ABSTRACT: The range of solar radiation reaching the air-water inter-phase, medium wave UV radiation, i.e. UVB 290–320 nm and UVA 320–400 nm, is of the highest biological importance due to its harmful effects. Radiation within this range causes DNA damage (lethal effect) or limits the growth of organisms by inhibiting enzyme synthesis, reducing active transport, or by inducing mutations.

The studies were carried out in 2007 based on samples water collected from surface microlayer (SM) (up to 150 μm) and subsurface water (SSW) (25 cm) of pelagic zone of eutrophic lake. The representative collection of bacterial strains was isolated from collected samples. The following are measured: the DNA and cellular protein synthesis activity, respiration activity of the bacteria and activity of hydrolytic enzymes in control cultures, subjected to UVB radiation (applied dose 100 mW cm\(^{-2}\)) and with and without humic substances (HS) (final concentration 100 mg L\(^{-1}\)) playing role of compounds potentially protective from UV radiation. UVB irradiation had the strongest inhibiting impact on production of DNA in bacterial cells (12–23% of that in non-irradiated samples). UVB radiation also inhibits the synthesis of cellular protein (27–43% of that in non-irradiated samples) and bacterial respiration activity (44–48%). UVB radiation had by far the lowest impact on the activity of hydrolytic enzymes. HS may function as a protective agent against UV radiation only in DNA synthesis. No significant differences in response to UVB were found between planktonic and neustonic bacteria.

KEY WORDS: surface microlayer, heterotrophic bacteria, UV radiation, humic substances

1. INTRODUCTION

In a vertical profile of a water body, the surface microlayer (SM) constitutes its outermost boundary. This layer forms a particular chemical and physical environment, which differs substantially from subsurface water (Hillbricht-Ilkowska and Kostrzewska-Szlakowska 2004). In general, surface microlayer contains an increased number of bacteria, which are referred to as bacterioneuston. Due to the specificity of its habitat, bacterioneuston is more exposed to stressful ecological factors than microorganisms inhabiting deeper layers of the water column.

Insolation is one of the primary factors affecting the number and activity of the bacterioneuston. Intense solar radiation reduces the abundance of all microorganisms in a water body, but the amount of harmful UV radiation reaching water below the surface microlayer is significantly lower due to absorption and diffusion. Surface microlayer is
by far the most irradiated layer of a water body, and the amount of solar radiation, including UV, reaching SM is basically the same as that reaching land surfaces.

Considering the entire range of solar radiation penetrating the water–air interphase, medium-wave UV radiation, that is UVB 290–320 nm and UVA 320–400 nm, is of the highest biological significance due to its harmful effect. This range of radiation is responsible for DNA damage (lethal effect) or inhibition of organism growth disturbing enzyme synthesis by limiting the active transport and inducing mutation, causing the sublethal effect (Cockell 2000, Goosen and Moolenaar 2008).

Numerous studies demonstrated that solar radiation, particularly UVB radiation, is detrimental to the production of bacterial biomass and the activity of exoenzymes (Kaiser and Herndl 1997, Chröst and Faust 1999, Sommaruga 2001). It is also noteworthy that photo-oxidation of Dissolved Organic Matter (DOM) and Particulate Organic Matter (POM) is induced by UV radiation, and results in the release of considerable quantities of easily assimilable organic matter to the environment, which may increase the activity of bacterioplankton (Herndl et al. 1997, Jorgensen et al. 1998). Furthermore, the surface microlayer contains a considerable amount of humic substances (HS), which constitute up to 50% of DOM (Sieburth 1983). And it is known that humic substances effectively absorb UV radiation, thus protecting microorganisms against its adverse effects (Górniak 2004).

It is extremely difficult to obtain accurate measurements of metabolic activity of bacterioneuston under in situ conditions. This difficulty arises from the fact that under natural conditions it is impossible to control specific physicochemical parameters that affect the activity of organisms. For that reason, present study investigated the impact of UVB on the bacterial activity, namely, DNA synthesis, cellular protein synthesis, activity of hydrolytic enzymes, and respiration under controlled laboratory conditions. Another purpose of the study was to examine whether humic substances can shield bacteria from harmful UVB radiation.

2. MATERIALS AND METHODS

The study analyzed strains of heterotrophic bacteria isolated from surface microlayer and subsurface water.

Water samples were collected in the summer of 2007 in the pelagic zone of eutrophic
Table 1. Morphometric and trophic characteristics of study lake – Lake Brzeźno (Jańczak 1997).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (ha)</td>
<td>71.6</td>
</tr>
<tr>
<td>Maximum depth (m)</td>
<td>9.7</td>
</tr>
<tr>
<td>Mean depth (m)</td>
<td>4.4</td>
</tr>
<tr>
<td>Length of shoreline (m)</td>
<td>5550</td>
</tr>
<tr>
<td>pH (1)</td>
<td>7.9–8.4</td>
</tr>
<tr>
<td>Electrolytic conductivity (μS cm⁻¹) (1)</td>
<td>300–390</td>
</tr>
<tr>
<td>Water transparency (m) (1)</td>
<td>0.6–1.0</td>
</tr>
<tr>
<td>Total organic carbon (mg L⁻¹) (2)</td>
<td>5–15</td>
</tr>
<tr>
<td>Chlorophyll a (μg L⁻¹) (2) (1)</td>
<td>5–26</td>
</tr>
</tbody>
</table>

(1) data supplied by Department of Environmental Microbiology and Biotechnology, Nicolaus Copernicus University

Impact of UV on lake bacteria

Lake (Table 1) (Lake Brzeźno) (53°57.5’N, 17°48.6’E) located in forested region (Tuchoła Forest) (Northern Poland).

The samples were taken in June July and August 2007 year. Surface microlayer (SM) water samples were collected using the Garrett (1965) technique with a plexiglas plate capturing 150 mm layer of water. Subsurface water (SSW) with plankton bacteria was collected from the depth of ca 25 cm with an automatic pump. Collected samples (10 replications) were transferred to sterile glass containers.

Bacterial strains were isolated by surface inoculation into TSA medium plates (Difco). After a 6-day incubation at 20°C, representative strain samples were inoculated into TSA slants.

Strains of isolated bacteria (60 from SM and 20 from SSW) were cultured for 3 days at 20°C in 50 mL of liquid TSB medium. Subsequently, 30 mL samples were collected from each culture and centrifuged for 5 minutes at 10 000 rpm at 10°C. After centrifuging, the supernatant was used as an unpurified solution of extracellular enzymes, and the bacterial sediment was suspended in 30 mL of sterile physiological salt solution. Optical density of bacterial suspension of each strain was adjusted to the same value of 0.5, using sterile solution of physiological salt as diluter.

The bacterial suspension and a solution of extracellular enzyme of a given strain were divided into three 10 mL parts and trans-
ferred to three parallel sterile Petri dishes in tree replications. The first dish was the control, and was not exposed to UVB radiation. The remaining dishes were UVB-irradiated (Philips Lamp, 15 minutes, 100 mW cm\(^{-2}\)), but prior to exposure, a sterile solution of humic substances (Fluca) (final concentration 100 mg L\(^{-1}\)) was added to one of them. DNA synthesis was investigated by measuring the incorporation rate of \(^{3}\)H-methyl thymidine in bacteria according to the Fuhrman and Azam (1982) method. Thus, the activity of DNA synthesis was determined based on the amount of absorbed thymidine, knowing that
there was an average of $2.5 \times 10^{-15}$ g of DNA per one bacterial cell.

Synthesis of cellular protein was examined using labeled $[^3H]$ leucine according to a method described by Kirchman et al. (1985). The amount of produced protein was calculated based on the quantity of incorporated leucine, assuming that leucine constituted an average of 0.073% of bacterial protein mass (Kirchman et al. 1989).

Activity of hydrolytic enzymes was investigated by measuring the rate of fluorescein release from fluorescein diacetate (FDA) (Gillian and Duncan 2001). The quantity of released fluorescein was measured with a spectrofluorimeter Hitachi f-2500 using an excitation wavelength of 480 nm and an emission wavelength of 505 nm.

Respiration activity of bacteria was examined using an OxiTop Control 12 system. Biochemical oxygen demand was determined according to the instruction manual (WTW 1998). Cultures were incubated for 12 hours at 22°C. Respiration activity was expressed in $\mu$g O$_2$ g$^{-1}$ bacterial protein h$^{-1}$. The protein content was determined according to the Bradford (1976) method. All the investigations were done in three replications.

3. RESULTS

UVB radiation had a strong inhibiting effect on DNA synthesis in all examined bacterial strains from both SM and SSW. Experiments, in which humic substances were used as a protective agent, showed that DNA synthesis was also inhibited, but the inhibition rate was significantly lower than in samples without HS (Fig. 1, the presented on figure values are average of at least 180 measures). The inhibiting effect of UVB radiation on DNA synthesis was stronger in planktonic strains than in neustonic strains (Fig. 5).

The present study demonstrated that UVB radiation also inhibits the synthesis of cellular protein (Fig. 2). But, the experiments with HS as a potentially protective agent yielded completely different results than the experiments investigating DNA synthesis. The addition of HS did not only reduce the inhibition rate of protein synthesis, but the opposite, even greater decrease in this activity was observed in the presence of humic substances (Fig. 2). This effect was observed in both planktonic and neustonic bacterial strains, but the inhibition rate of protein production was higher in planktonic strains, in both irradiated samples with HS and without (Fig. 5).

UVB irradiation of hydrolytic enzyme solution caused a slight, statistically insignificant reduction of their hydrolytic activity. Also, the study showed no statistically significant impact of HS on the activity of UVB-irradiated enzymes (Fig. 3).

Investigations of bacterial respiration activity conducted with the OxiTop Control sys-

![Fig. 5. The activity of neustonic (SM) and planktonic (SSW) bacteria after exposure to UVB radiation expressed as % of control (non-irradiated) sample. UV – UVB irradiation, UV (HS) – UVB irradiation in the presence of HS.](image)
tem pointed to a significant reduction in oxygen demand in the UVB-irradiated samples as compared to the non-irradiated cultures (Fig. 4). Oxygen demand in the UVB-irradiated samples constituted on average 44–47% of that in non-irradiated samples (Fig. 5). Inhibition of respiration rate was observed in both planktonic and neustonic bacteria. There were no statistically significant differences in respiration activity of UVB-irradiated bacteria in the presence and absence of HS (Fig. 4).

UVB irradiation had the strongest inhibiting impact on production of DNA in bacterial cells. DNA production in UVB-irradiated samples constituted only 12–23% of that in non-irradiated samples, and only in the case of this process, the protective function of humic substances was clearly observed. In samples irradiated in the presence of HS, DNA production constituted 63% (neustonic strains) and 38% (planktonic strains) of DNA production in non-irradiated samples. UVB radiation had by far the lowest impact on the activity of hydrolytic enzymes (Fig. 5).

4. DISCUSSION

It is widely accepted that intense solar radiation reduces abundances of all microorganisms in a water body, but the amount of the harmful UV radiation that penetrates the water column below the surface microlayer is significantly reduced as a result of absorption and diffusion. Particularly in the presence of high concentrations of dissolved organic carbon (DOC), harmful UVB radiation reaches to a depth of a mere several centimeters (Hessen et al. 1997). According to Zaitsev (1971), the upper 10 cm of water bodies absorbs ca 75% of UV radiation with a wavelength of $\lambda = 254$ nm. Numerous studies pointed to an inhibiting effect of solar radiation, and particularly UVB radiation, on the enzymatic activity and production of bacterial biomass (Kaiser and Herndl 1997, Christ and Faust 1999, Somaruga et al. 1999, Walczak 2008a, b). Furthermore, Vosjan and Zdanowski (2001) reported that UV radiation significantly inhibited bacterial metabolic activity measured by the amount of synthesized ATP.

On the other hand, however, numerous authors reported that the impact of UV and visible light on a total activity of bacterioneuston was low (Williams et al. 1986, Garabetian 1991). After all, UV radiation induces photooxidation of DOM and POM, which results in the release of considerable amount of easily assimilable organic matter to the environment, which in turn may stimulate the activity of bacterioplankton (Häder et al. 1998, Jorgensen et al. 1998, Winter et al. 2001).

The present study demonstrated that UVB-induced inhibition was highest in the case of DNA production in bacterial cells, which, in UVB-irradiated samples, constituted only 12–23% of the production in non-irradiated samples. Davidson (1998), Herndl et al. (1997) and Kaiser and Herndl (1997) obtained similar results regarding the impact of UV radiation on the activity of DNA synthesis and/or secondary bacterial production. These studies showed that following the exposure of bacteria to UV radiation, the activity of DNA synthesis decreased by over 50% in comparison to the control. Moreover, in situ analyses demonstrated that the activity of DNA synthesis in bacterioneuston was significantly reduced by UVB radiation (Walczak 2008b). Sommaruga (2001) reported that during short-term (3–4 h) experiments the rate of thymidine incorporation was reduced by nearly 70%.

The present study also demonstrated that UVB radiation had an inhibiting effect on the synthesis of cellular protein. Sommaruga et al. (1997) obtained similar results. But, the comparison of UVB impact on the activity of DNA and protein syntheses clearly showed that protein synthesis was inhibited to a much lesser degree than DNA synthesis. This observation pertained to both neustonic and planktonic strains. Denwart et al. (1999) reported even that the process of leucine incorporation was not significantly inhibited by UV radiation.

Jorgensen’s et al. (1998) study indicated that protein synthesis in bacteria was more intense after UV radiation, but in this study, bacteria were irradiated in water collected from a lake. It is certain that under these conditions, UVB-induced photooxidation of organic matter was much more important due to its role in production of particulate matter, easily accessible to bacterioplankton. This
process could be responsible for an increase in total cellular activity, including protein synthesis.

Earlier studies conducted directly in lacustrine water showed significant differences in the activity of hydrolases depending on solar radiation (Walczak 2008a). Similar results were obtained by Boavida and Wetzel (1998) in a study on the activity of phosphatase and Jorgensen et al. (1998) during investigations of the activity of urease and glucosidase. All enzymes belong to exocellular hydrolases, and are secreted to the external environment with no protection against the harmful effect of UV radiation. Earlier studies showed that inhibition of extracellular enzymes not bonded to organic matter was significantly higher (50–60%). In contrast, the inhibition rate of enzymes bonded to organic matter was only 30% (Herndl et al. 1993). In the present study, UVB irradiation of a hydrolytic enzyme solution was responsible for only a slight, statistically insignificant reduction of their hydrolytic activity. Furthermore, a protective impact of organic matter (HS) on the activity of UVB-irradiated enzymes was not observed.

Due to the absence of data describing the impact of solar radiation on the activity of these enzymes in available literature, it was impossible to provide a broader comparison of the presented results. Earlier study (Walczak 2008b) on the activity of cellular dehydrogenases participating in electron transport within the respiratory chain showed no significant impact of solar radiation on the activity of these enzymes. In addition to dehydrogenases, many other enzymes participate in the process of cellular respiration, and UVB-induced inhibition of their activity may lower the respiratory activity of bacteria.

The obtained results also pointed to a significant reduction of the UVB-induced inhibition of DNA synthesis in the presence of SH. During experiments with various fractions of organic matter used as a protective substance, Herndl et al. (1997) observed only 20% decline in the activity of thymidine incorporation after UV irradiation and in the presence of SH. But, Scully et al. (2003) reported that exposing bacteria to UVB radiation significantly reduced their survivorship; at the same time, the author indicated that the decline in survivorship was less substantial when a solution of fulvic acid was added to the bacterial suspension.

The analysis of the rate of cellular protein synthesis in the presence of HS as a potentially protective agent against UVB radiation yielded completely different results from those obtained during experiments on DNA synthesis. The addition of HS did not increase the activity of protein synthesis, quite the opposite, a further decline in this activity was observed. The same results were obtained basically for all analyzed bacterial strains. Furthermore, the study demonstrated that the effect of HS on the respiration activity and the activity of hydrolytic enzymes was not unambiguously protective.

The results showed that HS may function as a protective agent against UV radiation, but only in certain types of metabolic activity (DNA synthesis). In other processes, HS in connection with UV radiation not only did not provide protection, but rather served as an inhibiting factor (cellular protein synthesis). Benner and Biddanda (1998) also demonstrated that the exposure of DOM in surface water to UV radiation may inhibit bacterial activity. On one hand HS do absorb part of UV radiation, but on the other hand photochemical reactions produce numerous compounds, which are detrimental to bacteria. According to Scully et al. (2003) reactive forms of oxygen play an important role in UV-induced decomposition of organic matter. These compounds are produced in water in the presence of UV radiation and organic matter, and subsequently, may inhibit the activity of extracellular enzymes in an indirect manner.

The present study demonstrated that no significant differences in response to UVB were found between planktonic and neustonic bacteria. Agogue et al. (2005) didn't noted the differences in the sensibility of bacterio-neuston and bacterioplankton on the UV radiation. Also Arrieta et al. (2000) didn't found correlation between sensibility on UV radiation (determined as the decrease of incorporation of thymidine and leucine) and the place from which the samples were taken.

The present study confirmed and elaborated the findings of earlier reports on the significance of solar and UV radiation for the microbial activity in an aquatic environment.
It is noteworthy, however, that the effect of solar radiation including UV is not limited to a straightforward and direct impact on bacterial cells. Radiation affects bacteria through a wide spectrum of indirect interrelations, contributing to matter photooxidation or affecting phyto- and zooplankton, which also have a considerable effect on activity of aquatic bacteria.

5. REFERENCES

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