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UV RADIATION IMPACT ON ENZYMATIC AND RESPIRATORY ACTIVITY OF NEUSTONIC AND PLANKTONIC BACTERIA

WPŁYW PROMIENIOWANIA UV NA AKTYWNOŚĆ ENZYMATYCZNĄ I ODDECHOWĄ BAKTERII NEUSTONOWYCH I PLANKTONOWYCH

Abstract: The surveys were carried out in the pelagic zone of Brzezno lake (the Tuchola Forest). Water samples were collected from the surface microlayer and the subsurface water. The surveys covered respiratory activity of bacteria, using a measurement system OxiTop Control. Also, general activity of hydrolytic enzymes was estimated measuring rates of fluorescein release from fluorescein diacetate. The research was conducted in two experimental layouts: with and without humic substances. Conducted surveys proved substantially lower respiratory and hydrolytic activity of bacteria influenced by UVB radiation compared with bacteria without UVB radiation. The experiments did not prove unambiguous protective operation of HS – only for selected strains with bacteria subjected to UVB radiation in presence of HS demonstrated lower decrease of respiratory and hydrolytic activity.

Keywords: UV radiation, surface microlayer, bacterioneuston, enzymatic activity, respiratory activity

In the vertical plane, the external layer of water body is so-called surface microlayer. This layer constitutes a particular chemical and physical environment, which differs substantially from the subsurface water. The surface microlayer is formed by adhesion forces, which are a result of intermolecular attraction and surface tension on the interface of two media: air and water. This leads to the accumulation of organic and inorganic compounds within the layer. The surface microlayer often contains an elevated number of bacteria, called bacterioneuston. Due to the fact that bacterioneuston inhabits the surface microlayer, its members are exposed to stressful ecological factors to a greater degree than organisms inhabiting the water column.

The insolation is one of the primary factors affecting the number and activity of the bacterioneuston. The highest levels of solar radiation, including UV, that reach a water body are concentrated in surface layers. The most insolated water layer in aquatic

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systems is certainly the surface microlayer. The amount of radiation penetrating the surface microlayer affects organisms living within that water layer as well as decomposition and matter circulation processes.

Considering the entire range of solar radiation reaching the air-water interface, medium wave UV radiation, ie UVBλ = 290-320 nm and UVA λ = 320-400 nm, is of the highest biological importance due to its harmful effects. Radiation in this wavelength range causes DNA damage (lethal effect) or inhibits the growth of organisms by inhibiting enzyme synthesis, reducing active transport and inducing mutations, all of which are sublethal effects [1].

There are numerous studies that demonstrate that solar radiation, in particular UVB radiation, is detrimental to the production of bacterial biomass and exoenzyme activity [2–4]. It is also noteworthy that photooxidation of *dissolved organic matter* (DOM) and *particulate organic matter* (POM), which results in the release of considerable quantities of easily assimilable organic matter to the environment and may increase bacterioplankton activity [5, 6], occurs under the influence of UV.

Experimental

The surveys were based on heterotrophic bacteria strains, isolated from the surface microlayer water and subsurface water.

Water samples collection. Water meant for analyses was collected in the summer, in pelagic zone of the Brzezno lake (53°57.5′ N; 17°48.6′ E), which lies within the Tuchola Forest area. The surface area of the lake equals 71.6 ha, with a maximal depth of 9.7 m, length of 2405 m, and width of 560 m. It is situated at 139.8 m above sea level and is rated among eutrophic water bodies.

Surface microlayer water samples were collected by a Garrett [7] technique using a Plexiglas plate, which collects a 150 μm water layer. Subsurface water was sampled from a depth of 25 cm using an automatic pump. Taken water samples were poured into sterile glass containers.

Isolation of bacterial strains. In order to isolate bacterial strains there was a surface screening carried out on the *tryptone soy agar* (TSA) (Difco) medium surface. After 6 days of incubation at 20 °C a representative strains collection was detached and transferred onto TSA medium bevels.

Preparation of test bacterial strains suspension. Isolated bacteria strains were generated for 3 days at 20 °C in 50 cm^3 of liquid *tryptone soy broth* (TSB) medium. Afterward, from each culture taken was 30 cm^3 and spun for 5 min at 10 000 rpm, temperature of 10 °C. The supernants was used as crude enzyme solution, bacterial deposit was suspended in 30 cm^3 of sterile Ringer's solution. Optic density of each strain bacterial suspension was driven to equal value of 0.5 applying sterile Ringer's solution as a diluting agent.

Exposure to UVB radiation. Prepared bacterial suspension and crude enzyme solution of given strain was divided into 3 parts, 10 cm^3 each, and transferred into three parallel sterile Petri dishes. First one was a controlling agent and was not subjected to

UVB radiation. Two other dishes containing bacterial suspension were exposed to UVB radiation (lamp Philips; 15 min., 50 $\mu W/cm^2$), while before exposure to radiation there was sterile solution of humic substances (Fluca) added to one of them (final concentration HS 100 mg/dm^3).

Activity of hydrolytic enzymes – by measuring the rate of *fluorescein released* from fluorescein diacetate (FDA) [8]. The quantity of released fluorescein was measured using a Hitachi f-2500 spectrofluorometer at an excitation wave of λ = 480 nm and emission wavelength of λ = 505 nm.

The respiration activity of bacteria was determined with the measurement system OxiTop Control 12. The measurement of *biochemical oxygen demand* (BOD) with OxiTop®-Control was carried out according to the operating instruction provided by the supplier [9]. The incubation was carried out for 12 hours at 22 ^oC. The respiration activity was expressed in mg O_2/dm^3 of bacterial suspension.

Results and discussion

Exposure of hydrolytic enzymes solution to UVB radiation brought about reduction of their hydrolytic activity to 88–76 % of not exposed samples value. Stronger inhibition of enzymatic activity UVB radiation caused for enzymes produced by planktonic strains (Fig. 1). In case of 9 for 20 investigated strains found was minor protective effect of humic substances (Table 1). Enzymes produced by these strains demonstrated higher activity exposed to radiation at HS presence.

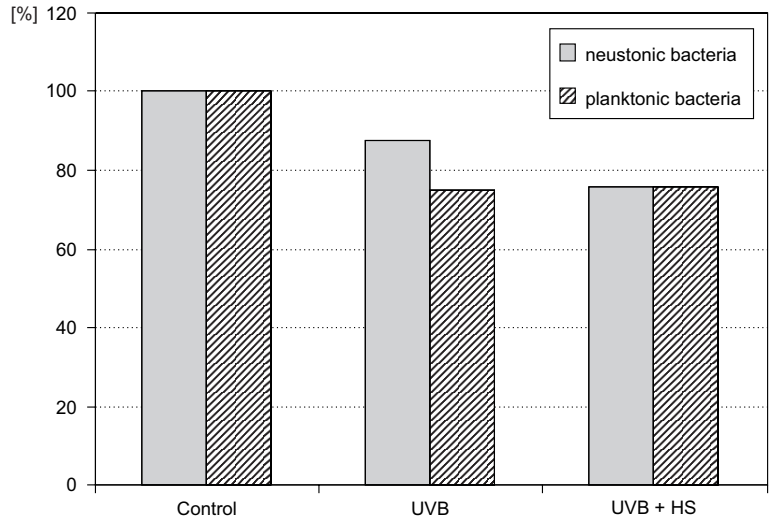

Fig. 1. The activity of hydrolytic enzymes after exposure to UVB radiation (average)

Prior to this study Walczak [10] noticed also directly in the lake water significant differences of hydrolases activity depending on solar radiation. Similar results obtained

Boavida and Wetzel [11] who surveyed phosphatases activity as well as Jorgensen [4] who investigated urease and glucosidase.

Table 1

UVB radiation impact on enzymatic activity of bacteria

Number of strain	Control	UVB	UVB in presence of HS
Neustonic bacteria			
1N	0.365*	0.243	**0.271**
2N	0.714	0.672	0.432
3N	0.183	0.152	0.151
4N	0.347	0.261	**0.300**
5N	0.262	0.210	0.207
6N	0.628	0.467	**0.511**
7N	0.095	0.100	0
8N	0.073	0.072	**0.079**
9N	0.493	0.548	0.427
10N	0.381	0.354	**0.385**
Planktonic bacteria			
1P	0.025	0.026	0.023
2P	0.530	0.372	0.231
3P	0.248	0.116	**0.173**
4P	0.341	0.235	0.229
5P	0.552	0.577	0.511
6P	0.116	0.030	**0.076**
7P	0.396	0.384	0.347
8P	0.134	0.114	**0.116**
9P	0.488	0.328	**0.401**
10P	0.415	0.334	0.320

Expanations: * – concentration of fluorescein (μg/cm^3); HS – humic substances; ■ – protective operation of HS.

All of these enzymes (urease, phosphatase, and glucosidase) are categorized as extracellular hydrolases. Therefore, they are secreted to the external environment, where there is no protection against the harmful effect of UV. Furthermore, it was also observed that extracellular enzymes unbound to organic matter are inhibited to a much higher degree (50–60 %). In contrast, inhibition of enzymes bound with organic matter equaled only to 30 % [3].

Experiments on respiratory activity of bacteria with application of measurement system OxiTop Control proved significant reduction of oxygen use in samples exposed to UVB radiation compared with not expose bacteria (Fig. 2). Oxygen use for exposed samples made up 50–66 % of bacteria not subjected to UVB radiation values on average

(Fig. 3). Inhibition of respiration rate was found for both, planktonic and neustonic bacteria. No statistically significant difference was observed in respiratory activity whether or not HS accompanied bacteria exposed to UVB radiation (Fig. 2).

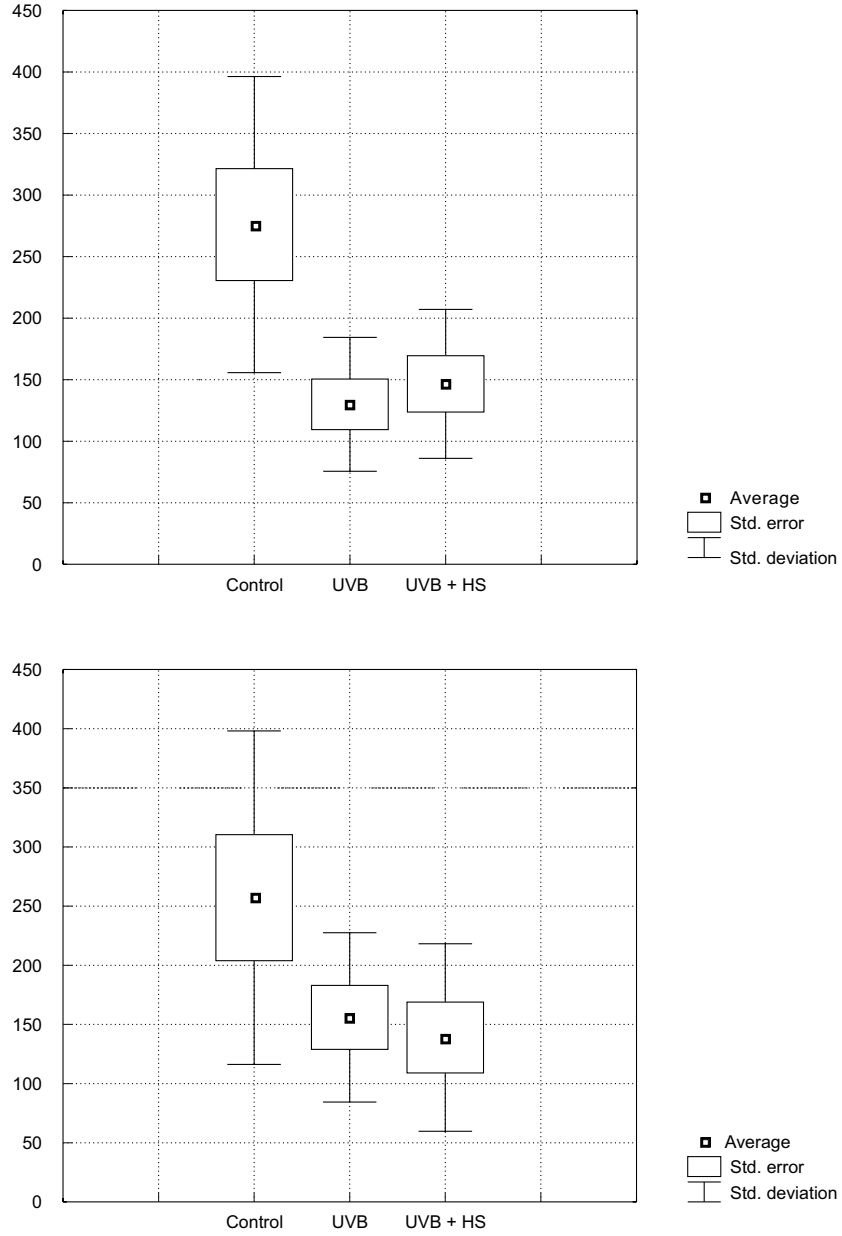

Fig. 2. UVB radiation impact on respiratory activity of bacteria (the *average respiration activity* was expressed in [mg O_2/dm^3 of bacterial suspension])

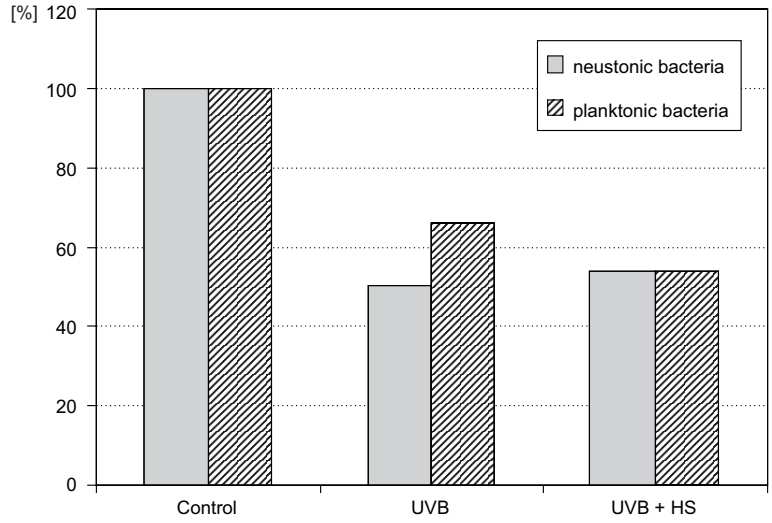

Fig. 3. The respiratory activity of bacteria after exposure to UVB radiation (average)

Unfortunately, no prior results of studies analyzing the effect of solar radiation on respiratory activity of neustonic bacteria have been found in the available literature, which prevents broader comparison of the presented results. Former Walczak study [10] regarding cellular dehydrogenases activity participating in electrons' transport within respiratory chain did not prove significant impact of solar radiation on these enzymes activity. Beside dehydrogenases, in cellular respiration take part also many other enzymes. Hindering their activity by UVB radiation can result in reduction of bacteria respiratory activity.

The experiments did not prove unambiguous protective operation of HS. Only for selected bacteria strains exposure to UVB radiation at HS presence resulted in lower reduction of respiratory and hydrolytic activity. On one hand, HS absorb some part of radiation. On the other hand, photochemical reactions produce many compounds that are harmful for bacteria. According to Scully et al [12], reactive forms of oxygen also play an important role in the decomposition of organic matter under the influence of UV. These compounds are produced in water in the presence of UV and organic matter and may indirectly inhibit the activity of extracellular enzymes.

Summing up, the conducted research confirmed and expanded earlier reports regarding the importance of solar radiation and UV on the activity of bacterial enzymes in aquatic environments. However, it should be noted that the effect of solar radiation, including UV, is not limited to simple and direct impacts on bacterial cells. Radiation affects the environment through a wide range of indirect means, eg organic matter photooxidation or the impact on phyto- and zooplankton, which also has a considerable impact on the activity of aquatic bacteria.

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WPŁYW PROMIENIOWANIA UV NA AKTYWNOŚĆ ENZYMATYCZNĄ I ODDECHOWĄ BAKTERII NEUSTONOWYCH I PLANKTONOWYCH

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Abstrakt: Badania prowadzono w strefie pelagialu jeziora Brzeźno (Bory Tucholskie). Próbki wody pobierano z mikrowarstwy powierzchniowej (MP) i wody podpowierzchniowej (WPP). W trakcie badań oznaczono aktywność oddechową bakterii z zastosowaniem systemu pomiarowego OxiTop Control oraz ogólną aktywność enzymów hydrolitycznych, mierząc tempo uwalniania fluoresceiny z dioctanu fluoresceiny. Badania prowadzono w dwóch układach doświadczalnych: bez *substancji humusowych* (SH) i w obecności tych substancji. W wyniku przeprowadzonych badań stwierdzono zdecydowanie niższą aktywność oddechową i hydrolityczną bakterii po naświetleniu UVB, w porównaniu z bakteriami nie naświetlonymi. Nie stwierdzono jednoznacznie ochronnego działania SH, jedynie w przypadku niektórych szczepów naświetlanie bakterii UVB w obecności SH powodowało mniejszy spadek aktywności oddechowej i hydrolitycznej.

Słowa kluczowe: promieniowanie UV, mikrowarstwa powierzchniowa, bakterioneuston, aktywność enzymatyczna, aktywność oddechowa