

IMPACT OF OIL CONTAMINATION AND HUMATES
ON THE GROWTH OF POACEAE

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Abstract. The article examines the impact of oil contamination and humates (*Humifield Forte* and *Fulvital Plus*) on the growth characteristics of Poaceae, such as corn (*Zea mays* L.) of Dostatok 300 MV hybrid, spring barley of the Karat variety and spring wheat of the Diana variety. Oil contamination has proved to inhibit plant growth and affect a decrease in the amount of photosynthetic pigments in the leaves, which can be explained by oil toxicity and the acquired hydrophobicity of soil. Soaking seeds in humate solution proved the effectiveness of their use for corn (*Zea mays* L.) of Dostatok 300 MV hybrid and spring barley of the Karat variety: the plants developed well and accumulated biomass actively. The use of humates for spring wheat of the Diana variety did not produce the desired effect: growth characteristics were lower, and the studied plants were weaker. An increase in the number of photosynthetic pigments, especially carotenoids, in the leaves of Poaceae plants indicates the feasibility of using humates to increase the stress resistance of plants in the early stages of growth and development in conditions of oil contamination of soil. A stimulating effect of the combination of oil contamination and Fulvital Plus on the growth of Poaceae was proved. Fulvital Plus is a growth stimulator and deficiency corrector of plant nutrition elements. The results obtained from the research prove the effectiveness of using humates for plants and can be used to increase the stress resistance and yield capacity of Poaceae in man-made conditions and to develop phytoremediation technologies for restoring oil-contaminated areas.

Keywords: oil-contaminated soils, humates, Poaceae, plant growth, photosynthetic pigments, phytoremediation.

1. Introduction

Pollution of the natural environment with oil and petroleum products is one of the multifaceted issues of environmental protection. Due to contamination, the ability of soil to self-clean is disrupted. No other pollutant can compete with oil in terms of the scale of distribution associated with its production, transportation, processing and storage, as well as with the level of chemical loads on all components of the environment. Oil contamination of the soil is closely related to the increased negative impact of harmful substances on the soil biota (Dzhura, 2011; Dzhura, Podan, 2017; Horova et al., 2012; Klimova, 2006; Tumanyan et al., 2017).

The negative effect of oil on the soil is that it disrupts the water-air balance, reduces the moisture capacity, blocks the availability of mineral nutrition elements due to hydrophobization of the soil surface particles with heavy fractions of hydrocarbons, and inhibits biological processes with toxic components of the oil. Restoration of soil fertility and its protection from pollution is one of the most complex

scientific problems of our time and thus requires taking a complex of physical, chemical and biological measures. Research on the participation of plants in the processes of destruction of oil components and development of innovative technologies for remediation of contaminated soils remain relevant and important in both theoretical and applied aspects (Banks, Schultz, 2005; Dzhura, 2011; Gupta et al., 2011).

We analyze the issues of phytoremediation of oil-contaminated areas and possible ways to increase the stress resistance of plants in man-made conditions. Fertilizers based on humic and fulvic acids which have high biological activity and are low in impurities have appeared in the Ukrainian market. These products are in high demand all over the world and are known as anti-stress agents and growth stimulants, as they provide long-term mobilization of the main elements of mineral nutrition (N, P, K) in the soil and improve its water regime (Danalatos et al., 2007; Horova et al., 2015; Park et al., 2011; Podan, Dzhura, 2019). It is known that humic products in general and *Humifield Forte* and *Fulvital Plus* in particular, are multifunctional and have bioprotective properties that ensure active growth and development of plants, formation of high yields and high quality of crops, as well as increase stress resistance to adverse environmental conditions (Kim et al., 2012; Matsiuk, 2006; Medkov et al., 2017; Stefanovska et al., 2017). Studying the effectiveness of the use of these products in phytoremediation technologies is of great scientific interest. Taking into account the topicality of the problem, we were the first to investigate the effect of oil contamination of soil and humates on the growth of Poaceae in order to make further predictions about the effectiveness of their use for the restoration of oil-contaminated areas through expanding the species diversity by seeds.

2. Materials and Methods

The objects of research were Poaceae plants: corn (*Zea mays* L.) of Dostatok 300 MV hybrid, spring barley of the Karat variety and spring wheat of the Diana variety, grown in laboratory conditions on oil-contaminated soils under the influence of humates (*Humifield Forte* and *Fulvital Plus*). Experiments were conducted according to the following scheme: No. 1—control (soil without oil) + Poaceae plants; No. 2—soil

selected in the vicinity of the oil well on the Staryi Sambir oil field in Lviv region (repeatedly contaminated) + Poaceae plants; No. 3—soil No. 2 + oil, 10 g/kg (simulating an oil spill) + Poaceae plants; No. 4—soil No. 2 + oil, 10 g/kg + Poaceae plants + *Humifield Forte*; No. 5—soil No. 2 + oil, 10 g/kg + Poaceae plants + *Fulvital Plus*. Two weeks after oil insertion, seeds were planted. For variants 1, 2, 3, seeds were pre-soaked in water and for variants 4 and 5—in solutions of *Humifield Forte* and *Fulvital plus* (0.2 g per 1 litre of water) respectively. Figure 3 shows the studied plants in pots. This scheme applies to all the results of the studied variants presented in Fig. 1–5.

On the 15th and 25th days of vegetation, morphometric parameters and the content of photosynthetic pigments in plants were analyzed according to the generally accepted methodology. Chlorophyll A and B content and the total amount of carotenoids were quantified in the leaves from a single sample by the spectrophotometric method (Musiienko et al., 2012). The experiments were repeated three times. The obtained results were processed statistically. The reliability of the difference between the control and experimental variants was evaluated with Student's t-test, and differences where the significance level p was <0.05 were considered as probable.

3. Results and Discussion

It is known that oil contamination of the soil can change the morphological structure and physiological activity of plants. The degree of inhibition of plant growth and development depends on the intensity of contamination, which in turn depends on both oil toxicity and the acquired hydrophobicity of the soil. Taking into account the indirect impact of oil contamination of soils on plants (water stress, impaired absorption function of roots, significant lack of nutrients, etc), it can be argued that such conditions are unfavourable for plant growth (Dzhura, Podan, 2017).

Growth is one of the most important parameters that characterizes a plant's response to stress. Research results have shown that Poaceae, such as corn (*Zea mays* L.) of Dostatok 300 MV hybrid, spring barley of the Karat variety and spring wheat of the Diana variety show resistance to oil

contamination of the soil because even without the use of humates, seeds sprouted, but plants accumulated biomass more slowly (Fig. 1, 2, 3, variants 2, 3). For instance, the height of the

aboveground part in corn was half that of the control, and in barley and wheat – at the level of the control (Fig. 1, 2, variants 2, 3 as compared to variant 1).



Corn (*Zea mays* L.) of Dostatok 300 MV hybrid

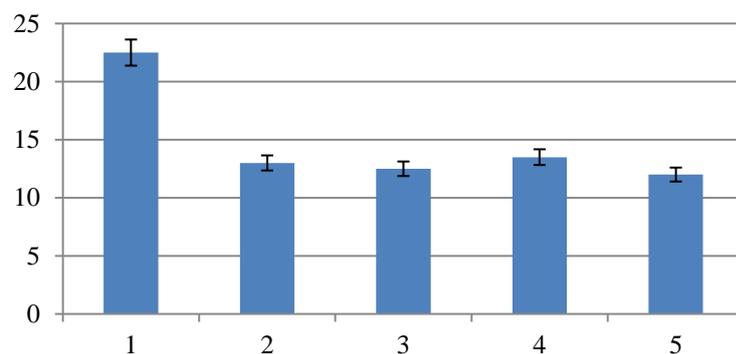
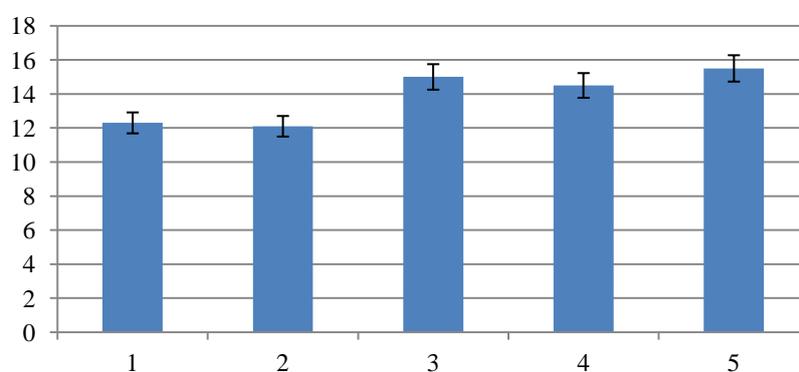


Spring barley of the Karat variety

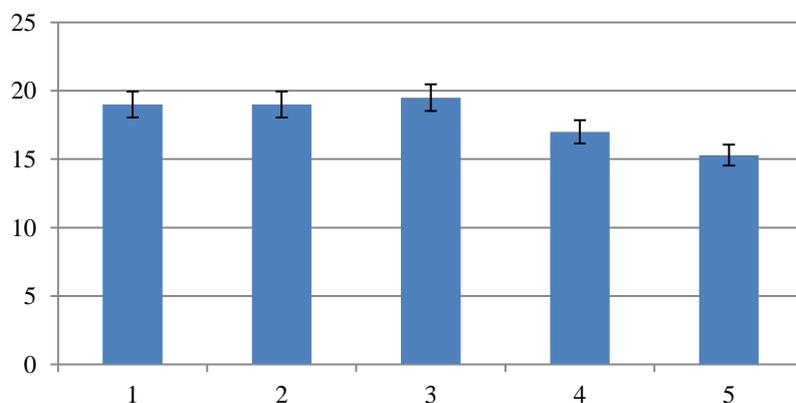


Spring wheat of the Diana variety

Fig. 1. Impact of oil contamination and humates on Poaceae growth

Corn (*Zea mays* L.) of Dostatok 300 MV hybrid

Spring barley of the Karat variety



Spring wheat of the Diana variety

Fig. 2. Height of the aboveground part of Poaceae under the effect of oil contamination and humates, cm

No. 1 – control (soil without oil) + Poaceae plants;

No. 2 – soil selected in the vicinity of the oil well in the Staryi Sambir oil field in Lviv region (repeatedly contaminated) + Poaceae plants;

No. 3 – soil No. 2 + oil, 10 g/kg (simulating an oil spill) + Poaceae plants;

No. 4 – soil No. 2 + oil, 10 g/kg + Poaceae plants + *Humifield Forte*;

No. 5 – soil No. 2 + oil, 10 g/kg + Poaceae plants + *Fulvital Plus*.

The use of *Humifield Forte* (variant 4) and *Fulvital Plus* (variant 5) somewhat stimulated the growth and development of corn and barley plants and contributed to a greater accumulation of biomass, the plants looked more stable and stronger (Fig. 3, variants 4, 5, as compared to variants 1, 2, 3).

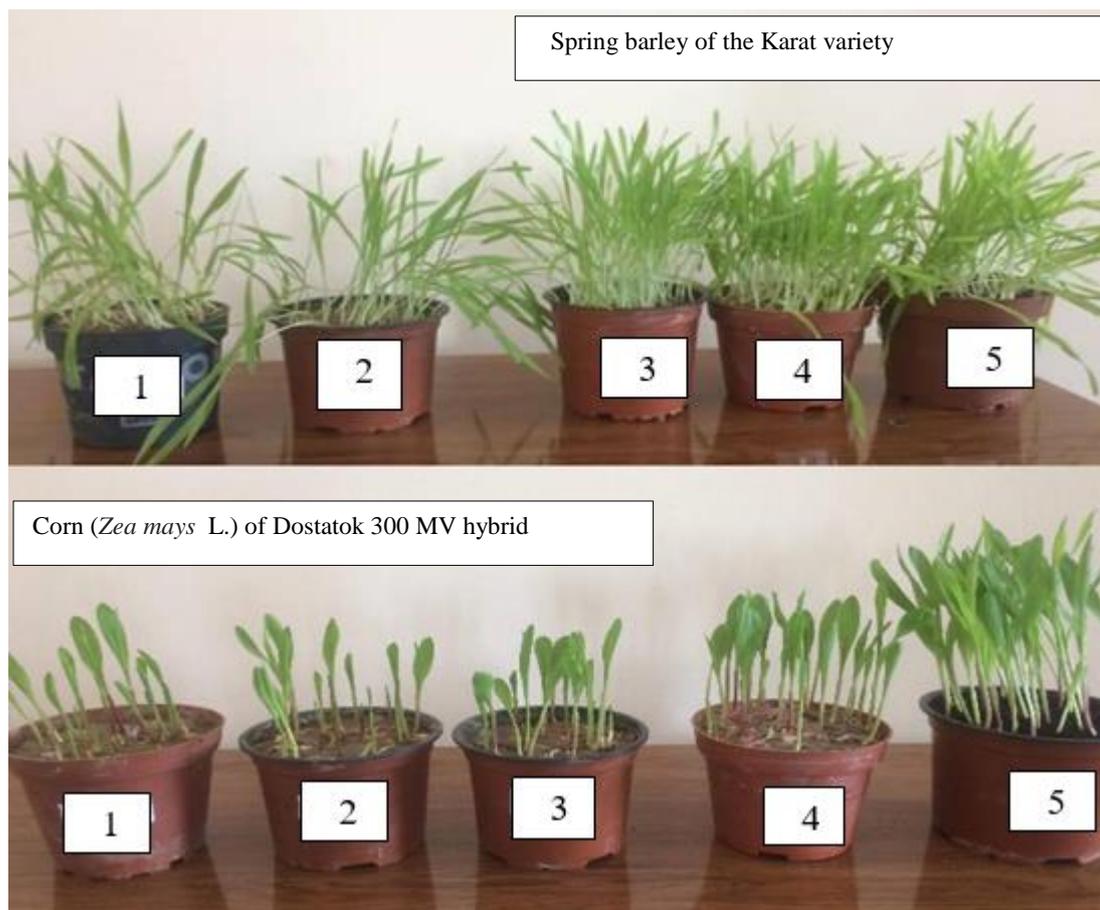


Fig. 3. Barley and corn plants under the effect of oil contamination and humates

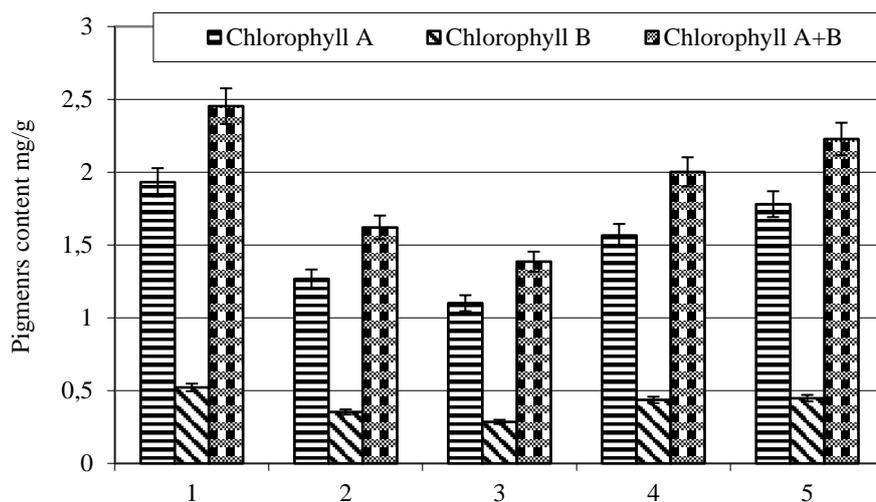
The experiment showed that spring wheat of the Diana variety proved resistant to oil contamination as well: seeds sprouted, plants accumulated biomass (see Fig. 1, 2, variants 2, 3), while the use of Humifield Forte (variant 4) and Fulvital Plus (variant 5) did not give the desired effect: growth parameters were lower, the studied plants were weaker.

The direct effect of oil on the vegetation cover is manifested in the fact that plant growth slows down, the functions of photosynthesis and respiration are disrupted, various morphological disorders can be noted, the root system, leaves, stems and reproductive organs suffer. Photosynthetic pigments, especially chlorophylls, are very sensitive indicators of plant health. Changes in the content and composition of pigments under the influence of stress factors are caused by changes in the ratio of the synthesis intensities and catabolism. At the same time, a number of scholars note bigger changes in the chlorophyll A content as compared to chlorophyll B and explain this process by the lability of the former, which serves as a substrate for the biosynthesis of the latter. Chlorophyll

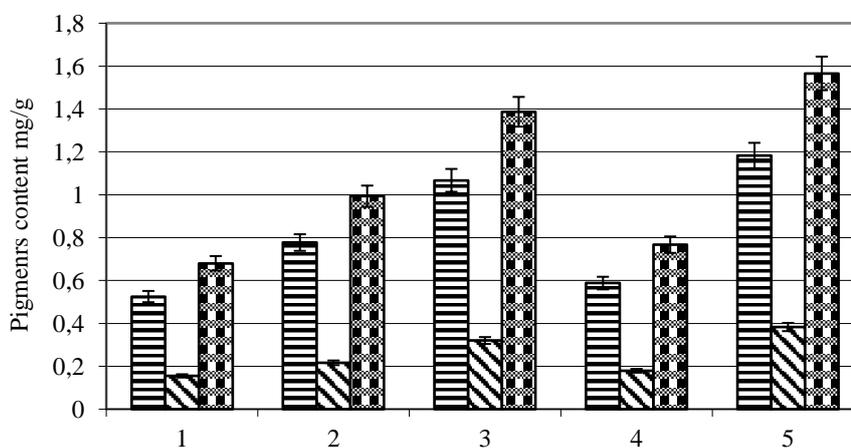
content in leaves is one of the most significant characteristics of adaptation of the photosynthetic apparatus of plants to adverse environmental conditions (Banks, Schultz, 2005; Dzhura, 2011; Dzhura, Podan, 2017).

We studied the content of photosynthetic pigments in the leaves of Poaceae plants (Fig. 4, 5). Thus, under the influence of oil, the content of chlorophylls in the leaves of *Z. mays* was decreased by 30–40 % (see Fig. 4, variants 2, 3 as compared to variant 1).

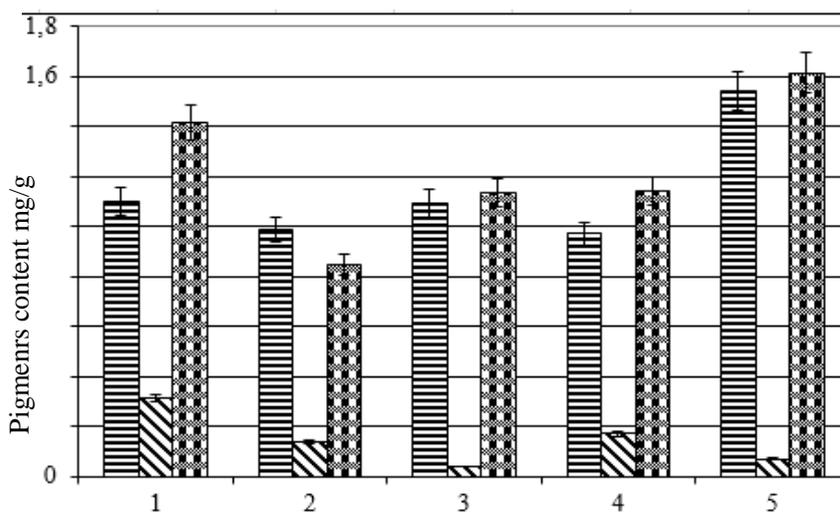
Obviously, the harmful effect of tarry asphaltene substances of oil on the soil changes its water-air regime and physicochemical properties, which leads to a deficiency of mineral nutrition elements and a violation of their bioavailability to plants. While the use of *Humifield Forte* and *Fulvital plus* – growth stimulators and deficiency correctors of plant nutrition elements (variants 4, 5) – contributed to an increase in the content of green pigments in the leaves of *Z. mays*, which may indicate the formation of stress resistance of corn at the molecular and cellular level.



Corn (*Zea mays* L.) of Dostatok 300 MV hybrid



Spring barley of the Karat variety



Spring wheat of the Diana variety

Fig. 4. Content of photosynthetic pigments in the leaves of Poaceae under the effect of oil contamination and humates

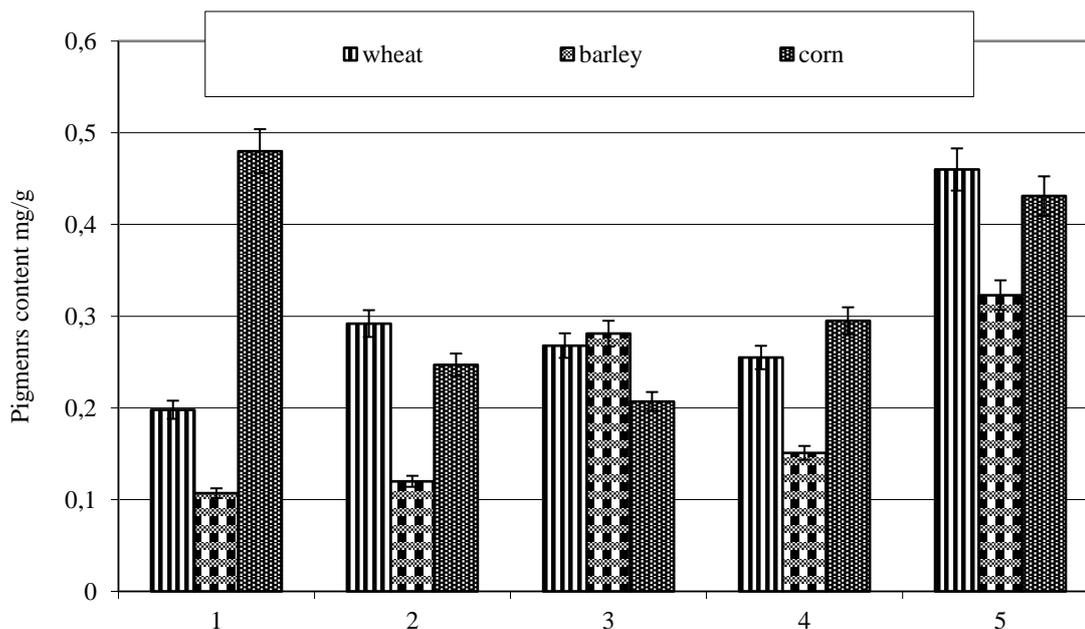


Fig. 5. Carotenoid content in the leaves of Poaceae under the effect of oil contamination and humates

In barley leaves, application of *Humifield Forte* contributed to a decrease in the content of green pigments (variant 4 as compared to variants 2, 3), but the studied indicators were at the level of control as compared to variant 1. The reasons for such an effect require further explanation and will be explored in future studies. At the same time, the stimulating effect of joint exposure to oil contamination and *Fulvital Plus* (variant 5) on the growth and content of photosynthetic pigments in spring barley of the Karat variety was shown.

The experiment showed that the content of photosynthetic pigments in the leaves of spring wheat of the Diana variety under the influence of oil contamination remained almost at the level of control (variants 2, 3 as compared to variant 1). It was proved that wheat leaves also experienced a stimulating effect of joint exposure to oil contamination and *Fulvital Plus* (variant 5) on the chlorophyll A content, the total amount of chlorophylls (A + B) and carotenoids (see Fig. 5).

Carotenoids are important components of the photosynthetic apparatus of plants. Not only do they capture and supply additional light energy to the reaction centres of photosystems I and II, but they also protect the photosystems from the destructive effects of light and oxygen. As unsaturated hydrocarbons, they contain a significant amount of conjugated double bonds between carbon atoms, so they can actively

intercept free radicals and inhibit free radical reactions. Carotenoids are polyfunctional pigments that are parts of the multicomponent antioxidant system and are considered as one of the factors that ensure the tolerance of plants to various stressful factors (Pidlisnyuk et al., 2016; Turgay et al., 2009).

Carotenoids are less sensitive to stress than chlorophylls. However, when exposed to oil, their content in the leaves of the studied plants also decreases (see Fig. 5, variants 2, 3). The use of *Humifield Forte* and *Fulvital Plus* (variants 4, 5) contributes to an increase in the carotenoid content, which may indicate an increase in the stress resistance of the studied plants.

Thus, pre-soaking seeds in the solutions of anti-stress agents (*Humifield Forte* and *Fulvital Plus*) increases the resistance of plants, which is manifested in an increase in the content of photosynthetic pigments, in particular carotenoids, and this confirms the feasibility of using humates to increase the stress resistance of plants in the early stages of growth and development in conditions of oil contamination of the soil.

4. Conclusions

Poaceae, such as corn (*Zea mays* L.) of Dostatok 300 MV hybrid, spring barley of the Karat variety, and spring wheat of the Diana variety, are

resistant to oil contamination because even without the use of humates, seeds sprouted and plants accumulated biomass. Oil contamination somewhat inhibited plant growth (variants 2, 3), affected the decrease in photosynthetic pigments content in the leaves due to both oil toxicity and acquired hydrophobicity of the soil. Pre-soaking Poaceae seeds in the solutions of *Humifield Forte* (variant 4) and *Fulvital Plus* (variant 5) proved the effectiveness of their use for corn and barley: the plants developed well, accumulated biomass intensively and looked stronger and more stable. The use of humates for spring wheat of the Diana variety did not give the desired effect: growth indicators were lower, the studied plants were weaker (variants 4, 5). The study showed the stimulating effect of joint exposure to oil pollution and *Fulvital Plus* (variant 5) on the content of carotenoids (variant 5) in the Poaceae leaves, which indicates the feasibility of using this humate to increase the stress resistance of plants in the early stages of growth and development in conditions of oil contamination of the soil.

The obtained research results can be used to increase the stress resistance and yield of Poaceae in conditions of oil contamination and in the development of phytoremediation technologies for the restoration of oil-contaminated areas.

References

- Banks, M. K., & Schultz, K. E. (2005). Comparison of plants for germination toxicity tests in petroleum-contaminated soils. *Water, Air, and Soil Pollution*, 167 (1-4), 211–219. doi: <https://doi.org/10.1007/s11270-005-8553-4>
- Danalatos, N., Archontoulis, S., & Mitsios, I. (2007). Potential growth and biomass productivity of miscanthus × giganteus as affected by plant density and N-fertilization in Central Greece. *Biomass and Bioenergy*, 31(2-3), 145–152. doi: <https://doi.org/10.1016/j.biombioe.2006.07.004>
- Dzhura, N. (2011). Perspektyvy fitoremediatsii naftozabrudnnykh gruntiv roslynamy Faba bona Medic. (Vicia faba L.) [Prospects for phytoremediation of oil-contaminated soils by Faba bona Medic plants. (Vicia faba L.)]. *Visnyk Lvivskoho Universytetu. Seriya Biologichna*, (57), 117–124. Retrieved from <http://prima.lnu.edu.ua/faculty/biologh/wis/57/6/15/15.pdf>
- Dzhura, N., & Podan, I. (2017). Ekologichni naslidky dohotryvaloho naftovydobutku na Starosambirskomu rodovyshchi [Ecological consequences of extended oil production at Staryi Sambir petroleum deposit]. *Visnyk Lvivskoho Universytetu. Seriya Biologichna*, 76, 120–127. Retrieved from <http://prima.lnu.edu.ua/faculty/biologh/wis/76/4/15/15.pdf>
- Gorova, A., Pavlychenko, A., Kulyna, S. and Shkremetko, O., (2012). Ecological problems of post-industrial mining areas. *Geomechanical Processes During Underground Mining*, 35–40. doi: <https://doi.org/10.1201/b13157>
- Gupta, V. K., Jain, P. K., Gaur, R. K., Lowry, M., Jaroli, D. P., & Chauhan, U. K. (2011). Bioremediation of petroleum oil contaminated soil and water. *Research Journal of Environmental Toxicology*, 5(1), 1–26. doi: <https://doi.org/10.3923/rjet.2011.1.26>
- Horova, A., Pavlychenko, A., & Vysochyn, L. (2015). *Vykorystannia huminovykh rechovyn dlia vidnovlennia gruntiv u hirnychodobuvnykh rehionakh [Use of humic substances for soil restoration in mining regions]*. II International Scientific and Practical Conference Restoration of Biotic Potential of Agroecosystems (pp. 50–51). Dnipropetrovsk; Arbuz.
- Kim, S., Da, K., & Mei, C. (2012). An efficient system for high-quality large-scale micropropagation of miscanthus × giganteus plants. *In Vitro Cellular & Developmental Biology - Plant*, 48(6), 613–619. doi: <https://doi.org/10.1007/s11627-012-9472-x>
- Klimova, N. (2006). Deiaki pytannia metodyky otsinky stanu zabrudnennia gruntiv unaslidok naftohazovydobutku [Some questions of the methodic of soil pollution state value as a result of oil and gas mining]. *Visnyk Lvivskoho Universytetu. Seriya «Heohrafiia»*, 33, 144–151. doi: <http://dx.doi.org/10.30970/vgg.2006.33.2674>
- Matsiuk, D. (2006). Ekonomiko-matematychni ta ekoloho-enerhetychni aspekty vykorystannia sukhoi biomasy yak alternatyvnoho dzherela enerhii [Economic-mathematical and ecological-power aspects of dry mass usage as alternative source of energy]. *Visnyk Vinnytskoho Politekhnichnoho Instytutu*, 5, 111–113. Retrieved from <https://visnyk.vntu.edu.ua/index.php/visnyk/article/view/348>
- Medkov, A., Stefanovska, T., Pidlisniuk, V., & Ponomarenko, S. (2017). Vplyv rehulatoriv rostu roslyn na adaptyvni vlastyvoli miskantusu hihantskoho (Miscanthus x giganteus) dlia vyrobnytstva biomasy na gruntakh, zabrudnnykh vazhkymy metalamy [Impact of plant growth regulators to Miscanthus x giganteus establishment while producing biomass at land contaminated by heavy metals]. *Biologichni Studii*, 11(3-4), 100–101. doi: <http://dx.doi.org/10.30970/sbi.1103>
- Musiienko, M., Parshykova, T., & Slavnyi, P. (2001). *Spektrofotometrychni metody v praktytsi fiziologii, biokhimii ta ekologii roslyn [Spectrophotometric methods in the practice of physiology, biochemistry and plant ecology]*. Fitosotsiotsentr, Kyiv.
- Park, S., Kim, K. S., Kim, J.-T., Kang, D., & Sung, K. (2011). Effects of humic acid on phytodegradation of petroleum hydrocarbons in soil simultaneously contaminated with heavy metals. *Journal of Environmental Sciences*, 23(12), 2034–2041. doi: [https://doi.org/10.1016/s1001-0742\(10\)60670-5](https://doi.org/10.1016/s1001-0742(10)60670-5)
- Pidlisnyuk, V., Trögl, J., Stefanovska, T., Shapoval, P., & Erickson, L. (2016). Preliminary results on growing second generation biofuel crop miscanthus x giganteus at the

- polluted military site in Ukraine. *Nova Biotechnologica Et Chimica*, 15(1), 77–84. doi: <https://doi.org/10.1515/nbec-2016-0008>
- Podan, I. I., & Dzhura, N. M. (2019). Influence of oil pollution and humates on growth of Miscanthus Plants. *Ecological Sciences*, 2, 182–186. doi: <https://doi.org/10.32846/2306-9716-2019-2-25-30>
- Stefanovska, T., Pidlisniuk, v, Bilyi, O., Kvak, V., Tsvihun, H., & Shapoval, P. (2017). Ahronomichni aspekty vyroshchuvannia miskantusu hihantskoho (*Miscanthus x giganteus*) yak syrovyny dlia vyrobnytstva tverdoho biopalyva na zabrudnenykh vnaslidok viiskovoi diialnosti gruntakh [Agronomic aspects of growing *Miscanthus x giganteus* as a raw material for the production of solid biofuels on soils contaminated because of military activity]. *Biologichni Studii*, 11(3-4), 99–100. doi: <http://dx.doi.org/10.30970/sbi.1103>
- Tumanyan, A., Tyutyuma, N., Bondarenko, A., & Shcherbakova, N. (2017). Influence of Oil Pollution on Various Types of Soil. *Chemistry and Technology of Fuels and Oils*, 53(3), 369–376. doi: <http://dx.doi.org/10.1007/s10553-017-0813-7>
- Turgay, O. C., Erdogan, E. E., & Karaca, A. (2009). Effect of humic deposit (leonardite) on degradation of semi-volatile and heavy hydrocarbons and soil quality in crude-oil-contaminated soil. *Environmental Monitoring and Assessment*, 170(1-4), 45–58. doi: <https://doi.org/10.1007/s10661-009-1213-1>