

## SPME IN THE DETECTION OF BioVOCs: MONITORING THE PANEVEGGIO NATURAL PARK

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We think that most of us have experimented a phenomenon that, at first sight, seems to be far from any logic thought: moving from 200 to 1500 meters the density in air decreases from 1.22 to 1.08 Kg/m<sup>3</sup>, which correspond to a decrease of oxygen of about 12%. In principle this would lead to a more difficult breathing; however trekking in forest make us to experiment an easier respiration.. This aspect is further confirmed by the past approaches in the cure of pulmonary disease: in the case of severe diseases (e.g. tuberculosis or pneumonia) a period past in mountain environment was considered high beneficial for the cure and convalescence. This suggests the presence, in hood environment, of substances in air at trace level which necessarily favour the respiration

The nonmethane volatile organic compounds (VOCs) can be subdivided in two different group: those of anthropogenic origin and those of natural origin. The former are represented mainly by aromatic compounds and chlorinated derivatives originating from both industry production and combustion processes, while among those of natural origin biogenic volatile organic compounds (BVOCs) are the most relevant ones [1]. These substances, originating from the metabolism of plants, can be classified in three different group: isoprene, monoterpenes and others (aldehydes, organic acids, alcohols, phenols) which contribute with levels of 44, 11 and 45% respectively to the total emission [2]. Isoprene is a very reactive compound and its biogenic emission plays an important role in atmospheric chemistry. It reacts rapidly with hydroxyl radical in the atmosphere, leading to hydroperoxides that, through anthropogenic NO<sub>x</sub>, can enhance ozone formation. On the other hand it reacts directly with ozone, which seems highly toxic for the metabolism of several kinds of plants, leading to a decrease of its concentration. Another effect of isoprene in atmosphere is the growth of aerosols. The biological role of isoprene (well described by F. Loreto et al and TD Sharkey et al) is mainly tied to its protective function against environmental stress. [3,4]

In the present investigation the most of our attention was focalized to the detection of terpenoids and the other secondary metabolites released from the plants, indicated as biologically

active compounds. The project is based on the determination of a map related to the plant metabolites present in the atmosphere of the Paneveggio Natural Park, a 19,711 hectares area placed in the Dolomite region (north-east region of Italy), in order to obtain an evaluation of the quality of the air present in the park (figure 1).

The study was based on the sampling of BVOCs by portable SPME devices placed in different points of the park, as show in figure 2 and in table 1. In the same table, the atmospheric conditions and the predominant plants in the different sites are also reported.

Considering the chemical nature of the compounds of interest, the stationary phase employed to BVOCs sampling was a bonded Carboxen/Polydimethylsiloxane (75  $\mu\text{m}$ ), particularly suitable for trapping both apolar and medium polarity volatile metabolites released from plant.

Considering the low concentration of BVOCs (at ppb level) the fiber exposition has been maintained for 5 hours. To prevent degradations and losses of the trapped compounds, each SPME device after the sampling was stored at 0°C and kept at the same temperature till the analysis (about 24 h later). The further analysis were performed by GC/MS and for this aim a Fison MD 800 instrument was employed. The SPME fiber was exposed inside the GC/injector at 250 °C in splitless mode (10 s) and the chromatographic conditions were the following: column, J&W DB 5MS (30m x 0.25 mm id x 0.25  $\mu\text{m}$  film thickness); temperature program, 40° C (5 min) 4°C/min to 150 °C (0 min) 15°C/min to 300 °C; head pressure, 10 psi. The EI spectra were obtained operating at 70 eV (200  $\mu\text{A}$ ) and by full scans in the range m/z 40-400.

The typical results so obtained are shown in the chromatograms of figure 3. By comparison with the NIST library the structure assignments reported in figure 2 have been obtained. When this approach was unsuccessful the structures have been proposed on the basis of an accurate study of the spectra.

By this approach about 90% of the adsorbed compounds have been identified. The results obtained in different zone are compared in table 2. The most the detected species can be related to the volatile fraction of the essential oils released by the vegetal organisms present in the zone self it.

As can be observed from these data, aside the volatile metabolites, some substituted aromatic hydrocarbon are also detected. Surely of anthropogenic origin (reasonably from gasoline engines) and, considering the low traffic level present in the valley under investigation, they could have been transported by wind from the close Primiero valley. However their levels are particularly low.

The analysis of chromatogram 1 (figure 3A) show a series of peaks due to the volatile terpenic species, while aldehydes and phenols are present in lower quantity. In this zone Norwegian spruce (*Picea Abies*) prevails together with silver firs (*Abies Alba*) and beech trees (*Fagus sylvatica*).

The results obtained are in perfect agreement with volatile metabolites typical of this kind of plants.

The sampling performed in zone 2, in which a larger distribution of broad-leaved plants is present, leads to slightly different results: some monoterpenic species ( $\beta$ -pinene and limonene) exhibit lower concentration, while a clear increase of C<sub>12</sub>-C<sub>13</sub> hydrocarbons is observed.

The results show a further change moving to higher altitude, where the change in vegetation is more marked. In fact, as shown by the data obtained by sampling in zone 3 (1600 m of altitude) a clear decrease of the peaks related to phenolic and aldehydic compounds is observed. Interestingly a clear increase of 3-carene become present. Considering that in this zone mugo pine (*pinus mugo*) is the most diffused plant, it could be proposed that this plant is the most relevant source of this metabolite.

Zone 4 shows high concentration of larch and the related chromatogram (figure 3B) put in evidence a clear increase of  $\alpha$ -pinene and 3-carene. Finally the last sampling (zone 5) was performed in a wide grass field surrounding by beech tree and Norwegian spruces. In this region significant decrease of monoterpenic species has been observed, accompanied by an increase of aldehydes concentration.

The experimental data so obtained allow to the following general consideration:

1. the atmospheric sampling by SPME and the further GC/MS analysis is highly effective for the qualitative determination of the BVOCs released from plants and present in a forest environment;
2. the semiquantitative approach adopted in these preliminary studies allows to establish the relative concentration of different BVOCs for each zone under investigation.

The results summarized in table 2 show that the BVOCs content in air exhibit clear changes in function of altitude and of surrounding vegetation.

Terpenoids are a class of secondary phyto-constituent particularly wide. They have a common biosynthetic origin, due to the linking of moieties constituted by five carbon atoms, called isoprenic units, based on the isooctane skeleton. They are volatile at low temperature and their mixture constitute the plant essential oil. Furthermore terpenes, aside their therapeutic effect which will be discussed below, exhibit a relevant ecological role: in fact they constitute a biochemical screen against the attack of insects, fungi and exhibit a protective function with respect to chemical stress. From the therapeutic point of view, terpenoids represent the most interesting class of the BVOCs, due to their relevant anti-inflammatory and anti-microbial activity for man and in general on animals. It is more than a century that essential oils (e.g. trementine, pine extract) are employed in the cure of disturbs at bronchial level, as well as of rheumatic and neuralgic disturbs [5, 6]. These essences are constituted by a volatile monoterpenic part, containing  $\alpha$ -pinene,  $\beta$ -pinene,  $\Delta^3$ -carene, terpinolene,  $\gamma$ -terpinene, mircene, and by a less volatile part constituted by diterpenes and sesquiterpenes. Trementine of larch, pine essences, trementine medicinal essences are the most common and described in the international pharmacopoeia.

The therapeutic effect is mainly due to the most volatile constituent of oil and can be related to their antioxidant activity. Considering that pulmonary and bronchial diseases and some skin infection are associated to the formation of the reactive oxygenated species (ROS), the antioxidant action of terpenes can lowering as well as prevent the inflammatory state [6, 7]. Recent studies have demonstrated that terpenes, through their antioxidant activity, are active in the prevention of arteriosclerosis, stroke and heart attack. They inhibit the low density lipoprotein (LDL) oxidation, limiting the formation of products which represent one of the causes of the above cited pathologies. These volatile constituents of essential oil are present, obviously, even in air and their concentration in forest can reach also concentration higher than 10 ppb.

Trough these considerations it is possible to recognize how healthy and therapeutic can be the atmosphere of the forest mainly covered of conifers as that of Paneveggio Natural Park is. It is to emphasise that the quality of the breathed in air is not only related to the absence of toxic pollutants in the atmosphere, but also to the presence of volatile plant metabolism products which, for their biological proprieties, have a beneficial effect.

## References

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Figure 1. Map of the Paneveggio Natural Park.

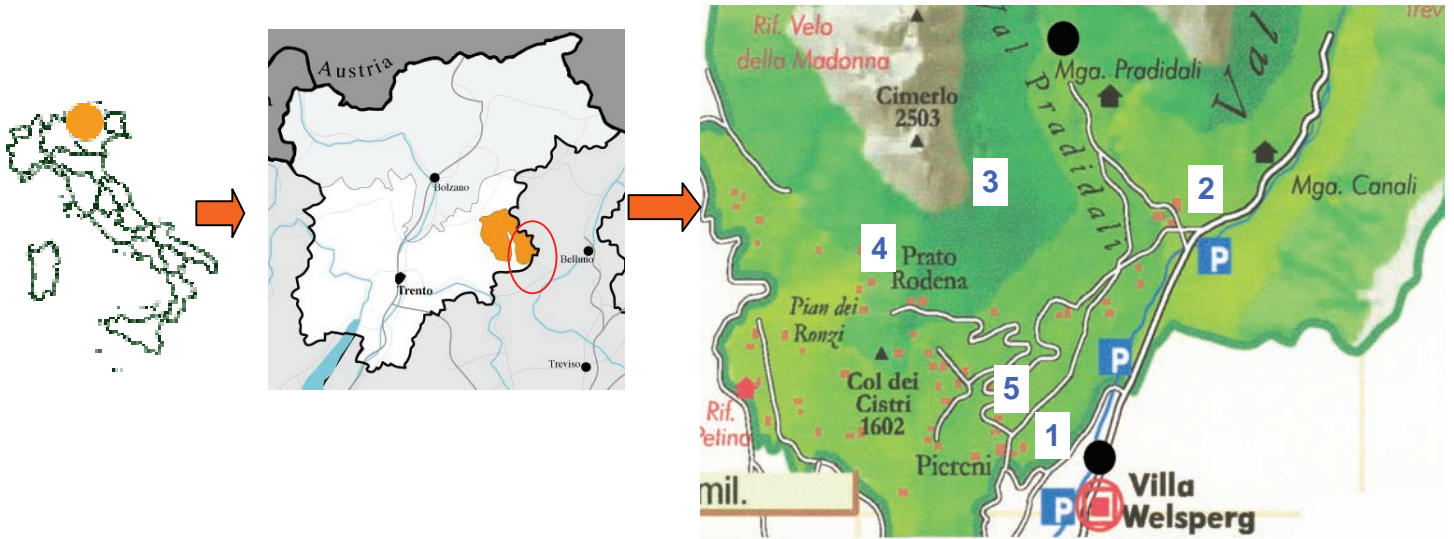
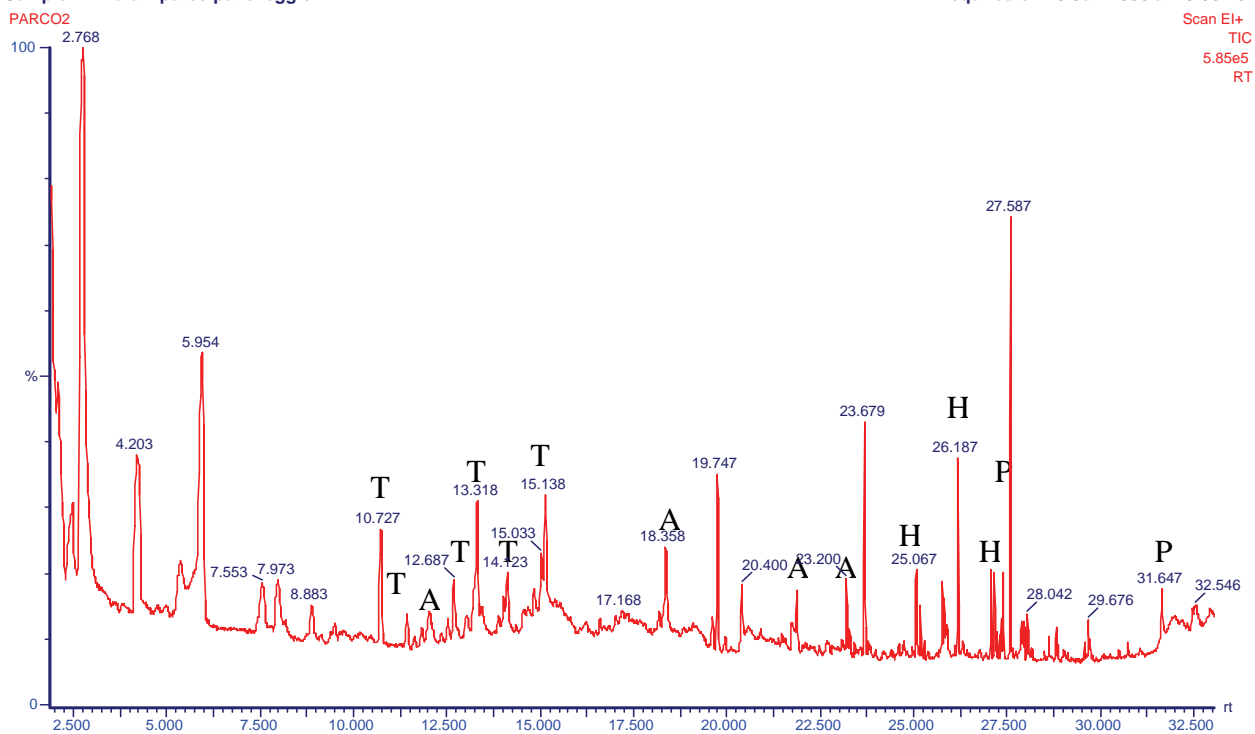


Figure 2. Portable SPME employed to sampling the VOCs in the park.

Sample ID: fibra 2 parco paneveggio

Acquired on 15-Jun-2006 at 13:30:18



Sample ID: fibra 8 parco paneveggio

Acquired on 15-Jun-2006 at 12:08:03

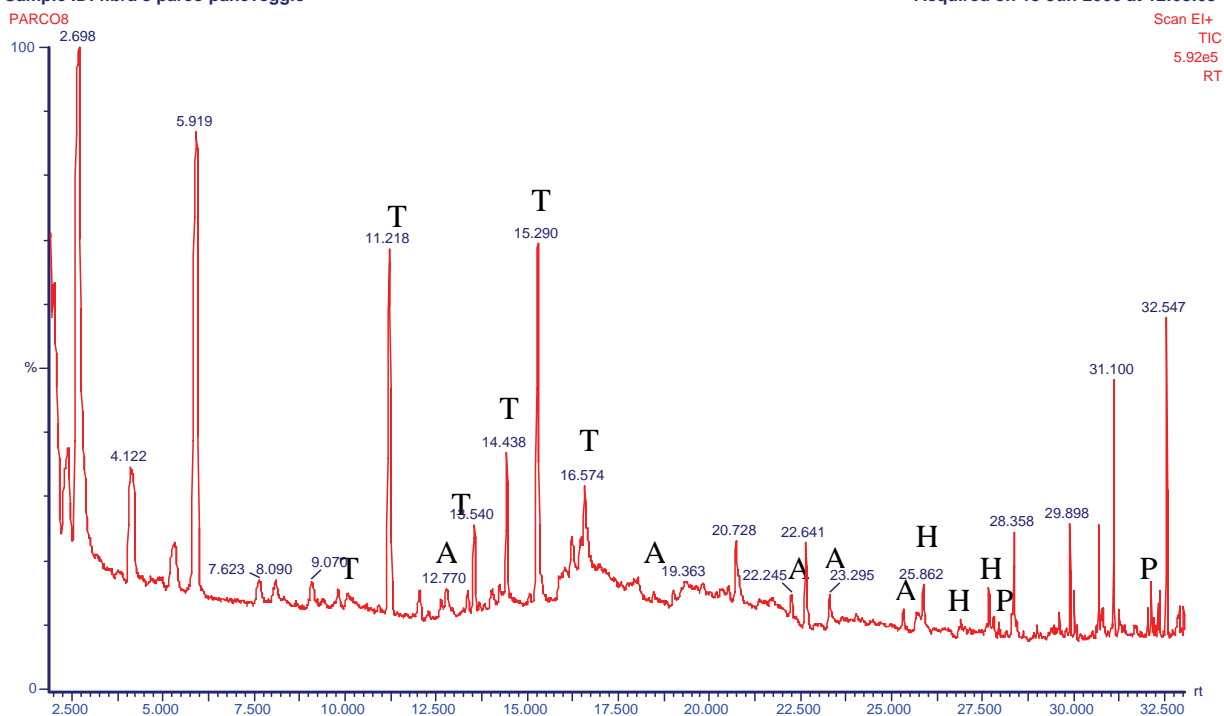


Figure 3. A) Chromatogram related to the sampling of zona 1. B) Chromatogram related to the sampling of zona 4; T (terpene species), A (aldehydes), H (Hydrocarbons C10-C15), P (substituted phenols).

**Table 1.** Environmental parameters of the sampling zones.

| <b>ZONE</b> | <b>CONDITIONS</b>   | <b>PREVALENT PLANTS</b>                                       |
|-------------|---|---|
| <b>1</b>    | Temperature: 21 °C<br>Altitude: 1050 m<br>Wind: absent        | Norwegian spruce,<br>Silver fir, European<br>Beech            |
| <b>2</b>    | Temperature: 22 °C<br>Altitude: 1250 m<br>Wind: absent        | broadleaf plant,<br>European Beech,<br>Norwegian spruce .     |
| <b>3</b>    | Temperature: 22 °C<br>Altitude: 1600 m<br>Wind: gentle breeze | Mugo pine   |
| <b>4</b>    | Temperature: 22 °C<br>Altitude: 1520 m<br>Wind: absent        | Larch   |
| <b>5</b>    | Temperature: 22 °C<br>Altitude: 1200 m<br>Wind: absent.       | Grass surrounded of<br>European Beech and<br>Norwegian spruce |

**Table 2.** The most abundant VOCs identified and their relative abundances. (“+++“ VOC most abundant; “++” VOC less abundant; “+” VOCs under 5%). The abundance assignment is related to the percentage area, calculated only on the volatile organic compounds below reported.

| N° | R.T.<br>(min) | Compounds  | Relative Abundance |        |        |        |        |
|----|---------------|--|--------------------|--------|--------|--------|--------|
|    |               |  | Zone 1             | Zone 2 | Zone 3 | Zone 4 | Zone 5 |
| 1  | 2.488         | Benzene  | +                  | +      | -      | -      | -      |
| 2  | 4.203         | Toluene  | ++                 | +      | +      | +      | +      |
| 3  | 7.553         | o-Xilene   | +                  | +      | +      | +      | +      |
| 4  | 7.973         | m,p-Xilene   | +                  | +      | +      | +      | +      |
| 5  | 8.883         | Benzene substituted                                | +                  | +      | +      | +      | +      |
| 6  | 10.725        | $\alpha$ Pinene                                    | ++                 | ++     | ++     | +++    | ++     |
| 7  | 11.427        | Camphene   | +                  | +      | +      | +      | +      |
| 8  | 12.034        | Benzaldehydes                                      | +                  | +      | +      | +      | +      |
| 9  | 12.687        | $\beta$ Pinene                                     | ++                 | +      | +      | ++     | +      |
| 10 | 13.434        | Cumene   | +                  | +      | +      | +      | +      |
| 11 | 14.123        | 3-Carene   | +                  | +      | +++    | +++    | +      |
| 12 | 14.834        | ?  | +                  | +      | ++     | +      | +      |
| 13 | 15.033        | Limonene   | ++                 | +      | ++     | +      | +      |
| 14 | 15.138        | 1-8 Cineole  | ++                 | ++     | +      | +      | +      |
| 15 | 18.358        | Nonanal  | ++                 | +      | +      | +      | ++     |
| 16 | 20.400        | Bicycle 3,10 hexan-2one                            | +                  | +      | +      | +      | +      |
| 17 | 21.870        | Aldehydes C <sub>10</sub> -C <sub>12</sub>         | +                  | +      | +      | +      | +++    |
| 18 | 23.200        | Aldehydes ramified C <sub>10</sub> C <sub>13</sub> | +                  | +      | +      | +      | +      |
| 19 | 25.067        | Hydrocarbons C <sub>13</sub> -C <sub>12</sub>      | +                  | ++     | ++     | -      | +      |
| 20 | 25.184        | Tetradecanal                                       | +                  | -      | +      | +      | +      |
| 21 | 26.187        | Pentadecane  | ++                 | ++     | ++     | +      | +      |
| 22 | 27.074        | Pentane-1,3-dioldiisobutirrate,                    | +                  | +      | +      | -      | ++     |
| 23 | 27.156        | Hexadecane substituted                             | +                  | ++     | +      | +      | +      |
| 24 | 27.401        | Phenol substituted A                               | +                  | +      | +      | +      | ++     |
| 25 | 27.587        | Compound not identified                            | +++                | +++    | +++    | +      | ++     |
| 26 | 29.676        | Esters > C6  | +                  | +++    | -      | +      | +      |
| 27 | 31.647        | Phenol substituted B                               | +                  | +      | +      | -      | +      |