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INFLUENCE OF HUMIC ACIDS (HA), DCMU, AND LIGHT ON THE ULTRAWEAK LUMINESCENCE (UL) OF *CHARACEAE* CELLS

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ABSTRACT

Ultraweak luminescence (UL) within the visible range accompanies physiological processes taking place in Native non-stressed *Nitellopsis Obtusa* cells. Intensity of UL emission is several times higher than the intensity of an environmental medium. The influence of Humic acid (HA), diuron-urea herbicide (DCMU) and photosynthetic active radiation (PAR) on UL intensity and spectral composition for algae cells was investigated. Measurements were made both in neutral and reactive media. An artificial pond water (APW) served as a neutral medium. Algae cells in APW were used as the control samples. Concentrations of specific chemical agents were equal to $8 \cdot 10^{-2}$ mg·mL^{-1}, $4.2 \cdot 10^{-2}$ mmol·L^{-1} for HA and DCMU, respectively, whereas PAR intensity reached 900 µE·s^{-1}·m^{-2}. The influence of HA and light on UL intensity was studied. It was found that by light alone doubles UL intensity, whereas it remains unaffected by DCMU acting alone or even in connection with light. A spectral composition of UL emitted by ***Nitellopsis Obtusa*** cells is modified by HA and DCMU. Both of them caused an increase in the shortwave UL emission and a delay in the central range of the VIS spectrum. DCMU and light acting together, similarly to HA and light, caused the emission to increase significantly at 480 and 660 nm. The only difference between DCMU and HA while acting with light was an increase found in UL intensity at 540 nm under a common DCMU + light influence. This phenomenon was not observed for HA + light action. In the longwave part of the emission spectrum the bands of chlorophyll, dimoles O_2, NADH, FMN and Q_{10} can be taken into consideration. The shortwave part of the spectrum may be related to phytochrom excited molecules, though such a hypothesis requires further investigations.

Key words: ultraweak luminescence, algae (Characeae), humic acids, DCMU, light.

INTRODUCTION

Some biochemical processes in living organisms lead to creation of radicals and molecules in the excited state. Energy necessary for exciting these molecules is obtained from the part of energy liberated from series of exoergic red-ox reactions. Photon stream emission from the excited molecules provides information about their states and the mechanism of energy transfer.

Intact plant cells, placed in darkness, spontaneously emit electromagnetic radiation both in the visible and UV ranges of spectrum [16]. This quasi-stationary radiation has an intensity range $10\text{-}10^4$ photons$\cdot s^{-1}\cdot cm^{-2}$ [14] and it has been named the ultraweak luminescence (UL). This luminescence may indicate that a short-living free radical process of lipid peroxidation has been generated [1]. It has been confirmed that UL in plants is specific to intact chloroplast though it has not observed in thylakoid membranes [2]. It was found that UL emitted from a mitochondria fraction (derived from field pea roots) are of great intensity in the range between 400-650 nm. In that range fluorescence of coenzymes (NADH, FMN, Q10) of a respiration chain [10] was observed. Physiological processes in *Characeae* cells and impact of compounds such as phenols and humic acids lead to changes in both the intensity and spectral composition within the range of 340-700 nm. Such results suggested that at least part of the excited electron transmitters in red-ox processes were deactivated via an emission process.

In the present study, cells of the algae *Nitellopsis obtusa (Characeae)* placed in neutral and reactive media were used as UL sources. Influence of humic acids (HA), diuron- urea herbicide (DCMU) [3-(3,4-dichloro-phenyl)-1,1–dimethylurea] and light [photosynthetically active radiation (PAR)] were studied.

Humic substances, such as humic and fulvic acids affect mitochondria respiration and oxidative phosphorylation [9,17]. Diuron affects hyper polarisation of membranes [8]. It inhibits photosynthesis [7] and may diverse oxidation-reduction reactions in the respiration process.

The aim of this work was to study UL emitted from *Characeae* cells. The study aims at characterising both intensity changes and spectral composition of UL emitted from the cells that exposed to HA or DCMU solutions and light.

MATERIALS AND METHODS

The cells of *Nitellopsis obtusa* used in the experiments were taken from Zagłębocze Lake, near Lublin in Poland, and cultured in an air-conditioned laboratory in an artificial pond water (APW) under a natural day/night regime at room temperature. One day before the experiment, ecorticate internodal cells (about 4-6 cm long and 0.5-0.8 mm in diameter) were separated from the culture and after being rinsed with tap water they were kept for about 1h in a solution containing streptomycin and penicillin G in the amount of 10 $\mu g\cdot cm^{-3}$ and 20 $\mu g\cdot cm^{-3}$, respectively. Such treatment was applied to eliminate artefacts [6] due to bacteria that are likely to be living at the surface of *Characeae* cells. To eliminate artefacts due to long-lasting luminescence from a sensitised chlorophyll, which was observed to last a few hours [7], prior to UL measurements all the samples were kept in complete darkness for about 20 hours. APW composed of NaCl, KCl, $CaCl_2$ in the amount of 0.1 mmol/L each and a 20 mmol/L Hepes buffer was prepared. To achieve pH equal to 7.2 NaOH (10%) was used.

Humic acids were purchased from Fluka AG (Buchs AG), DCMU diuron-herbicide [3-(3,4 – dichlorophenyl)–1,1–dimethylurea] from RdH Laborchemikalien (Riedel-de Haen).

Hepes from Aldrich and Sigma (Poznań, Poland) antibiotics from Polfa (Tarchomin, Poland) and other agents from POCh (Gliwice, Poland).

Light emission in the range of 340-700 nm was detected by a high-sensitive photon-counting system, utilising 9558A photomultiplier tube (PMT) with a S-20 photocathode. Both PMT and the preamplifier were cooled down to 260°K. The amplified signal from the photomultiplier tube was fed into a counter (Camac Electronic System, Polon, Poland) interfaced (PC111, Polon) with a personal computer. The spectral distribution of UL could be measured with ‘cut-off filters’; a set of 20 Russian cut-off filters was used. The filters were rotated stepwise by a microprocessor-controlled stepping motor. This electromagnetic response of the tested organism coded in the set of photocount time series was recorded and analysed by a specially developed computer program. The spectral distribution of UL was calculated and corrected for the integral intensity changes in time, $I_{UL} = f(t)$, transmittance of filters, spectral sensitivity of PMT and a long-lasting phosphorescence of some filters, as described elsewhere [3, 15].

Samples were irradiated with LRFR 400 W and WLSW 330 W lamps made in Poland. During exposition of *Characeae* cells the PAR photon stream density of 900 $\mu Es^{-1}m^{-2}$ covering the range between 400-700 nm was applied. UL intensity was studied in two experimental arrangements:

a) immediately after introducing HA or DCMU to *Characeae* cells solutions,
b) 24 hours after exposing the cells to the chemical agents.

Integral UL intensity (ΣI_{UL}) was calculated on the base of surface under the kinetic curve

$$[I_{UL} = f(t)]. \quad \Sigma I_{UL} = \int_a^b I_{UL}(t)dt \quad \text{where}: a = 10\,s,\ b = 1800\,s.$$

UL spectra of *Characeae* intact control cells and submitted to agent actions (HA or DCMU) were investigated after being placed for 24 hours in darkness in APW, HA or DCMU solutions, respectively. Subsequently, all the samples were irradiated for 5 hours with a photosynthetic active radiation. All the irradiated cells were left in darkness for about 14 hours. Finally, the intensity and spectral distribution of UL were measured again. The measurements were taken in four series. Each sample contained 25 cells of *Nitellopsis obtusa.* The applied concentrations of HA and DCMU solutions amounted to $8 \cdot 10^{-2}$ mg·cm^{-3} and $4.2 \cdot 10^{-2}$ mmol·L^{-1}, respectively.

RESULTS AND DISCUSSION

Living processes of *Nitellopsis Obtusa* cells, like most living organisms, are accompanied by ultraweak luminescence (UL) in the visible spectral range. The presented paper reports the results of the studies dealing with intensity and spectra composition of the radiation emitted by *Nitellopsis Obtusa*. Both studied factors varied during cells interaction with HA, DCMU and light. UL intensity for control cells and the ones subjected to HA and DCMU activity diminishes with time. Probably, it was caused by the cells reacting violently to the occurred change in the environmental conditions that required more time to adapt to new conditions. The relation between the integral intensity obtained for the studied structures and the control one are presented in Tab.1. DCMU activity did not change UL to control integral intensity relation, neither at the start of the experiment, nor 24 hours later, although implicit values of this intensity diminished with time. HA influence on cells of the studied algae is far stronger and leads to an increment in the integral intensity while related to the control sample (1.45) at the beginning of the experiment; 24 hours later every value diminished to one half (0.73) of the previous magnitude. In order to estimate the real influence of the environment on the spectral UL composition of *Nitellopsis Obtusa* cells the measurements were performed 24 hours after cells have adapted to the given conditions. UL spectrum of alga cells in the neutral solution (APW) is presented in Fig.1. It may be divided into two parts. In the shortwave part (340-450 nm) a strong emission was observed at 380 nm. Although it is surprising and difficult to explain, we would like to underline the reproducibility of this result. In its longwave part (450-700 nm) almost constant intensity of UL was observed. It is a range of wavelength that corresponds to many known, biologically important emitters. However, ultraweak luminescence within 450-600 nm range may correspond to the fluorescence of electron carriers in a respiration cycle [11]. The band at 634 nm is known as the band originating from the excited oxygen (dimers) molecules [4], while the next at 685 nm may be emitted by excited molecules of chlorophyll [7].

Table 1. Relative integrated UL intensity from *Nitellopsis Obtusa* cells affected by HA and DCMU solutions action: α-directly after injection, β- 24 hours after exposition to HA / DCMU action

$\left(\frac{\sum I_{c+HA}}{\sum I_c}\right)_\alpha$	1.45±0.05
$\left(\frac{\sum I_{c+DCMU}}{\sum I_c}\right)_\alpha$	1.02±0.06
$\left(\frac{\sum I_{c+HA}}{\sum I_c}\right)_\beta$	0.73±0.07
$\left(\frac{\sum I_{c+DCMU}}{\sum I_c}\right)_\beta$	1.12±0.09
$\frac{(\sum I_c)_\beta}{(\sum I_c)_\alpha}$	0.64±0.07
$\frac{(\sum I_{c+HA})_\beta}{(\sum I_{c+HA})_\alpha}$	0.43±0.03
$\frac{(\sum I_{c+DCMU})_\beta}{(\sum I_{c+DCMU})_\alpha}$	0.73±0.05

The influence of HA and DCMU on I_{UL} emission was studied for a selected wavelength. That influence was estimated by examining the relation of I_{UL} of algae kept in HA or DCMU solutions to I_{UL} of algae cells in the control sample. The results of the studies are presented in Fig.2. Points in the plot are connected by a dashed line to emphasise that not a full spectrum is represented but the relative changes of I_{UL} intensity for a selected λ.

Fig. 1. UL Spectral composition of alga *Nitellopsis obtusa* cells in an artificial pond water (APW)

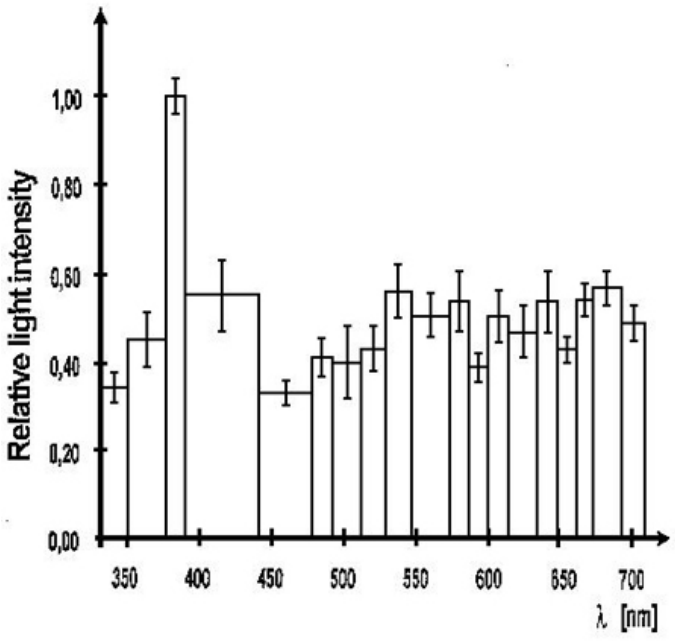
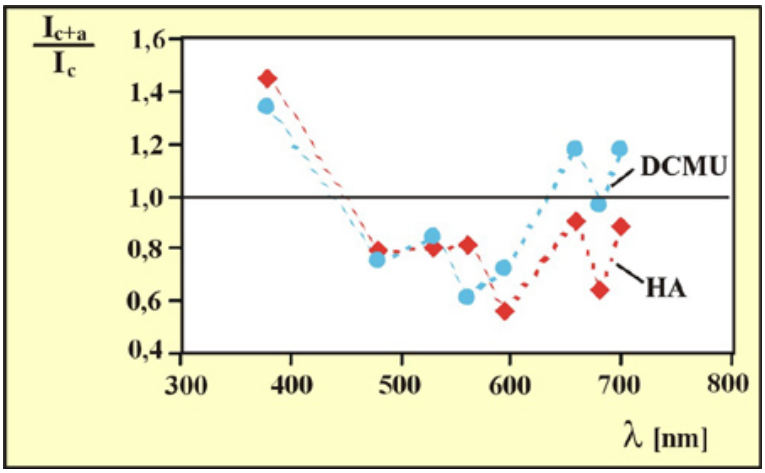


Fig. 2. Relative changes of UL spectral distribution of algae *Nitellopsis Obtusa* cells influenced by HA or DCMU. I_c – Intensity of the control sample (UL intensity of cells in APW), I_{c+a} – UL intensity for cells after agents action. Concentrations: HA – $8 \cdot 10^{-2}$ mg·mL^{-1}, DCMU-$4.2 \cdot 10^{-2}$ mmol·L^{-1}



The results obtained from the studies show that HA and DCMU cause very similar changes in the investigated ranges of spectra. The changes occurs though the values of intensity relations for alga I_{UL} in HA or DCMU at λ>600 nm are different. It may be concluded that both compounds effect in the same way the transport of electrons related to the main living processes in the investigated cells. Diminishing I_{UL} intensity may be interpreted either as an improvement in cells condition, e.i. the improvement of transport conditioning their living processes, or a significant lowering of their living activity.

Our previous experiments showed [11] that the intensity of ultraweak luminescence of algae *Nitellopsis obtusa* in HA solution of 0.1 mg·cm^{-3} concentration caused an increase in the cytoplasm cyclosis rate for alga cells that may be regarded in terms of faster physiological processes. We showed also that the increased cyclosis rate is followed by an increase in the oxygen consumption by *Characeae* cells. Such information in turn allows to make a supposition that the debasement effect of I_{UL} in relation to the control under the influence of low HA concentration (0.08 mg·cm^{-3}) (see Fig.1) is related more to stimulated living processes in cells rather than to a significant impediment.

While studying the influence of cells irradiation with PAR on the intensity change and on spectral composition of the investigated UL, it was found that the integral UL intensity of irradiated cells measured 14 hours after irradiation increased at least twice (Tab.2).

Table 2. Relative integrated UL intensity from *Nitellopsis Obtusa* cells affected by common action HA/DCMU solutions and light

$\frac{(\sum I_c)h\nu}{(\sum I_c)}$	2.48±0.29
$\frac{(\sum I_{c+HA})h\nu}{(\sum I_{c+HA})}$	2.00±0.23
$\frac{(\sum I_{c+DCMU})h\nu}{(\sum I_{c+DCMU})}$	2.61±0.22

Relative UL intensities between 450 and 610 nm (see Fig.3) in relation to the ones from the cells that have not been irradiated (see Fig.1) decrease distinctly with the exception of emission at λ = 540 nm. Contribution of UL emission within the 650-700 nm range increases only slightly. It should be emphasised that light does not influence relative intensity of UL at 380 nm. Table 2, Fig.4 and Fig.5 illustrate the light effect on algae cells placed in the reactive solution. As it was mentioned before, in all the studied samples, light causes integral UL

intensity to increase (Tab.2). DCMU does not contribute to this increase, and HA influence is relatively low. On the contrary, changes of UL spectral distribution after cells irradiation were significant and differences between HA and DCMU were found (Fig.4 and Fig.5). For the cells placed in HA solutions (Fig.4) light affected the central part of spectrum (500-610 nm) where emission decreased but it was distinctly stimulated at 480 nm and 660 nm.

Fig. 3. Spectral composition of UL of algae *Nitellopsis obtusa* cells in a neutral medium (artificial pond water, APW) 5 hours after irradiation by photosynthetic active light (PAR) of 900 µE·m^{-2}·s^{-1} intensity and after being kept in darkness for 14 hours

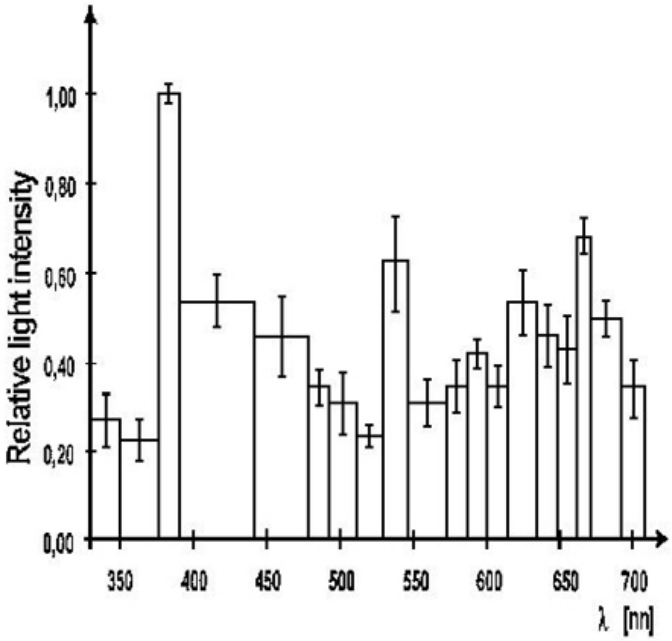


Fig. 4. Relative changes of UL spectral distribution of algae *Nitellopsis Obtusa* cells influenced by HA and light 5 hours after irradiation and after being kept in darkness for 14 hours, I_c – Intensity of the control sample (UL intensity of cells in APW); I_{c+a} – UL intensity of cells after agents action (HA or HA+ hµ). Concentration HA – 8·10^{-2} mg·mL^{-1}, intensity of photosynthetic active light (PAR) 900 µE·m^{-2}·s^{-1}

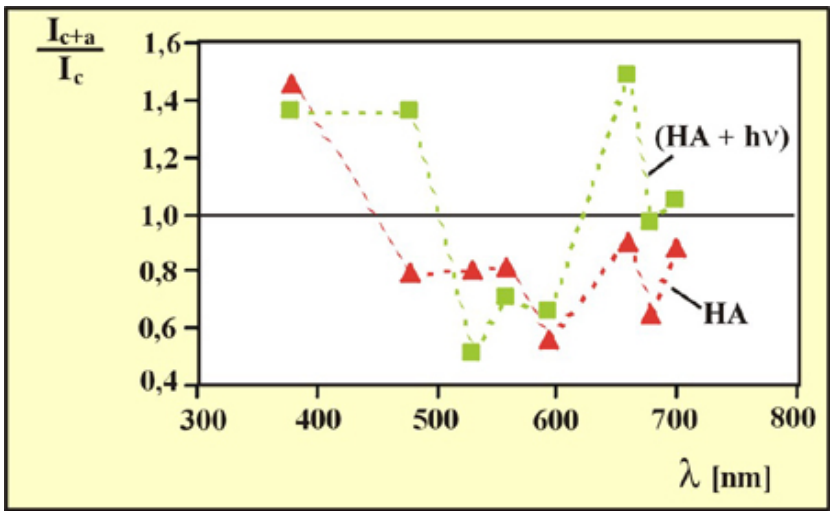
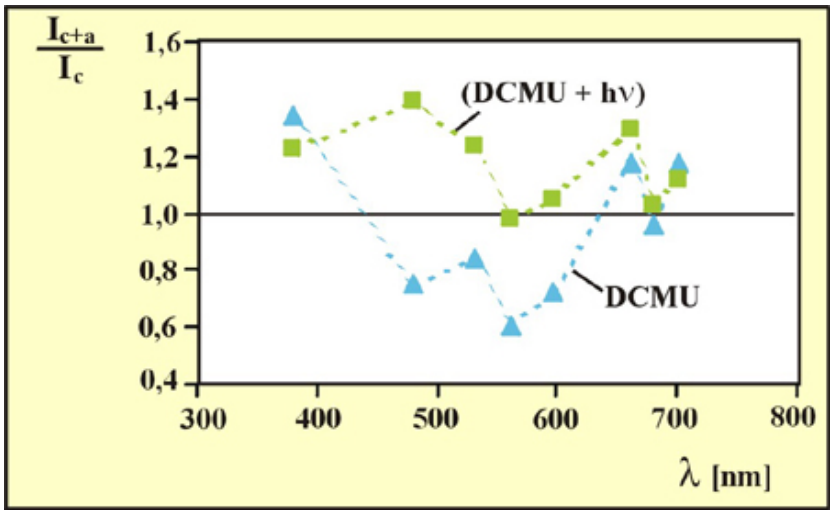


Fig. 5. Relative changes in UL spectral distribution of algae *Nitellopsis Obtusa* cells influenced by DCMU and light 5 hours after irradiation and after being kept in darkness for 14 hours, I_c – Intensity of the control sample (UL intensity of cells in APW), I_{c+a} – UL intensity of cells after agents action (DCMU or DCMU+hν). Concentration DCMU·4.2 mmol·L^{-1}, intensity of photosynthetic active light (PAR) 900 µE·m^{-2}·s^{-1}



For the cells in DCMU solutions, light (PAR) causes an increase of relative UL intensity almost in all the researched ranges with an exception of λ= 570 nm and λ =680nm.

It is known that light causes activation of photosynthetic enzymes, for which not only chlorophyll and its precursor, but also fitochrom is responsible [5, 12]. Increased UL intensity at wavelengths 480 and 660 nm originating from PAR radiation may be linked to the emission of excited phytochrome molecules. Activation absorption spectrum of phytochrome has its maxima at 360 and 440 nm, and at 660 nm and 730 nm, in the shortwave and in long-wave part of the spectrum, respectively.

CONCLUSIONS

Spontaneous UL has been observed in a great variety of plants tissues. Both size and suitability of algae *Characeae* cells proven by serving as a model material for electrophysiological investigation over many years, contributed greatly to our choice of plants to be investigated. The UL intensity from such cells shows a measurable signal which can be easily modified by many factors [4, 11]. UL spectra from these cells manifest a broad emission within 480-610 nm range and emission remains significant even at 380 nm. Such a phenomenon is unique for green plants whose ultraweak emission is as a rule greater than 650 nm [14].

In the presented paper three factors, namely HA, DCMU and light, were examined with regards to their different influence upon UL intensity and spectral composition of *Nitellopsis obtusa* cells.

Light (PAR) causes a significant increase in the integral UL intensity, even 14 hours after the irradiation has been interrupted. DCMU influences this intensity neither alone nor in connection with light. Spectral UL composition of *Nitellopsis Obtusa* changes both under HA and DCMU influence. Both factors lead to the increased UL emission in the shortwave part of spectra, at 380 nm, and at the same time, decrease this emission in the longwave part. Joined activity of both chemical factors and light evokes increased luminescence at 480 nm and 660 nm. The difference concerned the stimulation of luminescence at 540nm after DCMU-light common action.

REFERENCES

1. Boveris A., Cadenas E., Reiter R., Filipowski M., Nakase Y., Chance B.; 1980. Organ chemiluminescence: noninvasive assay for oxidative radical reactions. Proc. Natl Acad. Sci. USA (Plant Biol.); 77: 347-351.
2. Hideg E., Inaba H.; 1991. Biophoton emissions (ultraweak photoemissions) from dark adapted spinach chloroplasts. Photochem. Photobiol.; 53: 137-142.
3. Inaba H.; 1988. Super-high sensitivity systems for detection and analysis of ultraweak photon emission from biological cells and tissues. Experientia; 44: 550-559.

4. Jaśkowska A., Borc R., Milczarek i., Dudzial A. and Śpiewla E.; 2001. Kinetics studies of ultraweak luminescence induced by ascorbic acid in characeae cells and their structures. Luminescence; 16: 51-56.
5. Kopcewicz J.; 1980. Rola fitochromu we wzroście i rozwoju roślin. [Phytochrome role in growth and development of plants]. Wiadomości botaniczne; 24; 1; [in Polish].
6. Krauss W.; 1962. Physiology and biochemistry of algae. Lewin R.A. (ed.), New York, Academic Press, ; 67-84.
7. Lavorel J.; 1980. A study of dark luminescence in chlorella; background, luminescence, 3-(3,4-dichlorophenyl)-1,1 dimethylurea-triggered luminescence and hydrogen peroxide chemiluminescence. Biochim. Biophys. Acta; 590: 385-399.
8. Lucas W.J., Ogata K.; 1985. Hydroxyl - and Bicarbonate - Associated Transport Processes in Chara corallina. Studies on the Light Dark Regulation Mechanism. J. exp. Bot.; 36: 1947-1958.
9. Milczarek I. 1983. Vlianie 1,4-benzochinonov i gumusovych vieščestv na bioenergetičeskie processy w mitochondriach.[Influence of 1,4-benzoquinons and humus matter on bioenergetic processes in mitochondria] VIII International Symposium "Humus et Planta"; Prague, CSSR; 423-426; [in Russian].
10. Milczarek I.;1994 Spontaniczna i modyfikowana ultrasłaba luminescencja z korzeni peluszki oraz izolowanej z niej frakcji mitochondrialnej [Spontanoeous and modified UL. luminescence from peluszka roots and its isolated mitochonrial fraction]. Abstracts, 30th Polish Biochemical Society Session.; 270-271; [in Polish].
11. Milczarek I., Jaśkowska A., Gołębiowska.D.; 1994. Effect of humic acid and polyphenols on ultra-weak luminescence from Characeae cells. In: Humic Substances in Global Enviroment and Implications on Human Health; Senesi N., Miano T.M. (eds.) Elsevier Science: Amsterdam; 323-328.
12. Nejfach S.A.;1973. Mechanisms of cell transformations PWN; Warsaw: Poland; 1973. (Polish translated from Russian).
13. Sławińska D., Polewski K., Sławiński J.; 1992. The stress - induced elektromagnetic emission from biosystems: chemiluminescence response of plants to mechanical and chemical damage. Bioelectrochem. Bioenerg, 28: 483-488.
14. Sławiński J.; 1988. Luminescence research and its relation to ultraweak cell radiation. Experientia; 44: 595-5 71.
15. Tryka S.; 1998. Cut– off filter method for light – induced photon emission spectra estimation. Computers Chem; 22: 113-118
16. Van Dyke K.; 1985; Bioluminescence and Chemiluminescence. Instruments and Applications, (ed.) CRC Press, Boca Raton, Fla.
17. Visser S.A.; 1987. Effect of humic substances on mitochondrial respiration and oxidative phosphorylation. The Science of the Total Environment; 62: 347-354.

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