Changes in the P700 chlorophyll-a content (microgram per gram d.w.) of cells of *Scenedesmus opoliensis* originating in 3 different sectors of the Mureş River, cultivated in unpolluted (O) and cadmium-polluted (Cd) media

The equilibrium between biosynthesis and degradation of chlorophyll-*b* is also influenced by polluting agents. The algal populations which had become tolerant, are able to maintain this equilibrium under stress conditions, while the other populations show an obvious decline in the amount of chlorophyll-*b* in the presence of Cd (Fig.2). Even under a constant illumination, the size of the light-harvesting pigment-protein complexes varies on a large scale if different stressors are present in the algal cells. Chlorophyll-*b*, which in greea algae is the main accessory photosynthetic pigment, is present mainly in the very dynamic peripherical regions of the light-harvesting complexes.

Pollutants the inhibit any segment of the biosynthetic pathway of this pigment, or enhance its degradation, will reduce the overall amount of chlorophyll-*b*, thus causing a decreased efficiency of energy absorption by the algal cells, which finally may result in the decline of biomass production. This suggests that monitoring the quantity of chlorophyll-*b* under specific light conditions may represent a useful tool for detecting the disturbance of energy input into the aquatic communities, caused by polluting agents (such as cadmium). In contrast, the amount of one of the most important types of chlorophyll-*a* (known as the P700 pigment) is highly stable under different conditions, being not suitable for the detection of unfavorable water quality (Fig. 3).

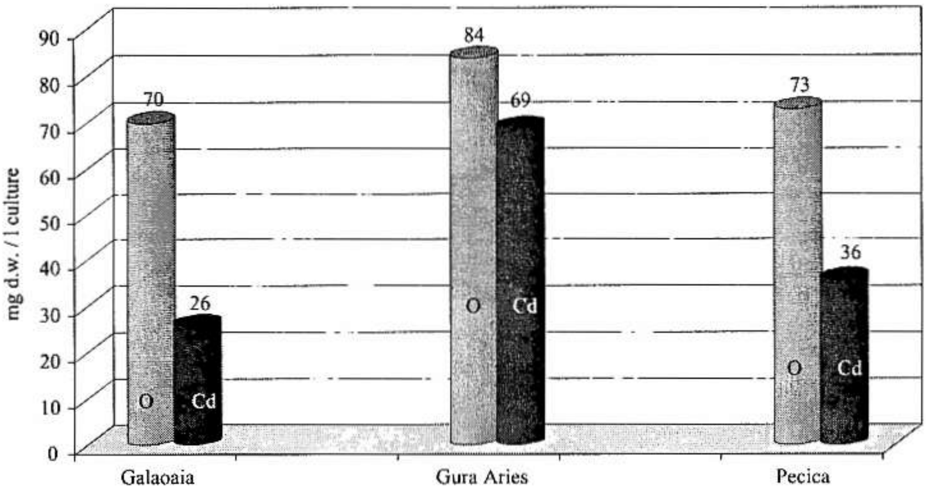

Fig. 4. Dry biomass production after 10 days of cultivation of *Scenedesmus opoliensis* populations, originating in 3 different sectors of the Mureş River, cultivated in unpolluted (0) and Cd-polluted (Cd) media

The dry biomass production is mostly affected by heavy metal contamination in the algal populations isolated from the unpolluted upper sector of the Mureş River (Fig. 4). This suggests that acclimation to harmful conditions is a prerequisite of the survival of microalgae in polluted environments. Small variations of the dry biomass can be registered even in the absence of the polluting agent, but the presence of dissolved cadmium induces a much more pronounced decline in dry weight of the unacclimated populations than in the case of the representatives of the same algal species isolated from a heavily polluted sector of the Mureş River.

These results reflect that by examining the different populations of the same algal species, their physiological sensitivity or tolerance to polluting agents directly indicates the quality of the aquatic environment.

The above presented invastigations can be completed with the study of different physiological categories of heterotrophyc microorganisms that are strongly interconnected with the planktonic primary producers, and in the same time provide us with useful information about hygienical parameters of the water. The chemical pollutants also affect the growth and diversity of these heterotrophic bacteria that decompose the body of dead organisms and many xenobiotics, as well (Gauthier and Archibald 2001). In this regard one suggestive example will be given bellow, while the influence of water pollution on other bacterial groups will be presented separately.

Accumulation of soluble heavy metals in the water leads to a decreased number of bacteria, while the number of coliforms and faecal streptococci is the highest in the lower sector of the river, which is loaded with organic substances from household wastewaters. This reflects that the chemical nature of the pollutants is the main determinant of the development of different prokaryotic microorganisms alon the different sectors of the river (Tab. 1).

Table 1. The number of different types of bacteria at 3 sampling sites along the Mureş River (in 1 ml of water)

Sampling sites	Total number of bacteria	Coliforms	Faecal streptococci
Gălăoaia Sept. 1999	632	3	0.1
Gălăoaia Dec. 1999	620	3	0.1
Gălăoaia May 2000	584	3	0.1
Gălăoaia Aug. 2000	654	3	0.1
Gura Arieş Sept. 1999	300	27	0.8
Gura Arieş Dec. 1999	204	54	9.2
Gura Arieş May 2000	600	28	7.0
Gura Arieş Aug. 2000	324	27	7.0
Pecica Sept. 1999	6000	110	0.7
Pecica Dec. 1999	660	348	6.1
Pecica May 2000	4820	140	5.1
Pecica August 2000	5800	173	5.1

The values of total number of bacteria and the number of coliforms and streptococci indicate clean, unpolluted water in the upper section of the Mureş river (Gălăoaia) in all sampling periods. In this region the river flows far from the human settlements and the polluting sources, and the life of the aquatic communities is not perturbed. The water brought by the Arieş River pollutes the water of the Mureş, the unfavourable effect being reflected by the presence of coliforms and especially of streptococci in high number at the confluence site of the Mureş with the the Arieş in every sampling period, excepting September, when the values were lower. The total number of bacteria and the number of indicators increase significantly in the lower region of the river, indicating strongly polluted water. The household wastewater and the by-products coming from agriculture and animal husbandry that are released in the river determine the contamination of the water with faecal materials and increase the amount of the indicator microorganisms. The highest values were registered in the last sampling site along the Mureş (Pecica). The presence of pathogens in a number above of the admissible values makes the water unsuitable even for bathing or for irrigation of crops that serve as food or fodder.

Conclusions

1. Functional parameters of algal cells, such as H_2O_2-scavenging enzyme activity, chlorophyll-*b* and net bioproductive potential, are good indicators of tolerance or sensitivity to water pollution with heavy metals.
2. By examining the physiological tolerance of different ecotypes of the same *Scenedesmus opoliensis* species, collected from the different sampling sites along the

Mureş, and cultivated under controlled experimental conditions in culture media enriched with cadmium-chloride, very elocvent conclusions can be drawn concerning the pollution of the aquatic environment to which the examined ecotypes belong. Under controlled experimental conditions, the algal populations originating in polluted water exhibit a higher capacity to cope with stressful conditions.

3. The most sensitive populations to heavy metal pollution are those inhabiting the upper and the lowermost sections of the river (Gălăoaia and Pecica).

4. The algae that are mostly tolerant to high concentrations of heavy metals were identified at Gura Arieş, where the Arieş river brings highly polluted waters.

5. The amount of coliforms and faecal streptococci from the total number of bacteria is an indicator of the degree of water pollution with organic compounds of anthropic origin.

6. The water brought by the tributary streams (Arieş and Târnava), loaded with organic materials, perturbs the natural microbial communities, increasing the number of faecal indicators, especially of streptococci.

Acknowledgement

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