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Obtaining of metallic nanoparticles by plasma-erosion electrical discharges in liquid mediums for biological application

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Abstract: *Obtaining of metallic nanoparticles by plasma-erosion electrical discharges in liquid mediums for biological application.* The process of electrical discharge in the water of the plasma discharge channel was investigated and its diagnostics was performed. The specific features of the energy redistribution were shown, together with the physical model of the dispersed phase. The biological functionality of the metal nanoparticles and the prospects for their use in biotechnology were found.

Key words: plasma, electrical discharge physics, biological functionality of nanoparticles.

INTRODUCTION

The development of physical methods of high-energy treatment of materials allows to obtain the substance in nano-dispersed state. Application field of the materials goes beyond traditional technical use and now nanoparticles (nano-sized state of matter) usage is considered in biology and medicine [1]. Analysis of existing methods for producing metallic nanomaterials shows their limitations in terms of getting metal nanoparticles not in the ion, but in atomic (molecular) state, because in atomic (molecular) state the problem of toxicity and biocompatibility of nanometals can be solved. One of the most promising methods for producing nanomaterials is spark erosion treatment of the metal in water and formation of colloidal solutions nanoparticles of the respective metals [2]. Thus obtained particles have size of 20–100 nm and competitive advantages compared to similar solutions of metal salts, given that the ions have a much greater toxicity for the pathogen and plants. In addition, the use of water as a carrier of metal, simplifies the application of nanoparticles, which is a serious problem for ultrafine powders at the treatment of biological objects.

We have obtained suspensions of submicron metal particles in water by a method of volumetric electric-spark destruction of metal granules [3]. The method consists in simultaneous formation of spark channels in contacts between the metal granules which layer is immersed in a liquid, owing to a pulse

TABLE 1. Parameters of the discharge circuit

Inductance circuit L, μH	Charging voltage U, V	Capacitor C, μF	Camera's conductivity, mS/cm	Camera's resistance R_C,	Pulse frequency f, kH
~ 1	40…200	25…100	0.001…0.02	0.15…1.5	0.2…2

supply to them electric energy from the special generator. As a result of spark erosion the part of metal of granules evaporates and, becoming tempered in a liquid, forms fine dispersion fraction of spark-erosive particles. In our researches we used thyristor generators of discharge pulses with capacitor stores of energy (Tab. 1). The simplified scheme of a power part of one of them is given on Figure 1 and the principle of its work in detail is described in [3].

Synchronous oscillograms of voltage *u* on a layer of granules *Cu* and a current in it *i* for one of conditions of obtaining of suspensions are given on Figure 2. From the analysis of synchronous oscillograms *u*(*t*) and *i*(*t*) follows that resistance of load is essential nonlinearity and is subject to stochastic fluctuations. This fact is necessary for considering at support of stability of the generator work.

The measurement of plasma temperature in discharge local volume by optical emission spectroscopy is realized. The Boltzmann plot technique in this case was used. As an example, in plasma diagnostics of discharge between copper granules in liquid environment the spectral lines CuI were used. Additionally structure phase analysis of nanoparticles was carried out by X-ray techniques. It was found that correlation between discharge circuit parameters and nanoparticles composition takes place in such systems. The obtained colloidal systems are suggested in biology and medicine application.

The pulse power source was used to initiate the discharge between metal (copper, silver, iron or zinc) granules dipped into the deionized water.

Optical emission spectroscopy technique was used to investigate plasma properties of such discharge. Spectral components of discharge emission radiation were studied by the specially developed diffraction spectrometer. Spectral emission was recorded by CCD array (3000 × 2000 pixels). The tungsten

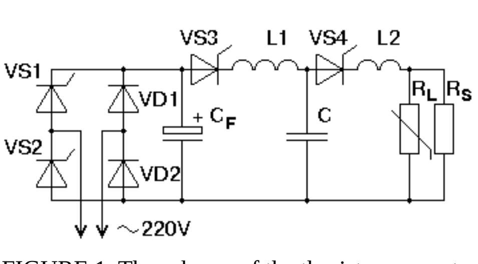
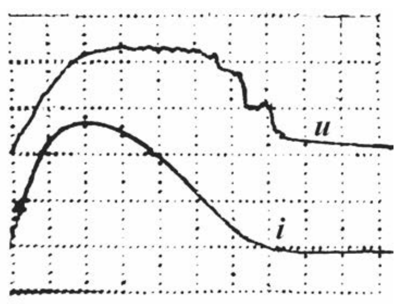


FIGURE 1. The scheme of the thyristor generator of discharge pulses



u: 50 *V/div*; *i*: 157 *A/div*; *t*: 20 μ*s/div*

FIGURE 2. Oscillograms of voltage on a layer of granules *Cu* and a current in it

ribbon lamp was used to define a spectral sensitivity of the experimental device. As an example we measured the temperature in the discharge between copper granules. The excitation temperatures of copper atoms in plasma were determined by Boltzmann plot technique using CuI spectral lines 510.5, 515.3, 521.8 and 578.2 nm. Spectroscopic data for these lines were used from [4].

Spectrum emitted by pulse discharge plasma is shown in Figure 3. As one can see there are some copper atom lines in this spectrum. It allows us to suppose that copper atoms are significant part of plasma volume. We must note that in a DC arc discharge in air between rod copper electrodes at arc current 3.5 A we have similar emission spectrum at copper content in plasma of few percent [5].

In an assumption of Boltzmann distribution of atom's energy levels population in studied plasma it can be possible to obtain excitation temperatures. In Figure 4 an example of Boltzmann plot for copper atom is shown. The slope in Figure 4 gives excitation temperature 7500 ±500 K.

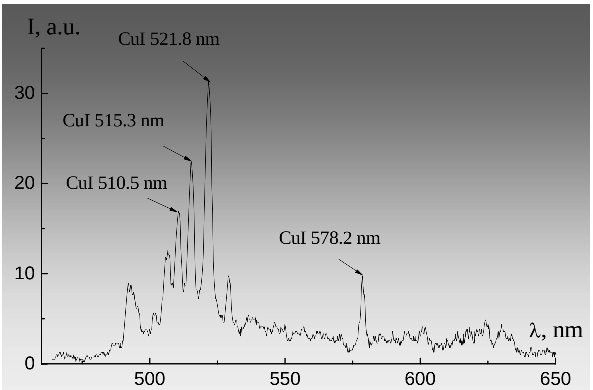


FIGURE 3. Visible range of the spectrum emitted by pulse discharge between copper granules

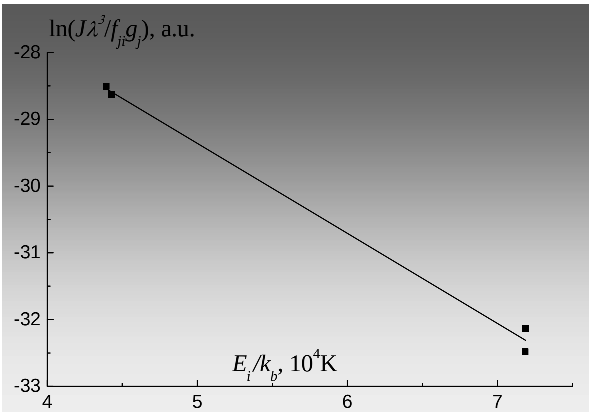


FIGURE 4. Boltzmann plot based on the emission spectrum of the pulse discharge between copper granules

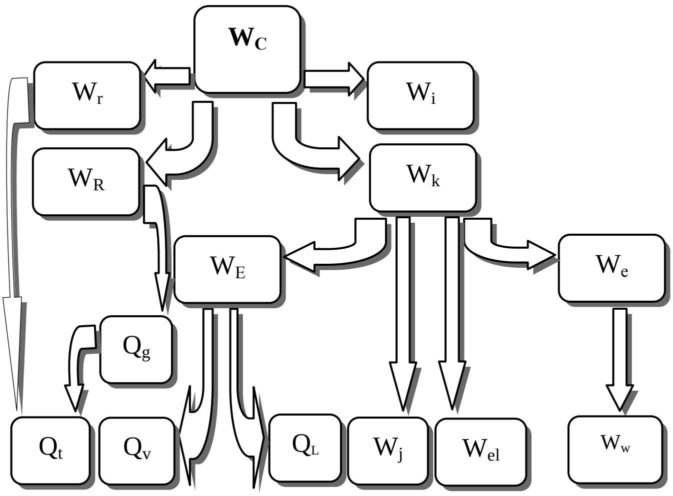


FIGURE 5. Physical processes and transformation scheme of energy discharge: W_C – accumulated energy of capacitive element; W_i – ionization energy of electrode gap; W_r – losses energy of the discharge circuit; W_R – heating of contacts energy; W_k – injection energy of plasma channel; W_e – expand energy of channel; W_E – the energy it takes to erosion of the electrodes; Q_g – heat volumetric energy of contacts; Q_t – temperature heating of the working environment; Q_L – heat melting the surface of the granules; Q_v – heat of vaporization of liquid metal grains; W_j – energy emission of channel; W_{el} – energy electrolysis; W_w – shock wave energy generate

On itself and the numerous data of other researchers, shows the features of the process and the proposed improved model of redistribution of energy discharge capacitor (Fig. 5).

The physical model of the dispersed phase

At the time of the formation of the conduction channel, the energy stored in the capacitor is injected into the plasma channel. Current increase to a maximum value I_{max}, is achieved with a minimum of resistance of the plasma channel r_{min} (Fig. 6). The maximum temperature of the gas in the channel increases to 7000–8000°C, causing intense local heating and melting of some areas on the granules surface.

Rapid expansion of the channel leads to a drop in its pressure to a level less than atmospheric. At this point, a part of liquid metal in the form of small droplets is evacuated out of the hole, and some – transform in a vapor state, fill the volume of the channel and ionize (Fig. 7). The subsequent drop in voltage causes the termination of the discharge, thermolysis

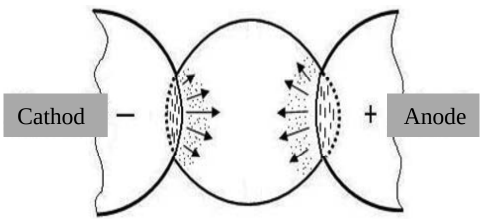


FIGURE 6. Phase of intensive expansion of the channel

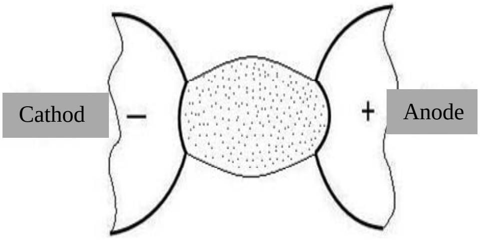


FIGURE 7. Phase of vapor of liquid metal

of plasma and the gradual cooling of the total reaction volume.

The conditions for the heterogeneous metal vapor condensation on the inner surface of the channel wall and the solidification of the melt as a result of heat removal are created (Fig. 8). Ending process – the destruction of the gas gap and filling by water, which leads to rapid cooling of formed metal nanoparticles.

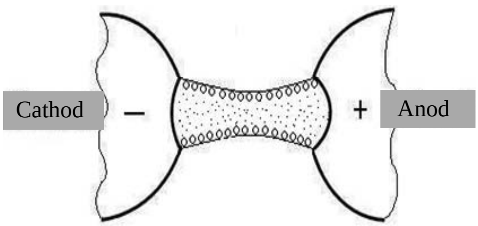


FIGURE 8. Phase of condensation

During electrical erosion of granules, as a result of melting of some surface areas, the fraction of the metal is formed with a diameter of 10 to 500 microns, and at the transformation of liquid to vapor, and its condensation and crystallization – fraction by size from 10 to 100 nm (Fig. 9).

Based on the fact that the basic properties of nanomaterials are formed at the stage of their forming, their effectiveness are closely related to the kinetics of the processes occurring in the reaction zone of the discharge chamber. The combination of intense heat and force action on the metal, which occur during the ultrashort time intervals, a prerequisite to obtain nanomaterials with non-equilibrium structure and increased level of free energy. As a result the high ratio of surface area to volume nanoparticles have new physico-chemical properties in comparison with the bulk samples of the same materials. For example, the Gibbs energy of nanoparticles can be ten times and more greater than the bulk material parameters. Nanoparticles can contain a specific phases observed in bulk materials only in cases impact of high temperatures and pressures. Electrokinetic potential of such particles can differ significantly from the potential of microparticles.

The study of the fine structure showed that in the process of electrical discharge treatment, granules are formed nanoparticles with defects in the crystal structure as dislocations, twins and sources of generation of dislocations. Around the nuclei and the dislocation loops observed distortion of the crystal lattice. Analysis of defects in the crystal structure (Fig. 10) shows that in the α-Fe nanoparticles observed random distribution of dislocation in quantity from $2{\cdot}10^{14}$ to $6{\cdot}10^{14}$ cm^{-2}. Resulting in the electric-processing level of dislocation density (~10^{14} cm^{-2}) is close to the limit and is achieved by the joint action of shock waves, generated in the process of pulsed expansion electric spark channel, at the pressure in several hundred atmospheres, and high, more than 10^3–10^4 ºC/s cooling rate. The level of the lower limit of the cooling rate of nanoparticles determined from the condition that the fixation of γ-Fe at room

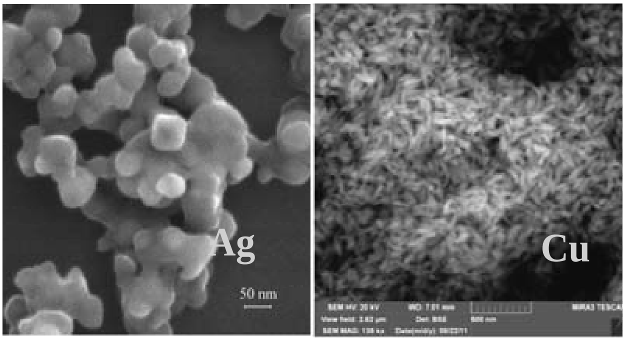


FIGURE 9. Nanoparticles Ag and Cu (SEM)

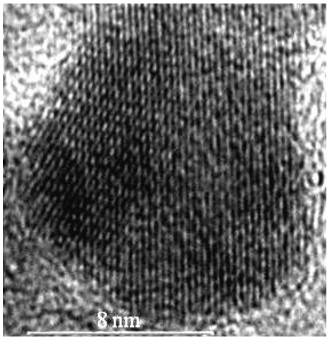


FIGURE 10. Nanoparticles Fe (TEM)

temperature and normal pressure is possible at a cooling rate more than of 10^3–10^4 ºC/s.

Comparison of the density of dislocations in and bulk materials obtained by known methods shows that the level of defects in the crystal structure of nanoparticles, after spark processing, significantly higher than the values achieved by known methods of hardening metals and alloys. The high level of dislocation density in the nanoparticles determines their high energy saturation.

New environmentally friendly methods of fighting with pathogens can be created on base of nanoparticles. At the same time, depending on the concentration of the element is radically changing the properties of matter, which makes them biocidal to pathogenic microorganisms and nutrient complex of microelements for plants.

It is shown that the plants resist infection by mobilizing a complex of protective reactions of the organism. One of the earliest responses of plant organism to the effects of phytopathogen is the local generation of Reactive oxygen species (ROS). According to modern researchers found that a number of pathogens capable of generating active form oxygen outside the plant organism. One possible mechanism to reduce the level of ROS by plants and fungi are the activation by enzymes of antioxidant protect. The virulence of many pathogens is correlated with their catalase activity. We have previously shown that colloidal solutions nanoparticles Mn, Fe, Mg, Mo, Ag, Cu, Zn in water by concentrations from 10^{-10} to 10^{-3} M stimulate plant growth.

Now the great interest to such group of biologically active substances, as nanosolution of metals (colloidal solutions of metals) is shown, which thanks to the structurally-phase condition get a number of properties which are useful to biological objects [6].

The nanoparticles of metals can serve the activators of antioxidant protective mechanism of plants in various stressful conditions: cultivation on “adverse” soils.

It is necessary by working out of preparations which are directed on increase of plants productivity, to establish their influence on accumulation of plant matter. Besides, a very important point is also increase of their stability to natural phytopathogens, which, as it is clearly shown by many authors, their antioxidant system is played an important role in [9, 10]. In relationship between the host plant and the pathogen an essential role plays both the production of ROS, and the processes that lead to their destruction. The special role in these processes belongs to pathogens enzymes: superoxide dismutase, catalase, which activation leads to decrease toxic concentration of ROS to microorganisms.

The most promising in this regard will be preparation that could lead to increase the antioxidant status of plants or reduce the level of antioxidant activity of pathogenic fungi [12, 13].

The purpose of this study was to investigate the protective role of metals nanosolution to plant resistance to the effects of stress factors of biogenic and abiogenic origin.

MATERIALS AND METHODS

The object of study was test culture of watercress. Seeds treatment of nanosolutions metals is for 12 hours, for 5 days with further during germination.

Intracellular enzymes were determined in the leaves disintegrated by destroying cells in 50 mM Tris-HCl buffer (pH 7.8) at the 0–4ºC, and pelleted by centrifugation for 10 min at 5000 r/min.

Fungi biomass were destroyed like plants biomass, and in the resulting disintegration there was determined activity of catalase according to the method [7]. Coefficient of molar extinction of catalase at 410 nm is 22.2×10^3 $mM^{-1} \cdot cm^{-1}$.

The protein concentration in the samples was determined by the method of Bradford, using as a standard albumin of bovine serum [8].

As test solutions were used colloidal solutions of metals Mn, Mg, Mo, Ag with a wide range of concentration 10^{-3} (10^{-4}), 10^{-6}, 10^{-8}, 10^{-10}M: The effect of the colloidal solution of Zn to phytotoxic effect of herbicide Esteron 60 (ethylhexyl ester 2,4-D) are investigate. The test object were pea plants (*Pisum sativum* L.) of “Gotivsky” varieties – a model dicotyledonous weeds, which are sensitive to this herbicide. As control are used plants which are treated with water. Phytotoxicity were measured on index of growth inhibiting of fresh root weight and aerial parts of the plant compared to the control without treatment, the duration of the experiment was 36 days, they were treated with nanosolution during the growing season. The concentration of colloidal solution of Zn was 1 mg/l. Statistical analysis was performed using the software package Sigma Stat – 6.0 and was represented graphically using the program Microsoft Excel.

DISCUSSION OF RESULTS

We conducted a comparative study on the effect on the growth of the stem test culture of watercress of colloids nanoparticles of four metals which concentrations varied by 7 orders of magnitude.

In the study of a wide range of concentrations from 10^{-4} and 10^{-10} M on the growth of the stem was revealed a number of action patterns: Mn stimulated stem growth at concentrations between 10^{-4} and 10^{-10} M, Mo and Mg, and Ag at concentrations of 10^{-4} and 10^{-6} M, Ag 10^{-8} and 10^{-10} M was shown the effect the same as the control (Fig. 11).

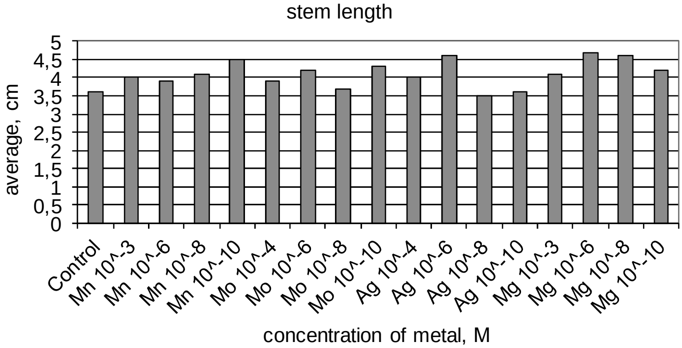


FIGURE 11. The effect of colloidal solutions of nanoparticles on the length of the stem

These data suggest that the investigated colloid solutions have expressed phytostimulation action that depends on their concentration. We found the growth stimulatory effects were observed at low concentrations of colloidal solutions, which is comparable to the concentrations where certain growth factors are acted in, which makes them very promising both from an economic and environmental points of view. Also the effect of nanosolution metals on the activity of catalase in the test culture was investigated (Tab. 2).

It was shown that the colloidal solution of Mo at all studied concentrations of 2–2.5 times increased catalase activity relative to control (without nanosolution action) in the study of colloidal solutions influence on catalase activity – one of the major antioxidant enzymes of plants.

In the presence of Ag stimulation of catalase activity increased with the increase of solution concentration from 10^{-10}M to 10^{-4}M, Mg significantly increased the activity of catalase, however, directly related of the enzyme activity from the solution concentration was found. Only colloid of Mn in the whole range of the investigated concentrations inhibited the catalase activity.

TABLE 2. The influence of metals colloidal solution on the catalase activity

Type of metal	Concentration, Mol	Catalase activity, μMol/mg protein
Control		0.47 ±0 .0235
Mn	10^{-3}	0.37 ±0.0185
	10^{-6}	0.45 ±0.0225
	10^{-8}	0.34 ±0.017
	10^{-10}	0.32 ±0.016
Mo	10^{-4}	0.91 ±0.0455
	10^{-6}	1.23 ±0.25
	10^{-8}	1.23 ±0.25
	10^{-10}	0.92 ±0.046
Ag	10^{-4}	1.11 ±0.05
	10^{-6}	0.78 ±0.039
	10^{-8}	0.67 ±0.03
	10^{-10}	0.56 ±0.028
Mg	10^{-3}	2.22 ±0.11
	10^{-6}	1.22 ±0.06
	10^{-8}	0.76 ±0.038
	10^{-10}	1.29 ±0.06

Three species of phytopathogenic fungi *Botrytis cinerea*, *Alternaria alternate*, *Ulocladium* sp. was analysed the influence of Ag colloidal solution on the catalase activity as an enzyme which participates in the pathological process causing hyphomycetes and promotes the pathogen survival. It is shown that all tested fungi decreased intracellular catalase activity, and the degree of effect severity they were located as follows: *Botrytis cinerea* (25%), *Ulocladium* sp. (33%), *Alternaria alternate* (by 36%) (Tab. 3).

TABLE 3. Effect of Ag colloidal solution on the activity of catalase in fungus

Type of fungus	Catalase activity, μMol/mg protein	
	Control	Option with the addition of Ag
Ulocladium sp.	3.75 ±0.17	2.51 ±0.11
Alternaria alternate	6.43 ±0.31	4.097 ±0.21
Botrytis cinerea	4.81 ±0.22	3.62 ±0.13

The obtained data testifies to decrease in aggression investigated phytopathogens as a result of Ag nanosolution influence.

The effects of Zn colloidal solution on phytotoxic effect herbicide Esteron 60 (ethylhexyl ester 2,4-D) were investigated. Complex 2-ethylhexyl ester, like all phenoxy compound, the active ingredient is 2,4-D acid, acts as a auxin growth inhibitor. Activity of this ethereal forms of 2,4-D is in several times higher than in the amine salts, mainly due to the speed of penetration and defeat the forces of sensitive species. (http://www.agronome.info).

It is established that addition of Zn colloid solution in concentration of 1 mg/l, in the mix with Esteronom 60 weakened damaging action of herbicides on plants. This fact gives the grounds to believe that the Zn colloid solution can be used as an antipillbox at damage of cultural plants by herbicides.

Analysis of the results on the effect of simulated experience Zn nanosolution on pea plants with herbicide injury revealed the following pattern: the processing of Zn colloidal solution mass aerial part of the control plants in 36 day becomes 33.4%, and the mass of roots, by 21.7%, adding Zn colloidal solution in mix with Esteron lead to weight above-ground parts of 37%, roots, by 23.6%, compared to the control without the addition of Zn solution.

Unlike drugs that contain microelements in the form of salts, preparations that contain colloidal solutions of microelements are non-toxic and have a prolonged effect on the plant [6, 11]. This is particularly important for plants growing on problem soils (soils with a high content of sodium chloride and/or calcium carbonate, which is typical for soils of the *Crimean peninsula*), on soils, which are necessary for plant mineral elements are available for plants, which leads to inhibition of plant growth and decline in yields.

Our studies have shown the protective effect of these drugs on cultivated plants damaged by herbicides, which makes them promising as an antidote. Thus, it was found that the investigated colloid solutions exhibit fitostimulation action, and also increases the catalase activity – one of the antioxidant enzymes of plants. The degree of the effect depends on the type of colloidal solution and its concentration. It was revealed the inhibitory effect of Ag colloidal solution to the catalase activity in three species of pathogenic fungi *Botrytis cinerea*, *Alternaria alternate*, *Ulocladium* sp. was shown a reduction (25–36%), which reduces their pathogenic potential.

These results indicate the prospects of study drugs and their necessity of their detailed research.

CONCLUSIONS

The study and physical modeling of a pulsed electric discharge in a weakly conducting medium, the diagnosis of the plasma channel parameters allowed to specify a sequence of transformation and redistribution of energy storage and to identify the main stages of the synthesis of nanoparticles. Numerous experimental data on the kinetics of the process became the basis for the development of technology for production of stable colloidal solutions of metal nanoparticles by electro- physical method. The possibility of controlling the phase-structural composition and morphology of metal nanoparticles. Colloidal solutions of bio metals can be widely used in biology and medicine because of low toxicity and biologically available form.

It was found that vegetative treatment crops colloidal Zn, Cu, Mn, Fe, Mg, Mo, Ag promotes strong root system and greatly enhances the growth of above-ground parts of plants. The result of vegetative treatment of crops with aqueous solutions of preparations of “colloid +” is to increase crop yield by 10–30% and improve the quality of plant material. In contrast, preparations are containing trace elements in the form of salts, preparations containing colloidal solutions of trace elements, non-toxic and have a prolonged effect on the plant and biologic objects.

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Streszczenie: *Uzyskiwanie nanocząstek metali poprzez wyładowania elektryczne w zakresie erozji plazmy w środowisku wodnym w zastosowaniach biologicznych.* Przeprowadzono badania wyładowań elektrycznych w środowisku wodnym w celu uzyskania nanocząstek metali. Określono specyficzne cechy redystrybucji energii oraz przedstawiono fizyczny model fazy rozproszonej. Określono funkcjonalność biologiczną nanocząsteczek metali oraz perspektywy ich wykorzystania w biotechnologii.

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