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PHOTOSYNTHETIC PIGMENTS CONTENT IN THE URBAN HABITAT HERBACEOUS PLANTS LEAVES UNDER THE VOLATILE ORGANIC COMPOUNDS EFFECT

Abstract. It is necessary to find and develop new methods for evaluating open air pollution by volatile organic compounds due to the current changes in the structure of industrial production. While applying these methods, plants can be used quite efficiently. Therefore, the purpose of the article was to study the nature and conditions of change of chlorophylls *a*, *b* and carotenoids content in the urban habitat herbaceous plants leaves at different doses of volatile organic compounds and their mixtures effect under the specified experimental conditions.

The results of the experiment show that our hypothesis has been confirmed. It is the following: there are features and conditions of formation in reed fescue plants photosynthetic pigments of different levels under the effect of different amounts of pollutants having various volatility at different stages after their appending. Groups of substances which have the equal effect on pigment content have also been identified: pentane and hexane (have a strong effect) at early stages after treatment; benzene, benz(a)pyrene and o-xylol (have a weaker effect); butylacetate has practically no inhibitory effect; later: benz(a)pyrene, o-xylol and pentane (have a strong effect); butylacetate, hexane and benzene (have a weaker effect).

The results of the studies can be used to develop a method for evaluating open air pollution by volatile organic compounds using herbaceous plants pigments. They contribute to the study of the toxic effects of volatile organic compounds on the change in photosynthetic pigments content.

Keywords: photosynthetic pigments, reed fescue *Festuca arundinacea* Schreb., volatile organic compounds

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СОДЕРЖАНИЕ ФОТОСИНТЕТИЧЕСКИХ ПИГМЕНТОВ В ЛИСТЬЯХ ТРАВЯНИСТЫХ РАСТЕНИЙ ГОРОДСКОЙ СРЕДЫ В УСЛОВИЯХ ВОЗДЕЙСТВИЯ ЛЕТУЧИХ ОРГАНИЧЕСКИХ СОЕДИНЕНИЙ

Аннотация. В связи с происходящими в настоящее время изменениями в структуре промышленного производства необходимы поиск и разработка новых методов оценки загрязнения атмосферного воздуха летучими органическими соединениями. Для достижения этой цели достаточно эффективно могут быть использованы растения.

Изучены характер и закономерности изменения содержания хлорофиллов *a*, *b* и каротиноидов в листьях травянистых растений городской среды при разных дозах воздействия летучих органических соединений и их смесей в заданных условиях.

Результаты эксперимента подтвердили гипотезу о наличии особенностей и закономерностей формирования различных уровней пигментов фотосинтеза растений овсяницы тростниковой при действии неодинаковых по степени летучести загрязнителей через 1 и 3 сут после их введения. Также выявлены группы одинаково действующих на содержание пигментов веществ: на ранних этапах после обработки – пентана, гексана (сильнодействующие), бензола, бенз(а)пирена, о-ксилола (действуют слабее), бутилацетата (практически не проявляет ингибирующий эффект); на более поздних сроках – бенз(а)пирена, о-ксилола, пентана (сильнодействующие вещества), бутилацетата, гексана, бензола (действуют слабее).

Результаты проведенных исследований вносят вклад в изучение токсического действия летучих органических соединений на изменение содержания пигментов фотосинтеза и могут быть использованы для разработки методов

оценки загрязнения атмосферного воздуха летучими органическими соединениями с использованием пигментов травянистых растений.

Ключевые слова: фотосинтетические пигменты, овсяница тростниковая *Festuca arundinacea* Schreb., летучие органические соединения

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Introduction. Modern industrial production dramatically expands the range of sources of effect and the volume of the environmental impact. The sources of volatile organic compounds are industrial enterprises of thermal power engineering, fuel, chemical and petrochemical industry, mechanical engineering and non-ferrous industry. Their emissions can now be of a significant amount due to changes in technological processes. As a result, alkanes, cycloalkanes, non-organic and aromatic hydrocarbons, alcohols and esters reach environment. All these toxicants are very dangerous in terms of their effects on living organisms. Depending on the dose of effect they can cause allergic reactions in humans and animals, hypoxia, reduction of blood capillary tone, neurotoxic action and suppress soil biota. The study of volatile organic compounds effect on the activity intensity of the plants photosynthetic apparatus, on the one hand, is little studied when compared to the effect of oxides of nitrogen, carbon, sulphur, ammonia, particulate, heavy metals and microplastics [1–11], on the other hand, it is relevant in order to compare the their toxicity level to the parameters of plants life activity [12–17]. It is necessary to find and develop new methods for evaluating open air pollution by volatile organic compounds due to the current changes in the structure of industrial production. While applying these methods, plants can be used quite efficiently.

For this purpose it is necessary to obtain data on change of photosynthetic pigments content in leaves of the most common herbaceous plants of urban habitat at different doses of volatile organic compounds and their mixtures effect. The purpose of the article was to study the nature and conditions of change of chlorophylls *a*, *b* and carotenoids content in the urban habitat herbaceous plants leaves at different doses of volatile organic compounds and their mixtures effect under the specified experimental conditions.

Materials and research methods. The plants of reed fescue *Festuca arundinacea* Schreb. were the study object because it is one of the most common herbaceous plants in the urban habitat. When setting up an experiment on treating reed fescue plants with saturated, aromatic hydrocarbons and ester, the main scientific idea was to establish the nature of changes in the photosynthetic pigments content while setting artificially certain levels of hydrocarbon intake by leaf blades. Based on this, a comparative analysis of the photosynthetic pigments content in reed fescue leaves treated with various doses of hydrocarbons (pentane, hexane, benzene, o-xylene, benz(a)pyrene, butylacetate and their mixtures) was made after certain periods of time after the effect (one day later and three days later after treatment).

For the experiment, reed fescue plants were grown for a month in plastic containers with soil at an illumination intensity of 120 μmol quanta·m^{-2}·s^{-1}, a temperature of 22 °C, a light mode of 14 h. Leaves of the same size were selected for treatment with solutions of volatile organic compounds, visually controlling the absence of damage and the degree of formation of the pigment apparatus.

Leaf blades of reed fescue *Festuca arundinacea* Schreb. were treated with aqueous hydrocarbon solutions. The hydrocarbon doses used were calculated on the basis of the maximum permissible concentrations (MPC) of pollutants set for open air [18]. According to standards, the maximum permissible concentrations of pentane in open air is 100,000.0 μg/m^3; hexane – 60,000.0 μg/m^3; benzene – 100.0 μg/m^3; xylols – 200 μg/m^3; butylacetate – 100.0 μg/m^3; benz(a)pyrene – 5.0 ng/m^3. A maximum single permissible concentration value was used for all compounds except for benz(a)pyrene; average daily permissible concentration value was used for benz(a)pyrene [18]. Untreated plants of reed fescue *Festuca arundinacea* Schreb were used as the control ones. The plants selected for the experiment were treated with aqueous solutions of the studied compounds in the following concentrations: 0.0001–0.03 mg/ml pentane; 0.00006–0.018 mg/ml hexane; 0.0001–0.03 μg/ml benzene; 0.0002–0.06 μg/ml o-xylol, 0.000005–0.0015 ng/ml benz(a)pyrene, 0.0001–0.03 μg/ml butylacetate (acetic acid butyl ester). The leaf blades were treated by aqueous solutions spraying (50 ml of aqueous solution of injected compound each dose). The mixtures of the following concentrations 0.01 μg/ml butylacetate + 0.02 μg/ml o-xylol; 0.02 μg/ml butylacetate +

0.04 μg/ml o-xylol; 0.01 μg/ml benzene + 0.02 μg/ml o-xylol; 0.02 μg/ml benzene + 0.04 μg/ml o-xylol; 0.01 mg/ml pentane + 0.006 mg/ml hexane; 0.02 mg/ml pentane + 0.012 mg/ml hexane; 0.01 mg/ml pentane + 0.006 mg/ml hexane + 0.0005 ng/ml benz(a)pyrene; 0.02 mg/ml pentane + 0.012 mg/ml hexane + 0.001 ng/ml benz(a)pyrene were used to detect the combined effects of the studied compounds.

Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Japan) was used to determine the chlorophylls *a*, *b* and carotenoids content in the leaf blades of reed fescue *Festuca arundinacea* Schreb. The photosynthetic pigments content was determined one day later and three days later after treatment. The leaves with a wet weight of 30–40 mg were used for the extraction of photosynthetic pigments. Chlorophylls and carotenoids were extracted by 99.5 % acetone in 3 biological replications. The pigment content of the extracts was calculated by the extinction coefficients given in work [19] for the corresponding solvent according to formulas

$$C_a = 9.784D_{662} - 0.99D_{644},$$

$$C_b = 21.426D_{644} - 4.650D_{662},$$

$$C_a + C_b = 5.134D_{662} + 20.436D_{644},$$

$$C_k = 4.695D_{440.5} - 0.268C_{a+b},$$

where C_a, C_b, C_k – the average concentration of chlorophylls *a*, *b* and carotenoids in the leaves extract of the study objects (μg/ml); $D_{440.5}$, $D_{644,}$ D_{662} – optical density at wavelengths of 440.5, 644 and 662 nm.

A weight of wet leaves and a volume of the obtained pigment filtrate were used to convert the photosynthetic pigments content to the wet weight. The pigments content is given in mg/g of wet weight. The validity of the difference between the pigment content in the experimental and control samples was evaluated by a single factor dispersion analysis. Mathematic processing of the digital material was made using *M. Excel* and *Statistica* software applications.

Results and its discussion. The alkanes effect on plants is dangerous because they have a sufficiently high resistance and low chemical activity, since high temperature or ultraviolet radiation are necessary for reactions involving alkanes. This does not contribute to reducing their toxicity when interacting with other compounds. Complete destruction of alkanes occurs only with the participation of carbon-assimilating microorganisms (mainly bacteria and yeast), which live in water and soil [20].

When pentane was effected reed fescue plants in our studies, there was a decrease in the content of all photosynthetic pigments at both the early and later stages of the experiment. At the same time all the doses used in the experiment caused an inhibitory effect. Under the effect of the maximum pentane dose a decrease in the content of all studied pigments was observed by 1.51–1.97 times one day later and by 2.0–2.18 times three days later as compared with the control samples (Tab. 1).

This may be a consequence of pentane oxidation to acid and a negative effect on chlorophyll formation. However, a clear correlation of chlorophylls *a*, *b* and carotenoids content reduction and pentane solution concentration increase was observed only at the last two maximum doses (0.02–0.03 mg/ml) one day after treatment and the last three doses (0.01–0.03 mg/ml) three days after treatment.

Lower pentane concentrations effect caused a different quantitative hopping impact. It was possibly due to the existence of some threshold level to which the plant's protective mechanisms act. At the same time higher dose levels have had a pronounced toxic effect that can be effectively tested with the help of reed fescue plants if they are in the air.

When compared to pentane, leaf blades spraying with hexane solutions having a longer carbon chain caused a less, but natural, decrease in pigment content at all post-treatment stages (Tab. 2).

This trend was particularly characteristic of chlorophyll *a*. At maximum hexane concentrations (0.012–0.018 mg/ml) there was a slight increase in chlorophyll *b* and carotenoids acting as additional light-harvesting and protective components. The hexane solution effect of a minimum concentration (0.00006 mg/ml) at late stages of the experiment did not cause a decrease in the content of all the pigments studied. In general, the maximum dose of hexane resulted in a reduction of the pigment content by 1.21–1.79 times after one day as compared to the control one and by 1.39–2.03 three days after treatment. At this stage the maximum hexane concentration effect was almost as twice as the minimum dose effect.

Table 1. **Photosynthetic pigments content in leaves of reed fescue *Festuca arundinacea* Schreb. after treatment with pentane**

Concentration of pentane solution, mg/ml	Pigment content, mg/g wet weight			chl *a*/chl *b*	chl (*a* + *b*)/car
	chl *a*	chl *b*	car		
1 day later					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.0001	0.71 ± 0.03*	0.28 ± 0.01*	0.45 ± 0.02*	2.55	2.19
0.005	0.88 ± 0.02*	0.31 ± 0.02*	0.55 ± 0.03*	2.89	2.17
0.01	0.69 ± 0.02*	0.27 ± 0.01*	0.44 ± 0.02*	2.54	2.19
0.02	0.80 ± 0.03*	0.30 ± 0.01*	0.53 ± 0.03*	3.13	2.08
0.03	0.63 ± 0.02*	0.26 ± 0.01*	0.39 ± 0.02*	2.44	2.27
3 days later					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.0001	0.94 ± 0.04*	0.35 ± 0.02*	0.57 ± 0.03*	2.69	2.27
0.005	1.09 ± 0.04*	0.33 ± 0.02*	0.60 ± 0.03*	3.43	2.40
0.01	1.03 ± 0.04*	0.37 ± 0.02*	0.62 ± 0.03*	2.93	2.27
0.02	0.76 ± 0.04*	0.31 ± 0.02*	0.47 ± 0.01*	2.46	2.29
0.03	0.65 ± 0.03*	0.26 ± 0.01*	0.40 ± 0.02*	2.50	2.28

Note. Here and in Tab. 2–8 * – values of photosynthetic pigments content are given as authentically other than the control one at $p \leq 0.05$; chl *a* – chlorophyll *a*; chl *b* – chlorophyll *b;* car – carotenoids.

Table 2. **Photosynthetic pigments content in leaves of reed fescue *Festuca arundinacea* Schreb. after treatment with hexane**

Concentration of hexane solution, mg/ml	Pigment content, mg/g wet weight			chl *a*/chl *b*	chl (*a* + *b*)/car
	chl *a*	chl *b*	car		
1 day later					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.00006	0.94 ± 0.04*	0.31 ± 0.02*	0.56 ± 0.02*	3.05	2.21
0.003	0.90 ± 0.05*	0.28 ± 0.01*	0.53 ± 0.02*	3.28	2.22
0.006	0.80 ± 0.03*	0.24 ± 0.01*	0.48 ± 0.01*	3.34	2.20
0.012	0.72 ± 0.03*	0.27 ± 0.01*	0.45 ± 0.01*	2.71	2.18
0.018	0.69 ± 0.02*	0.29 ± 0.01*	0.61 ± 0.02*	2.41	1.61
3 days later					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.00006	1.43 ± 0.06	0.75 ± 0.03*	0.95 ± 0.04*	2.79	2.27
0.003	0.99 ± 0.04*	0.41 ± 0.02*	0.57 ± 0.02*	2.43	2.46
0.006	0.97 ± 0.04*	0.27 ± 0.01*	0.56 ± 0.02*	3.64	2.21
0.012	0.78 ± 0.03*	0.30 ± 0.02*	0.45 ± 0.01*	2.69	2.41
0.018	0.70 ± 0.04*	0.31 ± 0.01*	0.61 ± 0.02*	2.28	1.66

In general, among the alkanes that were used, the hexane effect at all stages is more susceptible to chlorophyll a due to the sharpest decrease in the content as compared to the control one, and pentane – to chlorophyll a at early stages and to chlorophyll a and carotenoids – three days after treatment.

A feature of benzene behavior in the environment is that the increase in its concentration in the air can be wave-like at a maximum level in the first 30 minutes and further after 4 h [20]. In addition, benzene is characterized by poor oxidation in the external environment. Xylols are able to inhibit algae growth, and their activity can be enhanced in the presence of solid particles. Benz(a)pyrene, like some other polyarenes, is highly toxic, capable of transboundary transfer, biological transformation, accumulation in natural objects and has a mutagenic property [12, 13, 20].

The results of the determination of the pigment content in the benzene test show a correlation between their amount and the benzene concentration, both one day and three days after treatment of the leaf blades. This indicates to the active benzene effect within all the doses used (Tab. 3).

Table 3. **Photosynthetic pigments content in leaves of reed fescue *Festuca arundinacea* Schreb. after treatment with benzene**

Concentration of benzene solution, μg/ml	Pigment content, mg/g wet weight			chl *a*/chl *b*	chl (*a* + *b*)/car
	chl *a*	chl *b*	car		
1 day later					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.0001	1.05 ± 0.04*	0.36 ± 0.02*	0.41 ± 0.02*	3.0	2.30
0.005	1.02 ± 0.04*	0.32 ± 0.01*	0.61 ± 0.02*	3.21	2.20
0.01	0.92 ± 0.03*	0.29 ± 0.01*	0.57 ± 0.02*	3.14	2.12
0.02	0.91 ± 0.04*	0.29 ± 0.01*	0.57 ± 0.03*	3.16	2.09
0.03	0.84 ± 0.03*	0.27 ± 0.01*	0.51 ± 0.02*	3.16	2.14
3 days later					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.0001	1.40 ± 0.06	0.43 ± 0.02*	0.83 ± 0.03	3.28	2.20
0.005	1.16 ± 0.05*	0.39 ± 0.01*	0.70 ± 0.02*	3.0	2.24
0.01	0.92 ± 0.04*	0.36 ± 0.02*	0.56 ± 0.02*	2.57	2.29
0.02	0.90 ± 0.04*	0.29 ± 0.01*	0.55 ± 0.03*	3.12	2.18
0.03	0.82 ± 0.03*	0.25 ± 0.01*	0.49 ± 0.02*	3.26	2.18

At the same time its maximum concentration effect resulted in reduction of chlorophylls *a*, *b* and carotenoids content by 1.44–1.48 times one day and by 1.71–2.07 times three days after treatment of leaf blades. However, the benzene increased doses effect (0.01 and 0.02 μg/ml) resulted in substantially the same content of all the pigments studied at both early and late stages of the experiment, whereas the minimum doses (0.0001 and 0.005 μg/ml) had the strongest effect on the reduction of the amount of pigments one day after injection. Thus, it is possible to detect the duration of low levels of benzene effect with the help of reed fescue plants. The minimum dose treatment (0.0001 μg/ml) caused a decrease in the amount of pigments in comparison with the control one by 1.09–1.20 times one day later and by 1.20 times three days later, that is, the maximum dose effect was about as twice as the minimum concentration effect.

When compared to benzene, a stronger inhibitory effect on pigments is observed for maximum concentrations of o-xylol. It is possibly associated with more active oxidation processes of o-xylol in the side chain (Tab. 4).

Table 4. **Photosynthetic pigments content in leaves of reed fescue Festuca arundinacea Schreb. after treatment with o-xylol**

Concentration of o-xylol solution, μg/ml	Pigment content, mg/g wet weight			chl *a*/chl *b*	chl (*a* + *b*)/car
	chl *a*	chl *b*	car		
1 day later					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.0002	1.09 ± 0.04*	0.46 ± 0.02*	0.68 ± 0.02*	2.37	2.29
0.01	1.07 ± 0.04*	0.45 ± 0.02*	0.66 ± 0.03*	2.40	2.32
0.02	1.01 ± 0.04*	0.42 ± 0.01	0.62 ± 0.03*	2.38	2.31
0.04	0.84 ± 0.03*	0.28 ± 0.01*	0.55 ± 0.02*	3.16	2.04
0.06	0.60 ± 0.03*	0.25 ± 0.01*	0.40 ± 0.02*	2.33	2.14
3 days later					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.0002	1.43 ± 0.06	0.52 ± 0.02	0.88 ± 0.03	2.74	2.22
0.01	0.91 ± 0.04*	0.39 ± 0.01*	0.55 ± 0.03*	2.33	2.37
0.02	0.59 ± 0.03*	0.26 ± 0.01*	0.37 ± 0.02*	2.29	2.30
0.04	0.51 ± 0.02*	0.23 ± 0.02*	0.31 ± 0.02*	2.17	2.36
0.06	0.48 ± 0.02*	0.22 ± 0.01*	0.31 ± 0.01*	2.19	2.25

O-xylol effect, as benzene, resulted in a uniform reduction of photosynthesis pigments content, except for a minimum dose (0.0002 µg/ml) three days later. At the same time, minimal doses of o-xylol (0.0002 and 0.01 µg/ml) caused approximately the same pigments content one day after treatment, and maximum concentrations (0.04 and 0.06 µg/ml) had the same effect three days later. Thus, minimum doses of o-xylol at early stages and maximum doses of o-xylol at late stages are able to effect equally on pigment levels in the leaves of reed fescue plants. In general, o-xylol had a more negative effect on photosynthetic pigments than alkanes and benzene, especially three days after injection. There was a reduction of photosynthetic pigments by 1.57–2.07 times one day later and by 2.35–2.93 times three days later at its maximum concentration. In addition, the maximum dose of o-xylol had such an effect on the amount of pigments by 2–3 times more than the minimum concentration effect.

Despite the evidence of severe toxic effects on living organisms, benz(a)pyrene did not show the most intense negative effect on the photosynthetic pigments content during the experiment (Tab. 5).

Table 5. **Photosynthetic pigments content in leaves of reed fescue *Festuca arundinacea* Schreb. after treatment with benz(a)pyrene**

Concentration of benz(a) pyrene solution, ng/ml	Pigment content, mg/g wet weight			chl *a*/chl *b*	chl (*a* + *b*)/car
	chl *a*	chl *b*	car		
1 day later					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.000005	1.25 ± 0.04	0.41 ± 0.01	0.71 ± 0.02	3.07	2.32
0.00025	0.99 ± 0.04*	0.34 ± 0.02*	0.44 ± 0.02*	2.94	3.0
0.0005	0.97 ± 0.05*	0.32 ± 0.01*	0.51 ± 0.03*	3.0	2.50
0.001	0.94 ± 0.04*	0.36 ± 0.02*	0.59 ± 0.02*	2.72	2.16
0.0015	0.61 ± 0.02*	0.25 ± 0.01*	0.37 ± 0.02*	2.43	2.33
3 days later					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.000005	1.10 ± 0.05*	0.38 ± 0.02*	0.63 ± 0.02*	2.93	2.34
0.00025	0.86 ± 0.03*	0.30 ± 0.01*	0.50 ± 0.02*	2.85	2.31
0.0005	0.82 ± 0.03*	0.31 ± 0.01*	0.52 ± 0.02*	2.65	2.17
0.001	0.69 ± 0.02*	0.29 ± 0.01*	0.43 ± 0.02*	2.34	2.31
0.0015	0.59 ± 0.02*	0.24 ± 0.01*	0.36 ± 0.02*	2.52	2.30

This may have caused a decrease in the solutions toxicity due to their air contact and oxidation of the benz(a)pyrene, resulting in the formation of quinones and further carboxylic acids. As hexane, benz(a)pyrene was characterized by a natural decrease in chlorophyll *a,* which was most clearly observed three days after treatment.

In general, chlorophyll *a* is the most sensitive pigment to the aromatic hydrocarbon effect at early stages, and in case of benz(a)pyrene – chlorophyll *a* and carotenoids. Over time, benzene most actively affects the change in chlorophyll *b*, o-xylol – in chlorophyll *a* and carotenoids.

Butylacetate is the most common solvent during the preparation and use of paint-and-lacquer materials. Butylacetate dissolves cellulose esters, oils, fats, chlorine rubbers, vinyl polymers, carbiol resins, etc. Butylacetate is widely used in the pharmaceutical industry to separate primary substances in antibiotic production.

A natural decrease in photosynthetic pigment content as compared to the control one was observed at early stages only at maximum doses (0.02 and 0.03 µg/ml) in the butylacetate experiment, and at late stages – all doses except the minimum dose (0.0001 µg/ml) (Tab. 6). Chlorophyll *a* is most susceptible to the butylacetate effect at late stages.

It should be noted that industrial emissions are characterized not by a single element or compound, but by a whole spectrum, sometimes quite measurable. As a consequence, pollutants can affect plants as different mixtures. Taking into account that there are certain protective mechanisms in living organisms to neutralize the toxic substances effects, it is possible to mutually influence these mechanisms by different compounds with their combined effects.

Table 6. **Photosynthetic pigments content in leaves of reed fescue *Festuca arundinacea* Schreb. after treatment with butylacetate**

Concentration of butylacetate solution, μg/ml	Pigment content, mg/g wet weight			chl *a*/chl *b*	chl (*a* + *b*)/car
	chl *a*	chl *b*	car		
1 day later					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.0001	1.62 ± 0.07*	0.53 ± 0.03*	1.08 ± 0.04*	3.07	1.99
0.005	1.29 ± 0.05*	0.41 ± 0.02	0.76 ± 0.04	3.18	2.22
0.01	1.28 ± 0.05*	0.49 ± 0.02*	0.84 ± 0.03*	2.63	2.13
0.02	1.11 ± 0.06*	0.37 ± 0.01	0.71 ± 0.03	2.99	2.10
0.03	0.98 ± 0.04*	0.35 ± 0.01*	0.67 ± 0.02*	2.78	2.0
3 days later					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.0001	1.25 ± 0.05*	0.78 ± 0.04*	0.89 ± 0.03*	2.32	2.21
0.005	1.03 ± 0.04*	0.35 ± 0.01*	0.60 ± 0.02*	2.97	2.30
0.01	0.90 ± 0.03*	0.33 ± 0.02*	0.55 ± 0.02*	2.73	2.23
0.02	0.80 ± 0.03*	0.30 ± 0.01*	0.50 ± 0.01*	2.69	2.21
0.03	0.77 ± 0.04*	0.28 ± 0.01*	0.50 ± 0.02*	2.82	2.09

Table 7. **Photosynthetic pigments content in leaves of reed fescue *Festuca arundinacea* Schreb. after treatment with butylacetate + o-xylol; benzene + o-xylol mixtures**

Experiment option	Pigment content, mg/g wet weight			chl *a*/chl *b*	chl (*a* + *b*)/car
	chl *a*	chl *b*	car		
1 day later (butylacetate + o-xylol mix)					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.01 + 0.02 μg/ml	1.33 ± 0.05*	0.46 ± 0.01*	0.88 ± 0.03*	2.93	2.02
0.02 + 0.04 μg/ml	0.98 ± 0.04*	0.35 ± 0.01*	0.68 ± 0.02*	2.78	1.96
3 days later (butylacetate + o-xylol mix)					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.01 + 0.02 μg/ml	0.80 ± 0.03*	0.32 ± 0.02*	0.51 ± 0.02*	2.54	2.19
0.02 + 0.04 μg/ml	0.62 ± 0.02*	0.26 ± 0.01*	0.39 ± 0.01*	2.38	2.22
1 day later (benzene + o-xylol mix)					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.01 + 0.02 μg/ml	1.27 ± 0.05*	0.51 ± 0.02*	0.81 ± 0.03*	2.51	2.20
0.02 + 0.04 μg/ml	1.09 ± 0.04*	0.44 ± 0.01*	0.71 ± 0.03*	2.50	2.16
3 days later (benzene + o-xylol mix)					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.01 + 0.02 μg/ml	0.94 ± 0.04*	0.35 ± 0.01*	0.62 ± 0.05*	2.65	2.10
0.02 + 0.04 μg/ml	0.62 ± 0.02*	0.27 ± 0.01*	0.39 ± 0.03*	2.30	2.29

The results of the performed analysis showed the butylacetate ability to reduce toxic effect of xylol, and xylol – to strengthen toxic effect of butylacetate on the photosynthetic pigments content (Tab. 7).

Treatment of the leaf blades of reed fescue with a benzene and o-xylol mixture resulted in a reduction of the effect of these compounds separately on the amount of pigments in the reed fescue (Tab. 7). Mixtures of pentane with hexane, as well as pentane with hexane and benz(a)pyrene, on the contrary, increased the negative effect of these compounds as compared to their single effect on the pigment apparatus of reed fescue plants (Tab. 8).

The results of the dispersion analysis show the significant differences between the samples of control and experimental pigment values when treated leaf blades of reed fescue with single compounds (F_{act} (1, 6) = 6,44–227,95; F_{crit} (1, 6) = 5.99 at $p \leq 0.05$). The exception was as follows: one day later, while treated with 0.02 μg/ml o-xylol solution (chlorophyll *b*); 0.000005 ng/ml benz(a)pyrene (all pigments);

Table 8. **Photosynthetic pigments content in leaves of reed fescue *Festuca arundinacea* Schreb. after treatment with mixtures of pentane + hexane; pentane + hexane + benz(a)pyrene**

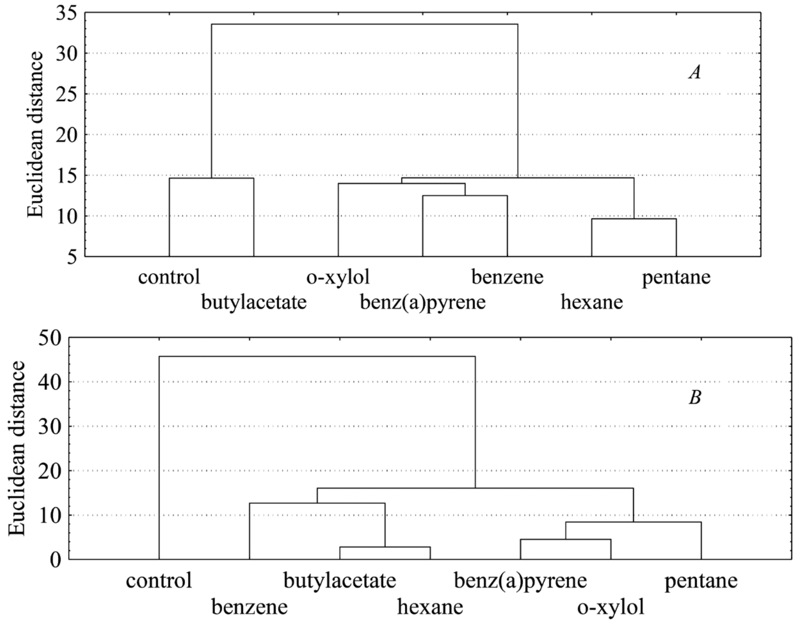
Experience options	Pigment content, mg/g wet weight			chl *a*/chl *b*	chl (*a* + *b*)/car
	chl *a*	chl *b*	car		
1 day later (mix pentane + hexane)					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.01 + 0.006 mg/ml	0.66 ± 0.02*	0.31 ± 0.01*	0.41 ± 0.03*	2.13	2.38
0.02 + 0.012 mg/ml	0.63 ± 0.01*	0.31 ± 0.02*	0.39 ± 0.04*	2.01	2.40
3 days later (mix pentane + hexane)					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.01 + 0.006 mg/ml	1.03 ± 0.04*	0.42 ± 0.01*	0.63 ± 0.05*	2.45	2.29
0.02 + 0.012 mg/ml	0.50 ± 0.01*	0.25 ± 0.01*	0.30 ± 0.03*	2.01	2.49
1 day later (mix pentane + hexane + benz(a)pyrene)					
Control	1.24 ± 0.05	0.39 ± 0.02	0.74 ± 0.04	3.21	2.21
0.01 mg/ml + 0.006 mg/ml + 0.0005 ng/ml	0.67 ± 0.02*	0.30 ± 0.01*	0.42 ± 0.02*	2.22	2.34
0.02 mg/ml + 0.012 mg/ml + 0.001 ng/ml	0.58 ± 0.02*	0.21 ± 0.01*	0.35 ± 0.02*	2.88	2.26
3 days later (mix pentane + hexane + benz(a)pyrene)					
Control	1.42 ± 0.05	0.52 ± 0.03	0.84 ± 0.03	2.74	2.29
0.01 mg/ml + 0.006 mg/ml + 0.0005 ng/ml	0.63 ± 0.02*	0.31 ± 0.01*	0.35 ± 0.02*	2.01	2.73
0.02 mg/ml + 0.012 mg/ml+0.001 ng/ml	0.62 ± 0.02*	0.23 ± 0.01*	0.38 ± 0.02*	3.85	2.19

0.005 µg/ml and 0.02 µg/ml butylacetate (chlorophyll *b* and carotenoids). Three days later incorrect differences were observed in the following cases: while treated with 0.00006 mg/ml hexane (chlorophyll *a*); 0.0001 µg/ml benzene (chlorophyll *a* and carotenoids); 0.0002 µg/ml o-xylol (all pigments). Thus, the number of unreliable differences between experimental and control samples was 7.8 % of variants compared to the control ones. In addition, most unreliable differences in most of experiment variants were observed after treatment with single compounds one day later as compared to the differences obtained three days later, and they were typical of samples treated with butylacetate. No false differences were observed for mixture treatment.

The results of the cluster analysis of photosynthetic pigments content in reed fescue leaves show that taking into account the similarity of their effects in the range of all doses at early stages after treatment the used compounds can be grouped as pentane and hexane (have a strong effect), benzene, benz(a)pyrene and o-xylol (have a weaker effect), that is alkanes and aromatic hydrocarbons were strictly distributed into different clusters (see Figure, *A*). Butylacetate did not cause significant changes in pigment content at early stages of effect as compared to the control one. Perhaps this effect is due to the fact that pentane and hexane have maximum volatility (the volumetric relative volatility of pentane and hexane on the filter paper is 12.3 and 7.43 at 25 °C, respectively), which may be the cause of stronger effect of these compounds at early stage after injection. Benzene belongs to the group of semi-volatile compounds (dimensional relative volatility on filter paper is 3.77 at 25 °C); o-xylol, butylacetate and benz(a)pyrene are small compounds (dimensional relative volatility of o-xylol and butylacetate on the filter paper is 0.565 and 1.0 at 25 °C, respectively). Perhaps that’s why their effect is not evident at once, but after a while.

At late stages the hexane effect was weakened and the remaining compounds were reduced; there has been a redistribution of alkanes and aromatic hydrocarbons, and benz(a)pyrene, o-xylol and pentane (have a strong effect); butylacetate, hexane and benzene (have a weaker effect) had an approximately equal effect (see Figure, *B*).

Conclusion. Our hypothesis has been confirmed. It is the following: there are features and conditions of formation in reed fescue plants photosynthetic pigments of different levels under the effect of different amounts of pollutants having various volatility at different stages after their appending. It has been found that there is a clear correlation of reduction in the amount of pigments only with increased doses of pentane (0.02–0.03 mg/ml). When compared to pentane all hexane solutions concentrations (0.00006–0.018 mg/ml) caused less severe but a more natural decrease in pigment content, especially chlorophyll *a*; a slight



Dendrogramma of chlorophylls *a*, *b* and carotenoids content in leaves of reed fescue Festuca arundinacea Schreb. one day after treatment (*A*) and three days after treatment (*B*)

increase in chlorophyll *b* and carotenoids is possible at increased doses of hexane (0.012–0.018 mg/ml). When there are increased benzene concentrations (0.01–0.02 μg/ml), pigment levels are almost the same. The o-xylol solutions of the minimum dose (0.0002 and 0.01 μg/ml) effect on the pigments equally at early stages and at late stages – in the maximum amount (0.04 and 0.06 μg/ml). Benz(a)pyrene as one of the most dangerous modern toxicants within doses 0.000005–0.0015 ng/ml does not have the most intense negative effect on pigment content; it is characterized by a natural decrease in the level of pigments within all doses three days after injection. Butylacetate whose emissions currently reach significant volumes only of maximum doses (0.02 and 0.03 μg/ml) causes a natural decrease in pigment content at early stages and at late stages – within all doses except the minimum dose (0.0001 μg/ml).

The most sensitive pigments to volatile organic compounds effect are: chlorophyll *a* for hexane; chlorophyll *a* at the early stages for pentane; chlorophyll *a* at the late stages for butylacetate; chlorophyll *a* for all aromatic hydrocarbons at the early stages, and chlorophyll *a* and carotenoids for benz(a)pyrene; at the late stages, benzene most actively effects the change in chlorophyll *b*, o-xylol – in chlorophyll *a* and carotenoids.

When effecting the pigment content together, butylacetate reduces the toxic effect of o-xylol, and o-xylol increases the toxic effect of butylacetate. Benzene and o-xylol in the mixture reduce each other's negative effects; mixtures of pentane and hexane, as well as pentane, hexane and benz(a)pyrene, in contrast, increase each other's negative effects.

The following groups of substances which have the equal effect were obtained: pentane and hexane (have a strong effect) at the early stages after treatment; benzene, benz(a)pyrene and o-xylol (have a weaker effect); butylacetate has practically no inhibitory effect; later: benz(a)pyrene, o-xylol and pentane (have a strong effect); butylacetate, hexane and benzene (have a weaker effect).

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