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METAL CONTENT MONITORING IN THE BIOLOGICAL STRUCTURES

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Abstract. *The authors propose a monitoring method for defining metal content in the biological structures, such as plant leaves, tissue samples of animal origin, human skin, etc. The authors used dielectric barrier discharge (DBD) in air at atmospheric pressure as a diagnostic medium. According to the research, at the optimal selection of gas discharge parameters it will not have destructive effect on tissues of biological structure. Indeed, generation of chemically active particles in the plasma will be minimal one. The dielectric barrier separates the investigated sample from the electrode of the discharge system. DBD activation proceeds at frequencies close to the sound range (not more than 15 kHz). It was due to the requirement of ionic component emission only from cells on the surfaces of the structures under study. The conditions of low thermal effect of atmospheric pressure plasma on plant and animal sample provide the choice of DBD frequency range.*

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Introduction

Nowadays, there is a steady tendency to merge certain methodological techniques, methods, and research practices of different scientific and technological centres in order to obtain new results in terms of interdisciplinary knowledge [1]. Many studies fields have developed and tested research methods for obtaining new original results in related fields. One of the promising trends is plasma chemistry and plasma-related technologies: processes of polymerisation in plasma, synthesis of new materials using plasma stimulation, modification of surface states of solid inorganic materials and materials of natural origin, etching of metals (semiconductors), and a number of other technologies are currently implemented [2-4]. At the same time, plasma is used as a source of high-energy and chemically active particles (radicals and ions) [5], as an effective diagnostic tool [6].

According to many researches, plasma is widely used for diagnostic purposes. These include probing of crystalline structures for identifying defective samples, technologies of material atomisation for studying their elementary composition, etc. These technologies are most widespread in mechanical engineering, light, and electronic industries. In this regard, there even appeared a common term uniting all these technological approaches - elion processing of materials.

With the development of nanotechnology, interdisciplinary trends in basic research and applications occurred. Technological methods originally oriented only at industry began to spread into other spheres: health care, fine organic synthesis, etc. For example, plasma has become used for sterilisation and fine cleaning of various surfaces from pathogens, disinfection of water and biological media from harmful substances, etc. [7, 8]. The techniques of transferring a liquid cathode ionic component to the region of the positive column of the glow discharge, i.e., to the gas phase, turned out to be quite promising [9]. Heterogeneous processes at the interfaces have become decisive [10].

Problem statement and experimental equipment description

However, in terms of DBD, AC current will flow even in the presence of barrier generally non-conductive structures when DC current is used. In biological samples, these may be elements of epithelium, cellular structures, keratinised formations, etc. Thus, in discharges supported by an alternating electric field, a reliable means of visualising the internal structure of cellular structures remains in the form of characteristic radiation of gas discharge components.

When igniting DBD at atmospheric pressure, the air components and various particles related to the degradation products of its components should be fixed. Fig. 1 shows the emission spectrum of a spark discharge on air.

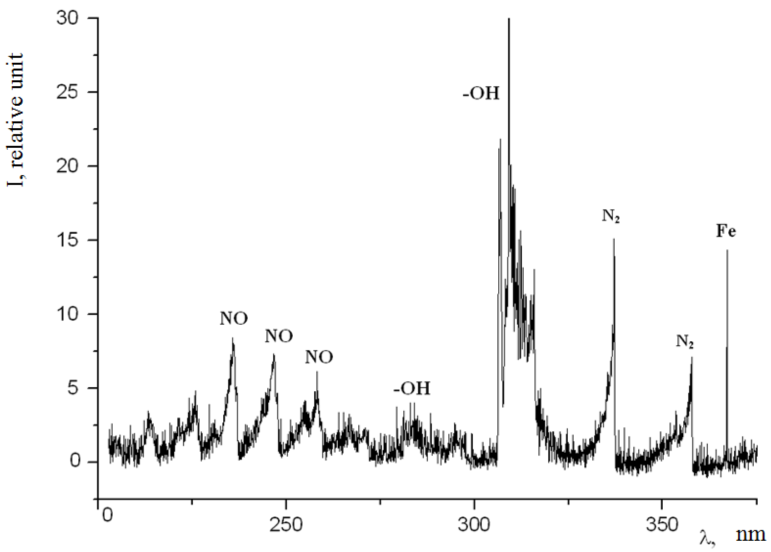


Fig. 1. Emission spectrum of a spark discharge on air (spectrum obtained using an industrial high-resolution spectrometer "Avaspec")

The spectrum clearly shows the nitrogen (second positive system) bands at 337 and 357 nm ($C_3\Pi_u$ transition → $B_3\Pi_g$), the bands of the -OH radical ($A_2\Sigma$ transition → $X_2\Pi$), and the γ-system bands of the NO molecule ($A^2\Sigma$ transition → $X^2\Pi$) at 236, 247 and 259 nm, respectively. Oxygen usually dissociates well in the discharge; the lines of atomic oxygen are in the red region of the spectrum. Therefore, they were not included in the spectrum shown in Fig. 1. The analysis of the air component emission in the discharge was not purpose of this study. Such spectra are well studied and described in the literature. Moreover, the presence of decomposition products of air components in the spectrum depends significantly on the type of discharge and the method of its activation. Thus, the probability to detect NO particles and -OH radicals in low-pressure glow discharges and in DBD at atmospheric pressure is significantly lower. It is explained by the low specific powers invested in the discharge, especially at low discharge currents and low gas temperature. It is known that the main channel of NO formation is a sequence of reactions involving air components:

$$O_2 + e \rightarrow O + O \quad (1)$$

$$N_2 + O \rightarrow NO + N \quad (2)$$

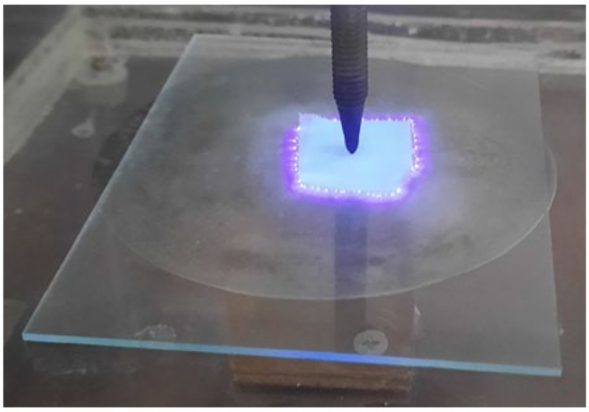
$$N + O_2 \rightarrow NO + O \quad (3)$$

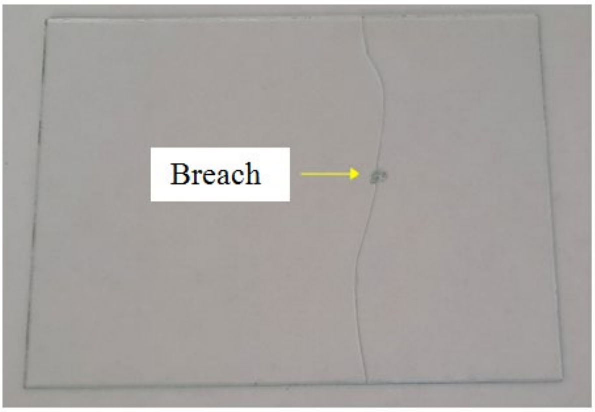
The average temperature for recording an appreciable concentration of NO is 1900 K [11]. The hydroxyl radical is formed mainly by thermal dissociation of residual water vapour on air:

$$H_2O \rightarrow H + OH^- \quad (4)$$

The probability of processes 1-4 significantly increases with increasing discharge current (increasing the power invested in the discharge) and the transition of the discharge from quiet diffusion to spark discharge. The region of transition from one type of discharge to another does not have a clear line, but it can be determined by plasma emission. In this case, processes 1-4 will be initiated in the discharge, and plasma chemical degradation products will be recorded spectrally (see Fig. 1). This mode of discharge combustion is unacceptable, as it can cause the destruction of the cell structure on the surface of the material under study. The reliability of the dielectric barrier was a guarantee of the absence of DBD transition to sparking. The electrical strengths of various materials claiming to be dielectric barriers were studied. Different materials were analysed: glass of different thicknesses, ceramic plates (thickness 0.3 mm), mica and silicone coatings. Relatively porous materials such as paper and cardboard were not considered. We required a barrier with the following characteristics: high electrical strength, low sensitivity to overheating and mechanical shocks, optimum thickness. An important requirement was the possibility of mechanical processing.

There are unsuccessful operation results of the setup using barriers with low reliability for our experiments. Fig. 2, *a* shows a discharge with a sample of woven material placed on a glass dielectric barrier. Fig. 2, *b* shows the barrier destruction after several sessions of operation.





a *b*

Fig. 2. Experiments on the role of glass as a barrier for DBD: *a* - appearance of the discharge in the presence of woven material; *b* - glass sample, the barrier has cracked along its entire length

Glass can be used as a dielectric barrier under certain conditions. Although, during long burning of the discharge there were local overheating, breakdown was formed (see Fig. 2, *b*), and the glass was destroyed. The mechanism of barrier destruction always was similar. First, a local breach was formed; it causes the local overheating, and further destruction of the barrier material. The thickness of the barrier will determine the burning efficiency of the discharge. The thicker the barrier, the more significant part of the power supply power was lost and could not participate in the initialisation of the target processes. Therefore, no optimal ratio between its thickness and its effectiveness as a barrier has been found for glass.

In order to find an optimal dielectric variant, we performed test experiments with a 2 mm thick silicone heat dissipating mat with the following characteristics: density ρ = 3.1 g/cm^3; thermal conductivity γ = 16 W/m·K; electrical strength σ = 10 kV/mm. This mat was ideal spacer material between powerful transistors and heat sinks. Indeed, in our test showed insufficient results. High field strengths in the discharge zone led to its breach.

The best option was proved to be sheet mica for magnetrons in microwave ovens. Such sheets could withstand voltages on the electrodes of the discharge system up to 20 kV at varying frequencies in the range of 1-50 kHz. We have not yet operated with higher frequencies within the framework of this study. It is explained by the absence of a suitable high-frequency high-voltage generator at this stage.

The generator for our experiments was self-made according to the classical two-cycle ZVS-driver scheme with a fixed frequency (if necessary, the frequency could be changed within 1-50 kHz by making minor changes in its design). The generator output voltage could be varied up to 10 kV, which was by a large margin for the purposes of our experiments. We operated at frequencies not exceeding 15 kHz. The output voltage was selected experimentally each time considering the specifics of the research object. The requirement to avoid damage to biological structures due to discharges destructive action was mandatory for us.

The qualitative composition of near-surface layers liquid phase of biological structures was monitored by the corresponding emission spectra. A portable small diffraction 55 mm spectrometer CLMG-7206 by Gain Express was used. The spectrometer did not require a separate power supply, had small dimensions and, therefore, could be used even in the field. Logitech HD Webcam C270 (1.3 Mpix) was used as a detector. It performs automatic illumination correction, provides natural colour reproduction. Moreover, the resolution of this camera was quite sufficient for obtaining resonance lines of metals in the emission spectra. Fig. 3 shows the schematic diagram of the equipment.

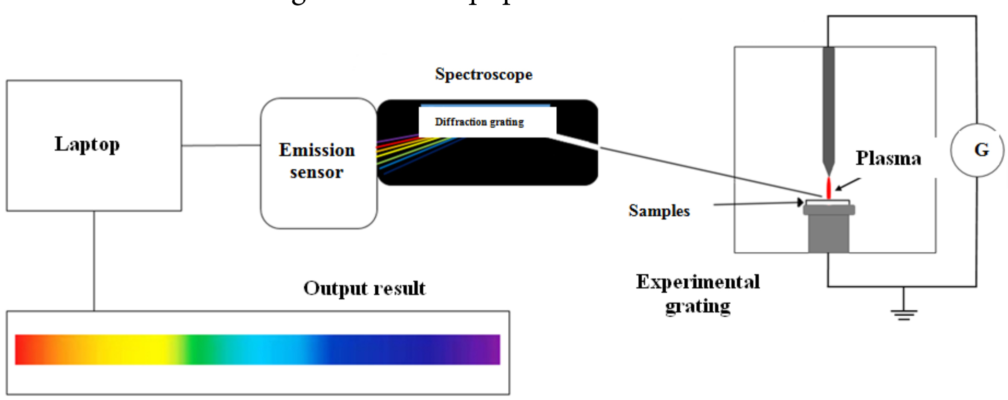


Fig. 3. Schematic diagram of experimental equipment

We conducted the experiments in a sealed transparent experimental cell; radiation was discharged through a side hole in the cell. We placed the studied samples on a grounded table inside the experimental cell. Beforehand, we covered the table with a dielectric barrier. The table itself and the second electrode were made of stainless steel. For processing of spectral data and obtaining spectra the software of the public laboratory "Spectral Workbench" was used.

The peculiarity of the laboratory software was that the obtained spectra were not correlated with real values of wavelengths (relative scale was used). In this regard, we specifically used illumination from a radiation source with a known spectrum. In our case it was a mercury lamp DRSH-250, giving well-distinguishable mercury lines 404, 436, 545, and 546 nm. Figure 4 shows the spectrum of a mercury lamp. Indeed, for calibration any reference signals equally reproduced under different conditions could be used.

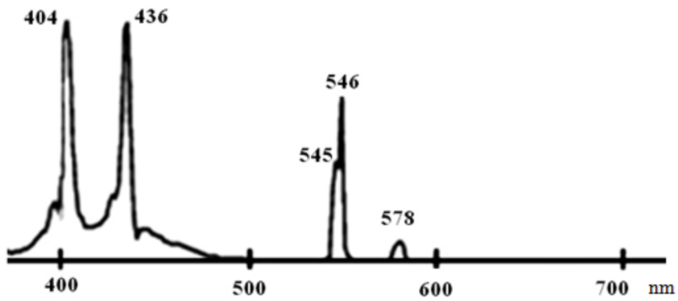


Fig. 4. Emission spectrum of the DRSH-250 lamp. Insignificant peak at 578 nm refers to background illumination from fluorescent light bulbs (refers to phosphor emission)

Peaks related to the background illumination of fluorescent lamps used to illuminate the laboratory could be observed on the operating spectra in a number of cases. This is the emission of phosphor in the visible region of the spectrum with wavelengths of 488 and 578 nm. In our test, the mercury lines were used to identify signals in the plasma spectra in the presence of the studied objects. In fact, having reproducible lines in the spectra, we calculated the wavelength scale, and identified new lines in the spectra. Hence the spectrum shown in Fig. 4 was artificially fitted with an abscissa scale on which the wavelengths were plotted. This was the only drawback of the software used and the CLMG-7206 spectrometer. The resulting spectrum was free of weak signals (noise). In addition, this equipment was not affected by electromagnetic fields.

Results and discussion

We chose leaves of plants with a well-developed leaf plate as the objects of our study. The influence of some hypothetical negative factors should cause a sharp change in the concentration of certain chemical elements or compounds in the composition of cells. Therefore, we artificially simulated such a situation. Therefore, we saturated plants with metal salts. The choice of the salts was determined by the presence of these metals in biological structures under study. Indeed, their overabundance can cause different disorders in the organism. Although the experiments were conducted with plant leaves, we consider its main conclusions could be implemented to animal and human structures. The reason is the similarity of the surface structures of all biological structures. Fig. 5 shows a schematic representation of the cuticle. It is a set of rigid, but flexible, non-mineral external coverings of an organism or its parts and protecting it. All living organisms and plants have a similar structure of protective coverings.

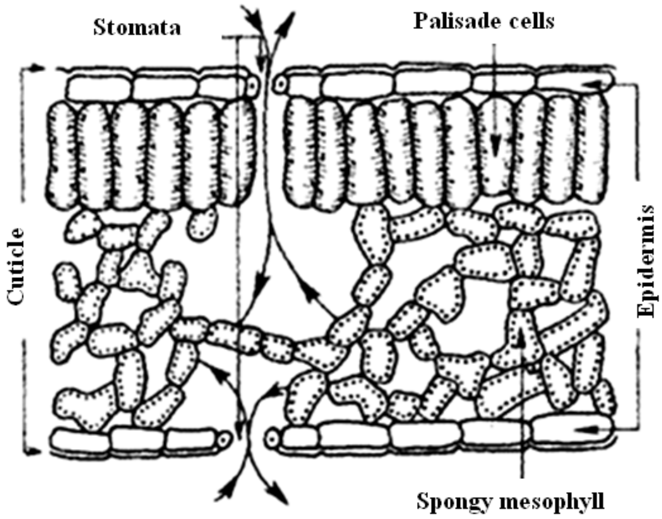


Fig. 5. Cuticle structure (arrows show mass transfer channels)

Biological structures, in particular leaves, are protected externally by a dense covering tissue, the epidermis (see Fig. 5). Underneath is the leaf pulp (mesophyll). The palisade cells absorb most of the light energy. Spongy mesophyll participates in the process of photosynthesis, providing gas exchange. The mesophyll cells are environmentally surrounded by water containing various anions and cations. The epidermis contains so-called stomata, formed by two interlocking cells. Through these stomata, water evaporation and gas exchange with the environment is ensured. When a discharge is ignited near a biological structure, high electric field strengths can be realised in them, also due to the inhomogeneity of the object. The liquid phase ions in such inhomogeneous fields are capable of exerting effects comparable to the threshold values of ion energies at which materials are atomised at industrial plants [12]. Vibrations with different frequencies are formed in the cells of a living organism under the action of plasma, and wave processes are initiated under the action of electromagnetic waves. Therefore, metals in the ionised state in biological structures can be subject to displacement under the action of electromagnetic fields. In other words, one can expect emission of contaminated particles into the plasma volume with their subsequent excitation and production of characteristic radiation. However, an alternating electric current of appropriate shape, amplitude, and frequency affects the organism. Under its frequencies corresponding to the spontaneous biopotential activity of the tissues of the organism, the phenomenon should also contribute to the emission of the internal contents of the cell into the gas phase. A number of diseases or disorders can be detected by such techniques. Indeed, the presence of non-natural components in the biological structures, the abrupt change in the ratio of the necessary ones, can cause acute intoxication, and regarded as a sign of disease or poisoning.

The salts selected for the research, we saturated the leaves of the plants with, are shown in Table 1. The saturation time was always the same - 12 h. The saturation technique consisted in immersing the stem of a freshly cut plant with leaves into the salt solution.

The salt concentration was the same in all experiments and corresponded to 10% salt concentration. We also tried to make the saturation conditions the same. The liquid volume in the jars was unchanged, the stem of the plant was immersed into the liquid for 5 cm. Diffuse lighting was provided in the laboratory during the whole time of the experiment; the influence of sunlight was excluded. The contact of leaves with the solution was also excluded.

Table 1. Salt samples and their corresponding characteristic emission wavelengths

Sample (salt)	Wave length λ, nm
$NaCl$	589
$FeCl_3$	585
$MnCl_2$	602
$CuCl_2$	510 and 523
$ZnCl_2$	472, 481 and 636
KCl	583
$MgCl_2$	571

Figs. 6 and 7 show examples of DBD emission spectra in the presence of leaves saturated with iron and copper salts, respectively.

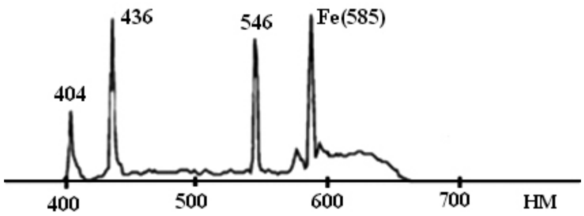


Fig. 6. Radiation spectrum of DBD in the presence of samples saturated with $FeCl_3$

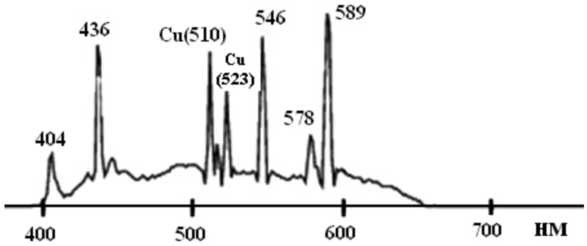


Fig. 7. Radiation spectrum of DBD in the presence of samples saturated with $CuCl_2$

Similar spectra were obtained for all samples given in Table 1. The conditions of discharge excitation were selected experimentally and required by the conditions under which there was no damage to plant leaves after their treatment in plasma. The leaves were examined microscopically. The experiment was considered unsatisfactory if black dots were observed on the leaf plate, indicating inadmissible currents flowing in the sample. It was also necessary to ensure the condition of stable discharge combustion to record the discharge emission spectra. The conditions of safe processing of plasma samples were determined as a result of generalisation for the results obtained. Hence, the voltage at the electrodes of the discharge system should not exceed 4 kV at frequencies from 1.5 to 15 kHz on average (the discharge current did not exceed 1 mA). An important aspect for the experiment was the samples quality. Leaves should be fresh, without signs of damage, breakage; have a natural moisture level. Long-term storage of leaves before the experiment is highly undesirable.

Conclusion and recommendations on the results application

Hence, using DBD it is possible to emit (exit into the volume of the discharge combustion zone) ionised components from the near-surface layers of biological structures with the possibility of their spectral detection. This technique can become the basis for the manufacture of a portable mobile unit for monitoring the composition of biological structures both in the laboratory and in the field. At the same time, not only plants but also animals can be used as the structures under study. The main constraint may be unstable discharge combustion at low frequencies. In the future, it is planned to expand the frequency ranges of DBD activation, simultaneously solving the problem of the depth of probing biological structures.

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