Recently, new promising, economically viable and environmentally safe biotechnological methods of remediation of contaminated areas, are becoming particularly important. Continuously growing influence is attracted to phytoremediation technologies with application of stable plants and growth stimulators [1]. Growth regulators can have stimulating effect on plant growth and influence their resistance to adverse conditions, in particular soil contamination of various nature [2, 3]. According to the literature, the use of preparations Emistim C and Agrostimulin in pre-sowing treatment of seeds helped to accelerate the development of plants and improve their adaptation to stressful environmental conditions [4].

In our opinion, biogenic surfactants (biosurfactants) can be promising agents for the improvement of plant resistance to adverse conditions due to their high efficiency and unique physical, chemical and biological properties. Biosurfactants have several advantages over synthetic surfactants, since they are biodegradable and low toxic, which determines their use in environmentally safe technologies [5]. The ability of biosurfactants to influence the permeability of cell membranes, activity of enzymes, and other biologically active substances promotes plant growth [6].

It is known that biosurfactants can be used for remediation of contaminated areas, including soil, contaminated with petroleum and petroleum products [7]. Not only petroleum but also the pathogenic fungi, present in the contaminated soils, inhibit plant growth and the use of biocides...
is also appropriate to protect plants in these conditions [8]. Biocide ethylthiosulfanilate (ETS) — a synthetic analogue of phytoncide allicin of garlic or onion — belongs to disulfur-containing organic compounds and has a broad spectrum of antimicrobial activity [9]. At low concentrations ETS may act as plant growth regulator [10]. However, its effect on the growth of plants in petroleum contaminated soils was not investigated.

An important role in the adaptation of plants to unfavorable conditions belongs to photosynthesis pigments and antioxidant biochemical reactions [11]. They participate in neutralizing reactive oxygen species (ROS), which excessively accumulate in plant cells under the influence of stressors and initiate the processes of oxidative degradation of membrane structures with formation of hydrogen peroxide and malondialdehyde (MDA) — a product of lipid peroxidation (LPO) which affects proteins and DNA. However, ROS and LPO products may play the role of signaling molecules involved in the activation of protective systems in cells.

In turn, hydrogen peroxide is considered as a messenger in the transmission of cellular signals, because it is a small, short-lived, freely diffusing molecule that is generated as a result of enzymatic reactions in response to the stress signal and affects thiol groups of proteins [12]. Hydrogen peroxide is accumulated in plant cells due to photorespiration and redox processes and in excess amounts has a toxic effect on cells. In this regard, the destruction of hydrogen peroxide is important for preventing toxic effect on cells and for the formation of water and oxygen, vital for plants.

Based on the above, the aim of the work was to study the influence biosurfactants of rhamnolipid nature and biocide ethylthiosulfanilate on growth (morphometric) and biochemical parameters of plants, which characterize the adaptive processes when growing on contaminated soils.

**Materials and Methods**

Seeds of field pea (Pisum arvense L.) and sorghum sudangrass (Sorghum bicolor subsp. drummondii), and biological preparations — ethylthiosulfanilate (a synthetic analogue of garlic phytoncides from the Department of Technology of Biologically Active Compounds, Pharmacy and Biotechnology of the Lviv Polytechnic National University) [9, 10], and rhamnolipid biocomplex (RBC), which is the product of microbial synthesis of the strain Pseudomonas sp. PS-17 containing rhamnolipid surfactants and polysaccharide (4:1) obtained in the Department of Physical Chemistry of Fossil Fuels of the Litvinenko Institute of Physical-Organic and Coal Chemistry of the National Academy of Sciences of Ukraine) were used in the work [6, 13]. The work was carried out with artificially spiked soils with different petroleum content (5%, 8% and 10%) as absolute control with clean garden soil.

Pre-sowing seed treatment was carried by the conventional method [14]: the seeds were soaked for 3 hours in solutions (0.01 g/l) of RBC and ETS, the control variant was soaked in water, then planted in containers, each containing 300 g of soil.

Plants were grown in the laboratory conditions at 18–20 °C under fluorescent light (fluorescent lamp spectrum maximum — 0.50–0.66 nm) during 21 days, photoperiod was 12 hours of light and 12 of darkness. After that morphometric (weight of shoot and root, length of the plants) and biochemical parameters (content of photosynthetic pigments, hydrogen peroxide, malondialdehyde) were determined.

Plant photosynthesis pigments were determined by spectrophotometric method, extraction of pigments was carried out with acetone. Absorbance was determined at 662 nm (for chlorophyll a), 644 nm (for chlorophyll b) and 440.5 nm (for carotenoids) using the spectrophotometer Shimadzu UVmini-1240 (Shimadzu Corporation, Japan), their content was calculated using Holm-Wettstein formulas [15] in mg/g of wet matter weight.

Malondialdehyde content in plants during lipid peroxidation (LPO) was determined by its interaction with 2-thiobarbituric acid, which resulted in formation of colored product with maximum absorption at 532 and 600 nm [16]. MDA content was expressed in mol/g of wet matter weight.

Malondialdehyde content in plants during lipid peroxidation (LPO) was determined by its interaction with 2-thiobarbituric acid, which resulted in formation of colored product with maximum absorption at 532 and 600 nm [16]. MDA content was expressed in mol/g of wet matter weight.

The hydrogen peroxide content was measured by spectrophotometric method in plant homogenate after centrifugation [17]. 1 ml of the supernatant was supplemented with 3 ml of 0.1% Ti(SO₄)₂, the color intensity was assessed at 410 nm, H₂O₂ content was expressed as mM/g of wet matter weight. The experiments were performed in triplicates. Statist and Microsoft Exel software were used for for statistical data processing.
Results and Discussion

To study the influence of ETS and RBC on plants the artificially spiked soil with different petroleum content (5%, 8% and 10%) was used. Such model pollution was selected according to a range of petroleum concentrations occurring in the soil at sites subject to reclamation (including the territories of petroleum companies in Western Ukraine).

The optimum concentration of RBC and ETS solutions by their effect on plants were identified in previous studies [6, 10].

In the first stage the influence of ETS and RBC on morphometric parameters of the growth of plants on soil with different petroleum concentrations were studied. When comparing growth parameters of the studied plants the stimulating effect of the action of both RBC and ETS was observed, but the best results were shown for field pea: the mass of plants which is an integral factor of their growth and development, increased for shoot on 39% and for root — on 26% (Fig. 1, A).

Plant dimensions also increased: shoot length — on 35% in average, the root length — on 48% (Fig. 1, B).

In experimental variants with sorghum the increase of morphometric parameters under the influence of ETS and RBC was observed (Fig. 2).

As it is seen from the results the stimulating effect of ETS and RBC for sorghum was rather lower than for field pea.

The influence of rhamnolipid surfactants on plants can be explained primarily by their effect on the permeability of cell membranes, which helps to improve the absorption of water and nutrients from the soil. Biosurfactants may also stimulate the growth of plant cells by stretching, which was shown earlier in the biotests on wheat coleoptile cuts [6]. It is known that the effect of increase in cell stretching and weight is associated with better water absorption, activation of membrane enzymes, including H+ATPase and acid hydrolases [18]. Thiosulfanilates are highly reactive compounds interacting with nucleophiles, electrophiles, radicals.

Nucleophilic substitution reactions occur with an opening of –S-S-bond due to redistribution of electron density in thiosulfogroup that determines the direction of nucleophile attacks [9]. Presumably, ETS can contribute to the metabolism of RNA and DNA, which contain amino groups, as well as metabolism of proteins and amino acids with disulfide and sulfide fragments, which are the components of plant cells. This can cause accelerated cell division affecting enzymatic processes. The ETS influence on plants is obviously closely related with its protective action, participation in redox processes, contributing to violations of respiratory system of pathogenic microorganisms. At that thiosulfanilates have low toxicity (LD$_{50}$ = 2000 mg/kg). In our opinion, in the case of ETS application for pre-sowing treatment of seeds it doesn’t accumulate in the soil. It was shown that at low concentrations ETS can improve seed germination, weight of seedlings, reduce the number of diseased plants [10].

Under the influence of RBC and ETS the content of photosynthetic pigments increased in both plants (Fig. 3, 4), which are important indicators of plant metabolism both in normal and in stress conditions [19, 20].

![Fig. 1. Morphometric parameters of field pea under the influence of rhamnolipid biocomplex and ethylthio-sulfanilate when growing on petroleum contaminated soil: A — weight of shoot and root; B — length of shoot and root; C — absolute control — garden soil; * — $P \geq 0.05$ if compared with control](image)
Thus, increase in total chlorophyll a+b content in field pea under the influence of RBC and ETS when growing in petroleum contaminated soil was established: 5% contamination — on 9% and 5% respectively; 8% contamination — 34% and 27%; 10% contamination — 14% and 7% if compared to control (Fig. 3), which may indicate an increase of shade-tolerance of plants. This is confirmed by the literature data on the relationship between the total chlorophyll content in plants and their tolerance to low insolation [19] and is of great importance for plant adaptation, particularly in the Western region of Ukraine, which is characterized by a significant amount of dark, overcast days.

For sorghum the increase of total chlorophyll a+b content under the influence of RBC and ETS was observed: 5% contamination — on 20% and 10% respectively; 8% contamination — on 21% and 7%; 10% contamination — 22% and 16% if compared to control (Fig. 4). The established increase of chlorophyll a+b content in both plants may indicate the photosynthesis stimulation, which is probably connected with improvement of germination conditions caused by the action of RBC and ETS.

To evaluate the intensity of redox processes that characterize the negative impact of environmental factors on plants, the content of hydrogen peroxide and malondialdehyde were determined (Table 1, 2).

Thus, it was found that the content of these indicators in field pea (in the case of seed treatment with RBC and ETS solutions) has decreased: hydrogen peroxide — on 23% and 22% respectively, malondialdehyde — on 16% and 18% respectively compared to control (Table 2).

The reduction of the studied parameters was observed in sorghum plants after the treatment of seeds with RBC and ETS solutions: hydrogen peroxide content — on 8% and 10.6%, respectively, malondialdehyde —
Experimental articles

Table 1. Content of MDA and hydrogen peroxide in field pea plants grown on petroleum contaminated soils

<table>
<thead>
<tr>
<th>Experimental variants</th>
<th>$H_2O_2$, mcM/g of wet weight</th>
<th>Malondialdehyde, mcM/g of wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.92 ± 0.06</td>
<td>0.694 ± 0.032</td>
</tr>
<tr>
<td>Petroleum 5%</td>
<td>2.50 ± 0.05</td>
<td>0.909 ± 0.029</td>
</tr>
<tr>
<td>Rhamnolipid biocomplex</td>
<td>2.37 ± 0.07</td>
<td>0.753 ± 0.044</td>
</tr>
<tr>
<td>Ethylthiosulfanilate</td>
<td>2.30 ± 0.01</td>
<td>0.742 ± 0.024</td>
</tr>
<tr>
<td>Petroleum 8%</td>
<td>3.19 ± 0.08</td>
<td>1.011 ± 0.030</td>
</tr>
<tr>
<td>Rhamnolipid biocomplex</td>
<td>2.31 ± 0.10</td>
<td>0.882 ± 0.011</td>
</tr>
<tr>
<td>Ethylthiosulfanilate</td>
<td>2.48 ± 0.03</td>
<td>0.844 ± 0.010</td>
</tr>
<tr>
<td>Petroleum 10%</td>
<td>3.54 ± 0.07</td>
<td>1.140 ± 0.037</td>
</tr>
<tr>
<td>Rhamnolipid biocomplex</td>
<td>2.78 ± 0.11</td>
<td>0.985 ± 0.021</td>
</tr>
<tr>
<td>Ethylthiosulfanilate</td>
<td>2.72 ± 0.13</td>
<td>0.993 ± 0.013</td>
</tr>
</tbody>
</table>

Note: $n = 30$; all values are significantly different from control $P \geq 0.05$.

Table 2. Content of MDA and hydrogen peroxide in sorghum plants grown on petroleum contaminated soils

<table>
<thead>
<tr>
<th>Experimental variants</th>
<th>$H_2O_2$, mcM/g of wet weight</th>
<th>Malondialdehyde, mcM/g of wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.04 ± 0.05</td>
<td>0.613 ± 0.032</td>
</tr>
<tr>
<td>Petroleum 5%</td>
<td>2.06 ± 0.09</td>
<td>0.839 ± 0.056</td>
</tr>
<tr>
<td>Rhamnolipid biocomplex</td>
<td>1.91 ± 0.11</td>
<td>0.753 ± 0.047</td>
</tr>
<tr>
<td>Ethylthiosulfanilate</td>
<td>1.86 ± 0.06</td>
<td>0.704 ± 0.035</td>
</tr>
<tr>
<td>Petroleum 8%</td>
<td>2.58 ± 0.15</td>
<td>1.086 ± 0.075</td>
</tr>
<tr>
<td>Rhamnolipid biocomplex</td>
<td>2.39 ± 0.06</td>
<td>0.914 ± 0.023</td>
</tr>
<tr>
<td>Ethylthiosulfanilate</td>
<td>2.34 ± 0.05</td>
<td>0.903 ± 0.032</td>
</tr>
<tr>
<td>Petroleum 10%</td>
<td>3.03 ± 0.20</td>
<td>1.168 ± 0.031</td>
</tr>
<tr>
<td>Rhamnolipid biocomplex</td>
<td>2.81 ± 0.03</td>
<td>1.120 ± 0.025</td>
</tr>
<tr>
<td>Ethylthiosulfanilate</td>
<td>2.70 ± 0.09</td>
<td>1.103 ± 0.038</td>
</tr>
</tbody>
</table>

Note: $n = 30$; all values are significantly different from control $P \geq 0.05$.

On 11.3% and 15%, respectively, if compared to control (Table 2).

According to the results, when cultivating the plants in contaminated soil oxidative reactions were activated (increase of MDA and $H_2O_2$), but after the seed treatment with RBC and ETS solutions, these parameters were significantly reduced, which could point to reduction of the impact of pollution. Since the intensity of oxidative reactions in field pea has decreased under the influence of RBC and ETS more than in sorghum, it can be assumed that field pea has better adaptive capacity for soil contamination.

Thus, the stimulating influence of RBC and ETS on the growth of field pea and sorghum on petroleum contaminated soils was determined: in field pea the shoot weight increased on 39%, root weight — on 26%, shoot length — on 35% and root length — on 48% if compared with control. The content of photosynthetic pigments increased in field...
pea and sorghum under the influence of RBC and ETS: chlorophyll $a$ — on 12.8% and 6.6% respectively, chlorophyll $b$ — on 28.1% and 17.8%, chlorophyll $a+b$ — on 19.5% and 11%, carotenoids — 8.3% and 6.4%. The decrease of lipid peroxidation parameters in field pea and sorghum as a result of RBC and ETS application was established: the of hydrogen peroxide content — on 15% and 16% respectively, malondialdehyde content — 13.5% and 16%, indicating increased resistance of plants to unfavorable conditions of contaminated soil.

The obtained results indicate the prospects of application of biosurfactants and thiosulfonate biocides as efficient and environmentally friendly substances for stimulation of phytoremediation of technological contaminated areas.


REFERENCES
ВПЛИВ ПОВЕРХНЕВО-АКТИВНОГО РАМНОЛІПІДНОГО БІОКОМПЛЕКСУ ТА ЕТИЛТИОСУЛЬФАНІЛАТУ НА РОСТОВІ ТА БІОХІМІЧНІ ПОКАЗНИКИ РОСЛИН НА ГРУНТІ, ЗАБРУДНЕНОМУ НАФТОЮ

А. Р. Баня1, О. Я. Карпенко3, В. І. Лубенець3, В. І. Баранов2, В. П. Новиков3, О. В. Карпенко1

1Відділення фізико-хімії горючих копалин Інституту фізико-органічної хімії та углехімії ім. Л. М. Литвиненка НАН України, Львів
2Львівський національний університет імені Івана Франка, Україна
3Національний університет «Львівська політехніка», Україна

E-mail: e.v.karpenko@gmail.com

Метою роботи було дослідження впливу рамноліпідного біокомплексу та етилтиосульфанилату на ростові показники (маса, довжина рослин) і біохімічні показники (вміст пігментів фотосинтезу, пероксиду водню, малонового діальдегіду) рослин гороху польового та сорго, вирощуваних на ґрунті, забрудненому нефтою.

Показано стимулювальне впливі рамноліпідного біокомплексу на ростові показники гороху польового: маса побега достовірно збільшилася на 39%, кореня — на 26% порівняно з контролем. Для сорго спостерігався різні приріст показників росту. Под впливу рамноліпідного біокомплексу збільшувався вміст пігментів фотосинтезу. Установлено, що при дії рамноліпідного біокомплексу збільшується вміст пігментів фотосинтезу. Встановлено, що при дії рамноліпідного біокомплексу збільшується вміст пігментів фотосинтезу.

Ключові слова: рамноліпідний біокомплекс, етилтиосульфанилат, горох польовий, сорго, нафта.