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# Gas Chromatographic Determination of Extractable Compounds Composition and Emission Rate of Volatile Terpenes from Larch Needle Litter

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**Abstract.** A combination of solid-phase microextraction (SPME) and gas chromatography can be successfully used both for establishing the qualitative composition of volatile organic compounds (VOC) emitted by leaf litter and for determining their emission rates. Taking as an example European larch litter, it is shown that "dead" plant material contains considerable amounts of volatile components as well as non-volatile compounds that can be VOC precursors formed as a result of enzymatic reactions. It is proposed to include the determination of extractable compounds into the methodology of studying litter as a source of atmospheric VOC. Some data on litter mass are reported and it is concluded that this data may be included into special models for emission evaluation. In this work the distribution coefficients of monoterpene hydrocarbons between the gas phase and polydimethylsiloxane fiber coating necessary for quantitative determinations in SPME were estimated.

**Key words:** needle litter, terpenes, emission, extractable compounds, HS-SPME, distribution coefficients, calculation

## 1. Introduction

The high content of photooxidants, e.g. ozone and peroxides, in the boundary layer of the atmosphere is due to a great scale of anthropogenic emission of nitrogen oxides. In the presence of volatile organic compounds (VOC), nitrogen oxides are involved in gas-phase reactions leading to the formation of these secondary pollutants (Atkinson, 2000; Fuentes *et al*., 2000). The continental vegetation is a predominant source of reactive VOC (Zimmerman *et al*., 1978; Isidorov, 1990; Guenther *et al*., 1995, 2000), just as microorganisms (Wilkins, 1996; Kuzma *et al*., 1997; Wagner *et al*., 2000; Fall and Copley, 2000). The recognition of this fact has led to intensive study of biogenic VOC emission.

Much less attention has been devoted to VOC emission into the atmosphere by leaf litter, the mass of which is $(5–80)\times10^{15}$ g (Zavarzin, 1984). The potential importance of this source has been pointed out rather long ago. According to Zimmerman *et al.* (1978), VOC emission rate from litter was on the average

162 $\mu$g/(m$^2$ $\times$ h) at 30 °C. The role of leaf litter as a VOC source has recently been again confirmed in two works (Lindinger *et al*., 1998; Warneke *et al*., 1999). According to the latter, as a result of nonenzymatic termochemical reactions 6–8 Tg of acetone and 18–40 Tg of methanol can be emitted into the atmosphere from litter. It has been reported later that more than a 100 of organic compounds of various classes were detected in litter emissions of seven tree species. They include aliphatic hydrocarbons, mono- and sesquiterpenes, carbonyl compounds, and alcohols, as well as sulfur- and chlorine-containing compounds (Isidorov and Jdanova, 2002; Isidorov *et al*., 2003). All these facts confirm the suggestion that "much more research should be devoted to identifying and quantifying these sources of VOCs" (Warneke *et al*., 1999).

The success achieved in the last decades in studying VOC emission by the live vegetation was caused by the development of effective methodology of these studies. Some investigations were carried out by using grab sampling air with a stainless steel canister and subsequent concentration of VOCs in a cold trap (Greenberg and Zimmermann, 1984; Greenberg *et al*., 1992; Camel and Caude, 1995). Other studies are based on the concentration of VOCs over a sorbent bed with subsequent thermal desorption followed by separation with gas chromatography and detection with a flame ionization detector (GC-FID) or a mass spectral detector (GC-MS) (Holzer *et al*., 1977; Ioffe *et al*., 1977; Holdren *et al*., 1979; Yokouchi *et al*., 1983; Ciccioli *et al*., 1984; Isidorov *et al*., 1985; Helming and Arey, 1992; Hoffmann, 1995; Steinbrecher *et al*., 2000).

A relatively new technique of sampling and preparation for analysis is solid phase microextraction (SPME) (Pawliszyn, 1997). This method provides a significant advantage over "traditional" methods named above. This is due to its rapidity, precision, reproducibility, and relatively low cost of the equipment used. Therefore, SPME is being extensively applied in analytical practice (Martos *et al.*, 1997; Peñalver *et al*., 1999; Alpendurada, 2000). One of its variants is the headspace solid phase microextraction (HS-SPME): determination of volatile components in the gas phase being in contact with the investigated (liquid or solid) material. Volatile components from the gas phase are directly adsorbed onto a fused-silica fiber coated with adsorbent, and then thermally desorbed into a GC injection port. In particular, the effectiveness of HS-SPME was demonstrated in flavour analysis (Bicchi *et al*., 2000; Pérès *et al*., 2001; Jirovetz *et al*, 2002; Kim and Lee, 2002; Hamm *et al*., 2003), as well as in the analysis of VOCs from coniferous needle litter (Isidorov *et al*., 2003). However, in most of these works, the investigation of volatile compounds was made on the qualitative or semi-quantitative level, when the results of identification and relative content of components adsorbed on a SPME fiber are reported.

In this communication we propose a possible variant for investigating the leaf litter as a source of atmospheric VOC's. This methodology includes qualitative and quantitative HS-SPME analysis of VOCs and the evaluation of their emission rate, as well as exhaustive liquid extraction of low-molecular weight organic compounds.

The aim of the latter is to evaluate the store of volatile compounds (and their potential precursors) in the litter. Needle litter of larch, which contains mainly terpene compounds, is taken as an example.

## 2. Experimental Section

### 2.1. MATERIALS

Commercial $\alpha$-pinene, 3-carene, limonene, and myrcene were purchased from Roth (Warsaw, Poland). Hexane (Baker, HPLC grade), diethyl ether and pyridine (Gliwice, Poland) were used without additional purification. Bis(trimethylsilyl) trifluoroacetamide, BSTFA with addition of 1% trimethylchlorosilane was purchased from Sigma-Aldrich.

Intact European larch (*Larix decidue*) needles were collected from two trees in July 2003 in the park zone of Białystok, Poland. Brown litter was collected from the same trees at the end of November and at the beginning of December 2003 at a temperature of $+5\,^{\circ}$C. Humidity and extractable compounds were determined immediately after the plant material was delivered into the laboratory. To determine humidity, three needle feeds of about 4 g each were dried to a constant weight at $105\,^{\circ}$C. Weight loss of living needles and litter were $63.3 \pm 1.0\%$ and $42.5 \pm 0.5\%$, respectively.

### 2.2. EXTRACTION PROCEDURE AND SAMPLE PREPARATION FOR ANALYSES

For the determination of extractable non-polar compounds, needle feeds (3–4 g) were placed in vessels, 50 mL of *n*-hexane was poured on them and the mixtures were stirred (2 h) at temperatures of 50–55 °C. The extraction with fresh solvent portion was repeated. The combined extracts concentrated on a rotor evaporator to 2.5 mL were subjected to GC and GC-MS analysis. After this the solvent was removed under slight vacuum on a rotor evaporator and the mass of non-polar organic compounds extracted from live needles and litter was determined.

After extraction with hexane, the needles were dried in air and crushed. 30 mL of diethyl ether was added to the powder and the resulting mixture was stored for 1.5 h with periodic stirring. The extraction with fresh ether portion was repeated three times. The combined ether extracts were filtered through a paper filter and evaporated on a rotor evaporator to a volume of about 5 mL. This concentrate was transported into a previously weighed pea-shaped flask 10 mL in volume, evaporated to dryness, and the extracted material mass was determined. Pyridine (50 $\mu$L) and BSTFA (100 $\mu$L) were added to the dry residue. The resulting solution was sealed and heated for 1 h at 60 °C to form trimethylsilyl derivatives (TMS).

For the determination of water-soluble compounds, the residue of powder was extracted at 85–90 °C twice by portion of 25 mL of distilled water. Total extraction time was 2 h. Water was removed to dryness from filtered extracts under vacuum

and the mass of organic material was determined. A part of this material (15–20 mg) was transported into a 2 mL flask, pyridine and BSTFA were added, and TMS were prepared.

### 2.3. HS-SPME SAMPLING OF VOLATILE TERPENES

A part of the collected litter was used to determine the composition of terpene hydrocarbons emitted from them into the gas phase and to estimate their emission rate. Before the experiments, this litter was maintained at room temperature for 1 h. The litter used for repeated experiments was stored in a sealed glass vessel at 4 °C. Before the beginning of the experiments, it was also kept at room temperature for 1 h.

A schematic drawing of the "closed loop" system for the sampling of terpenes emitted by litter is shown in Figure 1. The air in the system is circulated (200 mL/min) through a glass vessel sealed hermetically with a lid. Inlet and

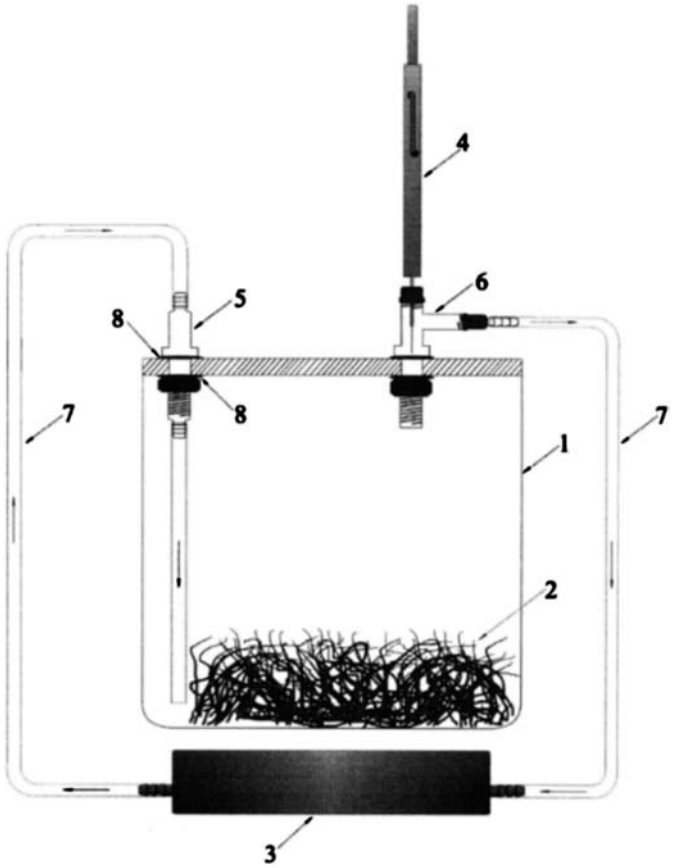


*Figure 1.* "Closed loop" system for HS-SPME sampling of VOC. 1 – glass vessel, 2 – sample of the litter, 3 – pump, 4 – SPME holder, 5 – gas inlet port, 6 – outlet port, 7 – connecting tubes, 8 – silicon rubber washers.

outlet ports as well as connecting tubes are made of inert materials (Teflon$^{TM}$). The glass vessel was prewashed with a 5% solution of BSTFA in toluene, followed by methanol washing and was dried at 80 °C. The aim of this operation was to decrease losses of volatile compounds due to their adsorption on vessel walls. The total volume of the gas phase in this system is 490 mL.

Naturally moist needles (4–5 g) were placed into the termostated glass vessel and air was pumped through the system. The rubber septum of the inlet port was picked by a needle of an SPME Holder 57330-U (Supelco Inc., Bellefonte, PA, USA) with fused silica fiber coated with polydimethylsiloxane (PDMS, 100 $\mu$m) stationary phase. The exposure time of the fiber in gas flow was 1 h. The adsorbed components were desorbed by introducing the SPME fiber into the gas chromatograph injection port.

### 2.4. GC SEPARATION AND DETERMINATION OF EXTRACTABLE COMPONENTS

Hexane extracts, TMS derivatives as well as volatile compounds adsorbed on the fiber were separated on a Hewlett-Packard HP-4890D gas chromatograph with flame ionization detector (FID) and an HP-5 fused silica column (30 m $\times$ 0.25 mm, film thickness 0.25 $\mu$m). The initial column temperature was 50 °C and a temperature rise to 280 °C was accomplished at a rate of 5 °C/min. The injector temperature was 260 °C. Helium flow rate through the column was 1 mL/min with a 1:50 split (hexane extracts and TMS derivatives separation) or a 1:10 split (FID calibration and HS-SPME experiments).

A mixture of $C_6$-$C_{27}$ *n*-alkanes was previously separated under the above conditions, and their retention times were determined. Retention indices (RI) were calculated from the results of the chromatography of this mixture and of extracts. Components were identified by comparison of measured and literature (Adams, 1995) values of the RI. The identification correctness was checked by the results of GC-MS analysis on a Perkin-Elmer Turbo Mass instrument. The components separated in the above conditions were identified with the aid of Wiley 7 and NIST mass spectra library.

The flame ionization detector was calibrated according to the results of chromatographic analysis of the series of terpene solutions ($\alpha$-pinene, 3-carene, limonene, and myrcene) in *n*-hexane at the concentration of each compound ca. 3, 30, 60, 150 and 300 ng/$\mu$L. Direct solution injections (1 $\mu$L) were performed with a microliter syringe (Hamilton), attaining reproducibility of less than 5%.

## 3. Results and Discussion

### 3.1. EXTRACTABLE SUBSTANCES IN LITTER

Leaf litter contains insoluble biopolymers with a phenol structure (lignins) and cellulose as well as some amount of extractable organic material. Some of these substances evaporate from litter relatively easily and pass into the atmosphere.

Semi-volatile and non-volatile compounds may serve as precursors of partly oxidized volatile organic compounds (OVOC). These OVOC are formed during microbiological decomposition and enzymatic oxidation of this organic fraction. Free carbohydrates and unsaturated carboxylic acids should be decomposed at the highest rate. It may be assumed that glucosides of phenolcarboxylic acids and flavonoids should also undergo biodegradation relatively readily.

From our viewpoint, the study of leaves litter as a source of atmospheric VOC should include the determination of not only VOC in the gas phase but also that of the nonvolatile organic material as a potential OVOC precursor. These investigations are necessary for understanding the dynamics of composition and the rate of VOC emission into the atmosphere.

Our approach includes the determination of fractional composition of the extractable substances. This is achieved by applying solvents of different polarities. Extraction by hexane makes it possible to determine the content of "neutral" compounds (terpenes and terpenoids as well as lipids). Diethyl ether extracts the more polar acidic part formed mainly by aliphatic, aromatic, and resin acids. In the third extraction stage, nonvolatile water-soluble compounds are isolated.

Table I lists the fractional composition of extracts from larch live needles and litter. The last line gives mass content of each fraction per dry weight of the investigated material. It can be seen that their fractions in the living foliage and in the litter differ considerably. Especially great differences were recorded in the case of hexane and water extracts.

The greatest part of "neutral" compounds extracted by hexane is formed by volatile monoterpenes and very slightly volatile lipids. The latter are mainly represented by higher odd alkanes $C_{27}$, $C_{29}$, and $C_{31}$ and by other compounds contained in cuticular waxes: a mixture of highest even alcohols and carbonyl compounds. It is noteworthy that in the period of active vegetation the content of terpene compounds in living needles was approximately twice less then in the fallen needles. It is known from the literature that during vegetation considerable variations in the composition of secondary metabolites are observed (von Rudloff, 1972). Moreover, the most intensive accumulation of terpenes takes place in autumn.

The most marked difference in the composition of ether extracts was the fact that living needles contained rather large amounts of hydroxyacids participating in Krebs and Kelvin cycles: lactic, succinic, and citric acids. These acids were almost completely absent in litter extracts. On the other hand, ether extracts of needle litter contained large amounts of unsaturated fatty acids and phytol. The precursors of the former are, evidently, triglycerol lipids and that of the latter is chlorophyll a. If phytol is formed as a result of hydrolytic decomposition of chlorophyll, then simultaneously the hydrolysis of the second ester group should take place, and VOC should contain a large amount of methanol. As already mentioned, the emission of a large amount of this alcohol from pine litter was reported by Warneke *et al*. (1999).

As was to be expected, the water-soluble fraction contained monosaccharides as well as considerable amounts of polyols. It is also natural that the contents

*Table I.* Fractional composition (%) of the extractive substances of larch live needles and litter

| | Hexane extracts | | Ether extracts | | Water extracts | |
|---|---|---|---|---|---|---|
| Group of compounds | Litter | Live needles | Litter | Live needles | Litter | Live needles |
| **Monoterpene $C_{10}H_{16}$ hydrocarbons** including: | **32.3** | **21.8** | Trace | Trace | – | – |
| $\alpha$-pinene | 15.3 | 5.7 | Trace | – | – | – |
| $\beta$-pinene | 6.8 | 2.5 | Trace | Trace | – | – |
| 3-carene | 6.0 | 3.2 | Trace | Trace | – | – |
| Limonene + $\beta$-phellandrene | 5.8 | 3.1 | Trace | Trace | – | – |
| **Monoterpenoids** including: | **2.5** | **3.1** | – | – | – | – |
| Bornyl acetate | 2.0 | 2.4 | – | – | – | – |
| **Sesquiterpene $C_{15}H_{24}$ hydrocarbons** including: | **13.0** | **10.4** | – | – | – | – |
| $\beta$-caryophyllene | 1.2 | 0.2 | – | – | – | – |
| $\gamma$-muurolene | 7.6 | 7.6 | – | – | – | – |
| **Sesquiterpenoids** including: | **13.0** | **4.9** | – | – | – | – |
| Caryophyllene oxide | 10.6 | 4.3 | – | – | - | – |
| **Diterpenes** | **2.6** | **4.8** | – | – | - | – |
| **Lipides** | **36.5** | **55.0** | – | – | - | – |
| **Aliphatic acids** including: | – | | **28.8** | **6.5** | **1.0** | **0.8** |
| Tetradecanoic | – | – | 3.0 | Trace | – | – |
| Hexadecanoic | – | – | 10.8 | 0.5 | – | 0.6 |
| Octadecanoic | – | – | 0.9 | 1.0 | – | Trace |
| Linoleic | – | – | 2.0 | – | – | |
| Oleic | – | – | 10.8 | 4.9 | – | 0.2 |
| Hydroxyacids Including: | – | – | **0.6** | **5.0** | **0.5** | **2.9** |
| Lactic | – | – | 0.2 | 1.5 | 0.4 | 0.9 |
| Succinic | | | 0.3 | 1.2 | Trace | 0.4 |
| Citric | | | Trace | 0.8 | – | Trace |
| **Resin acids** Including: | – | – | **22.1** | **2.0** | – | **0.3** |
| Isopimaric | – | – | 4.6 | Trace | – | – |
| Pimaric | – | – | 2.0 | 0.6 | – | – |
| Dehydroabietic | – | – | 3.4 | – | – | 0.3 |
| Abietic | – | – | 1.6 | 1.4 | – | – |
| Levopimaric? | – | – | 7.7 | – | – | – |
| **Alcohols** Including: | Trace | – | **24.0** | – | – | – |
| Nonacosanol | – | – | 6.0 | – | – | – |
| Phytol (two isomers) | Traces | – | 15.9 | – | – | – |
| **Polyols** Including: | – | – | **3.6** | **9.2** | **41.1** | **25.5** |
| Glycerol | – | – | 2.5 | 0.6 | 5.5 | 0.5 |

(*Continued on next page*)

*Table I.* (*Continued*)

| | Hexane extracts | | Ether extracts | | Water extracts | |
|---|---|---|---|---|---|---|
| Group of compounds | Litter | Live needles | Litter | Live needles | Litter | Live needles |
| Xylitol | – | – | – | – | 3.5 | – |
| Glucitol | – | – | – | – | 1.1 | – |
| Inositol (three isomers) | | – | Traces | 7.3 | 13.6 | 23.7 |
| **Carbohydrates** including: | – | – | Trace | **4.8** | **52.8** | **62.6** |
| Fructose | – | – | Trace | 3.3 | 19.2 | 17.3 |
| Galactose | – | – | Trace | 0.05 | 1.5 | 21.4 |
| Glucose | – | – | Trace | 1.5 | 31.5 | 24.1 |
| Average amounts of extractable matter, mg/g, d.w. | $30 \pm 4$ | $16 \pm 1$ | $37 \pm 3$ | $27 \pm 1$ | $157 \pm$`12 | $224 \pm 5$ |

of carbohydrates in living leaves is higher (approximately by 43%) because they are primary products of the photosynthesis. The principal monosaccharides were glucose and fructose, whereas glycerol and inositol (the latter presented by three isomers) prevailed among polyols.

More than 20% of the ether extracts and about 5% of the water extracts remained unidentified. They are mainly formed by glucosides and disaccharides mixtures that are difficult to separate.

The material presented in this section and also obtained by us for pine and spruce (Isidorov *et al.*, 2003) indicates that coniferous tree litter contains large amounts of volatile terpenes as well as easily biodegradable components, mostly carbohydrates and polyols. The presence of large amounts of fatty acids, glycerol and phytol in litter indicates that even in the early stages of litter formation some biomolecules, such as chlorophyll and cellular lipids undergo active decomposition. About 70% of the extractive substances mass in litter is formed by carbohydrates which most easily undergo biodegradation. These compounds serve as an excellent substrate for microorganisms, and their biodegradation by fungi should be accompanied by OVOC emission (Babich and Stotzky, 1974).

### 3.2. HS-SPME DETERMINATION OF TERPENES EMITTED INTO THE GAS PHASE BY LARCH LITTER

We have previously shown that the combination of sample collection by HS-SPME with gas chromatography can be an effective method for determining the qualitative composition of VOC in litter (Isidorov *et al.*, 2003). In principle, this method makes it possible to carry out not only qualitative but also quantitative characterization of

VOC emission. The ratio of the amount of substance $i$ adsorbed by the stationary phase of the fiber in a device for SPME to its content in the gas phase at a given temperature is expressed by the Equation (1):

$$K_{fg}^{i} = C_{f}^{i}/C_{g}^{i} = \left(m_{f}^{i}/V_{f}\right)\left(V_{g}/m_{g}^{i}\right), \tag{1}$$

where $K_{fg}^{i}$ is the distribution coefficient for compound $i$, $C_{f}^{i}$ and $C_{g}^{i}$ are its concentrations on the SPME fiber and in the gas phase, respectively, $m_{f}^{i}$ and $m_{g}^{i}$ are compound $i$ masses in the volume of the fiber ($V_f$) and in that of the gas phase ($V_g$), respectively.

Unfortunately, distribution coefficients and their temperature dependence are determined experimentally for few compounds. In particular, one can find in the literature the $K_{fg}$ values only for three monoterpene hydrocarbons: myrcene, $\alpha$-pinene and limonene (Nilsson *et al.*, 1996; Martos and Pawliszyn, 1997; Bicchi *et al.*, 2000).

In this communication, we attempted to decrease this lack of data by evaluating distribution coefficients on the basis of the known QS-CRR (Quantitative Structure-Chromatographic Retention Relationships) approach used for calculating chromatographic retention indices (Kaliszan, 1987). This approach has already been successfully used to evaluate distribution coefficients of aromatic hydrocarbons and esters in the *n*-hexane-acetonitrile system (Isidorov *et al.*, 2001).

For calculating the $K_{fg}$ values we used the two-parameter correlation equation

$$\log\ K_{fg} = a\ \log\ X + bY + c \tag{2}$$

in which descriptors $X$ and $Y$ are weakly inter-correlated physicochemical characteristics: boiling temperature (K) and GC retention indices (*RI*) on the HP-5 non-polar column. Initial data for calculation were the $K_{fg}$ values between the gas phase and the fiber with PDMS coating (100 $\mu$m) of $\alpha$-pinene, limonene, and five aromatic hydrocarbons experimentally determined by Martos and Pawliszyn (1997). The results of calculation are given in Table II.

The first part of Table II contains the initial data for calculating the coefficients $a$, $b$, and $c$ from Equation (2). The last column gives the corresponding errors. It can be seen that there is good agreement between the calculated and experimental values of $K_{fg}$: in the case of $\alpha$-pinene and limonene the calculation error is lower than 5%. The $F$-test value is larger than the value tabulated for significance level $\alpha = 0.01$, which means that Equation (2) is significant at the 99% significance level. The second part of Table II lists the results of $K_{fg}$ evaluation for some monoterpene hydrocarbons. The correctness of the results is confirmed by good coincidence between the experimental (Nilsson *et al.*, 1996) and calculated values of $K_{fg}$ for myrcene.

Martos and Pawliszyn (1997) have also established that the temperature dependence of distribution coefficients is described by a linear Equation (3),

$$\log\ K_{fg} = a'/T + b', \tag{3}$$

*Table II.* Estimation of $K_{fg}$ values for monoterpene hydrocarbons according to Equation (2)

| Hydrocarbon | $X = T$ (K) | $Y = RI$ | $K_{fg}^{exp}$ (296 K) | $K_{fg}^{calc}$ | Error, % |
|---|---|---|---|---|---|
| Benzene | 353.15 | 660 | 345 | 344 | 0.3 |
| Toluene | 383.78 | 760 | 967 | 976 | 0.9 |
| Ethyl benzene | 409.30 | 857 | 2468 | 2507 | 1.7 |
| *p*-Xylene | 411.50 | 866 | 2731 | 2729 | 0.07 |
| *o*-Xylene | 417.56 | 888 | 3388 | 3381 | 0.2 |
| $\alpha$-Pinene | 429.15 | 936 | 5500 | 5290 | 4.7 |
| Limonene | 451.15 | 1031 | 12362 | 12609 | 2.0 |
| | | Results of estimation | | | |
| Tricyclene | 426.15 | 926 | – | 4781 | – |
| Camphene | 431.65 | 953 | – | 6084 | – |
| $\beta$-Pinene | 438.15 | 980 | – | 7812 | – |
| Myrcene | 440.15 | 991 | 8511* | 8594 | 1.0 |
| 3-Carene | 445.15 | 1011 | – | 10352 | – |
| $\alpha$- Terpinene | 447.15 | 1018 | – | 11074 | – |
| $\beta$- Phellandrene | 448.65 | 1030 | – | 12221 | – |
| $\beta$-Ocimene | 451.15 | 1040 | – | 13389 | – |
| $\gamma$- Terpinene | 456.15 | 1062 | – | 16334 | – |
| Terpinolene | 459.15 | 1088 | – | 20102 | – |

*Note.* $a = 4.2257$; $b = 0.00300$; $c = -10.214$; $n = 7$; $R^2 = 0.998$; $F_{2,5} = 998$; $F_{2,5,\,\alpha=0.01} = 13.3$.
*Nilsson *et al.* (1996).

and have determined experimentally the coefficients $a'$ and $b'$ in the 16–38 °C temperature range. We also used this data to evaluate these coefficients for a wider group of monoterpenes. The results of this evaluation are listed in Table III.

### 3.3. EVALUATION OF MONOTERPENE EMISSION RATE FROM LARCH LITTER

Experiments for evaluating monoterpene emission rate were carried out with a unit shown schematically in Figure 1. A glass vessel with larch litter was placed in a water bath with a thermo regulator (not shown in Figure 1). Figure 2 shows a typical chromatogram of terpene hydrocarbons in needle emission.

The quantity of monoterpenes adsorbed on the fiber ($m^i_f$) was determined from the results of chromatographic analysis of VOC and GC detector calibration. The concentration of individual components in the gas phase of the vessel was calculated from Equation (4):

$$C^i_g = C^i_f / K^i_{fg} = m^i{}_f \big/ \left(V_f \times K^i_{fg}\right) \tag{4}$$

*Table III.* Estimated $a'$ and $b'$ values for monoterpene hydrocarbons from slope and $y$-intercept of log $K_{fg}$ as a function of inverse temperature

| Terpene hydrocarbon | $a'$ | $b'$ | $R^2$ |
|---|---|---|---|
| Tricyclene | 2337 | −4.216 | 0.998 |
| $\alpha$-Pinene* | 2302 | −4.071 | – |
| Camphene | 2388 | −4.276 | 0.996 |
| $\beta$-Pinene | 2428 | −4.300 | 0.993 |
| Myrcene | 2447 | −4.322 | 0.989 |
| 3-Carene | 2474 | −4.334 | 0.986 |
| $\alpha$-Terpinene | 2483 | −4.334 | 0.985 |
| Limonene* | 2454 | −4.199 | – |
| $\beta$-Phellandrene | 2507 | −4.368 | 0.981 |
| $\beta$-Ocimene | 2522 | −4.378 | 0.979 |
| $\gamma$-Terpinene | 2555 | −4.403 | 0.974 |
| Terpinolene | 2608 | −4.488 | 0.958 |

*Martos and Pawliczyn (1997).

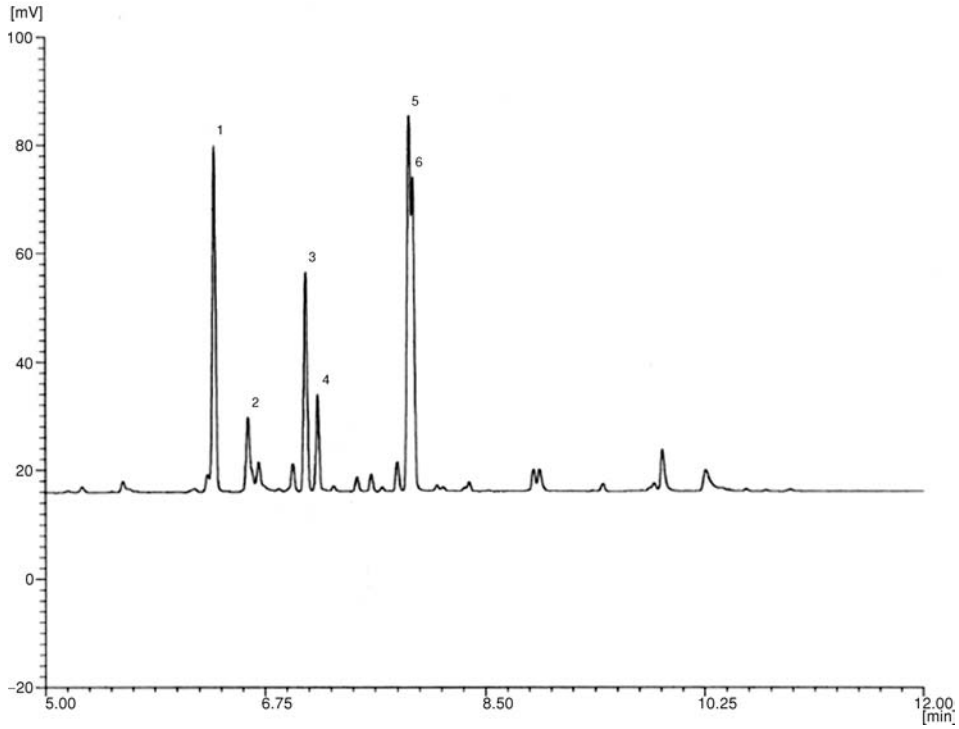


*Figure 2.* HS-SPME chromatogram of terpenes from larch needle litter concentrated on PDMS (100 $\mu$m) fiber. Identified components: 1 – $\alpha$-pinene, 2 – camphene, 3 – $\beta$-pinene, 4 – myrcene, 5 – $\beta$-phellandrene, 6 – limonene.

*Table IV.* Estimated emission rates of monoterpenes from larch litter (295 K)

| Terpene | $K_{fg}$ | $E_{dw}$, $\mu$g/(g × h) |
|---|---|---|
| $\alpha$-Pinene | 5358 | 0.50 ± 0.10 |
| Camphene | 7062 | 0.10 ± 0.04 |
| $\beta$-Pinene | 9164 | 0.18 ± 0.04 |
| Myrcene | 9374 | 0.05 ± 0.03 |
| $\beta$-Phellandrene | 15200 | 0.12 ± 0.06 |
| Limonene | 15812 | 0.113 ± 0.08 |
| | Total: 1.08 ± 0.11 | |

The volume of the stationary phase ($V_f$) of PDMS at a layer thickness 100 $\mu$m is 0.690 $\mu$L (Martos and Pawliszyn, 1997). Terpene emission rate was calculated from the Equation (5):

$$E^i[\mu g/(g \cdot h)] = \left(C_g^i - C_g^{i0}\right) \cdot V/(t \cdot m) \quad (5)$$

where $V$ is the gas phase volume in the unit, $t$ is the litter's residence time in the unit, $m$ is the litter dry weight, and $C_g^{i0}$ is the initial terpene concentration in the gas phase (it was taken to be equal to zero). Table IV contains the averaged results of four experiments on the emission rate into the gas phase of five terpene hydrocarbons for which the experimental or evaluated $K_{fg}$ values are available. It would be interesting to compare the obtained value with terpenes emission rate of living needles. Unfortunately, we have not encountered in the literature the corresponding data for European larch. However, one of the authors of this paper obtained experimentally at the beginning of the 1990s the temperature dependence (in the 12–30 °C range) of the emission rate of the terpenes sum by Siberian larch (*Larix sibirica*) (Isidorov, 1994). This dependence was expressed by the equation $\log V = 23.9–6884/T$. Hence, at 295 K the rate of $C_{10}H_{16}$ hydrocarbons emission is about 1.76 $\mu$g/(g × h) and has the same order of magnitude as the emission rate of litter of European larch found by us.

To evaluate the role of the leaf litter in the emission of the organic compounds into the atmosphere it is necessary to have data on VOC composition and emission rate under environmental conditions as well as data on the mass of "dead" plant material. It is known that the rate of litter decomposition of different species of arboreous plants is different and in the case of most coniferous trees it is 5–7 yr (Berg and Ekbohm, 1991; Kurz *et al*., 2000). During this period, the environment conditions change many times. For instance, the temperature on the soil surface even under the conditions of temperate climate can change from below zero to 70–80 °C. It is evident that the composition and rate of VOC emission should depend on this parameter.

*Table V.* The coefficients of the Equation (6) for different type of larch forests (Krasikov, 1985)

| Forest type | $a''$ | $b''$ | $c''$ |
|---|---|---|---|
| Grassy forest | 0.837 | −2.154 | 0.00038 |
| Green moss forest | 1.159 | −3.457 | 0.00068 |
| Swampy forest | 0.834 | −3.640 | 0.00077 |

It may seem that the data on the mass of leaf litter are difficult to formalize and, therefore, it is difficult to include them in appropriate models. However, forestry science has long ago established the regular character of changes in all elements of forest ecosystems (including the ground cover) during their development. Hence, there is a possibility of establishing relationships between the main forest characteristics and the leaf litter mass.

In some works empirical equations are proposed describing the relationship between leaf litter mass and the age of forests of certain types. For instance, for three types of forests in Evenkija (North-eastern Siberia) consisting of Siberian larch, Krasikov (1985) has proposed the exponential Equation (6):

$$m = A^{a''} \cdot e^{(b''+c''A)} \tag{6}$$

in which $m$ is the leaf litter mass (t/ha), $A$ is the forest age (yrs). Table V gives the values of the coefficients $a''$, $b''$, and $c''$.

It follows from Equation 6 that when these forests of low productivity attain 50 yr of age, the leaf litter mass attains 3.1 t/ha. This "dead" plant material can contain about 600 kg of VOC and their precursors. The mass of leaf litter in more productive forests is even greater. The dynamics of leaf litter mass in pine forests of the Archangelsk region (North-western Russia) indicates that it makes up almost 50% of the total overgrown biomass of trees in forests 20–60 yr of age and attains 21 t/ha (at 60 yr). Then the litter fraction gradually decreases, and in forests older than 100 yr it is 8–10% (Zyabchenko, 1984). According to Kurz *et al.* (2000), the mean litter-fall in 100 yr old maritime pine (*Pinus pinaster*) forest for 1994–1996 was $4.46 \pm 0.72$ t/ha. The litter mass of the forest floor was $43.1 \pm 12$ t/ha, and the needle compartment represented 39% of the total decomposing litter mass.

## 4. Conclusions

Leaf litter has already for a long time been considered as an important source of minor inorganic gases in the atmosphere, such as $CO_2$, COS, and $H_2S$ (Swift *et al.*, 1979). Data obtained in recent years also confirm the role of leaf litter as an important VOC source. It was suggested that the highest VOC emission rates by litter are observed at the beginning of autumn and in late spring (Warneke *et al.*, 1999). The investigation of the composition of VOC emitted into the atmosphere

from leaf litter is very important. To solve this problem rapidly, special methodology is required.

The above methodology based on the combination of sample collection by HS-SPME method and GC analysis can be used for determining not only the qualitative composition of VOC but also terpene emission rates. Broadening of the range of components investigated in this way (mainly lower alcohols and carbonyl compounds) requires the estimation of coefficients of distribution between the gas phase and the fiber coatings for SPME.

It is reasonable to include into the methodology the determination of a store of volatile components in the litter as well as of such substances that can be precursors of OVOC formed during microbiological decomposition of plant material.

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