Acta Agrophysica, 2002, 63, 63-86

USE OF ULTRAWEAK PHOTON EMISSION IN AGRICULTURE

J. Sławiński

Department of Radio-and Photochemistry, Institute of Chemistry and Technical Electrochemistry
Faculty of Chemical Technology, University of Technology
Piotrowo 3, 60-965 Poznań

S u m m a r y. All biota spontaneously emit ultraweak electromagnetic radiation ($<10^{-16}$ W/cm^2 for ca 600 nm, from UV to near IR) during the metabolic reactions associated with physiological state. The emission originates from internal exergonic biochemical reactions mainly of peroxyradical character and hence related to homeostasis and its perturbations by stress factors. Detection and analysis of ultraweak photon emission makes it possible to acquire immediate information on biological activities non-invasively. The review reports and discusses recent progress and results of the application of ultraweak photon emission for analytical and diagnostic purposes in agriculture and related areas. Photonic responses from plants, animals and biogenic materials to environmental physical, chemical and microbial influences acquired by means of advanced optoelectronic technology are described and shown to be a new very sensitive and fast channel of vital information.

K e y w o r d s: photon emission, photosynthetic luminescence, oxidative stress, immunoluminescent methods in agriculture, biotechnology and ecology.

INTRODUCTION

This paper reviews an ultraweak photon emission (UPE) i.e. an endogeneous, spontaneous or induced ultraweak luminescence generated by living systems. The review is limited to these aspects which are relevant to applications in agriculture and related fields. It is curious that this universal phenomenon accompanying life processes was discovered half a century ago by Italian physicists who first used photomultipliers for the observation of germinating seeds [16]. Since then researches on UPE have been enormously developed and gained a lot of applications in physics, biomedical and material sciences, agriculture and environment protection. The review has a general character, covers applications of spontaneous and white light-stimulated ultraweak emissions and is based mainly on recent literature.

CHARACTERISTICS OF ULTRAWEAK PHOTON EMISSION

Ultraweak photon emission (UPE, also called biochemiluminescence or biophotons) is the emission of electromagnetic radiation in the spectral range 180-1500 nm from biological systems. This kind of emission is to be distinguished from bioluminescence and is a naturally occurring phenomenon associated with normal, pathological and lethal biochemical and biophysical processes. Such an endogeneous spontaneous emission is observed from all sorts of organisms and their tissues, cells and subcellular components in the living state. The flux of UPE is of the order of $1\text{-}10^4$ photons/s cm^2; in general less than 10^{-16} W/cm^2 (λ = 600 nm). UPE is inherently associated with fundamental biological processes, first of all with the metabolism, the cell division, fertilization and development of an egg, photosynthesis, stress and death of an organism. The basic requirement for UPE is the potential for formation of excited molecules which emit rather than decay to equilibrium. The excited state species P^* must be formed in an elementary reaction which has sufficient free energy ΔG to excite a product P:

$$R_1 + R_2 + \ldots \xrightarrow{excitation\ k_R} P^* \xrightarrow{emission\ k_{fP}} P + h\nu_{p.} \qquad (1)$$

k_R, k_{fP} and k_{fA} are the rate constants of the corresponding processes. The generation of the excited molecule P^* requires an exergonic metabolic reaction with

$$|\Delta G| > hc/\lambda \qquad (2)$$

where c and λ are the velocity and wavelength of light emitted and G is the Gibbs free enthalpy. The value of G is 5-15 times higher than the free energy of the ATP hydrolysis ($\cong$ 36 kJ/mol). Such a high energy event can be considered as a bioener- getic catastrophe and has rather a low probability. The quantum efficiency (Φ) of UPE is defined as the ratio of the number of photons (N_h) to the number of molecules (N_r) that react:

$$\Phi = N_{h\nu}/N_r \qquad (3)$$

Φ - values of UPE are extremely low, of the order of 10^{-15}-10^{-9}. Therefore, the analytical potential of UPE is seriously limited and mainly the processes with intrinsically high Φ - values, e.g. bioluminescence or with specially added chemiluminescent enhancers or sensitizers are used in agriculture, biotechnology, environment protection and biomedical practice. Analytical applications of UPE are based on the proportionality between the intensity of the CL (I) and the rate (w) of the limiting reaction step:

$$I = \Phi w. \tag{4}$$

The reaction rate w may be proportional to the concentration of the analyte to be determined. Thus, measurements of UPE parameters can provide information about: (i) the reaction rate, (ii) its energetics, and (iii) analyte concentration which is proportional to the reaction rate. This relationship is the basic for a variety of bioluminescent analyses employing highly efficient enzymatic luminescent systems of fireflies, bacteria, jelyfish and other. The determination of ATP is the most common and important [10,34,85]. Despite low Φ - values of majority UPE processes, quantitative analytical assays are possible due to extremely sensitive methods of the photon counting technique (see section Methods and Instrumentation below). As a representative example of such analyses; a continuous determination (monitoring) of humic acid in natural water may be cited. Peroxidation of trace amounts of humic acid with MnO^{-}_{4} [56] or $H_2 O_2$ + HCHO [41] generates weak chemiluminescence the intensity of which is proportional to the concentration of humic acid. The detection limit is 50 ppb [41].

Many processes with very low Φ - values can be brightened dramatically upon the addition of: (*i*) highly fluorescent acceptor molecules (*A*) which transfer the excitation energy and reemit it with higher probability than P^*, e.g. rhodamine, chlorophyll, riboflavine etc:

$$P^* + A \xrightarrow{k_{et}} P + A^* \tag{5}$$

$$A^* \xrightarrow{k_{fa}} A + h\nu_A \tag{6}$$

when $k_{et} >> k_{fp}$ and $\Phi_A >> \Phi_P$.; or (ii) chemiluminescent enhancers which react with reactive oxygen species (ROS), e.g. 3-amino-1,4-phthalhydrazide (luminol), 9,9'-dimethylbisacridinium nitrate (lucygenin), and specially designed and synthetized chemiluminescent probes.

This sensitized or enhanced UPE is commonly exploited for numerous sensitive assays in biomedical and environmental sciences, but unfortunately, much less in agriculture.

MECHANISMS AND SOURCES OF UPE

Mechanisms and sources underlying UPE are not yet fully understood. Three hypotheses trying to explain the origin, mechanism and functional role of UPE are currently proposed.

1. Radical redox reactions stimulated by the electron leakage from the respiratory chain (bioenergetic imperfections), mainly oxidation of lipids in biomembranes produce electronic excited states (*) of carbonyl compounds and oxygen - the products of ROO• recombination (dismutation):

$$2\ ROO\bullet \rightarrow {}^3(>C=O)^* + ROH + {}^1(O_2)^* \qquad \text{(chemiexcitation)} \qquad (7)$$

$${}^3(>C=O)^* \rightarrow {}^1(>C=O) + h\nu\ (\text{blue})\ {}^1(O_2)^* \rightarrow {}^3O_2 + h\nu\ (\text{red})\ \text{(emission)} \qquad (8)$$

In the living organism under stationary conditions (the steady state far from thermal equilibrium) radical redox reactions are counterbalanced by reactions scavenging detrimental ROS that results in a stationary flux of UPE. Any perturbation disturb this fine balance which leads to changes in parameters of UPE. Majority of experimental data relatively well prove this hypothesis of imperfections [10,34,77,85,93,95].

2. The hypothesis of coherent photon field within double helice of DNA (photon storage). This field coupled to the conformational (superhelical) states of DNA is claimed to control regulatory processes in the cell, especially proliferation and differentiation. The concept is theoretically well elaborated in terms of quantum mechanics and non-linear optics, but only few experimental data seem to support it [11,13,72].
3. A molecular dynamic model connecting the free energy of ATP hydrolysis with supersonic motion of soliton within polypeptide chains. Energy fluctuations of the conformational states of polypeptide chains can be coherently enhanced by in-phase ATP energy and produce electronic excited states. This relatively new concept is mainly based on quantum mechanical calculations and still waits for falsification [116,117].

METHODS AND INSTRUMENTATION

UPE-based methods are generally capable of the low detection limits, do not require external photoexcitation, and the emitted light carries vast information on the spatial, temporal and energetic characteristics of the light-producing systems. The most commonly used technique for UPE measurements is single photon counting (SPC) with the use of high – sensitivity, low – noise photomultiplier tubes (PMT) cooled to ca -270 K. PMTs with broad spectral sensitivity from UV (180 nm) to NIR (1100 nm) and high signal – to – noise ratio (S/N) have GaAs(Cs), InGaAs(Cs) or multialkali Na – K – Sb – Cs photocathodes [21,35,39].

Methods of measurements of the spectral distribution of UL employ usually a cut-off filters technique or recently, a diffraction grating incorporated into a position – sensitive photocathode and specially designed anode, and are described in detail elsewhere [39,104]. A new detection system based on a dual charge integrating amplifier circuit that employs two GaInAs – InP pin photodiodes for UPE in the near infrared has been setup. The minimum detectable power is 0.9 exp(–15) W that enables to detect $^1O_2$ ($^1\Delta g$) (λ = 1268 nm) from the HCl-H_2O_2-reaction at the concentration of H_2O_2 as low nmolar [55].

Recently, the application of low light level imaging technology allows the spatio (2D)-temporal visualisation of UPE from samples ranging in the size from ca 20 mm to 20 cm. Imaging systems have the spatial resolution ranging from 512–512 to 1024–1024 pixels, read – out noise as low as 3 e^- per pixel which means that a signal as low as 6 e^- can be detected for a S/N ratio of 2. Since the pixel can contain as many as $6x10^5$ photoelectrons at saturation, the ratio between the maximum and minimum detectable signals, i.e. the dynamic range, would be greater than $5x10^4$. Cooled (cryogenic) charge coupled devices (CCD) slow scan cameras allow the image acquisition time from seconds to days, thus to detect and analyse light from a single cell or from very small amounts of analyte. The spectral sensitivity of a CCD sensor covers the 180-1100 nm range and the quantum yield ca 40% at the maximum sensitivity in 500-800 nm. CL and bioluminescence (BL) – based applications may be assessed qualitatively and quantitatively, both characteristic at the micro level (e.g. single mammalian cell) and at the macro level (e.g. petri dish, plants or animals with the size less than 20 cm). The simltaneous measurement of multiple samples (e.g. microtitre tray) significantly increases the efficiency of routine analyses. The problems pertaining to digital low light-level imaging are comprehensively discussed elsewhere [1,24,42,73]. Recent progress and trends in search into the techniques of measuring and analyzing UPE are comprehensively reported in Advances in Biophoton and Biophotonics Research issued by Biophotonic Information Research Center Tohoku Institute of Technology, Japan and monographs and proceedings published by International Institute of Biophysics, Neuss, Germany, pointed in the references. Several selected examples discussed further will illustrate the analytical – diagnostic potential of CL and UPE methods in the field of agriculture and environment protection.

EXAMPLES OF APPLICATIONS OF UPE IN AGRICULTURE AND RELATED AREAS

Spontaneous UPE from spores, grains and seeds

Twelve species of fungi growing on the Sabouraud medium in darkness and illumination (220 lx, 360-780 nm) were tested for their ability to emit UPE. *Boletus edulis, Pestalotia funerea* and *Microsporum gypseum* exhibited UPE, whose intensity exceeded by 100-130% that of the control, i.e. the Sabouraud medium. Other tested fungi did not revealed UPE, but even quenched it. The illumination resulted in the decrease of UPE intensity [100]. Resting spores of *Entomophthora virulenta* with 0 and 97% of germination ability emitted UPE in two distinct phases: a physicochemical one associated with water imbibition (0-24 h) and physiological phase when germination occured. The intensity of UPE correlated with germination potential [98].

UPE from grains and their homogenates of wheat, rye, barley and field pea in the dried phase and during water imbibition was shown to correlate with grains germination capacity. The correlation was better for homogenates [59-61]. Mechanical damage to wheat seeds was shown to increase the light output [105-107]. Also, an excellently good approximations were obtained for imbibing wheat grain between the intensity of UPE, the rate of water uptake and temperature [106,108, 109]. An ample experimental and theoretical elaboration of the biophoton emission as a measure of the germinative ability is given elsewhere [4,96,119]. UPE accompanying germination of soya seeds is the subject of many studies [8,44]. Rice seeds new and aged as well as genetically modified exhibit different characteristics (the rise rate, peak value, attenuation tendency) of UPE in early imbibition period [69,113,114]. There is a consensus on the metabolic source of energy for chemiexcitation. Particularly, oxidative deamination and transamination by flavoprotein dehydrogenase can produce ROS [36]. Therefore, application of chemiluminogenic enhancers such as luminol, lucygenin, adamantyl compounds would be probably helpful for practical applications. On the other hand, detailed studies on UPE observed during different phases of microsporogenesis of larch suggest the nuclear chromatine origin of the emission [13-15]. The process of mitosis can be monitored in its own light using 2-dimensional UPE imaging technique [44,76].

Photo – induced delayed luminescence of plant and animal organisms

Photo – induced delayed luminescence (PDL) is a phenomenon of long – lived photon emission (ms-min) by organisms after their illumination with white light

[103,112]. There are numerous experimental evidence showing a close connection between the state of the organism (homeostasis) and the parameters of PDL.

Many works have been devoted to the application of PDL to determine the germination capacity of seeds.

The PDL of barley seeds shows anti–correlation to the three physiological parameters: germination capacity, water content and temperature. This result suggests that PDL reflects the molecular structure of seeds [4,76,111,112,118].

The kinetics of PDL of tomato seeds can be fitted to a function which is the sum of two independent hyperbolic decay curves:

$$I(t) = t^a b + t^c d \tag{9}$$

where: a and c are the slope factors at the power of t multiplied with the intensity b and d at time $t = 1$ s. This model offers the most predictive value for the germination capacity [67,68,75,101].

PDL of the green plants fits best the hyperbolic decay $I = I_0 (t + t_0)^m$ with 0.82.2. The parameter I_0 depends on the size of the emission system and sensitivity of the light detector. Parameter t_0 depends on the excitation of the system; lower t_0 means higher excitation. The kinetics of PDL can be analyzed in terms of the composite electron band model. According to this model different molecules form an energy–coupled system with composite electron band structure and unique emission properties [67].

PDL of spruce seedlings (*Picea abies)* was shown to be correlated with the development of ethioplasts to chloroplasts [18]. The general stress syndrome induced by $AlCl_3$ (general adaptation syndrome) consisting of inhibition of the cell proliferation and slower uptake of Ca(II), Mg(II), Zn(II), Fe(II) and other divalent cations was also correlated with the m parameter [37].

In studies of detrimental stress factors affecting plants, application of luminescent methods allow fast, simple and sensitive monitoring of the resistance potential. For example, differences in the luminescence of regenerated cucumber plants caused by plant hormones in the medium [9], triazine resistance of wheat and rape plants [62,63], of chilling resistance of cucumbers [78] and other quick tests for plants growing in the natural environment [64] have been elaborated. PDL of Acetabularia acetabulum has appeared to be a sensitive and fast test for herbicide residues in water and soil [65,66].

A great advantage of PDL method is its 10-1000 times higher intensity than that of spontaneous UPE and a short measurement time and possibility to manipulate with the illumination parameters. However, the lack of understanding of the

origin and mechanism of PDL impedes significant step toward highly promising applications.

UPE in the environment protection

Environmental analyses are required to assess the prevalence of chemical or microbiological pollution, to monitor the extent of pollution and an aid to prevent further contamination. Sensitive and specific asays which are also cost–effective are required. Because of the different sample martices involved in environmental monitoring, e.g. soil, water and air, the assays must also be robust and adaptable.

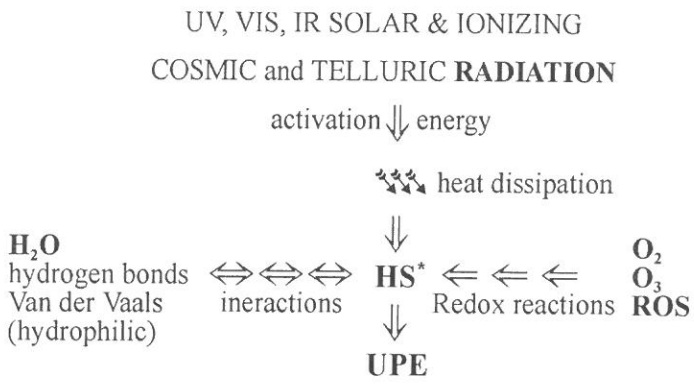
Determination of pollutants in water, soil and organisms

Monitoring of water quality using conventional analysis such as HPLC, BC and GC-MS, although sensitive and specific, requires extensive sample extraction techniques and is often time consuming. Recently, the potential of combined luminescence (high sensitivity) with immune assays (high specificity) in environmental monitoring has been widely recognized [2].

Halogenated organics are probably the most prevalent class of environmental contaminants worldwide (herbicides, insecticides, fungicides, refrige-rants, solvents, water chlorination products). The maximum admissable concentration (MAC) for any single pesticide in water supplies is 100 ng/L and for total pesticides is 500 ng/L – the legal limit set by the Council of the European Communities (with permanent MAC-decreasing tendency). The competitive enhanced CL immunoassay (ECLIA) allows to determine triazine herbicides in this concentration range as well as paraquat (>10 ng/L) [2]. For example, assays for triazine and paraquat determination can be performed using SPC photomultiplier standard luminometers, plate reading luminoscans, image–intensifier–based instruments or CCD cameras. With microtitre plate technique a large number of samples can be handled simultaneously and prepared for endpoint detection in immunoassays, or enzyme enhanced CL (enzyme labels of horseradish peroxidase and xanthine oxidase) and transient labels enhanced CL (e.g. luminol, acridinium compounds). Other BL and CL reactions employed in environmental toxicology and microbiology use luminous bacteria, fireflyy luciferase–luciferin, NADH and cytochrome P–450 isoenzymes [110]. CL immunoassays have many attributes: they combine specificity and sensitivity with ease of use, large sample throughputs, minimal sample cleanup and also offer the possibility of developing assays which can be used away from traditional analytical laboratories. Other applications of chemiluminescent techniques to environmental sciences are reviewed in [83].

UWL and humus substances in soil and aquatic environment

It was shown 30 years ago that during the oxidative degradation of humus substances electronic excited states and free radicals are produced which leads to UPE. In model photochemical and dark chemical oxidation reactions of humus substances generation of reactive oxygen species (ROS: $^1O_2$*, $(^1O_2$*$)_2$, $\bullet O^-_2$, $\bullet$OH, H_2O_2, ROOH, etc.) concomitant with ultraweak light emission was proved [82,91,94-120]. These results have latter been confirmed by many authors [3,17, 33,120] and they revealed previously unknown a vital ecological role of the interaction of humus substances with main components of biosphere: solar radiation, oxygen and water (Scheme 1):



Scheme 1. A simplified representation of interactions of humus substances (SH) with main components of the biosphere

UPE embraces a deep interconnectedness among these four elements. Therefore a concise discussion on the use of UPE as a tool to reveal mechanisms and ecological role of humus substances seems to be justified.

Degradation of humus substances by UV radiation and ozone

There is no doubt that humus is the most efficient target or antenna for solar radiation ever designed by Nature. Therefore, photochemical production of ROS may have significant ecological consequences.

Since ROS are generated on surface and/or inside a spongy colloidal macromolecule of humus and are rather short living, the nearest substrate is just a susceptible moiety of the humus itself, especially o-quinone rings [99]. Thus, humus material also undergo slow photooxidation and still slower auto– and enzymatic

and mineral–catalyzed dark degradative oxidations [30]. Quite recently a tropospheric concentration ozone (O_3)-induced UPE concomitant with humus degradation has been reported [25,26,54]. All these reactions are accompanied by UPE, the intensity, kinetics, energetics (emission spectra) and luminescence decays provide information about the dynamics, rate and mechanism of humus degradation. Degraded material is more: hydrophillic, acid and water soluble, and have lower spin concentration (paramagnetism) and molecular weight than the parent material. The low–molecular products: CO_2 and organic hydroxy– and keto–acids exert significant impact: (i) contribute to the global warming effect (CO_2), (ii) increase the pool of easily assimilated foodstof for microorganisms and (iii) the total humus–degradation process decreases slowly albedo of the surface of Earth and antioxidative capacity of humus substances [12]. Little is known about physiological effect of humus degradation products on higher plants [31,57,58] and still less on the physico–chemical and microbiological changes of the soil.

Humus substances as environmental photosensitizers

Absorption of solar radiation by humus substances can lead to the following reactions:

$$HS + hv \rightarrow {}^1HS^* \xrightarrow{\text{isc}} {}^3HS^* \tag{10}$$

$${}^3HS^* + {}^3O_2 \begin{cases} \rightarrow {}^1HS^+ + \cdot O_2^- \\ \rightarrow {}^1HS + {}^1O_2^* \end{cases} \tag{11}$$

where isc is the intersystem crossing and ^{1,3} indexes are multiplicities; singlet and triplet states, respectively. The generated ROS attack xenobiotics that undergo degradation and mineralization. Such processes are observed in natural waters like lakes, rivers and even see regions close to rivers estuaries containing humus material. Slow degradation/mineralization reactions are accompanied by UPE, the intensity I and kinetics $I=f(t)$ of which reflects the rate and kinetics of the reaction(s) [25,30,94]. An increasing tropospheric UV-radiation intensity resulting from the stratospheric ozone depletion strongly accelerate these processes [17,31,82].

Photoprotective and antioxidative role of humus and its correlation with UPE

Humus substances and closely related dark biopigments – melanins, not only absorb all kinds of radiation and efficiently convert it into heat, but also act as active scavengers of free peroxyradicals induced in electrochemical [12] and photochemical

[90] processes. It has been proven that humus substances (HS) quench electro– and photoinduced chemiluminescence of many model reactions just by scavenging ROO radicals transforming them into less active ones:

$$ROO\bullet + HS \rightarrow ROOH + S\bullet . \quad (12)$$

Such radicals (S•) due to a high resonance energy do not support progression of radical chain reactions and are stable. Thus, HS and melanins reveal a double face: they are strong antioxidants which protect biota against the detrimental effect of ROS leading to the oxidative stress and can also act as photosensitizers and/or substrate of ROS. Our knowledge about these two opposite functions of HS and melanins and their role in agriculture is very limited and requires further exploration. Quantitative chemiluminescent assay of HS in natural water has been elaborated as mentioned previously [41,56].

Photosynthetic delayed luminescence and bioluminescence as an indication of stress and gene expression

One of the most universal indicator of stress in photosynthetic plants is a reduction in the rate of photosynthesis, which is reflected by an increase in delayed fluorescence (Strehler radiation, ns-ms time-regime) and ultraweak chemiluminescence (ms-min) from the plants chlorophyll [103,112]. Chlorophyll fluoresces from approximately 680 to 780 nm and at the same region there are two oxygen absorption bands at $\lambda = 690$ and 760 nm. The absorption cross section of the lines in these bands are sufficiently strong to ensure that little sunlight at these λ reaches the Earths surface through the atmosphere. The plant fluorescence sensor operates as a Fraunhofer line discriminator, detecting light at the cores of the lines comprising these atmospheric O_2 bands. The sensor works at the principle that, as the light collected from fluorescing plants passes through a cell containing O_2 at low pressure, the gas will absorb some of the light and re-emit it as photons that can be detected with a PMT. The induced fluorescence signal provides an immediate, absolute measure of the plants fluorescence intensity in the narrow bands in which the device responds. In the field, the sensor can remotely collect two-band fluorescence information from target plants from as far as 200 m. This direct measurement of light emission from plants offers the most reliable way to probe plants biophysical processes. Thus, the fluorescence device can early detect plant stress resulting from water and nitrogen deficiencies, as well as other applied

stresses, before there are any visible signs. This allows timely intervention minimizing damage to a crop [22].

Studies of photoinhibition of cucumber, wheat, rape and triazine-resistant rape line growing in high irradiance and chill have been performed using the chlorophyll a fluorescence induction and PDL [18,64]. The results indicate that the luminescence parameters of PDL kinetics inform about the structure and function of the primary quinone acceptors Q_A and Q_B in photosystem II. They can be also used for estimation of the degree of photoinhibition, especially the regularity of primary photosynthetic reactions course.

Bioluminescent proteins – luciferases from bacteria, jellyfish and fireflies are useful tools in molecular biology and agriculture not only as reporter proteins, but also because of the sensitivity, reproducibility and simplicity of the corresponding detection assays. An exciting example of bioluminescence application is its use as an *in vitro* indicator of denaturation processes. It is possible to treat living cells with environmental contaminants or altered physical conditions such as light, temperature, pH, osmotic shock, electrical field, oxygen deprivation and assess their effects on physiological response by monitoring real–time changes in the BL activity. Luciferase can be also used as an *in vivo* reporter of gene expression: luciferase gene has to be brought to the promoter region of the gene of interest. Due to the null background luminescence of luciferase, the quantification of very weak promoters is possible [71,102,115]. In another project promoters of dehalogenases (cleavage of the C-Cl bond) were selected, amplified and inserted into a multiple cloning site on a plasmid, upstream from *Vibrio fisheri lux* CDABE. These plasmids were used to transform several bacterial strains to generate bacterial constructs which respond by increased bioluminescence to the pre–determined target haloorganics (detection of 4-Cl-benzoic acid, 4CBA). The bacterial construct displays a marked specificity towards haloderivatives of 4CBA that offers a powerful tool for the selective determination of haloorganics [2,110].

The use of UPE to the determination of food quality and deterioration of food products

There are permanent attempts to apply UPE, bio- and chemiluminescence for evaluation of food quality. This approach is justified as these luminescences, particularly UPE, represent a holistic integrated responses of properties and structure of a biogenic material to external influences (see Table 1). In recent years compelling evidence has been accumulated that spontaneous or photoinduced UPE of certain

Table 1. Basic features of the ultraweak–luminescence–response to internal and environmental detrimental stress factors

Holistic response	Combines the effect of stress on the homeostatic system (the cell, tissue or organism); presents a synergetic superposition of positive/negative feedbacks of processes influencing luminescence.
Homeostasis-dependent sensitivity of the photonic (luminescence) response	High or low depending on the efficiency of regulation mechanisms (amplification or damping), quantum yield of excitation/emission and the rate of processes. Can be enhanced and specified by e.g. chemi-fluore-scent probes.
Multiparametric information	Contains information about: species-specificity of a stress factor, the rate and energetics of chemiexcitation process, its heterogeneity and dynamics.
Discrete character of the photonic signal (luminescent response)	Inherently quantified signal (photon count series) is ideal for statistical (numerical) procedures, e.g. analysis of stochastic processes, correlation analysis etc.

food products can provide information about the food quality and its resistance to microbial, physical and chemical deterioration. Chemiluminescence–based methods have been developed for monitoring of the deterioration of food [40,51,74], detection of irradiated food [7,53] and investigation of reactions of water with cereal products [28,86].

Holistic response combines the effect of stress on the homeostatic system (the cell, tissue or organism); presents a synergetic superposition of positive/negative feedbacks of processes influencing luminescence

Homeostasis-dependent sensitivity of the photonic (luminescence) response-high or low depending on the efficiency of regulation mechanisms (amplification or damping), quantum yield of excitation/emission and the rate of processes can be enhanced and specified by e.g. chemi–fluorescent probes.

Multiparametric measurements provide information about: species–specificity of a stress factor, the rate and energetics of chemiexcitation process, its heterogeneity and dynamics.

Discrete character of the photonic signal (luminescent response) i.e., inherently quantified signal (photon count series) is ideal for statistical (numerical) procedures, e.g. analysis of stochastic processes, correlation analysis etc. The oxidative deterioration of food products has received much attention in connection with its pathological effects. It is intrinsically associated with slow auto- and peroxidative exergonic reactions in which electronically excited light-emitting molecules are generated.

Therefore, the rate, energetics and mechanisms of oxidative deterioration have been recently successfully examined using UPE measurements [28,40,70,82].

An ultrasensitive slow scan charge coupled device (CCD) camera Night Owl Molecular Light Imager was employed to monitor the surface intensity I distribution (single photon counting imaging, SPCI) and kinetics $I=f(t)$ of UPE from a variety of plant oils subjected to oxidative deterioration stimulated by ozonation and photooxidation [76]. It was found that the increase in the intensity I of UPE was correlated with the rate of oxidative deterioration of oils exposed to oxygen ozone or UV-VIS radiation. The effect of chlorophylls as a photosensitizer, vitamin C and E as a radical chain-breaking antioxidants and Fe and Ni salts as catalysts of hydroperoxides decomposition has been proved [28,82].

UPE from 7 species and varieties of cereal grains and rye bread was monitored as a successiveSPCI by means of a high sensitivity slow scan CCD camera Night Ow_l. The interaction of water with air-dried intact and damaged grains enhanced CL intensity and revealed interspecies differences in SPCI. UPE of rye bread slices exposed to the UV-VIS radiation or ozone was monitored by the SCPI and found to reflect the rate of cereal food deterioration [28,29,82]. The results illustrate potential use of the SPCI technique as a fast, sensitive and noninvasive method for monitoring kinetics and spatial distribution of oxidative reactions in plant food products, the field of cereal food sciences.

UPE and wood weathering

The weathering process of the surface of wood exposed to out of doors results in defibration, loss of original colour and greying and has serious consequences for wood technology. Pollutants, singlet oxygen, ozone and UV radiation enhanced by the ozone layer depletion, moisture and acid rains are the major weathering factors. Freshly cut specimens of wood do not exhibit any detectable photoinduced UPE or EPR signal. The kinetics $I=f(t)$ and emission spectra $I=f(t)$ of photoinduced (irradiation of wood samples with a xenon lamp) UPE were measured [79,81]. The similar kinetics for different types of wood can be best fitted by two-exponential sum:

$$I(t) = a_1 \exp(-t/a_2) + a_3 \exp(-t/a_4) \qquad (13)$$

where: a_1 and a_3 are initial I-value of components and a_2 a_4 are time constants, respectively. All emission spectra have three spectral regions: 450–550, 600–640 and 700-720 nm. Such similarity of the time constants between different types of wood and spectra is suggesting a common mechanism connected with observed

luminescence. Imaging of wood UPE by means of Night Ow_1 CCD camera revealed that wood samples not irradiated with UV+VIS do not emit UPE flux higher than about 100 photons/mm^2 s (λ = 650 nm). A satysfying spatial resolution of the UPE inhomogeneity pattern and textural details were achieved. A significant role of water, polyphenols and cellulose photooxidation contributing to the weathering was well proven [79]. Therefore, the photon imaging of surface luminescent phenomena may be important for searching new and more effective antioxidants and retardants for protection of wood and natural fibers against environmental deterioration.

Evaluation of the viability of domestic animals spermatozoa

There have been made permanent attempts to employ UPE as a fast and sensitive biophysical method for the evaluation of the structural and functional integrity of membranes in domestic animals spermatozoa. The problem is relevant to sperm cryoconservation for artificial insemination. Spermatozoa cells were incubated at temperatures higher than the optimum temperature in the presence of prooxidants.The process of lipid peroxidation was intensified by the redox-cycling Fe(II)-ascorbate system and pure O_2. After various time of incubation $FeSO_4$ solution was injected into cell suspension in order to decompose accumulated hydroperoxides. This resulted in a rapid formation and recombination of radicals accompanied by light flash. Using the single photon counting method and optical cut-off filters, spectra of the intrinsic UPE were measured for the first time without luminescent probes (chemiluminescence enhancers). The spectra (300-850 nm, λ_{max} 630 nm) provided information about energetics of the radical recombination and photon emission. The motility, vitality, O_2-consumption and ATP production in the cells were simultaneously determined. The time course and light sum of UPE was correlated with physiological and biochemical indices. A drop in moti-lity and vitality of spermatozoa was accompanied by accumulation of ATP, increase of UPE intensity and light sum, and decrease in oxygen consumption [19,32,52]. In the presence of μM mM concentrations of α-tocopherol the intensity of UPE decreased while the vitality of bull spermatozoa cells increrased in comparison with the cells incubated with ascorbic acid + Fe(II). Results of these investigation confirm the radical character of UPE and indicate that the main source of radiation emitted are singlet molecular oxygen species and electronically excited carbonyls. Attempts to enhance a very weak signal of UPE and to increase the S/N ratio to the values required for practical applications by the use chemi- and fluorogenic probes are ac-

tually performed [6,23,27]. UPE from developing eggs and embryos has only been sporadically investigated [5,20,38].

UPE as the measure of homeostasis perturbation

The transition from one level of the organisms homeostasis to another under stress is accompanied by changes in intensity I, kinetic pattern $I = f(t)$, spectral distribution $I(\lambda) = f(\lambda)$ and statistical photocount distribution $P = f(n, \Delta t)$ of a stationary UPE. A new conceptual and methodological approach which combines bioenergetic, kinetic and spectral aspects of open systems far from thermal equilibrium (i.e. a living state) with corresponding properties of UPE has been proposed [93,95,97]. It takes into consideration the control biofeedback mechanisms governing energy sources and the activity of electrogenic pumps which maintain homeostasis and are mainly localized in membranes. The energy dissipation in a living system is:

$$\delta = (dS_i/dt)dV \tag{14}$$

where: dS_i/dt stands for the rate of entropy production within the system (organism) per volume of unit. It can be related to the rate of metabolic processes:

$$d = \sum I_i X_i > 0 \tag{15}$$

where the flux (rate) is:

$$I_i = {}_{k=1}\sum{}^{N} L_{ik} X_k \qquad i = 1,2, \ldots, N \tag{16}$$

and the gradient

$$X = 1/T \, {}_1\sum v_1 \mu_1 \tag{17}$$

where: μ_1 is the chemical potential and v_1 is the molecular fraction of reactant l. Ion–radical processes in mitochondrial membranes involving exergonic steps of the metabolic peroxidative reactions underline UPE. Under homeostatic conditions (the steady state) the flux I_r of ion-radicals, ROO•, peroxides or $^1O_2$* is minimal owing to the radical-scavenging reactions. These reactions are generated by inhibitors (mainly antioxidants) present in the membrane, e.g. α-tocopherol, β-carotene and substituted polyphenols and flavonoids as well as enzymes, e.g. SOD, catalase etc. The stationary condition for open systems δ = constant $\rightarrow$ minimum, requires that fluxes I_r, I_a (the flux of antioxidants/inhibitors) and I_f

(unsaturated membrane lipids which serve as the source of substrates for I_r) are counterbalanced. The value of I_r is coupled to UPE by the following equation:

$$I_r = I/\Phi \tag{18}$$

where Φ is the total quantum yield of UPE. The spectral properties of UPE are associated with the free energy ΔG of the elementary step of exergonic reactions by $\Delta G = hc/\lambda$ (Eq. 2), where λ is the short–wave limit of the emitted radiation. Thus, all factors influencing control mechanisms in the membranes have to affect parameters of UPE. The solution of phenomenological equations:

$$I_i = \sum_{k=1}^{N} L_{ik} X_k \quad \text{for } i = 2 \text{ and } i = 3 \tag{19}$$

gives two-dimensional or three–dimensional (hyper) surfaces (a parabola) typical of a self–regulating oscillatory system. The living state emitting electromagnetic radiation (UPE) may be treated as such a system. Therefore, this matematical model describes well homeostatic control (feedbacks) of living organisms and perturbations of homeostasis by stress factors [74,93,95,97]. It is also in agreement with the so called Quality Factor Q:

$$Q = \text{Energy stored in the system/ Energy lost per period } 2\Pi \tag{20}$$

analogous to the energy storage and dissipation in an oscillating electric circuit LC.

The UPE response to stress factors may be also analyzed by statistical procedures: integrated moving average (IMA), autoregression integrated moving average (ARIMA) and systemic analysis using the experimentally aquisited photocount time series [45-48]. The analysis based on photon statistics and correlation analysis (the time–interval measurement of photoelectrons) elaborated quite recently [43] has merit for for noninvasive detection of an UPE signal coupled with physiological and pathological states. It will contribute to more efficient extracting information from faint UPE signals.

REFERENCES

1. Advances in biophoton and biophotonic research. Tohoku Inst. Technol., Biophotonics Information Research Center and Photonics Research Institute, Sendai, 1, 109-204, 2000.
2. **Aherne G.W., Hardcastle A., Saleem P., England N.:** Enhanced chemiluminescent assays for environmental monitoring. In: Bioluminescence and Chemiluminescence current status (Eds P.E. Stanley, L.J. Cricka). John Willey & Sons, Chichester, New York, 91-98, 1991.
3. **Backlund P.:** Degradation of aquatic humic material by UV light. Chemosphere 25, 1869-1878, 1992.
4. **Bajpai R.P., Bajpai P.K., Roy D.:** Ultraweak photon emission in germination. J. Biolumin. Chemilumin., 6, 227, 1991.

5. **Beloussov L.V., Louchinskaia N.N.:** Biophoton emission from developing eggs and embrtos. In: Biophotons (Eds J.J. Chang, J. Fisch, F.A. Popp). Kluwer Acad. Publ., Dordrecht, 121-141, 1998.
6. **Bochenek M., Laszczka A., Sławiński J., Skrzyszowska M., Szcześniak-Fabiańczyk B.:** Integrity of bull sperm membranes double-stained with SYBR14 and PI assessed by fluorescent microscopy and flow cytometry. Ann. Anim. Sci., 26(4), 115-125, 1999.
7. **Boegel K.W., Heide L.:** Luminescent measurements for detection of irradiated food. In: Biological Luminescence (Eds B. Jeżowska-Trzebiatowska, B. Kochel, J. Sławiński, W. Strek). World Scientific, Inc., Singapore, 600-629, 1990.
8. **Boveris A., Varsavsky A.I., Goncalves da Silva S., Sanchez R.A.:** Chemiluminescence of soybean seeds: spectral analysis, temperature dependence and effect of inhibitors. Photochem. Photobiol., 38, 99-104, 1983.
9. **Burza W., Murkowski A.:** Differences in the luminescence of regenerated cucumber plants caused by plant hormones in the medium. Gartenbauwis., 59, 105-108, 1994.
10. **Cambell A.K.:** Chemiluminescence: Principles and applications. Ellis Horwood, Chichester and Weinheim, 2,1988.
11. **Chang J.J., Fisch J., Popp F.A.:** Biophotons, Kluwer Acad. Publ., Dordrecht, 1998.
12. **Chmura J., Sławiński J.:** Assay of antioxidants by the quenching of the anthracene-sensitized electrochemiluminescence. J. Biolumin. Chemilumin., 9, 1-6, 1994.
13. **Chwirot W.B.:** Ultraweak luminescence studies of microsporogenesis in larch. In: Recent advances in biophoton research and its applications (Eds F.A. Popp, K-H Li, Q. Gu). World Scientific, Singapore, 259-285, 1992.
14. **Chwirot W.B., Dygdała R.S., Chwirot S.:** Ultraweak photon emission during microsporogenesis i *Larix europea*. Mill. Cytobios., 44, 95-101, 1985.
15. **Chwirot W.B., Dygdała R.S.:** Ultraweak photon emission in UV region during microsporogenesis i *Larix europea*. Cytobios 65, 25-31, 1991.
16. **Colli L., Faccini U., Guidotti G., Dugnani-Lonatti R., Orsenigo M., Sommariva O.:** Further measurements on the bioluminescence of the seedlings. Experientia, 11, 150, 1955.
17. **Corin N., Backlund P., Kulovaara M.:** Degradation products formed during UV-irradiation of humic waters. Chemosphere, 33, 245-255, 1996.
18. **Drinovec L., Jerman I., Lahajnar G.:** The influence of growth stage and stress on kinetics of delayed ultraweak bioluminescence of *Picea abies* seedlings. In: Biophotonics and coherent systems (Eds L. Beloussov, F.A. Popp, V. Voeikov, R. Van Wijk). Moscow, 439-445, 2000.
19. **Ezzahir A., Godlewski M., Kwiecińska T., Sitko D., Rajfur Z., Sławiński J., Szczęśniak-Fabiańczyk B., Laszczka A.:** Iron-induced chemiluminescence of bull spermatozoa. Appl. Biol. Comm., 2, 133-137; The influence of white light on photoinduced luminescence of spermatozoa, Appl. Biol. Comm., 2, 139-144, 1992.
20. **Foerder C.A., Klebanoff S.J., Shapiro B.M.:** Hydrogen peroxide production, chemiluminescence and the respiratory burst of fertilization. Proc. Nat. Acad. Sci., USA, 74, 3183-3187, 1978.
21. **Gall D., Fisch J., Nolte R., Walkling A.:** Measurement of low-level light emission under lab-conditions. In: Biophotons (Eds J.J. Chang, J. Fisch, F.A. Popp). Kluwer Acad. Publ., Dordrecht, London, 159-181, 1998.
22. **Gaughan R.:** Fluorescence sensor watches over plants. Biophotonics Int., 8(1), 18, 2001.
23. **Godlewski M., Kwiecińska T., Laszczka A., Sławiński J., Szczęśniak-Fabiańczyk B, Wierzuchowska D.:** The effect of DNA fluorophores on the ultraweak photon emission from spermatozoa. In: 8-th Int. Symp. Molecular and physiological aspects of regulatory processes of the organism (H. Lach). Cracow, 132-133, 1999.

24. **Górski Z.:** An attempt to use a CCD camera for imaging of the low-energy -emitters. In: Natl. Symp. Nuclear Technique in the industry, medicine, agriculture and environment protection, Polish Nuclear Soc., Kraków, 277-282, 1998.
25. **Górski Z., Grobelny P., Sławiński J.:** The effect of UV-radiationj and ozone on humus substances. Cur. Topics Biophys., 20, 128-133, 1996.
26. **Górski Z., Gwoździński K., Manikowski H., Sławiński J.:** Chemiluminescence and free radicals of humic acid solutions exposed to tropospheric concentrations of ozone. Clin. Lab., 7/8, 409, 2000.
27. **Górski Z., Laszczka A., Szczęśniak-Fabiańczyk B., Sławiński J.:** The use of luminescence probes and a charge-coupled device (CCD) camera Molecular Light Imager for recording photon emission from bull spermatozoa. In: Molecular and physiological aspects of regulatory processes of the organism (Ed. H. Lach). Int. Symp. Polish Network of Molecular and Cellular Bio-logy UNESCO/PAS, Kraków, 131-133, 2000.
28. **Górski Z., Sławińska D.:** Application of chemiluminescence imaging in food sciences. Clin. Lab., 7+8, 409, 2000.
29. **Górski Z., Sławińska D.:** Imaging of the water-induced chemiluminescence in various grains and cereals In: Properties of water in foods (Ed. P. Lewicki). Proc. 9th seminar, Warsaw Agricultural University, Dept. Food Eng., Warsaw Univ. Press, 209-220, 1998.
30. **Górski Z., Sławińska D.:** Chemiluminescence imaging of humic acid interactions with UVradiation and ozone. Clin. Lab., 7+8, 410, 2000.
31. **Grabikowski E., Pieniawski R., Puzyna W., Sławiński J.:** The influence of photooxidation products of humic acids on germination and growth of wheat seeds. Zesz. Nauk. Akad. Roln., Szczecin, Roln. XVI, 64, 117-127, 1977.
32. **Gumińska M., Kedryna T., Laszczka A., Godlewski M., Sławiński J., Szczęśniak-Fabiańczyk B., Kwiecińska T., Rajfur Z., Wierzuchowska D.:** Changes in ATP level and iron-induced ultraweak photon emission in bull spermatozoa caused by membrane peroxidation during thermal stress. Acta Biochem. Polon., 44, 131-138, 1997.
33. **Haag W.R., Hoigne J.:** Photosensitized oxidation in natural water via OH radicals. Chemosphere, 14, 1659-1671, 1985.
34. **Hasting J.W., Kricka L.J., Stanley P.E.:** Bioluminescence and Chemiluminescence Molecular Reporting with Photons. John Wiley and Sons, Chichester, New York, 1997.
35. **Heering W.:** Measuring weak light signals not far from noise-level. In: Biophotons (Eds J.J. Chang, J. Fisch, F.A. Popp). Kluwer Acad. Publ., Dordrecht, London, 1998, 143-157.
36. **Hideg E., Inaba H.:** Dark adopted leaves of paraquat-resistant tabacco plants emit less ultraweak light than susceptible ones. Biochem. Biophys. Res. Commun., 178, 438, 1991.
37. **Hiramatsu M.:** Biophotons and defense response in plants. In: Biophotons (Eds J.J. Chang, J. Fisch, F.A. Popp). Kluwer Acad. Publ., Dordrecht, London, 45-55, 1998.
38. **Ho M.W., Xu X., Ross S., Saunders P.T.:** Light emission and rescattering in synchronously developing populations of early *Drosophila* embryos. In: Recent advances in biophoton research and its applications (Eds F.A. Popp, K-H Li, Q. Gu). World Scientific, Singapore, 287-306, 1992.
39. **Inaba H.:** Super-high sensitivity system for detection and analysis of ultraweak ohoton emission from biological cells and tissues. Experientia, 44, 550-559, 1988.
40. **Kaneda H., Kano Y., Osawa T. Kawakishi S. Kamimura M.:** Role of free radicals in chemiluminescence generation during the beer oxidation proces. Agr. Bio. Chem., 54, 2165-2166, 1990.

41. **Kitano M., Ogasawara Y., Xincheng Hu, Takenaka N, Bandow H., Maeda Y.:** Continuous determination of trace amounts of humus acid in natural water by chemiluminescent method. Microchem. J., 49, 265-274, 1994.
42. **Kobayashi M., Devaraj B., Usa M., Tanno Y., Takeda M., Inaba H.:** Development and applications of new technology for two-dimensional space-time characterization abd correlation analysis of ultraweak biophoton information. Frontiers Med. Bio. Eng., 7, 299-309, 1997.
43. **Kobayashi M., Inaba H.:** Photon statistics and correlation analysis of ultraweak light originating from living organisms for extraction of biological information. Appl. Optics, 39, 183-192, 2000.
44. **Kobayashi M., Devaraj B., Usa M., Tanno Y., Takeda M., Inaba H.:** Two-dimensional imaging of ultraweak photon emission from germinating soybean seedlings with a highly sensitive CCD camera. Photochem. Photobiol., 65, 535-537, 1997.
45. **Kochel B.:** Luminescence of perturbed living organisms: memory function approach based on linear stochastic models of nonstationary UPE processes. In: Photon emission from biological systems (Eds B. Jeżowska-Trzebiatowska, B. Kochel, J. Sławiński, W. Strek). World Scientific, Singapore, 101-116, 1900.
46. **Kochel B.:** Time-resolved luminescence of perturbed biosystems: stochastic models and perturbation measures. Experientia, 48, 1059-1069, 1992.
47. **Kochel B.:** Perturbed living organisms: a cybernetic approach to oscillatory luminescence. Kybernetes, 24, 53-76, 1995.
48. **Kochel. B.:** New aspects of ecotoxicology through a systemic approach. Kybernetes, 22, 69-77, 1993.
49. **Kochel B., Sajewicz W.:** A new measure of xenobiotic toxicity from the time-resolved luminescence. Bull. Math. Biol., 59, 897-910, 1997.
50. **Kochel B., Sajewicz W.:** Entropy of photon-counting processes with infinite history: An approximation based on the process memory. Clin. Lab., 7/8, 393, 2000.
51. **Koehler B., Lambig K., Nagl W., Popp F.A., Wahler J:** Photoemission - Eine neue Methode zur Erfassung der Qualitaet von Lebensmitteln. Deutsche Lebensmittel-Rund., 3, 78-83, 1991.
52. **Laszczka A., Ezzahir A., Godlewski M., Kwiecińska T., Rajfur Z., Sitko D., Slawiński J., Szczęśniak-Fabiańczyk B., Wierzuchowska D.:** Iron-induced ultraweak chemiluminescence and vitality of bull spermatozoa. In: Bioluminescence and Chemiluminescence: Status Report. (Eds A.A. Szalay, L.J. Kricka, Stanley P.E.). John Wiley & Sons, Chichester, 523-527, 1993.
53. **Lewin G., Schreiber G.A., Helle N., Popov I.N.:** Nachweis einer -Bestrahlung von Lebensmitteln. Zur Anwendung der H_2O_2 und Photostimulierten Chemilumineszenz I. Frisches Obst, Bundesgesundhbl., 2, 61-62, 1993.
54. **Lipski M., Sławiński J., Zych D.:** Luminescence of the oxidated humus acids. J. Lumines. 9, 133-138, 1999.
55. **Makiuchi M. Miyazaki J., Shirashi T., Kakinuma K., Inaba H.:** Dual charge integrating amplifier circuit using two GaInAs- InP pin photodiodes for UPE in the near infrared region. IEEE Photonics Technol. Lett., 9, 677-679, 1997.
56. **Marino D.F., Ingold D.:** Determination of humus acids by chemiluminescence. Anal. Chim. Acta, 124, 23-30, 1981.
57. **Milczarek I.:** Effects of humic and ascorbic acids on ultraweak luminescence of field pea seedlings and roots. In: The role of humic substances in the ecosystems and in environment protection (Eds J. Drozd, S.S. Gonet, M. Senesi, J. Weber). PTSH-Polish Soc. Humic Substances, 973-980, 1997.
58. **Milczarek I., Jaskowska A., Gołębiowska D.:** Effects of humic acids and polyphenols on ultraweak luminescence from *Characeae* cells. In: Humic substances in the global environment

and implications on human health (Eds M. Senesi, T.M. Miano). Elsevier, Amsterdam, 1245-1258, 1994.

59. **Milczarek I., Sławiński J., Grabikowski E.:** Studies on seeds vitality by means of the single photon counting of ultraweak luminescence. Zesz. Nauk. AR, Szczecin, 39, 249-263, 1973.
60. **Milczarek I., Sławiński J., Grabikowski E.:** The influence of temperature on the ultraweak luminescence of germinating seeds. Zesz. Nauk. AR, Szczecin, 42, 306-322, 1974.
61. **Milczarek I., Sławiński J., Grabikowski E.:** The effect of free radical inhibitors on ultraweak biochemiluminescence of germinating seeds. Zesz. Nauk. AR, Szczecin, 48, 97-110,1974 (in Polish).
62. **Murkowski A.:** Photosynthetic luminescence assay for determination of triazine resistance of rape plants. Proc. 7th Int. Rapeseed Cong. Poznan, 4, 830-836, 1987.
63. **Murkowski A., Skorska E.:** The application of the luminescence method to evaluation of wheat and rape susceptibility to certain herbicides. Hodowla Roślin, Aklimatyzacja i Nasiennictwo, 32, 171-174, 1988.
64. **Murkowski A., Skorska E.:** Chlorophyll A luminescence - an index of photoinhibition damages. Current Topics Biophys., 21, 72-78, 1997.
65. **Musumeci F., Godlewski M., Popp F.A., Ho M.W.:** Time behaviour od delayed luminescence in Acetabularia acetabulum. In: Recent advances in biophoton research and its applications (Eds F.A. Popp, K-H Li, Q. Gu). World Scientific, Singapore, London, 327-344, 1992.
66. **Musumeci F., Scordino A., Triglia A.:** Delayed luminescence and structure of simple biological systems. In: Biophotonics and coherent systems (Eds L. Beloussov, F.A. Popp, V. Voeikov, R. Van Wijk). Moscow University, 141-150, 2000.
67. **Musumeci F., Scordino A., Triglia A.:** Coherence and biophoton emission as investigated on *Acetabularia acetabulum*. In: Biophotons (Eds J.J. Chang, J. Fisch, F.A. Popp). Kluwer Acad. Publ. Dordrecht, London, 109-120, 1998.
68. **Musumeci F., Triglia A., Grasso F., Scordino A., Sitko D.:** Relation between delayed luminescence and functional state in soya seeds. Il Nuovo Cimento D, 16,65-73, 1994.
69. **Neurohr R.:** Non-linear optical properties of delayed luminescence from cress seeds. In: Recent advances in biophoton research and its applications (Eds F.A. Popp, K-H Li, Q. Gu). World Sci., Singapore, 375-392, 1992.
70. **Orlov D.S., Demin V.V., Zavgorodnaia Yo. A.:** The effect of molecular parameters of humic acids on their physiological activity (in Russian). Dokl. Akad. Nauk, 354(6), 843-845, 1997.
71. **Pasini P., Musiani M., Roda A., Zerbini M., Gentilomi G., Baraldini M., Gallinella G., Venturoli S.:** Chemiluminescence in situ hybridization for the detection of viral genomes. In: Bioluminescence and Chemiluminescence Molecular reporting with photons (Eds J.W. Hastings, L.J. Kricka, P.E. Stanley). John Willey & Sons, Chichester, New York, 509-512, 1997.
72. **Popp F.A.:** Photon storage in biological systems. In: Electromagnetic Bioinformation (Eds F.A. Popp, G. Becker, H.L. Koenig, W. Peschka). u. Schwarzenberg, Munchen., 123-141, 1979.
73. **Roda A., Pasini P., Musiani M., Robert C., Baraldini M., Carrea G.:** Chemiluminescence imaging as a bioanalytical tool. In: Bioluminescence and Chemiluminescence Molecular Reporting with Photons Hasting (Eds J.W. Kricka, L.J. Stanley). John Wiley and Sons, Chichester, New York, 307-310, 1997.
74. **Rohner F.:** Biophoton emission as an indicator of Biological Quality. In: Recent advances in biophoton research and its applications (Eds F.A. Popp, K-H Li, Q. Gu). World Scientific, Singapore, 1992, 415-420.
75. **Scordino A., Triglia A., Musumeci F., Grasso F., Rajfur Z.:** Influence of the presence of atrazine in water on *in vivo* delayed luminescence of *Acetabularia acetabulum*. J. Photochem. Photobiol, B. Biol., 32, 11-17, 1996.
76. **Scott R.Q., Usa M., Inaba H.:** Ultraweak emission imagery of mitosing soybeans. Appl. Phys. B 48, 183-185, 1989.

77. **Seliger H.H.:** Applications of bioluminescence and chemiluminescence. In: Chemiluminescence and Bioluminescence (Eds M.J. Cormier, D.M. Hercules, J. Lee). Plenum Press, New York, 1973, 461-478.
78. **Skorska E., Murkowski A.:** Photosynthetic luminescence detection as the quick test of chilling resistance of cucumber plants. Hodowla Roślin, Aklimatyzacja i Nasiennictwo, 32, 285-289, 1988.
79. **Godlewski M., Kwiecińka T., Laszczka A., Sławiński J., Szczęśniak-Fabiańczyk B, Wierzuchowska D.:** The effect of DNA fluorophores on the ultraweak photon emission from spermatozoa. In: 8-th Int. Symp. Molecular and physiological aspects of regulatory processes of the organism (Ed. H. Lach). Cracow, 132-133, 1999.
80. **Sławińska D. Górski Z., Sławiński J.:** Chemiluminescence imaging of oxidative changes in plant food. Pol. J. Food Nutr. Sci., 7/48 (2), 67-72, 1998.
81. **Sławińska D, Polewski K.:** Chemiluminescence as a new evidence for singlet oxygen participation during the weathering of wood. Polish J. Med. Phys. Eng., 1, 249-250, 1995.
82. **Sławińska D., Puzyna W., Sławiński J.:** Chemiluminescence during photooxidation of melanins and humic acids arising from a singlet oxygen mechanism. Photochem. Photobiol., 28, 459-463, 1978.
83. **Sławinska D., Richetta M.:** Chemiluminescence techniques in environmental sciences. In: Monitoring Black Sea environmental conditions. World Federation of Sciences, Linda Kruger, Lousanne, 241-257, 1999.
84. **Sławińska D., Sławiński J.:** Chemiluminescence of humic acids. Nature, 213, 902-903, 1967.
85. **Sławińska D., Sławiński J.:** Low-level luminescence from biological objects, 495-531; Applications of bioluminescence and low-level luminescence from biological objects, 533-601. In: Chemi-and Bioluminescence (Ed. J.G. Burr). Marcel Dekker, Inc., New York, Basel, 1985.
86. **Sławińska D, Sławiński J.:** Chemiluminescent methods of the evaluation of food quality. In: I Natl. Conf. Food, Nutrition and Health (Ed. A. Lewicki). Warsaw University Press, Warsaw, 280-285, 1998.
87. **Sławińska D, Sławiński J.:** Chemiluminescence of cereal food products. I. J. Biolumin. Chemilumin., 12, 249-253, 1997.
88. **Sławińska D, Sławiński J.:** Chemiluminescence of cereal food products. II. J. Biolumin. Chemilumin., 13, 13-19, 1998.
89. **Sławińska D, Sławiński J.:** Chemiluminescence of cereal food products. III. J. Biolumin. Chemilumin., 13, 21-24, 1998.
90. **Sławińska D., Sławiński J., Cieśla L.:** The inhibition of peroxyradical-induced chemiluminescence by melanins. Physiol. Chem. Phys & Med. NMR., 15, 209-212, 1983.
91. **Sławińska D., Sławiński J., Sarna T.:** Photoinduced chemiluminescence and ESR signals and polyphenols and quinone polymers. Photochem. Photobiol., 21, 393-396, 1992.
92. **Sławińska D., Sławiński J., Sarna T.:** Effect of light on EPR spectra of humic acids. J. Soil. Sci., 26, 93-99, 1975.
93. **Sławiński J.:** Ultraweak luminescence and perturbations of biohomeostasis. In: Photon emission from biological systems (Eds B. Jeżowska-Trzebiatowska, B. Kochel, J. Sławiński, W. Strek). World Scientific, Singapore, 49-78, 1986.
94. **Sławinski J.:** Dark paramagnetic biopolymers - the emitters or traps of photons? In: Biophotonics and coherent systems (Eds L. Beloussov, F.A. Popp, V. Voeikov, R. Van Wijk). Moscow University, Moscow, 189-202, 2000.
95. **Sławiński J., Ezzahir A., Godlewski M., Kwiecińska T., Rajfur Z., Sitko D., Wierzuchowska D.:** Stress-induced photon emission from perturbed organisms. Experientia, 48, 1041-1058, 1992.
96. **Sławiński J., Grabikowski E, Majchrowicz I.:** Ultraweak photon emission generated by germination. Bio-Photon Physics, 1, 71-80, 1979.

97. **Sławiński J., Kochel B.:** Stochastic models of nonstationary photon emission from chemically perturbed living organisms. In: Photon emission from biological systems (Eds B. Jeżowska-Trzebiatowska, B. Kochel, J. Sławiński, W. Strek). World Scientific, Singapore, 78-100, 1986.
98. **Sławiński J., Majchrowicz I., Grabikowski E.:** Ultraweak luminescence from germinating and resting spores of *Entomophthora virulenta*. Acta Mycol., 17, 137-139, 1982.
99. **Sławiński J., Mańkowski H., Górski Z.:** EPR spectra of humic acids and melanins exposed to UV radiation and ozone. Cur. Topics Biophys., 23(1), 103-112, 1999.
100. **Sławiński J., Sławińska D., Majchrowicz I.:** Biochemiluminescence of certain fungi. Acta Mycol., 12(2), 289-299, 1977.
101. **Souren J.E.M., Bonn-Niermeyer E., Van Wijk R.:** Germination capacity of tomato seeds & ultraweak photoinduced delayed luminescence. In: Biophotonics and coherent systems (Eds L. Beloussov, F.A. Popp, V. Voeikov, R. Van Wijk). Moscow University, Moscow, 419-430, 2000.
102. **Souren J.E.M., Van Wijk R.:** Luminometry in cellular research. In: Biophotons (Eds J.J. Chang, J. Fisch, F.A. Popp). Kluwer Acad. Publ., Dordrecht, London, 65-78, 1998.
103. **Strehler B., Arnold W.:** Light production by green plants. J. Gen. Physiol., 34, 809-820, 1951.
104. **Tryka S.:** Cut-off filter method for light-induced photon emission spectra estimation. Computers Chem., 22, 113-118, 1998.
105. **Tryka S.:** Ultraweak luminescence from mechanically damaged wheat seeds during imbibition. In: Biological Luminescence (Eds B. Jeżowska-Trzebiatowska, B. Kochel, J. Sławiński, W. Strek). World Scientific, Singapore, 630-646, 1990.
106 **Tryka S.:** The effect of temperature on the relationship between ultraweak luminescence and the water absorption rate by imbibing grain. Acta Agrophysica, 2000.
107. **Tryka S., Koper R.:** Luminescence of cereal grain subjected to the effect of mechanical loads. In: Photon emission from biological systems (Eds B. Jeżowska-Trzebiatowska, B. Kochel, J. Sławinski, W. Strek). World Scientific, Singapore, 248-254, 1986.
108. **Tryka S., Koper R., Grundas S.:** Investigation of the influence of mechanical damages in diversified types of wheat grain structure on the intensity of ultraweak biochemiluminescence. Zesz. Probl. Post. Nauk Roln., 354, 177-183, 1989.
109. **Tryka S., Woźniak W.:** Effect of moisture treatment on the ultraweak luminescence from imbibing wheat grain. Int. Agrophysics, 12, 127-132, 1998.
110. **Van der Berg R.H., Jansen E.H.J., Reinerink E.J., Zomer G.:** Automated chemiluminescence measurements of transient and enzyme labels in microtitre plates. In: Bioluminescence and Chemiluminescence current status (Eds P.E. Stanley, L.J. Cricka). John Willey & Sons, Chichester, New York, 211-214, 1991.
111. **Veselova T.V., Veselovsky V.A., Rubin A.B., Bocharov V.Z.:** Drelayed luminescence of air-dry soybean seeds as a measure of their viability. Physiol. Plant., 65, 493-497, 1985.
112. **Veselovsky V.A., Veselova T.V.:** Luminescence of Plants (in Russian). Nauka, Moscow, 1990.
113. **Wenli C., Da X., Tonghong T.:** A study of ultraweak biophoton emission of new and aged rice seeds during early imbibition. SPIE 2000 Bejging, Biomedical Photonics & Optoelectronics, Imaging., 424, 214-217, 2000.
114. **Wenli C., Da X., Tonghong T.:** Observation of ultraweak biophoton emission from new, aged rice seeds and rice seeds with different gene during early imbibition period. J. South China Normal Uni. Natur. Sci. Edition, 2, 50-54, 2001.
115. **Woodhead J.S. Herbert S.A., Weeks I.:** Immunochemiluminometric screening assays based on acridinium labels and microtitre plate luminometers. In: Bioluminescence and Chemiluminescence current status (Eds P.E. Stanley, L.J. Cricka). John Willey & Sons, Chichester, New York, 167-170, 1991.

116. **Xiaofeng P.:** A molecular dynamical theory of ultraweak biophoton emission in living systems and its properties. Chinese J. Atom. Mol. Phys., 12, 411-419, 1996.
117. **Xiaofeng P.:** A molecular dynamical theory of ultraweak biophoton emission in living systems and its properties. II. Chinese J. Atom. Mol. Phys., 13, 70-78,1996.
118. **Yan Yn.:** Biophoton emission and germinating capacity of barley seeds. In: Biophotonics and coherent systems (Eds L. Beloussov, F.A. Popp, V. Voeikov, R. Van Wijk). Moscow University, Moscow, 431-438, 2000.
119. **Zeiger B.F.:** Photon emission of cereal seeds, Biophotons, as a measure of germinative ability and vigour. In: Biophotons (Eds J-J Chang, J. Fisch, F.A. Popp). Kluwer Acad. Publ., Dordrecht, Boston, 251-297, 1998.
120. **Zeep R.G.:** Environmental photoprocesses involving natural organic matter. In: Humic substances and their role in environment (F.H. Frimmel, R.F. Christian), Willey-Interscience, Bath, 193-214, 1988.

ZASTOSOWANIE ULTRASŁABEJ EMISJI FOTONOWEJ W ROLNICTWIE

J. Sławiński

Zakład Radio- i Fotochemii, Instytut Chemii i Elektrochemii Technicznej,
Wydział Technologii Chemicznej, Politechnika Poznańska, ul. Piotrowo 3, 60-965 Poznań

S t r e s z c z e n i e. Wszystkie żywe organizmy emitują niezwykle słabe świecenie (o mocy 10^{16} W dla długości fali = 600 nm lub ca $10\text{-}10^4$ fotonów/scm^2, od UV do IR) podczas metabolicznych przemian. Promieniowanie to zwane ultra słabą luminescencją, biochemiluminescencją, emisją fotonową lub "biofotonami" jest ściśle związane z natężeniem, kinetyką, energetyką i charakterem procesów biochemicznych oraz stanem fizjologicznym organizmu (komórek) tj. homeostazą. Parametry ultra słabego świecenia dzielących się komórek oraz kiełkujących nasion roślin uprawnych. dostarczają informacji o intensywności procesów metabolicznych i dość dobrze korelują ze zdolnościa kiełkowania zarodników i nasion. Pozwalają szybko i nieinwazyjnie oszacować zdolności adaptacyjne roślin do niskich i wysokich temperatur, wytrzymałość na suszę, zasolenie i inne czynniki agrofizyczne i meteorologiczne. W przeglądzie przedstawiono i przedyskutowano ostatnie osiągnięcia w zastosowaniu ultra słabej emisji fotonowej do celów analitycznych i diagnostycznych w rolnictwie i pokrewnych dziedzinach. Metody te w powiązaniu z zaawansowaną optoelektroniką stanowią bardzo cenne i czułe źródło istotnych informacji.

S ł o w a k l u c z o w e: emisja fotonowa, fotosyntetyczna luminescencja, stress oksydacyjny, metody immunoluminescencyjne w rolnictwie, biotechnologii i ekologii.