UNIVERSITÀ DEGLI STUDI DI CAMERINO

School of Advanced Studies

DOCTORAL COURSE IN

CHEMICAL AND PHARMACEUTICAL SCIENCES AND BIOTECHNOLOGY

XXI cycle

Monitoring of Biogenic Volatile organic Compounds (BVOCs) in Air with Analytical Techniques

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ACADEMIC YEAR 2017-2018

Abstract

Biogenic volatile organic compounds (BVOCs), are a heterogeneous group of molecules with a wide range of functions useful for plants and, consequently, for the whole ecosystem and the environment. The general aim of this dissertation is the study and monitoring of BVOC emissions in different place and time and the correlation between emissions and climate changing, atmospheric reaction and behaviour of birds. This dissertation concerns 4 main chapters in which we discuss 4 different aspects of the study of BVOC emissions. The first one regards a rapidly and inexpensive analytical method that is based on the use of thermal gas-chromatography (TD-GC-MS) to monitor BVOCs released by Juniperus Communis in Sibillini National Park. Hexanal, Toluene, α-Pinene, 4-Terpineol and β-Caryophyllene have been used as standard compounds. This method showed a good sensibility (limit of detection ranges between 10-19 pptv for all compounds except for Hexanal that is 100 pptv), repeatability (RDS% within 11%), precision (recovery higher than 85%) and selectivity. We collected samples in situ from April 2016 to September 2017 almost once for months. The compounds have been identified using Kovats retention indices (RI) and quantified with the response factor (RF) for different class of compounds. All samples have been correlated with temperature and humidity values. The results show higher emission in April (flowering period) and at particular condition of temperature (24°C). These results are comparable to the data found in the literature about emissions of Juniperus Communis, confirming the validity of the developed method. The second aspect of our research project regards the study of all parts of Juniperus Communis and Juniperus Oxycedrus using a different analytical technique: Solid Phase-Microextraction (SPME). The study was performed in June, during the blowing period, different plant parts were sampled (branches, leaves, fruits) and analyzed in the laboratory; the study was conducted to complete the monitoring of emissions in the National Park of Sibillini to understand which BVOCs come from different parts of the plant. From the results obtained, there is a greater emission from the Juniperus Oxycedrus, this confirms the data obtained during the air monitoring lasted two years. In fact, while the red juniper emits more monoterpenes and sesquiterpenes, the Juniperus Communis is rich in oxygenated terpenes. Moreover, during my PhD I spent 6 months abroad in Mainz at the Max Planck Institute for Chemistry in Jonathan Williams's research group. I studied oxygenated BVOC emissions in

Amazon forest during dry season. The site chosen for this study was the Amazon Tall Tower Observatory, ATTO, the site is equipped with a 325m tall tower and sampling was performed on this tower at four different heights (40, 80, 150 and 320 m). the analysis were performed using a thermal desorption coupled with a TOF-MS equipped with a cyclodextrin column to observe chiral compounds. The use of the chiral column allowed the separation of the enantiomers R and S and, in the case of 1-Hexanol-2-Ethyl it was possible to evaluate the relationship between the two enantiomers. In this case, a change of chirality is observed between 40 and 80m and again between 80m and 320m. Moreover, we found that Nonanal is the compound higher emitted by plants as defence mechanism after bacterial or insect attack. In the case of the MEK, however, it was confirmed that it is directly emitted from the vegetation in fact shows a concentration greater than 40m. Subsequently it tends to decrease until it reaches 320m where an increase can be observed, this has become due to the formation of MEK as an oxidation product of alkanes or other compounds. Then we found that trend of isoprene's degradation products are in accordance with the results in the literature and that, at times when the isoprene is most emitted, the MVK and the MACR are lower but they increase in height, in fact they get higher concentrations at 320m. The last study concerns a field campaign in Arnino (Pisa, Italy), this project is a collaboration between Max Planck Institute for Chemistry, Max Planck Institute for Ornithology and University of Pisa. The objective of the project is to analyze the emissions correlating them to the wind direction and then to create an olfactory map of the pigeons with the aim of connecting the emissions to the orientation of the pigeons. . In particular, I studied compounds emitted primarily from anthropogenic sources such as Benzene, Toluene but also Acetone, and were later correlated with the wind direction figures to understand where the major contribution of these compounds comes from. In the case of compounds such as Benzene and Toluene (emitted by vehicles or industries), it is possible to observe a daily profile with the highest emissions during the day which then decrease in the night and moreover, there are greater concentrations when the wind comes from the city of Pisa and then from East / South-East. The Acetone study has encountered major problems, because this compound comes from different contributions.

Contents

1. INTRODUCTION

Organic compounds are important atmospheric components. In these last years the study of biogenic volatile organic compounds (BVOCs) emitted by plants has become very important to understand their impact on ozone formation and climate change. Thus, the general aim of this dissertation was to find an analytical method, relatively simple and inexpensive, to evaluate plant emissions and then to correlate the emissions with climate change and seasonal variation.

1.1. Definition of Biogenic Volatile Organic Compounds

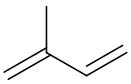
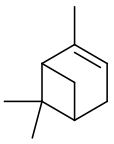
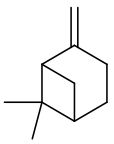
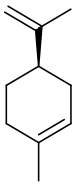
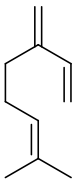
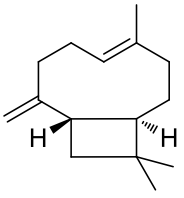
Biogenic volatile organic compounds (BVOCs) are a large group of heterogeneous molecules having a high vapour pressure at room temperature. The majority of BVOCs are emitted by plants, they are over 400 molecules including aldehydes, ketones, organic acids, alcohols, alkanes, isoprene and terpene derivatives (Zimmerman 1979) but the knowledge on the performance of emissions and on the behaviour of them are limited (Karl et al., 2009). Some BVOCs are very reactive and form a link between the biosphere, the atmosphere and the climate (Laothawornkitkul et al., 2009).

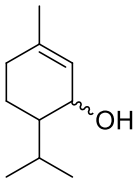
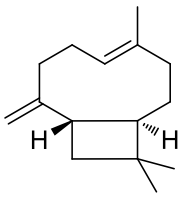
All living organisms produce and release biogenic volatile organic compounds. Plants emit BVOCs for different reasons, for communication within and between plants and across trophic levels (Kessler and Baldwin, 2001; Shiojiri et al., 2006), to attract pollinators and seed dispersal animals (Maffei, 2010), and to protect themselves from biotic and abiotic stresses (Peñuelas and Staudt, 2010).

1.2. BVOC chemical speciation

As mentioned previously, the term biogenic volatile organic compounds includes organic atmospheric trace gases other than carbon dioxide and monoxide. Global emissions inventory as recent as the 1990s categorised BVOC emissions into isoprene, monoterpenes and a general grouping of “other VOCs” (Guenther et al. 1995). Now, it is possible to re-categorise biogenic VOCs to reflect the most recent research by adding two categories: oxygenated VOCs (oxVOCs) and very reactive biogenic VOCs (VR-BVOC) (Holzinger et al. 2004). The Table 1.1 below shows chemical formulas and structures of these categories (Koppmann 2007).

Table 1.1: Categories of biogenic volatile organic compounds.

Category	Chemical formula/definition	Structure	Other compounds
Isoprenoids	C_5 compounds	 isoprene	Methylbutenol
Terpenoids			
Endocyclic	$C_{10}H_{16}$ Double bonds inside ring structure	 α-pinene	Δ^3 carene α-terpinene y-terpinene
Exociclyc	$C_{10}H_{16}$ Double bonds outside ring structure	 β-pinene	Camphene Sabinene p-Cymene
Combination	$C_{10}H_{16}$ Double bonds outside and inside ring structure	 D-Limonene	β-Phellandrene Terpinolene
Open-ended	$C_{10}H_{16}$ No ring structure present	 myrcene	Ocimene
Sesquiterpenes	$C_{15}H_{24}$	 β-caryophyllene	α-Humulene

Oxygenated	C_{10} compounds containing oxygen	 4-terpineol	Methyl chavicol
oxVOC	C and O containing compounds	 acetone	Acetaldehyde, methanol, ethanol, methylbutenol
VR-BVOC	Terpenoid or oxVOCs with lifetimes on the order of minutes to hours	 β-caryophyllene	β-Farnesene α-Terpinolene

The most studied group of biogenic VOCs are the isoprenoid and terpenoid compounds. The terpenoids are a group of extremely widespread compounds, in fact, are found in all living organisms, as well as being the largest group of natural compounds existing in nature, with more than 40,000 structures (Bohlmann and Keeling., 2008). These include C_{10} compounds known as monoterpenes, C_{15} compounds or sesquiterpenes and the larger C_{20} (diterpenes), C_{25} (sesterterpenes) and C_{30} (triterpenes) molecules. They possess many biological properties, which have enabled a wide range of uses as drugs, fragrances and perfumes, pesticides, food additives and antibacterial agents.

1.3. BVOC synthesis

In the past twenty years, a significant amount of progress has been made in understanding the isoprene synthesis process. Isoprenoid biosynthesis central intermediates are isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Their formation is divided into two cell compartments: (1) cytosolic MVA-dependent pathway and (2) plastidial nonmevalonate pathway (MVA-independent pathway) (Laule et al., 2003). The MVA-dependent pathway is responsible for the synthesis of sesquiterpenes (SQTs), while the recently discovered MVA-

independent pathway is involved in providing the precursors for MTs (Monoterpenes), certain SQTs (Sesquiterpenes) and diterpenes (Laule et al., 2003). The reason why the sesquiterpene synthesis is separated from isoprene and monoterpene synthesis is not yet clear (Li and Sharkey, 2013). Finally, isoprene and MTs are formed in plastids, while SQTs are formed in the cytosol (Figure 1.1)

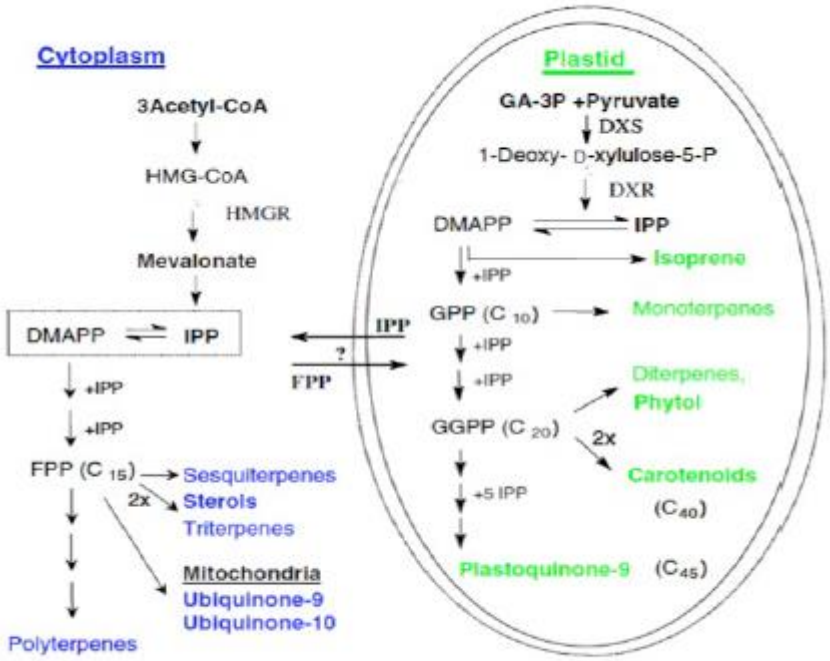


Figure 1.1: Overview of isoprenoid biosynthesis in plants according to plant cell compartment division.

Other compounds emitted by plants are non-terpenoids; some examples of non-terpenoids are green leaf volatiles (GLVs), which are formed following the lipoxygenase (LOX)- pathway (Grechkin, 1998), and benzene derivates which production often, but not always, follows the shikimate pathway (Herrmann, 1995; Misztal et al., 2015). These compounds, especially GLVs, are not as abundant as terpenes in plant emissions under non-stressed conditions, but may constitute a larger part when plants are under stress and can also be emitted from the soil (Misztal et al., 2015; Peñuelas et al., 2014; Scala et al., 2013).

1.4. BVOC emissions

Once an organism has overcome its biochemical constraints on production, biogenic VOCs must overcome the physiological constraints and travel from the intercellular air spaces and storage structures into the atmosphere. There are three possible foliar release mechanisms:

1. exit of gases via the stomates (compounds that are not stored in specialised structures in the leaf can exit from the intercellular air space to the atmosphere through the stomata) (Fall and Monson 1992);
2. non-stomatal exit via diffusion through the leaf cuticle or woody portions of the plant (emissions from leaf wounding) (Loreto et al. 1996b);
3. release of compounds from the storage pools in plants (via mechanical wounding such as insect damage or herbivore feeding) (Litvak et al.1999) .

The most common mechanism is the stomata (Harley 2013). The emission is driven by a concentration gradient with the highest concentration inside the leaves to lower concentration in the atmosphere (Niinemets et al., 2014). When the stomata closes, the BVOC concentration inside the leaves builds up and the gradient will be strong enough to force compounds through the stomata, that is if production still occurs (Niinemets et al., 2004). However, if a closed stomata results in shut down of production, the emission stops whenever the gradient strength weakens. If the gradient switches direction, when the concentration is higher in the atmosphere than in the leaves, an uptake of compounds will occur (Niinemets et al., 2014). Some BVOCs are released as soon as they are produced and some compounds are stored in specific storage parts in the plants (Kesselmeier and Staudt, 1999). The emissions are driven by temperature and light, on the base of compound. The isoprene and some monoterpenes are released directly after production and the emissions depend on light and temperature (Laothawornkitkul et al., 2009; Taipale et al., 2011) and are affected by enzymatic regulation (Li and Sharkey, 2013). Emissions from specific storage structures are not directly dependent on photosynthesis and on light (Laothawornkitkul et al., 2009) and the release is more under physical than biological control (Li and Sharkey, 2013).

1.5. Emission source of BVOCs

The predominant source of biogenic VOC emissions is from the foliage of terrestrial vegetation. This includes natural vegetation such as trees, leaves, leaf litter, stems, and roots, (Lin et al., 2007; Leff et al., 2008; Aaltonen, et al., 2011; Noe et al., 2012) as well as anthropogenically induced vegetation such as crops and urban landscapes. Other minor sources such as oceanic and soil emissions can also contribute to global totals of biogenic VOCs (Koppmann 2007). On a global scale, approximately 90 % of annual VOC emissions are derived from biogenic sources (Table 1.2), with an estimated amount of about 1000 Tg year^{-1} (Guenther et al., 2012). This is significantly greater (ca. 10 times) than the emission of anthropogenic VOCs.

Table 1.2: Estimated annual global VOC and methane emissions from different sources (Guenther 1999).

	Annual emission (TgC a^{-1})			
Source	**Isoprene**	**Monoterpenes**	**Other VOCs**	**Methane**
Canopy foliage	460	115	500	<1
Terrestrial ground cover and soils	40	13	50	175
Flowers	0	2	2	0
Ocean and freshwater	1	<0.001	10	15
Animals, humans and insects	0.003	<0.001	0.003	100
Anthropogenic (incl. biomass burning)	0.01	1	93	220
Total	**~500**	**~130**	**~650**	**~510**

The predominant VOCs emitted by vegetation belong to terpenoids, isoprene is copiously emitted, then the other important terpenoid classes are the monoterpenes and sesquiterpenes (Table 1.3).

Table 1.3: Chemical species that dominate the annual global VOC emission from vegetation (Wiedinmyer et al. 2004).

Annual emission ($TgC\ a^{-1}$)	Compound
250-750	Isoprene
50-250	Methanol, α-pinene
10-50	Acetaldehyde, acetone, β-pinene, Δ-carene, ethanol, ethene, hexenal, hexenol, hexenyl-acetate
2-10	Propene, formaldehyde, hexanal, butanone, sabinene, limonene, methyl-butenol, butene, β-phellandrene, p-cymene, myrcene
0.4-2.0	Formic acid, acetic acid, ethane, toluene, camphene, terpinolene, a-terpinolene, α-thujene, cineole, ocimene, γ-terpinene, bornyl acetate, β-caryophyllene, camphor, piperitone, linalool, tricyclene

In addition to terpenoids, plant emissions also comprise alkanes, other alkenes, carbonyls, alcohols, esters, ethers, and acids. Despite the overwhelming multitude of individual organic compounds found in plants only a relatively small number are considered relevant to atmospheric chemistry, either due to their large emissions or/and high reactivity.

1.6. Factors affecting BVOC emissions

Environmental affecting factors of photosynthesis and BVOC emissions can be classified into two major categories: (1) internal factors (genetical and biochemical) and (2) external factors, subdivided into biotic and abiotic factors (Marin-Loaiza and Cespedes, 2007). Here, we focus on light, temperature and seasonality.

1.6.1 Light dependencies

Light is vital for plant growth and development and plants utilize it in two distinctive ways: as a source of energy and as a source of spatial and temporal information (Heldt, 2005). Isoprene emission is strongly dependent on photosynthetically active radiation (PAR; the portion of the spectrum (400–700 nm) that activates photosynthesis). The response of isoprene to PAR is hyperbolic, and emissions increase with increasing light until they reach a saturation point (Fig 1.2) (Koppmann 2007).

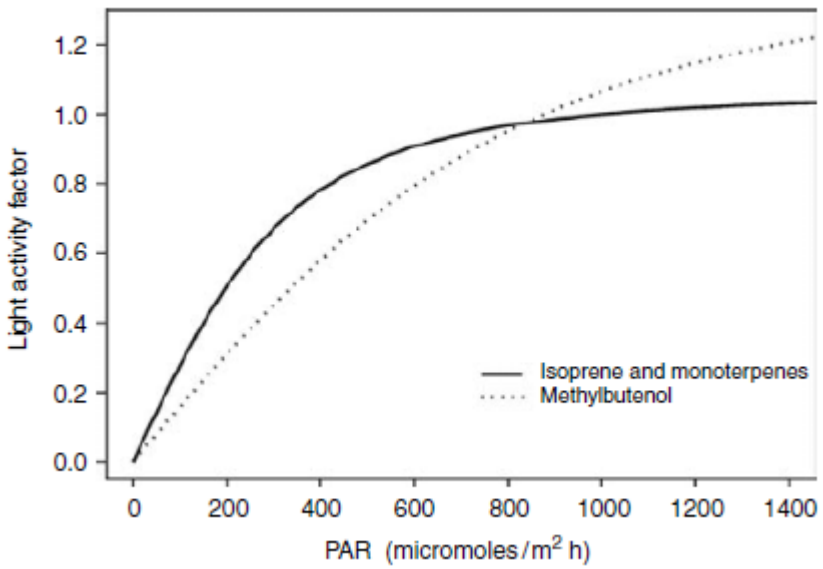


Figure 1.2: Light dependencies of biogenic VOC emissions (Koppmann 2007).

Many studies have found that monoterpene emissions are dominantly a function of temperature and are not impacted by PAR (Dement et al., 1975; Guenther et al.,1993) others have reported MT emissions are a function of both light and temperature (Koppmann, 2007). Isoprene and MT are differently emitted on the base of two mechanism: (1) temperature-dependent only and (2) temperature and light-dependent emitters. Isoprene emission is strongly light dependent while Mt emissions are indirectly influenced by light by (1) providing biosynthetic energy, (2) controlling leaf anatomical changes, and (3) photoregulating MT synthesis (Koppmann, 2007)

1.6.2 Temperature dependencies

The temperature dependence of biogenic VOC emissions is known since early experimental work (Dement et al. 1975) and can influence all categories of biogenic VOC emissions because temperature affects all biochemical reactions, including those involved in the photosynthesis process. Tingey et al. (1981) first described how isoprene emissions increased with increasing temperature but later studies showed that isoprene emissions increased with temperature until 35–45°C (Guenther et al. 1993; Harley et al. 1996), above this value the emissions decreased (Figure 1.3). This implies enzymatic dependence in emissions. Recently, Singsaas and

Sharkey (2000) explained this phenomenon by a regulatory mechanism rather than isoprene synthase destruction (Koppmann, 2007).

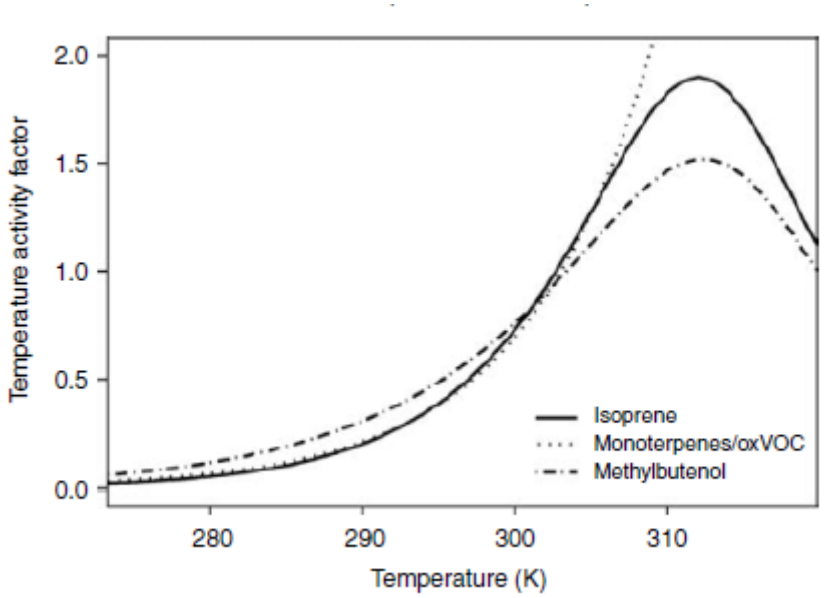


Figure 1.3: Temperature dependencies of BVOC emissions (Koppmann 2007).

The temperature dependences isoprene and light-dependent MT emitters have a different storage and release mechanism than non-light dependent MT emitters is different as well (Koppmann, 2007). Emission rates of temperature-dependent MT emitters increase exponentially with increasing temperature. This could be explained by the MT storage pool (Figure 1.4Figure 1.3) linking the emissions to MT volatility and Henry's law constant. Temperature increases the emission rate of most BVOCs exponentially by enhancing the enzymatic activities of synthesis, by raising the BVOC vapour pressure and by decreasing the resistance of the diffusion pathway (Peňuelas and Llusia, 2003).

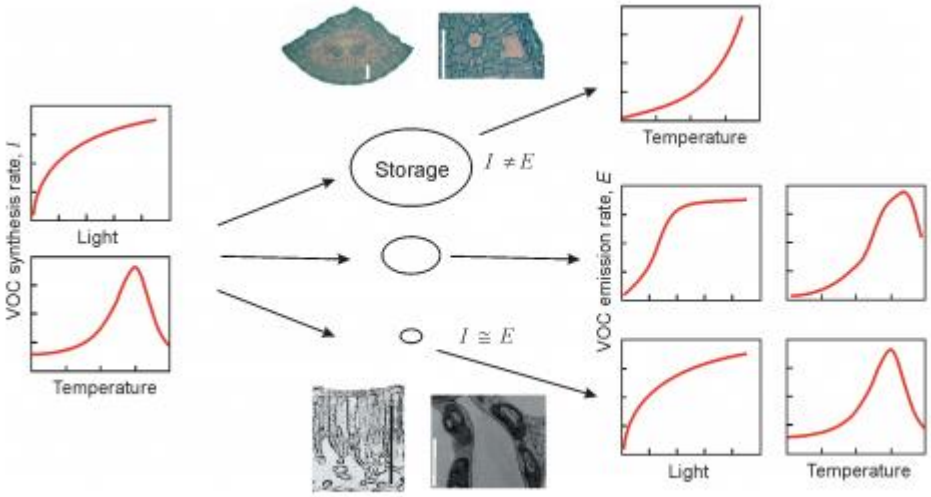


Figure 1.4: Light and temperature effects on BVOC emissions for storing and non-storing plant species (Grote and Niinemets, 2008; Laothawornkitkul et al., 2009).

The temperature dependence of oxVOC and some VR-BVOC emissions have been measured for a few plant species and ecosystems. MBO emissions exhibit a similar temperature dependence as that of isoprene (shown in Figure 1.3). Schade and Goldstein (2001) noted that oxVOC emissions are typically represented using a terpene-like temperature dependence in global and regional emission models, while Tarvainen (2005) shown that VR-VOC such as sesquiterpenes have strong temperature and seasonal dependence.

1.6.3 Humidity and drought dependencies

Guenther et al. (1991) showed slight humidity dependence of isoprene emissions; 2.3 % increase in emission per 10 % increase in relative humidity (Figure 1.5)

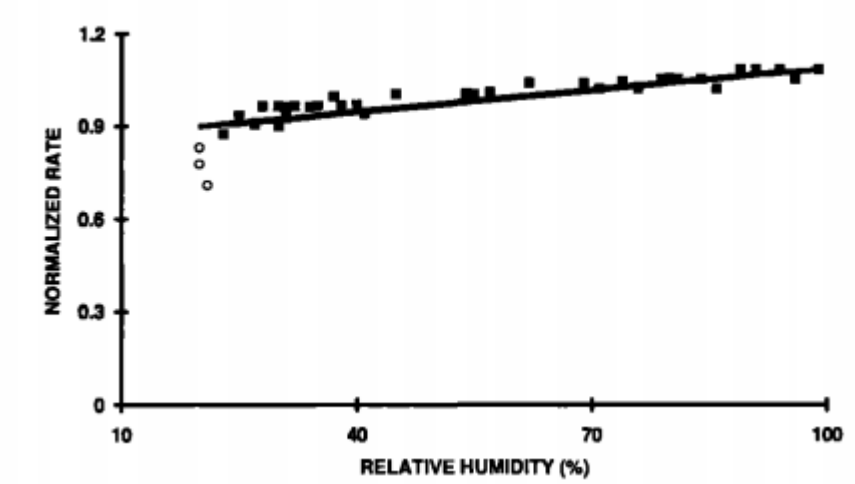


Figure 1.5: Normalized isoprene emissions from eucalyptus leaves (closed squares) as function of relative humidity. Emission rates are normalized by the observed isoprene emission at a relative humidity of 40%. (Guenther et al. 1991).

Monoterpene and some oxVOC emissions have been found to respond to changing moisture conditions. In general, emissions of monoterpenes increase during and following rain events (Helmig et al. 1998a; Schade et al. 1999). Other studies have found that acetone increases with increasing humidity, and ethanol varies as a function of the ambient relative humidity, this may have been driven by changes in stomatal conductance as a function of vapour pressure deficit (Schade and Goldstein 2001).

Photosynthesis and BVOC emissions are both affected by drought (Delfine et al., 2005; Hummel et al., 2010; Pinheiro and Chavez, 2011). Drought reduces photosynthesis by stomatal closure, leading to large morphological and physiological changes (Hummel et al., 2010), increasing leaf temperature and metabolic impairment (Escalona et al., 2002). All these processes affect the BVOC emissions because the plant decreases the carbon supply for BVOCs biosynthesis pathways.

1.6.4 Seasonal variation (phenology)

The seasonal cycle of vegetation can also influence biogenic VOC emissions. Phenological changes in leaves include physical, biological, and chemical changes and these changes can impact BVOC emissions. Biogenic VOC emissions respond to seasonal factors such as the outbreak, growth, aging and loss of foliage. Emissions are known to begin 2-4 weeks after budburst (Owen and Peňuelas, 2005). Another important factor regarding phenology is leaf

age and development. Many coniferous trees retain leaves longer than the typical growing season as defined by deciduous forests, and recent experimental work has indicated that leaf age can be important for some monoterpene and oxVOC emissions. While emissions of monoterpenes were unaffected by leaf age (Loreto et al. 2001a). Moreover, MBO (2-methyl-3-buten-2-ol) emissions were found to decrease with increasing leaf age (Harley et al., 1998) and leaf MT concentrations changed greatly during the first six months of growth and then gradually stabilized (Sharkey et al., 1991). Other studies indicate that other oxVOCs such as methanol may have decreasing emissions with increasing leaf age (Nemecek-Marshall et al. 1995).

1.6.5 Infestation

It is well known that infestations have an impact on both photosynthesis and BVOC emissions. Many authors have demonstrated infestation influences on photosynthesis (Ellsworth and Reich, 1993; Staudt and Lhoureillier, 2007; Velikova et al.,2010) and, consequently, the BVOC emissions. A few studies have examined and quantified monoterpene concentration and emission changes under insect attack for specific tree species including lodgepole pine, ponderosa pine. These studies, include beetle infestation studies and fungal inoculation studies, have found significant increases in monoterpenes due to beetle attack (Gara et al., 1993; Litvak and Monson, 1998; Jost et al., 2008; Amin et al., 2012; Prieme et al., 2000; Blande et al., 2007; Brilli et al., 2009; Berg et al., 2013). Insect herbivory can induce both substantial increases in total monoterpene emissions from vegetation and changes in the emission profile, with implications for atmospheric composition. Sesquiterpene emission rates are also elevated in conifers experiencing bark beetle attack (Heijari et al., 2011). Plant defences can be induced by pathogens and herbivores (Walling, 2000). The mechanisms involved in these defences are being elucidated rapidly (Kessler and Baldwin, 2002; De Vos et al., 2005; Kant and Baldwin, 2007; Pieterse and Dicke, 2007). Leaves normally release small quantities of BVOCs, but when herbivores damage the plant, more volatiles are being released (Paré and Tumlinson, 1997b). There are two classes of compounds emitted after insects attack: (1) green leaf volatiles (GLVs), emitted immediately after wounding (minutes) and not specifically linked to infestations (Davidson et al., 2008; Brilli et al., 2009; Arneth and Niinemets, 2010;

Niinemets, 2010); and (2) compounds that are emitted hours-days after infection, indicating induction of specific genes (Brilli et al., 2009). They consist of, for example, MTs, SQTs, MeSA (methyl salicylate) and methyl jasmonate (MeJA).

1.6.6 Other factors

Other factors might influence BVOC emissions, such as an increased atmospheric CO_2 (Koppmann, 2007) concentration, tropospheric O_3 , flooding (Copolovici and Niinemets, 2010), leaf wetness (Kim, 2001), nutrient status (Sharkey et al., 1991), circadian rhythms (Yuan et al., 2009), leaf oil content (Lerdau et al., 1994), genetic variability, and cultivar type. The different emissions could also exist in different cultivars and ecotypes even if they belong to the same species (Yuan et al., 2009).

1.7. Reaction pathways

When BVOCs are emitted from organisms they may enter the atmosphere, where they are subject to different processes depending on the specific compound and the chemical conditions in the atmosphere. They can react with oxidants (such as OH or NO_3 radicals or ozone O_3 or a chlorine atom) by one of these two mechanisms (Koppmann 2007):

1. the addition of O_3 or OH/NO_3 radicals to the double carbon bond in the biogenicVOC;
2. the substraction of an H atom from the hydrogen–carbon bond by OH or NO_3 (Finlayson-Pitts and Pitts 2000).

Most BVOCs follow the addition mechanism rather than the abstraction mechanism, with the exception of double carbon bond aldehydes that tend to react via subbstraction (Atkinson and Arey 2003). The main product of BVOC oxidation is alkyl radical (R•), which react rapidly with oxygen to form an alkyl peroxy radical (ROO•) (Koppmann 2007). The alkyl peroxy radical then can react with NO, NO_2, HOO• or another alkyl peroxy (ROO•). Reactions with NO_2 and HO• lead to the formation of peroxy nitrates ($ROONO_2$) or peroxides (ROOH). Reactions with NO can contribute to the formation of NO_2, and consequently to the formation of ozone O_3. Reactions of HOO• lead to the creation of stable products and act as a radical sink (Figure 1.6).

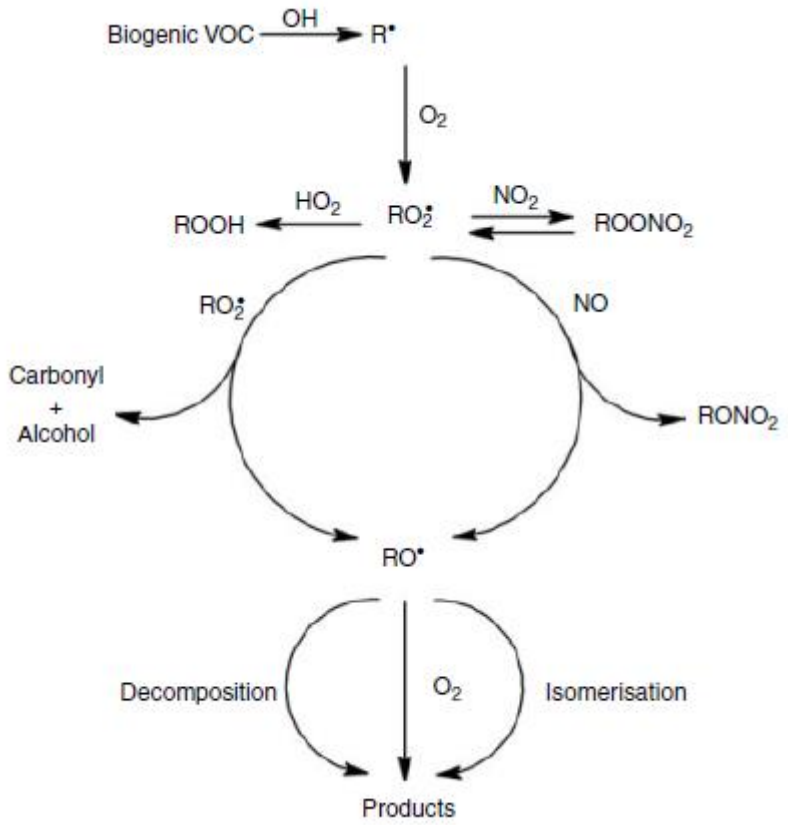


Figure 1.6: General biogenic VOC reaction pathway (Koppmann 2007).

In general, reactions with HOO• or ROO• dominate when level of NO in air is relatively low. This results in the removal of the radicals from the circulation and inhibiting the production of O_3. The reaction product of BVOC with HOO• is peroxide (ROOH), which may be deposited, photolyzed (HO• regeneration) or react with HOO• (Koppmann 2007). Reactions with NO or NO_2 dominate when the level of NO in air is high. This reaction produces alkyl nitrate (RONOO•) or alkoxy radical (RO•). Alkyl nitrate is a more stable compound and can remove NO_x from the circulation and alkoxy radical may be isomerized or decomposed to form different products. These reactions may contribute to the formation of NO_2, and an increase in the ozone level in the air (Koppmann 2007). However, the reactions with NO_2 lead to the formation of peroxy nitrates ($ROONO_2$), which, due to their durability, may play the role of "storage" of NO_x in the air (Bogacki and Sygula 2013).

1.7.1 Isoprene oxidation

An example of biogenic VOC oxidation is the reaction of isoprene and the OH radical. When NO concentrations are sufficiently low, isoprene oxidation can proceed by a HOx-mediated (HO•+HOO•) mechanism, which until recently was largely unexplored (Kuhlmann and Lawrence, 2004; Rosenstiel et al., 2003; Wiedinmyer et al.,2006). HO• addition to isoprene, followed by O_2 addition and the peroxy radical + HO_2 reaction, leads to formation of isoprene hydroxyhydroperoxide (ISOPOOH) in yields exceeding 70% (Crutzen et al., 2000; Lelieveld et al., 2008; Ren et al., 2008), with approximately 2.5% forming methacrolein (MACR) and 3.8% forming methylvinylketone (MVK) (Liu et al., 2013; Navarro et al., 2011). In general, the primary products of the isoprene–OH reaction are methyl vinyl ketone, methacrolein and formaldehyde (Figure 1.7)

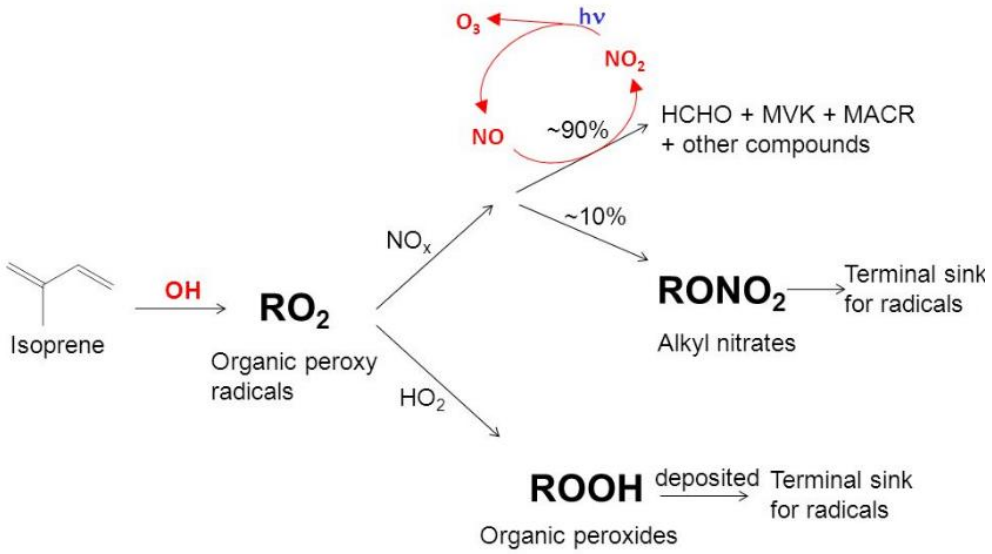


Figure 1.7: Isoprene and OH reaction pathway.

1.7.2 Monoterpene oxidation

Oxidation of monoterpenes in the atmosphere occurs as a result of the three possible reactions (Atkinson and Arey, 2003; Calogirou et al., 1999:

1. reaction with HO• radicals, during the day
2. reaction with NO_3• radicals, during the night
3. reaction with O_3 (ozonolysis), during the day and the night.

Addition of the HO• radical to the double carbon bond is the first yet preferential step in the mechanism of the oxidation of the monoterpenes. The mechanism of oxidation based on the separation of the hydrogen atom from the monoterpene is a reaction of a lesser importance. In both cases. The alkyl radical R• is formed, which is then subject to other reactions. Similarly, to the case with the HO• radical, also NO_3 radical oxidizes monoterpenes by attaching to the double carbon bond, as a result of this reaction, the nitroxyl or alkyl radical is created. In the case of reaction of monoterpene oxidation with ozone, the double carbon bond in monoterpene is decomposed.

An important biogenic VOC oxidation sequence is the α-pinene-HO• reaction, shown in Figure 1.8. This is one of the dominant loss mechanisms for α-pinene and is representative of the type of reactions typically occurring between terpenes and HO•.

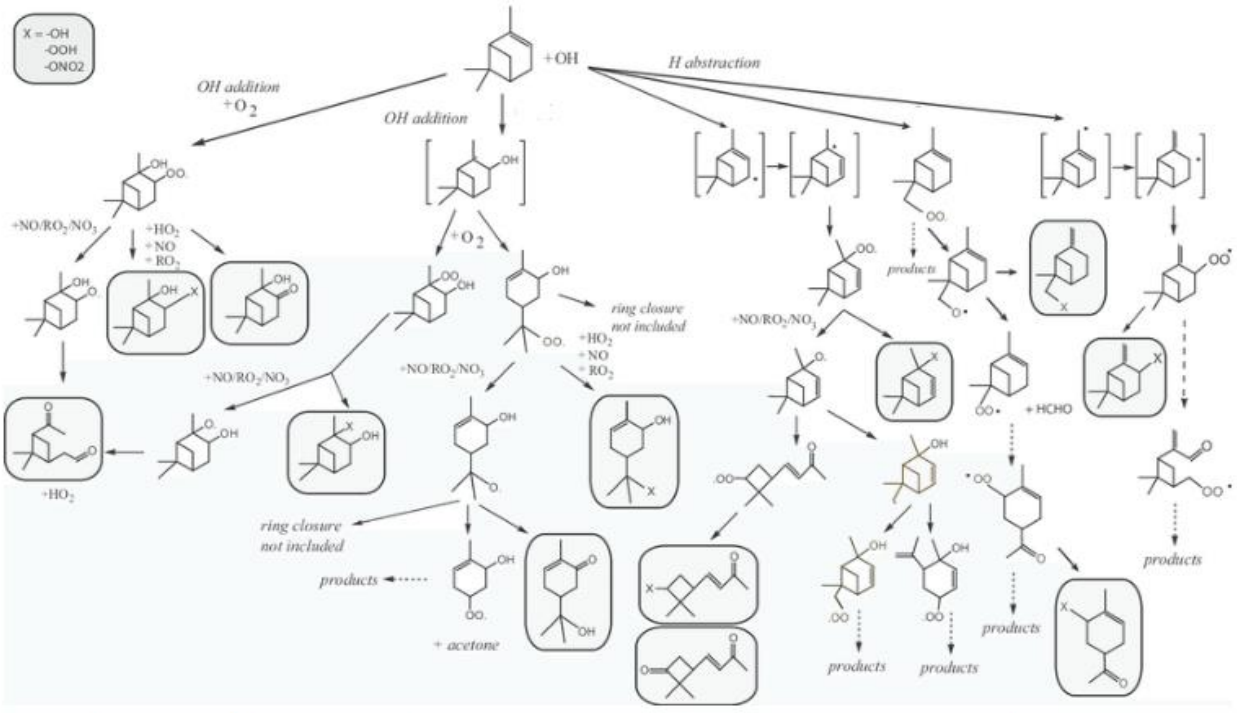


Figure 1.8: First oxidation steps of the α-pinene oxidation by OH. Framed compounds represent stable species (Valorso et al., 2011)

1.7.3 Lifetimes

As described before, the presence of double bonds in the chemical structure of BVOCs causes high tropospheric reactivity and makes chemical reactions their dominant loss mechanism. Table 1.4 lists the calculated atmospheric lifetimes for many biogenic VOC species derived from

laboratory-measured reaction rate constants and typical mixing ratios of OH, O_3 and NO_3 (Atkinson and Arey 2003; Atkinson et al. 1990, 1995, 1999; Calvert et al. 2000; Corchnoy and Atkinson 1990; Grosjean and Grosjean 1994; Meyrahn et al. 1986; Papagni et al. 2001; Reissell et al. 2001; Rudich et al. 1996; Smith et al. 1996).

Table 1.4: Calculated atmospheric lifetimes of biogenic volatile organic compounds (Atkinson and Arey 2003).

Biogenic VOC	Lifetime[a] for reaction with		
	OH[b]	O_3[c]	NO_3[d]
Isoprene	1.4 h	1.3 days	1.6 h
Monoterpenes			
Camphene	2.6 h	18 days	1.7 h
2-Carene	1.7 h	1.7 h	4 min
3-Carene	1.6 h	11 h	7 min
Limonene	49 min	2.0 h	5 min
Myrcene	39 min	50 min	6 min
cis-/*trans*-Ocimene	33 min	44 min	3 min
α-Phellandrene	27 min	8 min	0.9 min
β-Phellandrene	50 min	8.4 h	8 min
α-Pinene	2.6 h	4.6 h	11 min
β-Pinene	1.8 h	1.1 days	27 min
Sabinene	1.2 h	4.8 h	7 min
α-Terpinene	23 min	1 min	0.5 min
γ-Terpinene	47 min	2.8 h	2 min
Terpinolene	37 min	13 min	0.7 min
Sesquiterpenes			
β-Caryophyllene	42 min	2 min	3 min
α-Cedrene	2.1 h	14 h	8 min
α-Copaene	1.5 h	2.5 h	4 min
α-Humulene	28 min	2 min	2 min
Longifolene	2.9 h	>33 days	1.6 h
Oxygenated VOCs			
Acetone[e]	61 days (Atkinson et al. 1999)	>4.5 year[f]	>8 year (Atkinson et al. 1999)
Camphor	2.5 days (Reissell et al. 2001)	>235 days (Reissell et al. 2001)	>300 days (Reissell et al. 2001)
1,8-Cineole	1.0 days (Corchnoy and Atkinson 1990)	>110 days (Atkinson et al. 1990)	1.5 year (Corchnoy and Atkinson 1990)
cis-3-hexen-1-ol	1.3 h (Atkinson et al. 1995)	6.2 h (Atkinson et al. 1995)	4.1 h (Atkinson et al. 1995)
cis-3-hexenyl acetate	1.8 h (Atkinson et al. 1995)	7.3 h (Atkinson et al. 1995)	4.5 h (Atkinson et al. 1995)
Linalool	52 min (Atkinson et al. 1995)	55 min (Atkinson et al. 1995)	6 min (Atkinson et al. 1995)
Methanol	12 days (Atkinson et al. 1999)	>4.5 year[f]	2.0 year (Atkinson et al. 1999)
MBO	2.4 h (Papagni et al. 2001)	1.7 days (Grosjean and Grosjean 1994)	7.7 days (Rudich et al. 1996)
6-methyl-5-hepten-2-ol	53 min (Smith et al. 1996)	1.0 h (Smith et al. 1996)	9 min (Smith et al. 1996)

[a] From Calvert et al. (2000) unless noted otherwise.

[b] Assumed OH radical concentration: 2.0 × 106 molecules/cm3, 12-h daytime average.

[c] Assumed O_3 concentration: 7 × 1011 molecules/cm^3, 24-h average.

[d] Assumed NO_3 radical concentration: 2.5 × 108 molecules/cm^3, 12-h nighttime average.

[e] Photolysis will also occur with a calculated photolysis lifetime of ~60 days for the lower troposphere, July, 40◦N Meyrahn et al. (1986).

[f] Estimated.

Isoprene lifetimes with respect to OH and NO_3 are on the scale of hours and with O_3 on the scale of days. On the other hand, monoterpenes and sesquiterpenes react more quickly than isoprene, with lifetimes with respect to OH and NO_3 on the scale of minutes to hours and with O_3 on the scale of minutes to days. OxVOCs have much more variable lifetimes, ranging from minutes for some compounds to days for other compound. VR-BVOCs have lifetimes that are estimated to be on the order of minutes or less.

1.7.4 Research project goals

BVOC emissions have been extensively studied in the last twenty years but their impact on the atmosphere and their implications are still a subject of research. The general aim of this dissertation is the study and monitoring of BVOC emissions in different place and time and the correlation between emissions and climate changing, atmospheric reaction and behaviour of birds. This dissertation is divided in 4 main chapters in which we discuss 4 different aspects of the study of BVOC emissions.

1. BVOC emissions in National Park of Sibillini are scarcely studied and the characteristic landscape provides a high amount of organic compounds and emission of specific BVOCS. This is a perfect site to develop an analytical method and to monitor the BVOC emissions. This first chapter concerns the monitoring of emissions from April 2016 to September 2017 almost every months. Here is also discussed the development and validation of the analytical method we used to quantify BVOC emissions using thermal desorption coupled with gas chromatography (TD-GC-MS).
2. Solid phase-microextraction (SPME) was applied to study BVOC released by bush species of Juniperus Communis and Juniperus Oxycedrus. Different plant parts were sampled (branches, leaves, berries) and analyzed in the laboratory using the SPME-GC-MS analysis. Samples were collected in July 2016 and the study was conducted to complete the monitoring of emissions in the National Park of Sibillini to understand which BVOCscome from different parts of the plant.
3. The third chapter looks at the study of BVOC emissions in Amazon rainforest during dry season. This study was performed during my abroad period at the Max Planck

Institute for Chemistry, Germany under the supervision of Prof. Dr. Jonathan Williams and Dr. Nora Zannoni. MPIC developed and manages the ATTO (Amazonian Tall. Tower Observatory) project in cooperation with INPA (National research institute of Amazonia). Specifically, I participated in the method characterization, samples analysis and data processing of VOC samples through GC-TOF-MS from the Amazonian dry season. The chapter describes terpenoid compounds and their oxidation products in the Amazons with a specific focus on chiral molecules. The site is equipped with a 325m tall tower and sampling was performed on this tower at four different heights (40, 80, 150 and 320 m). In this way, it is possible to study the variation of emission at different heights and the oxidation products of terpenoids creating a vertical profile of emission. Moreover, the analysis were performed using a thermal desorption coupled with a TOF-MS equipped with a cyclodextrin column to observe chiral compounds.

4. The fourth chapter was performed in collaboration with Max Planck Institute for Chemistry, Max Planck Institute for Ornithology and University of Pisa. The study shows results of field observations conducted in a rural site in Tuscany (Arnino, near Pisa) in the frame of the campaign HOMING. The chosen site is the aviary from the Department of Biology of the University of Pisa. Forty years of olfactory research on birds conducted by the University of Pisa have reported the importance of the sense of smell for pigeons to orientate back home. We measured VOCs with weather parameters in order to investigate which odours help pigeons orienting and which are the main source of emissions of such VOCs. Samples were collected every day for 3 weeks in the period from May to June 2018. For this goal, in the sampling site there are several cages with homing pigeons who have the opportunity to fly during the day. In addition, the site is surrounded (compared to the four cardinal points) by four completely different landscapes (sea to the west, city to the east, pine forest to the north and characteristics plants to the south).

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2. Development and validation of a cheap and fast analytical method for monitoring BVOC emissions in Sibillini National Park

2.1. Introduction

Natural sources, in particular vegetation and plants, emit BVOCs, the amount, the relative proportion and the kind of molecules depend on the vegetation species and are conditioned by the environmental condition (Raveane et al., 2013). The plant emissions affect atmospheric photochemistry because they interact both with anthropogenic emissions and humidity (Teng et al., 2017). Until now, different sampling techniques and methods have been proposed for the analysis of VOCs depending on the complexity and variability of organic vapours in air mainly developed for pollution surveillance (Wang and Austin, 2006). Many sampling methods include the use of canister or air sampling bags (Tedlar, Teflon, Melinex) (Hansen et al., 2013) but, these techniques, where a fixed amount of air is trapped in a container, show different disadvantages; canisters are very expensive and have a limited sample volume (Jayanty, 1989); moreover, some compounds present in BVOC have low stability in them (Stuart et al., 1998) while sampling bags are inexpensive but the compounds may not stay stable for more than 24-48 h. A valid alternative for sampling is the use of sorbent tubes (Woolfended, 2010) especially if coupled with forced controlled flux. They provide a more versatile alternative to canisters or sampling bags for most BVOCs monitoring application and are easy to transport (Bianchi et al., 1993, Watson et al., 2011). They offer a low cost choice and can be reused approximately 100 times, after cleaning, before replacement (Harper 2000, Woolfended 1997). These tools permit to concentrate the compounds on an absorbing support material however the absorption selectivity of the support with respect to molecules of different affinity is a risk of the techniques. The samples anyhow obtained have to be analysed by methods able to separate and quantify the different molecules. The technique of choice for this is the gas-chromatography. If sampling involves absorption, the substances must first be desorbed; they can be extracted with a solvent or thermally desorbed and then analysed by gas chromatography with a wide range of detector like mass spectrometry (MS) or flame ionization (FID). Thermal desorption method permits to reach low detection limits in the order

of pptv (Ras et al., 2009, Wu and Chang, 2012) that make the technique eligible for monitoring the presence of BVOCs in atmosphere.

Multibed sorbent tube is the sampling tool selected in this study because it has high sampling versatility, it is compatible both with non-polar and polar compounds in air, it is even easily stored (Seeley and Broadway 1994).

In this chapter I describe the development and validation of a method to determine BVOCs in air using TD-GC-MS by using one reference substance for each class of molecules. In the next chapter I describe the use of the method for outdoor measurements in order to monitor, for about 2 years, the BVOCs in some sites with different environmental characteristics. That in order to correlate them with seasonal variation, temperature and humidity.

2.2. Materials and method

An automated thermal desorption system (TD-20, Shimadzu) coupled with a gas chromatography mass spectrometer (GCMS-QP2010Plus, Shimadzu) was used for the analysis of BVOCs (Figure 2.1a). The gas-chromatograph was equipped with a capillary column (Rxi-624SilMS, 30m x 0.25mm x 1.4 µm, fused silica column, Restek). Samples were collected using a personal sample pump LIFE XP sold by Mega System S.r.l. (Figure 2.1b).

a b

Figure 2.1: a) TD-GC-MS used for BVOC analysis; b) Sampling pumps.

In order to analyse the broad range of BVOCs emitted by plants and vegetation, we chose a multi-sorbent cartridge suitable for the determination of a wide range of compounds. The

selection of the cartridge was based on previous studies (Harper, 2000, Woolfended, 1997 Ras, 2009). The multisorbent cartridge Carbotrap C 300 (17.8 cm long, 0.6 cm outside diameter and 0.4 cm internal diameter) was selected for the analysis. It is filled with VOC adsorbents Carbopack C (graphitized carbon black, 80/100 mesh, weak sorption strength, hydrophobic) Carbopack B (graphitized carbon black 60/80 mesh, medium sorption strength) and Carbosieve S-III (spherical carbon molecular sieve, 60/80 mesh, very strong adsorptive strength) (Figure 2.2). The adsorbents are packed in order of increasing adsorbent strength, from sample inlet to sample outlet. The largest molecules in the sample are trapped by the first bed of adsorbent. Smaller molecules are trapped by the succeeding, stronger beds (Seco, 2013, Magnusson, 2015). Cartridges were conditioned, before the use, by thermal cleaning (trap cool temperature of 50°C, trap heat temperature of 250°C for 10 minutes, interface temperature 280°C) under a flow rate of nitrogen of 60 ml/min for 30 minutes.

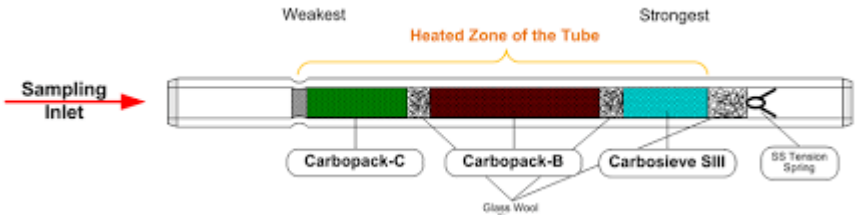

Figure 2.2: Sampling Tube: Carbotrap C 300.

2.2.1 Desorption and analytical method

The sampling tubes were thermally desorbed at 250 °C under He flux of 60 ml min^{-1} for 8 min (primary desorption). After primary desorption, the cold trap was rapidly heated from -10°C to 250°C and carrier gas (He 60 ml/min) transfers the desorbed analytes into the gas chromatograph for separation (secondary desorption), identification and quantification. The volatiles in the samples go into the capillary column via a transfer line heated at 260 °C. The oven was initially set to 35 °C for 5 min, increased to 220°C at a rate of 11°C min, maintained for 2 minutes and then increased again to 280°C at a rate of 20°C min^{-1}, maintained for 10 min and, at the end, increased to 310 °C at a rate of 20°C min^{-1} and then maintained for 5 min.

2.2.2 Chemical and materials

Hexanal, Toluene, α-Pinene, Limonene, 4-Terpineol and β-Caryophyllene were purchased from Sigma-Aldrich and Carlo Erba with purity of not less than 95%. Glass tubes Carbotrap C 300 were obtained from Supelco (Bellefonte, PA, USA).

2.3. Development of the analytical method and its Validation

2.3.1 Calibration Curves

A calibration curve for every of the molecules: Hexanal, Toluene, α-Pinene, 4-Terpineol and β-Caryophyllene was obtained. The procedure to obtain the calibration curve is as follow: pour 0.0200 g of the pristine chemical in a 1 ml flask and dissolve with hexane to obtain a 20,000 µg/mL standard stock solution. Dilute appropriate aliquots of this standard stock solution to obtain further three standard solutions of 2, 20 and 200 µg/mL (working standards). These three standard solutions were used to pick 5 aliquots so to inject in tubes 10, 100, 300, 400 and 600 ng of substance respectively. For each calibration point 10 replicas were made and the value of standard deviation and repeatability of the method was calculated. The quantities above are analysed versus the peak area from the chromatograms. Every sample was homogenized, inside the tube, by an N_2 flux at 100 ml min^{-1} for 3 minutes. The measurements were executed setting the Gas Cromatograph interface temperature to 250°C. Electron impact spectra were obtained with electron energy of 70 eV. For standards analysis, a 8 min solvent delay was applied in order to avoid the saturation of mass spectrometer detector with the hexane. Figure 2.3 shows as an example the calibration curve for α-Pinene.

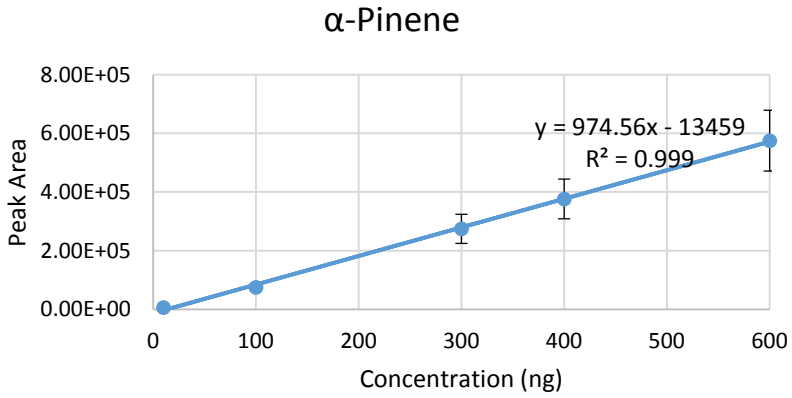

Figure 2.3: Calibration curve of α-Pinene.

2.3.2 Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were calculated on the base of standard deviation of the response and slope of calibration curve, using the expressions:

$$LOD = \frac{3.3\sigma}{slope}$$

$$LOQ = \frac{10\sigma}{slope}$$

Where σ is the standard deviation of the calibration curve at lowest point (at 10 ng). The thermal desorption allows to eliminate the use of solvents or other intermediate purification processes and increases the sensitivity of the method reaching low values of LOD and LOQ. In particular, with this technique, we detect less than 1.00 ng per tube for all the target compounds excluding Hexanal at 3.23 ng per tube. Table 2.1 shows the results of sensitivity and values of LOD and LOQ.

Table 2.1: Method target BVOCs analytes. CAS number (CAS no), retention time (RT min), boiling point (BP, °C at 760 mmHg), Limit of Detection (LOD, ng), Limit of Quantification (LOQ, ng) Sensitivity (slope of calibration curve).

Compound	CAS no.	RT (min)	BP (°C)	LOD (ng)	LOQ (ng)	Sensitivity
Hexanal	110 54 3	12.27	129	3.23	9.80	239041
Toluene	108 88 3	11.29	111	0.57	1.72	772814
α-pinene	80 56 8	14.52	155	0.44	1.34	974565
4-terpineol	562 74 3	19.01	209	0.78	2.37	802955
β-caryophyllene	87 44 5	22.30	268	0.89	2.70	234585

2.3.3 Linearity

In order to conduct an accurate investigation of the method linearity, a good knowledge and definition of the working range is essential. Samples with standard BVOC amount ranging from 5 to 1000 ng approximately were used to evaluate the linearity. Linearity was performed at different concentrations in the calibration curve range and at lower and higher concentrations to evaluate the ability of our analytical method to give results that are directly proportional to the concentration of the analytes in the samples within a given range of validity. The linearity of multi-point calibration was considered acceptable when R^2 (linear regression square coefficient) ≥ 0.99, S/N (signal to noise ratio) >10, and peaks showed a Gaussian shape. The

S/N value is calculated using the GC/MS software by Shimadzu GC/MS instruments packages. Each of the standard compounds shows an $R^2 \geq 0.99$ until 1000 ng.

2.3.4 Repeatability

Experiments with replicate tubes were carried out in our laboratory to evaluate the repeatability of the method, considering instrumental and standard preparation errors to evaluate the method, six replicates at 3 different amounts (10, 100, 600 ng) have been tested. The relative standard deviation (Table 2.2) for each series of replicates is less than 11%, this demonstrates the validity of the instrument and the method in accordance with EPA performance criteria (U.S. EPA, 1999).

2.3.5 Accuracy

Accuracy was measured as the percentage recovery; for this goal we measured the response after an injection of 200 ng of standard solution of BVOCs in a new tube. Recoveries were greater than 85% for all the considered compounds, the results are shown in detail in Table 2.2.

2.3.6 Calculation of the uncertainties

Total uncertainty was calculated from precision, systematic errors and random errors. The total uncertainty is given by the formula:

$$U_{tot} = \sqrt{U_{rip}^2 + U_{cal}^2 + U_{glass}^2 + U_{std}^2}$$

Where:

U_{rip} is the uncertainty due to absolute repeatability; calculated on the basis of standard deviations of the replications performed at a given concentration within the calibration range

U_{cal} is the uncertainty due to instrumental calibration; calculated on the basis of the average of the replications performed at a given concentration within the calibration range

U_{glass} is the uncertainty due to the variability of glassware and all materials used to prepare calibration curve declared by the producer company;

U_{std} is the uncertainty due to the standard purity declared by chemical company.

The total uncertainty U_{tot} (see Table 2.2) was lower than 20% for all BVOCs studied

Table 2.2: Relative standard deviation (RSD%) for 3 different concentrations, Recovery at 200 ng (Recovery %), Total uncertainty (Utot %).

Compound	**RSD %**			**Recovery %**	**U_{tot} %**	**R^2**	**RF (area/µg)**
	10 ng	**100 ng**	**600 ng**				
Hexanal	10.7	10.7	6.6	85.5	14.2	0.995	244567
Toluene	6.2	8.2	5.9	87.5	16.1	0.991	626749
α-pinene	5.3	7.5	4.3	91.0	18.7	0.997	834827
4-terpineol	8.3	7.1	10.7	92.0	15.2	0.996	641452
β-caryophyllene	4.0	7.1	10.9	95.0	14.6	0.995	202350

2.3.7 Method performance evaluation in the laboratory

The main object of the project is to develop an analytical method to determine a class of compounds using a single standard of that class; for this reason, I studied the validity of the method in the laboratory. In order to evaluate the performance of the method and to demonstrate its validity, 5 standard solutions of D-Limonene at the same concentration of calibration curve (from 10 to 600 ng) were prepared and were quantified using the α-Pinene RF (α-Pinene is the reference standard for all monoterpenes). In this way, it is possible to evaluate the percentage recovery at each concentration and, subsequently, demonstrate the validity of the use of RF to quantify a class of compounds. The results are shown in Table 2.3 below; it shows a recovery error proportional to the actual amount. The recovery difference varies from 30% to only 1.5% when passing from 10 ng to 600 ng of substance. The obtained results suggest that the use of class reference (α-Pinene) underperform with respect to the use of pristine standard if used for very low concentrations but it is comparable when the concentration increases.

Table 2.3: Quantification of D-Limonene. Actual amount of standard (ng), measured amount of standard (ng) and recovery percentage of D-Limonene using both D-Limonene and α-Pinene as standards.

Actual amount of standard (ng)	Measured amount (ng) with D-Limonene as standard	Measured amount(ng) with α-Pinene as standard	Recovery (%) with D-Limonene as standard	Recovery (%) with α-Pinene as standard
10	7	4	70.0	40.0
100	82	74	82.0	74.0
300	276	256	92.0	85.3
400	365	354	91.3	88.5
600	542	533	90.3	88.8

The recovery is greater than 85% for concentrations of 300 to 600 ng, these values are consistent with the accuracy calculated in the validation method for standard calibration curves.

2.4. A case study of BVOCs Monitoring

2.4.1 Site

The site chosen for this study was a rural area located at 1000m above mean sea level in the Sibillini National Park (Macereto site (Macerata) of Marche region in Italy latitude 42° 57' 41''N; longitude 13° 07' 13''E) (Figure 2.4)., in order to evaluate the analytical method. The experimental site is a plateau where there is a large presence of *Juniperus Communis* and *Juniperus Oxycedrus* (Figure 2.5)

Figure 2.4: Sampling site, Macereto. Landscape with Juniperus Communis and Juniperus Oxycedrus bushes.

Figure 2.5: On the left Juniperus Communis and on the right Juniperus Oxycedrus.

2.4.2 BVOC emission measurements

The measurements of BVOC emissions were performed using a personal pump positioned near Juniperus bushes (Figure 2.6). The samples were collected during the day almost once a month and then analysed in the laboratory using the TD-GC-MS. Samples were taken for 40 min at a flow of 200 ml min^{-1}, leading to a collection of 8 L of air in each cartridge. The glass tubes (with trapped VOCs) were closed with brass endcaps and brought to the laboratory. When delivered to the laboratory, the glass tubes were stored in desiccator at room temperature before analysis (no longer than 2 weeks).

Figure 2.6: Sampling method. On the left sampling pump near Juniperus Communis, on the right sampling pump near Juniperus Oxycedrus.

The samples were collected in the period from April 2016 to September 2017 in the same site. In Table 2.4 the sampling days and the temperature and humidity conditions are summarized.

Table 2.4: Overview of when samples were collected in different months (from 22th to 24th at 10 am) and meteorological conditions.

Month/Year	Temperature (°C)	Humidity (%)
April 2016	24.5	37.0
May 2016	24.9	36.9
June 2016	25.1	31.6
July 2016	26.9	35.7
August 2016	27.8	43.0
September 2016	21.2	37.4
October 2016	15.5	33.1
March 2017	9.1	30.5
April 2017	16.8	44.1
May 2017	21.6	46.0
June 2017	23.5	58.1
July 2017	25.8	48.7
August 2017	26.7	40.5
September 2017	20.8	42.6

2.4.3 Qualitative evaluation of BVOC emissions

Qualitative identification of molecules present in the field samples was conducted on the base of Kovats retention index using values found in literature (Kaban, 2010, Kiralan, 2015, Flores et al., 2004) and NIST mass spectrum library. Table 2.5 shows the compounds found in the in situ samples and their identification indexes.

Table 2.5: KI^A: Kovats Index calculated for Rtx-624Sil-MS (Restek; 30 m, 0.25 mm id, 1.4 µm film thickness) installed on GC-MS; KI^B: Kovats Index found for Rtx-624Sil-MS (Restek; 30 m, 0.25 mm id, 1.4 µm film thickness); RI^B: Reliability of Identification: a, mass spectrum and retention time identical with an authentic standard sample; b, mass spectrum.

Compounds	KI^A	KI^B	RI^B
Acids			
Acetic Acid	723	717	b
Propanoic Acid	819	817	b
Butanoic Acid	890	890	b
Hexanoic Acid	1068	1068	b
Esters			

Bornyl Acetate	1337	1341	b
Terpinyl Acetate	1389	-	c
Aliphatic Hydrocarbons			
Heptane	700	-	a
Octane	800	-	a
Nonane	900	-	a
Undecane	1100	-	a
Dodecane	1200	-	a
Tridecane	1300	-	a
Tetradecane	1400	-	a
Pentadecane	1500	-	a
Hexadecane	1600	-	a
Heptadecane	1700	-	a
Aldehydes			
3-Methylbutanal	686	687	b
Pentanal	735	735	b
Hexanal	840	-	a
2-Hexenal	905	905	b
Heptanal	945	941	b
2-(E)-Octenal	1119	1116	b
Octanal	1043	1039	b
Nonanal	1150	1151	b
2-(E)-Nonenal	1221	1226	b
Decanal	1253	-	c
4-(Z)-Decenal	1301	-	c
Aromatic Hydrocarbons			
Toluene	789	791	b
Napthalene	1260	1253	b
p-Xylene	893	892	b
Terpenes			
α-Pinene	952	-	a
Sabinene	996	991	b
β-Pinene	992	988	b
3-Carene	1024	1022	b
D-Limonene	1048	-	a
O-Cymene	1057	1059	b
Eucalyptol	1065	1064	b
γ-terpinene	1078	1079	b
4-terpineol	1229	-	a
Copaene	1406	1403	b
Isolongifolene	1469	1465	b
Thujopsene	1470	-	c
β-caryophyllene	1476	-	a

2.4.4 BVOC emissions

Quantification was performed after calculation of standard curves and mean response factors (RF) for each of the selected standard compounds. Here the response factor of every of the selected standard molecules is used for all the molecules of the same class hypothesizing that similar molecules have a similar response factor (for example the RF of the Hexanal is used for all the aldehydes in the chromatogram). The molecules that do not belong to the classes represented by the standards (i.e. acids, ketones, hydrocarbons etc) are all quantified using Toluene RF. Table 2.6 to Table 2.9 summarize the values of BVOCs found during the monitoring in different season and years for sampling near the Juniperus Communis and near the Juniperus Oxycedrus. The values indicate the total amount of all the molecules of every class.

Table 2.6: Class concentration of the major volatile components of air samples obtained close to Juniperus Communis. The results are expressed in pptv (considering Molar Volume at room temperature and pressure of 1 atm) and relative calculated value of uncertainty. The results are for the period from April 2016 to October 2016.

Compounds	April	May	June	July	August	September	October
Acids	14.88 ± 2.19	25.74 ± 4.14	30.51 ± 4.49	12.59 ± 2.07	4.98 ± 0.80	0.63 ± 0.10	<LOD
Esters	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Aliphatic Hydrocarbons	167.92 ± 27.04	184.69 ± 29.74	206.57 ± 33.26	124.32 ± 20.02	25.04 ± 4.03	1.84 ± 0.30	2.21 ± 0.36
Aromatic Hydrocarbons	53.27 ± 8.58	48.96 ± 7.88	36.76 ± 5.92	24.96 ± 4.02	3.23 ± 0.52	1.54 ± 0.25	0.89± 0.14
Alcohols	<LQ	<LQ	<LOD	<LOD	<LOD	<LOD	<LOD
Ketones	5.17 ± 0.83	3.82 ± 0.62	0.45 ± 0.07	<LQ	<LOD	<LOD	<LOD
Aldehydes	103.41 ± 14.68	752.14 ± 106.80	1455.84 ± 206.73	354.19 ± 50.29	51.79 ± 7.35	103.84 ± 14.75	9.39 ± 1.33
Monoterpenes	954.95 ± 178.58	638.981 ± 119.49	171.27 ± 32.03	88.36 ± 16.52	57.69 ± 10.79	10.70 ± 2.00	<LOD
Sesquiterpenes	<LOD	367.25 ± 53.62	668.90 ± 97.66	157.20 ± 22.95	13.09 ± 1.91	<LOD	<LOD
Hydroxyl-terpenes	130.63 ± 19.86	79.54 ± 12.09	23.96 ± 3.64	15.77 ± 2.40	10.53 ± 1.60	<LOD	<LOD

Note: **Acids, Esters, Aliphatic Hydrocarbons, Aromatic Hydrocarbons, Alcohols** and **Ketones** have been quantified using the response factor of **Toluene**;

Aldehydes have been quantified using the response factor of **Hexanal**;

Monoterpenes have been quantified using the response factor of **α-Pinene**;

Sesquiterpenes have been quantified using the response factor of **β-Caryophyllene**;

Hydroxyl-terpenes have been quantified using the response factor of **4-Terpineol**.

Table 2.7: Class concentration of the major volatile components of air samples obtained close to Juniperus Communis. The results are expressed in pptv (considering Molar Volume at room temperature and pressure of 1 atm) and relative calculated value of uncertainty. The results are for the period from March 2017 to September 2017.

Compounds	March	April	May	June	July	August	September
Acids	<LOD	5.67 ± 0.91	16.51 ± 2.66	31.52 ± 5.07	30.51 ± 4.91	17.39 ± 2.80	3.47 ± 0.56
Esters	<LOD	<LOD	<LOD	<LOD	<LOD		<LOD
Aliphatic Hydrocarbons	<LOD	9.84 ± 2.01	189.52 ± 30.51	285.29 ± 45.93	308.17 ± 49.61	157.23 ± 25.31	6.84 ± 1.10
Aromatic Hydrocarbons	<LOD	12.48	56.76 ±	42.56	35.61 ±	26.52	4.18
Alcohols	<LOD	<LOD	0.52 ± 0.08	<LQ	<LOD	<LOD	<LOD
Ketones	<LOD	<LOD	7.45 ± 1.20	4.85 ± 0.78	1.38 ± 0.22	<LQ	<LOD
Aldehydes	<LOD	85.27 ± 12.11	145.57 ± 20.67	392.99 ± 55.80	958.88 ± 136.16	584.19 ± 82.95	103.84 ± 14.75
Monoterpenes	<LOD	47.79 ± 8.94	996.27 ± 186.30	738.981 ± 138.19	281.38 ± 52.62	158.76 ± 29.69	10.79 ± 2.02
Sesquiterpenes	<LOD	<LOD	12.24 ±	297.95	528. 02 ±	189.29	<LQ
Hydroxyl-terpenes	<LOD	18.63 ± 2.83	173.96 ± 26.44	85.97 ± 13.07	43.69 ± 6.64	23.42 ± 3.56	<LQ

Note: **Acids, Esters, Aliphatic Hydrocarbons, Aromatic Hydrocarbons, Alcohols** and **Ketones** have been quantified using the response factor of **Toluene**;

Aldehydes have been quantified using the response factor of **Hexanal**;

Monoterpenes have been quantified using the response factor of **α-Pinene**;

Sesquiterpenes have been quantified using the response factor of **β-Caryophyllene**;

Hydroxyl-terpenes have been quantified using the response factor of **4-Terpineol**.

Table 2.8: Class concentration of the major volatile components of air samples obtained close to Juniperus Oxycedrus. The results are expressed in pptv (considering Molar Volume at room temperature and pressure of 1 atm) and relative calculated value of uncertainty. The results are for the period from April 2016 to October 2016.

Compounds	April	May	June	July	August	September	October
Acids	18.80 ± 3.03	31.54 ± 5.08	45.98 ± 7.40	38.29 ± 6.16	32.91 ± 5.30	<LOD	<LOD
Esters	32.91	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Aliphatic Hydrocarbons	103.42 ± 16.65	517.86 ± 83.38	874.39 ± 140.78	569.21 ± 91.64	414.10 ± 66.67	329.07 ± 52.98	56.41 ± 9.08
Aromatic Hydrocarbons	131.63 ± 21.19	168.98 ± 27.21	216.25 ± 34.81	189.47 ± 30.50	63.51 ± 10.23	47.01 ± 7.57	42.31 ± 6.81
Alcohols	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ketones	42.31 ± 6.81	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Aldehydes	1817.54 ± 258.09	2756.38 ± 391.41	4811.13 ± 638.18	1958.11 ± 278.05	962.23 ± 136.63	320.74 ± 45.55	107.14 ± 15.21
Monoterpenes	2215.39 ± 414.28	2869.42 ± 536.58	3834.30 ± 717.01	1268.37 ± 237.19	85.21 ± 15.93	3.85 ± 0.72	<LOD
Sesquiterpenes	<LOD	<LOD	955.08 ± 139.44	<LOD	<LOD	<LOD	<LOD
Hydroxyl-terpenes	76.66 ± 11.65	90.48 ± 13.75	101.09 ± 15.37	119.52 ± 18.17	62.78 ± 9.54	<LOD	<LOD

Note: **Acids, Esters, Aliphatic Hydrocarbons, Aromatic Hydrocarbons, Alcohols** and **Ketones** have been quantified using the response factor of **Toluene**;

Aldehydes have been quantified using the response factor of **Hexanal**;

Monoterpenes have been quantified using the response factor of **α-Pinene**;

Sesquiterpenes have been quantified using the response factor of **β-Caryophyllene**;

Hydroxyl-terpenes have been quantified using the response factor of **4-Terpineol**.

Table 2.9: Class concentration of the major volatile components of air samples obtained close to Juniperus Oxycedrus. The results are expressed in pptv (considering Molar Volume at room temperature and pressure of 1 atm) and relative calculated value of uncertainty. The results are for the period from March 2017 to September 2017.

Compounds	March	April	May	June	July	August	September
Acids	<LOD	<LOD	23.81 ± 3.83	43.12 ± 6.94	59.35 ± 9.56	27.49 ± 4.43	6.17 ± 0.99
Esters	<LOD	24.36	<LOD	<LOD	<LOD	<LOD	<LOD
Aliphatic Hydrocarbons	5.97 ± 0.96	69.87 ± 11.25	194.72 ± 31.35	385.49 ± 62.06	238.14 ± 38.34	177.83 ± 28.63	101.37 ± 16.32
Aromatic Hydrocarbons	37.08 ± 5.97	84.98 ± 13.68	198.96 ± 32.03	102.66 ± 16.52	58.11 ± 9.36	41.52 ± 6.68	34.88 ± 5.62
Alcohols	<LOD	<LOD	<LOD	<LQ	<LOD	<LOD	<LOD
Ketones	<LOD	<LOD	37.75 ± 6.08	<LOD	<LOD	<LQ	<LOD
Aldehydes	93.71 ± 13.31	169.97 ± 24.14	985.17 ± 139.89	2799.53 ± 397.53	1438.24 ± 204.23	731.19 ± 103.83	418.45 ± 59.42
Monoterpenes	<LOD	157.99 ± 29.54	1856.64 ± 347.19	2798.891 ± 523.39	1571.86 ± 293.94	188.23 ± 35.20	40.39 ± 7.55
Sesquiterpenes	<LOD	<LOD	269.28 ± 39.31	799.85 ± 116.78	301. 09 ± 43.96	<LQ	<LQ
Hydroxyl-terpenes	<LOD	51.79 ± 7.87	73.14 ± 11.12	115.37 ± 17.54	124.31 ± 18.90	43.82 ± 6.66	<LQ

Note: **Acids, Esters, Aliphatic Hydrocarbons, Aromatic Hydrocarbons, Alcohols** and **Ketones** have been quantified using the response factor of **Toluene**;

Aldehydes have been quantified using the response factor of **Hexanal**;

Monoterpenes have been quantified using the response factor of **α-Pinene**;

Sesquiterpenes have been quantified using the response factor of **β-Caryophyllene**;

Hydroxyl-terpenes have been quantified using the response factor of **4-Terpineol**.

From these results it is possible to observe that the emission trend during the years is quite constant with high emissions during spring and summer and low emissions in autumn and winter; this annual profile is in according to the seasons and the temperature. It is very interesting to see that the emissions close to the two species of Juniper are different, especially as regards to the concentrations of the aldehyde and monotorpens and the trend of the emissions of oxygenated terpenes. The aldehyde emissions near the *Juniperus Oxycedrus* are four times higher than emissions near the *Juniperus Communis* while the monoterpene emissions are two times higher (Seghetti C., et al, 2019). This fact suggests that with our method it is possible to estimate, roughly, the emission of a single specie if close enough to it. This hypothesis is confirmed by the comparison of literature data and some measures with SPME that I performed on parts of the plant, better described in the next chapter.

2.4.5 Dependence on temperature

Figure 2.7 shows the dependence of the BVOC emissions from the temperature, there, the percentage indicates the total percentage emissions observed during the annual sampling in which different temperatures were found. The temperature and humidity values were taken from the fixed weather station located in Visso (MC). Based on the results obtained in two years of monitoring, it is possible to state that, for the Juniper species, there is an optimal temperature for the emissions of biogenic compounds; in fact, the data obtained show that at temperatures below 18 °C the emissions are almost zero as well as when temperatures above 27°C. Instead, at temperatures between 23 and 24 °C, the maximum emission is observed.

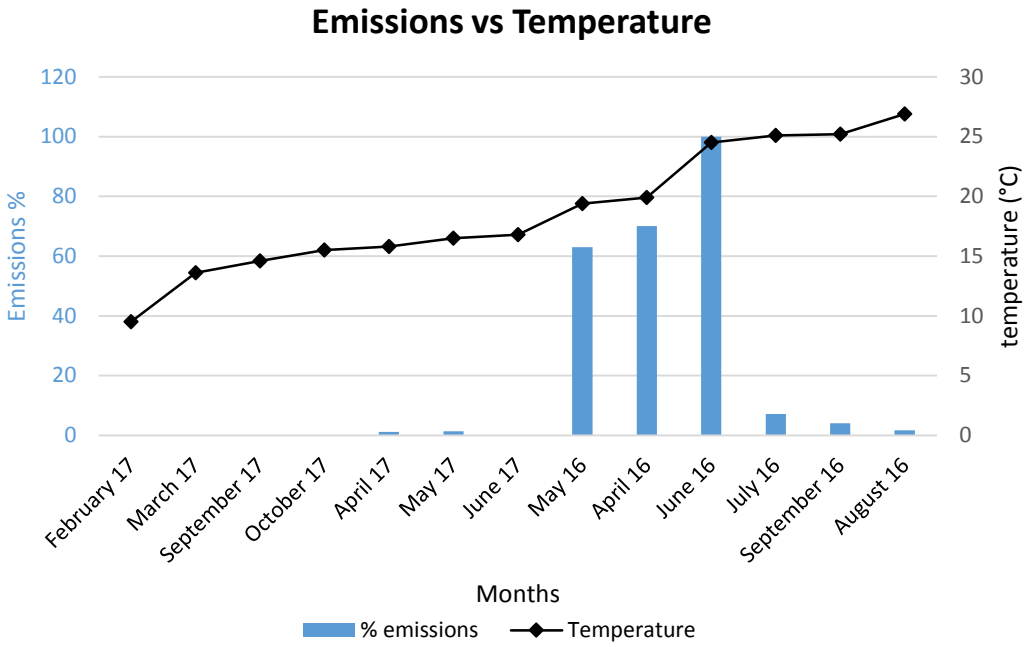

Figure 2.7: Correlation between emitted compounds and temperature.

Previous studies in the literature show similar results (Owen et al., 1997; Kesselmeier et al., 1996). Studies in the literature are carried out under different conditions, Owen sampled air with a system enclosing branches of plants directly on site, therefore, temperature and the intensity of light (like our project) influenced the emissions obtained. Kesselmeier, on the other hand, observed the dependence of emissions from the laboratory temperature and therefore excluded all other atmospheric factors. Both studies obtained the same results with respect to temperature: the two species of juniper attain the maximum emissions in the temperature range between 23 and 26 °C, a range very similar to our finding.

2.4.6 Dependence on relative humidity

Figure 2.8 shows the percentage of the emissions and the relative humidity measured over two years; there, the percentage indicates the total percentage emissions observed during the annual sampling in which different temperatures were found

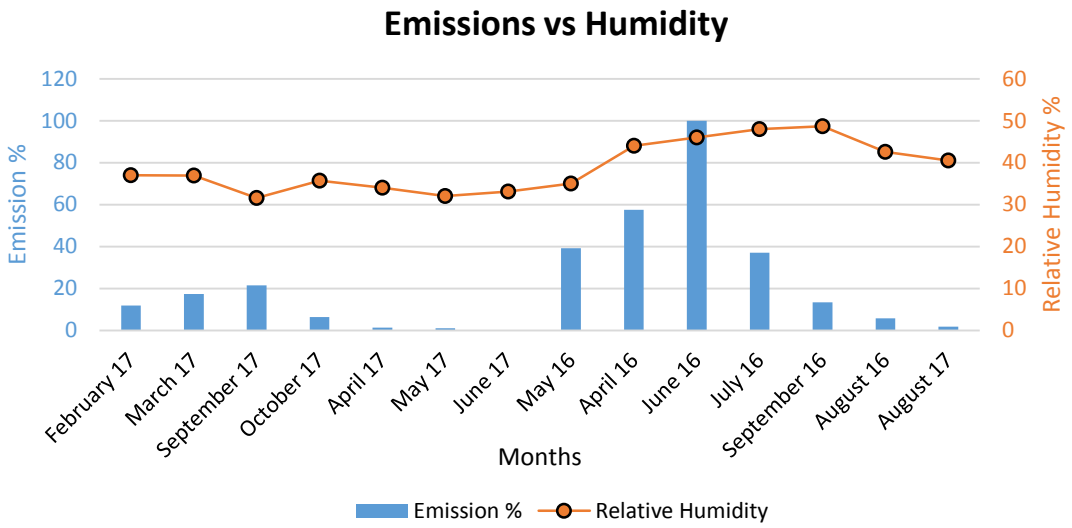

Figure 2.8: Correlation between emitted compounds and relative humidity.

Generally, biogenic emissions increase with the increasing of humidity (Guenther et al. 1991) but the contribution of temperature is higher than the contribution of humidity. Kesselmeier and coworkers found (Kesselmeier et al., 1997) that the emissions depend on the variation of humidity only when the relative humidity is greater than 80-90%. Shade et al. 1999 showed that the major contribution is due to the increase in temperature correlated with high humidity values, for this reason, the increasing of emission is not perfectly linear with respect to the increase in humidity. Figure 2.8 does not show, as expected, a clear correlation between emission and humidity because our data were always sampled at relative humidity much lower than the values indicated, in the literature, as affecting the emission.

2.4.7 Seasonal Variation

Results show (see Table 2.3 and Table 2.4) a higher emission of monoterpenes compared to other class of compounds in the months from April to July; this is perfectly in line with seasonal development. In fact, Juniper begins leader elongation in the spring. "Flowering" or cone development dates vary somewhat according to geographic location, but cone development generally occurs from April through June (McGregor et al., 1986, Hitchcock et al., 1969). The BVOCs emission, generally, shows a seasonal trend with the highest value during the end of

spring, most of the compounds follows the same pattern, but the sesquiterpenes emissions are higher only in early summer (Aaltonen et al., 2010). In fact, we found that sesquiterpene emissions are greater in June than April in according with literature.

2.5. Conclusion

The study of emissions at the site of the Sibillini National Park allowed to develop and validate a new qualitative and quantitative analytical method for the analysis of biogenic compounds in the air. This method is inexpensive and simple, its validation shows that it has good sensitivity, sensibility and reproducibility for all of target compounds. Compounds of different classes can be quantified without necessarily buying all the standards. The method permits an easy sampling even in places hardly accessible; a wide range of concentrations can be covered simply by adjusting the sampling time and flow. Furthermore, in this study, different atmospheric factors that influence the emissions of biogenic compounds have been considered. This has shown that, as regards the species of Juniperus Communis and Juniperus Oxycedrus, there are environmental conditions to obtain maximum emissions such as temperatures around 24 °C and during the spring period. The method permits to obtain, with respect to the emission of these species, results similar to those described in the literature without sampling the plants or using complex tools and procedure for sampling the emissions.

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3. Study of BVOC emissions of Juniperus Communis and Juniperus Oxycedrus by Solid Phase Microextraction (SPME)

3.1. Introduction

The exemplification of monitoring of BVOC emissions used in chapter 2 to validate the analytical method highlighted different composition of the samples obtained in proximity of the two species of the Cupressaceae. The main difference concerns the emission profile, mainly the concentrations of aldehydes and monoterpenes that close to the Juniperus Oxycedrus are higher than near to the Juniperus Communis. This study aims to analyse the differences of the emission of the two species of Juniperus, that is, their molecular difference, their concentrations difference, their seasonal evolution and even a tentative to disclose the part of the plant emitting the specific biogenic compounds. The study on the biogenic compounds from plants imply that those compounds have to be extracted from the plant tissue. Some of the compounds, at least partially, are naturally emitted in air by the plant so that they are naturally extracted but in the most case we need an extraction procedure to separate the solid part of the plant from the substance of interest.

Nowadays, several extraction methods are available for extracting essential oils from plant materials. Hydrodistillation (HD), distillation-solvent extraction (SDE), microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) are among the most used extraction techniques. After natural of forced extraction, we can get the BVOCs by solid-phase micro extraction (SPME). This is a sensitive preconcentration and extraction technique introduced in 1990 (Arthur and Pawliszyn, 1990), it has been applied to a wide variety of in vitro analyses of metabolites and similar compounds (Theodoridis et al., 2000). In general, SPME use a fused silica fiber coated with a thin sorbent phase that when it is exposed to the sample or its headspace concentrated the affine molecules on it. An analysis by SPME proceeds as follow: expose the fiber for a fixed time to adsorb the analytes, then introduce directly the fiber into the injection port of a gas or liquid chromatograph where the pre-concentrated molecules are directly desorbed into the chromatographic system (Pawliszyn 1997). Most of the application of SPME to living plants involves the cut of fresh or dry parts of them, or plants in vitro, and mainly the use of fibers coated with liquid polymeric phases (Maes et at., 2001, Schäfer et al.,

1995, Betts et al., 2000, Backman et al., 2001). For volatiles determination, the SPME fiber can be used as a passive sampler by exposing it to air containing compounds or to the headspace (HS) of vials containing the sample. SPME presents many advantages over other methods allowing sampling and preconcentration in a single step (Pawliszyn, 1997; Prosen and Zupancic-Kral, 1999; Sides et al., 2000; Kim and Lee, 2002; Augusto et al., 2003). Considering an optimum sampling time, compounds are concentrated in the fiber to amounts greater than the detection limits; after that, the fiber is desorbed directly into the injection port of the GC, transferring all compounds to the column and detection system. In addition, this technique does not require the use of solvents, which minimizes coelution with volatile compounds of interest and has environmental and economic advantages (Granero et al., 2005).

In this work, SPME was applied to study BVOC released by Juniperus Communis and Juniperus Oxycedrus. Berries, leaves and branches were analysed in order to understand which compounds are emitted from different parts of the plant. The compounds were identified using the Kovats indices and subsequently quantified according to the method validated in Chapter 2. The objectives were to identify the sources of each compound contributing to the whole profile of BVOC emissions.

3.2. Materials and Method

3.2.1 Plant samples

Leaves, berries and branches of Juniperus Communis and Juniperus Oxycedrus were collected in Macereto (Macerata, Italy, Sibillini National Park) in July 2016, when the blooming and crop were at their maximum level. Each part of the plants was stored in a weighed vial (in order to have the exact weight of the plant sample is known) and covered with parafilm. The samples were brought to the laboratory where they were stored at -4 °C, for no more than a week, until the analysis with GC-MS.

3.2.2 Analytical Instrumentation

BVOCs were analysed by total ion chromatograms (TIC) using GCMS-QP2010Plus, Shimadzu. The gas-chromatograph was equipped with a capillary column (Rxi-624SilMS, 30m x 0.25mm x 1.4 µm, fused silica column, Restek). Operational conditions for the chromatographic separation and detection were: initially set to 35 °C for 5 min, increased to 220°C at a rate of 11°C min^{-1}, maintained for 2 minutes and, at the end, increased again to 280°C at a rate of 20°C min^{-1} and maintained for 10 min. The conditions are shown in Figure 3.1.

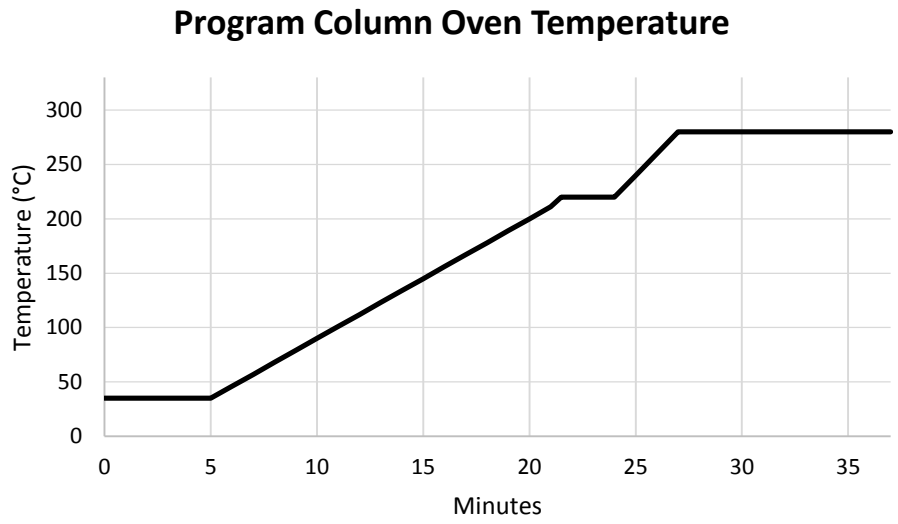

Figure 3.1: Temperature program for the chromatographic analysis of BVOCs using SPME.

Examples of the total ion current (TIC) chromatographic profiles recorded from the GC/MS analysis in scan mode of the headspace of Juniperus Communis berries are shown in Figure 3.2. Very good separation of compounds has been obtained using SPME for sampling the volatile fraction.

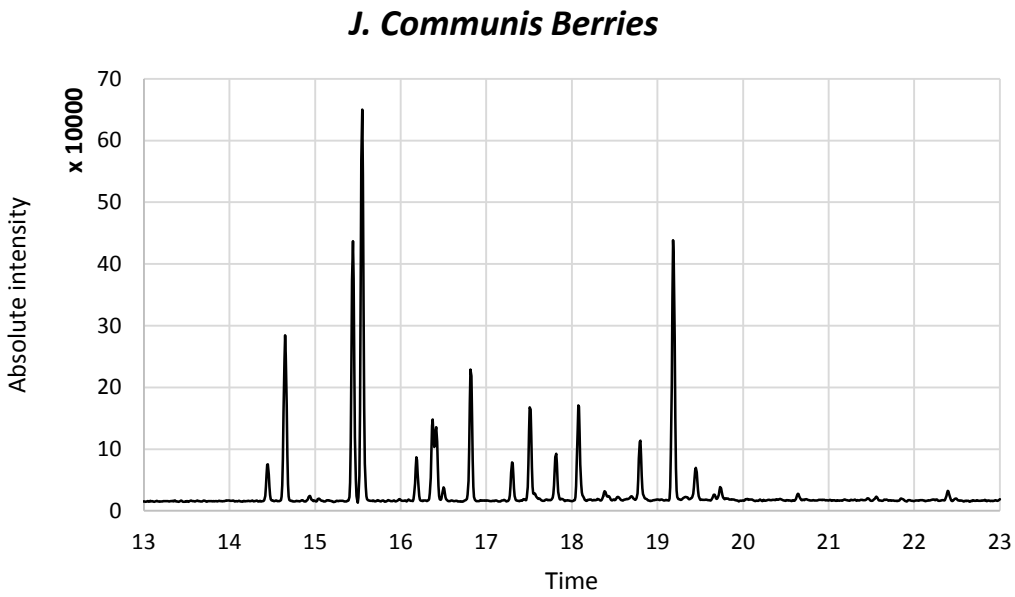

Figure 3.2: GC/MS chromatographic profiles of J. Communis berries obtained by SPME.

As can be seen from the chromatograms, the use of SPME together with GC/MS allowed tentative identification of many BVOCs that comprise mono- and sesquiterpenes as well as their oxygenated derivatives.

3.2.3 SPME Materials

We tested two SPME fibers for this study:

- 100 µm Polydimethylsiloxane (PDMS) fiber;
- Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber.

Both were supplied by Supelco (Oakville, ON, Canada). These fibers were chosen based on preliminary studies on literature (Zini et al., 2002, Zini et al., 2001, Barreira et al., 2015) and based on their chemical characteristics. DVB/CAR/PDMS fiber is recommended for volatiles and semi-volatiles flavour compounds, C3-C20 (MW 40-275) while the PDMS fiber is recommended for volatiles compounds (MW 60-275).

The fibers were conditioned, before use, according to the supplier specifications. The analytes were adsorbed exposing the fiber to the sample and equilibrating in a thermostatic bath at 60°C for 10 min. This setting is known to be suitable to achieve transfer of the analytes to the

fiber. After sampling, the fiber was desorbed into the GCMS-QP2010Plus split/splitless programmed-temperature injector operating at 260°C in split mode.

3.3. Results and Discussion

3.3.1 SPME choice

First of all, we performed experiments to compare the above mentioned fibers in order to choose the one best suited for our study. In this regard, two samples containing Juniperus Communis berries were analyzed using the two different fibers applying the same extraction and desorbing method. The obtained peak areas were normalized according to the weight of the sample (Figure 3.3).

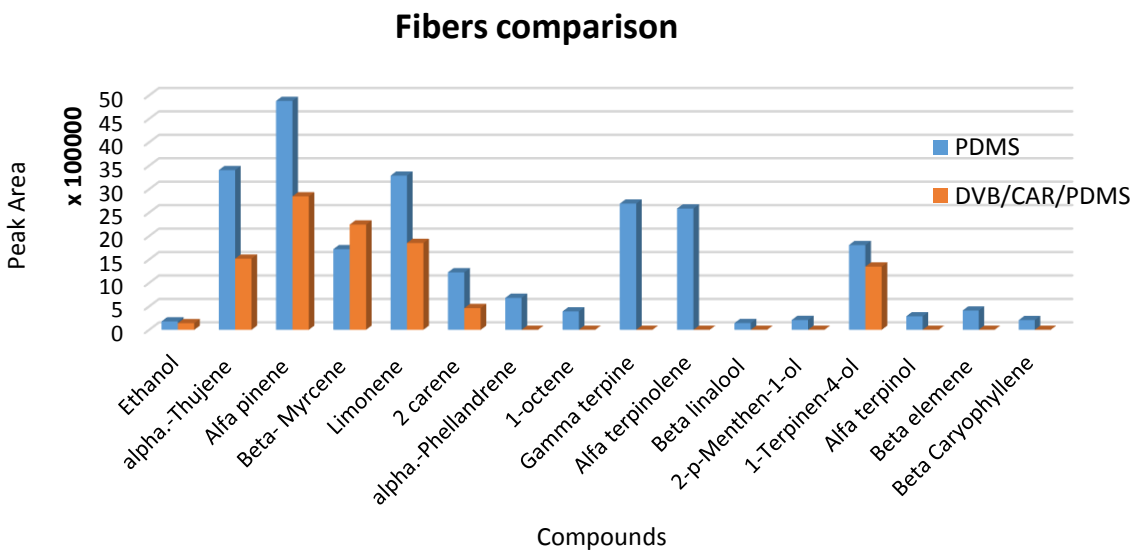

Figure 3.3: Analysis of Juniperus Communis berries using two different SPME fibers.

The Figure 3.3 shows that, as expected, both fibers are suitable for the analysis but, with our extraction procedure and analytical method, the PDMS fiber is more sensitive than the DVB/CAR/PDMS fiber for the biogenic compounds.

3.3.2 Qualitative evaluation of BVOC emissions

Qualitative identification of compounds present in samples was conducted on the base of Kovats retention index using values found in literature (Kaban, 2010, Kiralan, 2015, Flores et al., 2004) and NIST14 mass spectrum library. Table 3.1 shows the compounds found in the analysis and their identification indexes.

Table 3.1: KI^A: Kovats Index calculated for Rtx-624Sil-MS (Restek; 30 m, 0.25 mm id, 1.4 µm film thickness) installed on GC-MS; KI^B: Kovats Index found in literature for Rtx-624Sil-MS (Restek; 30 m, 0.25 mm id, 1.4 µm film thickness), RI^B: Reliability of Identification: a, mass spectrum and retention time identical with an authentic standard sample; b, mass spectrum and Kovats Index from literature in accordance; c, tentative identification by mass spectrum

Compounds	KI^A	KI^B	RI^B
Terpenes			
β-Myrcene	777	775	b
α-Thujene	948	944	b
a-Pinene	952	-	a
Sabinene	996	991	b
b-Pinene	992	988	b
Camphene	970	972	b
α-phellandrene	1019	1023	b
3-Carene	1024	1022	b
D-Limonene	1048	-	a
O-Cymene	1057	1059	b
β-Phellandrene	1066	-	a
α-Terpinolene	1116	1118	b
2-Carene	1060	-	c
Oxygenated Terpenes			
α-Terpineol	924	923	b
Eucalyptol	1065	1064	b
Cis sabinene hydrate	1130	-	c
(E)-p-2-menthen-1-ol	1160	-	c
Isopinocarveol	1213	-	c
4-Terpineol	1229	-	a
Cis-verbenol	1242	-	c
Verbenone	1794	-	c
Germacren-D-4-ol	1840	-	c
Piperitone	1852	-	c
Sesquiterpenes			
Cis-thujopsene	1361	-	c
Copaene	1406	1403	b
α-Cubebene	1435	1438	b
β-Elemene	1440	-	c

β-Panasinsene	1445	-	c
Longifolene	1469	1467	b
Thujopsene	1470	-	c
β-Caryophyllene	1476	-	a
α-Muurolene	1510	-	c
Germacrene B	1530	-	c
β-Bisabolene	1536	-	c
Isoledene	1547	-	c
β-Bourbonene	1734	-	c
Carvone	1808	-	c
Cedr-8-ene	1949	-	c

The use of Kovats indices allowed the identification of about 75% of the compounds, they were also compared with the results obtained in the literature to confirm the data collected (Foundil-Cherif et al., 2009, Foudil-Cherif et al., 2012, Owen et al., 2001). Foundil-Cherif et al studied the BVOCs on berries and leaves for both Juniperus Communis and Juniperus Oxycedrus. Their results show, for Juniperus Communis, a high concentration of Sabinene on leaves, while berries are characterized by α-Pinene and β-Myrcene; in the case of Juniperus Oxycedrus the most present compounds are α-Pinene and β-Pinene for both leaves and berries. We found more biogenic compounds than those of the aforementioned articles, this is probably due to the fact that our study include also the branches of the plant, we used different fibers and a different extraction process. BVOC emissions, furthermore, dependent on factors conditioned by the geographical position (Kesselmeier and Staudt, 1999) such as temperature and light intensity. This explains why the found compounds are different from the literature.

3.3.3 Quantitative evaluation of BVOC emissions

The quantification of the biogenic compounds emitted was made using the method validated in the previous chapter; we used the same calibration range and the same response factors obtained in the analytical method. The fiber was exposed inside the injector at 250 °C and subsequently desorbed and analyzed; the temperature ramp and the column for the chromatographic analysis are the same as the method validated in the previous chapter, for

this reason it was possible to use the same calibration curves to quantify our samples in SPME. From the qualitative analysis, it can be seen that the compounds emitted are monoterpenes, sesquiterpenes and oxygenated terpenes, the RF values of α-Pinene, β-Caryophyllene and 4-Terpineol were used respectively. Each sample of plants has been normalized on the basis of weight in order to find the amount of the compound as its weight. Figure 3.4 and Figure 3.5 show, in Juniperus Communis and Juniperus Oxycedrus respectively, the percentage distribution of every compound with respect to the part of the plant considered.

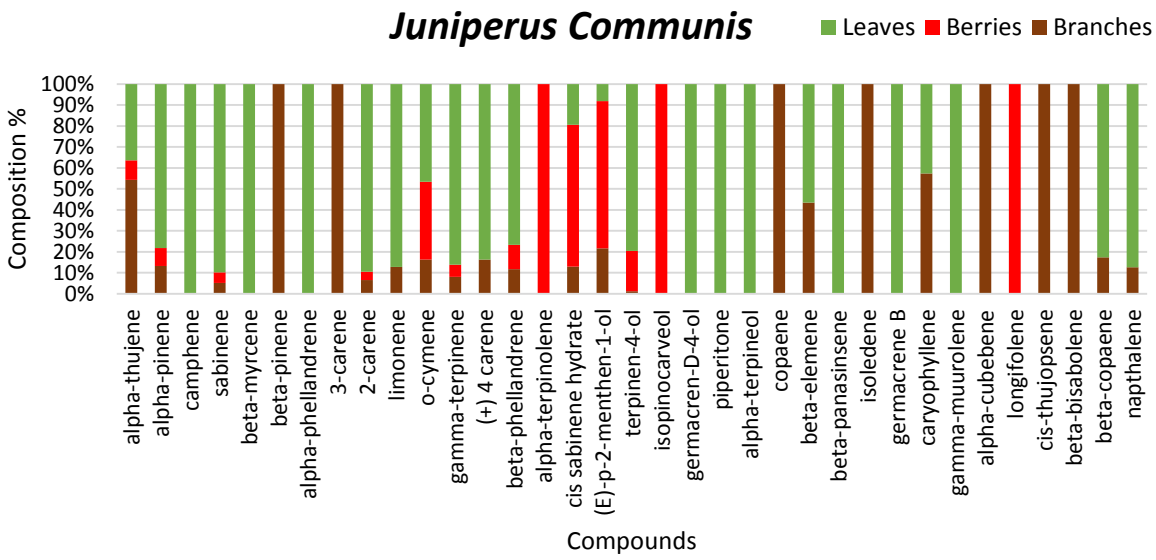

Figure 3.4: Percentage composition of BVOC emissions found in branches, berries and leaves of Juniperus Communis.

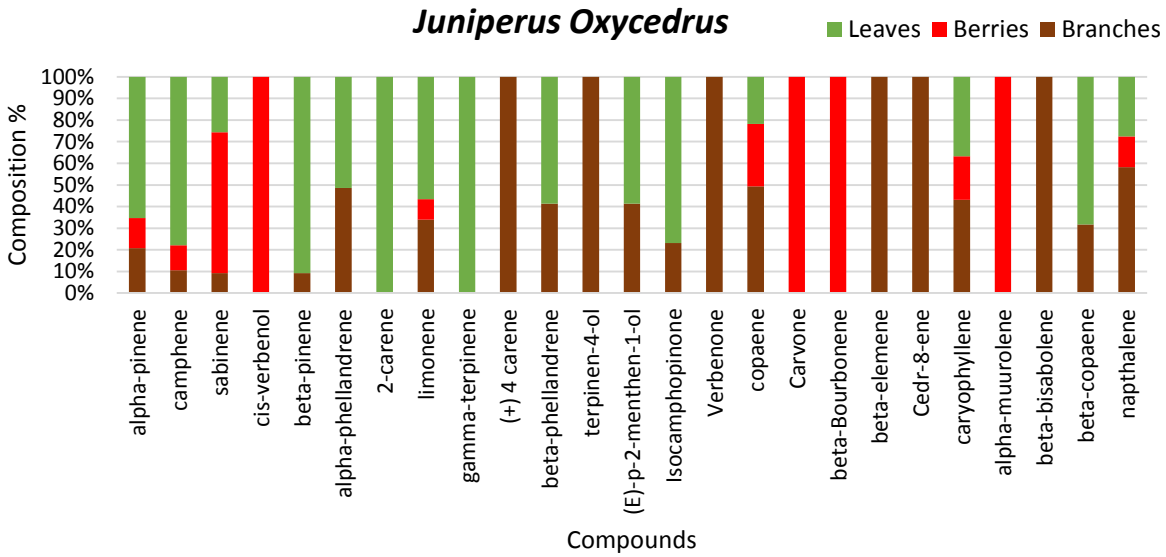

Figure 3.5: Percentage composition of BVOC emissions found in branches, berries and leaves of Juniperus Oxycedrus.

The comparison of Figure 3.4 with Figure 3.5 show that we found a larger number of compounds in the samples of Juniperus Communis than in those of Juniperus Oxycedrus, as for example α-Thujene, 3-carene, β-Panasinsene. It is also interesting to note that some biogenic compounds are characteristic of some parts of the plant; in the case of the Juniperus Communis, the characteristic compounds of the berries are the Longifolene, the Isopinocarvel and the α Terpinolene, while the compounds characterizing the leaves are Camphene, β-Myrcene, α-Phellandrene, Piperitone and others. In the case of the red juniper, the berries are characterized by the presence of cis-Verbenol, Carvone, β-Bourbonene and α-Muurolene that are mostly sesquiterpenes. The only common compound for both species present only in branches is β-Bisabolene, demonstrating that this is one of the main BVOCs emitted from the branches.

The emissions from the different parts of the plant were quantified and the percentage of monoterpenes, oxygenated terpenes and sesquiterpenes was evaluated.

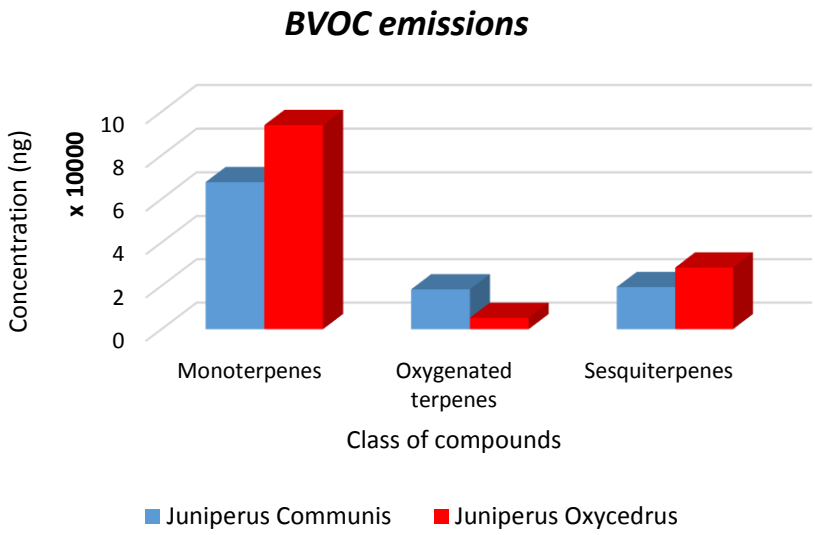

Figure 3.6: Percentage composition of the emissions of juniperus species.

Figure 3.6 shows that the Juniperus Oxycedrus emits a larger amount of monoterpenes and sesquiterpenes than the Juniperus Communis even if the numerosity of molecules is lower. The amount of oxygenated terpenes is larger in the Juniperus Communis. These results confirm the data obtained during air monitoring near to Juniperus species (Table 2.6 to Table 2.9) using the cartridges in the same site where the plant samples were taken. Obviously, the two types of analysis are influenced by different climatic and environmental factors. In fact, in the case of the study of the different plant parts with SPME, disturbance factors such as the reactions of the biogenic compounds in the atmosphere are eliminated and it is possible to concentrate only on the compounds that characterize the plant. This is also shown by the fact that in the SPME analysis there are no compounds such as aldehydes, ketones and aromatic compounds but only terpene derivatives. For this reason, it is not possible to compare the concentrations found with the two different methods but the general profile obtains the same qualitative results, confirming a good chromatographic separation and the complementarity of the two analytical techniques (Seghetti C. et al., 2016).

3.4. Conclusions

Headspace SPME has shown good potential to study composition of monoterpenes and therefore represent alternative to commonly used hydrodistillation technique. It is cheap,

simple, sensitive, fast and completely eliminates the use of organic solvent. Large variations in monoterpene and sesquiterpene compositions were observed between the two juniper species. Qualitative and quantitative analysis were performed, the use of Kovats indices allowed the recognition of biogenic compounds, this led to a comparison with the studies in the literature and to the demonstration of the emission difference due to the geographical position. Moreover, thanks to the qualitative analysis, it was possible to determine which compounds are emitted from the two species of juniper observing that, while the Juniperus Communis has a higher pattern of monoterpenes, the Juniperus Oxycedrus is rich in sesquiterpenes.

Quantitative analysis was performed using the analytical method validated in Chapter 2. The quantifications were made using the RF α-Pinene, 4-Terpneol and β-Caryophyllene for the three classes of compounds emitted. From the results obtained, there is a greater emission from the Juniperus Oxycedrus, this confirms the data obtained during the air monitoring lasted two years. In fact, while the red juniper emits more monoterpenes and sesquiterpenes, the Juniperus Communis is rich in oxygenated terpenes.

3.5. References

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4. Study of oxygenated terpenes and degradation products in Amazon rainforest during dry season.

4.1. Introduction

The main source of monoterpenes in the global atmosphere is emission from vegetation, with smaller contributions from soil, latter, oceans and other natural sources (Kesselmeier and Staudt, 1999; Kuhn et al., 2002; Ormeno et al., 2007). The Amazon rainforest is the largest, most productive, biodiverse, contiguous terrestrial ecosystem on Earth and of global importance in terms of carbon, water and energy fluxes (Davidson et al., 2012). The overall size of the Amazon rainforest is 5.4 million km^2; (Malhi et al., 2008) and the significant contribution of BVOC emissions from this vast forest to the global volatile organic compound (VOC) budget is 1000 Tg of carbon yr^{-1}; (Guenther et al., 2012). This is where a complex mixture of highly reactive biogenic volatile organic compounds (BVOC) is released to the atmosphere (Kesselmeier and Staudt, 1999), among them isoprene, monoterpenes, other isoprenoids and various oxygenated compounds. After entering the atmosphere, BVOC are oxidized primarily by the hydroxyl (OH) radical—typically within seconds to hours. Therefore BVOCs have a direct impact on ambient OH radical concentrations, or in other words the regional oxidation capacity of the atmosphere. For these reasons it is essential to study the emissions of the Amazon rainforest, to understand its function in atmospheric chemistry–climate interactions. Knowledge of these processes also serves to improve predictions of future changes in atmospheric composition and to assess the impact of changes in regional emissions and land use on the global climate caused by Amazon deforestation. In this study, all classes of oxygenated compounds have been studied, such as aldehydes, ketones, alcohols, carboxylic acids emitted by plants or deriving from oxidation reactions as function of height in the canopy (canopy height).

4.2. Material and Methods

4.2.1 The sampling site

The Amazonian Tall Tower Observatory (ATTO) site is located in central Amazonia (02°08.647’ S, 58°59.992’ W), 150 km north-east of the closest large city, Manaus, Brazil (Figure 4.1).

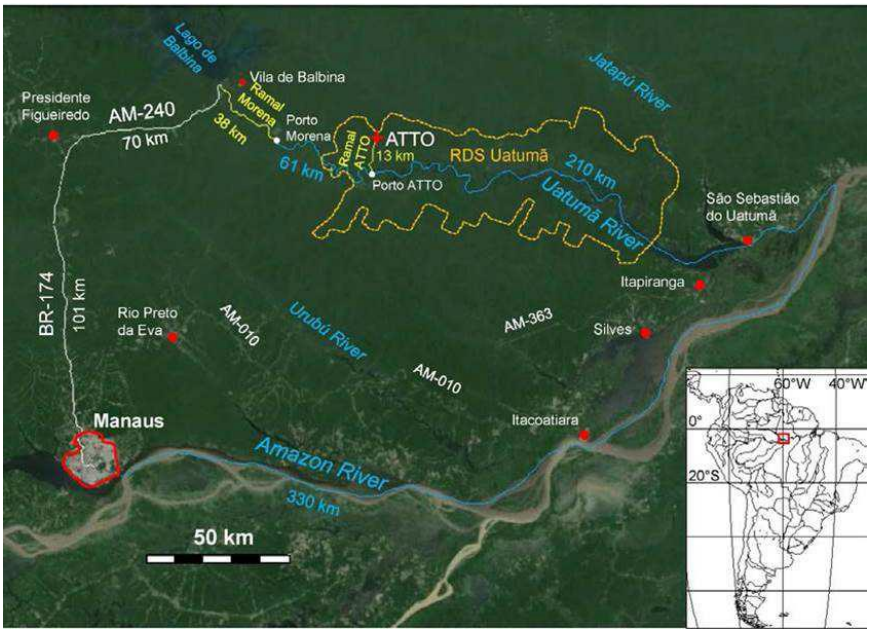


Figure 4.1: Map of the area of sampling site.

The immediate surroundings as well as large areas to the north-east, upwind of the site, are covered by an old-growth undisturbed forest, which permits natural process studies with minimal anthropogenic impact. The site is equipped with a 325m tall tower (Figure 4.2) and two 80-m smaller tower for the sampling of gases and particles in the atmosphere.

Figure 4.2: ATTO tower 325 m tall, used to support sampling instruments.

Sampling was performed on this tower above the canopy top (mean canopy height 35 m) at four different heights (40, 80, 150 and 320 m).

4.2.2 Air sampling

Collection of ambient air samples on adsorbent tubes, for subsequent analysis by a thermal desorption gas chromatography–mass spectrometry detector (TD-GC-MS), was conducted with four automated cartridge samplers, described previously (Kesselmeier et al., 2002; Kuhn et al., 2002, 2005) positioned at 40, 80, 150 and 320 m on the tower. In general, the autosampler consist in two main unites, a cartridge magazine that holds the adsorbent filled tubes (can provide a maximum of 20 sequential cartridges in a single sampling sequence) and the control unit timing the process and recording the data. In the latter unit are also present pumps, pressure gauges, mass flow controllers and power supply. The system is a constant-flow device, with one cartridge position per loop used as a bypass for purging the system.

The adsorbent tubes used for VOC sampling were filled with 130 mg of Carbograph 1 ($90 m^2 g^{-1}$) followed by 130 mg of Carbograph 5 ($560 m^2 g^{-1}$) sorbents. The size of the Carbograph particles was in the range of 20– 40 mesh. Carbographs 1 and 5 were provided by L.A.R.A s.r.l. (Rome, Italy) (Kesselmeier et al., 2002). The samples were collected from 21 of October to 9 of November. Samples were taken for 10 min every hour at a flow of 200 $cm^3 min^{-1}$, leading to a collection of 2 L of air in each cartridge using the automatic .

4.2.3 Analytical Instrumentation

After collection, the adsorbent tubes were analysed at the Max Planck Institute for Chemistry (MPIC) in Mainz, employing the gas chromatography method, using a time of flight mass spectrometry detector (TD-GC-TOF-MS, TD and Bench Tandem Ioniationn TOF-MS from Markes International, UK and GC 7890 from Agilent, USA), for identification and quantification of the BVOC species (Figure 4.3).

Figure 4.3: TD-GC-TOF-MS instrument used for BVOCs analysis in Max Planck Institute for Chemistry (MPIC).

Helium was used as the carrier gas, and separation occurred on Dimethyl TBS Cyclodextrin based column (0.15 µm, 0.15 mm ID, 25 m L). (Figure 4.4) The main characteristic of this column is that the substrate is chiral , in fact this column is a new generation of selective, fused silica capillary column, capable to separate efficiently both optical and positional isomers.

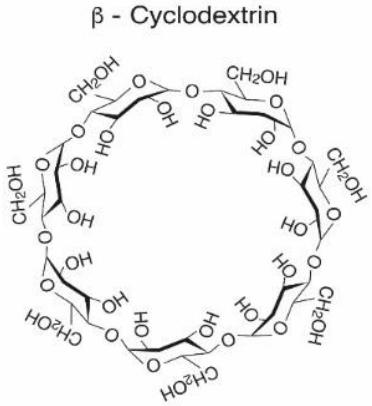


Figure 4.4: β-Cyclodextrin, chiral compound to separate optical and positional isomers.

The cartridges were thermally desorbed at 250 °C under He flux for 10 min (primary desorption). After primary desorption, the cold trap was rapidly heated from -10°C to 250°C and carrier gas (He 50 ml/min) transfers the desorbed analytes into the gas chromatograph

for separation (secondary desorption), identification and quantification. The oven was initially set to 40 °C for 5 min, increased to 150°C at a rate of 1.5°C min, and then increased again to 200°C at a rate of 30°C min-1, maintained for 1 min. Identification of separated compounds was achieved through injection of pure compounds and calibration gas curves; or with the use of the NIST library (similarity index greater than 75%). The analysis were performed in two different ionization mode, at -70eV and -14eV; but for the identification of the molecules, the spectra obtained with ionization at -70eV were considered.

4.3. Preliminary Results and Discussion

4.3.1 Calibration Curve

The calibration curves were prepared starting from certified standard gas cylinders. In particular, two different calibration gas cylinders were used, the first contains a mixture of 162NMHCs , while the second one contains 15 compounds, both from Apel Riemer Environmental Inc, USA, with certificate standards and accuracy better than ±5%. Two different standard cylinders were used because in the first one there are compounds like (+)(-)-Limonene; (+)(-)-α-Pinene; (+)(-)-β-Pinene and Benzene while in the second one are compounds like Methacrolein, Methyl Ethyl Ketone (MEK), Methyl Vinyl Ketone (MVK), DMS, Isoprene, Toluene and 1,3,5-Trimethylbenzene. Standard cartridges were prepared by pumping a known volumes of the gas standard with synthetic air and then was collected in a cartridge. The calibration curves vary according to the initial concentration of the compound inside the cylinders, the calibration ranges are summarized Table 4.1 below.

Table 4.1: Calibration ranges of the compounds.

Compounds	***Calibration range (ppb)***
(+)-Limonene	0.11-2.27
(-)-Limonene	0.015-0.28
(+)-α-Pinene	0.13-2.58
(-)-α-Pinene	0.15-2.85
(+)-β-Pinene	0.007-0.137
(-)-β-Pinene	0.27-5.24

Benzene	0.62-12.01
Methacrolein	0.51-5.04
MEK	0.52-5.19
MVK	0.48-4.78
DMS	0.50-4.95
Isoprene	0.50-5.00
Toluene	0.50-4.97
1,3,5-Trimethylbenzene	0.49-4.82

All calibration curves have a correlation coefficient (R^2) higher than 0.92. Figure 4.5 shows an example of calibration curve.

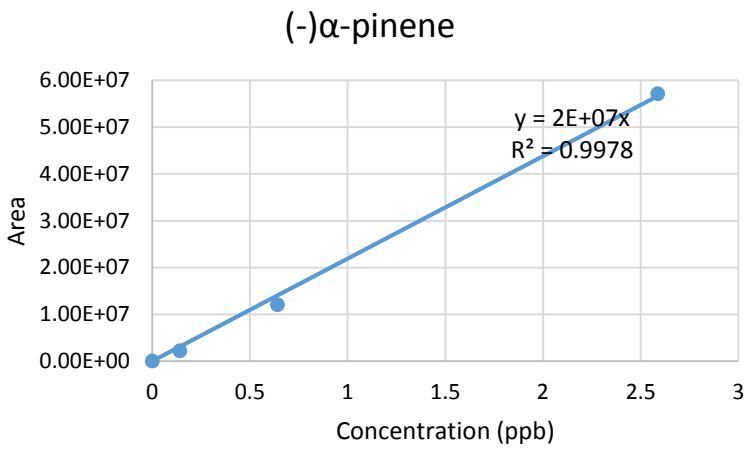


Figure 4.5: Calibration curve of (-)α-Pinene.

4.3.2 Chromatographic Resolution

The analysis of the cartridges taken in the Amazon forest, have detected the presence of hundreds of peaks of different compounds, as shown in the Figure 4.6 below.

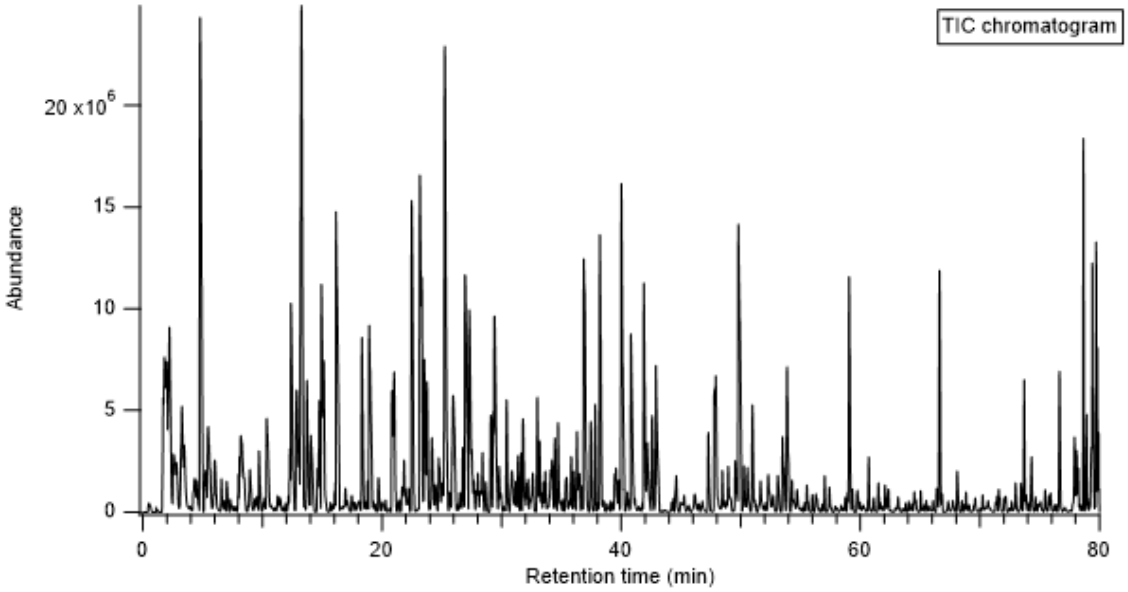


Figure 4.6: Example of a total ion chromatogram (TIC) observed during the analysis of samples collected in the Amazon forest.

In In all the samples there are different classes of compounds but the classes of molecules taken into consideration in this study are the oxygenated compounds emitted by plants or deriving from oxidation processes in air and consequently also isoprene and monoterpenes to be able to correlate them with the oxidation products. The different classes of compounds (isoprene, monoterpenes, sesquiterpenes and oxygenated compounds) were followed using a diagnostic ion in order to distinguish the compounds of interest from all the other peaks. In particular, the m/z 67 for isoprene, 93 for monoterpenes, 161 for sesquiterpenes and m/z 43, 59 and 71 for most oxygenated compounds. Figure 4.7 shows an example of the chromatograms obtained when the fragments of m/z 67, 93 and 161 are extracted from the total ion chromatogram.

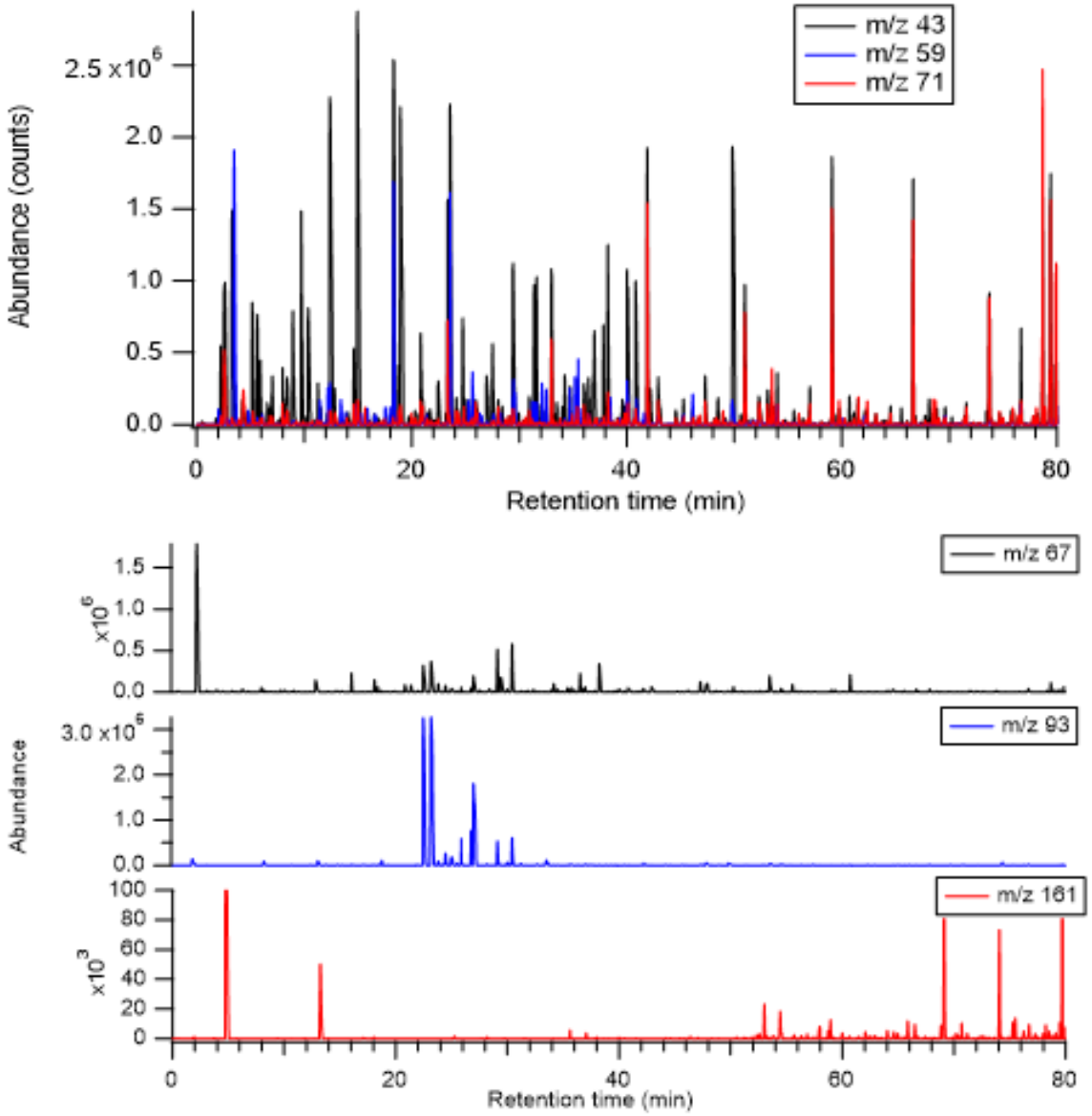


Figure 4.7: Diagnostic ions for different class of compounds and relative chromatograms (isoprene, monoterpene, sesquiterpene and oxygenated molecules).

4.3.3 Chiral Compounds in Air

As previously mentioned, the instrument used for the analysis of the cartridges has been equipped with a chiral column in such a way as to evaluate the presence of chiral compounds in the air and see how their relationship changes in the canopy and during different time of the day. Chirality is a geometric property of some molecules and ions. A chiral molecule/ion is non-superposable on its mirror image (Figure 4.8). The presence of an asymmetric carbon

center is one of several structural features that induce chirality in organic and inorganic molecules (IUPAC, 1997, Bruice, 2004).

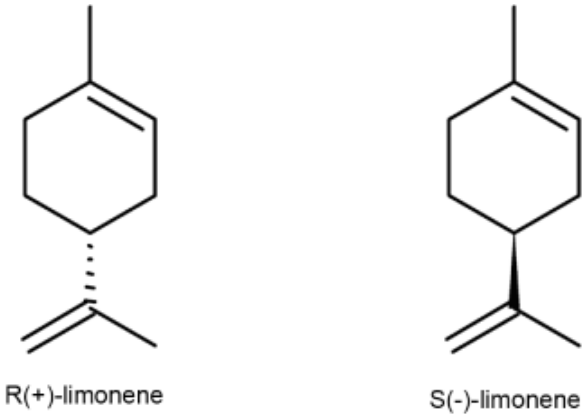


Figure 4.8: Example of chemical structures for both enantiomers of Limonene.

Many monoterpenes are produced in two enantiomeric forms: α-pinene, a common example, occurs as both (+)-α-pinene and (–)-α-pinene. The light-induced oxidation of natural and man-made volatile organic compounds affects the chemical and physical properties of the atmosphere (Andreau and Crutzen 1997, Kavouras et al, 1999). But although the importance of different enantiomers for the biological activity of a compound is well known, the implications for atmospheric chemistry have not been established (Nunes et al., 2005).

From the analysis of the samples collected in the Amazon, five chiral monoterpenes were found: α-pinene, β-pinene, Camphene, Limonene and 3-carene. Figure 4.9 shows the monoterpenes chiral pairs.

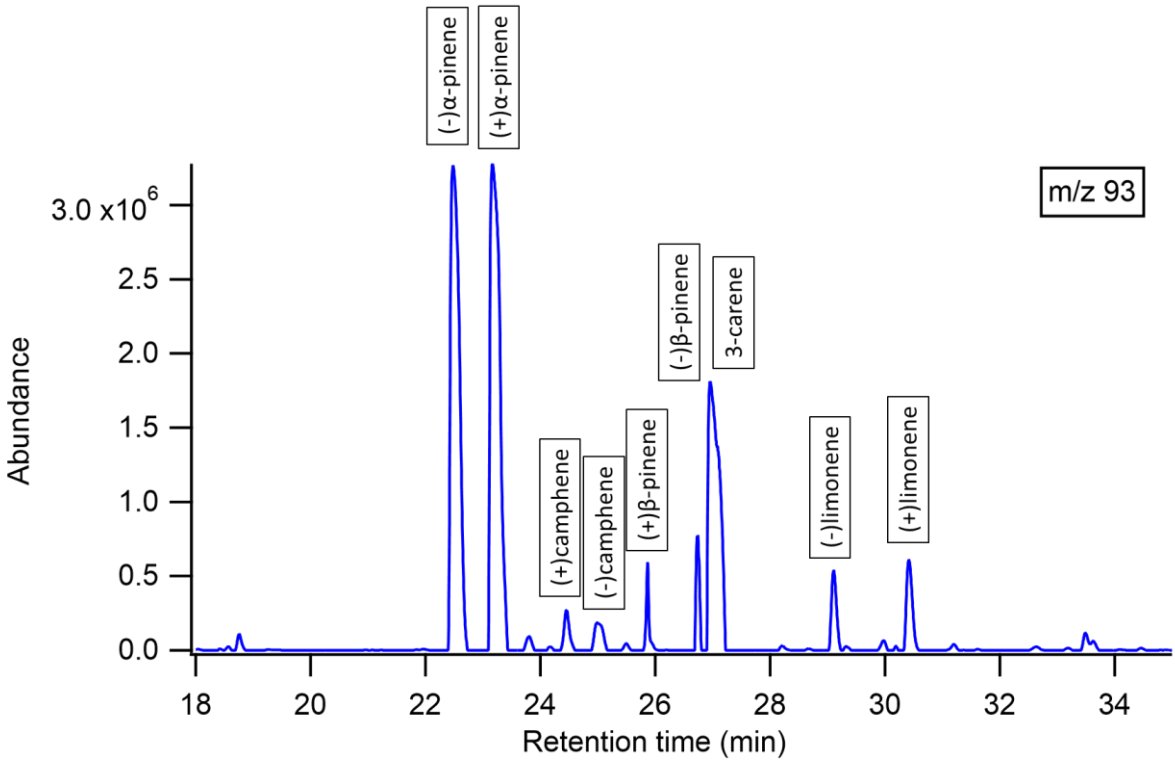


Figure 4.9: Monoterpenes chiral pair present in Amazonian rainforest samples.

From the Figure 4.9, it can be observed that the enantiomeric pairs do not have the same area and the same height, this is due to the fact that, when they react (for example with the hydroxyl radical), the chiral compounds produce 50% of the enantiomer R and 50% of the enantiomer S. When, on the other hand, a ratio different from 1 of the two enantioments (+)/(-) is observed, it means that they have been directly emitted on dependence of temperature or light (Williams et al., 2007). Furthermore, not only the terpenes have been followed but also the oxygenated compounds, in fact, in the analyzed samples there are chiral alcohols such as 2-ethylhexanol, or acetate compounds which have one or two stereocenters, thus forming two compounds and four enantiomeric compounds, respectively (Figure 4.10).

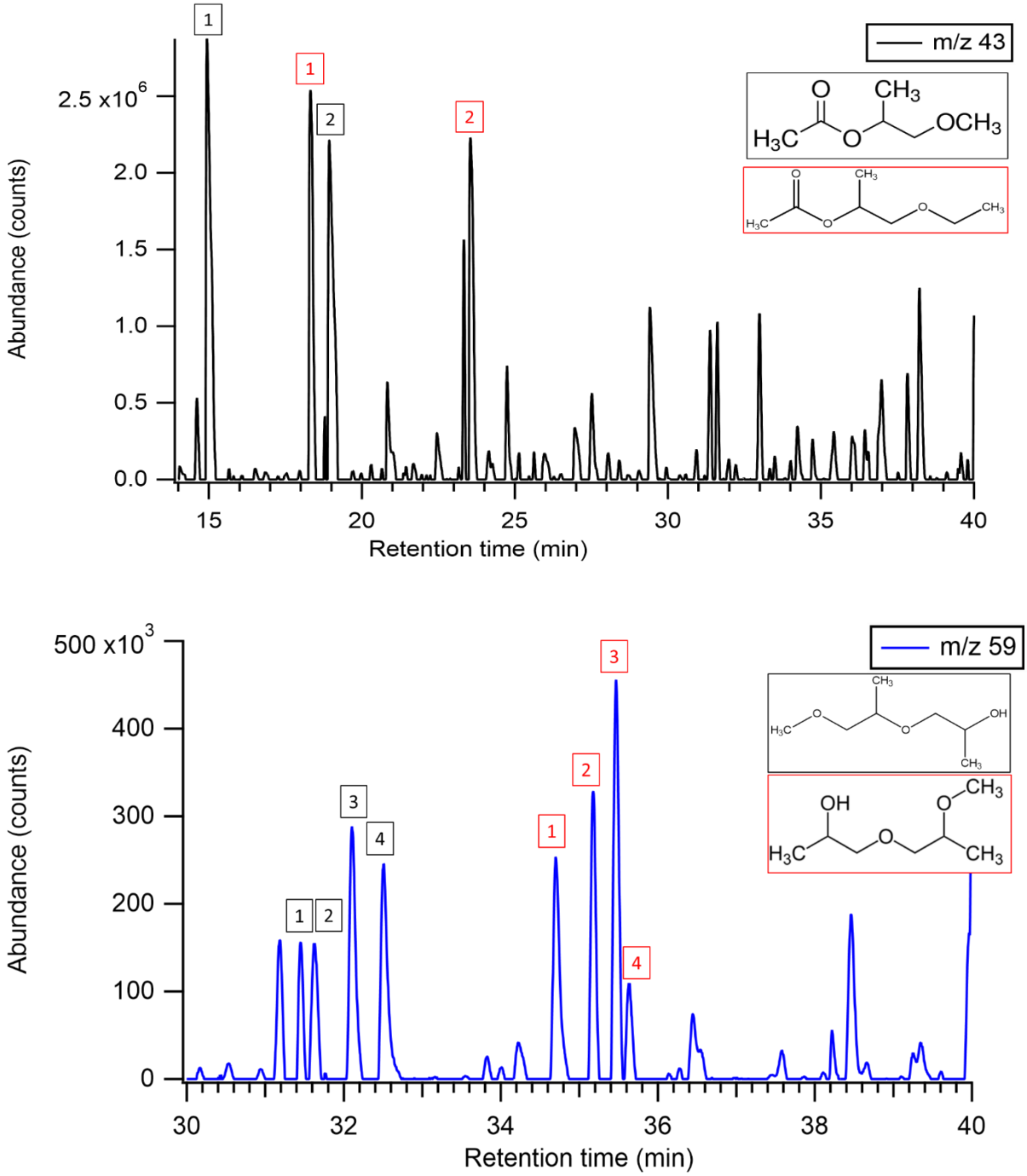


Figure 4.10: Chiral oxygenated compounds present in samples. The first one shows only two enantiomers while the second one has two stereocenters and, consequently, four enantiomers.

The emission of chiral compounds other than terpenes is a new topic and little studied, in fact there are few references in the literature.

4.3.4 Oxidation of isoprene

Once emitted isoprene reacts readily with the OH radical, resulting in an atmospheric lifetime of 1–2 h (Atkinson, 2000), thus affecting photochemistry on local, regional and global scales. The oxidation of isoprene results in the production of a wide range of stable products (Carter and Atkinson, 1996). One of the longer-lived by-products of this oxidation is carbon monoxide; besides carbon monoxide, the oxidation of isoprene in the atmosphere produces a wide range of compounds, including aldehydes, ketones, organic acids, organic nitrates, and other compound.

Two major compounds produced in the OH and ozone-initiated oxidation of isoprene are thought to be methyl vinyl ketone (CH_2=$CHCOCH_3$, MVK) and methacrolein (CH_2=$C(CH_3)COH$, MACR) (Pierotti et al., 1990; Martin et al., 1991). Figure 4.11 shows a scheme of oxidation products of isoprene.

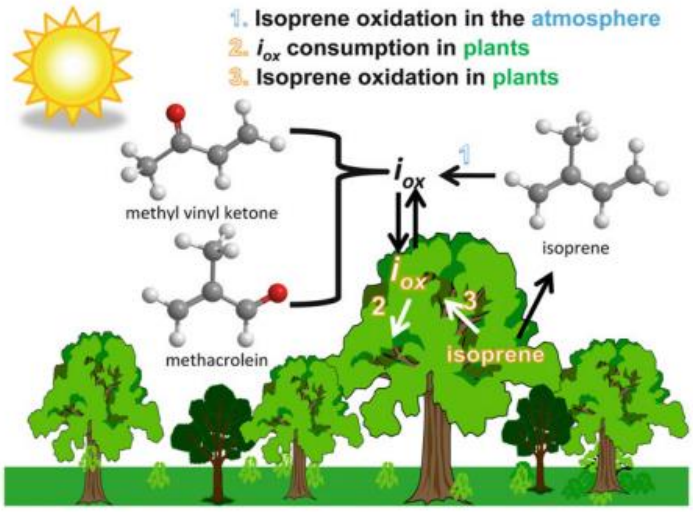


Figure 4.11: A simplified scheme of the production of isoprene and its oxidation products. (K. Jardine and A. Jardine).

Both MVK and MACR are removed from the atmosphere mainly through reaction with OH radicals (Table 4.2). Additionally they are photolysed and undergo dry and wet deposition (Helmig et al., 1999). Measurements of the ratio MVK/MACR and (MVK + MACR)/ISO have been utilised in field campaigns to derive information about the magnitude and location of isoprene sources and the photochemical processing of isoprene (Montzka et al., 1995; Biesenthal et al., 1998). Making use of the product yield for MVK given in literature (Tuazon and Atkinson, 1990) and the MVK/isoprene ratio measured in several field studies, Barket et

al. (2004) concluded that isoprene oxidation contributes mainly to ozone production for NOx mixing ratios in the range of ≈1–10 ppb.

Table 4.2: Lifetimes and rate constants of isoprene, methacrolein, and methyl vinyl ketone due to their reaction with OH, O_3, and NO_3 at typical ambient concentrations of the oxidants.

	OH		O_3		NO_3	
Reactant[1]	k^2 (cm^3s^{-1})	τ (h)	k^2 (cm^3s^{-1})	τ(h)	k^2 (cm^3s^{-1})	τ (h)
Isoprene	1.0×10^{-10}	1.7	1.27×10^{-17}	31	7.0×10^{-13}	0.8
MVK	2.01×10^{-11}	8.6	5.18×10^{-18}	77	$<6\times10^{-16}$	>920
MACR	2.86×10^{-11}	6.1	1.22×10^{-18}	326	3.4×10^{-15}	163

[1][OH] = 1.6×10^6 cm^{-3}, [O_3] = 7×10^{11} cm^{-3}, [NO_3] = 5×10^8 cm^{-3}
[2]Rate constant at 298 K according to the recent IUPAC recommendation (Atkinson *et al.*, 2005)

Oxidation of isoprene by OH in the presence of NOx produces MVK and MACR with yields of 32% and 23%, respectively (Tuazon and Atkinson, 1990). Thus the ratio MVK/MACR would be ≈1.4, if MVK and MACR would not undergo further reaction. Oxidation by OH, however, removes MACR more quickly than MVK and further increases the MVK/MACR ratio. In contrast, oxidation of isoprene by O_3 yields more MACR than MVK, and MVK reacts more quickly with O_3 than MACR. Thus ozone-dominated isoprene oxidation would result in MVK/MACR ratios lower than 0.4 (Carter and Atkinson, 1996).

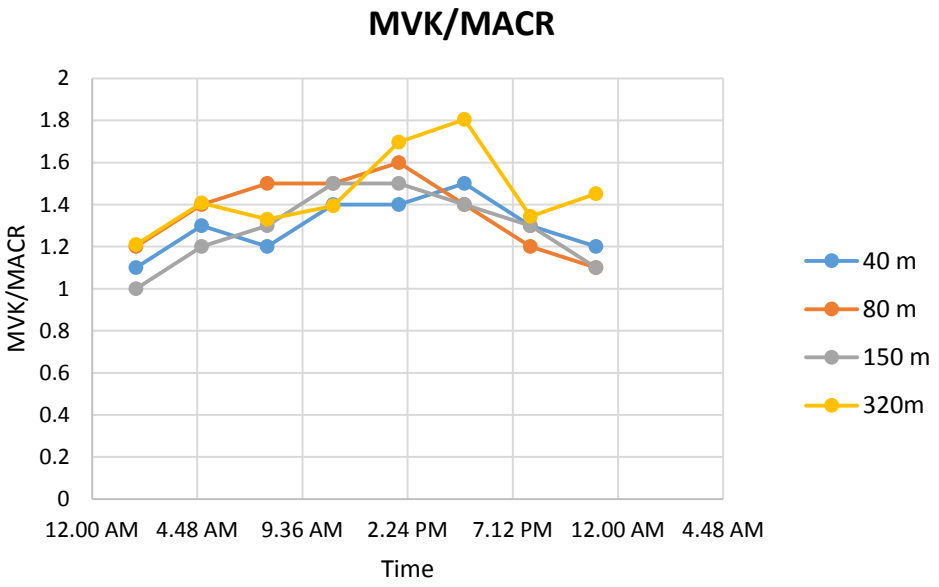


Figure 4.12: Observed ratios of MVK/MACR.

As shown in Figure 4.12, the MVK/MACR ratio showed a regular pattern, with mean daytime values above 1.4, indicative of OH-dominated isoprene degradation. Moreover, also the ratio (MVK + MACR)/ISOP was calculated to evaluate the effect of oxidation at different heights.

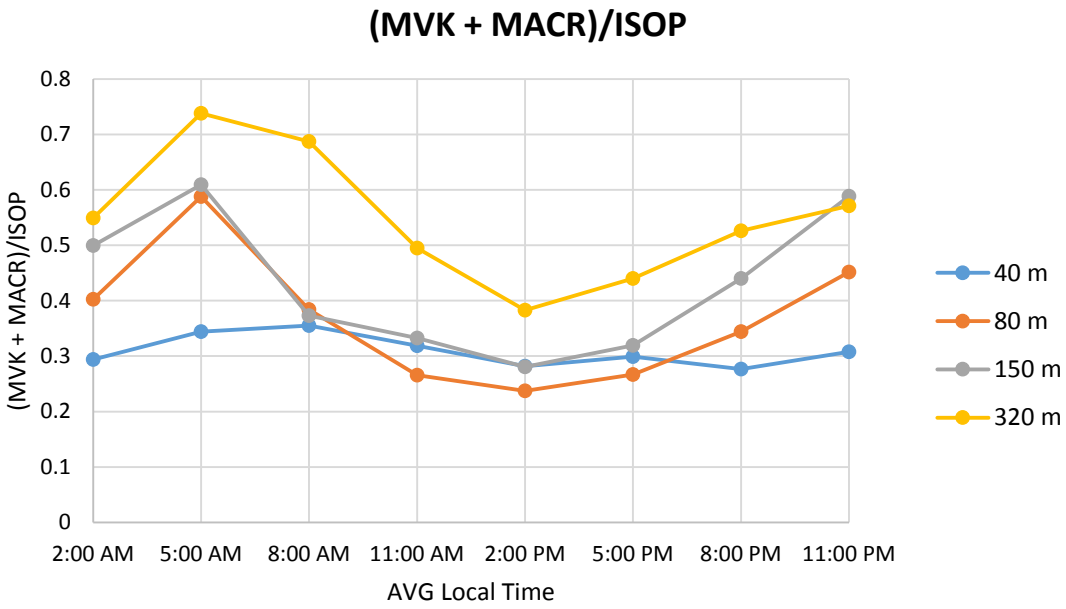


Figure 4.13: Observed variation of (MVK+MACR)/ISO at different heights.

Figure 4.13 shows that the values of (MVK+MACR)/ISOP are always < 1, this shows that these compounds are not directly emitted from plants or soil but derive only from the oxidation of isoprene. Furthermore, it is possible to observe that, when isoprene is at its maximum emission (at 12 and 2 pm), the ratio between the compounds is at its minimum value. Further information about atmospheric chemistry and transport can be derived from the ratio of the sum of MVK and MACR over isoprene, (MVK+MACR)/ISO (Kuhn et al., 2007). During the day, (MVK+MACR)/ISO is mainly driven by the OH chemistry, which destroys isoprene while producing (and concurrently destroying) MVK and MACR. As for every reactive scalar, the vertical profile of (MVK+MACR)/ISO is a function of turbulent mixing, surface deposition, distance from the emission source (transport time), and photochemical degradation (Montzka et al., 1995; Biesenthal et al., 1998; Barket et al., 2004).

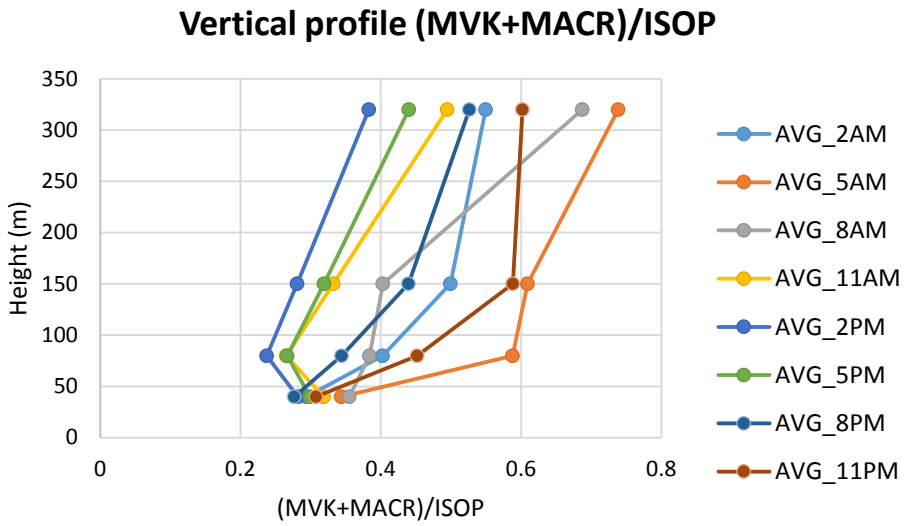


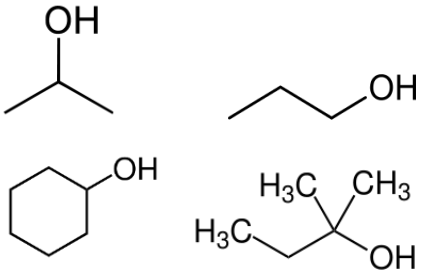
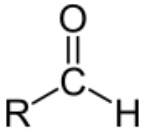
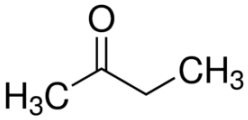
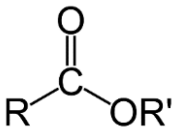
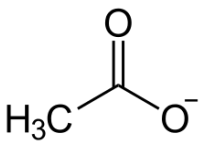
Figure 4.14: Observed vertical profile for (MVK+MACR)/ISOP.

Figure 4.14 shows the variation of the ratio of (MVK + MACR)/ISOP at different heights at different time of the day (the figure shows the correlation between the average (AVG) of all areas collected at different sampling time and heights), the profile is very similar in each sampling schedule; in fact, the oxidation products tend to increase with increasing height. This is in close agreement with Helmig et al. (1998) who reported an increase of the ratios MVK/isoprene and MACR/isoprene with height.

4.3.5 Oxygenated compounds

The aim of this research was not only the characterization of the isoprene oxidation compounds, but also the study of oxygenated compounds directly emitted by plants or the compounds of oxidation of terpenes. In this regard, different classes of compounds, alcohols, aldehydes, ketones, carboxylic acids and acetate compounds have been studied. Class of compounds and their emissions are summarized in Table 4.3.

Table 4.3: Compounds, their classes and main features.

Class of compound	Formula	Function
Alcohols		Produced in response to wounding and play an important role in the plants defense strategies and pest resistance (Matsui 2006). Directly emitted by plants as aroma compounds of fruits and other foodstuffs (Rettinger 1991).
Aldehydes		Components of plant fragrance, can be emitted after damage and are considered to be direct or indirect defense signals (Kishimoto et al., 2005).
Ketones (MEK)		Emitted by terrestrial vegetation (Brilli et al., 2014), fungi (Wheatley et al., 1997) and bacteria (Song and Ryu, 2013)
Carboxylic Acids		Produced for protection against fungal or bacterial diseases (Farmer, 2001)
Acetate		Volatile acetate esters have particularly pleasant odours and are key aroma compounds in flower (Shalit et al. 2003; Knudsen et al. 2004)

4.3.5.1 Alcohols

The alcohols are generally emitted from plants in order to protect themselves from the attacks of bacteria or microorganisms but, in some cases, the fruits and flowers of the plants directly emit them. Two compounds were monitored among the alcoholic compounds: the α- cumyl alcohol and the 1-hexanol-2-ethyl. In the case of α-cumyl alcohol, the vertical profile has been evaluated while in the case of 1-hexanol-2-ethyl the chiral pairs have been studied to observe the variation at different heights and emissions. In particular, young leaves emit α-cumyl alcohol (Bison et al., 2018) in fact, from the vertical profile, it can be observed that this compound is more abundant at 40 m and slowly decreases with increasing height, it is no longer present at 320 m (Figure 4.15).

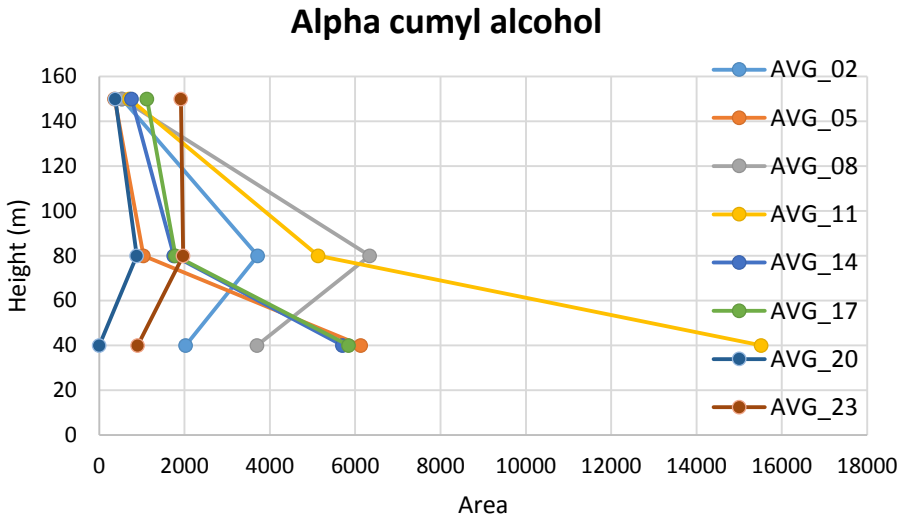


Figure 4.15: Vertical profile of α-cumyl alcohol from 40m to 150 m. Average of emissions depending at different sampling times.

Figure 4.15, shows the emissions according to the different heights, the values were obtained by averaging the areas obtained at the same time in the different sampling rates. The data obtained reflect the trend of the emissions of terpenes that are dependent on temperature and light, in fact, it can be seen that at 40 m in the daytime the emissions are higher than at night and, with increasing height, the α cumyl alcohol tends to decrease perhaps because it undergoes oxidation processes by the OH radical. In the case of 1-hexanol-2-ethyl, the two stereoisomers R and S have been found. The compound is encountered in natural plant fragrances, and the odour has been reported as "heavy, earthy, and slightly floral" for the R

enantiomer and "a light, sweet floral fragrance" for the S enantiomers (Rettinger 1991). Since the standard solutions of these two compounds are not available, literature studies have shown that the compound with the lowest retention time is the enantiomer R while the second is the S (Rettinger and Karl, 1991). In Figure 4.16 the vertical profiles of two enantiomers are shown.

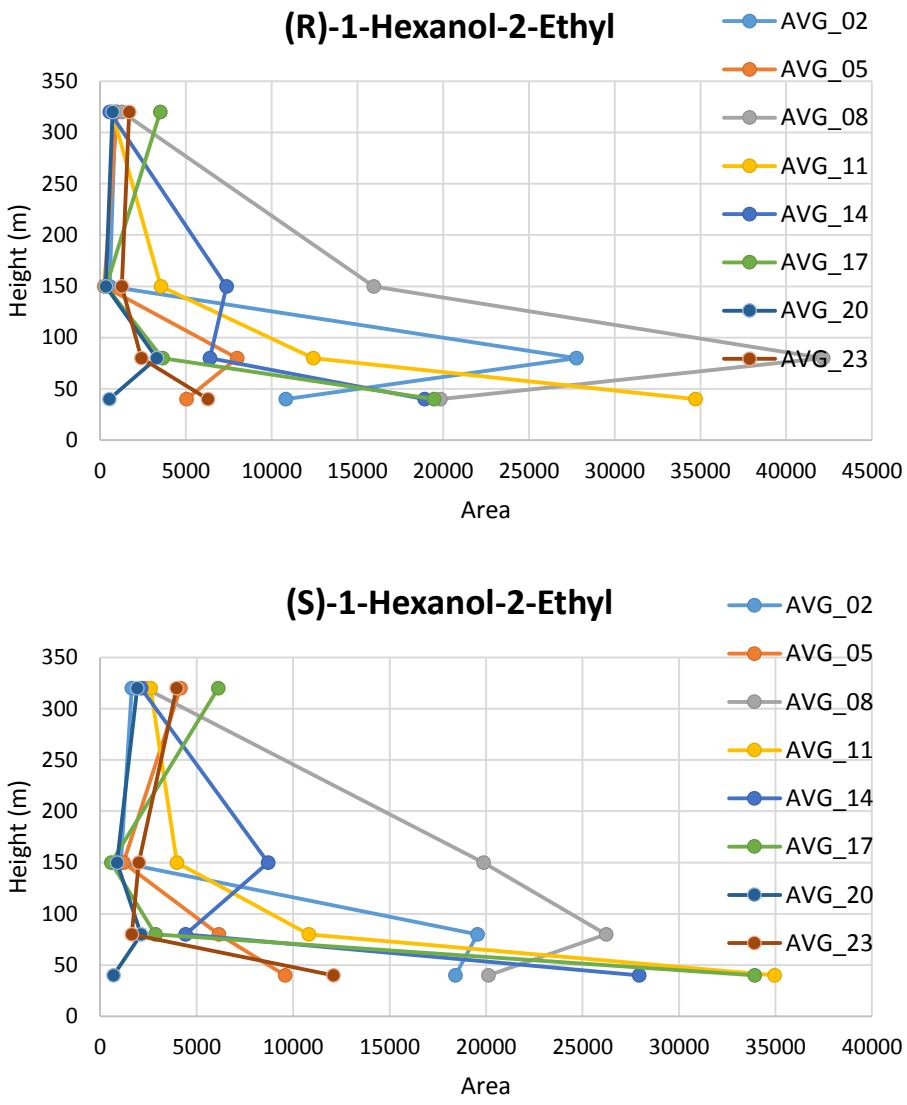


Figure 4.16: Vertical profiles of two enantiomers R and S of the 1-hexanol-2-ethyl.

The two profiles show a common trend, during the day the compounds are more present at 40 m and tend to decrease with increasing height, however, the behaviour at night is different.

The 1-Hexanol-2-Ethyl is more abundant at 80 m than at 40 m, then it decreases with increasing height.

Considering that the two chiral compounds are present, it is interesting to see how their relationship changes based on height.

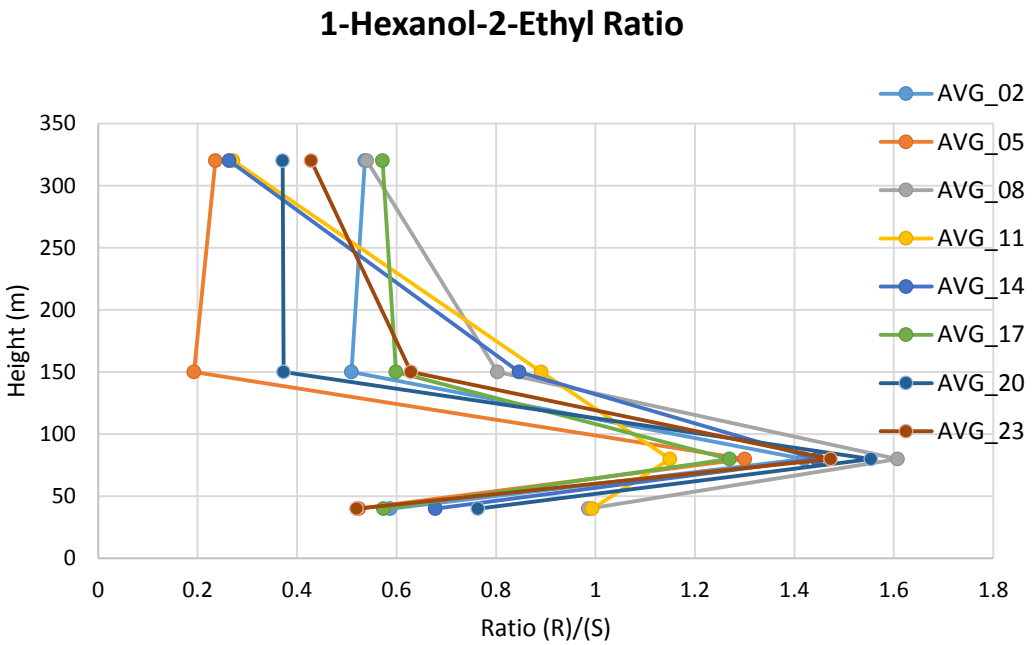


Figure 4.17: Ratio of the different enantiomers of 1-hexanol-2-ethyl versus the sampling height.

The relationship between the two enantiomers varies according to height; as can be seen from the Figure 4.17, at 40, 150 and 320 m, the S enantiomer is prevalent while the ratio reverses at 80m, this could be explained if we consider the deforestation carried out for the construction of the tower. The results obtained at 40m could be influenced by the presence of a few trees around the tower while the increase in emissions at 80m is due to the merging of BVOCs from all the underlying vegetation.

4.3.5.2 Aldehydes and Carboxylic Acids

Plants utilize special defence systems against environmental stresses (Zeng-hui et al., 2008). In response to mechanical damage, herbivore wounding, or pathogen infections, plants can release a range of VOC (Ping and Shen, 2001). Aldehydes belong to an important class of

volatiles that are indispensable to plants in response to environmental conditions. Nandi and Fries (1976) found that pentanal, hexanal, and heptanal exhibit strong antifungal activities against several fungi. Aldehydes C5-C12 were monitored to evaluate which is most emitted as a result of environmental stress or some damage.

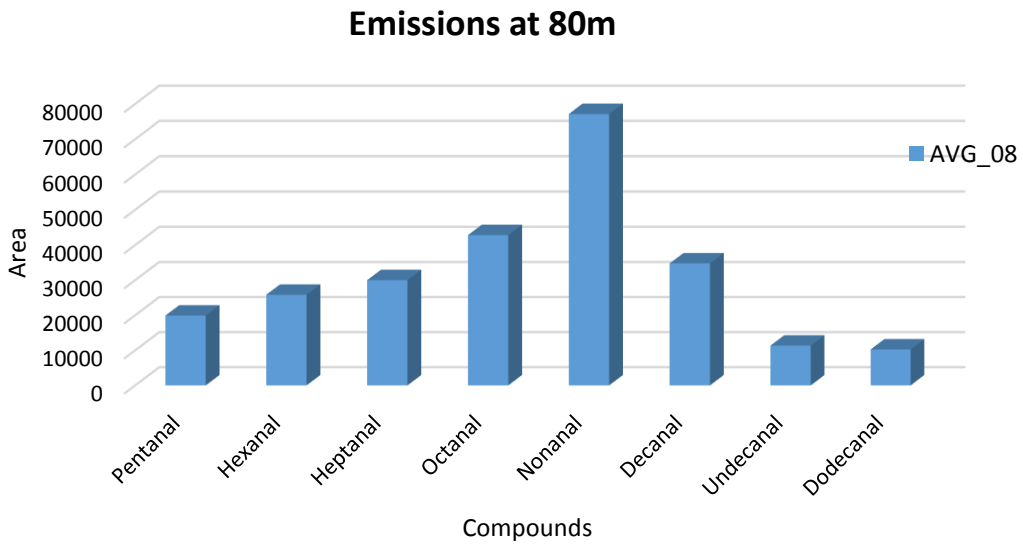


Figure 4.18: Aldehydes at 80m considering the average of the emissions monitored at 8 am.

The Figure 4.18 shows that in case of stress or damage, the most emitted compound is Nonanal. This study is preliminary in that it is not possible to state that the Nonanal is always the most emitted compound as surely the emissions of aldehydes depends on the type of environmental stress or the type of damage received (attack of bacteria or cut of leaves or branches) as reported in literature.

In addition to aldehydes, carbonyl compounds are also emitted from plants as a defence; it is, indeed, known that plants are able to release volatile compounds that also play a role in the control of defence, preparing neighbour plants for an enhanced response upon subsequent attack (Farmer, 2001; Erb et al., 2015). The release of volatile compounds, moreover, is a self-defence system by inducing defences in the same plant, preparing adjacent leaves that are not directly connected via the plant's vascular system (Heil and Ton, 2008). Carboxylic acids C3-C12 were monitored to evaluate the vertical profile of defence emissions; all carboxylic acids show the same trend.

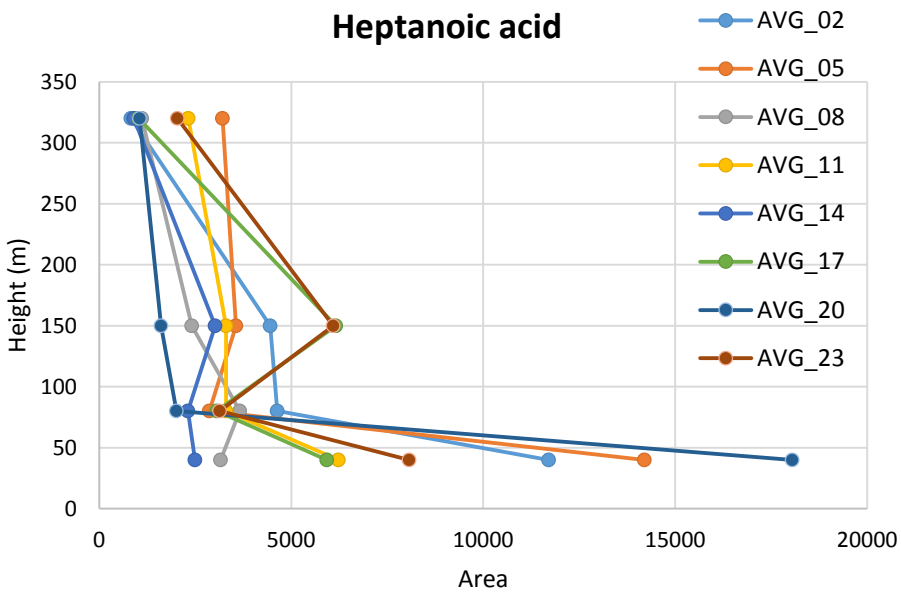


Figure 4.19: Dependence of Heptanoic Acid, at different time, from height.

The Figure 4.19 shows that the emissions are large at 40m and then decrease with increasing height, this is due to the fact that at 40m you are closer to the vegetation, then the compounds emitted are oxidized, for this reason the carboxylic acids decrease at 320m.

4.3.5.3 Ketones

Among the ketone compounds present in the samples collected in the Amazon, the MEK (Methyl Ethyl Ketone) was the one studied. Baraldi et al., in their studies, have shown that MEK is directly emitted by plants but, in addition to direct emission, it is also produced by the oxidation of other compounds such as s n-butane, 2-butanol, 3-methyl pentane and 2-methyl-1-butene (de Gouw et al., 2003; Sommariva et al., 2011). The vertical profile of the MEK shows how it varies from 40 to 320m (Figure 4.20).

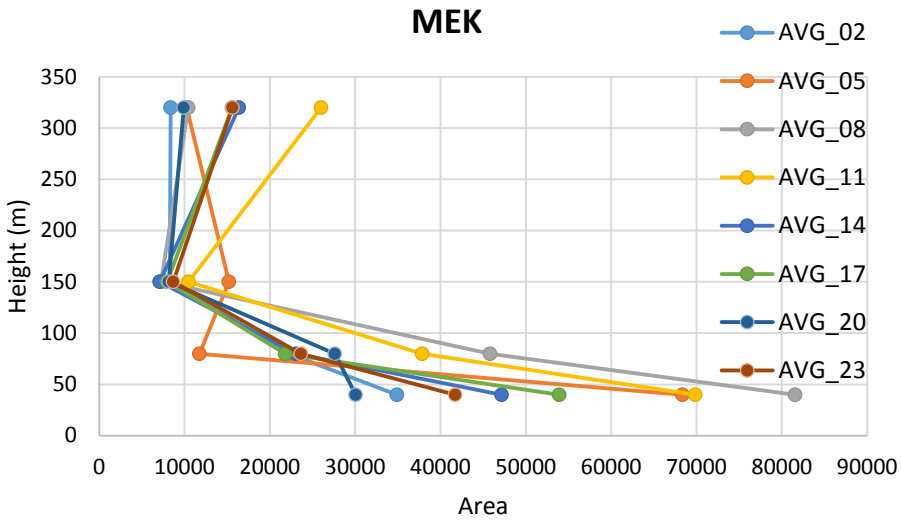


Figure 4.20: Dependence of MEK, at different time, from height.

At 40m there is the maximum emissions of MEK (demonstrating that it comes from vegetation), then, with increasing height, the concentration tends to decrease up to 150m, after 150m the profile changes and there is an increase, this is probably due to the oxidation of other compounds that have synthesized the MEK.

4.3.5.4 Acetate Compounds

One group of oxygenated compounds emitted by plants that play important ecological roles, including plant–plant and plant–insect interactions are volatile acetate esters (Engelberth et al.2004; Chehab et al. 2008, 2010). Different volatile acetate esters can be produced and emitted from plants, including short chain acetate esters, medium chain acetate esters, monoterpene acetate esters, aromatic acetate esters and fatty acid-derived acetate esters (Jardine et al., 2014). One of the most interesting acetate compounds to monitor is Geranyl Acetate, this compound is classified as oxygenated monoterpene and is directly emitted from plants (Hendel-Rahmanim, 2007). Figure 4.21 shows the vertical profile of Geranyl Acetate.

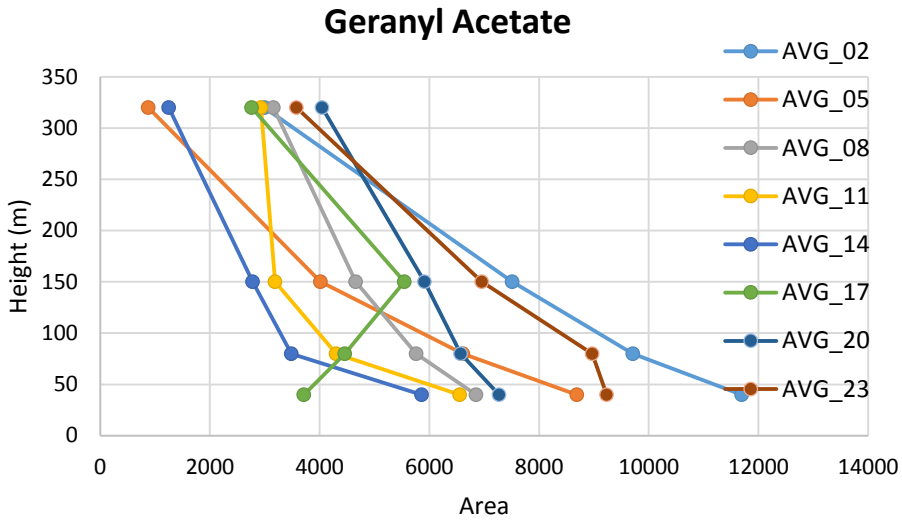


Figure 4.21: Dependence of Geranyl Acetate, at different time, from height.

This compound has a trend depending on the height; in fact the greatest emission is at 40m and decrease at 320m. The main characteristic is that it does not follow the classic daily profile but turns out to be on the contrary, there are more emissions during the night (Seghetti C. et al., 2018). The Geranyl Acetate is regulated by the Geraniol; in fact Hendel-Rahmanim et al demonstrated that Geraniol level limits Geranyl Acetate production under continuous light conditions. They suggested that once Geraniol is available, Geranyl Acetate emission is regulated at the level of gene transcription. However, under continuous light, Geraniol accumulation ceases, thereby inhibiting the production of Geranyl Acetate.

4.4. Conclusions

In the research of the biogenic compounds emitted by plants there is little information regarding oxygenated compounds either directly emitted or deriving from oxidation reactions. In this case, different classes of oxygenated compounds such as aldehydes, ketones, carboxylic acids, alcohols and acetate compounds have been studied; in addition to these, the isoprene oxidation compounds were also studied. The use of the chiral column allowed the separation of the enantiomers R and S and, in the case of 1-Hexanol-2-Ethyl it was possible to evaluate the relationship between the two enantiomers. In this case, a change of chirality is observed between 40 and 80m and again between 80m and 320m, probably due to the deforestation

below the sampling tower. Aldehydes and carboxylic acids are directly emitted by plants but in response to an attack by bacteria or fungi or to defend themselves from environmental stress. These compounds show a linear pattern, they decrease when height increases. Moreover, given the presence of aldehydes from C5 to C12, it has been observed that Nonanal is the main emitted aldehyde; plants do not directly emit this class of compounds but they are emitted after a bacterial or insects attack. In the case of the MEK, however, we found that it is directly emitted from the vegetation in fact shows an high concentration at 40m. Subsequently it tends to decrease until it reaches 320m where an increase can be observed, this has become due to the formation of MEK as an oxidation product of alkanes or other compounds. Finally, the degradation products of isoprene have been taken into consideration and studied. The data obtained have a trend in agreement with the results in the literature, at times when the isoprene has high emission, the MVK and the MACR are lower but they increase with height, in fact, they get higher concentrations at 320m. These results can be considered preliminary, the hereinafter of this research is to correlate the data obtained with the meteorological conditions during the sampling period and with the emission data of the main biogenic compounds such as α-pinene, limonene, β-pinene and others.

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5. The sense of smell for homing pigeons.

5.1. Introduction

One of the great mysteries related to the world of birds is how they navigate. There are two principal versions. There are two criteria needed to coordinate this task, a compass sense (a sense of direction) and a map sense (a sense of location). It is the ability to return from unfamiliar locations that posed the question of what sensory cues are used to determine locational information as well as directional information. It has been proposed that the compass sense can be derived from a number of perspectives. Magnetic orientation as a mechanism for directional sense was first put forward in the 19th century (Wiltschko W. and Wiltschko R., 1996). Equally, the sun could be used as a compass in order to navigate home (Kramer, 1959). Papi's mosaic model proposes that pigeons construct a map from the distribution of environmental odours, within a radius of 70-100 kilometres (Papi et al., 1971). Wallraff's gradient theory overcomes the problem of distance limitation by proposing the existence of long-range, stable atmospheric odour gradients (Wallraff, 1989a). However, about 40 years ago, Papi conducted in Tuscany a simple experiment that changed the course of research on bird navigation: a group of pigeons with their olfactory nerves sectioned were released at an unfamiliar site and never returned; their intact companions rapidly flew back to the loft (Papi et al., 1971). At about the same time, Wallraff and colleagues were investigating the behaviour of pigeons raised in confined aviaries and exposed to different environmental stimuli. Testing birds raised confined in an aviary provided with glass screens and birds raised in an aviary surrounded by a palisade, which prevented the full view of the horizon, they expected to observe an impairment only in the latter group. The pigeons allowed to view the horizon, but sheltered from the winds by glass screens, were unable to orient towards home. By contrast, birds exposed to the winds passing through the palisade were unimpaired, even if the view of the surroundings was obstructed. These first results, later confirmed by subsequent experiments, led to the hypothesis that an 'atmospheric factor' was likely to be involved in navigation (Wallraff, 1970). Both the results of Wallraff and Papi are explained by the 'olfactory navigation hypothesis' proposed by Papi (Papi et al., 1972): pigeons at their home area are able to learn windborne environmental odours in

association with wind direction; once at the release site they are able to recognise the prevalent local odours and recall from which direction these odours come from at the home area in order to determine the direction of displacement (Gagliardo, 2013).

A scheme of this theory is illustrated in Figure 5.1, there, the density of coloured dots symbolizes the idea that atmospheric odours are distributed along concentration gradients as hypothesized by Wallraff. Small dots of different colour represent different odour compounds distributed along the concentration gradients. Box A) schematizes the learning phase, the pigeon, at home, learns odours and their provenance direction. Box B) Operant phase: at the release site, the bird gathers information about local odours; the most abundant odour is the 'blue', this indicates that the bird has been displaced northwest. In fact, the bird has learned that the northwest wind was richer in 'blue' odour compounds. The bird orients southeast to fly home. (Gagliardo, 2013)

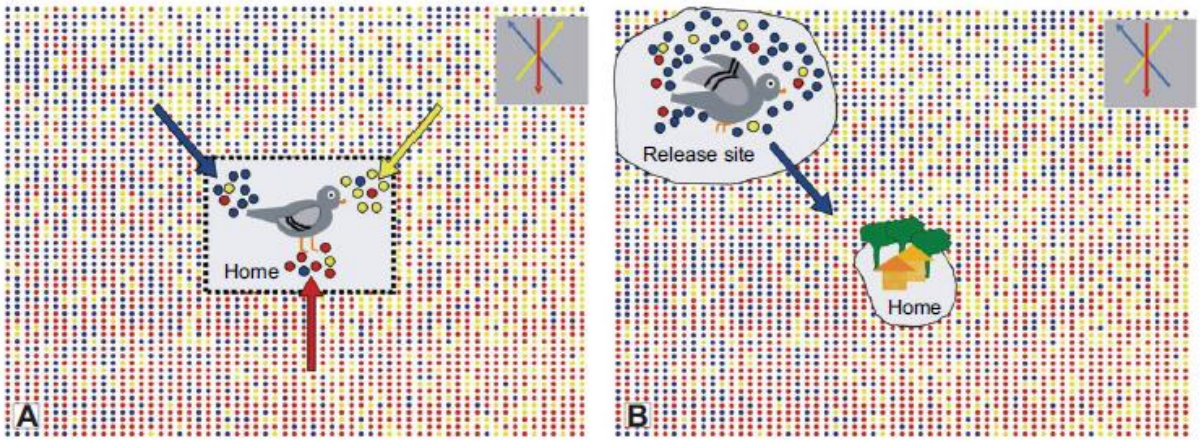

Figure 5.1: Scheme of the olfactory navigation hypothesis according to Wallraff's. Different colours of dots indicate different volatile molecules while the density of the dots schematize their concentration gradients. Box A) Learning phase, pigeon maps the odours. Box B) Operant phase, the pigeons when released use the odour mapped to return home. (Gagliardo, 2013).

A lot of experiments have demonstrated that birds deprived of their sense of smell with a reliable long-lasting method and released at unfamiliar locations are, in most cases, randomly scattered or oriented in a direction different from the home direction, but always consistently impaired in their ability to find their way home (Wallraff, 2005a). It is unknown which odours are preferentially learned by the pigeons in association with wind direction. Although it might be possible that any windborne odour might be incorporated in the map mechanism, it is reasonable to assume that biogenic odours are the most likely candidate. Interestingly, it has

been observed that orientation and homing performance of pigeons belonging to the same colony varied according to season, as the birds performed better in spring–summer than autumn–winter (Foà et al., 1984, Benvenuti et al., 1998; Benvenuti and Ranvaud 2004; Gagliardo et al., 2000).

One of the most convincing pieces of evidence in favour of olfactory navigation is that pigeons can incorporate artificial odours in their olfactory map if exposed to artificial air currents blowing artificial odours from specific directions (Ioalè et al., 1990; Papi et al., 1974). In one experiment (Figure 5.2), a group of birds received turpentine from the north and olive oil from the south, while for another group of pigeons turpentine was blown from south and olive oil from north. The birds were then displaced east and exposed to one of the two odours at the release site. When exposed to turpentine prior to release, the birds that had learned that turpentine was associated with the north wind oriented southward, while the group that had learned that turpentine was associated with the south wind headed north. Complementary results were achieved by stimulating the pigeons with olive oil. Therefore, one group of birds learned that northern areas were rich in turpentine and the southern areas were rich in olive oil; for the other group of pigeons this map was turned 180 degrees. Interestingly, stimulation with artificial odours produced initial orientation towards an expected direction only if the birds had been previously exposed to these odours in association with a wind direction at the home loft (Gagliardo, 2013).

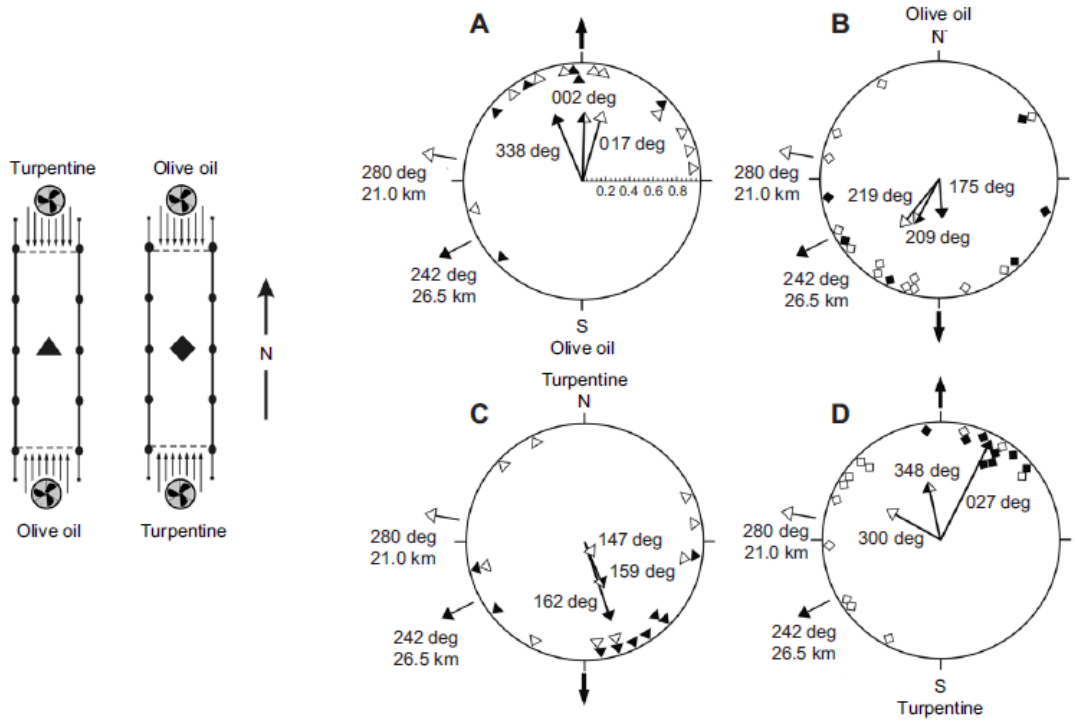

Figure 5.2: Scheme of the orientation of pigeons experiment, here they were exposed to odorous air currents. One group of birds (triangles) was exposed to olive oil from the south and turpentine from the north; another group of birds (diamonds) was exposed to olive oil from the north and turpentine from the south. The circular diagrams report the initial orientation of the birds when released from two sites. Before being released the birds were exposed to one of the two odours (olive oil, A and B; turpentine, C and D) learned in association with the artificial wind direction and oriented consistently to the expected direction (large outer arrow) predicted by the artificial odour current. The mean vectors of the initial orientation distributions are represented by the inner arrows (filled, open and half-filled/open symbols refer to the two different release sites and to the pooled distribution, respectively). Data are from Papi et al. (Papi et al., 1974) while scheme is from Gagliardo 2013.

These experiments have demonstrated the ability of pigeons to associate a specific odour to a direction, however, an olfactory map of the chemical compounds playing the role in the pigeons homing abilities has never been built. In this chapter, I will discuss the preliminary results of a field work conducted at the birds aviary of the University of Pisa in order to measure the VOC concentration in air and input these results in a model to build the birds olfactory map.

5.2. Materials and methods

5.2.1 Sampling Site

The sampling site chosen for this analysis study is the laboratory of ethology from the Faculty of Biology of the University of Pisa, located in Arnino, San Piero a Grado (Pisa, Italy, 43°39'25.0"N 10°18'16.7"E). This site is a rural site surrounded by different sources of biogenic and non-biogenic emissions. Land use management emissions are due to the presence of the

local coastal pine forest (mainly maritime and domestic pines), monti pisani with the Mediterranean maquis, the Tirrenian sea, the industrial harbour of Livorno, large urban agglomerate of Pisa and Livorno (nr. Inhabitants 249.793) and Pisa airports maritime pines and other types of plants, sea, urban and industrialized site as shown in Figure 5.3.

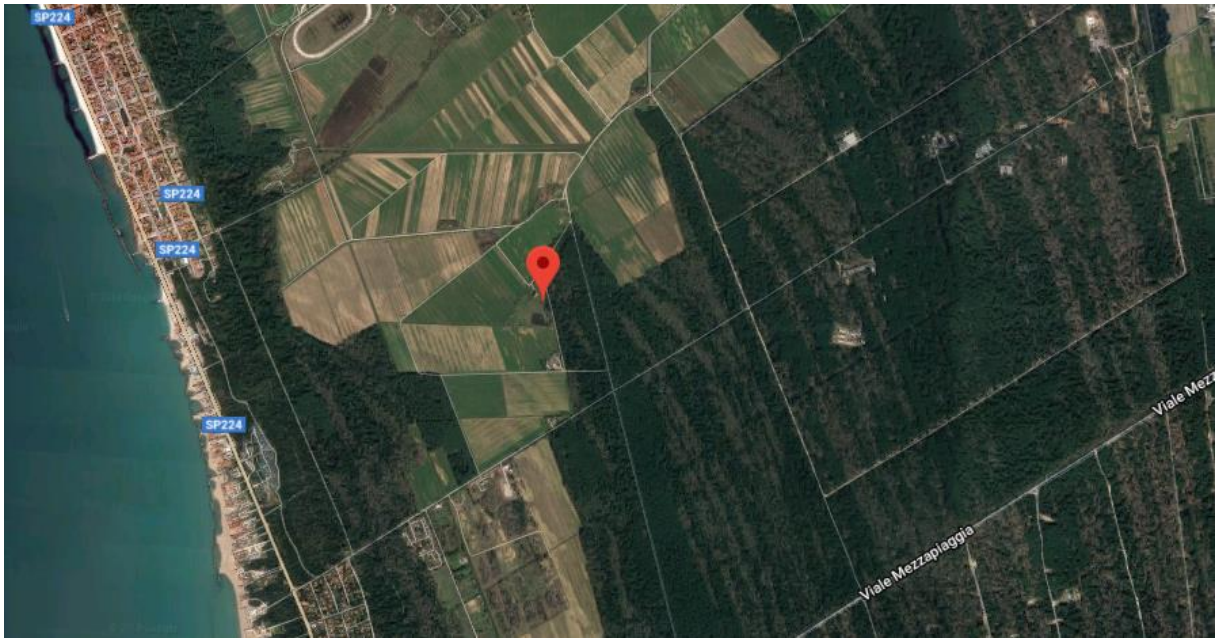

Figure 5.3: Sampling site in Arnino (Pisa).

Homing pigeons live in a loft (Figure 5.4) that allows them to take shelter at night but, at the same time, they are free to fly during the day. The birds aviary has an automatical opening which allows birds to fly free above the area (xxx) during the day and fly back there in the evening.

Figure 5.4: Homing pigeons cage in Arnino (Pisa).

5.2.2 Air sampling

The sampling system is completely innovative: it allows real-time analysis of the air, in fact, it is not necessary to use cartridges with adsorbent material, but the sampled air is injected directly to the gas chromatograph coupled with a mass detector. The samples were collected in the area near the loft where the homing pigeons are found; the instrument has been positioned inside a container; outside them a sampling line has been placed to take the air and analyze the samples on-line (Figure 5.5 and Figure 5.6).

Figure 5.5: Container for the measurement apparatus. At the top of the container, the weather station and the sampling line.

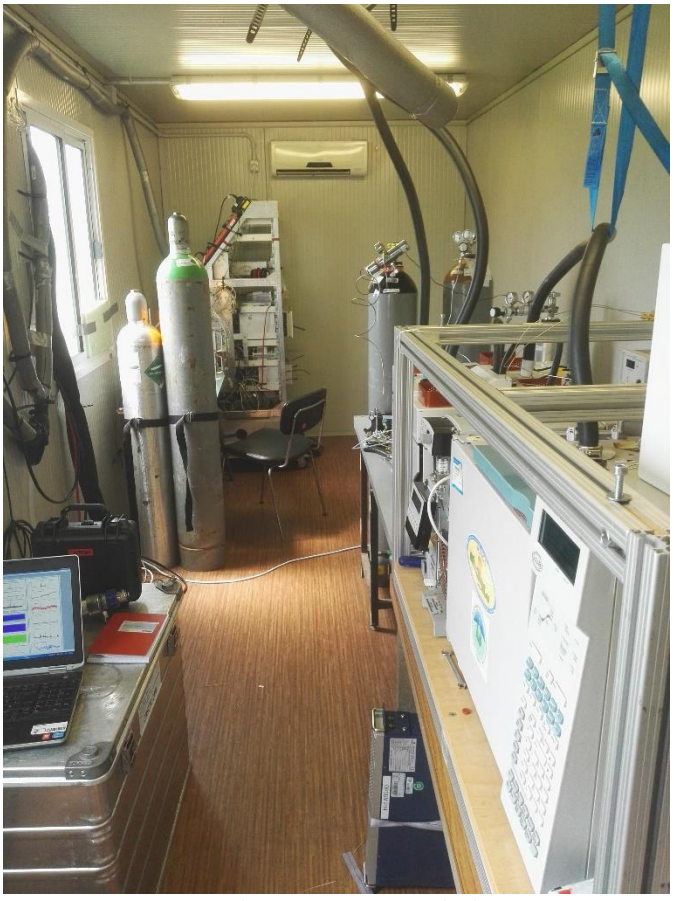

Figure 5.6: Sampling instruments inside the container.

The sampling line was made using inert PVC pipes connected to a pump to suck the air from the outside; moreover, the line has been insulated with polyurethane pipes. The samples were collected using a thermal desorption (TT24-7, Markes, UK) characterized by two traps that hold the sample alternately (collects the sample in Trap A and desorbs Trap B, then desorbs the sample collected in Trap B previously) and then send it to the GC-MS for analysis. In this way, a sample is analyzed when the previous chromatographic run ends (every 90 minutes). The sample flow is 200 ml/min for 15 minutes with a total of 3 L of air for each sample. In addition to the desorber, two instruments were used that allow the removal of moisture from the samples (Kori-xr, Markes, UK) in order to obtain greater sensitivity (Figure 5.7).

Figure 5.7: On-line thermal desorption (TT24-7) and accessories to remove the humidity (Kori-xr).

Moreover, the meteorological data were also collected using a portable weather station (WXT530, Vaisala, Finland) to correlate the emissions to different atmospheric agents but above all to correlate them with wind direction. The acquisition of data was made using software user friendly developed by the research group of Dr. Frank Drewik at the Max Planck Institute for Chemistry. The samples were collected from 15 May 2018 to 6 June 2018.

5.2.3 Analytical Instrumentation

As mentioned previously, the sampling and subsequent analysis was carried out online, therefore directly during the measurement campaign. To do this, the TT24-7 thermal desorption was used; it is a robust, transportable unit convenient for installation on-site. It interfaces to GC-MS and incorporates two electrically-cooled traps which operate in tandem

to ensure 100% sampling efficiency. Air is first sampled onto the channel A focusing trap at electronically controlled flow rates up to 1 L/min. At the end of the sampling time, channel B is switched in-line while channel A is pre-purged, desorbed and analysed. Trap heating rates approaching 100°C/sec combined with a reverse flow of carrier gas ensure efficient backflush desorption of analytes over a wide boiling range - giving narrow peak widths and optimum sensitivity. When sampling switches back to channel A, channel B is desorbed and analysed (Figure 5.8). An inert valves inside the TT24-7 isolate the sampling process from the analytical system allowing the analysis to overlap with collection of a subsequent sample. Sampling can proceed on the desorption system while GC-MS analysis of the previous sample continues.

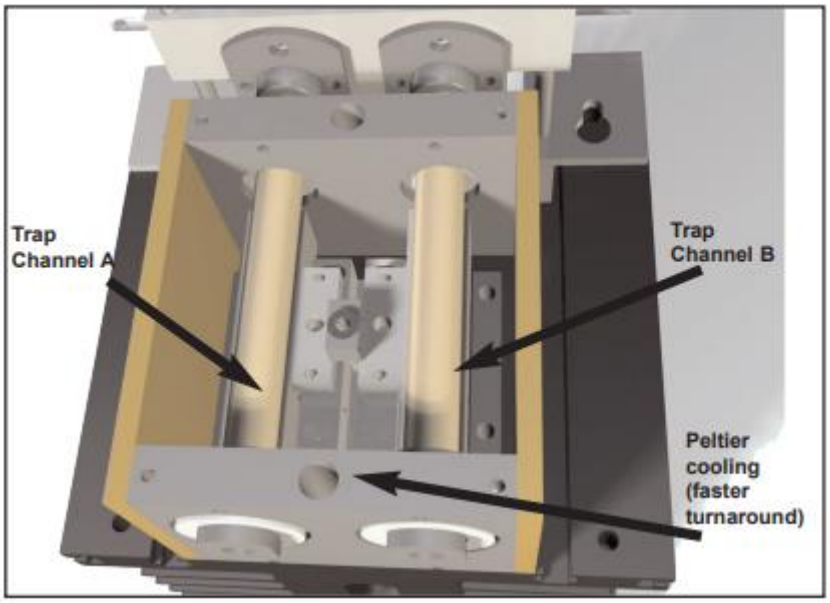

Figure 5.8: Scheme of the two traps for sampling.

Moreover, in order to avoid poor chromatography, it is necessary to remove the moisture before the gas flow reaches the GC column and detector, this procedure was done through the use of Kori-xr. The Kori-xr trap, held at –30°C, sits in-between the sample inlet and the sorbent-packed focusing trap, causing vapour-phase water in the air sample to be deposited as ice. During this process, collection of VOCs on the focusing trap continues unaffected. (Figure 5.9a). When sampling is complete, the analytes are transferred from the focusing trap to the GC, and ice is purged from the Kori-xr trap, to prepare it for the next sample (Figure 5.9b).

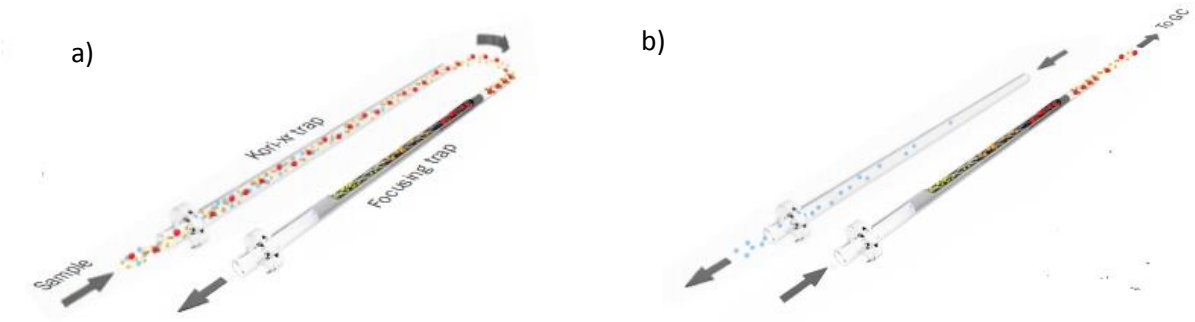

Figure 5.9: Scheme of air treatment: a) accumulating step with dehumidification b) analytical step with water trap desiccation.

The GC-MS used to quantify all compounds was a g6890A from Agilent (U.S.A.); Helium was used as the carrier gas, and separation occurred on Dimethyl TBS Cyclodextrin based column (0.15 µm, 0.15 mm ID, 25 m L). The oven was initially set to 40 °C for 5 min, increased to 150°C at a rate of 1.5°C min, and then increased again to 200°C at a rate of 30°C min-1, maintained for 1 min. Identification of separated compounds was achieved through injection of pure compounds and calibration gas curves; or with the use of the NIST library (similarity index greater than 75%). The analysis were performed at -70eV.

In addition to the analytical tools to qualify and quantify the compounds in the air, we have also used a weather station to follow the main atmospheric effects such as rain, wind direction, temperature (Figure 5.10).

Figure 5.10: Portable weather station

The portable weather station (WXT530, Vaisala, Finland) provides six of the most important weather parameters: air pressure, temperature, humidity, rainfall, wind speed and wind direction.

5.3. Preliminary Results

5.3.1 Calibration Curves

The calibration curves were prepared starting from standard gas cylinders. In particular, two different calibration gas were used, the first one is composed by 162 molecules in N_2, while the second one is composed by 15 compounds, both from Apel Riemer Environmental Inc, USA, with certificate standards and accuracy better than ±5%. Two different standard cylinders were used because in the first one there are compounds like (+)(-)-Limonene; (+)(-)-α-Pinene; (+)(-)-β-Pinene and Benzene while in the second one are compounds like Methacrolein, MEK, Methyl Vinyl Ketone (MVK), DMS, Isoprene, Toluene and 1,3,5-Trimethylbenzene.

The gas standard was prepared by diluting known volumes of the gas standard with synthetic air and then was connected to TT-24-7 to collect samples on Trap A and Trap B, alternatively. The calibration curves vary according to the initial concentration of the compound inside the cylinders, the calibration ranges are summarized in Table 5.1 below.

Table 5.1: Calibration ranges obtained from standard gases.

Compounds	***Calibration range (ppb)***
(+)-Limonene	0.11-2.27
(-)-Limonene	0.015-0.28
(+)-α-Pinene	0.13-2.58
(-)-α-Pinene	0.15-2.85
(+)-β-Pinene	0.007-0.137
(-)-β-Pinene	0.27-5.24
Benzene	0.62-12.01
Methacrolein	0.51-5.04

MEK	0.52-5.19
MVK	0.48-4.78
DMS	0.50-4.95
Isoprene	0.50-5.00
Toluene	0.50-4.97
1,3,5-Trimethylbenzene	0.49-4.82

All calibration curves have a correlation coefficient (R^2) higher than 0.90. The calibration curve for benzene trapped with line A is shown in Figure 5.11, it is different from the corresponding curve obtained with line B (see Figure 5.12).

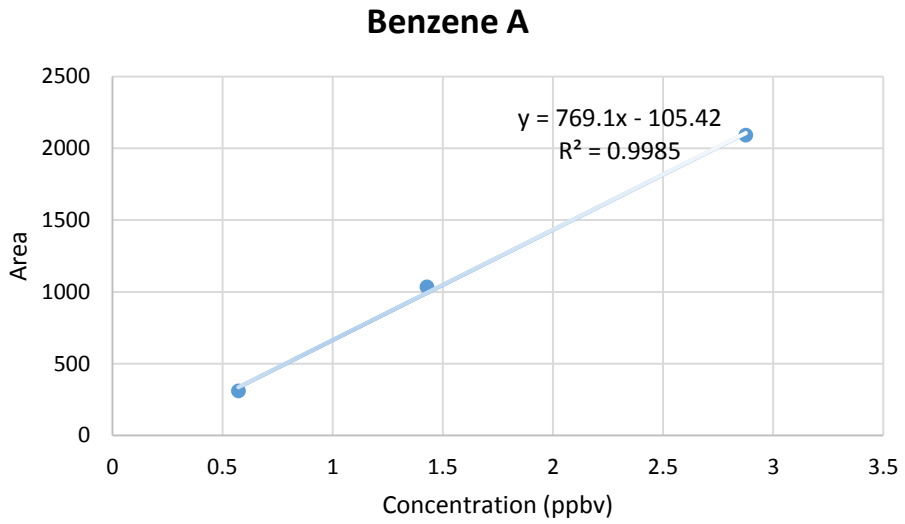

Figure 5.11: Calibration curve of Benzene on Trap A.

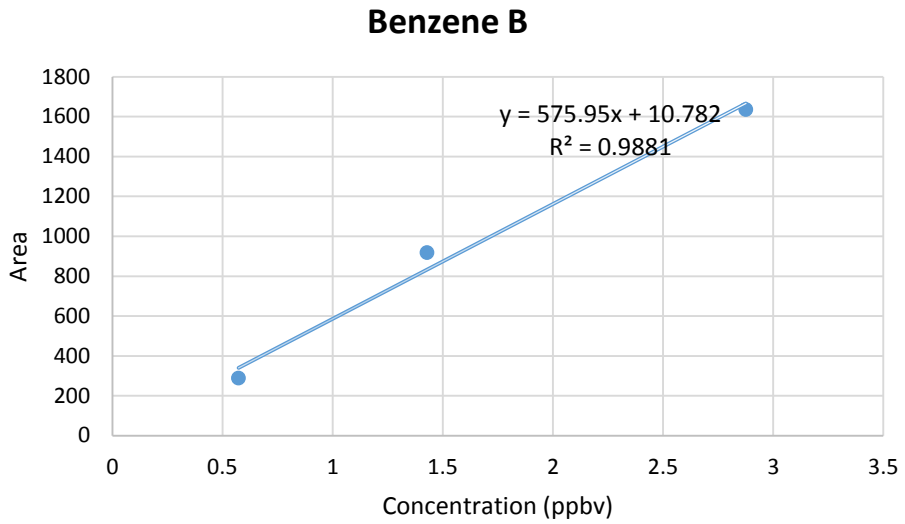

Figure 5.12: Calibration curve of Benzene on Trap B.

The values of the peak areas are lower than those obtained on line A for corresponding concentrations, for this reason two different response factors were estimated for the two lines and used accordingly.

5.3.2 Studied Compounds

In this chapter, I am focusing my attention on three different compounds in the air that originate both anthropological and biogenic: Acetone, Benzene and Toluene. These three compounds were chosen to observe the impact on homing pigeons of compounds generally classified as pollutants. Aromatic compounds is an important class of hydrocarbons taking a significant part in the formation of tropospheric ozone and the secondary pollutants, including organic aerosols, that can lead to photochemical smog (Wayne,2000; Baltaretu et al., 2009). A dominant source of aromatic compounds, particularly in urban environments, is automobile emissions with significant emissions being also from their use as solvents in various manufacturing processes (Wayne, 2000). Benzene and Toluene are often associated with air emissions from petroleum production industries (motor vehicle exhaust, incomplete combustion of fossil fuels, oil and gas service stations, and other industrial and human activities) (Filella and Peñuelas 2006, Skorokhod et al., 2017).

The concentrations of Benzene (Figure 5.13) and Toluene (Figure 5.14) found with this analysis are plotted with the wind direction to see if any correlation is present with the wind direction and with the different times of the day (from 31-05-2018 to 6-06-2018).

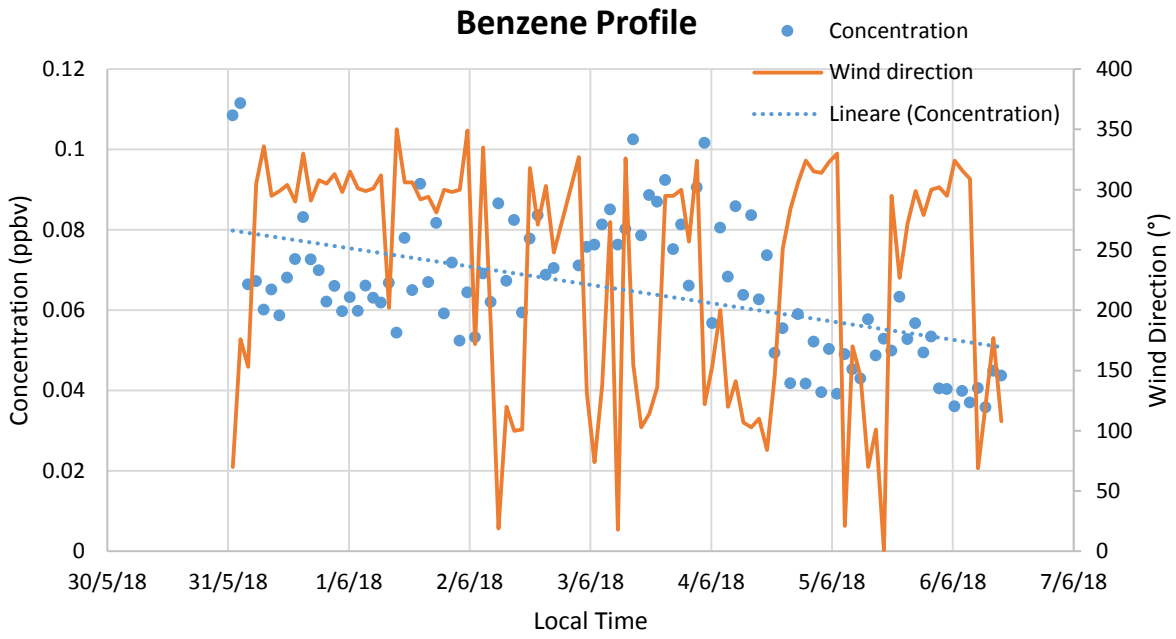

Figure 5.13: Correlation between Benzene emissions and wind direction from 31-05-2018 to 6-6-2018.

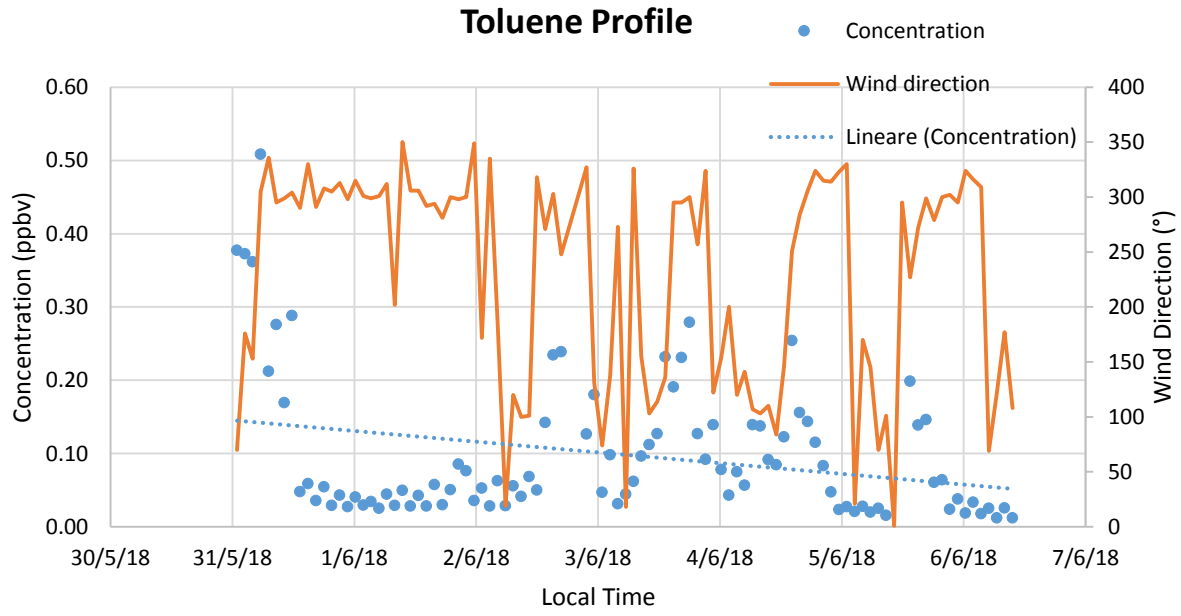

Figure 5.14: Correlation between Toluene emissions and wind direction from 31-05-2018 to 6-6-2018.

Figures 5.13 and 5.14 show the variability of concentration of benzene and toluene during the measurement time; generally the highest concentrations were measured during daytime. This result can be explained if one considers the presence of Benzene and Toluene due to traffic and pollution caused by vehicles, in fact, in general, there are many more cars during daytime than during night time. In addition, the profile of emissions in relation to the wind direction shows that there is a maximum of emissions during the day but especially when the wind is between 90 and 150 degrees, to indicate the origin of Benzene and Toluene from East / South East where most of the anthropogenic activities are taking place (i.e. the city of Pisa and where the industries are present).

One of the most abundant reactive oxygenated species in the remote atmosphere is acetone. It was first measured by Cavanagh et al. [1969] in the uncontaminated Arctic air of Point Barrow, Alaska. Acetone is emitted into the atmosphere from both natural and anthropogenic sources. Natural sources include direct emissions from vegetation, decaying organic material and secondary production by the oxidation of biogenic hydrocarbons (Singh et al., 1994; Jacob et al., 2002). Anthropogenic sources are vehicular emissions, solvent use and secondary production by the oxidation of man-made hydrocarbons (Singh et al., 1994; Jacob et al., 2002). In addition, biomass burning is found to emit acetone (Singh et al., 1994).

The concentration of Acetone (see Figure 5.15) found with this analytical method has been correlated with the wind direction and with the different times of the day (from 31-05-2018 to 6-06-2018).

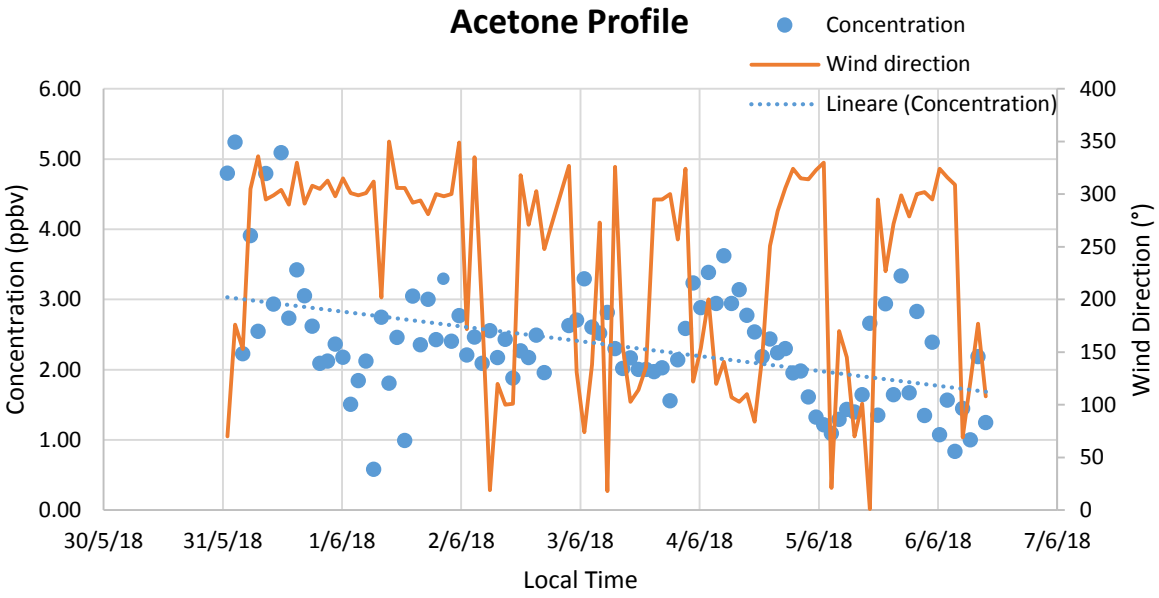

Figure 5.15: Correlation between Acetone emissions and wind direction from 31-05-2018 to 6-6-2018.

The daily profile turns out to be very particular as can be seen from Figure 5.15. It is not clear whether the dominant source for acetone is occurring during the day or during the night and if it has a preferred direction.

5.4. Conclusions and Next Experiments

The campaign to measure emissions in Pisa near the homing pigeon loft has allowed the study of different compounds and the correlation of them with the different atmospheric events with the aim of creating an olfactory map for the birds. The olfactory map of pigeons will be created by making a climate model where a series of variables will be taken into consideration such as direction, wind speed, temperature and light, atmospheric reactivity. In this project a new and innovative sampling and analysis method was used that allows online sampling and analysis of the compounds present in the air thus avoiding any type of contamination due to transport or storage. In particular, I studied compounds emitted primarily from anthropogenic sources such as Benzene, Toluene but also Acetone, and were later correlated with the wind direction figures to understand where the major contribution of these compounds comes from. In the case of compounds such as Benzene and Toluene (emitted by vehicles or industries), it is possible to observe a daily profile with the highest emissions during the day

which then decrease in the night and moreover, there are greater concentrations when the wind comes from the city of Pisa and then from East / South-East. The Acetone study (emitted from both anthropogenic and biogenic sources), on the other hand, has encountered major problems. In fact, from the profile collected, it is difficult to notice a linear profile and a specific direction of emissions; this is certainly, because this compound comes from different contributions. Moreover, to create a complete olfactory map, we are also studying other compounds of biogenic origin; at the end of this study, we will proceed to a practical study with the use of pigeons. The goal is to show that these birds return home using their olfactory knowledge so the next step is to create an artificial solution with the smells found in different directions and let the pigeons recognize the different smells and observe which direction they follow.

5.5. References

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