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BIOCHEMICAL COMPONENTS OF NITROGEN BALANCE IN SOIL UNDER CRUDE OIL CONTAMINATION AND PHYTOREMEDIATION

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Introduction. Nitrogen availability in oil contaminated soil is adversely affected due to an increase in the inorganic carbon (C) and nitrogen (N) ratio, unfavorable changes in physical, chemical and biological properties of the soil. Phytoremediation of oil contaminated soil may have a positive effect on N cycling. Changes in soil enzyme activity, content of organic and mineral N in the soil can be used as indicators of nitrogen balance. This paper aims to identify some biochemical elements of N cycling in oil contaminated soil and the effect of plants in recovering N balance in the soil.

Materials and Methods. In this study artificially oil contaminated soil (50 mL of crude oil per 1 kg of soil) was remediated by plants *Zea mays* L. and V*icia faba* var. *minor.* Soil samples were collected on the 10th day after oil pollution, on the 22nd day (seeds were sown), on the 65th day (30 days of plants' growth), and on the 95th day (60 days of plants' growth). Soil without oil and plant vegetation was used as control. We determined the nodulation ability of soil, activity of soil protease and urease, content of labile organic nitrogen and free amino acids accumulation in the soil.

Results. Our study revealed the absence of nodules on *V. faba* roots in oil contamination conditions. Protease activity was inhibited in oil contaminated soil compared to control. Under plant vegetation and oil contamination conditions, soil protease activity increased on the 65th day, and decreased on the 95th day compared to oil contaminated soil without plants. Amino acid concentration was significantly smaller for oil contaminated soil during the experiment than for soil without oil, but amino acid content was significantly greater for soil planted with *Z. mays* than for soil without plants. *V. faba* had a stimulating effect on amino acid accumulation only for uncontaminated soil compared to soil without plants. The results have shown that the urease activity decreased in oil contaminated soil during all experimental period. Results indicated that 22 days after oil pollution, the content of labile organic N decreased in oil contaminated soil, whereas on further stages of the experiment it was not significantly different compared to control. Significant reduction of labile N was revealed for oil contaminated soil with *Z. mays* plants on the 95th day and with *V. faba* plants on the 65th day compared to uncontaminated soil with plants.

Conclusions. Oil-contamination had a negative effect on all studied biochemical characteristics of soil: protease activity, urease activity, labile organic nitrogen, free amino acids accumulation. In oil contamination conditions the positive effect of *Z. mays* and *V. faba* plants was determined for protease activity on the 65th day, a positive effect of *Z. mays* plants on accumulation of free amino acids accumulation was observed on the 95th day. The shortage of labile N in oil contaminated soil increased during phytoremediation. Therefore application of *Z. mays and V. faba* plants for optimization of biochemical components of N balance in oil contaminated soil may be controversial and requires further exploration.

Keywords: oil contamination, phytoremediation, protease activity, urease activity, labile organic nitrogen, free amino acids

INTRODUCTION

When crude oil gets into the soil, it can disturb the balance of soil organic matter. Oil pollution causes an increased content of organic carbon in soil and immobilization of nitrogen in microbial biomass, which results in an increase in C:N ratio (Chaîneau *et al.,* 2003). Furthermore, hydrocarbons reduce N bio-availability due to changes in the physical, chemical and biological properties of soil (John *et al.,* 2016). Oil may have effects on all components of N cycling that are revealed in changes in soil enzyme activity, content of organic and mineral N in soil, activity and abundance of bacteria involved in N cycling (Bunio *et al.,* 2013; Dovgajuk-Semenuk *et al.,* 2014). Nitrogen dynamics and cycling play important roles in bioremediation and reclamation of hydrocarbon-contaminated soils, because nitrogen is an essential element for microbial activity and plant growth (Li *et al.,* 2023). The activity of protease and urease enzymes – the key agents in the transformation of labile organic N components and accumulation of free amino acids are important indicators of N dynamics in oil contaminated soils.

Phytoremediation of oil contaminated soil can have a positive effect on N cycling. This improvement could be attributed to nitrogen fixation by nodulation bacteria of legume plants or stimulation of activity of microbiota in the plant root zone (Bunio *et al.,* 2010; Diab, 2008; Velychko, 2014).

This study aims to identify important biochemical elements of N cycling in oil contaminated soil and effects of plants on recovering N balance in soil. In this study we have used *Zea mays* L. and *Vicia faba* var. *minor* plants for phytoremediation of oil contaminated soil. Their resistance to oil contamination and remediation efficiency were shown previously (Diab, 2008; Dzhura *et al.*, 2006; Marinescu *et al.*, 2011).

MATERIALS AND METHODS

Experimental design. We simulated soil pollution in a field experiment by adding 50 mL/kg of crude oil into the soil (oil density 0.87 g/cm^3). The dimensions of each plot were: length – 1m, width – 0.5 m, depth – 0.25 m. Plant seeds were sown 22 days after oil contamination of the soil. Unpolluted soil was used as the control. The experiment was performed in three replicates.

The experiment series:

1) non-contaminated soil (soil without oil contamination) – control;
2) non-contaminated soil planted with *Z. mays*;
3) non-contaminated soil planted with *V. faba*;
4) soil contaminated with oil;
5) soil contaminated with oil planted with *Z. mays*;
6) soil contaminated with oil planted with *V. faba*.

Soil sampling. Soil samples were collected on the 10th day after oil pollution, on the 22nd day (seed were sown), on the 65th day (30 days of plants growth), and on the 95th day (60 days of plants growth). On the 65th day, *Z. mays* was in V6 phase (6 leaves fully emerged), *V. faba* was at the flowering stage in control; on the 95th day, *Z. mays* plants were at the tassel formation stage (the branches of the tassel are visible), *V. faba* was at pod set stage (first pods visible) in control.

Five soil aliquots were randomly collected in each pot at the depth 5-7 cm. An average sample was prepared by homogenizing and mixing the aliquots of each replicate. Six replicate plants were destructively sampled from each experiment series for soil sampling. Loose soil was taken from the root zone (not attached firmly to roots) (Kumar *et al.,* 2021).

Soil analyses. *Nodulation ability of soil.* On the 65th and 95th days, 5 *V. faba* plants were carefully removed from the soil. The average number of nodules was counted on roots (Yu *et al.*, 2020).

Protease activity was determined as follows: 5 g of soil was weighed in a glass vial and 1.5 mL of toluene and 10 mL of a 2 % gelatin solution were added. The capped vials were incubated in thermostats at 37 °C for 20 h. After the incubation, 10 mL of filtrate was mixed with 2 mL of 10 % solution of $Cu(NO_3)_2$ in $CH_3COONa{\cdot}3H_2O$. The amino acids concentration was measured calorimetrically at 650 nm (spectrophotometer ULAB-101) (Hrytsaenko *et al.*, 2003; Gianfreda & Rao, 2014).

Free amino acid accumulation. A piece of sterile linen cloth was attached to a microscope slide and buried into the soil at a depth of 5 cm. After 10 days of exposure, the cloth was taken out and sprayed by 0.5 % solution of ninhydrin, then dried out in darkness. After that, 25 cm^2 of cloth was doused with 50 mL of 75% ethanol solution. The extract was analyzed calorimetrically at 570 nm (Hrytsaenko *et al*., 2003).

Urease activity was measured following the procedure: 20 g of the soil sample was dispensed into a Petri dish and treated with 4 mL of 1 % urea solution. Soil samples were incubated at 30 °C for 6 h with soil moisture content at 100 % of soil water-holding capacity in Petri dishes with pH indicating paper. Therefore, hydrolysis of urea resulted in emission of NH_3. The activity of the urea was measured by the pH level of NH_3 solution in Petri dishes (Hrytsaenko *et al.*, 2003).

Labile organic nitrogen was determined using the base hydrolysis method (Hamkalo, 2009). Soil hydrolysis with 0.2 M NaOH resulted in NH_3 emission. NH_3 was detected by H_3BO_3 absorption and titration by H_2SO_4.

Data analysis. Statistical analyses were conducted with the RStudio software. Main factors effects were determined by Two-way ANOVA. Mean comparisons were performed using the Tukey test. Effects were considered significant for $P < 0.05$ (McDonald, 2014).

RESULTS AND DISCUSSION

Nodulation of *V. faba* plants. The legume plants that associate with nitrogen-fixing bacteria have a significant selective advantage under conditions of limiting nitrogen in oil contaminated soil. This improvement could be attributed to an additional nitrogen source for plants due to nitrogen fixation activity of symbiotic bacteria. Moreover, some species of nitrogen-fixing bacteria exhibited variable degrees of oil degrading capacity (Jones *et al.,* 2005). Contamination of soil by crude oil could lead to a depression of microbial density and activity, including nitrogen fixers. The extent of the effect depends on the original soil properties and the plant exposed to contaminated soil (Adedeji *et al.,* 2022; Borowik & Wyszkowska, 2018; John *et al.*, 2016).

The average amount of nodules on *V. faba* roots was 7.5±1.3 on the 65th and 10.2±1.1 on the 95th day in control. Our study revealed the absence of nodules on *V. faba* roots in oil contamination conditions. This may be due to the fact that oil inhibits the ability of rhizobia to infect legume roots and form nitrogen-fixing nodules, which is shown in other studies (Borowik & Wyszkowska, 2018; Velychko, 2014).

The oil contaminated soil caused reduction in the nodulation of legumes *Calopogonium mucunoides* and *Centrosema pubescens.* It was observed that an increase in the level of pollution results in a decrease in the number of nitrogen fixers with time (John *et al.*, 2016; Sui *et al.*, 2021). It was shown that oil pollution at the level of 48 g/kg of soil had no effect on nodulation of *V. faba* (Dzhura *et al.*, 2010).

Protease activity. Organic forms of N predominate in soil, and approximately 40 % of total soil N is generally present in the form of proteins and peptides (Geisseler & Horwath, 2008). Protease enzymes are involved in the initial hydrolysis of protein components of organic nitrogen to simple amino acids (Bunio *et al.,* 2013), therefore protease activity has been assumed to be the rate-limiting step in the overall N cycle of soils (Rennenberg *et al.,* 2009). The thermodynamically “downhill” phase of the N cycle, in which N compounds are degraded, starts with the breakdown of organic matter and proceeds, via protein and peptide depolymerization, through the liberation of amino acids and (if these substances are not taken up by roots or microbes) further degradation to ammonium, which is often subsequently nitrified to nitrate (Jämtgård *et al.*, 2008). Proteases are produced by a wide range of bacteria, actinomycetes and fungi (Greenfield *et al.*, 2021).

Oil contamination of soil adversely affected protease activity: on the 22th day of experiment protease activity decreased by 25 %, and on the 65th day – by 16 %, whereas on the 95th day oil effect was not significant compared to control (**Table 1**).

Other studies have shown that protease activity decreased with an increase in oil concentration (8, 16, 25 L/m^2). The protease activity was inhibited for 7–8 years after soil pollution. It may be caused by toxicity of oil and an inhibitory effect of heavy metals as oil components (Kumar *et al.,* 2021). The studies have shown a strong increase in protease activity when proteins were added, which indicates that protease activity is substrate induced. However, addition of glucose resulted in protease repression in conditions of N deficiency (Geisseler *et al.,* 2008). Similar conditions are representative for oil contaminated soil with a high organic C content and a low N content.

The plants had a stimulating effect on protease activity in conditions of oil pollution on the 65th day resulting in a increase, but an inhibitory effect was determined on the 95th day – a 0.8-fold decrease compared to oil contaminated soil without plants. The observations suggest that the contribution of protease derived directly from plants is probably negligible (Fonseca-López *et al.,* 2020). The lower levels of protease activity in planted contaminated soil is in line with L. Bunio (Bunio *et al.*, 2010) who reported a decreased protease activity in the rhizosphere of plants *Carex hirta* L. This may be attributed to a competition between plants and bacteria for nutrients that resulted in a decrease in microbial activity. Significant variations in nitrogen species and phosphorus might be due to the joint effect of release and assimilation (Vavrek *et al.,* 2005; Vinolas *et al.,* 2001).

Table 1. **Protease activity and accumulation of free amino acids in soil under oil contamination conditions and phytoremediation**

Treatments	Protease activity (mg N/10 g of soil ×20 h)	Accumulation of free fatty acids (mg glycine/1 cm^2 of cloth)
22 days after soil contamination with oil		
Non-contaminated soil	0.88±0.061[a]	2.27±0.4[a]
Soil contaminated with oil	0.66±0.055[b]	0.97±0.064[b]
65 days after soil contamination with oil		
Non-contaminated soil	0.64±0.025[b]	3.53±0.37[c]
Non-contaminated soil + *Z. mays*	0.66±0.025[b]	3.21±0.11[c]
Non-contaminated soil + *V. faba*	0.61±0.025[ab]	2.63±0.44[bc]
Soil contaminated with oil	0.54±0.012[a]	1.35±0.16[a]
Soil contaminated with oil + *Z. mays*	0.99±0.016[c]	1.08±0.15[a]
Soil contaminated with oil +*V. faba*	0.96±0.31[c]	1.65±04.8[ab]
95 days after soil contamination with oil		
Non-contaminated soil	0.91±0.013[bc]	2.39±0.51[abc]
Non-contaminated soil + *Z. mays*	0.89±0.071[b]	4.27±0.44[c]
Non-contaminated soil + *V. faba*	0.95±0.014[bc]	3.17±0.46[bc]
Soil contaminated with oil	1.019±0.03[c]	0.68±0.02[a]
Soil contaminated with *oil* + *Z. mays*	0.83±0.17[ab]	2.0±0.54[abc]
Soil contaminated with oil + *V. faba*	0.75±0.04[a]	0.94±0.04[abc]

Comments: data marked by the same letter do not differ significantly (significance P<0.05)

Free amino acids accumulation. Amino acids either in a monomeric or polymeric (protein) state provide a readily available source of C and N for soil microorganisms. A large input of amino acids into soil may occur upon the lysis of root cells and through secretion of root exudates (Vinolas *et al.,* 2001; Wang *et al.,* 2022). Other sources include dead bacteria, fungal and animal tissues, excretions from microbes and animals (Jones *et al.,* 2005).

The contribution of amino acids to the N cycle (by redundancy analysis) was slightly lower than that of microorganisms, but stronger than that of soil enzymes (Li *et al.,* 2023).

As soon as free amino acids are released into the soil solution, several processes cause their disappearance including: (i) mineralization (ammonification, nitrification) to inorganic Nitrogen, (ii) binding in microbial biomass (immobilization), (iii) uptake by plants, (iv) losses by leaching, and (v) adsorption to charged surfaces (Jämtgård *et al.,* 2008).

A two-way analysis of variance yielded the main effect for oil, indicating that the mean amino acid content in soil was significantly lower for oil contaminated soil during all experiments than for soil without oil (**Table 1**). The decrease in the concentration of amino acids in oil contaminated soil may be caused by binding in microbial biomass (immobilization) or/and inhibited peptide depolymerization by proteaze (Geisseler & Horwath, 2008).

The main effect of plants was non-significant on the 65th day. On the 95th day, the main effect of *Z. mays* on accumulation of amino acids was shown: mean amino acid content was significantly higher for soil planted with *Z. mays.* plants than for soil without plants (**Table 1**). *V. faba* had a stimulating effect on amino acid accumulation only for soil without oil compared to soils without plants. A positive effect of plants may occur upon the lisis of root cells and through secretion of root exudate (Vinolas *et al.*, 2001).

Urease activity. Biochemical reactions in soil, including transformation and cycling of plant nutrients, depend on the activities of different enzymes. Urease activity deserves special attention since it plays a key role in transforming urea into ammonia for plant uptake. The activity of soil urease correlated positively with the densities of microorganisms, organic matter content, total nitrogen and available phosphorus (Fonseca-López *et al.,* 2020; Wyszkowska & Kucharski, 2000).

The results have shown that the urease activity decreased in oil contaminated soil during all experiments: a decrease in pH level to 18–20 % compared to control (**Table 2**). This is suggestive that hydrocarbon contamination may inhibit microbial activity.

Table 2. **Soil urease activity (pH of NH_3 solution) affected by oil-contamination conditions**

Treatments	Time since soil contamination with oil (days)		
	22	65	95
Non-contamineted soil	9.5±0.28[a]	9.0±0.39[a]	9.1±0.39[a]
Soil contaminated with oil	7.6±0.33[b]	7.0±0.36[b]	7.3±0.46[b]

Comments: Data marked by the same letter do not differ significantly (significance P<0.05)

Data revealed that the plants had no significant effect on the urease activity. The urease activity in the oil-contaminated soil (at levels 0.35, 10.8, 20.5 and 50 g/kg of soil) was higher than that in the control (uncontaminated) and nutrient supplemented soils (John *et al.,* 2016). The stimulating effect on soil urease activity was also determined for oil contamination conditions at the level of 50g/kg of soil and for contaminated soil planted with *Carex hirta* L. (Bunio *et al.,* 2010). Legumes also have a stimulating effect on the activity of soil urease. This may be due to larger microbial biomass (represented by microbial N) in the oil contaminated soil and stimulating microorganisms in the root zone. Furthermore, plant roots, as well as microorganisms, produce urease (Igiehon *et al.*, 2024; John *et al.*, 2011).

In another study, soil urease activity showed a significantly negative correlation (P = 0.001) with hydrocarbons content which could be used as a sensitive indicator of petroleum contamination (Li *et al.*, 2005).

Labile organic N. Nitrogen availability in soils is derived mostly from the labile organic N pool that is the major source of C and N for microbes and represent the major pathway through which N is transformed from crop residues to the soil (Vinolas *et al.*, 2001). On the other hand, the ammonia nitrogen and nitrate nitrogen affect nitrogen cycling bacteria of the soil (Elrys *et al.*, 2024). Nitrogen hydrolyzed by sodium base according Hamkalo (Hamkalo, 2009) is considered as labile fraction of organic matter and available to microorganisms utilization. Labile organic N is mainly composed of partially decomposed plant residues together with microbial products that are not closely associated with soil minerals (John *et al.*, 2016).

Our results indicated that 10 days after oil pollution, the content of labile organic N in soil decreased by 63 % in oil-contaminated soil, and after 22 days it decreased by 27 % compared to control (**Fig. 1**). However, on further stages of the experiment, N content was not affected by oil. Data analysis revealed that on the 65th day for soil planted with *V. faba,* N content was lower in oil-contamination conditions compared to uncontaminated soil with plants. On the 95th day, a significant reduction of labile N content was revealed in oil-contaminated soil with *Z. mays.* plants compared to uncontaminated soil with plants (**Fig. 2**). Significant changes in N content might be due to the joint effect of release and assimilation of nitrogen compounds by bacteria and plants (Igiehon *et al.,* 2024; Yu *et al.,* 2020). The ^{15}N tracer approach indicated that NO^-_3 immobilization rate was 2.5-fold higher than its reduction rate, indicating that NO^-_3 immobilization played a more important role during the process of NO^-_3 transformation (Liu *et al.,* 2023).

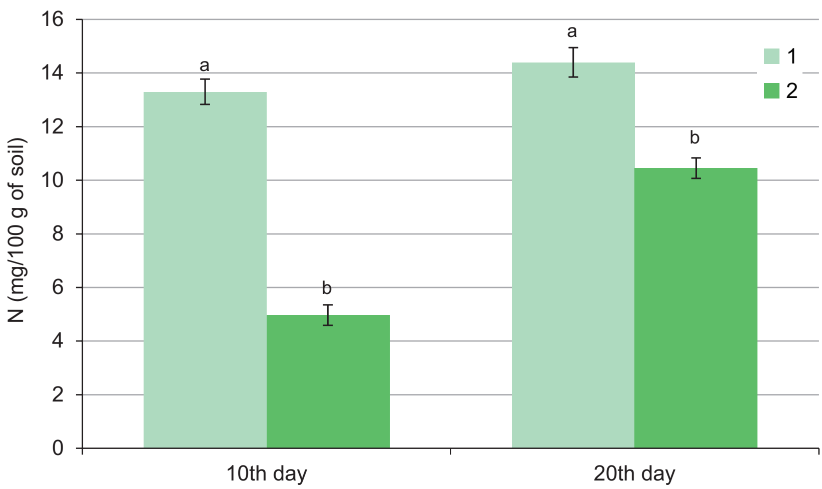

Fig. 1. Content of soil labile organic N in conditions of oil contamination before phytoremediation: 1 – soil without oil contamination; 2 – soil contaminated with oil
Comments: data marked by the same letter do not differ significantly (significance P <0.05)

In other studies, a decrease in sodium hydrolysed N in conditions of oil contaminated soil at a concentration of 5 % was shown (Dovgajuk-Semenuk *et al.,* 2015). The decrease in labile N on the 22th day may also be caused by N immobilization into microbial biomass. The studies have also shown that the microbial N increased while the mineral N decreased with an increase in the level of hydrocarbon contamination (John *et al.,* 2016). The decrease in labile fractions of nitrogen is in agreement with the low content of amino

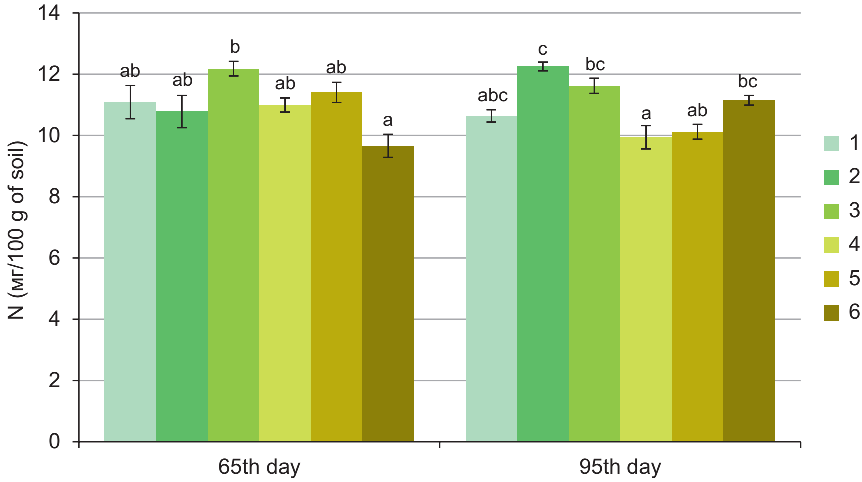

Fig. 2. Content of soil labile organic N in conditions of oil contamination and phytoremediation: 1 – soil without oil contamination; 2 – soil without oil contamination planted with *Z. mays*; 3 – soil without oil contamination planted with *V. faba*; 4 – soil contaminated with oil; 5 – soil contaminated with oil planted with *Z. mays*; 6 – soil contaminated with oil planted with *V. faba*
Comments: data marked by the same letter do not differ significantly, (significance P <0.05)

acids and reduced soil protease and urease activity from the previous analysis. Negative effect of plants is probably due to drastic competition between plants and microorganisms for all available forms of N, which are in deficiency (Henry *et al.,* 2002). Amino acids as labile organic N can also be taken up directly by plant roots, providing an alternative source of available soil N. The ability of plants to grow on an amino acid as the sole source of N in extreme environmental conditions: low temperatures, a short growing season, low precipitation, saline soil, was shown (Jones *et al.,* 2005). However, competition for amino acids between plants and microbes is not clear (Pan *et al.,* 2022; Vinolas *et al.,* 2001). Besides, a depletion of labile organic nitrogen pool resulted in a successive decrease in its products of mineralization (Rennenberg *et al.,* 2009).

CONCLUSIONS

1. In conditions of oil-contamination of soil, the development of nodules on roots of *V. faba* plants was totally inhibited.
2. Soil protease activity decreased in oil-contamination conditions on the 22th and 65th days of the experiment compared to control. The plant's effect in oil-contaminated soil conditions was stimulating on the 65th day, and inhibiting – on the 95th day compared to non-planted contaminated soil.
3. Accumulation of free amino acids in oil-contaminated soil was reduced compared to control. However, in conditions of oil-contamination on the 95th day *Z. mays* plants have a stimulating effect on free amino acids content compared to the contaminated non-planted soil.
4. Data reveals a decrease in soil urease activity in oil-contaminated soil, whereas the plant's effect was not significant.
5. Oil contamination causes reduction of soil labile organic N content on the 22th day. In oil contamination conditions, a shortage of the labile form of Nitrogen

for planted soil was revealed, so the remediation effect may be negligible. Therefore, application of *Z. mays and V. faba* plants for optimization of biochemical components of N balance and in oil-contamination soil may be controversial and requires further exploration.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights. This article does not contain any studies with human subjects performed by any of the authors.

Animal studies. This article does not contain any studies with laboratory animals performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [B.L.; T.O.]; methodology, [M.M.]; validation, [M.M.]; formal analysis, [M.M.]; investigation, [M.M.]; resources, [M.M.; B.L.]; data curation, [M.M]; writing – original draft preparation, [M.M.]; writing – review and editing, [B.L.; T.O.]; visualization, [M.M.]; supervision, [M.M.; B.L.]; project administration, [M.M.; B.L.]; funding acquisition, [–]. All authors have read and agreed to the published version of the manuscript.

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БІОХІМІЧНІ СКЛАДОВІ БАЛАНСУ НІТРОГЕНУ В ҐРУНТІ ЗА ДІЇ ЗАБРУДНЕННЯ СИРОЮ НАФТОЮ ТА ФІТОРЕМЕДІАЦІЇ

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Вступ. Доступність Нітрогену в нафтозабрудненому ґрунті погіршується внаслідок зростання співвідношення між органічним C та N, несприятливими змінами фізичних, хімічних і біологічних властивостей ґрунту. Фіторемедіація нафтозабрудненого ґрунту може мати позитивний вплив на колообіг N. Зміни у ферментативній активності ґрунту, вміст різних органічних форм N у ґрунті можуть бути використані як індикатори балансу Нітрогену у ґрунті.

Матеріали та методи. У нашому дослідженні ґрунт було штучно забруднено нафтою (50 мл нафти на 1 кг ґрунту) та проведено ремедіацію за участю рослин *Zea mays* L. і *Vicia faba* var. *minor*. Зразки ґрунту відбирали через 10 днів після забруднення нафтою та через 22 дні (висіяно насіння, 65 днів (30-та доба росту рослин), 95 днів (60-та доба росту рослин). Контролем слугував ґрунт без нафти та рослин. Визначали нодуляційну здатність ґрунту, активність протеази, уреази, вміст вільних амінокислот і лабільного органічного Нітрогену.

Результати. Дослідженням з'ясовано відсутність бульбочок на коренях рослин *V. faba* в умовах нафтового забруднення. Протеазна активність нафтозабрудненого ґрунту пригнічувалася, порівняно з контролем. За росту рослин протеазна активність нафтозабрудненого ґрунту збільшувалася на 65-ту добу, однак знижувалася на 95-ту добу, порівняно з нафтозабрудненим ґрунтом без рослин. Вміст вільних амінокислот був нижчим для нафтозабрудненого ґрунту протягом усього експерименту, проте був більшим для ґрунту з рослинами *Z. mays*, ніж для ґрунту без рослин. Рослини *V. faba* мали стимулюючий вплив на вміст вільних амінокислот лише для незабрудненого ґрунту, порівняно з ґрунтом без рослин. За результатами дослідження уреазна активність нафтозабрудненого ґрунту знижувалася, порівняно з контролем, протягом усього експеременту. На 22-й день після нафтового забруднення вміст легкогідролізованого N знижувався у нафтозабрудненому ґрунті, проте на пізніших етапах досліду не відрізнявся від контролю. Статистично значуще зниження вмісту лабільного органічного N було встановлено для нафтозабрудненого ґрунту з рослинами *Z. mays* на 95-ту добу та з рослинами *V. faba* на 65-ту добу, порівняно із незабрудненим ґрунтом з рослинами.

Висновки. В умовах нафтового забруднення ґрунту зафіксовано зниження усіх досліджуваних показників ґрунту: протеазна та уреазна активність, накопичення вільних амінокислот, вміст органічного легкогідролізованого Нітрогену. Встановлено збільшення протеазної активності для нафтозабрудненого ґрунту в умовах вирощування рослин *Z. mays* і *V. Faba* та збільшення акумуляції вільних амінокислот для ґрунту з рослинами *Z. mays*, порівняно з ґрунтом без рослин. Нестача лабільного N посилювалася для нафтозабрудненого ґрунту протягом фіторемедіації. Отже, використання рослин *Z. mays* та *V. faba* для оптимізації біохімічних компонентів балансу N у нафтозабрудненому ґрунті може мати неоднозначний вплив і потребує подальших досліджень.

Ключові слова: нафтове забрудненя, фіторемедіація, протеазна активність, уреазна активність, лабільний органічний Нітороген, вільні амінокислоти

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