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# Study of the Composition of Ether Oils from Pine Needles of *Pinus sylvestris* L. Growing in Various Edaphic Conditions of Kuzbass Surface Coal Mines Dumps

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**Abstract:** Composition of ether oils of *Pinus sylvestris* L. growing on surface of coal mine dumps. In the composition of ether oils 59 components have been identified: 14 monoterpenes, 23 sesquiterpenes and 22 terpenoids. In quantitative terms, terpene compounds dominate in pine needles: monoterpenes: α-pinene, sesquiterpenes - δ-cadinene + trans-kalamene. Composition of terpenoids in pine needles varies with edaphic conditions - in the inter-dump sag and on the graded dump without a potentially fertile layer, degradation of the composition of identified terpenoids and reduction of their number is observed. In needles of pines growing on graded dumps without a potentially fertile layer, the amount of sesquiterpenes increases, which obviously contributes to pines stability in adverse environmental conditions. Results of the studies performed provide a basis for considering planting *Pinus sylvestris* L on Kuzbass coal mines dumps as a resource base for ether oils.

**Key words:** Coal Mines Dumps · *Pinus sylvestris* · Ether Oils · Terpenes · Terpenoids

## INTRODUCTION

Kuzbass is the center of the coal mining industry in Western Siberia. It has about 5 thousand industrial enterprises, including over 50 pits and surface coal mines. As a result of coal mining, the area of disturbed soils in the Kuzbass region exceeds 100 thousand hectares. The specifics of coal dumps is that the environmental conditions are formed on embryo-earth that differs significantly from zonal ones towards oligotrophicity and xeromorphism, therefore, the number of plant species able to successfully grow there is very limited. It has been experimentally established that one of the species suitable for growing on rock dumps is *Pinus sylvestris* L. [1]. Out of forest plantations on Kuzbass dumps, *Pinus sylvestris* L. occupy about 11 thousand hectares [2].

*Pinus sylvestris L*. is a valuable medicinal plant with diverse biological action. Of particular interest to researchers is studying composition of *Pinus sylvestris L.* volatile compounds, which are by quantitative and qualitative composition superior to many other compounds found in conifers' compounds. Terpenes and terpenoids are widely used in manufacturing pesticides, disinfectants and in manufacturing fragrances. Medical use of terpenoids is based on their antiseptic and disinfectant properties [3-6].

In relation to the above, coal mines dumps can be considered as an additional resource base for introduction of *Pinus sylvestris* L. in order to obtain ether oils.

The purpose of this work is to study the composition of ether oils in needles of *Pinus sylvestris* L. that grows in various edaphic conditions of the Kedrovsky coal mine dump.

## MATERIALS AND METHODS

The Southern dump of the Kedrovsky coal open mine has plain and sloping terrain 58 m high with area equal to

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599.3 hectares. The age of the dump is 25 to 30 years; graded in 2004. Dump rock includes sandstone (60%), siltstone (20%), mudstone (15%), loam and clay (5%). Prevailing fractions are large aggregates (3 to 10 mm and more); content of fine particles is reduced.

The study was made at three plots on the Southern dump; (PN): PN No. 1 -graded dump with Potentially Fertile Layer (PFL), PN No. 2 - valley without PFL between dumps, PN No. 3 - planned dump without PFL. By their agrochemical indicators, embryo-earths of all PNs are characterized by high content of exchange potassium (100... 240 mg/kg) and low content of labile phosphorus (10...50 mg/kg). At PN No.1 and PN No. 2, medium content of nitrate nitrogen is observed (9.5...13.8 mg/kg). Embryo-earths of PN No. 3 have the lowest content of exchange phosphorus and nitric nitrogen (10... 20 and 6.0 … 3.6 mg/kg, respectively). Hygienic evaluation of dumps embryo-earths showed no excess of MPC for radionuclides and heavy metals adopted in the Russian Federation, MPC on the basis of world's generalized materials and regional background of ecologically clean areas [7-9].

Objects of study were *Pinus sylvestris L.*, 20-25 years old, which have grown on the three above-mentioned areas of the dump. Plant samples were collected in July 2012; and ether oils were extracted from two-years-old needles.

Preparation of samples for analysis. Raw material was prepared (with drying to air-dry state) and ether oils were obtained (steam distillation) using generally accepted methods [1]. The analyzed mixture (1-10 µl) was dissolved in 500 µl of acetone and 100 µl of hexane solution of a mixture containing equal shares by weight of normal hydrocarbons C8, C9... C24 with total concentration of 0.1% was added to the resulting solution.

Chromato-mass-spectrometry. Chromato-mass spectrograms were made using Agilent 5973N and Agilent5973N EI/PCI units. Components of studied ether oils were separated on gas chromatographs of Agilent 6890 series that are part of the said gas chromato-mass spectrometry systems. Separation was made in a HP-5ms quartz capillary column, 30m long with 0.25 mm inner diameter, stationary phase: copolymer 5% diphenyl-95% dimethylsiloxane, film thickness of stationary phase: 0.25 microns. Evaporator temperature: 280°C, sample volume: 1 µl, flow separation 100:1. Column temperature range: 50°C (2 min) – 50 to 240°C (4°C /min) – 240 to 280°C (20°C /min) - 280°C (5 min.). Carrier gas: helium at constant flow of 1 ml/min. Temperature of the interface between the chromatograph and the mass-selective detector: 280°C. Mass spectra were recorded using a HP 5971 MSD quadrupole mass spectrometer with ionization by electron impact with the energy of ionizing electrons 70 eV. Data were collected at the speed of 1.9 scans/sec in the range between 30 and 650 a.m.u. (Agilent 5973N) or 3 scans/sec in the range between 29 and 500 a.m.u. (Agilent 5973N EI/PCI). Delay between putting sample into the evaporator of the chromatograph and start of recording chromato-mass spectrogram was 3.0 min.

$J_x$ linear retention indices were calculated by the formula

$$J_x = J_n + 100k\frac{t_{Rx} - T_{Rn}}{t_{R(n+k)} - t_{Rn}}$$

where $J_n$ = 100n is the retention index of n-alkane comprising n carbon atoms in the molecule; $t_R$ is the absolute component retention times; $t_x$ is the retention time of the substance tested; and $t_n$ and $t_{n+k}$ are retention times of the nearest reference n-alkanes with number of carbon atoms, respectively, n and n+k, with that usually $t_n < t_x < t_{n+k}$.

Components identification. Components of the mixtures studied were identified by full mass spectra, values of linear retention indices, as shown in the manual [10].

Quantitative analysis was made using the method of internal normalization for areas of gas-chromatographic peaks without using correction factors. 100% were taken as the sum of components peaks with linear retention indices in the range between 850 and 2000.

## RESULTS AND DISCUSSION

Analysis of the results of chromatography-mass spectrometric study of *Pinus sylvestris L.* needles showed that ether oils composition included 59 components; of them, representatives of terpenes and terpenoids were been identified and the advantage (37 components) belongs to compounds of the first class (Table 1). Domination of terpenes (monoterpenes and sesquiterpenes) in *Pinus sylvestris L.* observed in our experiment are consistent with works of other researchers [11-15].

Table 1: Results of gas chromato-mass spectrometric study of *Pinus sylvestris L.* needles

| Name of the component | The observation site | | |
|---|---|---|---|
| | PN No. 1 | PN No. 2 | PN No. 3 |
| | Component Content % | | |
| TERPENES | | | |
| 1.Monoterpenes: | | | |
| *Acyclic monoterpenes:* | | | |
| β-myrcene | 0.68 | 0.62 | 0.58 |
| *trans*-β-ocimene | - | 0.30 | 0.53 |
| *Total acyclic monoterpenes* | *0.68* | *0.92* | *1.11* |
| *Carbocyclic monoterpenes:* | | | |
| limonene+ β-phellandrene | 0.50 | 0.42 | 0.44 |
| Terpinolene | - | 1.00 | 0.81 |
| α-terpinene | + | 0.05 | 0.06 |
| γ-terpinene | - | 0.13 | 0.11 |
| 3-thujene | 0.10 | 0.05 | 0.11 |
| α-pinene | 15.11 | 10.28 | 11.18 |
| β-pinene | 1.19 | 1.26 | 1.72 |
| Camphene | 2.22 | 1.49 | 1.42 |
| Sabinene | 0.21 | 0.20 | 0.14 |
| 3-carene | 7.24 | 7.57 | 7.11 |
| Tricyclene | 0.53 | 0.34 | 0.31 |
| *para*-cymene | 0.31 | + | + |
| *Total carbocyclic monoterpenes* | *27.41* | *22.79* | *23.41* |
| Total monoterpenes | *28.09* | *23.71* | *24.52* |
| 2.Sesquiterpenes: | | | |
| Caryophyllene | 3.01 | 2.53 | 2.61 |
| germacrene D | 1.06 | 6.35 | 4.22 |
| germacrene A | 0.39 | 1.02 | 0.83 |
| Humulene | 0.61 | 0.47 | 0.46 |
| β-element | 0.48 | 0.46 | 0.35 |
| β-cubebene | 0.35 | 0.18 | 0.33 |
| 4-epi-kubebol+ bicyclegermacrene | 2.37 | 4.08 | 5.71 |
| α-copaene | 0.72 | 0.36 | 0.67 |
| β-copaene | 0.23 | 0.17 | 0.21 |
| α-murolene | 2.39 | 2.19 | 2.26 |
| γ-murolene | 1.79 | 1.08 | 1.59 |
| *trans*-cadina 1(6),4-dien | - | 0.33 | 0.46 |
| *trans*-cadina-1,4-dien | + | 0.35 | 0.52 |
| *trans*-murola-3,5-dien | - | 0.16 | 0.25 |
| *cis*-murola-3,5-dien | - | 0.08 | 0.13 |
| *cis*-murola-4(14),5-dien | 0.24 | 0.28 | 0.43 |
| Bicyclosesquiphellandrene | 0.29 | 0.38 | 0.61 |
| γ-cadinene | 8.82 | 4.69 | 8.27 |
| α-cadinene | 0.48 | 0.41 | 0.52 |
| δ-cadinene+trans-kalamenene | 5.31 | 11.07 | 15.23 |
| β-bourbonene | 0.25 | 0.18 | 0.19 |
| Aromandrene | 0.59 | 0.18 | 0.53 |
| β-selinene | 1.91 | 0.87 | 1.71 |
| Total sesquiterpenes | *31.29* | *37.87* | *48.09* |
| *TOTAL TERPENES* | *59.38* | *61.58* | *72.61* |

Table 1: Continued

| Name of the component | The observation site | | |
|---|---|---|---|
| | PN No. 1 | PN No. 2 | PN No. 3 |
| | Component Content % | | |
| TERPENES | | | |
| TERPENOIDS | | | |
| 1.Spirits: | | | |
| Borneol | 0.07 | + | - |
| α-terpineole | 0.09 | + | - |
| 4-terpineole | 0.19 | 0.06 | + |
| *trans* verbenol | 0.23 | - | - |
| 1,10-di-epi-cubenol | 0.52 | 0.22 | 0.30 |
| 1-epi-kubenol | 0.85 | 0.53 | 0.69 |
| T-kadinol+T-murolol | 6.42 | 5.91 | 5.78 |
| α-kadinol | 5.96 | 7.24 | 5.47 |
| δ-kadinol | 0.82 | 0.93 | 0.76 |
| germacrene D-4-ol+spathulenol | 4.54 | 6.28 | 5.07 |
| Total spirits | *19.69* | *21.17* | *18.07* |
| Ethers: | | | |
| Bornylacetate | 2.67 | 2.10 | 0.54 |
| caryophyllene oxide | 1.52 | - | - |
| α-cubebene+ α- terpineol acetate | 0.62 | 0.38 | 0.31 |
| Manoiloxide | 0.06 | 0.10 | 0.06 |
| Total ethers: | *4.87* | *2.58* | *0.91* |
| 2.Aldehydes and ketones: | | | |
| Myrthenal | 0.17 | - | - |
| car-2-en-4-on | 0.12 | - | - |
| car-3-en-2-on | 0.11 | - | - |
| car-3-en-5-on | 0.55 | - | - |
| Verbenone | 0.15 | - | - |
| *trans*-2,3-epoxy-pinene (α-pinene epoxide) | 0.13 | - | - |
| caprolactam-2 | 0.21 | 0.09 | 0.05 |
| Oplopanone | 0.93 | 0.09 | + |
| Total aldehydes and ketones | *2.37* | *0.18* | *0.05* |
| *TOTAL TERPENOIDS* | *26.93* | *23.93* | *19.03* |

*Note: The "+" symbol indicates that the corresponding component is present, but its content does not exceed 0.05%; the "-" symbol indicated that the component is absent.*

Of monoterpenes, 14 compounds were identified; of them the following prevailed: α-pinene (10.28 to 15.11%), 3-carene (7.11 to 7.57%), camphene (1.42-2.22%), β-pinene (1.19 to 1.72%), β-myrcene (0.58 to 0.68%). Our studies are confirmed by works of Kainulainen and Holopainen [16].

Of sesquiterpene compounds, 23 compounds were identified. In quantitative terms, prevailing sesquiterpenes in samples of needles are: δ-cadinene+trans kalamenene (5.31 to 15.23%), γ-cadinene (4.69 to 8.82%), caryophyllene (2.61 to 3.01%) germacrene D (1.06 to 6.35%), 4-epi-kubebol+bicyclogermacrene (2.37 to 5.71%),

α-and γ-murolen (1.08 to 2.39%) and β-celinene (0.87 to 1.91%); remaining components of this class are found in small quantities.

Out of compounds of terpenoid class in test samples, 22 components were detected, among them spirit-containing compounds were found as well as ethers, aldehydes and ketones. From the terpenoids group, the highest content had T-kadinol+T-murolol (5.78 to 6.42%), α-kadinol (5.47 to 7.24%), germacrene D-4-ol+spatulenol (4.54 to 6.28%) and bornylacetate (0.54 to 2.67%).

Differences were found in qualitative and quantitative composition of pine ether oils, depending on the edaphic conditions. It was found that the qualitative composition of terpenes is less dependent on environmental conditions, especially that of dominant carbocyclic monoterpenes (α-pinene, 3-carene, camphene, β-pinene). It is obvious that the nature of biosynthesis of carbocyclic monoterpenes in *Pinus sylvestris* L. is a genetically determined characteristic. Qualitative composition of terpenoids in pine needles varies significantly depending on edaphic conditions. In particular, PN No. 2 and PN No. 3 showed total of 12 terpenoid components, among which aldehydes and ketones have the lowest content, compared to PN No. 1; this leads to reduction of terpenoids content. It has been experimentally established that despite more adverse edaphic conditions at PN No. 3, in needles of *Pinus sylvestris L.*, content of sesquiterpenes significantly increases (48.09%), compared to other observation sites (PN No. 1 - 31.29%, PN No. 2 - 37.87%). It is obvious that increase of synthesis of sesquiterpenes in pine needles in adverse environmental conditions contributes to its stability and is a manifestation of ecological plasticity.

## CONCLUSION

It has been experimentally established that needles of *Pinus sylvestris L.* that grows in various edaphic conditions of the Kedrovsky coal mine dump have high content of ether oils - 59 components were identified: 14 monoterpenes, 23 sesquiterpenes and 22 terpenoids (10 spirits, 4 ethers, 8 aldehydes and ketones). In quantitative terms, terpene compounds dominate in pine needles: monoterpenes: α-pinene, sesquiterpenes - δ`-cadinene+trans-kalamene.

Peculiarities of qualitative and quantitative composition of ether oils have been found, depending on the edaphic conditions of the dump. It was found that the composition of terpenes is less dependent on environmental conditions, especially that of prevailing carbocyclic monoterpenes (α-pinene, 3-carene, camphene, β-pinene).

Composition of terpenoids in pine needles varies significantly depending on the edaphic conditions. In needles of pines that grow at the valley between dumps and on graded dumps without a Potentially Fertile Layer, content of terpenoids is lower, mainly due to lack of aldehydes and ketones. This reduces the total amount of identified terpenoids. In needles of *Pinus sylvestris L.* that grows on graded dumps without a Potentially Fertile Layer, content of sesquiterpenes increases, which obviously contributes to pines' stability in adverse environmental conditions and is a manifestation of environmental plasticity.

Results of the made studies provide a basis for considering planting *Pinus sylvestris L.* at Kuzbass coal mines dumps as a resource base for ether oils in order to use them in pharmaceutical, chemical and other industries.

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