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CHEMICAL COMPOSITION OF *Pinus silvestris* ESSENTIAL OIL FROM CONTAMINATED AREAS

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The chemical composition of Pinus silvestris *essential oil from contaminated areas was studied. An apparent effect of radionuclides and toxic elements on the biosynthesis of terpenoids in common pine essential oil was found. Increasing contamination apparently increased the content of sesquiterpenes and* O*-containing substances and decreased the content of monoterpenes in the essential oil. The contents of* α*-pinene, camphene, and limonene increased and those of 3-carene, terpinolene, and* β*-pinene decreased in the monoterpene fraction.*

Key words: *Pinus silvestris*, essential oil, radionuclides, industrial pollutants.

The accident at the Chernobyl NPP spread radioactive contamination over ~25% of the forest areas or 1.7 million hectares [1].

Common pine is a plant that has little resistance to the effects of radiation. The extent and nature of radiation damage to pine depend on the absorbed dose. Externally this manifests itself as necrosis of active individual organs and tissues and ends with the death of the aerial part of the plant [2]. High concentrations of industrial pollutants have the same effect on common pine [3].

Changes on the chemical level in the metabolic system precede any morphological changes [4]. These include a change in the content of individual components of pine essential oil [5, 6].

Thus, the goal of this work was to study the chemical composition of essential oil of *Pinus silvestris* L. growing under contaminated conditions in the Republic of Belarus′.

Assimilatory organs play the role of a regulatory step in the functioning of the plant and are exceedingly sensitive to a change in growing conditions. A deficiency or toxicity of elements can affect the chemical composition of assimilatory organs of woody plants [7].

The principal source from which radionuclides migrate into the needles is the soil. The amount of radionuclides on the needle surface is inconsequential and, as a rule, is not considered. Therefore, the observed activities and dose rates are due to incorporated radionuclides and their decay products. Furthermore, the main biochemical processes in needle cells stop in autumn and winter whereas radioactive decay does not. This causes radioactive decay products to accumulate, as a result of which the stress on the wood biomass increases [8]. Table 1 gives the characteristics of radioactive contamination of pine needles.

The higher content of ^{137}Cs than ^{90}Sr in all samples is due to the different rates of migration of these radionuclides in soil. The results agree well with the transfer coefficients of the isotopes from soil into wood [9].

Absorption of metals by plants is determined primarily by the content of available forms in the soil levels occupied by the roots. The fraction of so-called contaminating elements that enters the ecosystem through the air increases substantially with atmospheric contamination. These elements are actively incorporated into the biogeochemical cycle, which can be seen in the elemental composition of pine needles (Table 2).

Thus, the source of stress for samples 1-3 is toxic elements; for 4-10, incorporated radionuclides.

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TABLE 1. Characteristics of Radioactive Contamination of Pine Needles

Sample No.	Specific activity A (^{90}Sr), Bq/kg	Specific activity A (^{137}Cs), Bq/kg	Gamma-irradiation dose rate, mSv/h
1	19	10	0.12
2	22	14	0.11
3	17	12	0.11
4	410	620	0.24
5	390	600	0.30
6	405	556	0.38
7	160	305	0.22
8	146	388	0.18
9	13	38	0.17
10	21	28	0.12
11	24	20	0.10
12	18	10	0.09

TABLE 2. Content of Toxic Elements in Pine Needles

Sample No.	Content of elements in needles, mg/100 g abs. dry wt.								
	S	Cd	Co	Cu	Ni	Pb	Zn	Mn	Cr
1	107.5	0.0023	0.0155	0.710	0.178	0.1866	2.614	6.526	0.2352
2	169.0	0.0040	0.0201	0.620	0.172	0.1422	2.418	6.810	0.1430
3	101.6	0.0068	0.0331	0.373	0.335	0.0701	3.086	39.044	0.0320
4	95.1	0.0044	0.0102	0.181	0.080	0.0129	3.410	20.942	-
5	96.9	0.0044	0.0104	0.238	0.075	0.0154	3.121	21.537	0.0002
6	83.2	0.0031	0.0101	0.164	0.078	0.0118	3.610	26.481	-
7	94.7	0.0043	0.0127	0.218	0.235	0.0160	2.666	37.989	-
8	94.1	0.0064	0.0129	0.316	0.101	0.0710	4.980	24.920	0.0001
9	90.0	0.0036	0.0076	0.297	0.134	0.0188	3.359	22.169	0.0091
10	97.3	0.0037	0.0079	0.273	0.113	0.0220	3.268	31.918	0.0120
11	94.2	-	0.0043	0.271	0.288	0.0046	3.062	6.321	-
12	92.3	-	0.0034	0.276	0.291	0.0029	4.020	13.210	-

Next, the mineral compositions of assimilatory organs were compared as metabolism proceeds using the change in chemical composition of essential oil released from needles of selected samples of green pine wood.

Table 3 presents data on the essential oil components of pine growing in areas with different types of contamination.

The observed variation in the composition of terpenoids confirms that biosynthetic processes are affected by pollutants. The results indicate that the total contribution of monoterpenoids decreases substantially as contamination by industrial and radioactive elements increases.

Table 3 shows that the contents of α-pinene, 3-carene, camphene, myrcene, β-pinene, limonene, and terpinolene dominate in the monoterpene fraction in all samples and undergo a complicated change depending on the contamination level of the area. As the contamination increases, the contents of α-pinene, camphene, and limonene increase whereas those of 3-carene, β-pinene, and terpinolene decrease.

In our opinion, the change in the contents of monoterpenoids should be related to protective mechanisms of trees that are evident, for example, as an increase of α-pinene in contaminated samples as a secondary metabolite with protective properties for stressed pine [10]. The dynamics of the change in the amount of α-pinene and 3-carene should also be noted. It can be seen that the contents of these components change in opposite directions. A decrease in the amount of 3-carene is, in our opinion, related to its increased reactivity in oxidative processes, the number of which, as mentioned earlier, increases with increasing contamination.

On the other hand, the mass fraction of *O*-containing compounds in pine essential oil increases insignificantly with increasing contamination level. This may be due to the occurrence of oxidative processes that are catalyzed by pollutants.

TABLE 3. Content of Terpene Hydrocarbons in Essential Oil of Pine Growing under Various Ecological Conditions

Compound	Sample No.											
	1	2	3	4	5	6	7	8	9	10	11	12
	Component content, %											
Tricyclene	1.1	1.3	1.2	1.9	2.1	2.2	1.5	1.9	1.3	0.8	0.8	1.0
α-Pinene	68.6	65.1	57.9	55.4	67.0	61.6	44.3	46.9	57.7	36.9	32.3	28.8
Camphene	4.9	4.6	3.6	7.6	5.5	7.1	5.5	6.3	5.4	2.6	3.1	3.4
Phenchene	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
Sabinene	0.2	0.2	0.7	1.0	0.2	0.5	0.6	1.0	0.4	0.7	1.2	1.3
β-Pinene	3.0	4.1	1.9	4.6	3.1	4.1	3.0	5.0	2.9	10.5	6.0	8.1
Myrcene	2.3	5.6	3.1	3.4	7.2	2.5	3.3	2.7	3.0	3.2	2.8	2.7
α-Phellandrene	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.4	0.1	0.1
3-Carene	2.7	6.4	21.9	16.8	3.8	16.6	30.0	19.3	18.1	33.1	41.0	45.0
α-Terpinene	0.1	0.1	0.2	0.3	0.1	0.1	0.3	0.2	0.2	0.2	0.3	0.2
p-Cymene	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.3	0.6	0.1
Limonene	12.0	5.6	2.0	5.8	9.6	2.7	3.1	4.8	4.1	5.3	4.2	3.0
γ-Terpinene	3.3	4.3	3.5	0.5	0.7	0.2	0.6	1.2	0.5	1.1	1.3	1.2
Terpinolene	0.8	1.0	2.8	2.1	0.4	1.6	3.7	1.9	2.9	3.9	4.3	4.2
Total monoterpenoids	45.6	44.8	44.4	46.1	46.0	45.6	48.1	48.1	50.0	57.4	62.2	59.9
Total *O*-containing compounds	2.6	2.6	3.0	2.7	2.5	2.5	2.4	2.3	2.4	2.1	1.9	2.0
Total sesquiterpenoids	51.8	52.6	52.6	51.2	51.5	48.3	49.5	49.6	47.6	40.5	35.9	38.1

The content of sesquiterpenoid hydrocarbons varies oppositely to that of monoterpenoids.

Thus, the research showed that the chemical composition of *P. silvestris* essential oil is changed by contamination. This is consistent with the disruption of biochemical processes in these trees by contamination and, therefore, of the general state of forests under these conditions. The nature of the changes in pine essential oil composition by toxic and radioactive elements indicates that they affect on biosynthetic processes of terpene hydrocarbons. The results can be used to monitor the state of forests.

EXPERIMENTAL

Common pine (*P. silvestris* L.), which is the principal forest species in Belarus′ and most sensitive to contamination [11], was studied. Samples of green wood were collected from wild trees 40 years old in autumn and winter when the content of essential oil in needles was maximum [12].

Samples 1-3 were collected in Minsk proper in industrial zones at Minsk Tractor Plant, TETs-3, and Minsk Automobile Plant [13]. Samples 4-10 were taken from radioactively contaminated woods. Samples 11 and 12 were from ecologically clean areas (Braslavsk Lakes National Park, Belovezhsk Forest Preserve). Such a set of collection sites enabled the action of various factors on the chemical composition of pine essential oil and the general condition of Belarus′ pine forests to be evaluated objectively.

We used whole needles that were not separated by age. The collected needle samples were made into a single sample from 35-40 trees that was used in further experiments using a literature method [14].

The homogeneity of the sample was checked at first by determining the content of gamma-emitters in the needles by measuring the dose rate with an RKSB-104 dosimeter. The collected samples were considered to have a homogeneous content of gamma-emitters if the measurements differed by less than 50% [15].

The level of contamination by radionuclides was determined from the specific activities of ^{137}Cs and ^{90}Sr. The ^{137}Cs content in the needle samples was monitored by a standard method on an RUG-91M radiometer; the specific activity of ^{90}Sr, on an RUB-91 radiometer [16].

The contents of toxic elements Pb, Cd, Zn, Cu, Co, Ni, Mn, and Cr in pine needles were established by atomic absorption [17]; of S, by nephelometry using a standard method [18].

Essential oils were isolated by steam distillation. The quantitative yield was determined volumetrically. Qualitative and quantitative analyses of essential oil from pine needles used GC on a Tsvet-800 chromatograph. Components were separated using quartz capillary columns 70 m in length with PEG-2000 and SE-70 liquid phases. Individual components were identified using standards and published retention indices [19]. Components in essential oil were quantified using an internal standardization method over peak areas.

All results were processed using statistics. The relative uncertainty of the measurements was ±5%.

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