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## EFFECT OF THIOSULFONATE-BIOSURFACTANT COMPOSITIONS ON PLANTS GROWN IN OIL POLLUTED SOIL

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### Abstract

The work is devoted to the search and investigation of the properties of the effective environmentally friendly phytoremediation activators. An integrated approach was used to evaluate the effect of the compositions of thiosulfonates with rhamnolipid biosurfactant on remediation plants. The biochemical and biophysical analysis of the rapeseed and ryegrass plants grown in the oil polluted soil - photosynthetic pigments, indicators of oxidative reactions and electrical impedance characteristics were determined. When applied via pre-sowing seed treatment, the thiosulfonate-biosurfactant compositions have contributed to the reduction of the indicators of oxidative reactions in plants (malondialdehyde and hydrogen peroxide content) and to the increase of photosynthetic pigments content. The electrical impedance characteristics of the plants were consistent with the data of the biochemical parameters. The composition of alythiosulfanilate with rhamnolipid biocomplex was the most effective preparation. The results testify to the effectiveness of the thiosulfonate-biosurfactant compositions for the improvement of the adaptive capability of plants to adverse conditions and prospects of their application as environmentally friendly means for soil phytoremediation.

*Key words:* biosurfactants, electrical impedance spectroscopy, polluted soil, phytoremediation, rhamnolipid, thiosulfonates

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### 1. Introduction

The contamination of soil and water with petroleum products causes significant changes in the environment, adversely affects crop production and poses a potential danger to the health of humans and animals. The persistence of petroleum pollution in the soil-water medium is dependent on chemical, physical, biological factors. Therefore, petroleum-contaminated areas should be treated in a short time using effective remediation methods.

The biological restoration of soil or water with plants and microorganisms (phytoremediation and

bioremediation) have a priority among the environmentally acceptable and promising methods. Plants are able to improve the structure of polluted soil and to secrete substances that stimulate the viability of the soil organisms (Klamerus-Iwan et al., 2015; Rusin et al., 2015).

The important role in the plant adaptation to the action of unfavorable environmental factors belongs to photosynthetic pigments and antioxidant protection systems. They are involved in the neutralization of the reactive oxygen species (ROS), the excessive accumulation of which in plant cells under the action of stressors initiates the processes of oxidative

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destruction of membrane structures with the formation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA), a product of lipid peroxidation (LPO), which affect proteins and DNA (Bellout et al., 2016; Choudhury et al., 2017; Luna et al., 2005). Therefore, in order to assess the effects of pollutants on plants, indicators of lipid peroxidation should be determined by the content of the product of lipid oxidation - malondialdehyde. Phytoremediation is often ineffective, due to low bioavailability of contaminants via sorption on soil, hydrophobicity and toxicity. So, to increase the effectiveness of remediation plants, various activators are used (Roy et al., 2015; Silva et al., 2014). Growth regulators can have stimulating effect on plant growth and influence their resistance to adverse conditions, in particular soil contamination of various nature (Bulak et al., 2014). Microbial surfactants (biosurfactants) deserve special attention as activators of plant growth and tolerance to adverse conditions, such as soil contamination (Karpenko et al., 2015). They are not inferior to the synthetic ones by their efficiency and, at the same time, are environmentally friendly. Due to their physico-chemical properties (desorption of hydrophobic substances from the soil, their solubilization, reduction of surface and interfacial tension of solutions) and biological activity biosurfactants may increase the degree of biodegradation of contaminants (Galabova et al., 2014).

The phytopathogenic fungi present in the polluted soils, also inhibit growth of plants, therefore application of biocides is advisable approach for plants protection in such soil conditions (Ilarionov et al., 2003). The special role for plants protection is played by thiosulfoesters - biocides with a wide spectrum of biological activity (biocides, preservatives, antioxidants, medicinal substances plant protectors and growth regulators). Thiosulfonates are sulfur-containing, biologically active compounds of the general formula RSO<sub>2</sub>SR' (Huang, 2004). It is reported that synthetically obtained thiosulfonates have a higher antimicrobial activity and are more stable than their analogue natural antibiotic alicin (*Allium sativum* L.) (Hu et al., 2002; Lubenets et al., 2019). Thiosulfonates exhibit a wide range of biological activity (Lubenets et al., 2017). They were reported to be effective as biocides for the protection of paints and varnishes; additives for cutting fluids; petroleum products, building materials and structures; algacides for the protection of surfaces, packaging materials, etc (Abad et al., 2017; Boldyrev et al., 1983).

Thiosulfonates are interesting as potential medicinal agents (Bolibrukh et al., 2015; Nawrot et al., 2012), plant protectors and growth regulators. However, thiosulfonates are characterized by low solubility in water, which limits their application. The increase of permeability of cell membranes by biosurfactants helps to increase hydrophilicity and permeability of cell membranes, which enhances the antimicrobial action of thiosulfonates (Lubenets et al., 2019).

Electrical impedance is a measure of the tissue ability to resist the flow of electrical current. The method of electrical impedance spectroscopy (EIS) (Grossi and Riccò, 2017) is used in biology to characterize the natural physiological properties of living organisms and to study the changes associated with functional behavior and deviation from normal course of physiological processes in cells and tissues. Non-destructive impedance measurements using surface electrodes is a powerful technique to investigate the electrical properties of a large variety of biological materials (Stout et al., 1987; Wu et al., 2008), as the response to an electrical stimulus applied to the tested sample in a wide range of frequencies provides an electrical fingerprint of the investigated material and can be used to monitor and estimate useful parameters of physiological state (Azzarello et al., 2006). Therefore, EIS a suitable technique for the studies of the influence of different preparations on plants in adverse conditions. In the work of Castro-Giráldez et al. (2010), prospective data of dielectric spectra were reported for certain key chemical components of apple in order to consider its potential application as a nondestructive control sensor for the prediction of climacteric fruit maturity. These assays were performed for considering the potential use of dielectric spectra (500 MHz to 20 GHz) to determine the optimal time for the fruit consumption. The total electrical resistance (R, impedance) of plant tissues was determined using a hardware/software module based on the AD5933 chip (USA) (Yusoff, 2016).

In the presented study, an integrated approach to evaluate the effect of thiosulfonate compositions with a biosurfactant on remediation plants, grown in oil polluted soil, was used. Finally, their biochemical parameters and electrical impedance characteristics were determined.

## 2. Material and methods

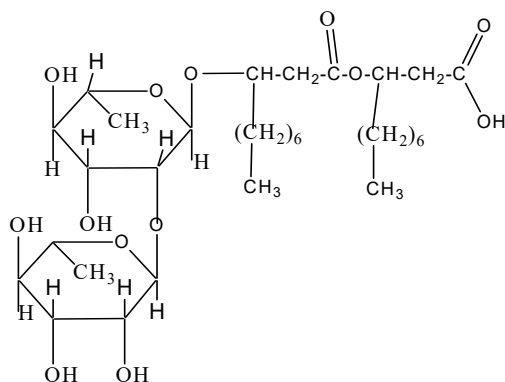
### 2.1. Sampling, sample preparation and characterization oil polluted soil

Clay loam soil (pH=5.3) was used in the experiment. The crude oil for the studies was obtained from Oil Gas Producing Department "Boryslavnaftogaz" (Lviv region, Ukraine). The crude oil characteristics: specific gravity - 0.87 g/cm<sup>3</sup>, freezing point - 130°C, paraffin fractions content - 48 %, naphthenes - 27 %, aromatic hydrocarbons - 20 %. The soil was spiked with oil to final content of 5 % by weight. Soil without oil was used as Control 1 and oil polluted soil - Control 2. The soil was placed in 5 kg containers for each experiment.

The rapeseed (*Brassica napus* L.) and ryegrass (*Lolium perenne* L.) plants were used as phytoremediation agents.

Rhamnolipid biocomplex (RBC), which is the product of microbial synthesis of the strain *Pseudomonas* sp. PS-17 containing rhamnolipids-polysaccharide 4:1 (Karpenko et al., 2009) obtained in the Department of Physical Chemistry of Fossil Fuels

of Litvinenko InPOCC, National Academy of Sciences of Ukraine, was used in the work (Fig. 1).



**Fig. 1.** Rhamnolipid biosurfactant - the product of microbial synthesis of the strain *Pseudomonas* sp. PS-17

*Biocidalthiosulfonates*: ethylthiosulfanilate (ETS), methylthiosulfanilate (MTS), allylthiosulfanilate (ATS) are synthetic analogues of phytoncides of garlic and onion which belong to disulfur-containing organic compounds (Boldyrev et al., 1983), were synthesized at the Department of Technology of Biologically Active Compounds, Pharmacy and Biotechnology of the Lviv Polytechnic National University (Fig. 2) (Banya et al., 2015; Lubenets et al., 2019).

Compositions of ATS, ETS or MTS with RBC were obtained by pre-dissolving the thiosulfonates in a minimum volume of ethanol (96%) and adding to an aqueous RBC solution (0.01 g/L) while stirring the mixture and keeping the temperature at 50°C and pH 7.0. Accordingly, the stable (at room temperature) water-soluble biocide compositions of thiosulfonates with rhamnolipid biocomplex were prepared.

## 2.2. Small lot experiment

The experiment was conducted according to the method (Dospheov, 1985) on the prepared oil polluted soils (Fig. 3). The rapeseed and ryegrass seeds were soaked in the solutions of thiosulfonate-RBC compositions (RBC - 0.01 g/L, thiosulfonates - 0.01 g/L, 3 hours, water was used as control) prior to

sowing. After the treatment, the seeds were sown in containers (50×35×25 cm) with oil contaminated soil. The soil in containers was watered daily (to maintain soil moisture of 70%, providing optimal conditions for each experimental option). Plants were grown outdoors during August-September at an air temperature of +22 - +25°C during the day and +12 - +16°C at night, the duration of daylight hours was 13-14 hours. After 30 days of growth, the physiological parameters of plants were analyzed: the content of photosynthetic pigments, lipid peroxidation activity, electrical impedance characteristics.

## 2.3. The content of photosynthetic pigments

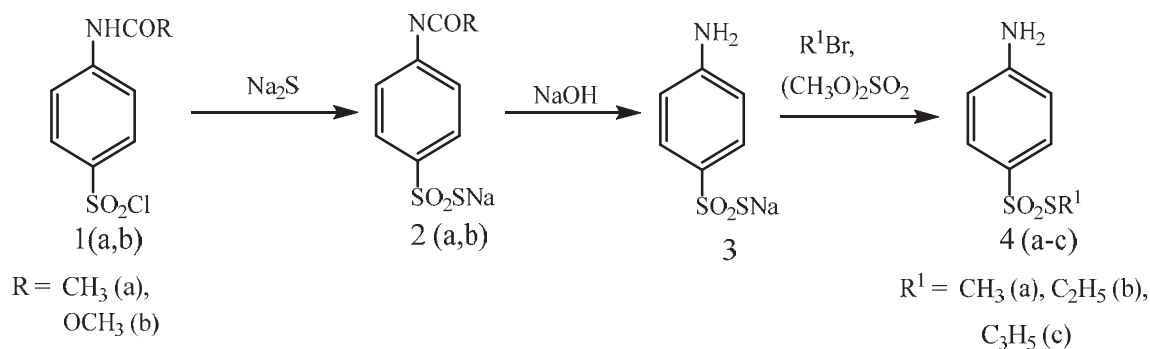
The content of photosynthetic pigments was determined by spectrophotometric method (Musienko et al., 2001); extraction of pigments was carried out with acetone. Absorbance was determined at 662 nm for chlorophyll *a*, 644 nm - chlorophyll *b*, 440.5 nm - carotenoids using the spectrophotometer Shimadzu UVmini-1240 (Shimadzu Corporation, Japan), their content was calculated using Holm-Wettstein equation in mg/g of wet weight.

## 2.4. Hydrogen peroxide content

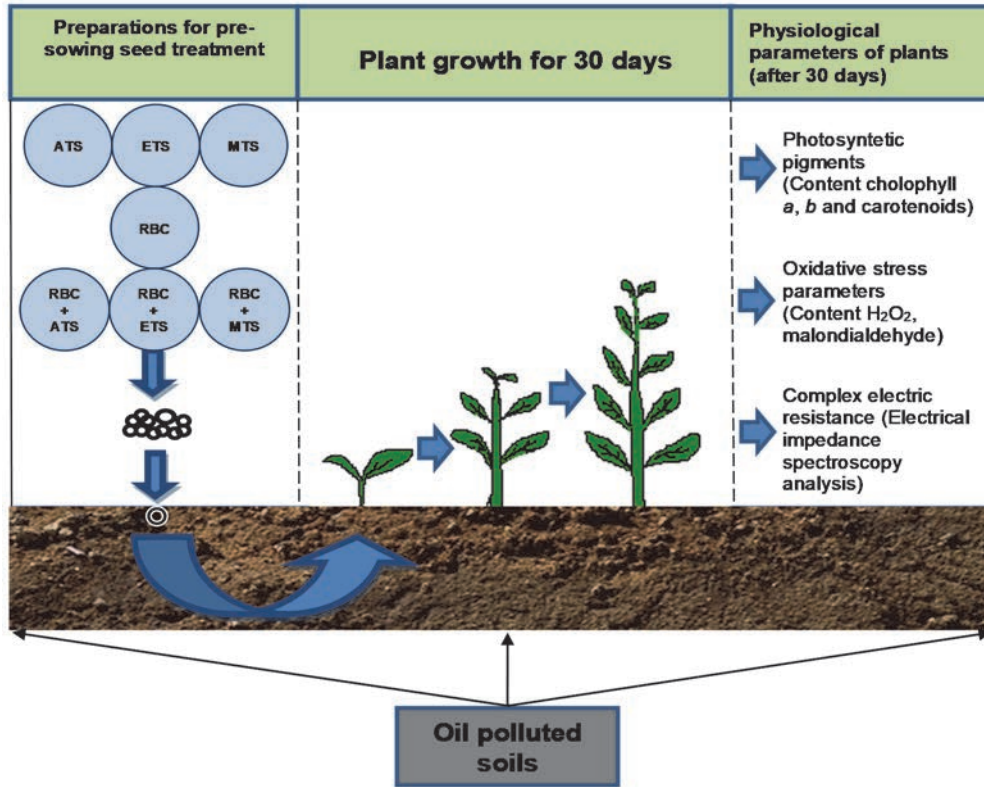
H<sub>2</sub>O<sub>2</sub> content was measured by spectrophotometric method in plant homogenate after centrifugation (Chen and Kao, 1999). 1 mL of the supernatant was supplemented with 3 mL of 0.1% Ti(SO<sub>4</sub>)<sub>2</sub>, the color intensity was assessed at 410 nm using the spectrophotometer Shimadzu UVmini-1240, H<sub>2</sub>O<sub>2</sub> content was expressed as mM/g of wet weight.

## 2.5. Intensity of lipid peroxidation (LPO)

The lipid peroxidation intensity was evaluated by the estimation of the malondialdehyde (MDA) content using its interaction with 2-thiobarbituric acid, as a result a colored product was formed with a maximum light absorption at a wavelength of 532 nm and measured spectrometrically (Bagnyukova et al., 2007).



**Fig. 2.** Scheme of the synthesis of the biocidal thiosulfonates



**Fig. 3.** Scheme for the investigation of the influence of rhamnolipid biocomplex (RBC), ethylthiosulfanilate (ETS), methylthiosulfanilate (MTS), alylthiosulfanilate (ATS) and their compositions on the plants grown in oil polluted soil

## 2.6. Electrical impedance spectroscopy (EIS) measurement

The impedance characteristics of rapeseed and ryegrass were measured below the first internal node of the plants stem with two parallel electrodes located at a distance of 10 mm from each other. Measurements for all samples were carried out under the same growing conditions: temperature and soil moisture. EIS was measured by applying the alternating current (AC) potential to the cell, and then measuring the current through the cell.

Alternating electric current at frequencies between 50-100 kHz was passed through the electrodes superimposed on the test setups, and the complex electrical resistance  $Z$  (Ohms) was measured. The frequency dependences of the complex electrical resistance were measured sequentially in triplicate.

## 2.7. Statistical analysis

All experiments were performed in triplicate and results were expressed as the mean  $\pm$  standard deviation ( $n=3$ ). Several methods were used to compare the treatments: one-way analysis of variance (ANOVA), Mann-Whitney U test, LSD and Tukey multiple comparison test. Conclusions on significant statistical differences among the group averages are based on the Tukey test (Morgan et al., 2012).

## 3. Results and discussion

### 3.1. Evaluation of oxidative stress indicators

The determination of the physiological and biochemical parameters of remediation plants for the evaluation of the effectiveness of potential activators is an important approach for the development of integrated soil remediation technologies (Jyoti et al., 2019; Rusin et al., 2015).

The peroxidation level was determined by the malondialdehyde accumulation (Table 1). The obtained data showed that the MDA content in ryegrass and rapeseed plants increased in the control variant on the polluted soil. This can be explained by the intensification of membrane phospholipids peroxidation under the action of a stressor - oil pollution in soil. At the same time, the reduction of MDA content was observed in variants with pre-sowing seed treatment with solutions of thiosulfonate-biosurfactant compositions. The best results for ryegrass were obtained with ATS-RBC - the MDA index was decreased by 48 %; for rapeseed: with ETC - by 59 %, with ATS+RBC - by 90 % if compared with the control 2 (Table 1). It was revealed that in the oil polluted soil the content of hydrogen peroxide in ryegrass and rapeseed has been increased, which is explained by the action of stress conditions (Ghosh et al., 2011; Gill and Tuteja, 2010).

**Table 1.** Biochemical characteristics of the remediation plants grown in oil polluted soil under the action of thiosulfonate-biosurfactant compositions

	Variants	Oxidative stress		Photosynthetic pigments, mg/g of wet weight			
		H <sub>2</sub> O <sub>2</sub> , μM/g	MDA, μM/g	Chlorophyll			Carotenoides
				a	b	a+b	
Rapeseed ( <i>Brassica napus</i> L.)	Control 1	25.31±1.74	11.818±0.755	0.81±0.06	0.44±0.06	1.25±0.12	0.59±0.04
	Control 2	30.64±1.73	15.786±0.882	0.49±0.007	0.36±0.02	0.85±0.03	0.52±0.04
	ATS	<b>26.71±0.35</b>	<b>11.041±0.475</b>	0.47±0.005	0.21±0.003	0.68±0.008	0.41±0.001
	ETS	<b>22.41±1.26</b>	<b>9.888±0.692</b>	<b>0.52±0.012</b>	0.24±0.005	0.76±0.02	0.45±0.001
	MTS	<b>28.66±1.06</b>	<b>10.462±0.235</b>	<b>0.56±0.07</b>	0.26±0.03	0.82±0.1	0.46±0.06
	ATS+RBC	<b>21.21±1.24</b>	<b>8.311±0.266</b>	<b>0.69±0.09</b>	<b>0.42±0.04</b>	<b>1.11±0.13</b>	<b>0.59±0.04</b>
	ETS+RBC	<b>28.48±1.60</b>	16.085±0.188	0.38±0.02	0.33±0.004	0.71±0.02	0.46±0.02
	MTS+RBC	<b>23.08±1.64</b>	<b>11.999±0.647</b>	0.47±0.007	<b>0.36±0.03</b>	0.83±0.04	0.51±0.001
	RBC	<b>24.00±0.90</b>	<b>10.718±0.292</b>	0.46±0.02	<b>0.58±0.14</b>	<b>1.03±0.12</b>	0.50±0.02
Ryegrass ( <i>Lolium perenne</i> L.)	Control 1	18.30±1.34	5.711±0.391	1.02±0.003	1.95±0.03	2.96±0.03	0.77±0.02
	Control 2	32.29±0.36	9.135±0.322	0.99±0.003	1.25±0.18	2.23±0.18	0.71±0.01
	ATS	31.26±1.39	<b>6.821±0.359</b>	<b>1.01±0.005</b>	1.07±0.11	2.08±0.11	0.71±0.01
	ETS	32.29±1.00	<b>7.021±0.510</b>	<b>1.01±0.002</b>	1.24±0.18	<b>2.26±0.18</b>	0.70±0.007
	MTS	33.59±0.87	<b>7.321±0.334</b>	<b>0.99±0.007</b>	<b>1.36±0.15</b>	<b>2.36±0.14</b>	0.71±0.01
	ATS+RBC	<b>22.01±0.62</b>	<b>6.149±0.313</b>	<b>0.99±0.03</b>	1.19±0.27	2.18±0.24	0.70±0.007
	ETS+RBC	<b>22.05±0.84</b>	<b>6.594±0.102</b>	<b>1.01±0.004</b>	1.12±0.13	2.13±0.13	0.70±0.007
	MTS+RBC	<b>27.97±1.04</b>	<b>6.406±0.226</b>	<b>1.00±0.001</b>	<b>1.33±0.01</b>	<b>2.33±0.01</b>	0.71±0.01
	RBC	<b>23.02±1.30</b>	<b>6.632±0.337</b>	<b>1.00±0.002</b>	<b>1.42±0.04</b>	<b>2.42±0.04</b>	0.71±0.01

Control 1: p<0.001 - \*\*\*; p<0.01 - \*\*; p<0.05 - \*; Control 2: p<0.001 - \*\*\*; p<0.01 - \*\*, p<0.05 - \*

After the pre-sowing seed treatment with thiosulfonate-biosurfactant solutions the H<sub>2</sub>O<sub>2</sub> content was decreased for both plants (Table 1). So, for ryegrass, the best results were in the variants with seeds treatment with RBC solution - by 40%, with ATS+RBC - by 47%, ETS+RBC - 46%; for rapeseed: under the ETS action - by 36%, RBC - by 27%, ATS+RBC - by 4%, MTS+RBC - by 32% compared to control 2.

### 3.2. Content of photosynthetic pigments in remediation plants

Oil pollution in the soil adversely affects the root nutrition of plants, which inhibits a number of processes, including photosynthetic activity. The contents of chlorophylls and carotenoids are the important characteristics of the photosynthetic apparatus adaptation to environmental conditions. They are involved in the formation of plant defense mechanisms under oxidative stress and provide increased resistance to adverse environmental factors (Bellout et al., 2016; Choudhury et al., 2017; Rusin et al., 2015).

It was shown that the content of chlorophylls for both plants lowered in the control variant (oil

polluted soil), which is explained by the negative effect of oil pollution (Table 1). The content of chlorophyll *a* in ryegrass practically didn't differ from the control; however, for rapeseed in the variant with ATS+RBC it was by 40 % higher than in the control 2. It is known that chlorophyll *a* is the main photosynthetic pigment. When exposed to stress factors, the synthesis of organic matter also increases due to an increase in the concentration of auxiliary chlorophyll *b*.

An increase in the chlorophyll *b* content in the variants with rapeseed and ryegrass was also established - in average by 58 % compared with the control 2. The content of carotenoids in ryegrass was within the control 2 (Table 1). Such changes of the pigments content are explained by the physiological adaptation, which includes internal mechanisms of protection and stress resistance.

### 3.3. Physiological state data of remediation plants by the electrical impedance spectroscopy analysis

The electrical impedance spectroscopy (EIS) (Grossi and Riccò, 2017) measurements *in vivo* were used to determine the effectiveness of the use of RBC, antimicrobial thiosulfonates and their complex action

on the physiological state of the rapeseed and ryegrass plants. This method is used in biological studies to characterize the properties of living organisms and determine the changes associated with functional behavior and disturbance of physiological processes. EIS analysis showed that the complex electric resistance ( $Z$ ) of rapeseed and ryegrass plants (Figs. 4-5) have the obvious dependence on the frequency of the alternating current ( $f$ ), therefore  $Z$  of all samples decreases with increasing the frequency from 50 to 100 kHz.

Such electrical impedance changes characterize the capacitive properties of the membrane and internal tissues of plants as biological objects. The curve showing the dependence of resistance on frequency displays the changes in the resistance of living systems. The impedance locus is dropping during functional changes and pathological processes in the object of interest. This criterion can serve as a test of tissue viability. A simpler evaluation of the test of resistance dispersion curve was given by Tarusov et al. (1968), which is determined by the

polarization coefficient ( $K_p$ ) (Eq. 1):

$$K_p = Z(10^4) / Z(10^5) \tag{1}$$

where:  $Z(10^4)$  - resistance at frequency  $10^4$ Hz;  $Z(10^5)$  - resistance at frequency  $10^5$  Hz.

Accordingly,  $K_p$  of the fresh intact tissues must be greater than 1.  $K_p$  of the dead tissue, or of that which has undergone the autolysis processes, is approaching or equal to 1. These criteria for all samples are presented in Table 2 by the numerical values of the resistance difference at the maximum and minimum frequencies  $Z(f_{max}-f_{min})$ , which determine the viability of the test plants. It was shown that the impedance locus steepness of the rapeseed and ryegrass plants grown in unpolluted soil are 35.9 kOm and 36.5 kOm respectively. The impedance locus steepness of the test plants grown in oil polluted soil are 30.1 kOm and 32.8 kOm. Such a reduction in the impedance locus steepness indicates changes in the functional behavior of both test plants and the violation of physiological processes in tissues.

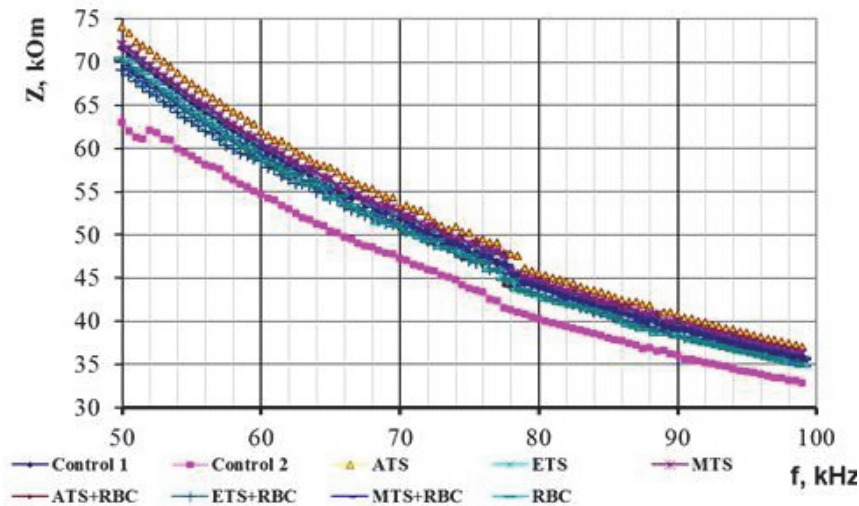


Fig. 4. Influence of thiosulfonate-rhamnolipid biocomplex compositions on the complex electric resistance ( $Z$ ) of the rapeseed plants grown in the oil polluted soil within the frequency range 50-100 kHz

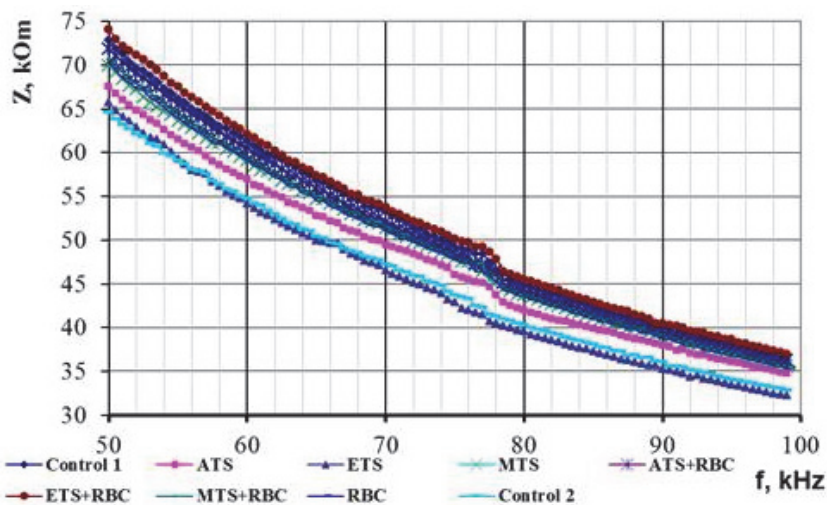


Fig. 5. Influence of thiosulfonate-rhamnolipid biocomplex compositions on the complex electric resistance ( $Z$ ) of the ryegrass plants grown in the oil polluted soil within the frequency range 50-100 kHz

These data correlate with the values of the polarization coefficients  $K_p$ . The polarization coefficient  $K_p$  of absolute control samples of rapeseed is 2.01, and of ryegrass is 2.00 (Table 2).

The values of these indicators were reduced to 1.92 for rapeseed and to 1.94 for ryegrass grown in soils with 5 % oil pollution. The values of polarization coefficient  $K_p$  in other samples have increased which indicates the restoration of the physiological characteristics of plants grown in oil polluted soil to the range of control 1 values as a result of the pre-sowing treatment with thiosulfonates and RBC can recover.

The influence of thiosulfonate-biosurfactant compositions on the electric resistance  $Z$  of the rapeseed and ryegrass plants when growing on the oil polluted soil are presented in Figs. 6-7. All experiments have been conducted at one electric current frequency of 50 kHz. Figs. 6-7 show that electric resistance  $Z$  of the plants grown on polluted soil (control 2 values), significantly decreased compared to control 1 by 12 % for rapeseed and 11.4% for ryegrass. These data indicate the effect of stress factors on the tissues of the studied plants.

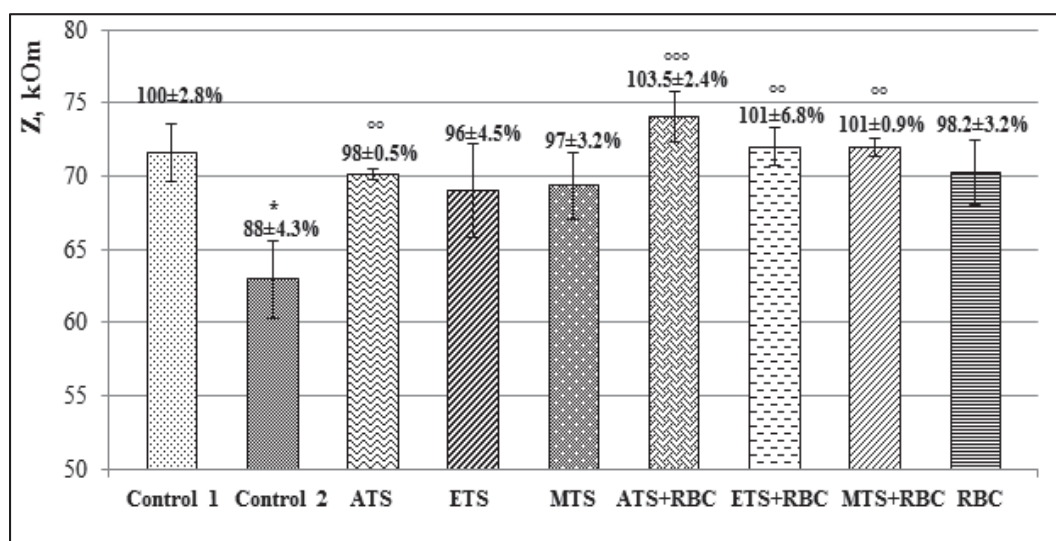
Comparison of mean values in groups with repeated use of the Tukey method reveals significant statistical differences between the mean values ( $P < 0.05$ ) in these groups.

It was shown that after treatment of rapeseed plants with thiosulfonate-RBC compositions, a significant increase in complex resistance was observed (within the range of 8–15.5%), which approached the indices of the plants grown in the unpolluted soil (control 1). This indicates an improvement ( $P < 0.05$ ) in the functional state of the seedlings and the restoration of their physiological properties (Fig. 6). It was found that the most effective composition (with a high degree of significance,  $P < 0.001$ ) was ATS + RBC. Similar results of the changes in the complex resistance were obtained for ryegrass with the compositions ATS + RBC and ETS + RBC (Fig. 7).

The values of the complex resistance of ryegrass have increased: under the influence of ATS + RBC - by 10%, and ETS + RBC - by 13.4% ( $P < 0.01$ ). This indicates an improvement in the physiological parameters of plants on contaminated soil under the influence of the new compositions.

**Table 2.** Impedance locus steepness, polarization coefficient and specific electrical conductivity of the plants grown in oil polluted soil

Test plants Samples	Rapeseed			Ryegrass		
	$Z(f_{max}-f_{min}), Om$	$K_p$	$\gamma, mSm/cm (50 kHz)$	$Z(f_{max}-f_{min}), Om$	$K_p$	$\gamma, mSm/cm (50 kHz)$
Control 1	35.9	2.01	0.349	36.5	2.00	0.343
Control 2	30.1	1.92	0.397	32.8	1.94	0.387
ATS	35.3	<b>2.01</b>	0.356	33.6	<b>2.04</b>	0.370
ETS	33.8	1.96	0.362	34.4	1.97	0.380
MTS	33.8	1.95	0.361	35.6	1.98	0.358
ATS+ RBC	<b>37.1</b>	<b>2.00</b>	<b>0.337</b>	<b>37.1</b>	<b>2.00</b>	0.348
ETS+ RBC	35.8	1.99	0.347	34.9	1.98	<b>0.337</b>
MTS+ RBC	35.8	1.99	0.347	34.9	1.99	0.354
RBC	35.6	2.02	0.356	31.7	1.97	0.356



**Fig. 6.** Influence of thiosulfonate-rhamnolipid biocomplex compositions on the complex electric resistance  $Z$  at 50 kHz of rapeseed plants grown in polluted soil (Control 1:  $p \leq 0.05$  - \*; Control 2:  $p \leq 0.001$  - °°°;  $p \leq 0.01$  - °°;  $p \leq 0.05$  - °)

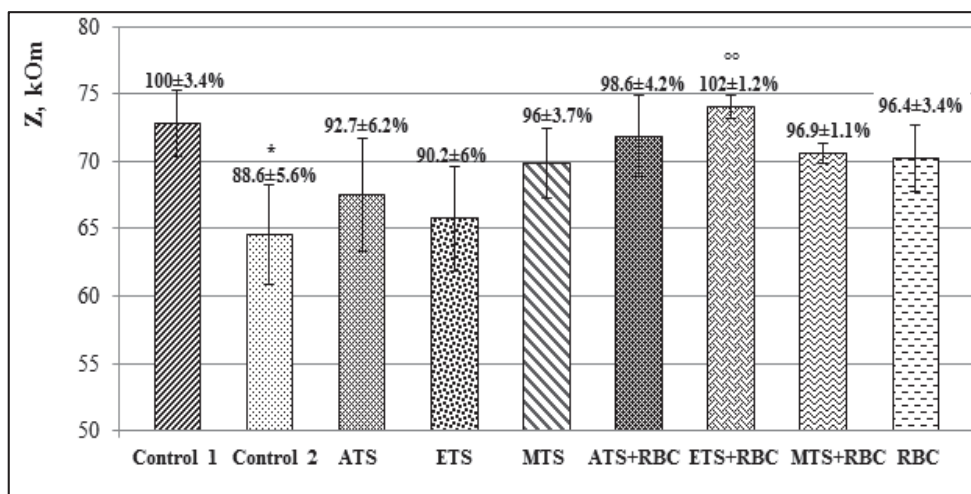


Fig. 7. Influence of thiosulfonate-rhamnolipid biocomplex compositions on the complex electric resistance Z at 50 kHz of ryegrass grown in oil polluted soil (Control 1:  $p \leq 0.05$  - \*; Control 2:  $p \leq 0.01$  - °;  $p \leq 0.05$  - °°)

The obtained results are consistent with published data. It is known that conductivity, as the inverse of the electrical resistance, influences the plant morphology and the ability of plants to absorb water (Zhang et al., 2012) and is resulting from the state of membranes. The higher conductivity is caused by the higher concentration of salts therefore plant absorbs water worse. Under stress conditions (polluted soil), the value of electrical conductivity in plants increases, which indicates deterioration of their physiological state and the ability to absorb water. Therefore, it was affirmed (Azzarello et al., 2006; Stout et al., 1987) that based on the electrical conductivity of plant tissues, it is possible to fix changes in their physiological functions. These changes can be accompanied by pathological processes in cells and membranes, fluid loss, water absorption, and changes in the content of intracellular and intercellular fluids (Azzarello et al., 2006).

The electrical resistance of test plants has increased if compared to the control 1 due to the action of thiosulfonate-biosurfactant compositions. The resistance increase can be explained by the effect of rhamnolipid surfactants on the permeability of their cell membranes, which contributes to the improvement of water and nutrients absorption from soil (Sotirova et al., 2008). It is also known that thiosulfonates positively influenced the condition of plant cells due to their protection against pathogenic yeasts of oil polluted soil.

#### 4. Conclusions

The article presents for the first time the results of the complex study of the effect of the thiosulfonate-biosurfactant compositions on the biological parameters of the rape and ryegrass when grown in oil polluted soil. The comparison of the results of the biochemical and biophysical studies has shown the effectiveness of the new preparations for the improvement of the resistance of the remediation plants to the adverse conditions of technologically

altered soil. This was evidenced by the significant reduction in the values of the oxidative stress indicators of rape and ryegrass, as well as an increase in the content of photosynthetic pigments.

The electrical resistance of the test plants - an important biophysical indicator - was restored to the values found in the plants, grown in the unpolluted soil.

The results indicate the effectiveness of thiosulfonate-biosurfactant compositions for improving plant adaptation to environmental conditions and, accordingly, their prospects for phytoremediation and restoration of contaminated soil and for agriculture as environmentally friendly activators.

#### References

- Abad P., Gil L., Arroyo-Manzanares N., Garcia-Campana A., (2017), Use of onion extract as a dairy cattle feed supplement: monitoring propyl propane thiosulfonate as a marker of its effect on milk attributes, *Journal of Agricultural and Food Chemistry*, **65**, 793-799.
- Azzarello E., Mugnai S., Pandolfi C., Mancuso S., (2006), *Stress Assessment in Plants by Impedance Spectroscopy*, In: *Floriculture, Ornamental and Plant Biotechnology*, vol. III, Global Science Books, 140-148.
- Bagnyukova T., Lushchak O., Storey K., Lushchak V., (2007), Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23°C, *Journal of Thermal Biology*, **32**, 227-234.
- Banya A., Karpenko O., Lubenetis V., Baranov V., Novikov V., Karpenko O., (2015), The influence of surface-active rhamnolipid biocomplex and ethylthiosulfanilate on growth and biochemical parameters of plants in oil polluted soils (in Ukrainian), *Biotechnologia Acta*, **8**, 71-77.
- Bellout Y., Khelif L., Guivarch A., Haouche L., Djebbar R., Carol P., Arous Belbachir O., (2016), Impact of edaphic hydrocarbon pollution on the morphology and physiology of pea roots (*Pisum sativum* L.), *Applied Ecology and Environmental Research*, **14**, 511-525.



- Boldyrev B., Bilozor T., Vljazlo R., Kozharskij A., Lubenec V., Luzheckaja O., Spas L., Solonin V., (1983), *Antimicrobial and Physiological Activity of Esters of Thiosulfonic Acids and Possible Ways of Their Practical Use in Various Sectors of the Economy*, In: *Biodeterioration in industry. Interuniversity Collection*, (in Russian), Anisimov A. (Ed.), Gorky State University, Gorky, 44-52.
- Bolibrukh K., Polovkovych S., Khoumeri O., Halenova I., Savchuk O., Terme T., Vanelle P., Lubenets V., Novikov V., (2015), Synthesis and anti-platelet activity of thiosulfonate derivatives containing a quinone moiety, *Scientia Pharmaceutica*, **83**, 221-231.
- Bulak P., Walkiewicz A., Brzezinska M., (2014), Plant growth regulators-assisted phytoextraction, *Biologia Plantarum*, **58**, 1-8.
- Castro-Giráldez M., Fito P.J., Chenoll C., Fito P., (2010), Development of a dielectric spectroscopy technique for determining key chemical components of apple maturity, *Journal of Agricultural and Food Chemistry*, **58**, 3761-3766.
- Chen L., Kao C., (1999), Effect of excess copper on rice leaves: evidence for involvement of lipid peroxidation, *Botanical Bulletin of Academia Sinica*, **40**, 283-287.
- Choudhury F., Rivero R., Blumwald E., Mittler R., (2017), Reactive oxygen species, abiotic stress and stress combination, *The Plant Journal*, **90**, 856-867.
- Dosphehov B., (1985), *The Field Experiment Method (with Basics of Statistical Processing of Research Results)*, (in Russian), Agroindustrial Publishing House, Moscow, Russia.
- Galabova D., Sotirova A., Karpenko E., Karpenko O., (2014), *Role of Microbial Surface-Active Compounds in Environmental Protection*, In: *The Role of Colloidal Systems in Environmental Protection*, Fanun M. (Ed.), Elsevier B.V., Amsterdam, 41-84.
- Ghosh N., Adak M., Ghosh P., Gupta S., Sengupta D., Mandal C., (2011), Differential responses of two rice varieties to salt stress, *Plant Biotechnology Reports*, **5**, 89-103.
- Gill S., Tuteja N., (2010), Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, *Plant Physiology and Biochemistry*, **48**, 909-930.
- Grossi M., Riccò B., (2017), Electrical impedance spectroscopy (EIS) for biological analysis and food characterization: A review, *Journal of Sensors and Sensor Systems*, **6**, 303-325.
- Hu Q., Yang Q., Yamato O., Yamasaki M., Maede Yo., Yoshihara T., (2002), Isolation and identification of organosulfur compounds oxidizing canine erythrocytes from garlic (*Allium sativum*), *Journal of Agricultural and Food Chemistry*, **50**, 1059-1062.
- Huang X., (2004), Study on the content and chemical constituents of volatile oil in welsh onions (*Allium fistulosum* L. var. giganteum Makino), *Food and Fermentation Industries*, **30**, 114-117.
- Ilarionov S., Nazarov A., Kalachnikova I., (2003), The role of micromycetes in the phytotoxicity of crude oil-polluted soils (in Russian), *Ecology*, **5**, 341-346.
- Jyoti R., Jyoti S., Pankaj S., (2019), *Biochar: A Sustainable Approach for Improving Plant Growth and Soil Properties*, In: *Biochar an Imperative Amendment for Soil and the Environment*, Vikas A., (Ed.), IntechOpen, London, 1-17.
- Karpenko O., Banya A., Baranov V., Novikov V., Kotwzan B., (2015), Influence of – biopreparations on phytoremediation of petroleum-contaminated soil, *Polish Journal of Environmental Studies*, **24**, 2009-2015.
- Karpenko E., Pokin'broda T., Makitra R., Pal'chikova E., (2009), Optimal methods of isolation of biogenic rhamnolipid surfactants (in Russian), *Russian Journal of General Chemistry*, **79**, 2637-2640.
- Klamerus-Iwan A., Błońska E., Lasota Ja., Kalandyk A., Waligórski, P., (2015), Influence of oil contamination on physical and biological properties of forest soil after chainsaw use, *Water, Air and Soil Pollution*, **226**, 389, <https://doi.org/10.1007/s11270-015-2649-2>.
- Lubenets V., Stadnytska N., Baranovych D., Vasylyuk S., Karpenko O., Havryliak V., Novikov V., (2019), *Thiosulfonates: The Prospective Substances Against Fungal Infections*, In: *Fungal Infection*, Silva de Loreto E. (Ed.), IntechOpen, London, 1-25.
- Lubenets V., Vasylyuk S., Monka N., Bolibruch K., Komarovska-Porokhnyavets O., Baranovych D., Musyanovych R., Zaczynska E., Czarny A., Nawrot U., Novikov V., (2017), Synthesis and antimicrobial properties of 4-acylaminobenzene-thiosulfoacid S-esters, *Saudi Pharmaceutical Journal*, **25**, 266-274.
- Luna C., Pastori G., Driscoll S., Groten K., Bernard S., Foyer C., (2005), Drought control on H<sub>2</sub>O<sub>2</sub> accumulation, catalase (CAT) activity and CAT gene expression in wheat, *Journal of Experimental Botany*, **56**, 417-423.
- Morgan G.A., Leech Nancy L., Gloeckner Gene W., Barrett Karen C., (2012), *IBM SPSS for Introductory Statistics*, In: *Use and Interpretation*, 4th Edition, Routledge Taylor & Francis Group, New York, 1-256.
- Musienko M., Parshykova T., Slavnyi P., (2001), *Spectrophotometric Methods in Practice of Physiology, Biochemistry and Ecology of Plants*, In: *Scientific Thought* (in Ukrainian), Kyiv, Ukraine, 49-50.
- Nawrot U., Zaczyńska E., Czarny A., Lubenets V., Karpenko E., (2012), Antifungal activity of synthetic derivatives of allicin - continued research, *Mikologia Lekarska*, **19**, 143-146.
- Roy S., Chandni S., Das I., Karthik L., Kumar G., Rao K., (2015), Aquatic model for engine oil degradation by rhamnolipid producing *Nocardiopsis* VITSISB, **3 Biotech**, **5**, 153-164.
- Rusin M., Gospodarek J., Nadgórska-Socha A., (2015), The effect of petroleum-derived substances on the growth and chemical composition of *Vicia faba* L., *Polish Journal of Environmental Studies*, **24**, 2157-2166.
- Silva R.D.C.F.S., Almeida D.G., Rufino R.D., Luna J.M., Santos V.A., Sarubbo L.A., (2014), Applications of biosurfactants in the petroleum industry and the remediation of oil spills, *International Journal of Molecular Sciences*, **15**, 12523-12542.
- Sotirova A., Spasova D., Galabova D., Karpenko E., Shulga A., (2008), Rhamnolipid-biosurfactant permeabilizing effects on gram-positive and gram-negative bacterial strains, *Current Microbiology*, **56**, 639-644.
- Stout D., Hall J., McLaughlin N., (1987), *In vivo* plant impedance measurements and characterization of membrane electrical properties: The influence of cold acclimation, *Cryobiology*, **24**, 148-162.

- Tarusov B., Antonov V., Burlakova E., Burdin K., Kozlov Ju., Kol's O., Kriger Ju., Kudrjashov Ju., Kurella G., Litvin F., Mamedov T., Parhomenko I., Petrusevich Ju., Jaglova L., (1968), *Electrical Conductivity of Biological Systems*, In: *Biophysics* (in Russian), Tarusov B., Kol's O. (Eds.), Hight School, Moscow, Russia, 188-193.
- Wu L., Ogawa Yu., Tagawa A., (2008), Electrical impedance spectroscopy analysis of eggplant and effects of drying and freezing-thawing treatments on its impedance characteristics, *Journal of Food Engineering*, **87**, 274-280.
- Yusoff. M.A.M., (2016), Development of AD5933-based impedance meter prototype for impedimetric sensor applications, *Procedia Chemistry*, **20**, 56-59.
- Zhang Q., Li Q., Zhang G., (2012), Rapid determination of leaf water content using VIS/NIR spectroscopy analysis with wavelength selection, *Spectroscopy: An International Journal*, **27**, 93-105.