Available online at www.sciencedirect.com
ScienceDirect
Environmental Pollution 155 (2008) 481–491
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

The essential oil qualitative and quantitative composition in the needles of *Pinus sylvestris* L. growing along industrial transects

Eugenija Kupcinskiene [a,*], Aida Stikliene [b], Asta Judzentiene [c]

[a] *Department of Biology, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania*
[b] *Department of Ecology, Lithuanian University of Agriculture, Studentu 11, LT-53361 Kaunas, Akademija, Lithuania*
[c] *Institute of Chemistry, A. Gostauto 9, LT-01108 Vilnius, Lithuania*

Received 1 February 2008; accepted 5 February 2008

Quantities of components of essential oil in the needles of Scots pine growing around two factories are described in relation to pollution.

Abstract

The aim of this study was to evaluate composition of the essential oils in the needles of *Pinus sylvestris* growing in the areas affected by a cement factory (CF), and an oil refinery (OR). Volatile components of the needles were analyzed by GC and GC/MS. The most heavily polluted CF stand had significantly higher concentration of γ-Terpinene, Caryophyllene oxide in the current-year needles, while higher concentration of δ-3-Carene, α-Terpinene, γ-Terpinene and Terpinolene was documented for 1-year-old needles. The most heavily polluted OR stand had a significantly higher concentration of Sabinene + β-Pinene, 1-epi-Cubenol in the current-year needles and a significantly higher concentration of Camphene, Sabinene + β-Pinene, Myrcene, α-Cadinene, 1-epi-Cubenol in the 1-year-old needles than the least polluted site. Along transects an increase in the amount of some diterpenes and a decrease in the components of the shorter chain essential oils was observed. These effects could be at least partially attributed to SO_2.

Keywords: Terpenes; Phytoindication; Conifers; Secondary metabolites; Air pollution; Cement dust pollution; Sulphur dioxide

1. Introduction

In many developed countries atmospheric pollution decreased in the 1980s (Emberson, 2003; Wieser et al., 2006) and in the Central and Eastern European countries in the 1990s (Mankovska, 1996; Staszewski et al., 1998; Sopauskiene et al., 2001). Despite this, during the past two decades there has been a growing interest in air pollution–vegetation effects (Bytnerowicz et al., 2006). Up-to-date field studies, monitoring, and modelling work document air pollution and climate change impacts on forests in Europe and North America (Manninen and Huttunen, 1995; Krupa and Legge, 2000; Bytnerowicz et al., 2002; Manning and Godzik, 2004; Huttunen 2005; Huttunen and Manninen, 2005; Paoletti, 2005, 2006; Günthardt-Goerg and Vollenweider, 2006; Allen et al., 2007; Grulke et al. 2007). It is particularly challenging to identify specific or unique indicators for stresses.

Recently greater attention is paid to the secondary metabolites including essential oils of various plant species (Lawrence, 1991; Shu and Lawrence, 1997; Barnola and Cedeňo, 2000; Lawrence, 2001, 2006). Terpenes in conifers are significant chemomarkers of environmental impact (Supuka and Berta, 1998). Diurnal (Barnola et al., 1997), seasonal and annual (Nault, 2003), climatic and edaphic (Kainulainen et al., 1992; Barnola et al., 1997), geographical–latitudinal–altitudinal (Manninen et al., 1998, 2002; Nault, 2003) variations of the concentration of terpenes in the needles of conifers have been documented.

Data concerning the effect of various anthropogenic factors on secondary compounds of the conifers is still controversial

* Corresponding author. Tel.: +1 370 6122 3391.
E-mail address: e.kupcinskiene@gmail.com (E. Kupcinskiene).

0269-7491/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2008.02.001

(Barnola and Cedeňo, 2000; Zavala and Ravetta, 2002; Turtola et al., 2006). Elevated CO_2 concentration caused an increase in the concentration of α-pinene (Heyworth et al., 1998; Salla et al., 2001), while influence of elevated UV-B radiation on secondary compounds of the conifers was not observed (Turtola et al., 2006). Increased limonene emission rates in ozone-fumigated woody plants were described (Klusia et al., 2002). Wider surveys of the conifer essential oil composition under acidic and alkaline pollutant effects are still scarce (Heller et al., 1990; Fuksman, 2002; Kainulainen et al., 1992; Supuka and Berta, 1998). The potential for air pollution effects on biogenic VOC emissions should be considered when assessing forest health (Erisman et al., 2005; Cape, 2007; Heath, 2007).

In Lithuania, since the 1990s the reduced level of emissions in a cement factory (operating since 1952), and an oil refinery (since 1980) caused further adverse changes in the surrounding forests (Kupcinskiene, 2001; Kupcinskiene and Huttunen, 2005) and some disturbances were registered in the ongoing decade (Ceburnis et al., 2002; Kupcinskiene, 2006; Judzentiene et al., 2007).

The present study was aimed to evaluate whether present levels of industrial pollution caused by the oil refinery and the cement factory affects the qualitative and quantitative characteristics of the essential oils in the needles of Scots pine (*Pinus sylvestris* L.) growing under different levels of pollution and at different distances from the pollution source.

2. Materials and methods

2.1. Study area

The Scots pine (*Pinus sylvestris* L.) growing along the transects from the cement factory (CF) and the oil refinery (OR) in Lithuania were investigated. In 2004–2005, total emissions from the CF comprised up to 3000 t year^{-1} and emissions from the OR 24,000 t year^{-1} (data provided by the Ministry of Environment of Lithuania). The total deposition of calcium and magnesium ions along the CF transect ranged between 19.1 and 2.6 kg ha^{-1} month^{-1} (Armolaitis et al., 1999). Elevated concentrations of heavy metals in the mosses were documented near CF and OR (Ceburnis et al., 2002). Sites near the OR differed in the amounts of sulphur dioxide up to 6.7 times (with the highest mean monthly concentration of 27 μg m^{-3}; in 2005, being lower, 9.4 μg m^{-3}), and in the amounts of nitrogen dioxide up to 2 times (with the highest mean monthly concentration of 6 μg m^{-3}, registered in 2005). Sites near the CF differed in the amounts of sulphur dioxide up to 9 times (with the highest mean monthly concentration of 8.3 μg m^{-3}, registered in 2005; Kupcinskiene, 2006), and in the amounts of nitrogen dioxide up to 1.9 times (with the highest mean monthly concentration of 3 μg m^{-3}). In both transects, the most polluted sites were the ones closest to the factories. The furthest stands were used as control sites. Before sampling severe drought was registered (Table 1).

Table 1
Precipitation (sum within decades, in mm) and temperature (mean values for the decades) in June–July 2005, according to the data of the closest to the transects Meteorological Station (Telsiai, Lithuania)

Month	Decade	Precipitation, mm	Temperature°C
June	I	3.1	11.9
	II	0.6	15.8
	III	2.0	16.0
July	I	1.1	19.0

Middle-aged pines (51–62 years old) growing on histosols (near the CF), and 46–75-year-old trees growing on luvisols (near the OR) were examined. Selection of sites was based on availability of the stands and the prevailing wind direction from each pollution source. Related to the CF, four stands belonging to *Carico-sphagno-Pinetum* (siccata) type in a 10-km transect (northeast direction) were studied. Near the OR, four stands of *Oxalido-Pinetum* type in a 5.4-km northeast transect and one stand (3.6 km away from the OR, east direction) was examined.

2.2. Plant material

Branches facing the wind coming from the factory were cut from eight pines at the height of 6–8 m above the ground (the third lowest part of the crown). From each tree, four shoots with the current-year (c) and 1-year-old ($c+1$) needles were used. July (10/07/2005) was chosen for sampling due to the most vigorous growth of the new needles that expanded in June. For analysis, four independent samples were prepared from each stand. A separate sample was made by mixing equal amounts of the needles collected from eight trees (Schulz et al., 1998) and drying the material at room temperature (20–25 °C). Defoliation (expressed in %) of the sampled trees was estimated according to EU standards (Anonymous, 1989).

2.3. Oil isolation and analysis

Oil yield was conducted by hydrodistillation of 50 g of dry needles. Pale yellow oils were obtained in 0.2–0.6 and 0.2–0.3% (v/w) of the yield, respectively, in the current-year and 1-year-old needles on a dry mass basis. GC analysis was done by an HP 5890(II) chromatograph equipped with FID and capillary column HP-FFAP (30 m × 0.25 mm i.d., film thickness 0.25 μm). Analyses by GC/MS were performed using an HP 5890 chromatograph interfaced to an HP 5971 mass spectrometer (ionization voltage 70 eV) and equipped with a capillary column CP-Sil 8 CB (50 m × 0.32 mm i.d., film thickness 0.25 μm). Other details of oil analyses were described earlier (Mockute et al., 2003; Mockute and Judzentiene, 2004). Qualitative analysis was based on a comparison of retention times, indices with mass spectra libraries (Wiley and NBS 54K), and other corresponding data (Adams, 2001). Quantity of each component of essential oils was calculated as relative concentration (peak area percentage) and amount (μg g^{-1} d.m., recalculated according to the internal standart).

2.4. Statistical analysis

To compare the stands along the transects, dispersion and correlation analyses were applied using EXCEL, SPSS, and SAS packages. Error bars in the figures indicate an interval of 95% confidence. The normality of the data distribution was assessed and log transformations were performed. Data were analysed with two-way analysis of variance. The significance of differences between sites was assessed by Tukey's multiple range test. Variances of concentration logarithms with their components (site, needle age and their interaction) were estimated by maximum likelihood (Schleppi et al., 2000).

3. Results

3.1. Needle essential oil general characteristics

There were no significant differences between the stands along separate transects in the total yield of the essential oils in the needles. Seventy identified components made up to 89.1–95.1% of total oil content. The qualitative composition of the main components appeared to be constant in the needles of all pine stands investigated. However, there were considerable differences in the amounts of separate components. The predominant fraction was found to be monoterpenes (19.0–40.0%), with the major constituents being α-pinene