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O. Berzenina ^{a,b}, I. Osinna ^b, N. Shtemenko ^{a,b}**PHYSICO-CHEMICAL METHODS IN ANALYSIS OF MONOCHLOROBENZENE INFLUENCE ON THE COMPOSITION OF SURFACE HELOPHYTES LIPIDS**^a Ukrainian State University of Chemical Technology, Dnipro, Ukraine^b National Mining University, Dnipro, Ukraine

The information value of physicochemical methods for determining the change in the composition of the plants surface under the influence of toxicants was shown. Monochlorobenzene (MCB) is one of the most widespread representatives among chlororganic aromatic pollutant. Helophytes are used for the treatment of contaminated waters and are good absorbers of aromatics. The quantity of surface lipids of the surface lipids of the plants under the influence of MCB were analyzed by IR-spectra and gas chromatography-mass spectrometry (GC-MS) data in comparison with control plants grown on the distilled water. It was shown that the quantity of surface lipids increased by 8.5–17.8% for all species. According to the influence of monochlorobenzene on the composition of fatty acids, two groups of plants were found as follows: an increase in the content of C_{24:0}–C_{32:0} was observed in the first group, and a sharp increase of C_{16:0} fatty acid took place in the second one. A decrease in the content of hydrocarbons in some species was noticed and changes in their heterogeneity were common for the most of exposed plants. The conclusion was drawn that FTIR spectra of surface lipids of *Acorus calamus* may be recommended for further investigations and the use in environmental monitoring.

Keywords: monochlorobenzene, infrared spectroscopy, Fourier transform infrared spectroscopy, gas chromatography-mass spectrometry, helophytes.

Introduction

Surface lipids (SLs) of plants (the term «epicuticular waxes» is close) are complex mixtures of highly hydrophobic substances, pivotal role of which for the plant survival is well-recognized [1]. The most important functions of this protective layer should be named here: control of water status; anti-adhesive, self-cleaning properties; protection against radiation, pathogens and chemicals penetration; maintenance of physiological integrity. It is commonly accepted that surface lipids are largely controlled by genetic programs [2]. This static view must be questioned due to at least one reason: the composition of SLs during plant development is changed dramatically [1,3]. It means that any environmental factor changing growth conditions could influence SL formation. Also, as plant SLs play pivotal physiological and ecological roles, it might be advantageous to adapt their composition and properties to environmental stresses, for example, for high concentrations of some exogeneous chemicals. Earlier we have found that three species, *Typha latifolia*, *Phragmites australis* and *Juncus effusus*,

had dramatically changed the composition and content of SLs during growing on highly contaminated water *in vivo* in comparison with control plants [4,5]. The questions are if one toxicant in high concentration could cause alterations in SLs biosynthesis and in what extent?

Helophytes (synonyms are «emergent water plants, marsh plants, etc.) are used for the treatment of contaminated waters and are good absorbers of aromatics [6]. Monochlorobenzene (MCB) is one of the most widespread representatives among chlorine-organic aromatic substances. Exposure to high concentrations of MCB is hazardous to the liver, kidney and especially for brain [7,8]. Thus, the aim of the present work was to analyze if SLs of a range of emergent plants were changed under the influence of MCB during planting *in vitro*, to establish if these alterations are common to all species and to determine if these changes may be used for environmental monitoring.

Materials and methods

Emergent plants *Typha latifolia* L. (Fam. Typhaceae), *Phragmites australis* L. (Fam. Gramineae),

Carex acuta L. (Fam. Cyperaceae), *Juncus effusus* L. (Fam. Juncaceae), *Acorus calamus* (Fam. Acoraceae) of the one month age were collected on the territory of the Dnieprovsko-Orelsky preserve and let to grow on distilled water during one month in the laboratory (120 plants of each specie) before experiment. The saturated solution of MCB was prepared in the following way: 100 mg of MCB were put to 1 liter of distilled water and the mixture was stirred during one night. The upper layers of the obtained mixtures were used as experimental solutions. The concentration of MCB in experimental solutions was determined by UV-spectroscopy [9] and was equal to 0.40–0.42 g/L.

All plants were divided on control ones (6 plants in each group, 5 groups for every specie), that grew on distilled water and exposed ones (6 plants in each group, 5 groups for every specie), that were put to grow on the solution of MCB). Each group was grown in separate vessel, i.e. every experiment was accomplished five times. In 10 days the solution of MCB in exposed plants were changed on freshly prepared one with the same high concentration of MCB. All plants were grown in the same light and temperature conditions, close to the natural summer period as follows: the day temperature was 25–30°C, and the night temperature was 18–20°C.

Top halves from mature fresh leaves were excised with clean razor blades from the control and exposed plants of each group 30 days after and weighted. SLs were extracted by hot chloroform, avoiding contact between chloroform and the open cuts, dried and weighted [10]. The results of weighting were expressed as the average of five trials with standard deviation. W-criterion of Wilcoxon's nonparametric tests was used to compare the parameters obtained from the control group without treatment and each group of exposition. The overall significance level was set at $p \leq 0.05$.

SL extracted from each group was dissolved in pure chloroform (Merk), 1/3 part from each sample was taken and united for each control and exposed plant to form one sample from each control and exposed specie for analysis by infrared spectroscopy. Fourier transform infrared (FTIR) spectroscopy of

SL in the h-ATR was applied [11].

SLs from three independent groups (randomly chosen from SL extracted from 5 groups) for each control and exposed plant were taken for mass-spectrometric analysis. SLs were derivatized with methanolic HCl (Supelco) to form methyl esters of fatty acids. The derivatized mixtures were analyzed by GC-MS using an Agilent 6890 gas chromatograph coupled to a 5973 mass selective detector (Agilent Technologies, Waldbronn, Germany). For GC separation, a 30 m long HP5 MS capillary column was used (0.25 mm i.d., 0.25 μ m film thickness) with the following oven temperature program: 50°C – 1 min – 50 K/min – 170°C – 4 K/min – 300°C – 4 min. One microliter of each derivatized extract with methylated carboxylic hydroxy functions was injected splitless (pulsed splitless) at an injector temperature of 300°C. The ion source of the mass spectrometer operated in electron impact mode at a temperature of 230°C. Full scan mass analyses were performed to get comprehensive information on the components contained in the extracts. Data were processed automatically using a database containing 178 GC retention times and mass spectral characteristics for identification of long chain alcohols, aldehydes and methylesters of fatty acids. In order to guarantee reproducible retention times, the GC-analysis was locked on hexadecanoic acid methylester as a time reference point [12].

Results

Plants grew well in the saturated MCB solutions without visible harmful influence. The total quantities of the surface lipids varied from 48 to 79 mg for control plants and 59–89 mg for exposed plants in extracts from the 100 g of the fresh leaves (Table 1). We have found that the quantities of SLs in plants under MCB exposition increased by 8.5–17.8%.

According to the GC-MS data (Table 2), the investigated plants have fatty acids and alkanes as prevailed chemical classes. The relative content of components in Tables 2–5 are given as percentages of compound classes ($n=3$; \pm SD).

The investigated species differ very much in the relative content of the main chemical classes. If to compare, for example, *J. effusus* had alkanes as

Table 1

Total quantity of SLs ($M \pm M$, mg in 100 g of the fresh weight of leaves) extracted from the leaves of control and exposed plants ($n=3$; \pm SD)

Experiment	<i>P. australis</i>	<i>T. latifolia</i>	<i>J. effusus</i>	<i>C. acuta</i>	<i>A. calamus</i>
Control	65.34 \pm 2.46	71.44 \pm 2.85	48.56 \pm 1.91	78.96 \pm 2.46	63.22 \pm 2.28
Exposed	74.34 \pm 3.48*	80.21 \pm 4.23*	59.05 \pm 4.02*	89.42 \pm 4.14*	69.08 \pm 2.46*

Note: * – $P \leq 0.05$, in comparison with control group.

Table 2

Content of the chemical classes of compounds in SL of investigated plants in % to total SLs (n=3; \pm SD)

No.	Plants	Content of compounds		
		Fatty acids	Hydrocarbons	Minor components
1	<i>Ph. australis</i> (control)	64.15 \pm 2.44	33.54 \pm 1.15	2.31 \pm 0.20
2	<i>Ph. australis</i> (exposed)	61.66 \pm 2.21*	33.42 \pm 1.01*	4.92 \pm 0.22*
3	<i>J. effuses</i> (control)	42.42 \pm 2.37	56.22 \pm 1.40	1.36 \pm 0.31
4	<i>J. effuses</i> (exposed)	43.75 \pm 2.58*	53.30 \pm 1.26*	2.95 \pm 0.24*
5	<i>C. acuta</i> (control)	85.06 \pm 2.87	13.70 \pm 1.17	1.24 \pm 0.18
6	<i>C. acuta</i> (exposed)	86.17 \pm 2.13*	8.98 \pm 1.21*	4.85 \pm 0.34*
7	<i>T. latifolia</i> (control)	61.18 \pm 2.41	37.80 \pm 1.30	1.02 \pm 0.27
8	<i>T. latifolia</i> (exposed)	67.18 \pm 2.70*	29.40 \pm 1.11*	3.42 \pm 0.21*
9	<i>A. calamus</i> (control)	51.22 \pm 2.55	47.14 \pm 1.24	1.64 \pm 0.19
10	<i>A. calamus</i> (exposed)	55.62 \pm 2.32*	35.46 \pm 1.29*	8.92 \pm 0.40*

Note: * – $P \leq 0.05$, in comparison with control group.

the prevailed components and *C. acuta* had fatty acids. The influence of MCB in common revealed in reducing of hydrocarbons, especially significant for *C. acuta*, *T. latifolia* and *A. calamus* and in increase of minor components for all experimental plants, especially for *A. calamus*.

In non-characteristic, individual part of the IR-spectra in the area from 1400 to 1000 cm^{-1} , SLs from exposed plants had slightly increased absorption than control ones (Fig. 1).

The FTIR spectra of SLs from investigated plants were dominated by three groups of characteristic absorption bands assigned to C=O and CH₂ groups. The area from 400 to 1900 cm^{-1} usually differs very much in control species and may be called a "specific area". The carbonyl stretching band profile from the second derivative regions spectrum clearly displayed two absorption bands at 1735 and 1725 cm^{-1} regions (Fig. 2).

Under the influence of MCB, the displacement of the maximums of the absorption bands took place. Such a displacement is common for all investigated plants, nevertheless their carbonyl profile is different from the shown one. Fatty acid composition of SLs of the investigated species is usual for the SL of plants: saturated and long-chained fatty acids prevailed (Table 3).

According to the obtained data, we have divided the investigated plants on two groups according to the different kind of influence of MCB on the fatty acids content. We found the significant increase of the long-chained (C₂₀–C₃₀) fatty acids in the first group (*P. australis*, *J. effusus*, *C. acuta*) (Fig. 3), and the increase of palmitic was found in another group (*T. latifolia* and *A. calamus*) (Fig. 4).

It is seen from the data given in Table 3 that the content of the C_{26:0}, C_{28:0}, C_{30:0} fatty acids increased in exposed species of the group of *P. australis*, *J.*

Table 3

Composition (%) of the main fatty acids in SL of *P. australis*, *J. effusus*, *C. acuta*, *T. latifolia* and *A. calamus* (n=3; \pm SD)

Fatty acids	<i>P. australis</i>		<i>J. effusus</i>		<i>C. acuta</i>		<i>T. latifolia</i>		<i>A. calamus</i>	
	control	exposed	control	exposed	control	exposed	control	exposed	control	exposed
C _{14:0}	3.17 \pm 0.15	3.13 \pm 0.14*	1.86 \pm 0.09	3.43 \pm 0.16*	2.10 \pm 0.09	1.53 \pm 0.07*	4.93 \pm 0.23	2.62 \pm 0.12*	8.09 \pm 0.37	4.76 \pm 0.22*
C _{15:0}	2.39 \pm 0.11	1.48 \pm 0.07*	4.45 \pm 0.22	1.71 \pm 0.08*	1.75 \pm 0.08	–	3.36 \pm 0.16	–	5.41 \pm 0.25	3.42 \pm 0.16*
C _{16:0}	18.60 \pm 0.89	18.16 \pm 0.8*	20.32 \pm 0.91	10.28 \pm 0.48*	8.40 \pm 0.38	3.31 \pm 0.16*	28.78 \pm 1.35	47.12 \pm 2.26*	36.62 \pm 1.68	52.16 \pm 2.45*
C _{17:0}	–	–	–	–	–	–	2.57 \pm 0.12	–	2.35 \pm 0.11	–
C _{18:0}	6.52 \pm 0.30	–	8.64 \pm 0.42	–	4.0 \pm 0.18	–	6.36 \pm 0.30	–	14.80 \pm 0.68	–
C _{20:0}	19.11 \pm 0.92	6.21 \pm 0.29*	5.92 \pm 0.29	8.63 \pm 0.41*	8.49 \pm 0.38	5.43 \pm 0.26*	6.07 \pm 0.29	17.37 \pm 0.83*	3.52 \pm 0.16	16.76 \pm 0.79*
C _{22:0}	13.16 \pm 0.63	20.60 \pm 0.95*	12.61 \pm 0.62	14.05 \pm 0.66*	15.43 \pm 0.60	9.83 \pm 0.47*	14.42 \pm 0.68	6.94 \pm 0.33*	6.01 \pm 0.28	5.67 \pm 0.27*
C _{24:0}	9.63 \pm 0.44	10.90 \pm 0.50*	11.60 \pm 0.57	18.22 \pm 0.85*	18.04 \pm 0.81	20.37 \pm 0.58*	15.05 \pm 0.71	7.43 \pm 0.36*	21.14 \pm 0.97	7.05 \pm 0.33*
C _{26:0}	8.24 \pm 0.10	13.06 \pm 0.60*	5.10 \pm 0.25	10.54 \pm 0.48*	4.18 \pm 0.19	15.11 \pm 0.73*	20.36 \pm 0.96	18.34 \pm 0.88*	2.06 \pm 0.09	10.18 \pm 0.48*
C _{28:0}	9.16 \pm 0.44	14.26 \pm 0.66*	16.36 \pm 0.80	18.08 \pm 0.85*	21.42 \pm 0.96	26.08 \pm 1.25*	–	–	–	–
C _{30:0}	10.02 \pm 0.48	12.02 \pm 0.55*	13.14 \pm 0.64	15.06 \pm 0.71*	16.12 \pm 0.73	18.34 \pm 0.88*	–	–	–	–

Note: * – $P \leq 0.05$, in comparison with control group.

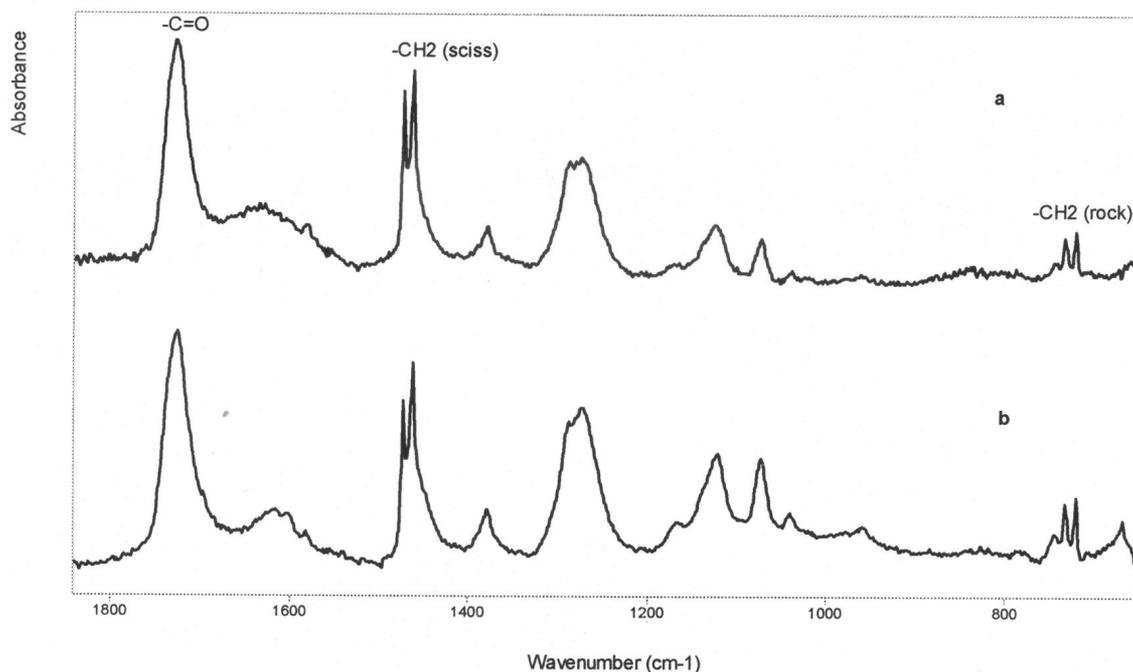


Fig. 1. IR-spectra of surface lipids of control (a) and exposed (b) plants *A. calamus*

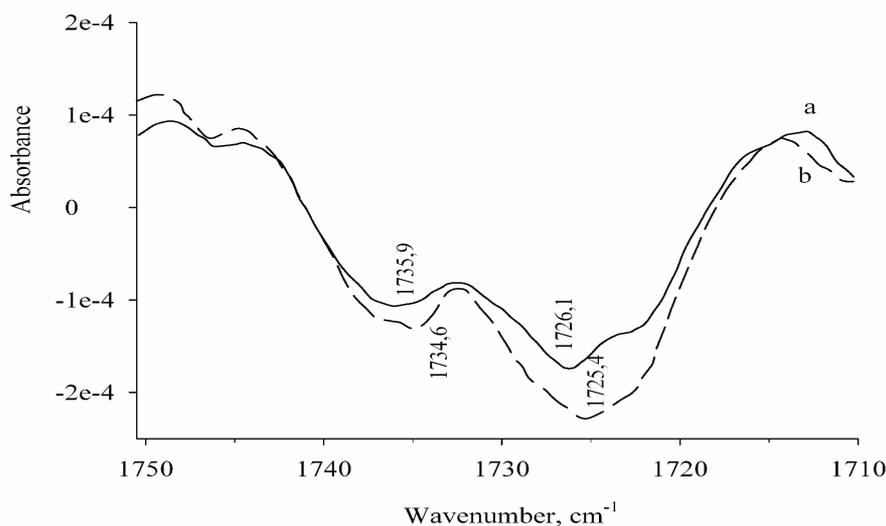


Fig. 2. Carbonyl area (second derivative) in spectra of surface lipids of control (a) and exposed (b) plants *A. calamus*

effusus and *C. acuta* as follows: C_{26} increases by 58–261%, C_{28} increases by 10–55% and C_{30} increases by 14–20% upon exposure to MCB. In the group *T. latifolia* and *A. calamus* palmitic acid increases in proportion by 42 and 63%, whereas stearic acid completely disappears.

Hydrocarbons of SL of the emergent plants are represented by odd-numbered chains mostly (Table 4).

There are no common directions of alteration of hydrocarbons in SLs of the investigated plants. In some species, we have not found significant changes (*J. effusus*); there is increase in the content of the

long-chained component C_{29} in *P. australis*, a decrease of long-chained components C_{29} , C_{31} took place in all other species. The content of even hydrocarbons that are usually in minor quantities, increased and the heterogeneity of hydrocarbons increased under the influence of MCB exposition in SLs of all species, excluding *P. australis*.

As SLs of *C. acuta* and *A. calamus* had maximal changes in increasing of the content of minor components (1.24 control – 4.85 exposed and 1.64 control – 8.92%, correspondingly) in comparison with other plants, where the increase took was significantly letter, we decided to present as an

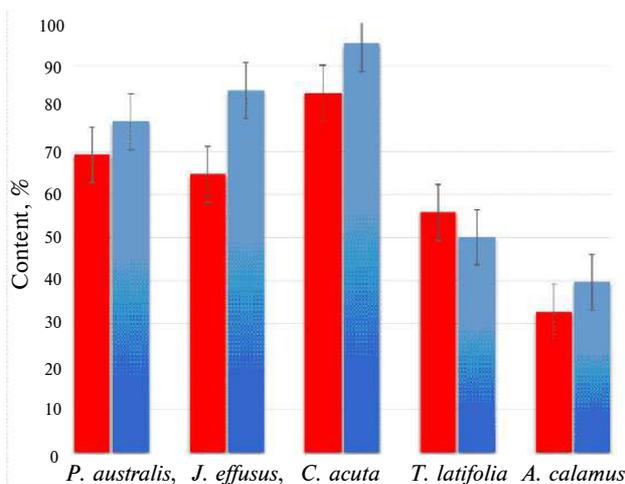


Fig. 3. Content of long-chained fatty acids in SL of control (red) and exposed (blue) plants

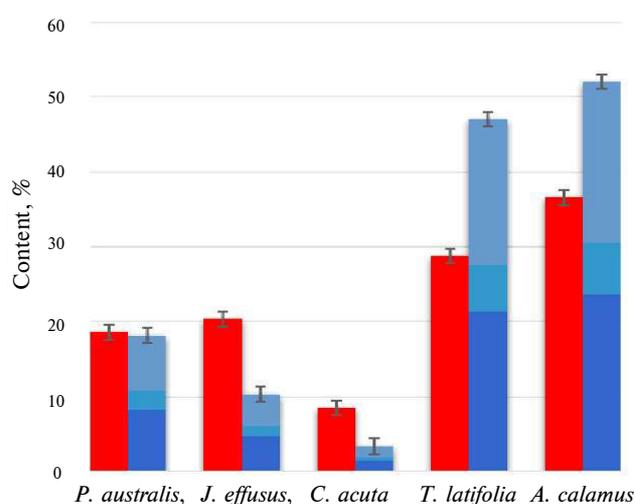


Fig. 4. Content of palmitic acids in SL of control (red) and exposed (blue) plants

Table 4

Composition (%) of the main hydrocarbons in SL of control plants (n=3; \pm SD)

Hydrocarbons	<i>P. australis</i>		<i>T. latifolia</i>		<i>J. effusus</i>		<i>C. acuta</i>		<i>A. calamus</i>	
	control	exposed	control	exposed	control	exposed	control	exposed	control	exposed
C ₂₃	4.75 \pm 0.22	2.21 \pm 0.10*	2.10 \pm 0.10	1.27 \pm 0.06*	–	–	14.44 \pm 0.68	27.82 \pm 1.00*	1.88 \pm 0.09	2.17 \pm 0.10*
C ₂₄	3.50 \pm 0.16	–	–	1.25 \pm 0.06*	–	–	–	–	–	6.21 \pm 0.30*
C ₂₅	9.08 \pm 0.43	4.18 \pm 0.20*	9.36 \pm 0.45	3.44 \pm 0.17*	4.52 \pm 0.21	6.32 \pm 0.29*	5.44 \pm 0.26	12.48 \pm 0.59*	4.68 \pm 0.22	9.43 \pm 0.45*
C ₂₆	4.94 \pm 0.23	1.73 \pm 0.08*	–	5.00 \pm 0.24*	–	–	–	1.01 \pm 0.05*	–	6.39 \pm 0.31*
C ₂₇	17.76 \pm 0.83	14.49 \pm 0.68*	20.34 \pm 0.98	18.51 \pm 0.89*	4.80 \pm 0.22	3.39 \pm 0.16*	22.16 \pm 1.04	14.24 \pm 0.67*	25.62 \pm 0.9	28.33 \pm 1.36*
C ₂₈	–	1.62 \pm 0.08*	2.26 \pm 0.11	3.65 \pm 0.18*	0.00	3.83 \pm 0.18*	3.24 \pm 0.15	4.62 \pm 0.22*	2.14 \pm 0.01	7.36 \pm 0.35*
C ₂₉	43.25 \pm 2.03	61.11 \pm 2.87*	50.28 \pm 2.41	44.76 \pm 2.15*	28.74 \pm 1.32	27.58 \pm 1.27*	58.82 \pm 2.76	39.63 \pm 1.86*	47.36 \pm 2.27	29.62 \pm 1.42
C ₃₀	1.87 \pm 0.09	–	–	2.44 \pm 0.12*	1.98 \pm 0.09	2.36 \pm 0.11*	–	–	–	4.56 \pm 0.22*
C ₃₁	15.85 \pm 0.72	14.65 \pm 0.69*	19.76 \pm 0.68	18.68 \pm 0.90*	59.96 \pm 2.76	56.42 \pm 2.60*	–	–	14.32 \pm 0.69	5.92 \pm 0.28*

Note: * – $P \leq 0.05$, in comparison with control group.

example the composition of the minor components of these two species (Table 5).

Usual minor components in SLs of plants are iso-, anteiso-fatty acids, as also in our experimental data presented in Table 5. Control species had only three or four minor components in SLs. Under the influence of MCB, the decrease or even disappearance of the content of “normal” components and appearance of plenty components with unusual structure was observed in SLs of *C. acuta*. In SLs of exposed plants *A. calamus*, the increase of the main component iso-pentadecanoic acid took place together with decreasing or disappearance of other three “normal” components; the appearance of only one new component was noticed.

Discussion

Recent investigations showed that SLs

quantities and compositions vary greatly between plant species and even between organs and developmental stages [13]. Analyzing the content of epi- and intracuticular waxes of eight plant species, these authors found that SLs of *Oreapanax guatemalensis* contained from 20 to 50% of fatty acids in the both kinds of SLs. But the most common features of the SLs content presented in this and other works was essential content of hydrocarbons, which in some species reached up to 90% of total SL.

We have found more total quantity of SLs in the exposed plants than in control. It possibly means that MCB activates biosynthesis of SLs. Some aromatic substances, including MCB, may be degraded in the cells of microorganisms and aromatic ring-derived carbon may be incorporated to fatty acids [14,15]. Plants are not known for such

Table 5

Minor components (% to total) in SL of *C. acuta*, *A. calamus* (n=3; \pm SD)

Components	<i>C. acuta</i>		<i>A. calamus</i>	
	Content, %		Content, %	
	control	exposed	control	exposed
C _{9:0} diMe	—	3.29 \pm 0.09*	—	—
C _{14:0} anteiso	39.80 \pm 1.59	—	—	—
C _{14:0} iso	—	0.67 \pm 0.02*	—	—
C _{14:1} w7c	—	0.31 \pm 0.09*	—	—
C _{15:0} iso	55.03 \pm 2.20	29.84 \pm 0.89*	57.04 \pm 1.99	70.44 \pm 2.11*
C _{15:0} anteiso	5.17 \pm 0.21	2.01 \pm 0.06*	7.86 \pm 0.27	6.54 \pm 0.19*
C _{15:1} w10c(d4)	—	0.58 \pm 0.02*	—	—
C _{16:0} iso	—	1.16 \pm 0.03*	—	—
C _{16:1} w11c	—	2.57 \pm 0.08*	—	—
C _{16:1} w9c	—	1.39 \pm 0.04*	—	—
C _{16:1} w8c	—	2.03 \pm 0.06*	—	—
C _{16:1} w7c	—	1.51 \pm 0.04*	—	—
C _{17:0} iso	—	0.60 \pm 0.02*	—	—
C _{17:0} anteiso	—	—	25.46 \pm 0.89	13.35 \pm 0.40*
C _{18:1} w9c	—	19.37 \pm 0.58*	—	9.67 \pm 0.29*
C _{18:2} w6c, w9c	—	16.95 \pm 0.51*	—	—
C _{18:1} w8c	—	17.12 \pm 0.51*	9.64 \pm 0.33	—
C _{20:0} iso	—	0.60 \pm 0.02*	—	—

Note: * – P \leq 0.05, in comparison with control group.

incorporation, but taking into account “green liver” model [16] (plant metabolism, conducting detoxification or elimination process of xenobiotics) this should not be excluded. Also, the formation of a thicker layer of protective molecules may be the result of the process of adaptation to contaminants.

Complex contamination of water in experiments described earlier [4,5] caused more alterations in the SLs content than MCB in large concentration in our recent experiment *in vitro*. This confirms the fact that the formation of SLs during the process of adaptation depends on the type of the toxicants, i.e. is highly specific to the structure of the xenobiotic(s).

As was stated above, the FTIR spectra of SLs showed three groups of characteristic absorption bands which are associated with C=O and CH₂ groups (Fig. 1). The area from 400 to 1900 cm⁻¹ presents the so-called “specific area”. In this specific area we found increase of absorption and sometimes even additional signals [5] that may be caused by the accumulation of a large quantity of the components called by us as minor. The second derivative of the spectrum in carbonyl area (Fig. 2) demonstrates the changes in composition of carbonyl-containing compounds in control and

exposed plants. This more detailed information about multiplicity of a carbonyl absorption in surface layer may be used in monitoring investigations, as rapid technique may be applied for preliminary comparative evaluation of plants from contaminated sites.

Some findings concerning the effects of stress on regulation of wax biosynthesis were described in work [17]. Under the influence of MCB the significant changings in biosynthesis of the main components of SL of the investigated plants occurred. According to the contemporary knowledge of the biosynthetic pathways of SLs components [18], two different systems of modification of the very long chained fatty acids exist: acyl reduction pathway (I) leading to the biosynthesis of even components and formation of the most high molecular weight components, esters; and decarbonylation pathway (II) leading to the odd carbon chained components, among which the dominant are hydrocarbons. According to this scheme, the investigated plants chose different ways for formation of adaptive surface layer: to intensify I biosynthetic system but with different extent of elongation, or to brake II system, or both. In any case, the synthesis of fatty acids was

activated by MCB in all species and led to the formation of more polar components of SLs with more deal of free or bound fatty acids. Especially essential shift in SLs composition was found for *A. calamus* where the process of elongation was disturbed in both I and II systems and the accumulation of minor components reached 8.92% of SL. These data support the idea that there may be found species among emergent or terrestrial plants with changeable system of the SLs formation and they could be good objects for monitoring investigations.

Little is known about synthetic pathways for minor components in surface layers of plants – branched and unsaturated fatty acids. These substances in SLs are poorly described. The only analogy and possible explanation that was found by us were surface lipids of human skin and their perversity [19]. Perversity manifests itself when one compares the lipids synthesized by skin with those synthesized by internal tissues. For example, skin makes odd instead of only even chains, branched instead of only straight chains, free instead of only esterified acids, places double bonds in unusual positions in the fatty chains, extends chains to extreme lengths, etc. Functions of these molecules were explained as these products may pose metabolic problems to potential pathogens and thus contribute to the survival of only compatible microorganisms. In the case of the investigated plants, the formation of SLs in the process of adaptation to xenobiotic exposure may be required the appearance of such protective molecules also.

Conclusions

All tested emergent plants (helophytes) changed composition of the SL under influence of maxima concentrations of MCB during planting *in vitro*. The most common directions of the alterations were: increase of fatty acids biosynthesis (long chained or palmitic fatty acids) that led to the change of the multiplicity of carbonyl absorption in FTIR spectra, the change in the heterogeneity of hydrocarbons content and the appearance of a large (up to 9% in some species) quantities of minor components: branch chained and unsaturated fatty acids of the SLs of a range of emergent plants changed their composition. Quantitatively and qualitatively the process of creation of the adaptive SLs differed very much among species and is highly specific. SLs of *A. calamus* were the most subjected to the influence of MCB that reflected in FTIR spectra. SLs of this specie may be recommended for following investigations of influence of other toxicants and for use in environmental monitoring. The epidermal tissue of plant plays a crucial role in survival of the

whole plant. The complexity of composition of SLs and our not full understanding of the mechanism of their synthesis and functions may explain the lack of information about influence of contaminants on their properties. This is very important for ecology of our planet, especially taking into account that SLs represent a defensive layer of the green life. Harmful contamination producing by a man may damage this defensive layer, thus leading to irreversible ecological catastrophe. In a whole, investigations of influences of chemicals on SL are of great importance and to our mind should be an intensively developing area of future research.

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REFERENCES

1. Yeats T.H., Rose J.K.C. The formation and function of plant cuticles // *Plant Physiology*. – 2013. – Vol.163. – P.5-20.
2. Busta L., Budke J.M., Jetter R. Identification of β -hydroxy fatty acid esters and primary, secondary-alkanediol esters in cuticular waxes of the moss *Funaria hygrometrica* // *Phytochem*. 2016. – Vol.121. – P.38-49.
3. Van Maarseveen C., Han H., Jetter R. Development of the cuticular wax during growth of *Kalanchoe daigremontiana* (Hamet et Perr. de la Bathie) leaves // *Plant Cell Environ*. – 2009. – Vol.32. – P.73-81.
4. Analytical methods to characterize the composition of surface lipids of helophytes exposed to VOC contaminant water / Macherius A., Haertig C., Kuschk P., Shtemenko N., Moeder M. // Dishovsky C., Pivovarov A. (eds): Counteraction to Chemical and Biological Terrorism in East European Countries, NATO Science for Peace and Security Series A: Chemistry and Biology. – 2009. – P. 95-100.
5. Influence of contaminant stress on the surface lipids composition of some helophytes / Shtemenko N., Kuschk P., Moeder M., Geyer W., Haertig C., Voevoda M., Shepelenko V., Alexeevskaya I. // In: Dishovsky C., Pivovarov A. (eds): Counteraction to Chemical and Biological Terrorism in East European Countries, NATO Science for Peace and Security Series A: Chemistry and Biology. – 2009. – P.101-108.
6. United States Public Health Service: Agency for Toxic Substances and Disease Registry // Toxicological profile for chlorobenzene. 1990. Available at: <http://atsdr.cdc.gov/toxprofiles/tp131.pdf>.
7. Degradation of 2,4,6-trinitrotoluene by selected helophytes / Nepovim A., Hebner A., Soudek P., Gerth, A.,

Thomas, H., Smreck, S., Vanek, T. // *Chemosphere*. – 2005. – Vol.60. – P.1454-1461.

8. *Detoxification* and antioxidant responses in diverse organs of *Jenynsia multidentata* experimentally exposed to 1,2-and 1,4-dichlorobenzene / Monferran M.V., Pesce S.F., Cazenave J., Wunderlin D.A. // *Environ. Toxic.* – 2008. – Vol.23. – P.184-192.

9. *Effect* of hydrotropes on solubility and mass transfer coefficient of chlorobenzene / Arunodhaya N., Jayakumar Ch., Gandhi N.N. Arunodhaya N., Jayakumar C., Gandhi N.N. // *Research Journal of Chemical Sciences*. – 2012. – Vol.2(8). – P.9-13.

10. *Buschhaus C., Jetter R.* Composition differences between epicuticular and intracuticular wax substructures: how do plants seal their epidermal surfaces? // *J. Exp. Bot.* – 2011. – Vol.62. – P.841-853.

11. *Dubis E.N., Dubis A.T., Morzycki J.W.* Comparative analysis of plant cuticular waxes using HATR FT-IR reflection technique // *J. Mol. Struct.* – 1999. – Vol.511-512. – P.173-179.

12. *Haertig C.* Rapid identification of fatty acid methyl esters using a multidimensional gas chromatography–mass spectrometry database // *J. Chromatogr. A*. – 2008. – Vol.1177. – P.159-169.

13. *Jetter R., Riederer M.* Localization of the transpiration barrier in the epi- and intracuticular waxes of eight plant species: water transport resistances are associated with fatty acyl rather than alicyclic components // *Plant Physiol.* – 2016. – Vol.170. – P.921-934.

14. *Naphthalene* degradation and incorporation of naphthalene – derived carbon into biomass by the thermophile *Bacillus thermoleovorans* / Annweiler E., Richnow H.H., Antranikian G. et al. // *Appl. Environ. Microbiol.* – 2000. – Vol.66. – P.518-523.

15. *Biodegradation* of chlorobenzene in a constructed wetland treating contaminated groundwater / Braeckevelt M., Rokadia H., Mirschel G. et al. // *Water Sci. Technol.* – 2007. – Vol.56. – P.57-62.

16. *Burken J.G.* Uptake and metabolism of organic compounds: green-liver model // *Phytoremediation: Transformation and control of Contaminants*. – S.C. McCutcheon and J.L. Schnoor (eds.). – In: John Wiley & Sons. – 2003. – P.59-84.

17. *Shepherd T., Griffiths D.W.* The effects of stress on plant cuticular waxes // *New Phytol.* – 2006. – Vol.171. – P.469-99.

18. *Samuels L., Kunst L., Jetter R.* Sealing plant surfaces: cuticular wax formation by epidermal cells // *Annu. Rev. Plant Biol.* – 2008. – Vol.59. – P.683-707.

19. *Nicolaidis N.* Skin lipids: their biochemical uniqueness // *Science*. – 1974. – Vol.186. – No. 4158. – P.19-26.

ФІЗИКО-ХІМІЧНІ МЕТОДИ АНАЛІЗУ ВПЛИВУ МОНОХЛОРБЕНЗОЛУ НА СКЛАД ПОВЕРХНЕВИХ ЛІПІДІВ ГЕЛОФІТОВ

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Показаний інформативний характер фізико-хімічних методів визначення зміни складу поверхні рослин під впливом токсикантів. Монохлорбензол (МЦВ) є одним з найбільш поширених представників серед хлорорганічних ароматичних забруднювачів. Гелофіти використовуються для очищення забруднених вод та є ефективними поглиначами ароматичних сполук. Методами ІЧ-спектроскопії і GC–MS було проаналізовано кількість поверхневих ліпідів рослин під впливом МЦВ у порівнянні з контрольними рослинами, вирощеними на дистильованій воді. Було показано, що кількість поверхневих ліпідів у всіх видів збільшилась на 8,5–17,8%. Залежно від впливу монохлорбензолу на склад жирних кислот були виявлені дві групи рослин: в першій групі спостерігалось збільшення вмісту C_{24:0}–C_{32:0}, у другому – різке збільшення жирної кислоти C_{16:0}. Було відмічено зменшення вмісту вуглеводнів у деяких видів, і зміни в їх гетерогенності були загальними для більшості експонованих рослин. Зроблено висновок про те, що спектри FTIR поверхневих ліпідів *Acorus calamus* можуть бути рекомендовані для подальших досліджень і використання в моніторингу навоколишнього середовища.

Ключові слова: монохлорбензол, інфрачервона спектроскопія, інфрачервона трансформація Фур'є, газова хроматографія-мас-спектрометрія, гелофіти.

PHYSICO-CHEMICAL METHODS IN ANALYSIS OF MONOCHLOROBENZENE INFLUENCE ON THE COMPOSITION OF SURFACE HELOPHYTES LIPIDS

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The information value of physicochemical methods for determining the change in the composition of the plants surface under the influence of toxicants was shown. Monochlorobenzene (MCB) is one of the most widespread representatives among chlororganic aromatic pollutant. Helophytes are used for the treatment of contaminated waters and are good absorbers of aromatics. The quantity of surface lipids of the surface lipids of the plants under the influence of MCB were analyzed by IR-spectra and gas chromatography-mass spectrometry (GC–MS) data in comparison with control plants grown on the distilled water. It was shown that the quantity of surface lipids increased by 8.5–17.8% for all species. According to the influence of monochlorobenzene on the composition of fatty acids, two groups of plants were found as follows: an increase in the content of C_{24:0}–C_{32:0} was observed in the first group, and a sharp increase of C_{16:0} fatty acid took place in the second one. A decrease in the content of hydrocarbons in some species was noticed and changes in their heterogeneity were common for the most of exposed plants. The conclusion was drawn that FTIR spectra of surface lipids of *Acorus calamus* may be recommended for further investigations and the use in environmental monitoring.

Keywords: monochlorobenzene; infrared spectroscopy; Fourier transform infrared spectroscopy; gas chromatography-mass spectrometry; helophytes.

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REFERENCES

1. Yeats T.H., Rose J.K.C. The formation and function of plant cuticles. *Plant Physiology*, 2013, vol. 163, pp. 5-20.
2. Busta L., Budke J.M., Jetter R. Identification of β -hydroxy fatty acid esters and primary, secondary-alkanediol esters in cuticular waxes of the moss *Funaria hygrometrica*. *Phytochemistry*, 2016, vol. 121, pp. 38-49.
3. Van Maarseveen C., Han H., Jetter R. Development of the cuticular wax during growth of *Kalanchoe daigremontiana* (Hamet et Perr. de la Bathie) leaves. *Plant, Cell & Environment*, 2009, vol. 32, pp. 73-81.
4. Macherius A., Haertig C., Kusch P., Shtemenko N., Moeder M. Analytical methods to characterize the composition of surface lipids of helophytes exposed to VOC contaminant water. In: Dishovsky C., Pivovarov A. (eds.): *Counteraction to Chemical and Biological Terrorism in East European Countries, NATO Science for Peace and Security Series A: Chemistry and Biology*, 2009, pp. 95-100.
5. Shtemenko N., Kusch P., Moeder M., Geyer W., Haertig C., Voevoda M., Shepelenko V., Alexeevskaya I. Influence of contaminant stress on the surface lipids composition of some helophytes. In: Dishovsky C., Pivovarov A. (eds.): *Counteraction to Chemical and Biological Terrorism in East European Countries, NATO Science for Peace and Security Series A: Chemistry and Biology*, 2009, pp.101-108.
6. United States Public Health Service: Agency for Toxic Substances and Disease Registry *Toxicological profile for chlorobenzene*. 1990. Available at: <http://atsdr.cdc.gov/toxprofiles/tp131.pdf>.
7. Nepovim A., Hebner A., Soudek P., Gerth A., Thomas H., Smreck S., Vanek T. Degradation of 2,4,6-trinitrotoluene by selected helophytes. *Chemosphere*, 2005, vol. 60, pp. 1454-1461.
8. Monferran M.V., Pesce S.F., Cazenave J., Wunderlin D.A. Detoxification and antioxidant responses in diverse organs of *Jenynsia multidentata* experimentally exposed to 1,2- and 1,4-dichlorobenzene. *Environmental Toxicology*, 2008, vol. 23, pp. 184-192.
9. Arunodhaya N., Jayakumar C., Gandhi N.N. Effect of hydrotropes on solubility and mass transfer coefficient of chlorobenzene. *Research Journal of Chemical Sciences*, 2012, vol. 2(8), pp. 9-13.
10. Buschhaus C., Jetter R. Composition differences between epicuticular and intracuticular wax substructures: how do plants seal their epidermal surfaces? *Journal of Experimental Botany*, 2011, vol. 62, pp. 841-853.
11. Dubis E.N., Dubis A.T., Morzycki J.W. Comparative analysis of plant cuticular waxes using HATR FT-IR reflection technique. *Journal of Molecular Structure*, 1999, vol. 511-512, pp. 173-179.
12. Hartig C. Rapid identification of fatty acid methyl esters using a multidimensional gas chromatography-mass spectrometry database. *Journal of Chromatography A*, 2008, vol. 1177, pp. 159-169.
13. Jetter R., Riederer M. Localization of the transpiration barrier in the epi- and intracuticular waxes of eight plant species: water transport resistances are associated with fatty acyl rather than alicyclic components. *Plant Physiology*, 2016, vol. 170, pp. 921-934.
14. Annweiler E., Richnow H.H., Antranikian G., Hebenbrock S., Garms C., Franke S., Francke W., Michaelis W. Naphthalene degradation and incorporation of naphthalene-derived carbon into biomass by the thermophile *Bacillus thermoleovorans*. *Applied and Environmental Microbiology*, 2000, vol. 66, pp. 518-523.
15. Braeckevelt M., Rokadia H., Mirschel G., Weber S., Imfeld G., Stelzer N., Kusch P., Kastner M., Richnow H.H. Biodegradation of chlorobenzene in a constructed wetland treating contaminated groundwater. *Water Science and Technology*, 2007, vol. 56, pp. 57-62.
16. Burken J.G. Uptake and metabolism of organic compounds: green-liver model. In: *Phytoremediation* (eds. J.L. Schnoor, A. Zehnder, S.C. McCutcheon, J.L. Schnoor), 2003, pp. 59-84. Available at: <https://doi.org/10.1002/047127304X.ch2>.
17. Shepherd T., Griffiths D.W. The effects of stress on plant cuticular waxes. *New Phytologist*, 2006, vol. 171, pp. 469-499.
18. Samuels L., Kunst L., Jetter R. Sealing plant surfaces: cuticular wax formation by epidermal cells. *Annual Review of Plant Biology*, 2008, vol. 59, pp. 683-707.
19. Nicolaidis N. Skin lipids: their biochemical uniqueness. *Science*, 1974, vol. 186, no. 4158, pp. 19-26.