

Influence of crude oil pollution on the content and electrophoretic spectrum of proteins in *Carex hirta* plants at the initial stages of vegetative development

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The role of proteins in the general adaptive response of *Carex hirta* plants to soil pollution by crude oil has been studied. It was established that a possible element of the process of adaptation of *C. hirta* plants to combined stress – conditions of soil polluted by crude oil – may be the synthesis of stress proteins – high molecular weight of more than 60 kD and low molecular weight, not exceeding 22–45 kD. The synthesis of all 5 HSP families was detected in the leaves and rhizomes, and only sHSP (starting from Mr 32 kD), Hsp 60 and Hsp 100 proteins were synthesized in the roots under the influence of crude oil pollution. The development of *C. hirta* adaptation syndrome under the influence of crude oil pollution of the soil was promoted by enhanced synthesis of proteins with Mr 85, 77, 64, 60 and 27 kD in the leaves, 118 and 41 kD in the rhizomes and proteins with Mr 105, 53, 50 and 43 kD in the roots of the plants. The decrease in the amount of proteins with Mr 91, 45, 28 kD in the leaves, proteins with Mr 85, 76 and 23 kD in rhizomes and proteins with Mr 64 and 39 in the roots of *C. hirta* plants under conditions of crude oil polluted soil could be a consequence of inhibition of synthesis or degradation of protein molecules providing the required level of low molecular weight protective compounds in cells. The root system and rhizomes of *C. hirta* plants undergo a greater crude oil load, which leads to increased protein synthesis in these organs and decreased in the leaves, correspondingly. However, a decrease in protein content in the leaves may indicate their outflow in the roots and rhizomes. Crude oil contaminated soil as a polycomponent stressor accelerated the aging of leaves of *C. hirta* plants, which could be caused by increased synthesis of ABA. ABA in its turn induced the synthesis of leaf-specific protein with Mr 27 kD. These proteins bind significant amounts of water with their hydrate shells maintaining the high water holding capacity of the cytoplasm under drought conditions. ABA inhibits the mRNA synthesis and their corresponding proteins, which are characteristic under normal conditions, and induces the expression of genes and, consequently, the synthesis of specific proteins including 27 kD protein. By stimulating the expression of individual genes and the synthesis of new polypeptides, ABA promotes the formation of protective reactions and increases the resistance of plants to crude oil pollution.

Keywords: adaptation syndrome; heat shock protein; abscisic acid (ABA); crude oil; soil.

Introduction

Studies of a plant's response to stress are usually monofactorial. The effect of hyperthermia or drought on the plant organism or another influencing factor is studied separately. Each abiotic stress causes a unique response to adaptation, taking into account the specific features of the plant. However, under natural conditions, plants are influenced not by one stress factor, but several at once. For example, when exposed to high temperatures, plants experience a lack of water. Recent studies have shown that the response of plants to a combination of drought and heat is unique and cannot be the same as the response of plants to each of these stresses separately. In addition, different stresses may have synergistic or antagonistic effects when combined (Takahashi et al., 2020; Zhang et al., 2020). Therefore, presently it is proposed to consider the combination of stresses as a new type of abiotic stress (Al-Wahaibi, 2011).

Such complex polycomponent stress for plants is crude oil pollution of the soil, which has become global (Smith et al., 2016; Dhanwal, 2017; Dickinson, 2017). Drilling and exploitation of crude oil and gas wells, the development of the oil refining industry are among the major factors of environmental contamination (Chizhevskaya et al., 2014). Pollution is virtually inevitable at the sites of crude oil extraction, storage and refining (Zahermand et al., 2020). According to the International Petroleum Industry Environmental Conservation Association, massive plant deaths do not occur during or immediately after oil spills. However, in the medium and long term, the effects of such incidents are extremely negative (Zaher-

mand et al., 2020). Decontamination of crude oil fields is an extremely complex task and a relevant issue for all oil production regions. Thus, there is an urgent need for the development of effective and environmentally friendly methods of cleaning the environment from crude oil pollution. Oil pollution is difficult to combat due to its complex chemical nature, high resistance and stability to environmental factors (temperature, solar radiation, humidity, etc.). Physical and chemical methods currently used to clean soils polluted with crude oil are labour intensive and costly, in addition much time is required for soil regeneration and restoration. Physical and chemical methods of cleaning contaminated soils have a one-time effect. They can be used both for one-time urgent cleaning of polluted soils and for prevention of land contamination in the areas that are often exposed to crude oil pollution (Tang et al., 2019). Biological methods have a longer effect and provide for a steady improvement in the environmental situation. Biological degradation of petroleum hydrocarbons can be conducted by bacteria, fungi and plants (Shevchyk & Romanjuk, 2017; Banarjee, 2018; Ite & Ibok, 2019). The economic, energy-efficient and environmental advantages of biological methods are commonly recognised. Currently, phytoremediation is considered to be the most promising method for cleaning industrial pollution in the developed countries. Plants are known for their ability to create strong resistance to negative environmental factors combined with valuable sedimentary and absorbing properties. They produce a number of adaptive and destructive reactions, the nature and intensity of which corresponds to the ecological state of the environment (Glibovytska & Mykhailiuk, 2020).

In the process of phytoremediation of soils, plant roots serve as a source of organic substances that stimulate the development of microorganisms, which in turn destroy hydrocarbons (Gorshkova et al., 2017; Shevchyk & Romanyuk, 2017). In addition, plants can absorb a number of organic pollutants and transform them into other environmentally safe products (phytotransformation). Considering this, it is increasingly important to study plant participation in the processes of oil components degradation and develop new approaches to phytoremediation of areas polluted with crude oil (Pakharkova et al., 2015; Shevchyk & Romanyuk, 2017).

It is believed that plants used for phytoremediation should be adjusted to the climatic and soil conditions of contaminated areas (Elekes, 2014; Tang et al., 2019; Peco, 2021) and capable of tolerating stress conditions (Ite & Ibok, 2019). Basically, phytoremediation should be carried out by means of native plants, especially those that grow in the contaminated areas, rather than foreign or genetically modified species (Nedjimi, 2021). Areas contaminated with crude oil and oil products are inhabited primarily by plant species capable of vegetative reproduction, whereby fully developed plants insensitive to crude oil in the soil are produced (Romaniuk et al., 2018).

Many researchers involved in studies of phytoremediation of oil-contaminated areas recommend the use of herbaceous plants (Hamzah & Priyadarshini, 2014; Laghlimi et al., 2015; Tang, 2019). The main advantage of grasses is the large fibrous root system, with a big root surface area, and, consequently, a numerous rhizosphere microbiota that plays an important role in soil detoxification (Malik et al., 2021). Grass roots have a large area of contact with the contaminant (Bhat et al., 2017). It is in the root zone of plants that the mass development of microorganisms takes place, including those that utilize or transform organic pollutants (Carvalho et al., 2013; Romaniuk et al., 2018; Tang, 2019). Therefore, the process of crude oil decomposition is faster in areas occupied by plants (under the influence of root secretions of plants and rhizosphere microorganisms) (Romaniuk et al., 2018).

In addition, a large group of perennial grasses can tolerate adverse conditions by means of underground shoots, or rhizomes. Species with long rhizomes are distinguished by high resistance to adverse conditions in ecotopes polluted with crude oil (Dzura et al., 2011). Underground shoots – rhizomes – provide for successful ontogenesis of herbaceous perennials. The family Cyperaceae occupies an important place within the plant system, while the genus *Carex* L. (Sedges), included in the family, refers to one of the largest genera of flora of Ukraine and the world (Jiménez-Mejías et al., 2015; Danylyk & Sosnovska, 2016; Danylyk & Sosnovska, 2017; González-Elizondo et al., 2018; Pereira-Silva et al., 2020; Koopman et al., 2021a). According to various sources, the genus *Carex* L. includes between 1800 and 2035 species (Danylyk & Kricsfalussy, 2020; Villaverde et al., 2020; Koopman et al., 2021b).

Carex hirta L. is a perennial herbaceous plant that forms rhizomes with long distinct internodes. It was found (Bunio & Tsvilynjuk, 2015; Tsvilynyuk et al., 2017; Rusyn & Hamkalo 2019) that *C. hirta* – an aboriginal herbaceous plant species on oil-contaminated soils of Boryslav – is able to grow on soils polluted with hydrocarbon. Consequently, this species can be potentially used as a phytomeliorant for the remediation of lands polluted with crude oil. This fact determined the choice of *C. hirta* plants for our research.

Crude oil pollution of the soil not only directly influences the plant organism due to the penetration of crude oil components through the root system or leaves and their subsequent inclusion into metabolism, but creates stressful growth conditions by changing water, air, biological and other soil properties. The response of plants to this complex influence has not yet been sufficiently studied (Dickinson, 2017).

One of the first non-specific responses of the plant organism to stress conditions is the formation of compounds (proteins, particularly) which are necessary for the protection of the plant cell (Al-Wahaibi, 2011). Abiotic stresses significantly influence plant proteomes including changes in the relative amounts of proteins, their cellular localization, posttranscriptional and posttranslational modifications, the interaction of proteins with other protein molecules and, finally, the biological functions of the proteins (Kosová et al., 2018). Clearly targeted mechanisms for regulation of protein metabolism in plant cells are an extremely important component of the cell responses to abiotic stressors (Al-Wahaibi, 2011; Wawrzyńska

et al., 2020). Different types of stress conditions may have different responses on components of the protein synthesis system (Mishra et al., 2018). Changes in protein synthesis affect the growth and development of plants and, consequently, influence the effectiveness of phytoremediation. Because of this, the role of proteins in the adaptation of crude oil resistant *C. hirta* plants was investigated.

Materials and methods

Field experiments were performed to study the influence of crude oil pollution of the soil on protein metabolism in *C. hirta* plants. Three phytoremediation sites with a total area of 90 m² were set at Boryslav crude oil and gas deposit field in the area with a low level of pollution in the south-eastern part of Boryslav city at the coordinates: 49°28'09" N, 23°42'69" E. Sod-podzolic type of soil is typical for these areas. A site with the background level of soil pollution typical for the industrial city was set as a control (conditional control). A site with soil artificially polluted by crude oil was set as a model soil (experiment).

Three ditches for control and model soils 0.25 m deep and 1x4 m in size were dug on the plots and their bottoms were lined with polyethylene film with perforations. Each ditch was filled in with 1000 kg of soil of 15% moisture. 50 kg of crude oil (making 5% of pollution) were poured into model soils and mixed. In 20 days after the artificial pollution (the required period for evaporation of volatile toxic crude oil products), 150 plants of *C. hirta* of virgin age selected from a homogeneous (according to ecological conditions) clean environment were planted in the soil of each ditch. They were planted in rows in a checkerboard pattern in holes 10–15 cm deep keeping the distance between plants at 15–25 cm (Bunio, 2014a). Soil moisture was maintained within 60% of the total moisture content.

25 plants were randomly selected from each site. Sampling was performed on the 30th (rosette phase) day of growth of *C. hirta* plants, which corresponds to the 50th day of destruction of crude oil in the soil.

The content and electrophoretic spectrum of proteins were determined in the leaves, rhizomes and roots of *C. hirta* plants. The total protein content was determined by the Bradford method (Bradford, 1976). To determine the qualitative composition of proteins, plant organs were frozen in liquid nitrogen. Frozen tissue samples were ground in a porcelain mortar under constant cooling with liquid nitrogen to a powdery state. The resulting homogenate was thawed, the proteins were extracted with constant stirring at +4 °C for 20 min with water containing protease inhibitors in RIPA buffer of the following composition: 50 mM Tris-HCl (pH 7.5), 1% NP-40, 0.5% DOC, 0.1% SDS, 5 mM EDTA, 1 mM PMSF, 5 mM benzamidine. The insoluble fraction was precipitated by centrifugation at 12,000 rpm for 25 min and the supernatant was transferred into a clean tube. 25% (V/V) of 50% trichloroacetic acid was added to the supernatant, then it was frozen at –70 °C for 60 min, thawed, precipitated by centrifugation at 12,000 rpm for 25 min. Then the precipitate was washed three times with 80% chilled acetone, dried and dissolved in RIPA buffer. The obtained samples were frozen in liquid nitrogen and stored until subsequent use at –70 °C. For electrophoretic analysis, samples were previously normalized to protein concentration, heated at 95 °C for 5 min in Laemmli buffer (62.5 mM Tris-HCl, pH 6.8, 1 mM EDTA, 2% SDS, 5% mercaptoethanol, 10% glycerin, 0.4% bromophenol blue) (Laemmli, 1970). Proteins were separated in a denaturing 12% SDS-PAGE in a Tris-HCl buffer system. 50 µg/mL of protein was applied. A set of standard proteins with a molecular weight of 200, 150, 100, 90, 70, 60, 50, 40, 30 and 20 kD was used a marker.

Electrophoregrams were painted with Coomassie R-250. Stained electrophoregrams were photographed, comparative densitometric analysis of proteins was performed with the program Gelpro 32. The protein content was determined in conventional units corresponding to each zone on the blots. Analysis of samples of each variant was performed three times.

Detection of abscisic acid (ABA) was performed by HPLC-MS method on liquid chromatograph Agilent 1200 LC with diode-matrix detector G 1315 B (USA) on a column Eclipse XDB-C18 4.6 × 250 with a particle size of 5 µm (The methodological recommendations..., 1988).

Data were analyzed using two-tailed Student's t-tests and differences were deemed significant at P < 0.05.

Results

On the 30th day of plant growth on crude oil polluted soil, the protein content was decreased by 20% with regard to control, and in underground organs it was increased by 7% and 8% with regard to control plants in roots and rhizomes, respectively (Fig. 1).

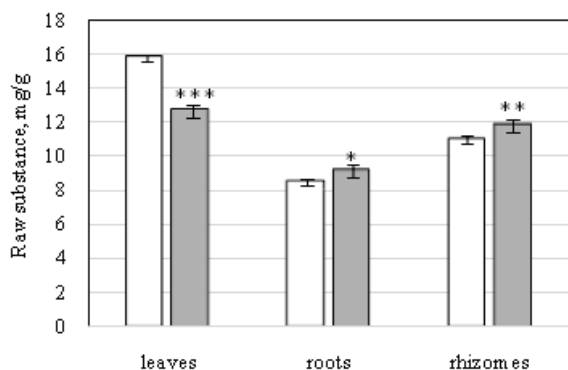


Fig. 1. Protein content in the organs of 30 days old *Carex hirta* plants under influence of crude oil pollution of the soil (mg/g of raw substance): white column – background soil (control); grey column – model soil (experiment – 5 % crude oil); the difference between control and experimental plants is statistically reliable (* – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$)

Simultaneously with changes in protein content, changes in its qualitative composition were observed. The results of electrophoretic studies showed that the spectra of proteins in plant organs of *C. hirta* under normal and stressful conditions had qualitative and quantitative differences, particularly, in the composition of components and the intensity of individual tracks (Fig. 2). At the same time, identical proteins are present in the profile of proteins synthesized during the growth of *C. hirta* plants on background (normal conditions) and model (stress conditions) soils (Fig. 2).

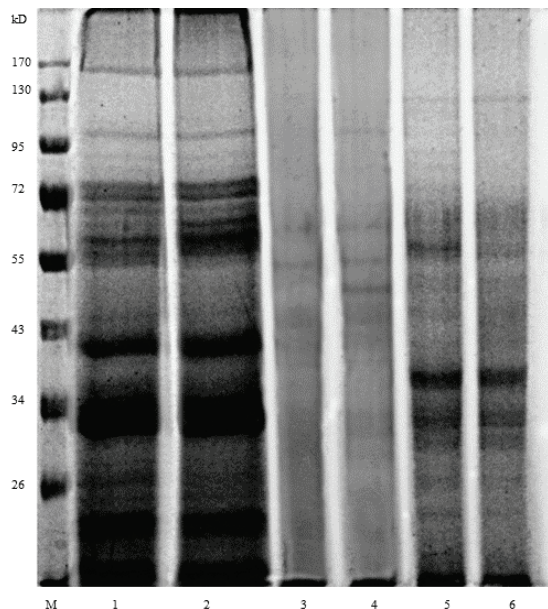


Fig. 2. Electrophoregrams of proteins of *Carex hirta* 30 days old plants on 30th day of growth on crude oil polluted soil (5%): M – markers of molecular weight; 1 – control (leaves); 2 – experiment (leaves); 3 – control (roots); 4 – experiment (roots); 5 – control (rhizomes); 6 – experiment (rhizomes)

Low molecular weight polypeptides (sHsp, 31–45 kD) were detected in electrophoregrams of all analyzed *C. hirta* plant organs both in control and under conditions of crude oil pollution of the soil, which confirms their participation in plant growth and development. Also, polypeptide sHsp with a molecular weight of 22 kD was found in leaves and rhizomes, however, such polypeptide was absent in the roots.

Under conditions of growth on crude oil polluted soil, sHsp proteins with the same molecular weight as in the control variant, but with some difference, were synthesized in the leaves of *C. hirta* plants. Proteins with a molecular weight of 26 kD were detected in the control, while in experimental plants – 27 kD (Table 1).

We found a 2.2-fold increase of ABA content in the leaves of *C. hirta* plants that grew on crude oil polluted soil (Fig. 3). At the same time the protein with a molecular weight of 27 kD was detected (Table 1).

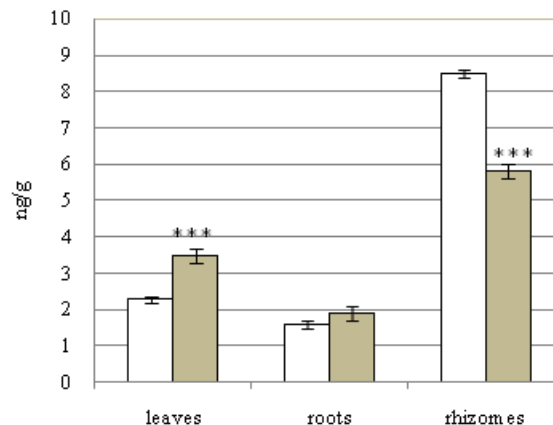


Fig. 3. The content of ABA (ng/g of raw substance) in the organs of *Carex hirta* plants on the 30th day of growth on crude oil polluted soil (5%): white column – background soil (control); grey column – model soil (experiment – 5 % crude oil); the asterisks indicate that there is statistically significant of ABA in plants (***) – $P < 0.001$)

Under conditions of crude oil polluted soil, up to 1.5 times more proteins with molecular weights of 41, 57, 73 and 77 kD were synthesized in sedge leaves (Table 1). Protein with Mr 60 kD was synthesized in *C. hirta* leaves both normally and under the influence of crude oil pollution but in the case of pollution, its expression was increased by 57%. There was also a significant increase up to 2.5 and 3.5 times of proteins with Mr 64 kD and 85 kD, respectively, during the growth of *C. hirta* plants on crude oil polluted soil. With regard to the control experiments, there was a slight decrease in the content of proteins with Mr 22, 25, 32, 69, 104 and 163 kD. The content of proteins with Mr 28, 45, 91 kD in the leaves of plants growing on crude oil polluted soil had more than halved.

Table 1

The relative protein content in the leaves of *Carex hirta* plants on the 30th day of growth on crude oil polluted soil (%); total mass proteins was recorded and used to calculate the relative mass (expressed as a percent of the value for the same line a marker proteins, set to 100%)

Mr, kD	Background soil (control)	Model soil (experiment)
163	12.15	10.48
104	6.41	6.11
91	2.16	1.09
85	8.80	31.54
77	18.92	25.12
73	22.40	25.43
69	14.71	12.65
64	12.81	31.86
60	31.87	50.14
57	24.66	29.66
45	7.79	2.76
41	47.05	48.11
32	55.02	48.36
28	2.33	1.09
27	0.00	4.00
26	12.14	0.00
25	6.59	6.37
22	42.15	31.79

The areas of the leaf plates varied almost along a symmetrical parabolic curve. Under the influence of crude oil, the descending part of the curve was steeper (Fig. 4).

Analysis of the fractional protein composition of the rhizomes of *C. hirta* plants under the influence of crude oil pollution revealed less significant, in comparison with the leaves, differences between control and

experimental plants. There were proteins with Mr 93 and 120 kD under normal conditions (control) and 118 kD in the experiment (Table 2). All other proteins detected by us were of the same electrophoretic mobility. Under the influence of crude oil polluted soil, a slight increase in the amount of low molecular weight proteins, most likely belonging to the Hsp 45, 65, 104 and 128 kD families, was observed in the rhizomes of *C. hirta*. The protein content of Mr 41 kD in the rhizomes of the experimental plants increased almost 6 times.

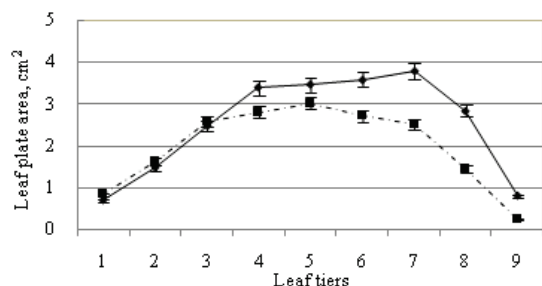


Fig. 4. Change in the leaf plates area of *Carex hirta* plants on the 30th day of growth on crude oil polluted soil (5%): background soil (control) and model soil (experiment – 5 % crude oil); values are means of three replicates $\bar{x} \pm SD$ (n = 3, 25 plants per repetition)

Table 2

The relative amount of protein in the rhizomes of *Carex hirta* plants on the 30th day of growth on crude oil polluted soil (%); total mass proteins was recorded and used to calculate the relative mass (expressed as a percent of the value for the same line a marker proteins, set to 100%)

Mr, kD	Background soil (control)	Model soil (experiment)
128	2.82	3.99
120	1.79	0.00
118	0.00	1.11
104	1.30	1.48
93	1.51	0.00
85	1.26	0.36
76	2.26	0.83
65	10.96	11.10
56	16.59	9.02
50	5.03	4.87
45	4.30	5.91
41	0.46	2.92
37	40.87	30.94
34	24.78	19.08
33	23.71	19.63
30	11.94	8.97
27	5.88	3.35
23	9.74	4.32

Proteins with a corresponding molecular weight of 56 kD, which probably belong to the HSP 60 family, were found under control and stress conditions, and, moreover, after growth on crude oil polluted soil, their content in the rhizome decreased almost two times (Table 2).

The amount of proteins with Mr 76 kD and 85 kD in the rhizomes of experimental *C. hirta* plants, belonging to the families HSP 70 and HSP 90, was 2.7 and 3.5 times less, respectively, compared to the control, which may be one of many ways of adaptation of plants to local conditions of existence.

In plants grown on crude oil polluted soil, proteins with a molecular weight close to 50, 53, and 105 kD were synthesized in the roots, which was not detected in the roots of control plants (Fig. 2, Table 3). The protein content in the roots with Mr 39 and 64 kD decreased more than 1.5 times compared to the control. An increase by 23% in the 43 kD protein content in the roots of experimental plants was observed. The thickening of *C. hirta* roots under conditions of growth on crude oil polluted soil was observed (Fig. 5).

Discussion

The adaptation syndrome includes a decrease of the intensity of total protein synthesis, which was exactly found in the leaves during the growth of *C. hirta* plants under conditions of crude oil pollution. This phenome-

non may be not so much the result of cell damage as a typical manifestation of the protective reaction, and partial inhibition of protein synthesis reduces the intensity of destructive processes (Waters & Vierling, 2020). It was found that one of the primary responses to stress is the selective degradation of the protein-synthesizing apparatus, proteosomal degradation of proteins and the formation of the necessary compounds for the urgent protection of plant cells (Katz & Orellana, 2012). At the same time, it is important to preserve the structure of protein molecules and prevent their aggregation (Al-Wahaibi, 2011). Termination or inhibition of protein synthesis, which is a characteristic response of plants to stressors, is an imperfect adaptation aiming to protect quickly the plant (although, in short-term) from death under adverse factors and to initiate the formation or mobilization of specialized mechanisms for long-term resistance. Most likely, all the power of the plant was directed to protection of the underground organs which are under greater stress load. We observed an increased protein metabolism in these organs. It is believed that the stress response is transitory and ensures the transition of the plant from normal to stressful metabolism by blocking non-essential metabolic pathways and triggering protective mechanisms, especially shock response systems (Waters & Vierling, 2020; Li et al., 2021). The increase of protein content in roots and rhizomes may be a consequence of their enzymatic adaptation and compaction of membrane structures in order to protect cells against the damaging factor. One of the possible detoxification reactions of pollutants is an increase of the content of proteins that are able to regulate cell osmosis (Murzin et al., 2011). Experimental data cited in the literature also suggest that protein accumulation is an important part of the mechanisms of stability in the event of various adverse factors (Jaber et al., 2013; Emad et al., 2018).

Table 3

The relative amount of protein in the roots of *Carex hirta* plants on the 30th day of growth on crude oil polluted soil (%); total mass proteins was recorded and used to calculate the relative mass (expressed as a percent of the value for the same line a marker proteins, set to 100%)

Mr, kD	Background soil (control)	Model soil (experiment)
105	0.00	2.54
64	6.89	4.29
54	5.02	4.38
53	0.00	2.62
50	0.00	9.05
43	5.82	7.14
39	2.53	1.46
32	5.40	5.16

There are three stages of the cellular response to abiotic stress: transition of signals to the nucleus, interaction with genes; induction or repression of mRNA translation; formation of stress proteins (Lario & Parra, 2013). Heat shock proteins (HSP) encoded in the nuclear, chloroplast and mitochondrial genomes (Ul-Hag et al., 2019) are synthesized in the cytoplasm, transported to all parts of the cell, to the nucleus and nucleolus, in mitochondria and chloroplasts, in the endoplasmic reticulum, even extra-cellular matrix, where they perform a protective function. Each family of HSP is encoded, as a rule, by several close genes, however regulation of their activity can differ. Also, the newly synthesized protein may be modified under certain circumstances and this may affect its molecular weight (Stoychev, 2013). HSPs protect other proteins against irreversible damages, prevent their aggregation in the cytosol and mitochondria, provide the correct conformation, which is necessary to maintain functional activity. The expression of these proteins is accompanied by stabilization of the cytoskeleton, in particular actin, which restores cellular structure. After the stress, the synthesis of proteins characteristic for the usual conditions of existence is resumed and the mRNA of stress proteins are destroyed. The ubiquitin protein provides proteolytic destruction of damaged HSPs (Liu & Chang, 2012; Tian et al., 2021; Zhao et al., 2021). Each stage of plant response to stress can be characterized by a unique protein composition. The phase of acclimatization and response to stress and adaptation of plants against adverse factors is accompanied by the synthesis of high-(60–100 kD) and low molecular weight (15–45 kD) stress proteins: chaperones, enzymes with antioxidant activity, protective proteins (LEA, PR) (Kosova et al., 2018). Stress proteins play an important role in maintaining

of cellular homeostasis under adverse conditions, in protecting and restoring the balance after stress (Al-Whaibi, 2011; Qu et al., 2013; Chen et al., 2014). The synthesis of stress proteins is accompanied by ultrastructural changes at the level of organelles. There is a breakdown of polysomes that

synthesize proteins inherent to the usual conditions of existence and formation of polysomes that synthesize heat shock proteins, segregation of nucleoli, disruption of organelle membranes and increase their contacts (Lario et al., 2013).

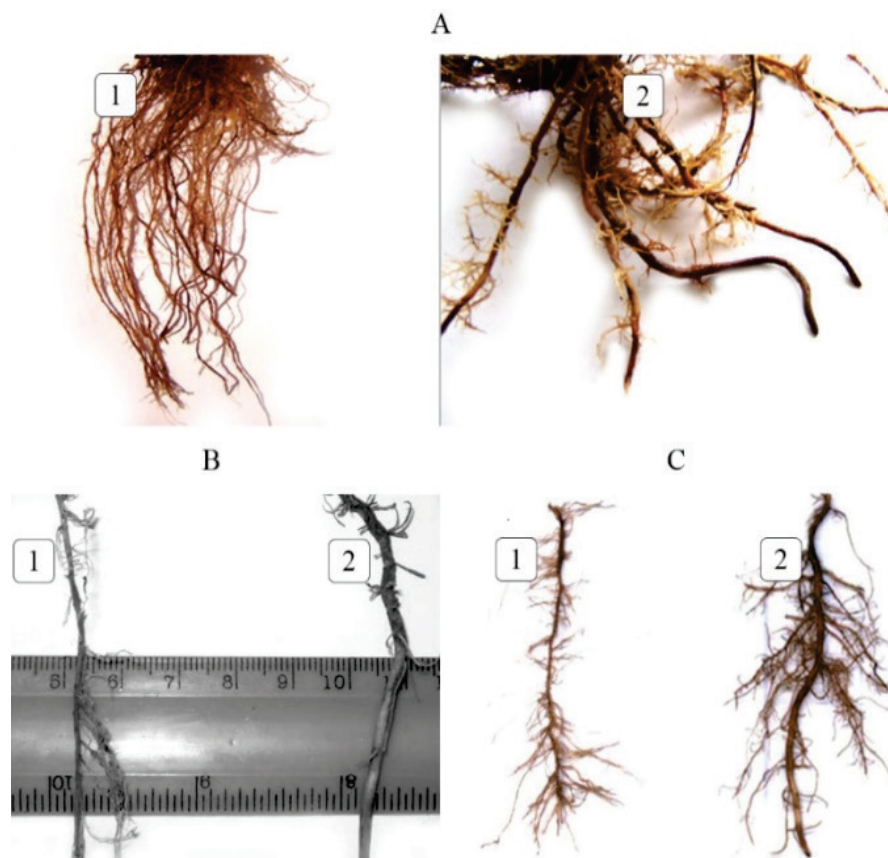


Fig. 5. The root system of *Carex hirta* plants on the 30th day of growth on crude oil polluted soil (5% of pollution): A – root system; B, C – individual fragments of roots; 1 – control, 2 – model soil

sHsp were detected in all plant organs of *C. hirta*. Their synthesis is associated with the development of resistance to stress. Proteins with a molecular weight from 15 to 45 kD are synthesized in the cells of all eukaryotes and are called low molecular weight heat shock proteins (small heat shock proteins, sHsp) (Mishra et al., 2018). Not only heat stress triggers sHsp gene expression and protein synthesis. Plants are characterized by an extraordinary diversity of sHsp due to the need for rapid adaptation to drastic changes in living conditions (Al-Whaibi, 2011; Mishra et al., 2018). The correlation between sHsp synthesis and stress response became the basis for formulating the hypothesis of the protective role of low molecular weight HSP. The mechanism – how sHsp act as cellular protectors – is not yet fully understood but it is clear that sHsp are capable of performing the function of ATP-independent chaperones binding partially denatured protein substrates and preventing their irreversible aggregation (Tian et al., 2021). Studies with purified plant CI (cytosolic class I), CII (cytosolic class II) and CP (chloroplast) sHSPs have confirmed this assumption. Upon completion of influence of damaging factors and restoration of normal conditions, sHsp are detected in the cell within the following 30–50 h, so it is assumed that the presence of sHsp is an important factor in the recovery of the plant organism (Mishra et al., 2018; Waters & Vierling, 2020).

Plants differ in the multiplicity of sHsp and their high intensity of synthesis under various stresses (Hasanuzzaman et al., 2013; Waters & Vierling, 2020). Low molecular weight sHsp biosynthesis indicates a protective function of these polypeptides under dehydration or rehydration (Cheng et al., 2008). The accumulation of low molecular weight hydrophilic proteins maintains the high water holding capacity of the cytoplasm under drought conditions. These proteins bind significant amounts of water with hydrate shells (Waters & Vierling, 2020). It is known that the synthesis of leaf-specific 27 kD protein is caused by water deficiency.

Water stress causes an increase in the content of so called stress hormones – abscisic acid (ABA), jasmonic acid and ethylene. ABA, for example, inhibits the synthesis of mRNA and their corresponding proteins which are typical under normal conditions, and induces the activity of genes and hence the synthesis of specific proteins, including 27 kD protein (Liu et al., 2016; Kamba et al., 2018; Li et al., 2021).

32 kD PsbO protein belongs to the triad of external proteins of Photosystem II (PSII). PSII possesses a membrane protein complex consisting of three tightly bound external proteins: PsbO (Mr 32–33 kD), PsbP (Mr 23–24 kD) and PsbQQ (Mr 17–18 kD) (Sasi et al., 2018). PsbO is one of the external PSII subunits located on the lamellar side of the thylakoid membrane and protects PSII from photodamage (Roose et al., 2016; Gururani et al., 2017). It is known that PsbO is a highly conserved protein. It is detected in 60–80% of higher plants and algae and about 40% of lower plants and cyanobacteria (Roose et al., 2016). Kosmala et al. (2009) noted a decrease in the amount of PsbO under cold stress, which affected the functional state of PSII and led to a decrease in the intensity of photosynthesis. A similar decrease in the productivity of photosynthesis was also found under other stresses (Dwyer et al., 2012; Gururani et al., 2017; Alyammahi & Gururani, 2020). Reduction in the content of PsbO also has a negative effect on rhizogenesis, accelerates the aging of the plant organism, stimulates tuber formation (Gururani et al., 2017; Alyammahi & Gururani, 2020). In our opinion, accelerated aging of *C. hirta* leaves during growth on crude oil polluted soil can be explained by the decrease in PsbO content, which was estimated by the change of leaf plates area on different tiers. Under the influence of crude oil, the descending part of the curve was steeper. This indicates on faster aging of leaves in plants that grew on crude oil polluted soil (Bunio et al., 2017).

Increased content of sHSP in the rhizomes may indicate significant stress load on this organ since chaperones are capable of forming bonds

with partially destroyed or denatured proteins and thus prevent their irreversible conformational changes and aggregation (Waters & Wierling, 2020). For example, it is known that the 33 kD Hsp protein content in plants increases in hydrophobic environments (Gow-Jen et al., 2011; Taguchi et al., 2020).

One of the biological functions of low molecular weight proteins is that they are capable of binding phytohormones and thus may affect the growth processes of plants and their adaptation to stressful conditions (Fernandes et al., 2013; Sliwiak et al., 2018). By stimulating the expression of individual genes and the synthesis of new polypeptides, ABA promotes the formation of protective reactions and increases plant resistance (Osakabe et al., 2014). ABA participates in the formation of responses acting as a trigger for the launch of signaling cascades. It has been found that a key role in signal transduction and the functioning of ion transport membrane systems in response to stress belongs to phosphatase and kinase ABA receptor proteins (Osakabe et al., 2014; Ali et al., 2020; Li et al., 2021). An increase in the concentration of endogenous ABA is a signal for the expression of genes sensitive to cold stress (Li et al., 2021). ABA activates COR genes (cold regulated) which include RAB (ABA-responsive), DHN (dehydrins) and genes from the family LEA (late embryogenesis abundant), whose products are directly involved in the formation of plant resistance against the action of low temperatures (Li et al., 2021). There were significant changes in the balance of other investigated phytohormones in both above-ground and underground organs of *C. hirta* plants under the influence of crude oil. The nature of these changes was not the same in different organs and depended on the impact of crude oil load on these organs (Bunio, 2014b).

Proteins of the HSP 60 family are found in mitochondria and chloroplasts in stress-induced and constitutive states (Al-Whaibi, 2011). Their main function is to provide conformational rearrangements of mitochondrial and chloroplast polypeptides. A significant increase of the protein with Mr 64 kD in the leaves, which belongs to the HSP 70 kD family, plays an important role in preventing aggregation and promotes the rearrangement of inactive proteins. It is well known that proteins of the HSP 70 kD family are mainly localized in the cytoplasm, where both stress-induced and constitutive polypeptides are expressed (Al-Whaibi, 2011), they are also present in the nucleus and mitochondria. This may indicate on a protective role of these proteins against adverse factors (Ul Haq et al., 2019).

The roots play a key role in the response of plants to water stress (WS). They are involved in the perception of WS and in signal transmission to the leaf (Bhakti et al., 2018). Proteins from the HSP 70kD (except 64 kD) and 90 kD families were not detected in the roots of either control or experimental plants of *C. hirta*. The reasons for not detecting HSP 70 in the organs of *C. hirta* plants under normal conditions or under conditions of pollution are not yet clear and require further investigation. Probably, the growth of *C. hirta* on soils with chronic crude oil loading (background soil) and soils with additionally added crude oil (model soil) affects the stability of HSP 70 mRNA, leading to inhibition of HSP 70 synthesis. At the same time, the fact cannot be excluded that the observed changes may be related to the natural, extremely low concentration (outside the sensitivity of the method) of HSP 70 in *C. hirta*. Although the induction of the synthesis of this stress protein is one of the universal, widespread components of the stress response to adverse conditions, it does not play an important role in the mechanism of stress adaptation in *C. hirta*. In some species, this process is reduced or not detected. Such phenomenon is known for some specialized and endemic species that are narrowly adapted to certain living conditions (Drozdova et al., 2019). The absence of induction of Hsp 70 in the roots of *C. hirta* plants may indicate their narrow specialization to certain living conditions (Kozeko et al., 2011). *C. hirta* is a mesohydrophyte usually growing in areas where ground waters are situated close to the surface. This species is found on sandy and clay substrates with pH from strongly alkaline to neutral and weakly acidic. The area of its distribution is not continuous, uneven, which reveals another specific feature of *S. hirta* – a complicated living strategy. This species does not withstand competition with other species, so it grows on short-lived ecotopes – inhabits artificial dumps along roads and railways, crude oil polluted areas. The 53 kD protein is called the protector of the genome due to its effect on DNA repair. It acts as a mediator between cell

proliferation, stress and cell death (Levine & Oren, 2009; Brady & Attardi, 2010). Expression of 53 kD in *Arabidopsis thaliana* induced premature aging and distortion of lateral shoots, the formation of thick stems, caused by increased homologous recombination of DNA. Hsp53 kD is also a super-suppressor of tumours (Ma et al., 2016). We assume that the thickening of *C. hirta* roots under conditions of growth on crude oil polluted soil is caused by an increase of the concentration of this protein.

Conclusion

Thus, the obtained results allow us to conclude that a possible element in the process of adaptation of *C. hirta* plants to combined stress – conditions of crude oil polluted soil – may be the synthesis of stress proteins: high molecular weight of more than 60 kD and low molecular weight not exceeding 22–45 kD.

While growing on crude oil polluted soil, the roots and rhizomes of *C. hirta* plants undergo more stress load than the leaves, as evidenced by the greater protein content in these organs. However, the increased protein content in these organs may as well indicate the outflow of this metabolite from the leaves where its decrease was noticed.

Under conditions of crude oil pollution of the soil, the synthesis of ABA increased in the leaves of *C. hirta* plants which, in turn, caused faster leaf aging and the synthesis of a specific protein with Mr 27 kD. ABA restricts plant growth with the aim of coordinating plant adaptation to stressful conditions.

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