UNIVERSITY OF MODENA AND REGGIO EMILIA

Research Doctorate in

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VOLATILE ORGANIC COMPOUNDS IN THE PLANT KINGDOM AND THEIR BENEFITS FOR HUMAN HEALTH AND URBAN ENVIRONMENT

PhD Candidate:

Dr. Vittoria Marsili

Tutors:

Prof. Laura Arru

Prof. Luca Forti

Coordinator:

Prof. Fabio Licciardello

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Abstract

Volatile Organic Compounds (VOCs) are organic molecules characterized by the ability to evaporate at room temperature and are produced by a variety of sources, including plants, microorganisms, and anthropogenic activities. In plants, VOCs act as inter- and intra-specific communication agents, in addition to participating in internal physiological processes. This form of "chemical language" allows plants to respond promptly to environmental stimuli and coordinate their responses in stressful situations. In this context, part of the experimental work had as its object the study of VOC emissions released by plants as a mechanism by which plants show a response to an acoustic stimulus. In particular, the responses were studied both in quantitative terms of VOC and qualitatively using GC/MS analysis. It was interesting to note that aromatic plants such as lavender and rosemary respond to sound provided as traditional Vedic chants at an intensity of 80 dB for 20 minutes a day. The plants responded to the stimulus with change in the relative abundances of the VOCs that make up their essential oils as if these were a code to communicate a message.

Natural VOC released by plants, such as essential oils, not only play a role in ecological interactions but also exhibit beneficial effects on human health when inhaled. This concept is linked to the study of the psychological and physiological effects of Shinrin-yoku, or "Forest Bathing." The research explored the psychophysiological effects of olfactory stimulation induced by essential oils in a controlled laboratory setting, utilizing a non-invasive approach known as VibraImage technology. VibraImage measurements were taken on subjects before and after inhalation of essential oils. Results indicated a statistically significant increase in positive behavioral parameters and a concurrent decrease in negative ones, contributing to an overall improvement in the subject's well-being.

Simultaneously, the study examined VOC from both natural and anthropogenic sources in different areas of Reggio Emilia, Italy. All VOC influence air quality as precursors to ozone (O3), secondary organic aerosols (SOA), and particulate matter (PM). While natural VOC contribute to atmospheric pollutant formation, they also have positive effects on human health. Furthermore, vegetation plays a crucial role in air purification and improvement, directly through leaf metabolic processes and indirectly through physical mechanisms. The experimental design involved the evaluation of the qualitative and quantitative variations of VOCs in different areas of the city of Reggio Emilia characterized by different percentages of vegetation cover and proximity to roads with high vehicular traffic. The data collected suggests that air quality can be influenced by the spatial distribution and type of urban area, with urban parks and green areas showing lower total VOC concentrations than

areas with less vegetation cover. These observations can help to formulate strategies to improve air quality in urban areas and highlight the importance of vegetation in an urban context.

Overall, the research work highlights the importance of understanding the complexity of interactions between plants, VOCs and the environment to develop sustainable strategies with the aim of preserving the health of both plants and humans.

1. Introduction

1.1. Volatile Organic Compounds (VOCs): Overview and Classification

Volatile Organic Compounds (VOCs) constitute a broad class of ubiquitous compounds in the environment, emitted daily into the atmosphere from a variety of sources, both natural and human activities (Carpenter et al. 2007). These molecules have different chemical and physical characteristics, but are united by high volatility at room temperature (vapor pressure ≥ 0.01 kPa at 20 °C) and low molecular weight (generally <300 Da) (Dudareva et al. 2006; Baldwin, 2010; Li et al. 2021).

The classification of VOCs may vary depending on the context. In this work, we chose to classify them based on the source of origin into anthropogenic VOCs and biogenic VOCs.

1.1.1. Anthropogenic VOCs

For anthropogenic Volatile Organic Compounds (AVOC), we refer to all low-molecular-weight chemical species originating from human activities. Vehicle traffic and industrial activities represent one of the main sources of VOC emissions. Processes such as internal combustion in gasoline and diesel vehicles, as well as industrial activities like the production of paints, solvents, and synthetic materials, release a wide range of volatile compounds into the atmosphere. The use of fertilizers, pesticides, and other chemicals in agricultural practices can also contribute to VOC emissions. Similarly, certain consumer products, such as cleaners, interior paints, personal care products, and sprays, contain solvents and other compounds that may release VOCs when used. Tobacco smoke also contains hundreds of VOCs (Friedrich et al. 1999; Duan et al. 2023; Zhou et al. 2023).

The main categories of VOCs of anthropogenic origin are described below:

- **Aliphatic Hydrocarbons**: chemical species containing carbon and hydrogen and which, depending on the type of bond, are classified into alkanes, alkenes, and alkynes. They primarily originate from the use of fossil fuels and the production processes of the petrochemical industry (Carpenter et al. 2007).
- **Aromatic Hydrocarbons**: cyclical organic compounds. This class includes BTEX compounds represented by benzene, toluene, ethylbenzene, and three isomers of xylene (ortho-xylene, meta-xylene, and para-xylene). BTEX are emitted mainly from petroleum

derivatives but also from other sources such as biomass combustion and coal distillation (Liu et al. 2008; Caselli et al. 2010).

- **Aldehydes**: organic compounds containing the functional group -CHO, mainly emitted by combustion processes. An example is formaldehyde, an organic compound characterized by a pungent odor. In addition to being a combustion product (tobacco smoke and other combustion sources), it is also emitted from urea-formaldehyde resins used for insulation and resins used for wood chipboard and plywood, upholstery, carpets, curtains, and other textiles subjected to wrinkle-resistant treatments (Araki et al. 2020).
- **Alcohols**: organic compounds containing the hydroxyl group (-OH). Alcohols can be released into the air as gases at room temperature and can originate from various sources such as industrial processes, motor vehicles, consumer products, and domestic activities. Common examples of alcohols include methanol (methyl alcohol), ethanol (ethyl alcohol), and isopropanol (isopropyl alcohol). These compounds can be present in solvents, detergents, personal care products, paints, adhesives (Friedrich et al. 1999; Van Thriel 2014).
- **Ethers**: compounds released into the air as gases, constituting a significant part of VOCs as they come from various sources such as industrial processes, consumer products, paints, solvents, and other materials (Williams et al. 2007; Cicolella et al. 2008).
- Other classes of anthropogenic volatile organic compounds include halogenated organic compounds, sulfur-containing organic compounds, and nitrogen-containing organic compounds.

Volatile organic compounds (VOCs) are highly reactive and can spontaneously react with any particle or substance present in the environment from the moment they are released into the air, leading to the formation of unwanted organic and inorganic derivatives in the atmosphere (Singh et al. 2022). Many of these compounds play an indispensable role in the production and maintenance of the stability of various products. Furthermore, the number of new volatile compounds entering the market is constantly increasing (Talapatra et al. 2011). However, the health impacts associated with prolonged exposure to these compounds are attracting attention, as recent research highlights their influence on a wide range of diseases and disorders. This emphasizes the need for government authorities, regulatory bodies, as well as chemical and research companies, to monitor their exposure and related toxic effects (Hernandez et al. 2019).

For further details, refer to **section 1.3**.

1.1.2. Biogenic VOCs

The Earth is an ecosystem composed of interconnected physical, chemical, and biological components. The terrestrial biosphere is one subsystem of this system and, acting as a source of biogenic volatile organic compounds (BVOCs) in the atmosphere, it establishes a strong connection between the Earth's surface, the atmosphere, and the climate (Laothawornkitkul et al. 2009). Organisms that synthesize and release BVOCs include plants, trees, soil microorganisms, and some species of bacteria.

BVOCs represent approximately 90% of total volatile organic compound (VOC) emissions (Guenther et al. 2006), and can be classified into Plant Biogenic Volatile Organic Compounds (PBVOCs) and Soil Biogenic Volatile Organic Compounds (SBVOCs) based on their producers, which are living organisms in terrestrial ecosystems (particularly vegetation and soil microorganisms).

Plant VOCs, primarily composed of isoprene, monoterpenes, and sesquiterpenes, are generally produced as a means of communication or as defense mechanisms against stress (Mäki et al. 2019; Cai et al. 2021). On the other hand, soil VOCs are mainly represented by monoterpenes and oxygenated volatile compounds. Since the soil contains large amounts of decomposing vegetation and serves as the primary habitat for microorganisms such as bacteria, actinomycetes, fungi, protozoa, and nematodes, soil VOCs primarily result from the microbial decomposition of soil organic matter and fallen leaves (Tang et al. 2019). In recent decades, many studies have been conducted to estimate biogenic VOC emissions and their contribution to pollutant formation (Wang et al. 2021).

Figure 1 illustrates a diagram of interactions between BVOCs and their derivatives in terrestrial ecosystems (Cai et al. 2021).

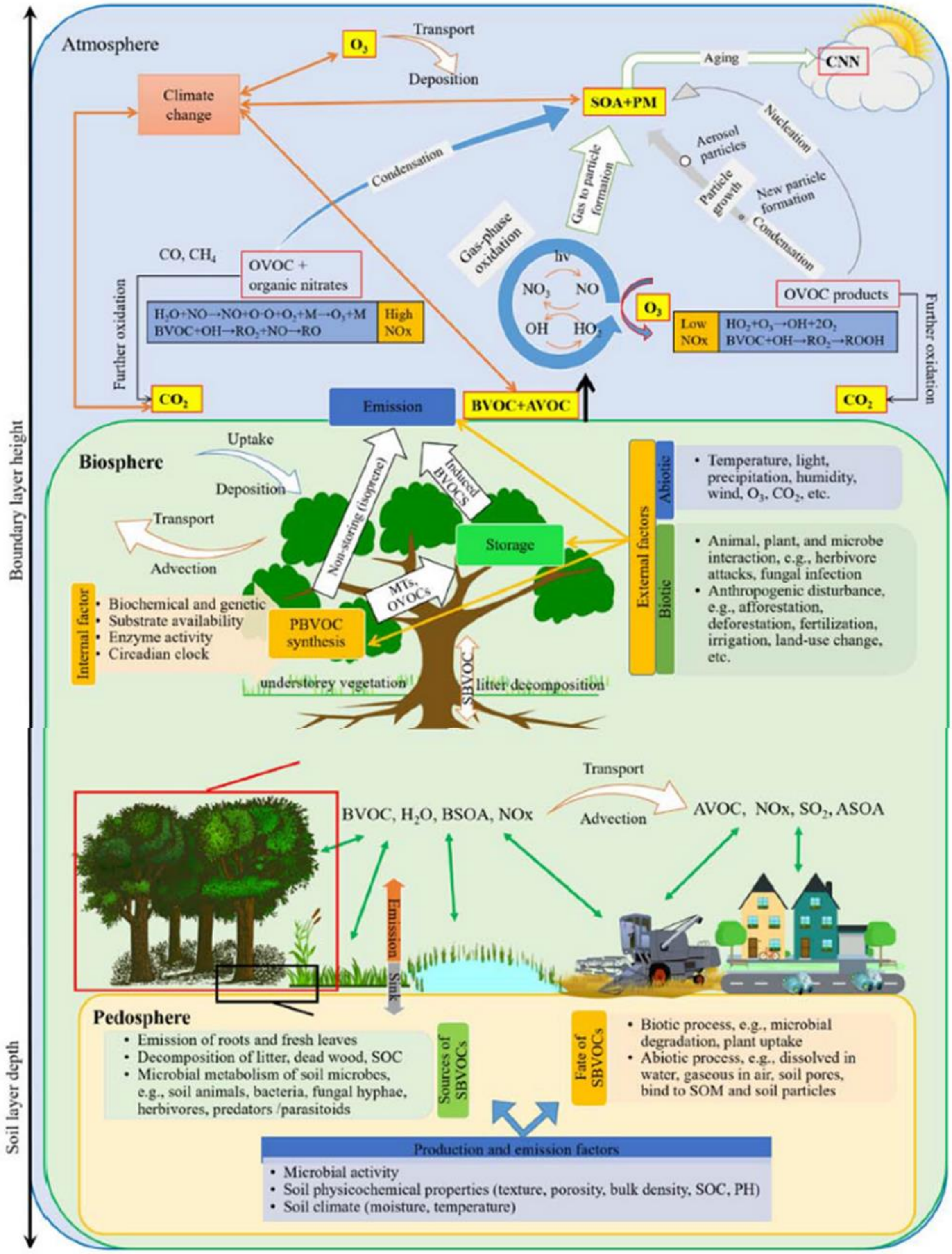

Figure 1: diagram of interactions of BVOCs and their derivatives in terrestrial ecosystems. AVOC: Anthropogenic VOC; BSOA: Biogenic Secondary Organic Aerosol; ASOA: Anthropogenic Secondary Organic Aerosol; OVOC: Oxygenated VOC; CCN: Cloud Condensation Nuclei (adapted from Cai et al. 2021).

1.2. VOCs and Plants

Plants release VOCs from both aerial and underground organs. Among these, leaves exhibit the highest emission rates in terms of mass, while the widest variety of VOCs is released from flowers and fruits, with emission rates peaking during maturation (Dixon et al. 2000; Knudsen et al. 2006; Soares et al. 2007).

The vegetative parts of woody plants release various mixtures of terpenoids, including isoprene, monoterpenes, sesquiterpenes, and some diterpenes (Owen et al. 2001; Keeling et al. 2006), while herbaceous species emit relatively high amounts of oxygenated VOCs and some monoterpenes (Fukui et al. 2000; Yang et al. 2021).

When plants suffer damage, VOC emissions can increase, and compounds called Green Leaf Volatiles (GLVs) can be produced, mainly represented by C6 aldehydes and ketones (Fall et al. 1999; Ameye et al. 2018). Biotic and abiotic stress can also induce the leaf production of terpenes, methyl jasmonate (MeJA), and methyl salicylate (MeSA), the quantity and quality of which depend on the type and extent of damage (Takabayashi et al. 1994; Mithöfer et al. 2005; Laothawornkitkul et al., 2008; Yu et al. 2018). The main classes of VOCs produced by plants are presented below.

1.2.1. Classes and Biosynthetic Pathways

Plant volatiles can be divided into four main classes: terpenoids, also known as isoprenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and amino acid derivatives. The latter are often present in the perfumes and aromas released by flowers and fruits. Although volatile compounds are synthesized through a few major biochemical pathways (**Figure 2**), various forms of enzymatic modifications such as hydroxylations, acetylations, and methylations contribute to the diversity of emitted volatiles, increasing their volatility in the final stages of their biosynthesis (Gang, 2005; Dudareva et al. 2007).

1.2.1.1. Terpenoids

Terpenoids constitute the largest class of plant secondary metabolites with many volatile representatives. Hemiterpenes (C5), many monoterpenes (C10), sesquiterpenes (C15), homoterpenes (C11 and C16), and some diterpenes (C20) indeed have high vapor pressure, enabling their release into the atmosphere (Dudareva et al. 2007). All terpenoids are synthesized from five-carbon precursors, isopentenyl diphosphate (IPP), and its allylic isomer dimethylallyl diphosphate (DMAPP), derived from two alternative pathways (**Figure 2**). In the cytosol, IPP is synthesized from three molecules of acetyl-CoA through the mevalonic acid pathway (MVA), while in plastids, it derives from pyruvate and glyceraldehyde-3-phosphate via the methyl-erythritol-phosphate pathway (MEP) (Eisenreich et al. 1998; Gershenzon et al. 2018; Newman et al. 2020).

In plastids, the DMAPP generated by the MEP pathway is used by isoprene synthases for the formation of isoprene (Rodriguez-Concepción et al. 2002). Additionally, the condensation of one molecule of IPP and one molecule of DMAPP, catalyzed by geranyl pyrophosphate synthase (GPPS), leads to the formation of GPP (C10), the precursor of all monoterpenes (Newman et al. 2020).

In the cytosol, the condensation of two molecules of IPP and one molecule of DMAPP, catalyzed by the enzyme farnesyl pyrophosphate synthase (FPPS), leads to the formation of FPP (C15), the precursor of sesquiterpenes (McGarvey et al. 1995; Zhou et al. 2020) (**Figure 2**).

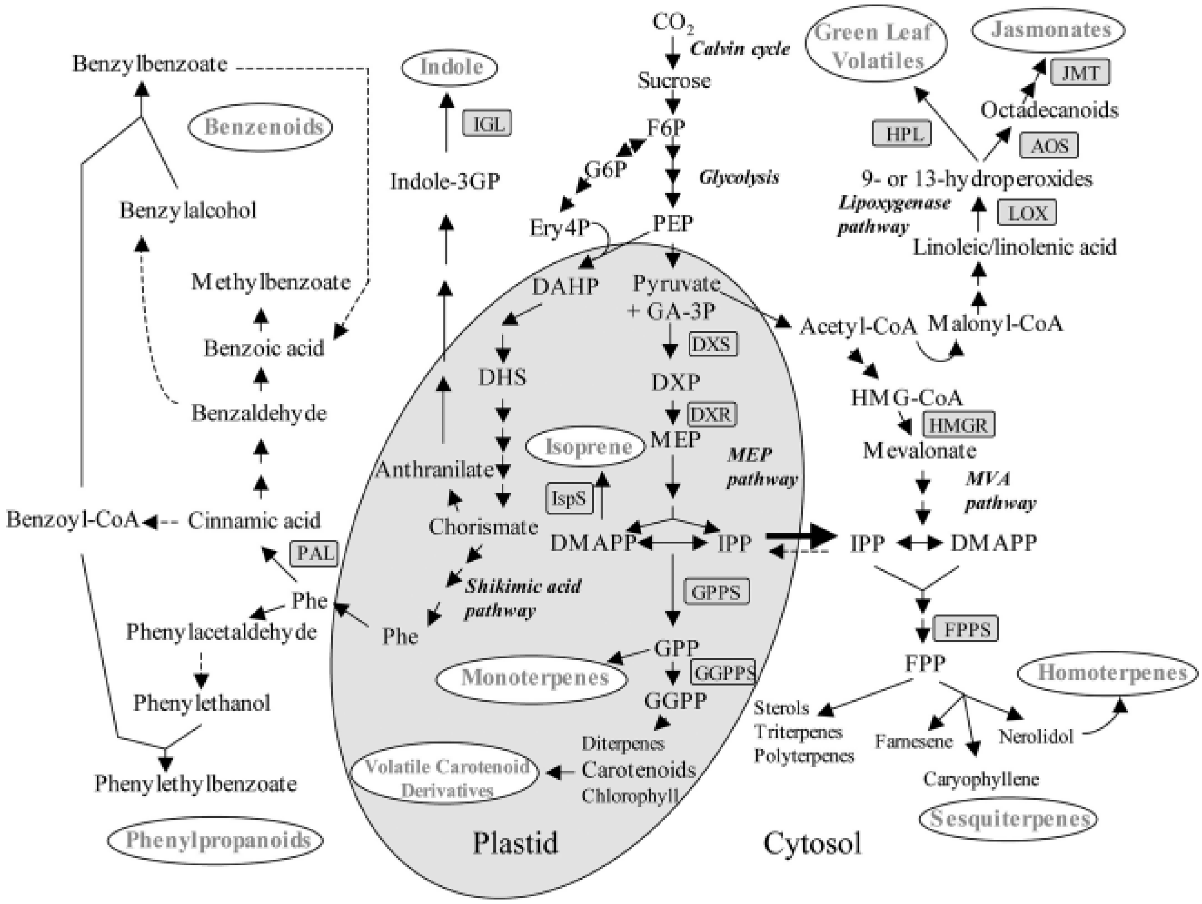

Figure 2: Metabolic pathways leading to the biosynthesis of various volatile compounds in plants. Pathway names are italicized, volatile compounds are in bold and enzymes are boxed. Abbreviations: Acetyl-CoA, acetyl coenzyme-A; AOS, allene oxide synthase; DAHP, 3-deoxy-D-arabino-heptulosonate 7-phosphate; DHS,3-dehydroshikimic acid; DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D xylulose

5-phosphate; DXR, DXP reductoisomerase; DXS, DXP synthase; Ery4P, erythrose 4-phosphate; F6P, fructose 6-phosphate; FPP, farnesyl diphosphate; FPPS, FPP synthase; GA-3P, glyceraldehyde-3 phosphate; G6P, glucose 6-phosphate; GGPP, geranylgeranyl diphosphate; GGPPS, GGPP synthase; GPP, geranyl diphosphate; GPPS, GPP synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; HPL, fatty acid hydroperoxide lyase; IGL, indole-3-glycerol phosphate lyase; Indole-3GP, indole 3-glycerol phosphate; IPP, isopentenyl diphosphate; JMT, jasmonic acid carboxyl methyl transferase; LOX, lipoxygenase; MEP, 2-C-methyl-D-erythritol 4-phosphate; MVA, mevalonate; PAL, phenylalanine ammonia lyase; PEP, phosphoenolpyruvate; Phe, phenylalanine (adapted from Dudareva et al. 2007).

- **Isoprene**

Isoprene is the most abundantly emitted volatile compound by plant species. The de novo synthesis of isoprene by terrestrial plants (about 2% of the carbon fixed during photosynthesis) contributes up to 0.5 Pg C/year to the global carbon cycle (Dani et al. 2014). This compound is never stored in plants after its production but is rapidly lost through volatilization. However, the contribution to emissions varies among different vegetation types in different areas of the Earth. Generally, woody species emit larger quantities of isoprene compared to herbaceous ones (Kesselmeier et al. 1999). Particularly high amounts are released by the Fagaceae family and the *Picea* genus (spruces) (Christianson et al. 2017). However, there are exceptions. For example, within the group of evergreen oaks that grow in the Mediterranean climate, some species (*Quercus agrifolia*) exclusively emit isoprene, others (*Quercus ilex* and *Quercus coccifera*) emit exclusively monoterpenes, while *Quercus suber* emits neither isoprene nor monoterpenes (Staudtet et al. 1995; Kaser et al. 2022; Mu et al. 2022).

- **Monoterpenes**

Monoterpenes constitute the primary fraction of essential oils produced and stored in the secretory organs of plants, such as trichomes or resin ducts, as shown in various studies conducted on conifers and members of the Lamiaceae, Apiaceae, Rutaceae, Myrtaceae, and Asteraceae families (Marchese et al. 2017). Monoterpenes like eucalyptol, borneol, camphor, bornyl acetate, carvacrol, menthol (−), γ-terpinene, (+)-α-pinene, (−)-β-pinene, and p-cymene are major constituents of essential oils (Crozier et al. 2006). Typically, plants that store monoterpenes are also emitters of monoterpenes; in particular, for some conifer species, a correlation has been observed between the concentration in the leaves and the emission from those leaves (Mermet et al. 2019). In other cases, this correlation has been less evident. For example, orange-colored leaves contain high amounts of sabinene and limonene but predominantly emit the sesquiterpene β-caryophyllene (Kesselmeier et al. 1999).

Conversely, some oak species emit high amounts of monoterpenes but do not store them (Fischbach et al. 2000; Mermet et al. 2019).

Structurally, monoterpenes can have acyclic or mono-, bi-, and tricyclic structures. They are typically non-oxygenated, with some exceptions such as 1,8-cineole and linalool. However, numerous oxygenated derivatives of monoterpenes exist (**Figure 3**) (Durareva et al. 2007).

- **Sesquiterpenes**

The emission of sesquiterpenes occurs in many plants that also emit other isoprenoids; however, the emission rates of sesquiterpenes among species cover a wide range of values and show substantial variability among individuals and species, as well as in different environments. The results of numerous studies indicate that temporal variations in sesquiterpene emissions seem to be mainly dominated by ambient temperatures, although other factors contribute (e.g., seasonal variations) (Holopainen et al. 2010). This implies that sesquiterpene emissions are more significant during certain periods of the year, especially from late spring to mid-summer (Duhl et al. 2008). The strong temperature dependence of sesquiterpene emissions also leads to an increase in emissions in warmer climates. Extreme weather events can disrupt short-term sesquiterpene emissions; however, the specific contribution induced by this type of disturbance is not yet known (Dada et al. 2023). Structurally, sesquiterpenes can be cyclic or acyclic, and numerous oxygenated derivatives exist (**Figure 3**) (Durareva et al. 2007).

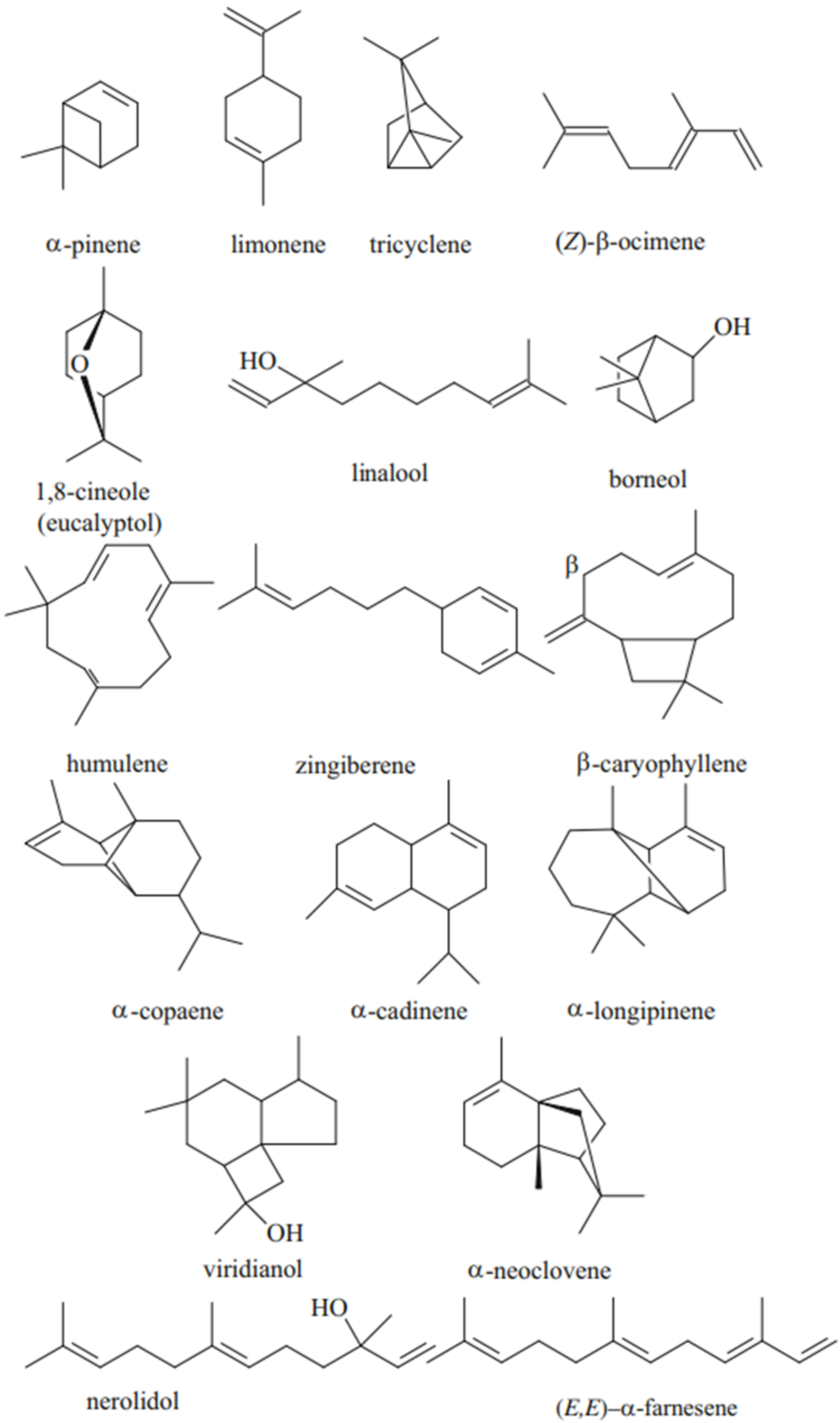

Figure 3: chemical structures of some monoterpenes, sesquiterpenes and derivatives.

1.2.1.2. Phenylpropanoids/Benzenoids

The class of phenylpropanoids and benzenoids includes various acids, aldehydes, and alcohols derived from L-phenylalanine. They are among the compounds responsible for the fragrance of flowers and the source of the pungent flavor in black pepper and chili peppers (capsaicinoids). Some, such as methyl salicylate, are induced by herbivores to attract predators and parasitoids of the herbivores (Van Poecke et al. 2001; Ament et al. 2004; Vogt et al. 2010).

They are synthesized from the shikimate pathway, which begins with the condensation of phosphoenolpyruvic acid from glycolysis and D-erythrose-4-phosphate from the pentose phosphate pathway (Widhalm et al. 2015; Santos-Sanchez et al. 2019) (**Figure 4**). Subsequently, six enzymatic steps lead to the formation of the key branching compound, chorismic acid, the final product of the shikimate pathway and the common precursor of all volatile phenols. These are represented by benzenoids (C6–C1), such as benzaldehyde, methyl salicylate, and benzyl benzoate, formed from cinnamic acid; C6–C2 compounds like 2-phenylethanol and phenylacetate, produced from phenylalanine; and C6–C3 compounds like eugenol, methyleugenol, and chavicol, produced from 4-coumaroyl-CoA (Widhalm et al. 2015).

1.2.1.3. Fatty Acid Derivatives

This class includes jasmonates and Green Leaf Volatiles (GLV). Jasmonates are compounds with plant hormone function involved in various physiological responses of plants. In particular, methyl jasmonate (MeJa) is a volatile hormone involved in plant-plant interactions (Kessler et al. 2006; Wang et al. 2020). MeJa is also among the main components of the jasmine flower scent, where it was first discovered (Demole et al. 1962).

GLVs are organic compounds that characterize the typical smell of freshly cut grass or damaged plant tissue (Hatanaka et al. 1987). Like jasmonates, they also play a key role in plant-plant, plant-insect interactions, and plant defense (Dudareva et al. 2007; Frost et al. 2008).

Volatile organic compounds derived from fatty acids (including GLVs) are produced from the unsaturated C18 fatty acids, linoleic acid, and linolenic acid. These are produced through the acetate pathway that obtains its substrate, acetyl-CoA, from glycolysis (Fu et al. 2017) **(Figure 4**). Through a series of oxidation, reduction, and acetylation steps, fatty acids are converted into aldehydes, alcohols, and acetates, such as (Z)-3-hexenol and (Z)-3-hexenyl acetate (**Figure 4**). The biosynthesis of methyl jasmonate originates from linolenic acid through multiple enzymatic steps. In particular, it is formed through the methylation of jasmonic acid by jasmonic acid carboxyl methyltransferase (Li et al. 2005; Bouwmeester et al. 2019).

1.2.1.4. Amino Acid Derivatives

This class includes volatile compounds derived from amino acids other than phenylalanine. It is a highly diversified group comprising acids, aldehydes, alcohols, esters, and nitrogen and sulfur-containing volatile compounds. Derivatives of branched-chain amino acids, such as leucine, isoleucine, and valine, are characteristic of fruits like bananas, apples, strawberries, and tomatoes (Espino-Díaz et al. 2016). Methionine derivatives, containing sulfur, are present, for example, in melons (Cannon et al. 2018) and are sometimes associated with defense functions (Sørensen et al. 2018).

In addition to canonical volatile biosynthetic pathways, occasionally in the literature, unexpected biosynthetic solutions emerge, demonstrating how extremely intricate the evolution of volatile compound biosynthesis can be (Sun et al. 2016). For example, the enzyme responsible for geraniol biosynthesis in roses has been found not to be a monoterpene synthase but rather a hydrolase (Magnard et al. 2015).

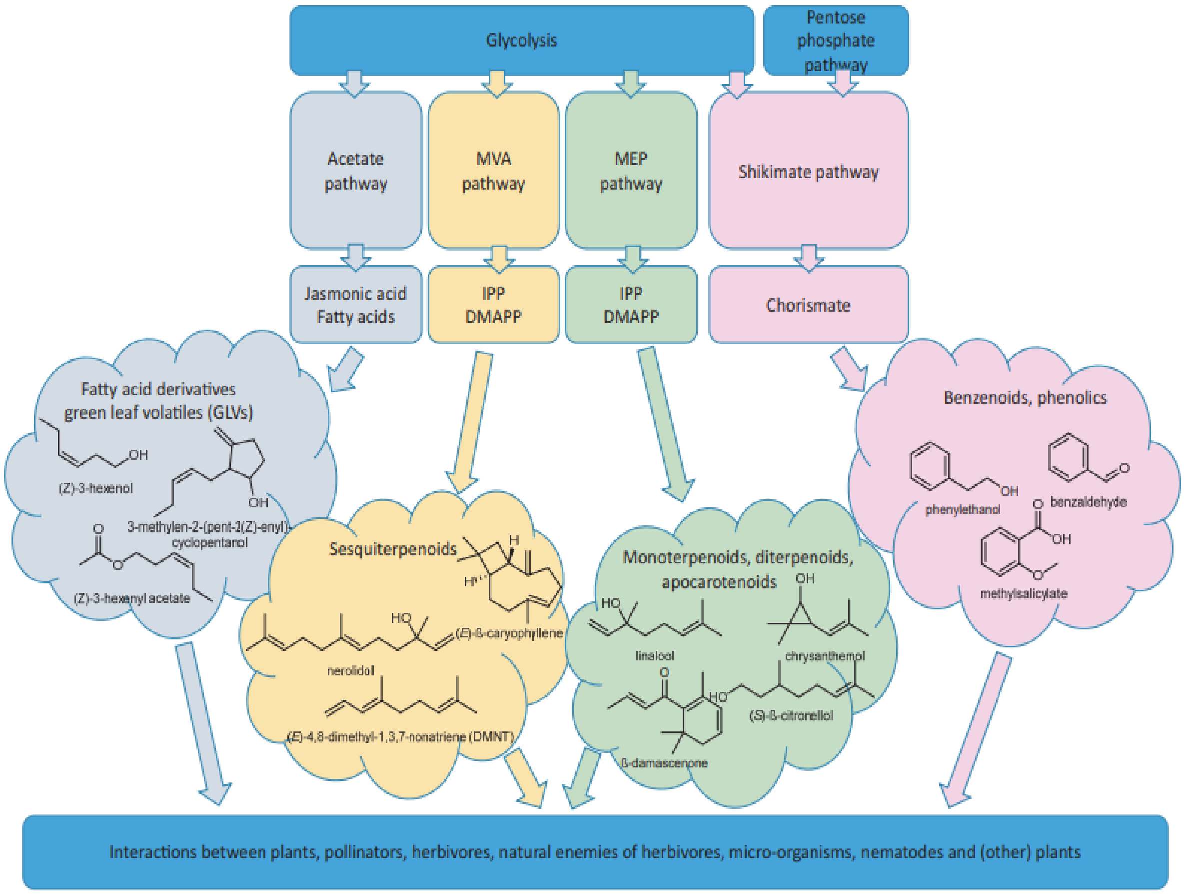

Figure 4: biosynthetic pathways of the major volatile compound classes and examples of volatiles. Fatty acid-derived VOCs (including GLVs and methyljasmonate) are produced from C18 fatty acids, produced through the acetate pathway that gets its substrate, acetyl-coA, from glycolysis. Acetyl-coA from glycolysis is also the substrate for the cytosolic mevalonic acid (MVA) pathway, while pyruvate from glycolysis is the substrate for the plastidic methylerythritol phosphate (MEP) pathway. The benzenoids and phenylpropanoids are produced through the shikimate pathway that gets its substrates from glycolysis and the pentose phosphate pathway (adapted from Bouwmeester et al. 2019).

1.2.2. Emission Mechanisms

The regulation of both the biosynthesis and release of volatile organic compounds is crucial for the functions that VOCs perform in signaling within the plant, as well as orchestrating interactions with other organisms (Blande et al. 2016). The diffusion of VOCs through plant membranes and cuticles is also likely to be an actively regulated process (Widhalm et al. 2015). Besides diffusion, emission can occur through stomata (Seidl-Adams et al. 2014), wounds (De Domenico et al. 2007), or specialized tissues such as flowers (Muhlemann et al. 2014) or glandular trichomes (Van Schie et al. 2007; Hare 2007).

At the cellular level, for VOCs to be emitted and enter the gaseous phase, they must cross membranes, the aqueous cell wall, and sometimes the cuticle. The movement of VOCs through each barrier is presumed to occur via passive diffusion. However, VOCs, which are mainly nonpolar compounds, will preferentially distribute into membranes, making diffusion slow in aqueous compartments (Sagae et al. 2008; Zvi et al. 2008).

In 2015, Widhalm and colleagues proposed a model describing VOC traffic within plant cells. Using Fick's law, they first observed that toxic levels of VOCs in membranes would be required to achieve the observed emission rates through diffusion alone. Thus, based on observations from Samuels et al. 2012; Weston et al. 2012; McFarlane et al. 2014; Mehrshahi et al. 2014, they proposed a model according to which biological mechanisms similar to those involved in the trafficking of other hydrophobic compounds would contribute to the emission of VOCs (Widhalm et al. 2015). Specifically, VOCs synthesized in the cytosol would preferentially distribute both in the plasma membrane and subcellular membranes (**Figure 5**). VOCs within compartments could be transported to the plasma membrane through endoplasmic reticulum (ER)–plasma membrane contact sites or through ER- and Golgi-associated vesicle trafficking processes, the trans-Golgi network (TGN) and/or the vacuole (**Figure 5**). Lipid transfer proteins (LTPs) might contribute to transporting VOCs through the plastid stroma, cytosol, and/or the cell wall, and transporters located in the plasma membrane could play a role in exporting VOCs from the cytosol or membrane into the cell wall (Widhalm et al. 2015). Additionally, VOCs synthesized in plastids, such as monoterpenes, can be transported to the ER through emifusion, a recently described mechanism involving the exchange of lipophilic metabolites between plastids and the ER (Mehrshahi et al. 2014). Subsequently, VOCs diffuse through the cuticle to be emitted into the environment (**Figure 5**).

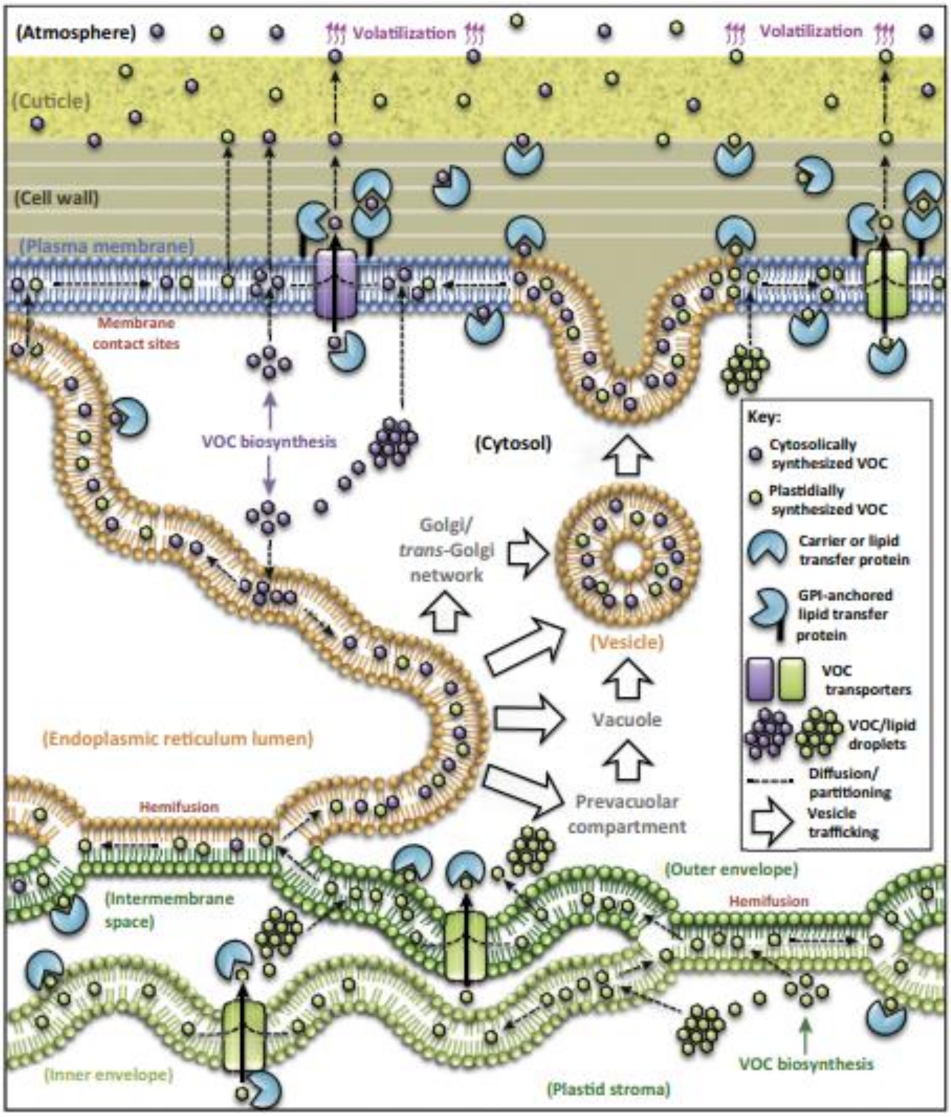

Figure 5: model for the trafficking of volatile organic compounds (VOCs) in plant cells (adapted from Widhalm et al. 2015).

1.2.3. Role of VOCs in Plant Physiological Processes

Volatile organic compounds play crucial roles within plant tissues in physiology, signaling, and defense. When emitted through the cuticle, stomata, or damaged tissues or specialized structures, they can be perceived by a range of other organisms, as well as remote parts of the plant (Dicke et al. 2010; Ninkovic et al. 2021). The composition of volatile mixtures can convey detailed information about the physiological and ecological state of plants, such as the presence of open flowers, herbivore attack, microbial infection, and ripe fruit production. Such information can be used by both harmful and beneficial microorganisms, animals, and other plants (Dicke et al. 2010).

The VOCs involved in all these functions can be produced constitutively or in response to specific interactions and external stimuli, both above and below the ground (Massalha et al. 2017). Although volatile compounds can travel long distances, plant-plant and plant-microbe communication usually occurs at relatively short distances, while plant VOCs with a role in plant-insect interaction can be perceived at distances of several hundred meters. The resulting extreme dilution and the vast variation in chemical structures and properties of VOCs pose a challenge to understanding the informative significance of VOCs, especially on larger spatial scales (Fu et al. 2017). Furthermore, as ecosystems are dynamic systems, the volatile mixture carrying information enriches during the journey, becoming increasingly complex (Aartsma et al. 2017; Kessler et al. 2018).

In this context, the timing of volatile compound emission becomes crucial for carrying out their functions in signaling within the plant, as well as orchestrating interactions with other organisms (Blande et al. 2016). Indeed, since different times of the day involve different abiotic conditions and different biotic communities, the rhythmic behavior of processes such as flower opening, leaf movement, and even the emission of volatile compounds can enhance plant fitness (Yerushalmi et al. 2009; Greenham et al. 2015).

The various physiological and ecological functions performed by mixtures of volatiles produced by plants are schematically represented in **figure 6**.

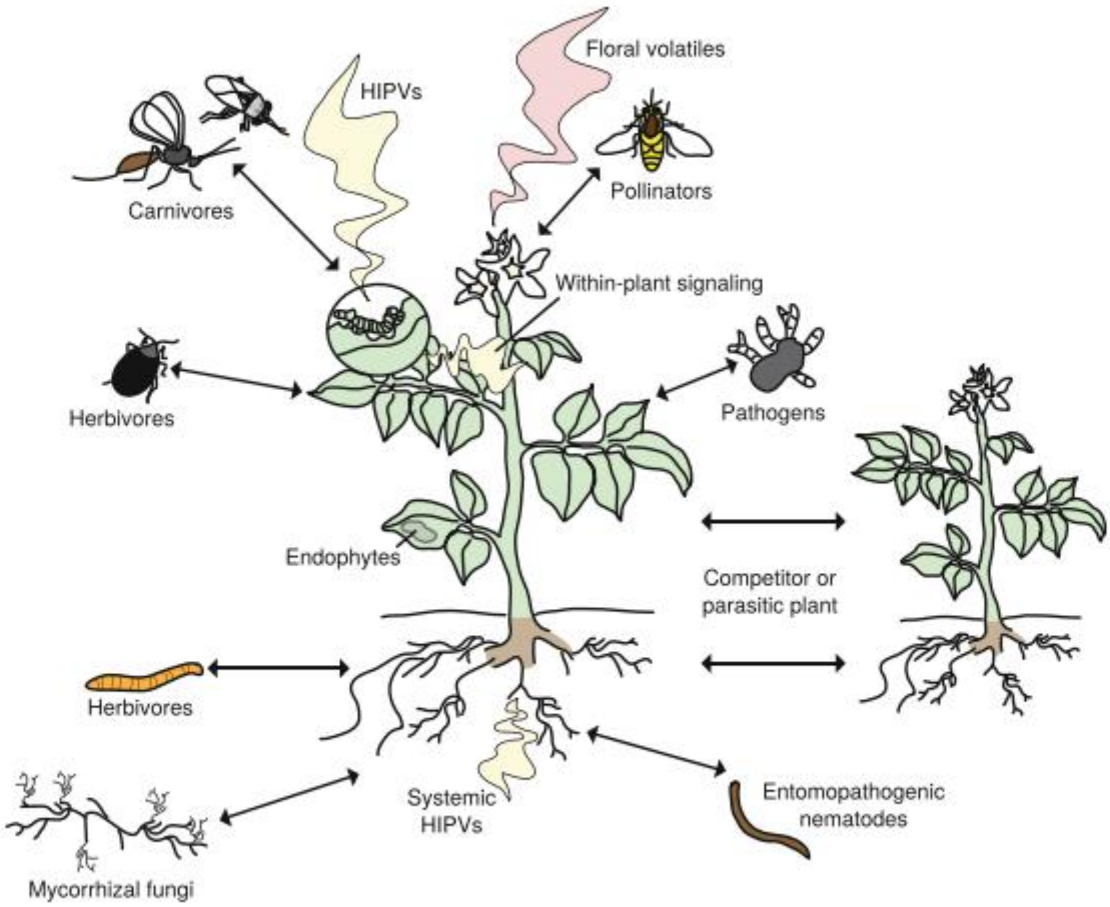

Figure 6: functional roles of plant volatile compounds (adapted from Dicke et al. 2010).

1.2.3.1. Role of VOCs in Interactions with Pollinators

Pollinators are often primarily attracted to floral scent over long distances (Hossaert-McKey et al. 2010). Floral scents comprise over 1700 compounds in various chemical classes (Mostafa 2022). Compounds that are attractive to pollinators can vary in concentration by orders of magnitude in different plant species (Schiestl 2015).

Pollinator attraction is often specific, which can be advantageous as specificity increases the efficiency of pollen transfer between plants of the same species. This specificity can be determined by a specific morphology of the flower that prevents access to the reward offered by the plant to most animals or it can be determined by signals that are detectable or of interest only to a few potential pollen vectors (Raguso et al. 2008). The chemical complexity of the floral scent involves a series of interactions between the olfactory neurons of insects, producing together the sensation of a bouquet that attracts them (Haverkamp et al. 2018).

An intriguing example is provided by the flowers of *Rafflesia cantleyi*, which emit an odor mimicking rotting flesh, attracting flies that normally lay eggs in such substrates as pollinators. The two main compounds in the floral bouquet of *Rafflesia* are dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS), produced by bacterial decomposition of methionine and cysteine in flesh, and are also present in the floral emission of *Rafflesia* (Wee et al. 2018).
Another well-known and highly specific example of floral mimicry is sexual mimicry, where flowers mimic mating signals to be pollinated by male insects (Johnson et al. 2016). This type of pollination system is commonly found among Australian orchids. For instance, methylthiophenols, previously known only as natural products produced by bacteria, have been identified as attractive signals for pollinators in the *Caladenia orchid* genus (Bohman et al. 2018).

1.2.3.2. Role of VOCs in Interactions with Herbivores

The ensemble of constitutive volatile compounds produced by plants is utilized not only by mutualists such as pollinators but also by antagonists like herbivorous insects, which exploit VOCs to identify and approach a host. Following the rupture of plant tissue by herbivores, plants release Herbivore-Induced Plant Volatiles (HIPVs), which are mixtures of volatile compounds, mainly GLVs, terpenes, and aromatic compounds (McCormick et al. 2012). HIPVs serve as signals to attract the natural enemies of herbivores, such as parasitoids and predators (Aartsma et al. 2017). For example, beetle larvae feeding on the roots of *Populus* spp. induce the emission of various monoterpenes, including 1,8-cineole, which has an inhibitory effect on *Phytophthora cactorum* (Lackus et al. 2018). These signals produced in response to herbivores are highly specific. In this regard, already in 1998, Moraes and colleagues observed that in tobacco, cotton, and maize, the de novo synthesis of VOCs involved in these interactions was triggered by the recognition of specific substances present in the oral secretions of predatory herbivores (De Moraes et al. 1998). HIPVs therefore provide plants with indirect resistance against attacking herbivores by attracting their antagonists. VOCs are also involved in direct resistance to herbivores, specifically deterring them through direct ingestion, being present in leaf tissues that come into contact with the air (Veyrat et al. 2016). Plants indeed produce a variety of chemicals with insecticidal activity, and some VOCs are directly toxic to invertebrates (Zhao et al. 2017; Laquale et al. 2018). Furthermore, blends of plant essential oils containing many HIPVs belonging to terpene classes have ovicidal and parasitic effects against various parasite classes such as lepidopterans (Isman 2016; Mossa 2016).

The hypothesis that HIPVs directly influence herbivorous insects is not new (Unsicker et al. 2009); however, the direct larvicidal or ovicidal effectiveness of HIPVs on herbivorous insects is poorly understood. The vast majority of insects have developed specialization in host range, feeding only on one or a few closely related species (Forister et al. 2015), while a minority of herbivorous insect species has a more generalist host range. Evolutionary theory suggests that chemical molecules shared among different plant taxa will be less toxic to generalist insects than to specialists (Cornell et al. 2003). HIPVs tend to be common across different plant taxa, and some of them may be pre-synthesized, stored in specialized cells in their original forms or conjugated in various types of plant tissues (Monson et al. 2012; Sugimoto et al. 2015) and released when herbivory disrupts cellular storage compartments (Niinemets et al. 2013).

1.2.3.3. Role of VOCs in Plant-Microorganism Interactions

Volatile organic compounds emitted by plants have various effects on microorganisms colonizing the root surface, leaves, and flowers, referred to as rhizosphere, phyllosphere, and anthosphere, respectively. VOCs influence the composition of microbial communities on emitting plants, promoting positive interactions and inhibiting the growth of harmful microorganisms (Dudareva 2006; Tripathi et al. 2011; Chagas et al. 2018).

At the root level, plant-produced VOCs can have short- and long-distance effects on rhizosphere microorganisms (Dudareva et al. 2006; Compant et al. 2021). Many of the VOCs released at the root level belong to the terpene class (Yeo et al. 2013). In this context, terpenes and other VOCs derived from roots likely play multiple roles as carbon sources, chemotactic agents, and defense metabolites (Junker et al. 2013). Particularly, the defensive functions of volatile terpenes in the rhizosphere have been described primarily in direct and indirect interactions with root herbivores (Johnson et al. 2012; Noman et al. 2021). For instance, the sesquiterpene β-caryophyllene, induced by insects, is known to promote indirect defensive responses in maize roots by attracting entomopathogenic nematodes (Turlings et al. 2012).

At the shoot level, emitted volatile compounds typically inhibit the growth of airborne bacteria, preventing their settlement on leaves (Gao et al. 2005). Monoterpenes like limonene and β-pinene are particularly effective in inhibiting bacterial growth (Farré-Armengol et al. 2015). Similarly, aldehydes such as acetaldehyde, benzaldehyde, and cinnamaldehyde can inhibit the growth of epiphytic bacteria at relatively low doses by affecting proteins associated with bacterial membranes (Shakir et al. 2021).

The role of microorganisms in plant volatile communication also extends to flower volatiles. Microorganisms can colonize nectar and modify a flower's volatile mixture, thereby altering floral visitation and, consequently, plant reproduction (Rering et al. 2018).
Volatile compounds are also involved in plant-fungus interactions, such as during the establishment of symbiosis with arbuscular mycorrhizae. The root and fungus can communicate not only through exudates, small RNAs, and phytohormones but also through VOCs released by both the plant and the fungus (Pineda 2013). For example, mycorrhizae can regulate the orientation of host roots by emitting VOCs that modify the branching angle of lateral roots, increasing the likelihood of encounter between mycorrhizal hyphae and plant roots in the rhizosphere. Since auxins regulate the branching angle of plant lateral roots, fungal VOCs can activate the auxin signaling pathway in the plant (Sun et al. 2015; Minerdi et al. 2021).

1.2.3.4. Role of VOCs in Plant-Plant Interactions

An intriguing function of volatile organic compounds is their role in within-plant communication and plant-to-plant communication, a phenomenon first described in 1983 (Baldwin and Schultz, 1983; Rhoades, 1983).
In the case of within-plant communication, unlike communication through vascular channels that do not effectively and uniformly connect all parts of a plant, volatile communication between different parts of the same plant is characterized by higher speed and might be a phenomenon regulating plant defense (Orians 2005; Karban et al. 2021). For example, in poplars, VOCs released by infested leaves "alert" nearby undamaged leaves, overcoming the absence of direct vascular connections (Brosset et al. 2022). Volatile compounds identified as inducers of within-plant defensive responses include volatile esters of salicylic acid and jasmonic acid (Blande et al. 2016).
Concerning volatile communication between neighboring plants, VOCs can be used as signals to compete for limited resources, enhance direct resistance against herbivores, microorganisms, and fungi, or attract predators (Turlings et al. 2018; Hammerbacher et al. 2019). However, the effectiveness of such volatile communication strongly depends on the context and the types of plants involved. A specific stress that disturbs a plant leads to the production of a specific VOC mixture that can trigger the activation or suppression of signaling pathways in a receiving plant, inducing a response in line with the imminent stress (Conrath et al. 2015; Martinez-Medina et al. 2016). However, when discussing the role of VOCs in plant-plant interactions, it is necessary to make a distinction between "cues" and "signals," both for emission and reception. "Cues" are pieces of

information used by a receiver that are not intentionally released by emitters for a specific reason. For example, constitutively released VOCs from a plant provide characteristic information about the emitter's identity, which may or may not be used by a receiver to detect and adapt to competitive neighbors (Ninkovic et al. 2021). "Signals," on the other hand, are released by emitters in response to an external disturbance and carry a specific message. For example, damaged plant cells release Damage-Associated Molecular Patterns (DAMPs) that activate the plant's immune response. The first biosynthetic pathways activated after recognizing DAMPs are those of jasmonic acid (JA), salicylic acid (SA), and ethylene (Pandey et al. 2017). These hormones can lead to a variation in the emitter's VOC profile. When these VOCs are perceived by the receiver, they induce the upregulation of defensive responses, preparing the plant for future herbivore attacks (Ninkovic et al. 2021). The transition from "cues" to "signals" occurs when external factors change the emitter's VOC profile, transmitting specific information in communication with neighbors. From an evolutionary perspective, the perception of volatile compounds is a crucial tool for a plant to adapt its growth strategy and enhance its fitness. However, decoding the language of communication between plants and understanding the potential intentionality of communication is not straightforward, and further studies are needed to comprehend how plants manage the vast array of VOCs in their environment.

1.2.4. **Environmental Factors Influencing VOC Emissions**

Plants in their natural environment are subject to a wide range of environmental conditions that influence the synthesis and release of volatile organic compounds (VOCs). Stressful situations can lead to the release of volatile compounds that are typically not emitted by unstressed plants. For example, ocimenes are characteristic stress-induced monoterpenes (Aros et al. 2012). The *de novo* production of VOCs in response to environmental stress, while representing a cost to the plant, provides greater phenotypic plasticity and makes it less susceptible to further damage (Antonelli et al. 2020). However, in response to disturbances in environmental conditions, not always new molecules are biosynthesized; often, what changes are the relative emission rates. In 2023, Bao et al. studied emission rates of volatile compounds from 357 species, including evergreen plants, deciduous plants, and conifers, compiling data from 159 studies conducted in 22 countries. In particular, they observed that a high concentration of ozone reduces the emission rate of isoprene by 18% and increases that of total monoterpenes by 43%. A high concentration of carbon dioxide, on the other hand, not only reduces the emission rate of isoprene but also decreases that of total monoterpenes.

The following explores responses in terms of changes in VOC emissions induced by variations in humidity, temperature, and light. Stress of different durations and severity can lead to complex emission responses with potentially significant consequences for plant communication with other organisms and for signaling from plant to plant.

1.2.4.1. Humidity Variations

Humidity variations have a significant impact on plant VOC emissions, and these variations show notable differences among different tree species. In some plants, the rate of VOC emission increases following an increase in environmental humidity, both atmospheric and soil moisture, while other plants are not sensitive to changes in environmental humidity or decrease the emission rate with rising humidity (Lun et al. 2020). For example, in *Salvia dolomitica*, drought conditions induce a modulation of the expression of genes involved in the synthesis of volatile compounds, leading to a particular increase in sesquiterpene production (Caser et al. 2019). Saunier et al. (2017) observed that under drought conditions, due to the closure of plant stomata, VOCs emitted by *Quercus pubescens* in spring and summer are 40%-50% lower compared to fall and winter rates. Similarly, in pine trees, under summer drought conditions, the release rate, net photosynthetic rate, and isoprene transpiration rate decrease (Lüpke et al. 2016).
Excessive water presence can also be a stress factor, causing hypoxia/anoxia conditions. Such environmental conditions lead to reduced cellular respiration, resulting in pH alterations that affect plant health (Pezeshki 2001). These anoxic conditions are correlated with the emission of ethanol and acetaldehyde (Holzinger et al. 2005).

1.2.4.2. Temperature Variations

Temperature is another crucial factor influencing the emissions of VOCs from plants. In particular, the synthesis and emission rates of isoprene and monoterpenes are closely linked to temperature, gradually increasing with rising temperatures. Niinemets et al. (2010) observed that even mild thermal stress can increase isoprene production through a positive and transient regulation of isoprene synthase, consequently modulating the entire isoprenoid biosynthetic pathway. However, the emission rate decreases when the temperature surpasses a certain threshold. For instance, Guidolotti

et al. (2019) measured VOC emissions from branches of *Eucalyptus robusta* exposed to a temperature increase (from 30 to 45°C) and found that isoprene emission increased while photosynthesis decreased. Li et al. (2019) conducted a long-term climate warming simulation on arctic plants and found that temperature increase led to a tripling of monoterpene emissions. Studies by Kivimäenpää et al. (2016) on the effect of high temperature on Scots pine trees also demonstrated that total emissions of monoterpenes and sesquiterpenes increased two to four times.
Regarding VOC emissions in response to low temperatures, fewer studies have been conducted. In *Solanum lycopersicum* exposed to different treatments with temperatures <1°C, an increase in monoterpene concentration was observed. Specifically, the presence of (E)-β-ocimene and β-caryophyllene was found to be directly correlated with the severity of the stress (Copolovici et al. 2012).

1.2.4.3. Light Variations

Light has an impact on the rate of photosynthesis in plants, as well as on transpiration rates and stomatal conductance. As early as 1993, Guenther et al. observed that leaf temperature and light could influence isoprene production by acting on the isoprene precursor, γ-dimethylallyl pyrophosphate (DMAPP), and isoprene synthase. Van Meeningen et al. (2017) conducted light gradient experiments on various European tree species and observed that isoprene, cymene, α-pinene, and others all exhibited a strong response to light. In the afternoon, with decreasing temperature, the emission of volatile compounds decreased, following the normal metabolic pattern of plants.

1.2.5. VOCs and Sound Waves

Although plants lack specialized sensory organs, they can detect a wide range of environmental stimuli, including sound (Appel et al. 2023). Recent studies have shown that sound stimulation can induce various physiological changes, including alterations in gene expression, epigenetic modifications, hormonal signaling, seed germination, growth, flowering, and defense (Del Stabile et al. 2022). Among these changes, the production of plant secondary metabolites has attracted increasing attention. Unlike primary metabolites, secondary metabolites are not essential for plant growth and life but generally regulate plant-plant and plant-environment interactions, playing crucial roles in defense against pathogens and stress (Yang et al. 2018). Many of these functions are carried out by volatile organic compounds, which, once emitted into the environment, provide information about the physiological and metabolic status of the plant (Kessler 2018).

Physically, sound is defined as a series of longitudinal pressure waves that propagate through a solid, liquid, or gaseous medium. Consequently, similarities can be recognized between the physical properties of sound and those of touch, and like gravity, wind, and rain, sound waves also have a mechanical influence (Telewski, 2006). Farmer et al. (2020) argue that mechanical stimulation due to touch induces electrical activity, leading to a rapid collapse of the membrane potential that can be linked to the production and/or action of jasmonate or ethylene.

Although it is not yet fully understood whether plants can respond to sound by perceiving it as it is or as a mechanical wave transmitted through the air or soil, some similarities in responses can be noted. Just as touch represents a stimulus in response to which plants can change their volatile emissions, serving as a signal to nearby plants (Ninkovic et al. 2021), sound might induce variations in the mix of emitted VOCs (Allievi et al. 2020). To date, there is no literature demonstrating a direct correlation between sound and changes in volatile compounds. However, some recent studies show variations in the content of secondary metabolites in response to sound wave treatments. For example, Kim et al. (2021) observed an increase in flavonoid content following treatments of radish, lettuce, and Chinese cabbage shoots for two hours a day with sound waves at different frequencies. Similarly, Altunas et al. (2019) observed effects on the content of lycopene, total acids, and total phenols in tomato plants in response to acoustic treatments. Razavizadeh et al. (2020) also observed an increase in the content of crocin, picrocin, and safranal in saffron plants treated with sound waves.

1.3. VOC and Human Health

1.3.1. Human Exposure to VOCs

Volatile organic compounds are naturally occurring molecules in the environment. However, average annual emissions of anthropogenic VOCs may have significantly increased in recent decades (Duan et al. 2023). Anthropogenic VOCs are synthetic chemicals widely used in the production of numerous everyday products for residential and commercial applications. While VOCs play an essential role in the production and maintenance of the stability of many products, the health impacts associated with their prolonged exposure are attracting increasing attention. Recent studies have linked VOCs exposure to a wide range of diseases and disorders (Soni et al. 2018; Pye et al. 2022).

Exposure levels also vary based on daily activities and the intensity of such activities in indoor environments such as offices, workplaces, residences, and outdoor environments like roadways. Although vegetation releases a certain proportion of VOCs, human activities are estimated to have a greater impact on the environment (Hanif et al. 2021). Specifically, industrial activities regularly produce numerous VOCs, but when traffic or industrial operations are limited or suspended for a certain period, the atmospheric condition improves not only in terms of the overall pollution rate but also in terms of the concentration of various VOCs (Zhou et al. 2023). Recently, during the early months of 2020, many restrictions were implemented to mitigate exposure to COVID-19, leading to a significant reduction in public gatherings and road traffic and the temporary shutdown of hundreds of industrial units. For example, during this period in Italy, a research team investigated whether the lockdown restrictions had led to an improvement in the seawater quality of the Venice Lagoon. Approximately 40 volatile compounds were detected in water samples before the lockdown, and only 17 were found after the lockdown. Furthermore, their concentration was lower than the pre-lockdown levels (Cecchi, 2021).

Inhalation is the predominant route of exposure to VOCs, but exposure can also occur through ingestion and skin contact (Kuranchie et al. 2019).

A study conducted in China aimed to monitor the contribution of VOCs to the air pollution in Beijing and assessed their environmental impact associated with health risks. In particular, levels of about 99 VOCs were measured, and the carcinogenic risk of some, such as benzene, butadiene, chloroform, acrolein, and acetaldehyde, exceeded USEPA and EPA standards for causing carcinogenic effects on human health (Li et al. 2020).

Other VOCs, such as perchloroethylene, chloroform, and chloroethane, widely used in solvents and paints, are soil and groundwater contaminants. Epidemiological studies suggest that these compounds can cause bladder, rectal, esophageal, cervical, and colon cancers (Jin et al. 2022). Exposure to chlorinated VOCs has also been associated with congenital defects and frequent miscarriages, posing a potential reproductive risk. Due to their multiple carcinogenic properties, many countries worldwide have designated several chlorinated VOCs for continuous monitoring by environmental agencies (Huang et al. 2014). Other VOCs like toluene and benzene-based derivatives may pose risk factors for obesity and diabetes. VOCs are not only related to carcinogenic, respiratory, and cardiovascular diseases but can also pose metabolic risks (Lee et al. 2022).

Many VOCs, if released into the environment in excessive quantities, can contribute to the formation of a polluted atmosphere, with severe consequences for air quality and human health. Their control is essential to preserve human health and the environment.

1.3.2. Effects of Biogenic VOCs on Human Health

A study conducted in 2019 by White and colleagues confirmed that spending at least two hours per week in green environments provides health benefits, including a lower likelihood of cardiovascular diseases, obesity, diabetes, asthma, mental distress, and enhanced cognitive development in children. Stress and psychophysical discomfort were significantly more prevalent in individuals who spent less time in contact with nature (White et al. 2019). These benefits are attributed to a series of VOCs produced and released into the air by plants. Specifically, certain terpenes, such as limonene and pinene, possess antioxidant, anti-inflammatory, analgesic, anxiolytic, and antidepressant activities associated with improved cognitive performance and mood (Antonelli et al. 2020).

In a 2020 review, the effects of the most abundant terpenes and terpenoids in northern hemisphere forested areas were studied, many of which were found to be effective against respiratory inflammation, atopic dermatitis, arthritis, and neuroinflammation. These compounds seem to act against inhibitors of pro-inflammatory mediators such as nitric oxide (NO), TNF-α, and PGE2, thereby modulating signal transduction pathways involving transcription factors like NF-κB and mitogen-activated protein kinase (MAPK) (Kim et al. 2020). Additionally, some terpenoids can interact directly with TRP receptors (involved in nociception and inflammatory responses), reduce oxidative stress, and stimulate cellular autophagy. The authors reported that, although these activities are not limited to forest bathing and can be exploited through skin applications or oral intake of essential oils, contact during forest bathing may be safer, although less beneficial (Kim et al. 2020).

Distilled essential oils, which result from the lipophilic distillation of aromatic plants, primarily consist of volatile terpenes and terpenoids (C10 and C15), and in specific cases, they contain phenylpropanoids, oxylipins, and, more rarely, nitrogen (N) and sulfur (S)-containing molecules (Rai et al. 2013). Moreover, essential oils contain a higher percentage of heavier compounds like sesquiterpenes, less present in the atmosphere due to the higher temperatures employed during distillation, allowing the volatilization of less volatile compounds (Rai et al. 2013). Many essential oils appear to be able to exert interesting pharmacological activities. Among these are:

- antimicrobial effects on antibiotic-resistant bacterial strains;

- antitussive, expectorant, bronchodilatory, and antispasmodic activities on the respiratory system;
- non-olfactory psychopharmacological effects on wakefulness, activation, memory loss, dementia, cognitive performance, anxiety, quality of life, and sleep quality;
- antioxidant activity;
- antinociceptive, anti-inflammatory, and cytotoxic activities;
- anti-nausea and spasmolytic effects on the intestine.

Furthermore, since essential oils are perceptively salient, they can have effects that influence mood, mediated not by classical pharmacological mechanisms but by olfactory imagery (Rai et al. 2013; Buckle et al. 2014; Hüsnü et al. 2015).

Similarly, the positive beneficial effects provided by natural environments, such as reduced heart rate and blood pressure, increased relaxation, overall well-being improvement, and enhanced depression scores, might be a multifactorial outcome, probably dependent not only on the presence of BVOCs in the atmosphere but also on the natural and green context as a whole (Hansen et al. 2017; Oh et al. 2017). However, preclinical studies on animal behavioral models have investigated the neurological role of naturally derived VOCs in central nervous system (CNS) depression, and it has been observed that such compounds have been able to increase muscle relaxation, resulting in improved sleep, pain, and anxiety in mice (Cheng et al. 2009; Franco et al. 2017). Recently, it has been reported that volatile compounds from pine trees, such as α-pinene and 3-carene, can improve sleep by acting as positive modulators for GABA-A-BZD receptors (Woo et al. 2020). Additionally, inhaled linalool and β-pinene have shown anxiolytic and antidepressant properties in various animal models (Guzmán-Gutiérrez et al. 2012; Guzmán-Gutiérrez et al. 2015).

Natural biogenic VOCs can also exert antioxidant and antiproliferative activities. In particular, limonene has been described as an antioxidant compound with free radical-scavenging properties, capable of reducing oxidative stress in diabetic rats and human epithelial cells as well as fibroblasts (Vieira et al. 2018). D-limonene has also been studied as an antiproliferative agent. *In vitro* experiments have suggested a series of possible mechanisms for this activity, such as reducing the proliferation of cancer cells through the inhibitory activity of prenyltransferase; increasing apoptosis of tumor cells through autophagy; reducing tumor-induced immunosuppression and inflammation, angiogenesis, and metastasis (Blowman et al. 2018; Kim et al. 2020; Vieira et al. 2018).

The beneficial effects of biogenic VOCs are not limited to actions on the respiratory system but, after inhalation absorption, exert systemic effects, such as those on the nervous system mentioned above.

Recent studies have also shown a correlation between the severity of diseases caused by the SARS-CoV-2 (COVID-19) virus, air pollution, and VOCs (Bashir et al. 2020; Zhu et al. 2020; Roviello et al. 2021). In particular, in some regions of southern Italy, such as Molise, Calabria, and Basilicata, where the forest area rate is > 0.34 hectares/inhabitant, a lower risk of death from COVID-19 has been observed compared to northern regions of Italy, such as Emilia Romagna, Lombardy, and Veneto, where the forest area rate is < 0.22 hectares/inhabitant (Roviello et al. 2021). Additionally, in these industrialized regions, environmental pollutants like fine particulate matter (PM) along with humidity increase the risk of respiratory infections such as SARS-CoV-2 (Xie et al. 2019; Jiang et al. 2020). In light of these considerations, Roviello and colleagues suggested that, in southern Italy, the evergreen vegetation belonging to the Mediterranean maquis exerts a protective effect against SARS-CoV-2 on the population, acting on two different fronts (Roviello et al., 2021). Firstly, the VOCs released by it act directly on the human body by stimulating the immune system to increase natural killer (NK) cell activity and reducing airway inflammation (Kim et al., 2020); furthermore, vegetation is involved in air purification by removing pollutants (Novak 2002; Novak et al. 2006).

1.4. VOC and Environment

Volatile Organic Compounds are chemical substances typically present in gaseous form, produced both by human activities (Anthropogenic VOCs) and natural sources such as plants (Biogenic VOCs) (Michanowicz et al. 2022). Despite the various sources of VOC emissions, human activities primarily contribute to pollution in both indoor and outdoor environments (Zalel et al. 2008). Particularly in urban areas, air pollution caused by traffic and vehicle engine combustion, industrial processes, waste decomposition, and solvent use has significantly increased in recent decades (Sicard et al. 2021). Among these sources, facilities producing petrochemical products, coal power plants, construction activities, and building painting contribute considerably to VOC emissions in urban areas (Banaszkiewicz et al. 2022). Furthermore, the number of new volatile compounds entering the market is constantly rising. Consequently, there is an increasing need for governmental bodies, regulatory agencies, chemical companies, and research organizations to monitor their exposure and the associated toxic effects.

1.4.1. Relationship between VOCs and Air Pollution

In the 1950s, Haagen Smit and his colleagues demonstrated that the oxidation of organic species in the presence of nitrogen oxides (NOx) and sunlight can lead to the formation of ozone (O3) (Haagen Smit, 1952). Ozone, toxic to both humans and plants, has become a significant issue in air quality, particularly in cities (Stevenson et al. 2013). Besides ozone formation, VOCs can significantly contribute to the formation of Secondary Organic Aerosols (SOA) through photochemical reactions, leading to the development of photochemical smog in the atmosphere and an increase in pollution rates, negatively impacting human health and ecosystems (Mochizuki et al. 2015). Additionally, ozone can become a secondary precursor, inducing a series of photochemical reactions between various primary oxidants and pollutants, resulting in the release of highly reactive gaseous compounds, complicating the ground-level atmosphere (Swamy et al. 2012).

These chemical reactions occur in the presence of light radiation (Kroll et al., 2008), but they are not solely dependent on it. The release of VOCs, both from biological and non-biological sources, is also influenced by temperature, and researchers have reported that ongoing global warming has a significant and increasing impact on VOC emissions (Ya´nez-Serrano et al. 2020; Ghirardo et al. 2020). To address air quality issues, stringent emission regulations have been continuously implemented. Identifying key precursors by assessing the ability of each VOC species and source to form O3 and SOA is crucial for implementing mitigation initiatives on their emissions. Some studies have emphasized that alkenes, along with aromatic hydrocarbons, contribute to more than 80% of photochemical ozone formation. Specifically, in the calculation of Ozone Formation Potential (OFP), ethylene, propylene, toluene, and xylene are the compounds that contribute the most (Song et al. 2021; Zheng et al. 2021). In the photochemical formation process of SOA, the contribution of aromatic hydrocarbons in urban areas has been reported to be up to 95% or more, especially with toluene, benzene, xylene, and ethylbenzene (Li et al. 2022; Qin et al. 2021). However, the characteristics of photochemical VOC pollution in different regions and at different times are not exactly the same (Li et al. 2022; Zhang et al. 2023). Furthermore, alterations in the chemical evolution of VOC emission levels depend on regional interactions between vegetation and climate, and these geographical variations lead to significant changes in the response of VOC emissions to global warming (Zhou et al. 2023).

With the global outbreak of the COVID-19 pandemic in early 2020, many countries implemented strict measures to control the epidemic's spread. In this context, several studies reported that COVID-19-related lockdowns improved environmental air quality (Lian et al. 2020; Pakkattil et al. 2021; Zhang et al. 2022). For example, Li et al. (2023) reported a 58% decrease in VOC concentrations in the Beijing-Tianjin-Hebei region during the pandemic control period in early 2020 compared to the pre-control period. Another study reported VOC concentrations on haze days before and during the lockdown were 154 ppb and 96 ppb, respectively, indicating a 37.7% decrease (Ma et al. 2022). However, these studies primarily focus on short-term comparisons before and after the COVID-19 lockdown, lacking long-term comparative research (Zuo et al. 2024).

In light of this, continuous attention to the role of various VOCs in secondary pollution formation is necessary so that local governments can implement long-term collaborative control of pollutants.

1.5. Research Objectives

The life of plants is extremely sophisticated and complex. As an integral and irreplaceable part of the environment, they are involved in multiple relationships at different levels. Evolutionarily, for their survival, they have become capable of perceiving information from the surrounding environment and interacting with it using a language unique to them, similar to insects, animals, and fish, each of which has evolved a personal way of relating to the environment and their peers. Furthermore, as humans have evolved in an environment long populated by plants, they have developed more or less conscious interrelationships with these organisms.
In this thesis, the aim was to delve into all these issues concerning both the sensitivity aspects of plants and their influence on humans.

The objectives of the research work are therefore multiple:

- The first part focuses on the fascinating field of Plant Acoustics, an emerging area of plant physiology that aims to study how plants can perceive and respond to sounds. This includes investigating variations in the content of secondary metabolites, particularly VOCs. The interest lies both in scientific knowledge and the hope of discovering elements that could lead to a better understanding of plant language, with potential applications in agriculture and floriculture practices.
- The second part centers on the beneficial effects of VOCs on human psychophysiological parameters. It specifically studies the effects of olfactory stimulation induced by essential oils with volatile compounds in a forest. A thorough understanding of the impacts of olfactory stimulation due to naturally occurring volatile molecules could provide the basis for practical applications in human health and well-being contexts.
- Finally, qualitative and quantitative variations of VOCs were monitored in 8 different zones of the city of Reggio Emilia, characterized by varying percentages of vegetation cover and proximity to busy roads. The goal is to assess differences in breathable VOCs in different environments, potentially laying the foundation for the development of sustainable strategies to preserve both environmental and citizen health.

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2. Plant Acoustics

Plants have long been considered passive, static, and unchanging organisms, but this view is finally changing. More and more knowledge is showing that plants are aware of their surroundings, and they respond to a surprising variety of stimuli by modifying their growth and development.

Plants extensively communicate with the world around them, above and below ground. Although communication through mycorrhizal networks and Volatile Organic Compounds has been known for a long time, acoustic perception and communication are somehow a final frontier of research. Perhaps surprisingly, plants not only respond to sound, they actually seem to emit sound as well. Roots emit audible clicks during growth, and sounds are emitted from xylem vessels, although the nature of these acoustic emissions still needs to be clarified. Even more interesting, there is the possibility that these sounds carry information with ecological implications, such as alerting insects of the hydration state of a possible host plant, and technological implications as well.

The importance of studying the effects of sound on plants lies both in basic research, to better delineate the dynamics of bioacoustic interactions in the plant kingdom, and in applied research, for instance, in optimizing agricultural techniques and promoting plant growth through targeted sound stimuli.

2.1. State of the art

Plants have been around for a long time, far longer than Homo sapiens. Homo sapiens arose approximately 300,000 years ago (Hubin et al. 2017), a minimal length of time compared with the age of Earth. The earliest land plants, on the other hand, first appeared in the fossil record millions of years prior to this. Non-vascular plants such as the true mosses, the Bryopsida, for example, first appeared in the Mississippian 340 million years ago and appeared well established by the Permian. However, the earliest bryophytes in the fossil record already have the basic thallus organization possessed by current forms, suggesting the possibility that the bryophytes evolved even earlier than the fossil record suggests (Taylor et al. 2009). *Baragwanathia*, which is a relatively complex vascular plant, was confirmed to be of Late Silurian origin, which was approximately 420 million years ago (Taylor et al. 2009). Fossils of the first flowering plants date back to around 130 million years ago, a milestone not only for their role in the evolution of plants but also for the relationship of plants with insects (Gomez et al. 2015). The appearance, enormous diversification, and ecological radiation of the angiosperms began during the Cretaceous, between 135 to 65 million years ago, and it represented a very significant alteration to the history of life on Earth. It had vast repercussions on the distribution of other groups of land plants and a great effect on the evolution of ecosystems and species. Today, there are more than 350,000 species of extant angiosperms, which is more than all the other groups of land plants combined (Friis et al. 2011). They occupy and dominate an astounding range of habitats, and, as autotrophs, the angiosperms are the base upon which most ecosystems are built (Friis et al. 2011).

Plants are sessile and photoautotrophic, which means that they produce new biomass from CO_2 using light energy in a process called photosynthesis. Most of the energy that enters terrestrial habitats is the result of photosynthesis. Plants are so important that they go so far as to influence the atmosphere and climate, and yet at the same time, the environment itself has a profound impact on photosynthesis and plants.

And therein lies the point. Plants are not passive organisms, though their sessile nature might make them appear to be static or unchanging. Though plants have an awe-inspiring impact on most life on earth, most people tend to underappreciate or not notice the plants living around them. Plants are alive, and like all things that are alive, they perceive many environmental and physiological signals, and through these, they perfect and modify their growth and development. Not only that, but recent scientific studies have also shown that plants are capable of exhibiting learning, memory, and even intelligence (although maybe not consciousness) (Gagliano et al. 2014; Gagliano et al. 2017; Toyota et al. 2018; Yokawa et al. 2018). It is reasonable to assume that plants have evolved to do so. After all, as stated before, land plants first appeared millions of years ago. As new plant forms evolved, so did the capacity to perceive stimuli and adapt to a changing environment (Sopory et al. 2019).

But plants do much more than just perceive and react. Plants communicate amongst themselves and with animals. Plants do so in many ways, both above and below ground; a growing body of research shows that plants can even detect and emit sounds (Jung et al. 2018; Allievi et al. 2021).

2.1.1. Communication through Sound

A growing body of research is showing that plants detect and emit sounds. This is unsurprising considering that there is no habitat colonized by plants that is without sound and taking into account their sessile nature and their age on Earth, it is reasonable to think that they have learned to do so.

2.1.1.1. What Is "Sound"

Sound is defined as a series of longitudinal waves of pressure that propagate through compressible media, such as air, liquids, or solids. Sound waves that fall in the range of frequencies between 20 Hz and 20 kHz belong to the audible sound, which is what a human ear can hear; frequencies below 20 Hz or higher than 20 kHz are defined as infrasound or ultrasound, respectively (Frongia et al. 2020). However, sound is not only vibration energy; it is also pressure generated by vibration waves that move through a suitable medium in the form of compression and rarefaction (Frongia et al. 2020). For a sound to be perceived, however, it is not sufficient that it consists of frequencies in the audible range; it must also have sufficient sound pressure, that is, the pressure variation produced by the acoustic phenomenon compared with the static value. For example, in the air a sound pressure of 20 μPa corresponds to a level of sound pressure of 0 dB (Christensen-Dalsgaard et al. 2019).

Application of sound at different frequencies, pressure levels, duration, and repetition of exposure periods has been proved to have an influence on plant growth, development, and germination (Jung et al. 2018). Taken together, these studies belong to the field of Plant Acoustics. Whether or not sound perception and/or emission are used in plant communication is a fascinating field of research. However, for acoustic plant communication to exist, there needs to be an emitter, in other words, a source of sound and a receiver not only able to perceive the signal but also able to decipher and eventually perform a sort of coherent response.

Sound stimulation has been proven to switch on stress-induced genes (Xiujuan et al. 2003a) or enhance genes related to disease resistance (Zhang et al. 2012), but there is also a sort of new age interest in growing plants with sound. Traditional ethnic music can positively affect the productivity and quality of plants. Many studies are heading in that direction: Javanese music has been applied to Chinese broccoli (*Brassica alboglabra*) plants (Hendrawan et al. 2020), and *Desmodyium girans* (Telegraph plant) (Munasinghe et al. 2018a) and rice (*Oryza sativa*) (Munasinghe et al. 2018b) show better growth performance when exposed to Buddhist pirith chants.

2.1.1.2. Sound at Cellular and Subcellular Level

On a cellular level, it seems that the most likely candidate for sound signaling is Ca^{2+}, which acts as a second messenger to Sound Vibrations (SVs). Although direct evidence is lacking, it is possible that SVs activate plasma membrane channels, evoking a membrane potential-based signaling cascade (**Figure 7**). Several studies have shown an efflux/influx of Ca^{2+} following SVs. It was shown, for example, that Chrysanthemum cells treated for one hour with SVs of 100 dB and 1000 Hz had an increase in H^+-ATPase activity (Yi et al. 2003). The upstream component of this increased activity was found to be Ca^{2+}. It appears that transient increases in cytosolic Ca^{2+} concentrations lead to an activation of calcium-dependent protein kinases, which then activate the H^+-ATPases. These kinases go on to regulate proteins and transcription factors, therefore altering gene expression (Zhao et al. 2002; Xiujuan et al. 2003b; Zhao et al. 2003; Mishra et al. 2016). For example, after treatment with SVs, plant cells showed increases in α-amylase activity and as a result, an increase in sugar levels. ROS scavenging enzymes have been shown to increase activity after SV treatment, and overall, the most common plant response to SV treatment is increased growth, for example, through increased cell division. All this, however, is just a general overview of what happens at the cellular level following sound perception, and it is well described in greater detail by Mishra and co-workers in 2016.

But there is another side to the coin. Sound is generated by vibrating objects, and the components of eukaryotic cells do just that. Following the hydrolysis of Adenosine triphosphate (ATP), motor proteins such as myosin generate vibrations (Gagliano et al. 2013). In addition, the nanomechanical motions of the cell wall of Saccharomyces cerevisiae, baker's yeast, are in the range of 800–1600 Hz, with amplitudes of 3 nm. Interestingly, exposing the cells of *S. cerevisiae* to a metabolic inhibitor caused the periodic motion to cease (Pelling et al. 2004). Cells are surrounded by other cells so that a cell can be influenced by the mechanical properties of its adjacent cells, and this can build up to a collective mode which results in amplification of the signal (Gagliano et al. 2013).

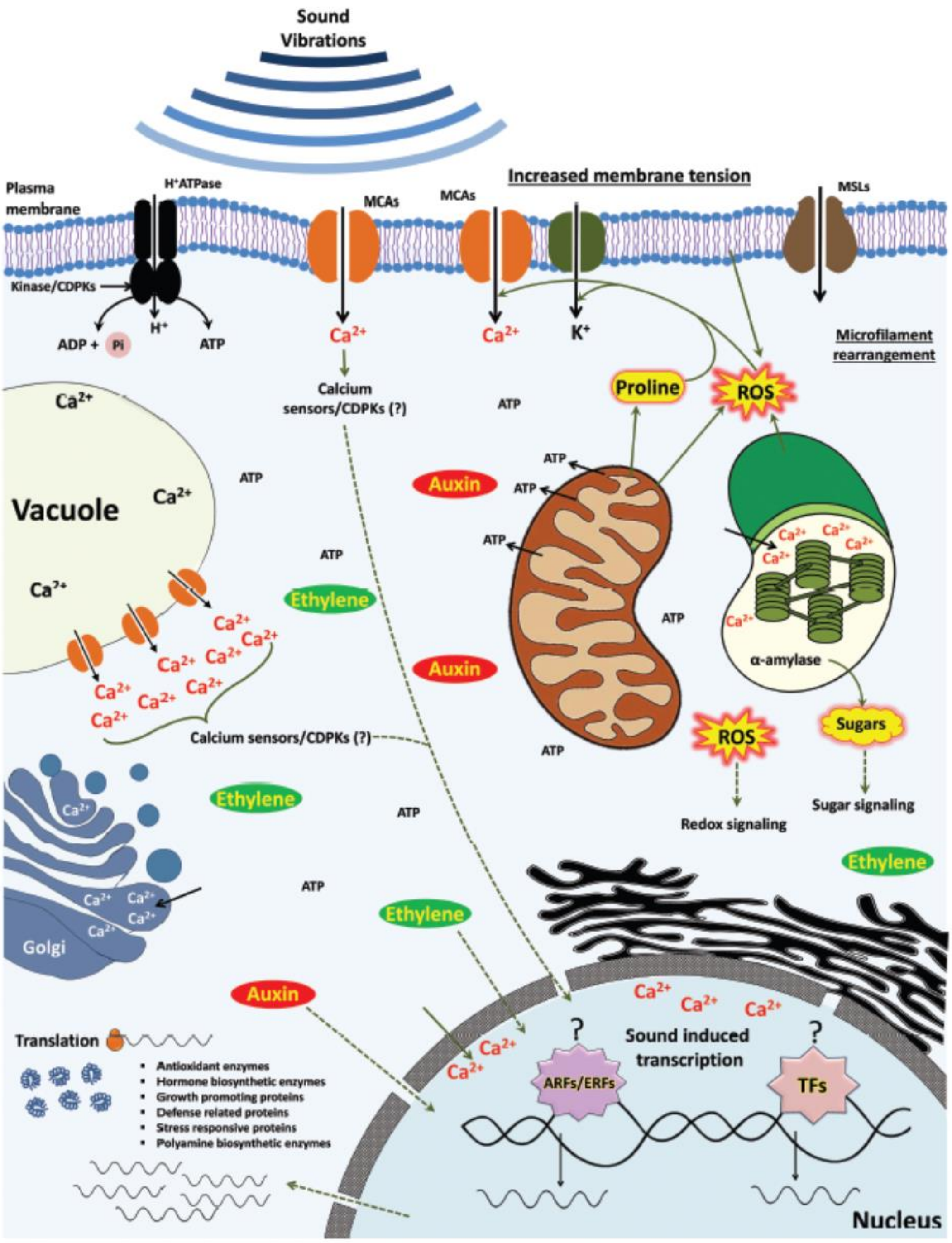


Figure 7: schematic representation of the possible molecular events triggered by treatment with SVs. After altering membrane tension, SVs go on to trigger an influx of Ca $^{2+}$. This influx possibly activates calcium sensors and calcium dependent protein kynases, which alter H^+-ATPase activity and regulate proteins and transcription factors to regulate gene expression (adapted from Mishra et al. 2016).

2.1.1.3. Sound like Touch

Sound and touch have similar physical properties, and yet plants cannot only properly distinguish between sound and touch, but they are also able to distinguish between relevant and irrelevant sound. The ability of plants to perceive touch has been well known for a long time. One need only to look at the carnivorous plant *Dionaea muscipula* or at *Mimosa pudica* to see plants reacting to touch. Since sound is generated by a vibrating body, and it propagates longitudinally by vibrating the particles of the medium, it passes through, when it reaches a body, it vibrates the body mechanically as well. In other words, sound waves mechanically impact an object they meet on their path (Sopory et al. 2019). The molecular basis for the perception of a mechanical stimulus in plants remains to be identified. However, touch sensitivity is not just limited to sensitive plant and carnivorous species. Every plant (or plant cell) perceives and accordingly responds to mechanostimulation (Mishra et al. 2019). Even plant roots are extremely sensitive to touch, being able to turn around an obstacle in their path. Like wind, light, rain, touch, sound is a pressure wave that translates into a mechanical influence. For the perception of a mechanical stimulus in plants, Telewski suggested a "unified hypothesis of mechanoperception in plants" (Telewski et al. 2006) with two models of mechano-receptors: (a) a cytoskeleton based on plasmodesma- plasma membrane- cellular network and (b) ion channels activated by stretching. Given the similarity of the sound stimulus to that of touch, it has recently been seen that signals and perception mechanisms of these two stimuli are common. However, the plants seem to distinguish between the two well in an extraordinarily sophisticated way (Mishra et al. 2019).

2.1.2. Effects of Sound Perception

Human conversation typically has an intensity of approximately 60 dB, and at this intensity, it can elicit vibrations, for example, in hearing organs, of just 10–50 nm. At these scales, the mechanical energy imparted by the vibrations is exceedingly small, and yet we have no problems hearing during conversation. Considering this, it really is reasonable to think that something as small as a trichome could vibrate in response to SVs and possibly convey information (Gagliano et al. 2012).

In much the same way plants have adapted to different pollinators, plants have adapted to different sounds in their environments. For example, flower morphology affects the efficiency of pollinators, affects the way pollinators visit flowers and the success of pollen import and export (Liu et al. 2019). Similarly, the carnivorous pitcher plant *Nepenthes hemsleyana* could possibly have evolved pitchers that reflect the echolocation of bats. The plant *N. hemsleyana* has a mutualistic interaction with bats, supplying a safe, parasite-free roosting spot, and the bats in return fertilize the plant with nitrogen-rich droppings, enhancing the nitrogen uptake of these plants by an average of 34% (Schöner et al. 2015).

2.1.2.1. Buzz Pollination

Insects, primarily Hymenoptera (De Luca et al. 2013), use vibrations to extract pollen from a wide variety of flower morphologies with poricidal anthers, that is, anthers where the pollen exits the anther through an apical pore or slit. This phenomenon is known as buzz pollination. In poricidal anthers, the pollen is not freely accessible, and its removal requires vibration. As many as 8-10% of angiosperms possess poricidal anthers that are pollinated through the use of vibrations. Interestingly, buzz pollination seems to have arisen independently several times in about 65 plant families (Mesquita-Neto et al. 2018).

Buzz pollination is not limited to a specific flower morphology, although it seems that the *Solanum* type flower has evolved specifically in response to sonicating bees. Flowers with poricidal anthers are visited by many insects, even non-sonicating insects that chew through the anthers to reach the pollen, but the primary visitors are sonicating bees (De Luca et al. 2013a).

Sonication seems to have arisen in a common ancestor of bees during the early Cretaceous (Mesquita-Neto et al. 2018). A bee lands on a flower and curls with the ventral side of the body around the anthers in a C shape, with the wings tightly folded back over the abdomen during sonication (King et a. 2003; De Luca et al. 2013a). The bee then rapidly contracts the thoracic muscles while preventing the wings from beating. The vibrations are transmitted to the anthers, which resonate, transmitting energy to the pollen, which is then expelled through the apical aperture (De Luca et al. 2013a). Centrifugal forces are generated, which eject the pollen (King et al. 1996).

There are both insect-related and plant-related variables that affect buzz pollination. Vibrations produced by sonicating bees can be characterized by duration, amplitude, and frequency. It was found that the greatest effect on pollen removal from anthers was given by duration and amplitude, while frequency had only a weak effect on pollen removal. Moreover, heavier bees produced buzzes with greater amplitude, ejecting more pollen (De Luca et al. 2013b). The magnitude of the vibration required to eject pollen from the anthers increased with frequency. The vibration frequency determines the time that a force may act on a particle, and therefore higher frequencies require higher amplitudes (King et al. 1996).

In terms of duration, bees increased the duration of their buzzing when visiting virgin flowers, and buzzes were shorter when returning to flowers that had already been visited. This could suggest that bees adjust the duration of their buzzing in relation to the pollen content of the flower (De Luca et al. 2013a). In theory, if a bee vibrated for a long enough time, it could extract all of the available pollen. The frequency of the buzzing is under physical and physiological control rather than behavioral control. This is because the vibrations depend on the muscles of the bee, and therefore there are limits to the frequencies they can achieve. The peak frequency, which refers to the frequency with the greatest relative energy within a buzzing vibration, varies between 100-400 Hz depending on the species of bee. Through harmonic frequencies, which are positive integer multiples of the original peak frequency (sound-standing waves), frequencies as high as 2000 Hz can be reached (De Luca et al. 2013a), but as stated before, this has very little effect on pollen removal. The optimal peak frequencies do, however, vary among plant species but still remain under 1000 Hz.

Plant traits also affect buzz pollination. Plant structures can either enhance or dampen the amplitude of the vibrations. For example, rigid, multi-layered anthers release more pollen compared with flexible anthers when vibrated. It’s reasonable to think that the size of the apical pore influences the amount of pollen released (De Luca et al. 2013).

2.1.2.2. Sweetened Nectar

Yet another example in the realm of pollination is the production of sweeter nectar within as little as three minutes following the perception of sound by flowers of *Oenothera drummondii*. The flowers of *O. drummondii* mechanically vibrated in response to recordings of bees and moths flying and also vibrated in response to the flight of a live bee, showing the same increase in nectar sugar content (Veits et al. 2019). The volume of nectar remained the same, meaning that an increase in sugar concentration was not a result of a drop in water content. The velocities of the oscillations of the flowers that in this experiment caused an increase in sugar concentration in the nectar was found in other experiments to be able to elicit defense responses by plants (Veits et al. 2019). Interestingly, the vibration of the flowers depended on the presence of petals, as flowers that had their petals removed or flowers covered by glass ceased to show a response to the sound vibrations.

2.1.2.3. Interpreting Relevant and Irrelevant Sounds

These examples show the important ecological role that sound can play in a plant's life. Plants don't live isolated from the rest of the world. Instead, there are extensive connections with other plants, animals, and microbes. Around plants, there are rich communities of arthropods, many of which use vibrations to find mates or prey. For example, vibrations caused by the chewing of *Plathypena scabra* worms caused predatory *Podisus maculiventris* stinkbugs to begin their search (Pfannenstiel et al. 1995). Chewing herbivores produce specific high-amplitude vibrations that travel quickly to other parts of the plant, and this can produce a local and systemic response in other parts of the plant. *Arabidopsis thaliana* leaves exposed to recordings of caterpillars chewing were proved to be primed for defense (Appel et al. 2014). The plants that had been exposed to chewing vibrations showed higher levels of glucosinolates and anthocyanins following herbivory, while there was no increase in anthocyanins in the plants that either received no vibrations or received vibrations from recordings of leafhopper singing or recordings of the wind (Appel et al. 2014). Interestingly, as with the greater amplitude of bee buzzing increasing pollen removal, higher amplitudes induced higher amounts of aliphatic glucosinolates (Appel et al. 2014). It is still to be understood how the response caused by the vibrations of a herbivore can generate an induced resistance or a systemic. One possibility is that the plant subject to herbivory integrates the vibrational signal with others coming from the herbivore's attack. As plants perceive warning signals via VOCs from nearby stressed plants, and VOCs can serve as a sort of chemical language in the communication between plants (Guerrieri et al. 2016), also vibrations can be used at least in some cases in plant communication (Eriksson et al. 2011; Gagliano et al. 2012b; Gagliano et al. 2013). This is yet another example of the ecological role that sound can play in a plant's life. The fact that plants perceive sound from so many different sources and adapt proves them to be ingenuously aware of their environment.

2.1.3. Sound below Ground

Sound travels easily and far in dense substrates, and soil is a wonderful example. Since the epigeal part of plants does respond to sound, it might be strange that the roots of plants would not be able to do the same, especially considering that vibrations in the soil are present at all times and places. A possible example of the hypogeal part of plants responding to sound vibrations could be the frequency selective behavioral response of *Zea mays* roots. When exposed to a continuous sound, the root tips very clearly bend towards the source of the sound (Gagliano et al. 2012c). Furthermore, the root tips very clearly show different responses to different frequencies, with the biggest response elicited by a continuous sound of frequency between 200–300 Hz. Interestingly, the root tips generated acoustic emissions, which could be measured at some distance in the hydroponic medium in which the roots are growing (Gagliano et al. 2012c).

Pisum sativum roots showed a behavioral response to sound. Even in the absence of moisture, the roots of *P. sativum* were able to locate water thanks to the vibrations induced by the movement of the water (Gagliano et al. 2017). Interestingly, in the presence of moisture, the authors showed that the roots preferred the moisture over the acoustic emissions, suggesting that plants could use the sound of water flowing to locate water and then more accurately find the water using moisture gradients. Interestingly, the roots showed avoidance behavior when in the presence of sound equipment, even when the sound equipment was broadcasting the sound of water flowing. The authors hypothesized that the roots were able to sense a cue, such as magnets in the speakers, that directed their growth away from the sound equipment (Gagliano et al. 2017).

2.1.4. Can We Communicate with Plants by Means of Sound?

Very clearly, sound has a very important ecological role in the lives of plants, but sound vibration treatment can also be used commercially. For example, treatment of harvested tomatoes with sound was shown to delay ripening. Mature green tomatoes were treated with sound waves of 250, 500, 800, 1000, and 1500 Hz for 6 h. All of the sound treatments except the 800 Hz and the 1,5 kHz delayed tomato ripening, with the 1 kHz treatment having the biggest effect. Seven days after treatment with the 1 kHz sound wave, 85% of the treated tomatoes were still green, whereas over 50% of the non-treated tomatoes had turned red (Kim et al. 2015).

The treatment with sound waves was shown to decrease both ethylene production in the treated tomatoes and the respiration rate. By the time the respiration rate of the non-treated tomatoes had begun falling after the completion of the ripening process, the respiration rate in the treated tomatoes was still increasing, suggesting that ripening was indeed delayed. Furthermore, the change in color from green to red was more gradual in treated tomatoes compared with non-treated tomatoes. Finally, the flesh firmness of treated tomatoes decreased more gradually, whereas the flesh firmness of the non-treated tomatoes dropped sharply after five days (Kim et al. 2015). This last result could possibly be explained with the help of a previous study which found that treatment with sound waves decreased the deformability of plant cell membranes and made them more rigid. The sound waves seemed to have an effect not on the cell membranes themselves but seemed to cause microfilaments to rearrange and become more rigid. Interestingly, different frequencies had different effects on the deformability of the cell membranes, with higher frequencies causing the deformability to decrease (Bochu et al. 2001). The possibility of delaying ripening through sound wave treatment has important ramifications for lengthening the shelf life of products such as tomatoes.

Treatment with sound can also act as a plant growth stimulant, although the underlying mechanisms for this increase in growth have not yet been properly identified. A possible explanation for this is the fact that sound treatment alters plant growth regulatory hormone levels. Sound treatment increases IAA and decreases ABA levels, and this could be a factor in promoting plant growth (Jung et al. 2018). It was found, for example, that following treatment of *Chrysanthemum* cells with 1000 Hz, 100 dB sound soluble protein content increased significantly compared with a control group. The treatment lasted for 60 min each day, and the treated plants were separated into groups treated for 3, 6, 9, 12, and 15 days. A rich content of soluble proteins is the basis for many physiological activities. Interestingly, soluble protein content increased significantly after six days and even more significantly after nine days but actually dropped back down when the treatment carried on too long, such as the 12- and 15-day treatments. Furthermore, sugar content increased following stimulation compared with the control group. Finally, amylase activity increased following stimulation compared with the control group. The importance of frequency in the response of plants to sound is shown time and time again. While 1000 Hz sound was shown to be beneficial by increasing soluble protein and sugar content and increasing amylase activity, 2000 Hz sound actually proved to be damaging for plant cells (Jia et al. 2003).

Other examples of sound treatment as a growth stimulant could be the increased yields in sound-treated tomato or the treatment of wheat with sound waves of 92 dB and 5 kHz to increase yield and dry weight. Photosynthesis was shown to increase following sound treatment in rice and strawberries, and photosynthesis-related proteins were highly expressed following 8-h sound treatments at 250 or 500 Hz in *Arabidopsis thaliana* (Junget al. 2018). Sound increased the resistance of strawberries to insects and disease (Mishra et al. 2016). SV treatment

brought about an increase in the length, number, and activity of Actinidia chinensis roots. Highly dormant seeds of Echinacea Angustifolia showed enhanced germination following treatment with sound vibrations of 1000 Hz and 100 dB (Mishra et al. 2016). SV treatment has possible applications in biotechnology, ultrasound being able to enhance Agrobacterium-mediated transformation of several plant species, or audible SVs showing to increase in vitro growth of many plant species (Bochu et al. 1998; Liu et al. 2003a; Liu et al. 2003b; Ananthakrishnan et al. 2007; Wei et al. 2012; Safari et al. 2013).

Sound treatment was also shown to induce drought tolerance in *Arabidopsis thaliana*, leading to a significant increase in survival rates compared with control plants (López-Ribera et al. 2017). At the end of the treatment, plants were sampled to determine changes in transcription. Eightynine genes were found to have had their expression altered, 87 of which upregulated, the remaining two downregulated. Of the 87 upregulated genes, 44 are involved in stress-related responses (López-Ribera et al. 2017).

2.1.4.1. The Case of Cavitation

Considering that plants do respond to sound, could plants themselves actually emit sounds? Going even further, if plants do emit sounds, could other plants, or perhaps the same plant, perceive these acoustic emissions and react to them? As mentioned previously, it was indeed found that corn roots grown hydroponically emitted sounds (Gagliano et al. 2012). However, prior to this discovery, it was already believed that in conditions of drought, cavitation in xylem vessels could be a source of acoustic emissions (Sopory et al. 2019). Cavitation is the mechanical breakage of the continuous water column in a xylem vessel that occurs when the tensile strength of the water column is exceeded. This is accompanied by the build-up of mechanical pressure, which, when released, leads to elastic wave propagation (Allievi et al. 2021). In other words, there is an abrupt release of tension in the xylem vessel lumen as the liquid water under negative pressure is replaced by water vapor (Zweifel et al. 2008).

Previously the measurement of acoustic emissions following cavitation was done through actual contact between sensors and the plant itself (Khait et al. 2018). Although still interesting, this method does not take into account whether these emissions could be sensed at a distance. However, plants do emit airborne sounds that can be detected from a distance. Different tomato (*Solanum Lycopersicum*) and tobacco (*Nicotiana tabacum*) plants were placed in an acoustically isolated anechoic box under different treatments and were recorded simultaneously at a distance of 10 cm by two directional microphones in order to eliminate false detections of clicks caused by the electrical equipment. The plants were either cut, placed under drought stress, or were in control conditions. The plants that were under stress or cut emitted significantly more sounds compared with the control plants. For the drought-stressed plants, the mean number of sounds emitted per hour was 35,4 for tomato and 11.0 for tobacco, while for cut plants, the mean number of sounds emitted per hour was 25,2 and 15.ì,2 for tomato and tobacco, respectively. Surprisingly, the control plants not subjected to either drought or cutting emitted less than one sound per hour (Khait et al. 2019).

Although the precise values differ slightly for tomato and tobacco, the mean peak frequencies of the emitted sounds, in other words, the frequencies with the maximal energy, were between 49 kHz and 58 kHz. These results not only indicate that the emitted sounds are ultrasonic, that is above 20 kHz and not detectable by the human ear, but also confirm that these emissions are detectable at least at a distance of 10 cm. This means that these emissions could be theoretically detected by other organisms, such as insects or other plants (Khait et al. 2019).

What makes this experiment especially interesting is the use of machine learning, which refers to a system's ability to improve and extend itself by learning new knowledge rather than being programmed with that knowledge (Khait et al. 2019), to determine whether it was possible to identify the condition of a plant based on the sounds it emitted. The regularized machine learning classifier, which was also trained to discriminate against the electrical noises made by the recording equipment, was able to correctly identify the condition of the plants based on the sounds they emitted. Not only could it distinguish between the control plants and the treated plants, but it could also distinguish between the cut plants and the drought-stressed plants. This is fascinating because it could mean that the sounds that plants emit when under drought stress could carry information; therefore these sounds could be intercepted by other organisms who could then respond and adapt. Finally, tomato plants were placed in a greenhouse to simulate more realistic conditions (Khait et al. 2019). The recording equipment was trained to discriminate between tomato sounds and greenhouse sounds. A consistent acoustic pattern was found in that the number of sounds emitted is very low when the plant has been recently irrigated, but the number of emissions drops as the plant becomes dry.

These ultrasound emissions could be detected at a distance of 3 to 5 m. This means that it is possible that these acoustic emissions could be perceived by other organisms. For example, many moths that use tomato and tobacco plants as hosts for their larvae can perceive sounds in the frequencies and intensities that were detected in this experiment. It is possible that the information contained in the acoustic emissions of drought-stressed plants could inform these moths not to lay their eggs on these plants (Khait et al. 2019). The emission of sound by drought-stressed plants has important implications in agriculture, as the detection of these sounds could be used to monitor the water status of crops, and this could, in turn, lead to more efficient and precise irrigation, therefore reducing water usage.

Interestingly, it seems that the acoustic emissions of plants can actually be distinguished between low-dB Ultrasonic Acoustic Emissions (UAE), which are below 27 dB, and high-dB UAE (above 35 dB) associated with cavitation. Most investigations on acoustic emission detection have focused on higher dB ranges under the assumption that low dB sounds cannot be distinguished from background noise. However, it was found that signals in the low dB range seem to have a consistent pattern. UAE remained in the low dB range on sunless days and at night and transitioned abruptly to the high dB range on sunny days. High dB acoustic emissions coincided sharply with decreased sap flow rate in *Quercus pubescens* (Zweifel et al. 2008).

Typically, low dB acoustic emissions increase in intensity as bark tissue expands with hydration, so at night or while it is raining, the intensity of the low dB emissions increases. During the day, as transpiration occurs, the diameter of the stem shrinks as water is lost, and the intensity of the low dB emissions decreases gradually until there is a very abrupt transition to the high dB emissions that are probably caused by cavitation. The highest low dB acoustic emissions occur before dawn, which is when the least water movement occurs. So low dB acoustic emissions closely follow stem radius changes de-trended for growth. There are different possible origins for the low dB emissions (Zweifel et al. 2008).

One possible source for the low dB sound could be the mechanical noise of the stem shrinking and expanding. Or it could be the respiration and metabolic growth activity of the cambium and ray parenchyma cells. These obviously produce diurnal courses of CO_2 efflux from the stem. If the water content is high enough, then the respiration rate follows the temperature. At low water contents, however, the missing water seems to inhibit biochemical activity, regardless of temperature. In conditions of drought, respiration follows stem water content more closely and is largely independent of temperature. Under such conditions, low dB acoustic emissions and stem water content match respiration. When the turgor pressure in the cambium increases, for example, at night, radial growth occurs; consequently, respiration increases, and low dB acoustic emissions increase (Zweifel et al. 2008).

For a long time, it was thought that sounds generated by plants were always a product of cavitation, but the overabundance of sound emissions by plants makes it highly unlikely that all sounds generated by plants are a product of cavitation, considering the limited number of water-conducting elements. Although it seems clear that cavitation can indeed emit sound, some authors believe that sounds generated from the xylem area are not caused by cavitation but by a stable bubble system capable of transporting water through peristaltic waves. Laschimke and colleagues believe that acoustic emissions are a result of sudden surface rearrangements of groups of wall-adherent microbubbles under positive pressure (Laschimke et al. 2006). These microbubbles, which have also been photographed, are largely stable and do not immediately result in embolism. Laschimke also found, in *Ulmus glabra*, that acoustic emissions are incessant during transpiration and re-hydration. Therefore it is very unlikely that all acoustic emissions are a result of cavitation. Acoustic activity is undiminished during the night. This means that acoustic activity is not solely a result of transpiration, although transpiration does modify the type of activity, as stated before. The authors analyzed the waveforms of the acoustic emissions in *U. glabra* during a testing period of 77 h. By analyzing the waveform, it is possible to better understand the underlying physiological processes that cause the acoustic emission (Vergeynst et al. 2015). It is reasonable to expect that a sound emitted by a cavitation event would have a very rapid fading of the acoustic signal, as the water column is rapidly and violently retracted along the vessel following the disruption. However, very few of the 2200 acoustic emission events had a waveform profile of this type. Instead, most acoustic signals showed great variability in the duration, amplitude, and frequency, which can hardly be explained by the cavitation theory of acoustic emission.

2.1.5. Plant Alerts

2.1.5.1. Communicating Drought Stress

At this point, it is clear that not only do plants respond to sound, but also that plants emit a wide variety of acoustic emissions, with varying frequencies, from audible to ultrasound, and varying durations and intensities. However, it is harder to actually pinpoint the source of these emissions, and the theory that acoustic emission was a result of cavitation has been put into question, or at least it has been shown that not all emission is a result of it.

As stated before, sound treatment increased drought tolerance in *A. thaliana*. The same was shown in *Oryza sativa*. Different rice plants were treated with single frequencies of 0,25; 0,5; 0,8; 1,0; and 1,5 kHz for 24 h. After this treatment, the plants were placed under drought stress for five days. Sound treatment with frequencies of 0.8 kHz and above increased stomatal conductance, relative water content, and quantum yield of PS II. Furthermore, hydrogen peroxide production was inferior in these plants, and the temperature of the sound-treated plants and leaves was inferior compared with control (Jeong et al. 2014). So, could it be possible that the acoustic emissions by plants could be perceived by other plants? It has already been mentioned that certain moths can detect sound in the frequencies emitted by drought-stressed tomato and tobacco plants and possibly avoid laying their eggs on those stressed plants; a machine learning tool could very clearly distinguish between stressed and control plants. Could a drought-stressed plant emitting cavitation sounds, among other sounds, alert other plants of impending drought stress? This is a possibility.

Freeze-thaw cycles are the second most important reason for inducing cavitation, so it was natural for studies to focus on the acoustic emission of plants following such cycles. A study found that ultrasonic acoustic emissions are detected during the freezing part of the cycle in conifers, occurring during the ice formation part of the cycle, and most UAE are perceived during the first freeze-thaw cycle, with lower emissions during subsequent cycles. It was also found that samples with water contents close to dehydration emitted UAE during temperature cycles, whereas very dehydrated samples or saturated samples showed few UAE (Mayr et al. 2010).

But why should plants communicate through the use of sound? What possible advantages could be obtained through the use of sound, as opposed to the use, for example, of VOCs? Firstly, physical signals such as sound can propagate very rapidly, as opposed to VOCs that need to diffuse through the air. Moreover, sounds can be analyzed quickly and can be sensed at very low intensities and over long distances. Sound not only propagates a lot faster than volatiles, but it also has the added benefit of allowing for more accurate source localization. This means that sound has features that degrade predictably over distance, allowing a receiver to estimate the distance from the emitter. Not only that, but acoustic emission is also the result of a physical process, at least in the case of cavitation, which means that there is little to no energy cost involved (Gagliano et al. 2013). VOCs, on the other hand, represents a significant loss of energy, and a substantial amount of the carbon fixed by plants is re-emitted into the atmosphere through VOC communication (Holopainen et al. 2004). A possible advantage of VOCs, however, could be their ability to linger in their environment after emission, whereas acoustic signals very obviously dissipate extremely quickly. However, it's also important to note that volatile signals depend on diffusion and wind direction and, therefore, also suffer from their dilution. This means that although VOCs can linger, they nevertheless need to be present in sufficient quantities to be able to be perceived. Sound, on the other hand, can be perceived by organisms even at very low intensities (Gagliano et al. 2013).

2.2. Preliminary *in vitro* studies

In this study, seeds of *Capsicum annuum* L., a plant native to the Americas and belonging to the Solanaceae family, were utilized.

Capsicum annuum has been domesticated for over 6,000 years and is currently cultivated in numerous varieties worldwide (Perry et al. 2007). The plant yields fruits of variable sizes, which can exhibit different colors depending on the degree of ripeness, often transitioning from green to red.

Sound vibration is one of natural stimuli trigging physiological changes in plants. Recent studies showed that soundwaves stimulated production of a variety of plant secondary metabolites, including flavonoids (Ozkurt et al. 2018; Altunas et al. 2019; Kim et al. 2021) e protein (Yi et al. 2003; Xiujuan et al. 2003; Xiaocheng et al. 2003). Flavonoids are one type of abundant secondary metabolites with different biological functions in plants, which may act as a free radical scavenger (Williams et al. 2004), a chelation compound for metals (Kidd et al. 2001), or a regulator for hormone (Lewis et al. 2011).

In this context, it has been studied the influence of classical music, either alone or combined with growth-promoting agents or stress factors, on the synthesis of proteins and secondary metabolites in pepper seedlings. The experiments were conducted in the "Cell Technology Laboratory" at the Agricultural University of Athens, Greece.

2.2.1. Materials and Methods

2.2.1.1. Preparation and sterilization of culture media

The culture media were prepared by dissolving the components of the MS medium (Murashige & Skoog, 1962) in distilled water in Pyrex containers placed on a magnetic stirrer. The composition of the medium is detailed in **Table 1**. The pH of the medium, measured using a pH meter, was adjusted to a value of 5.8 using 1M HCl or 1M NaOH. Subsequently, sucrose was added as a carbon source at a concentration of 30 g/L, and agar was added to create a solid medium at a concentration of 8 g/L.

After dissolving the agar for 10 minutes in a microwave oven, 33 mL of the medium were poured into each container. The culture media were sterilized in an autoclave at a temperature of 121 °C and a pressure of 1 atm for 20 minutes and allowed to solidify.

Table 1: composition of MS (Murashige & Skoog) Medium

		COMPONENTS	For 1 l of Media (mg)
INORGANIC SALTS	(Microelements)	$MgSO4 \cdot 7H_2O$	370
		$CaCl_2 \cdot 2H_2O$	440
		KNO_3	1900
		NH_4NO_3	1650
		KH_2PO_4	170
	(Macroelements)	$FeSO_4 \cdot 7H_2O$	27,8
		Na_2EDTA	37,3
		$MnSO_4 \cdot 4\ H_2O$	22,3
		$ZnSO_4 \cdot 7\ H_2O$	8,6
		$CuSO_4 \cdot 5\ H_2O$	0,025
		$COCl_2 \cdot 6\ H_2O$	0,025
		KI	0,83
		H_3BO_3	6,2
		$Na_2MoO_4 \cdot 2$ H2O	0,25
VITAMINS		Myo-inositol	100
		Nicotinic acid	0,5
		Pyridoxine HCl	0,5
		Thiamine HCl	0,1
CARBON SOURCE		Sucrose	3000

2.2.1.2. Seeding

Seeds of *Capsicum annuum L.* Espartano F1 (Sakata) were sterilized by immersing them for 10 minutes in a 10% (v/v) solution of sodium hypochlorite to which a few drops of Tween surfactant were added per 100 ml of solution. Subsequently, they were immersed in 70% (v/v) ethanol for 2 minutes and then rinsed four times with sterile water. The seeds were placed in Petri dishes on sterile cotton soaked with GA3 (gibberellic Acid), previously prepared at a concentration of 2 mg in 900 mL of water, and placed in a dark environmental chamber at a constant temperature of 25±1 °C for 48 hours.

Afterwards, the seeds were inoculated into the jars with previously prepared MS medium and placed in a growth chamber in continuous darkness for 3 days. On the fourth day, they were transferred to a growth chamber with a photoperiod of 8 hours of light and 16 hours of darkness, maintaining a temperature of 25±1 °C.

2.2.1.3. Treatments

After 20 days and 40 days of growth, the plants were transferred to pots (**Figure 8**) with fresh soil and divided into groups for different treatments:

- Control (6 pots)
- Treated with music (6 pots)
- Treated with added proline to the soil (6 pots)
- Treated with added proline to the soil and with music (6 pots)
- Treated with sodium chloride (NaCl) added to the soil (6 pots)
- Treated with NaCl added to the soil and with music (6 pots)

Specifically, proline was added to the soil at a concentration of 30 mM, while sodium chloride at a concentration of 75 mM. Plants treated with music were exposed every day for 15 days to 1 hour of auditory stimulation provided through Turbo-X M 100 speakers (Plaisio) with an intensity of 70-80 dB (from 11:00 am to 12:00 pm) in the morning. The chosen auditory stimulus is "The Four Seasons" by Antonio Vivaldi, a title that encompasses four solo violin concertos characterized by the alternation of allegro, largo, and adagio rhythms.

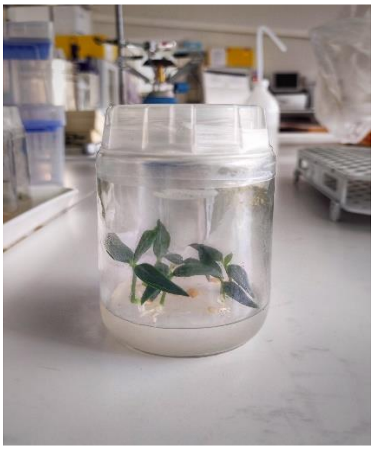


Figure 8: pot with peppers seedlings.

2.2.1.4 Spectrophotometric Analysis

- **Total Protein Content: Bradford Method**

The plants were collected and weighed separately in Eppendorf tubes. Subsequently, the tissues were ground to a fine powder in liquid nitrogen using a micropestle. The exact weight of each powdered sample was determined and thoroughly homogenized in 10 volumes of ice-cold phosphate buffer (50 mM, pH 7.0) containing 1% (w/v) polyvinylpolypyrrolidone. The extracts were incubated overnight with constant stirring. The samples were centrifuged at 10000 rpm for 20 minutes at 4°C, and the supernatant was collected. The protein content was estimated using the Bradford method with bovine serum albumin as a standard (Kondratiuk et al. 2015). The samples were then analyzed using a spectrophotometer with absorbance measured at 590 nm, and the protein quantification was performed using the standard curve: $y = 0{,}0006x + 0{,}0503$.

- **Total Phenolic Content: Folin - Ciocalteu Colorimetric Method**

Total phenolic content was analyzed using the Folin - Ciocalteu colorimetric method (Velioglu et al. 1998; Chlopicka et al. 2012) with some modifications. An aliquot of 0,3 mL of extract was mixed with Folin - Ciocalteu phenol reagent (2,25 mL). After 5 minuts, 6% sodium carbonate (2,25 mL) was added and the mixture was allowed to stand at room temperature for 90 min. The absorbance of the mixture was measured at 725 nm.
Standard calibration curve for gallic acid (or caffeic acid) (**Figure 9**) in the range of 0 - 200 µg/mL was prepared in the same manner and results were expressed as mg gallic acid equivalent (GAE) per gram of extract.

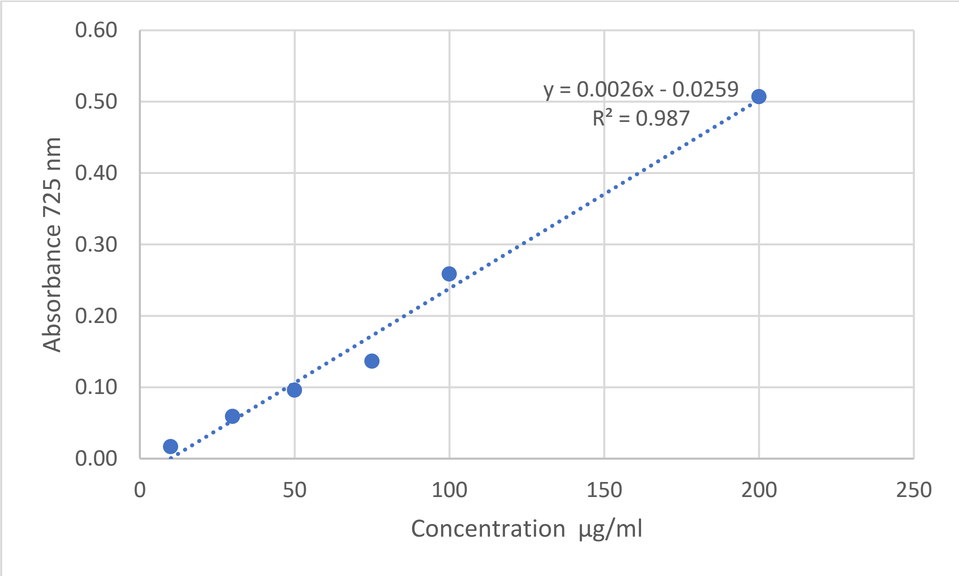


Figure 9: standard calibration curve for gallic acid.

- **Total Flavonoid Content: Aluminum Colorimetric Method**

Total flavonoid content was analyzed using the aluminum colorimetric method (Chang et al. 2002, Stankovic et al. 2011) with some modifications.

An aliquot of 0,5 mL of extract and 0,5 mL of standard were placed in different test tubes. Then were added 10% of aluminium chloride (0,1 mL), 1M of potassium acetate (0,1 mL), 80% methanol (1,5 mL) and distilled water (2,8 mL). A blank was prepared in the same manner but 0,5 mL of distilled water was used instead of the sample and 0.1 ml, the amount of aluminum chloride was also replaced by distilled water.

All tubes were incubated at room temperature for 30 min. The absorbance was taken at 415 nm. The concentration of flavonoid was expressed as µg quercetin equivalent (QE) per gram of extract.

In **figure 10** is represented the standard curve of quercetin in the range of 0–200 µg/mL.

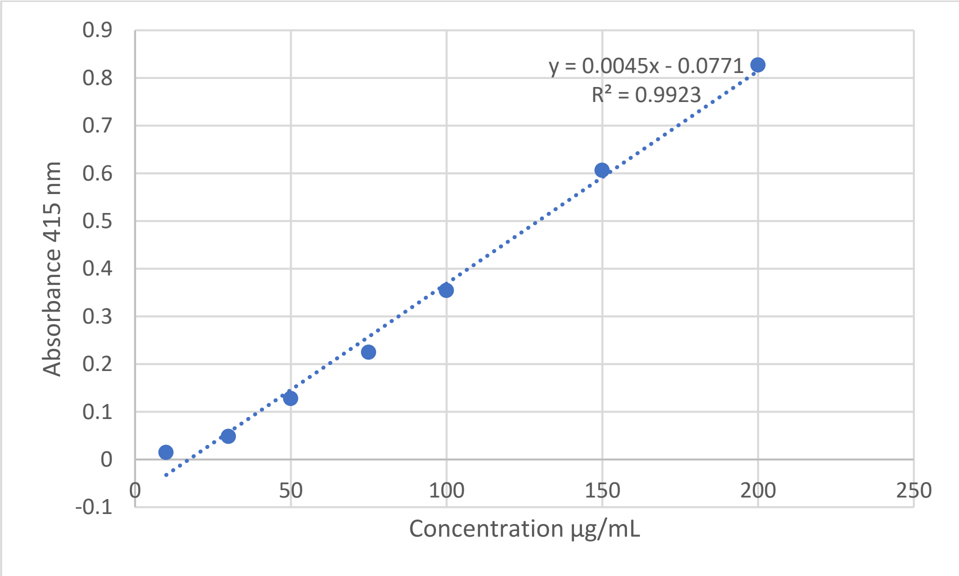


Figure 10: standard calibration curve for quercetin.

- **Free radical scavenging activity of the extracts: DPPH Method**

The free radical scavenging activity of all the extracts was evaluated by 1,1-diphenyl-2-picryl hydrazyl (DPPH) method. Specifically, 0,2 mM DPPH solutions were prepared in MeOH.
L-ascorbic acid, dissolved in distilled water, 0-100 µg/mL (0-5-10-20-30-50-75-100 µg/mL) were used as standard antioxidants. The DPPH assay was performed according to Prieto (2012) by mixing 180 µL of the DPPH solution and 20 µL of the antioxidant solution. As control, was used 20 µL of the extraction solution.
The mixture was reacted for 30 min in the dark at room temperature, and absorbance was measured at 517 nm using a spectrophotometer.
The capability of scavenging the DPPH radical was calculated by using the following formula:

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A0 - A1)/A0)*100\}$$

where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all the extract samples and reference.
In **figure 11** is represented the standard curve of DPPH.

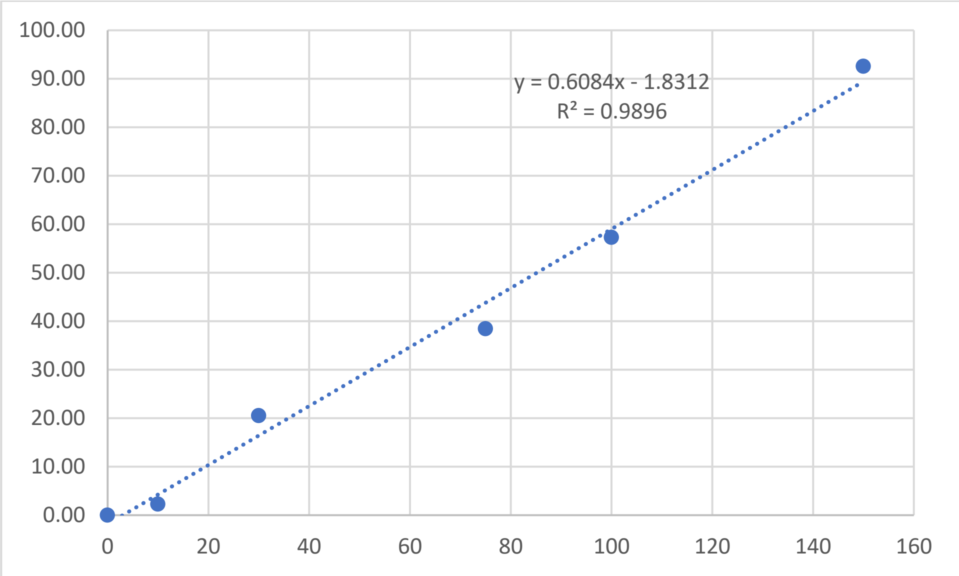


Figure 11: standard calibration curve of DPPH.

2.2.2. Results and Discussion

In this section of the research, the results of preliminary experiments on Plant Acoustics conducted on *in vitro* pepper plants (*Capsicum annuum* L.) are presented. Specifically, the influence of classical music, administered either alone or in combination with proline or sodium chloride, on the synthesis of proteins and secondary metabolites was examined.

2.2.2.1. Total Protein Content

Figure 12 illustrates the total protein content extracted from pepper plants treated with music (M), proline (P), sodium chloride (NM), and with combined treatments music + proline (PM) and music + sodium chloride (NM) for 14 days.

In the control (C), a total protein content of 151,15 µg/ml was detected. In the music-treated group, it was 199,91 µg/ml, in the proline-treated group 185,33 µg/ml, in the proline and music-treated group 155,16 µg/ml, in the sodium chloride-treated group 182 µg/ml, and in the sodium chloride and music-treated group 230,16 µg/ml. Some of the differences between these values were found to be statistically significant (Test t; $p < 0.05$). In particular, in the music-treated, sodium chloride-treated, and sodium chloride + music-treated groups, the protein content was significantly higher than in the control.

Although literature studies analyzing protein content following sound treatments are limited, an increase in total proteins is commonly observed. Yi et al. (2003) observed increased root division and growth in in vitro chrysanthemum plants treated with sound waves at 1000 Hz and 100 dB, explaining that these increases are closely related to protein content. The level of accumulation reflects not only the substances necessary for cell division but also enzyme content and their metabolism.

Yiyao et al. (2002) used a sound stimulation generator (SSG) to study the effects of the sound field on chrysanthemum callus growth, measuring soluble protein content, superoxide dismutase (SOD) activity, and indole-3-acetic acid (IAA) oxidase activity. It was observed that SOD activity and soluble protein content in the callus increased with the increase in sound pressure (dB) and frequency (Hz). This could be attributed to the influence of sound waves on the microstructure of chrysanthemum cell plasma membrane through enhanced lipid fluidity. The enhancement of cell wall fluidity is one of the mechanisms promoting plant growth through sound waves (Hassanien et al. 2014). Xiujuan et al. (2003) also analyzed the effect of sound waves on nucleic acids and proteins in chrysanthemum, observing that sound waves accelerated RNA and protein synthesis. Consequently, sound stimulation may increase transcription levels, mRNA content, and subsequently enhance the translation of multiple proteins. Sound stimulation also appears to increase the activity of some enzymes such as peroxidase and catalase (Azgomi et al. 2021) and induce differences in gene expression (Ye et al. 2023). However, further studies are needed to explore how the sound wave signal penetrates the cell and influences gene expression.

Both proline and sodium chloride also increase total protein content. However, while proline should promote plant growth, sodium chloride should act as a stress agent (Demir et al. 2002). Further studies are required to better understand which protein classes are predominantly produced and the effect of combined treatment with music.

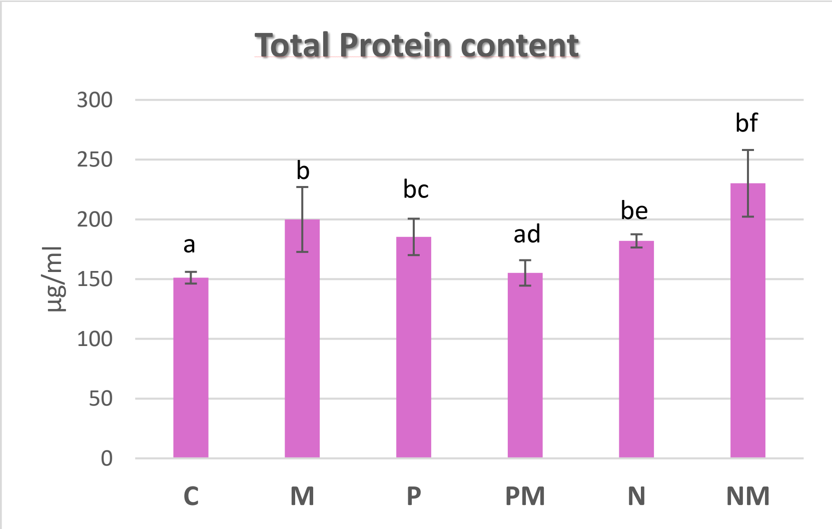


Figure 12: graph representing the Total Protein Content (µg/ml) in *in vitro* peppers plants. Treatments: Control (C); Music (M); Proline (P); Proline + Music (PM); NaCl (N); NaCl + Music (NM). Letters: a and b represent statistically significant differences between control and all other treatments; b and c between Proline and Proline + Music; d and e between NaCl and NaCl + Music ($p < 0.05$).

2.2.2.2. Total Phenolic Content, Flavonoid Content and Antioxidant Activity

Figure 13 represents the total phenolic content extracted from pepper plants treated with music, proline, sodium chloride, and with combined treatments of music + proline and music + sodium chloride. In the control 69,5 mg/g DW was detected; in the music-treated group 74 mg/g DW; in the proline-treated group 73 mg/g DW; in the proline + music-treated group 72,4 mg/g DW; in the sodium chloride-treated group 75,98 mg/g DW; and in the sodium chloride + music-treated group 84,05 mg/g DW, the highest value compared to all others. However, these variations among different treatments were not found to be statistically significant (t-test).

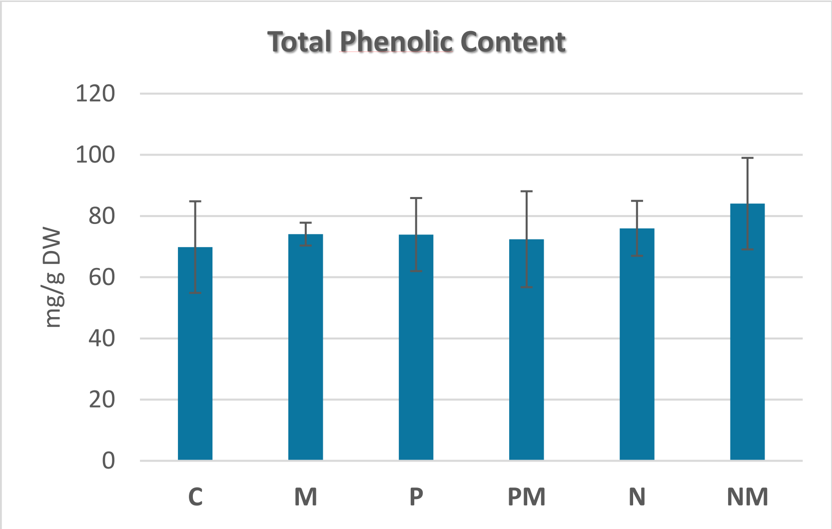


Figure 13: graph representing the Total Phenolic Content (mg/g DW) in *in vitro* peppers plants. Treatments: Control (C); Music (M); Proline (P); Proline + Music (PM); NaCl (N); NaCl + Music (NM).

Figure 14 represents the total flavonoid content. In this case, the control showed 6,85 mg/g DW; in the music-treated group, 6,67 mg/g DW; in the proline-treated group 6,13 mg/g DW; in the proline + music-treated group 6,04 mg/g DW; in the sodium chloride-treated group, 7,2 mg/g DW; and in the sodium chloride + music-treated group 6,8 mg/g DW, the highest value compared to all others. However, in this case as well, the slight variations among different treatments were not found to be statistically significant (t-test).

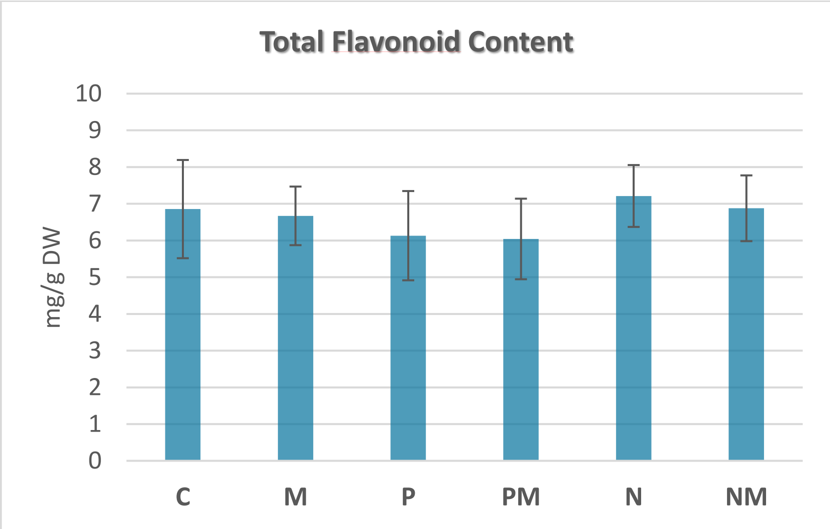


Figure 14: graph representing the Total Flavonoid Content (mg/g DW) in *in vitro* peppers plants. Treatments: Control (C); Music (M); Proline (P); Proline + Music (PM); NaCl (N); NaCl + Music (NM).

Figure 15 illustrates the free radical scavenging activity of all the extracts evaluated by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) method. The percentage of DPPH radical inhibition was found to be 88% in the proline-treated group, 79% in the proline + music-treated group, 79% in the sodium chloride-treated group, and 85% in the sodium chloride + music-treated group. These percentages were found to be statistically significant (t-test; $p < 0.05$) compared to the control (63%) and the music-treated group (66%).

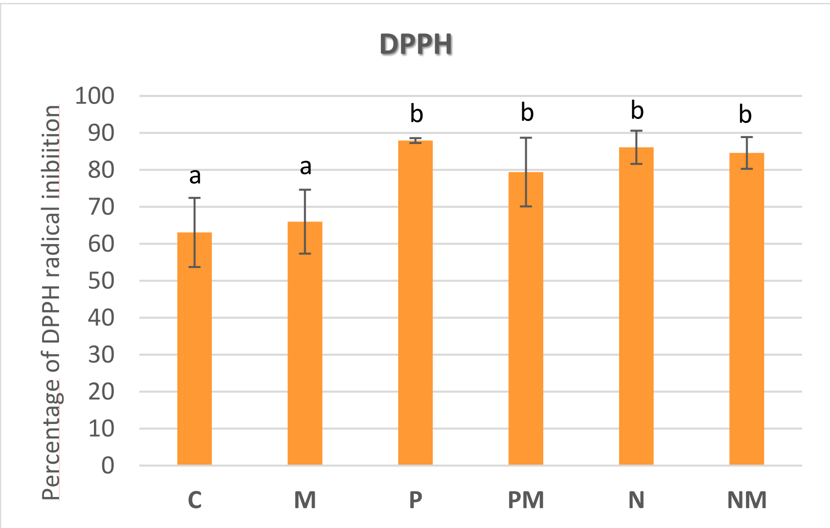


Figure 15: graph representing the free radical scavenging activity of the extracts evaluated by DPPH in *in vitro* peppers plants.

Treatments: Control (C); Music (M); Proline (P); Proline + Music (PM); NaCl (N); NaCl + Music (NM).
Letters: a and b represent statistically significant differences between control and all other treatments.

In 2021, Kim et al. conducted a study where they exposed radish, lettuce, and Chinese cabbage sprouts to sound waves of different frequencies (250; 800; 1000; 1500) for 2 hours a day for consecutive or alternate days. They observed that, through the DPPH test, the antioxidant activity of the sprouts was significantly higher in some sound wave treatments, and these antioxidant properties were correlated with the accumulation of flavonoids. However, the increase in flavonoid content following an up-regulation of genes for their biosynthesis produced different effects depending on the species, frequency, and exposure time.
In our case, the duration and intensity of the treatments may not have been sufficient to achieve increases in the content of secondary metabolites following exposure to sound waves. Furthermore, the production of secondary metabolites by in vitro plants is typically low, and to obtain appreciable quantities for practical and commercial purposes, biotic and abiotic elicitors are often used (Guru et al. 2022). In the latter category, stimuli such as UV radiation, high light intensity, and heavy metals are included, factors that represent a stress condition for the plant. On one hand, sound could be a stress factor, but on the other hand, it assumes a much broader ecological significance (**Par. 2.1.2.3.**), and to understand its role, it may be crucial to keep the plants immersed in their ecosystem rather than in isolated conditions like *in vitro*.

2.3. In plant studies

Lavender (*Lavandula officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) are two aromatic plants belonging to the Lamiaceae family, each with unique botanical characteristics. Lavender is a bushy shrub with grayish-green linear leaves and flowers gathered in aromatic spikes ranging in color from blue, purple, and azure to white or pink. It primarily grows in Mediterranean regions and is renowned for its rich volatile oils (Barret, 1996). Rosemary is an evergreen shrub with narrow, needle-like leaves, often used as an ornamental and culinary plant. It emits a distinct pine wood scent and is widespread in many parts of the world, particularly in the Americas and Europe (Flamini et al., 2002). Both plants are commonly cultivated for the production of their essential oils. Lavender essential oil is extracted from the flowers through steam distillation, resulting in an oil known for its relaxing and aromatic properties (Cavanagh et al., 2005; Guo et al., 2020). Rosemary, on the other hand, yields an essential oil obtained from the leaves and flowers, renowned for its stimulating and tonifying properties (Bozin et al., 2007). Both essential oils find wide applications in aromatherapy, cosmetics, and household products. Their production and usage remain crucial for both the perfume industry and herbalism (Cardia et al., 2021; Diass et al., 2021).

Numerous studies have demonstrated that different types of music, along with varying frequencies, pressure levels, and exposure durations, exert diverse effects on plant growth (Chowdhury et al., 2015; Ramekar et al., 2016; Hendrawan et al., 2020; Lai et al., 2020; Wang et al., 2023). For instance, Abdullah et al. (2019) observed that different genres of music had varying impacts on different parts of mung bean plants (Vigna radiata). "Soprano" music, for example, seemed to contribute to stem elongation, while recitation of the Quran induced an increase in the number of leaves. Chivukula et al. (2014) noted that Rosa Chinensis plants exposed to Vedic chants exhibited greater stem elongation, increased flower numbers, and larger flower diameters, whereas rock music appeared to hinder plant growth (Chivukula et al., 2014).

In this context, the response to music exposure, specifically Vedic chants, in *Lavandula officinalis* and *Rosmarinus officinalis* plants grown in the field was investigated. In particular, the contents of the leaf pigments, the quantitative variations of the VOCs released into the air and the qualitative variations of the VOCs in the essential oils of the plants under study, were analysed. The experiments were conducted at Villa Vrindavana in San Casciano in Val di Pesa, Florence, Italy.

2.3.1. Materials and Methods

2.3.1.1. Growing Conditions

The experiments were conducted in the garden of Villa Vrindavana, Florence, Italy. Controls and treated plant of lavender and rosemary were grown under similar environmental conditions with the same light exposure and south-facing orientation. Their positions were adequately spaced to ensure that the sound stimulus did not affect the control plants.

2.3.1.2. Sound Treatments

The plants were exposed every day for two months (April – June) to a 20-minute sound stimulus provided through an amplifier with an intensity of 70-80 dB (from 9:40 AM to 10:00 AM). Specifically, one of the mantras from the Vedic tradition, known to be effective in improving plant growth and development (Chivukula et al. 2014), was chosen. The mantra traditionally called 'Mahamantra' or 'Great mantra,' is also known in the West due to the spread of the Hare Krishna movement.

2.3.1.3. Leaf Pigment Analysis: PolyPen Instrument

The analysis of leaf pigments was carried out with the instrument PolyPen RP 410 PSI (Photon Systems Instruments), an handheld instrument that incorporates an internal Xenon incandescent lamp and measures spectral reflectance of leaves.

The selected indices detected by the PolyPen to assess leaf pigment content were:

- **MCARI** (**Modified Chlorophyll Absorption in Reflectance Index**): Provides a measure of the depth of chlorophyll absorption and is highly sensitive to variations in chlorophyll concentrations, as well as changes in the Leaf Area Index (LAI). MCARI values are not influenced by lighting conditions, soil background reflectance, and other non-photosynthetic materials.
- **ARI1** and **ARI2** (**Anthocyanin Reflectance Index 1 and 2**): Both indices are used to estimate the presence of anthocyanins in plants, differing in the considered wavelengths. ARI1 uses wavelengths at 550 nm and 700 nm, while ARI2 uses wavelengths at 550 nm and 735 nm. In both cases, the formula evaluates the normalized difference between reflectances at these wavelengths to obtain an index reflecting the presence of anthocyanins.
- **CRI1** and **CRI2** (**Carotenoid Reflectance Index 1 and 2**): Indices used to estimate the presence and concentration of carotenoids in plants using the spectral characteristics of reflected light at different wavelengths. The difference between CRI1 and CRI2 will depend on the specific wavelengths involved in their respective calculation formulas. Higher values of CRI1 and CRI2 indicate a greater presence of carotenoids.

2.3.1.4. Quantitative analysis of VOCs: Tiger LT Instrument

The investigation of variations in the concentration of volatile compounds emitted by the studied plants was monitored using the TIGER LT instrument (Ion Science-Italy, BO, IT). Tiger is a portable gas detector capable of detecting a wide range of VOCs, employing a Photoionization Detector (PID) to measure the relative gas concentrations in a range from 1 ppb to 20,000 ppb with a sensitivity of 1 ppb. The PID is equipped with a pump capable of drawing air at various time intervals (from 1 second to 59 minutes) and detecting the concentration; for the experiments, a two-second interval was chosen. The device has a lithium battery with a 24-hour autonomy, rechargeable in less than 7 hours, making it convenient for outdoor recordings. Moreover, it can record data that can be transferred to a PC and saved using the 'Tiger PC' software. For the experiments described in this chapter, Tiger was positioned based on the different locations and requirements of the experiments. Specifically, for the experiments conducted in open field conditions, Tiger was placed at a distance of 4-5 cm from the plant.

2.3.1.5. Essential Oils Extraction and Qualitative Analysis

In order to identify qualitative variations in the composition of essential oils between the treated and control groups, essential oils were extracted using the steam distillation method with a distiller still (Agristore, CS, Italy) (**Figure 16**).

This extraction technique relies on the physical property of essential oils being volatile, easily vaporizable, and carried away by steam. Fixed portions of fresh plant material weighing 200 grams were used, taking inspiration from essential oil producers who specifically chose and used only flowers in the case of lavender, and flowers and leaves in the case of rosemary.

A fixed amount of 250 ml water was placed in the boiler and heated over low flame to generate a slow boil. The passage of steam through the plant material makes the cell walls more permeable, leading to their rupture and the release of the essence, which, being volatile, is vaporized. The mixture of steam/essence is condensed in a coil cooled by a water recirculation system and returned to a liquid state, separating into essential oil and hydrosol. The essential oil settles on the surface as it has lower density than water.

Different samples were collected in glass bottles. Subsequently, the essential oil of each was separated from the hydrosol using Pasteur pipettes, and ethyl acetate was used as a solvent (3 ml) to extract the oil components. The extracts were then collected, filtered with glass wool inside glass pipettes, and finally injected for a volume of 3 µl into the GC/MS (Agilent Technologies, Inc) for analysis.

Figure 16: distiller still used for the extraction of essential oils.

2.3.2. Results and Discussion

The literature reporting the effects of sound (single frequency) or a varied musical repertoire on plants is quite diverse. It is challenging to find a sort of uniformity among the type of sound stimulus, the duration of administration and the overall treatment, the plant species studied, and the phase of the plant's life cycle under investigation, up to the observed effects, ranging from physiological, botanical, agronomic, to cellular and molecular.

2.3.2.1. Leaf Pigment Analysis

Measurements of reflected light at specific wavelengths and the calculation of Reflectance Indices (RIs) are widely used methods to estimate plant development, productivity, and stress-induced variations (Zarco-Tejada et al., 2005). RIs are often employed in agriculture to assess crop conditions and detect any signs of stress through non-invasive measurements. Reflectance in the visible light region is closely related to the content of photosynthetic pigments (Kior et al., 2021).

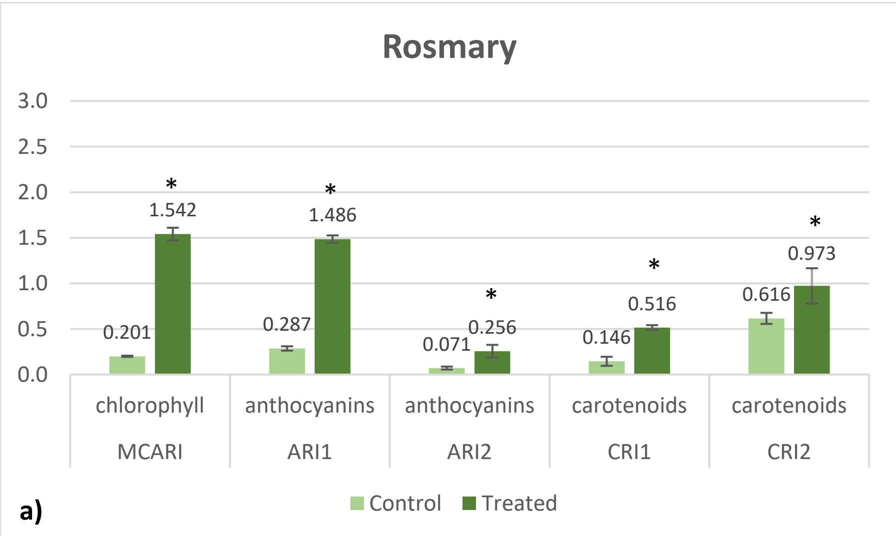
Chlorophylls are the most important pigments in plants for absorbing the solar energy needed for photosynthesis (Rabinowitch, 1965; Evans, 1989). The amount of chlorophyll per unit leaf area is a direct indicator of the plant's primary production and photosynthetic potential (Gitelson et al., 2006). Generally, healthy plants capable of maximum growth are expected to have higher chlorophyll levels than unhealthy ones. Therefore, determining the chlorophyll content of a leaf can be used to detect and study the nutritional status of plants, mutations, and both biotic and abiotic stress (Zarco-Tejada et al., 2004; Wu et al., 2008).

Anthocyanins are plant pigments responsible for red, violet, and blue colors in many parts of plants, such as leaves, flowers, and fruits. Anthocyanins are often produced in response to environmental stress, such as excessive exposure to sunlight, low temperatures, or other stress factors. Therefore, measuring ARI1 can provide information about the plant's response to stress and overall plant health.

Carotenoids are photosynthetic pigments that play various crucial roles in plants. They are part of the antenna complexes necessary for photosynthesis and are involved in photoprotection and light adaptation mechanisms (Frank et al., 1996). Additionally, they are involved in protection against environmental stress due to their antioxidant action (Zhou et al., 2017).

In the plant growth cycle, a decrease in chlorophyll typically indicates that plants are affected by environmental stress, while carotenoid variation reflects the physiological state of vegetation (Young and Britton, 1990).

In **figure 17** concentrations of chlorophylls, anthocyanins, and carotenoids, detected with the PolyPen RP 410 instrument, are depicted by the MCARI, ARI1, ARI2, CRI1, and CRI2 indices. Both in rosemary (**Figure 17a**) and lavender (**Figure 17b**), a significant increase in both chlorophylls and carotenoids is observed in plants subjected to sound treatment. Regarding anthocyanins, a significant increase in the treated group compared to the control was observed only in rosemary.



a)

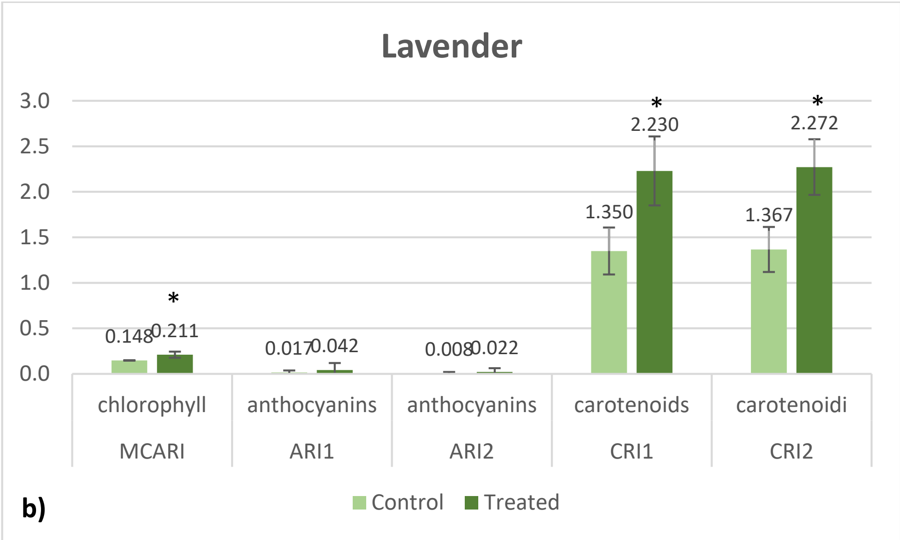


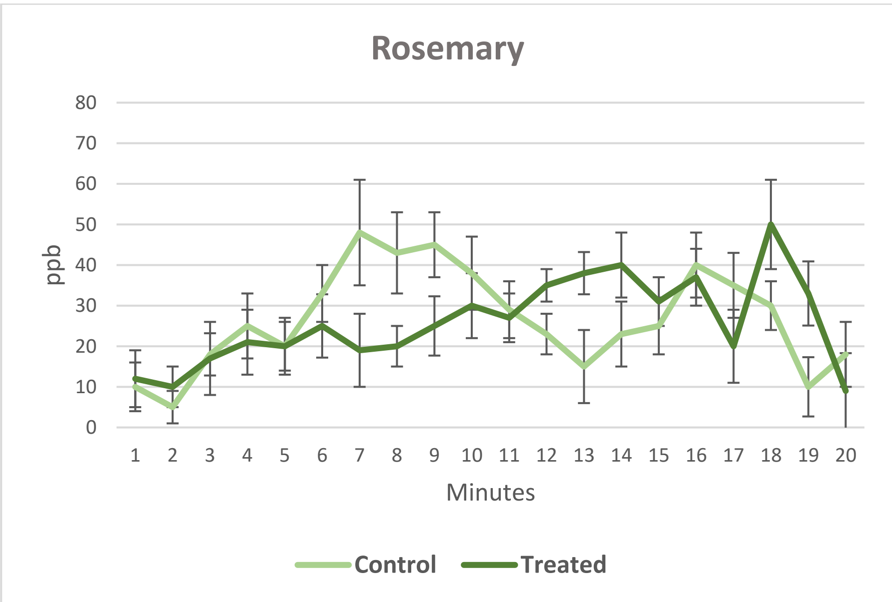
Figura 17: graphs representing the concentrations of chlorophyll (MCARI index), anthocyanins (ARI1, ARI2 indices), and carotenoids (CRI1, CRI2 indices) detected with the PolyPen RP 410 instrument in **a)** rosemary, **b)** lavender. Index values are dimensionless. Statistically significant differences calculated with a T-test ($p < 0.05$) are indicated by *.

Some research studies have demonstrated that different types of music have varying effects on plant growth (Chowdhury et al. 2015; Ramekar et al. 2016; Hendrawan et al. 2020; Lai et al. 2020; Wang et al. 2023). For instance, Abdullah et al. (2019) observed different effects of music types on various parts of mung bean plants (Vigna radiata). Specifically, 'soprano' music was found to contribute to stem elongation, while Quranic recitation led to an increase in the number of leaves (Abdullah et al. 2019). Chivukula et al. (2014) noted that Rose Chinensis plants exposed to Vedic chants exhibited greater stem elongation, increased flower count, and expanded flower diameter, while rock music appeared to hinder plant growth (Chivukula et al. 2014).

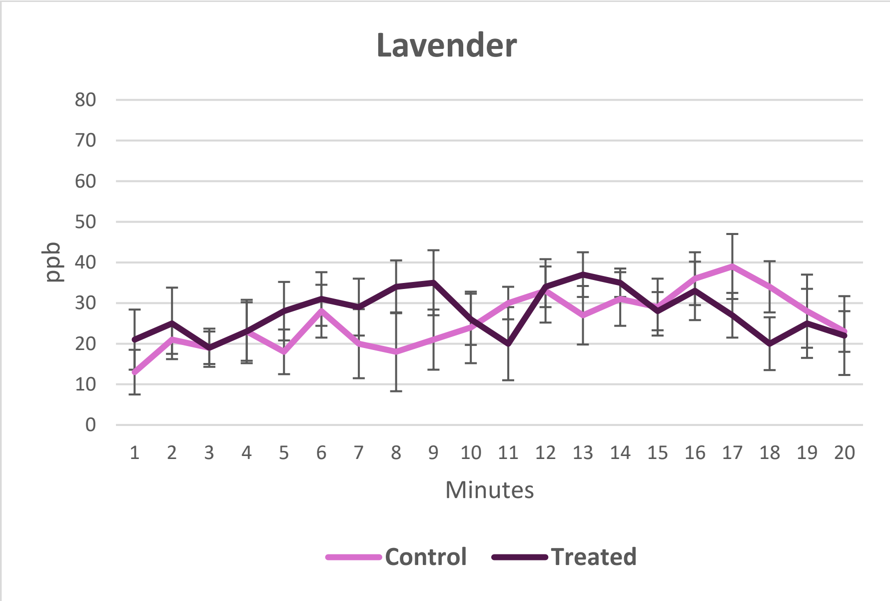
Certain types of music might promote plant growth primarily through the stimulation of sound waves, which, as vibrations, could act on stomata by increasing their opening. Consequently, the amount of absorbed carbon dioxide by the plant should increase, enhancing the photosynthetic rate and, consequently, the synthesis of organic material (Wang et al. 2023). This consideration seems to support the idea that sound not only influences plant growth but also affects various biological aspects, such as an increase in leaf pigment content, as found in our experiments.

2.3.2.2. Quantitative Analysis of VOCs

In **figure 18**, the average trend of VOC concentrations (ppb) in rosemary plants (**Figure 18a**) and lavender plants (**Figure 18b**) is depicted. In both cases, the concentrations fluctuate between 0 and 60 ppb throughout the monitoring period (20 minutes) in both the control and the treated group.
Specifically, the trend remains oscillatory within a range between 0 and 60 ppb in rosemary and 0 and 40 ppb in lavender.
The aim of this analysis was to explore the hypothesis that sound may influence the concentrations of VOCs released into the air by lavender and rosemary plants. However, the analysis conducted using the Tiger instrument revealed a lack of significant variations in VOC concentrations in response to sound stimuli.
The obtained data indicate quantitative stability of VOCs emitted by lavender and rosemary plants throughout the observation period, and even in response to sound treatment, the studied plants did not show a quantitative increase in VOC emissions despite environmental fluctuations and variations in biological cycles.



a)



b)

Figure 18: graph relating to VOC concentrations in ppb measured in a) rosemary plants and b) lavender plants.
The VOC concentration (ppb) is on the ordinate, the duration of the monitoring (minutes) is on the abscissa.

2.3.2.3. Essential Oils Analysis

Lavender and rosemary are well-known medicinal plants appreciated for their essential oils. They find applications in the cosmetic and aromatherapy industries, as well as in food and pharmaceutical industries, where they are used to flavor oral formulations or preserve products (González-Minero et al., 2020; Salehi et al., 2018).

Essential oils play crucial roles in plant physiology and ecology. They are involved in attracting dispersers and pollinators (Wagner et al. 2003), plant-to-plant communication (Dudareva et al. 2004), defense against herbivores (Oztekin et al. 2014), and protection against pathogens (Holopainen et al. 2003). The main compounds found in rosemary include α-pinene, camphene, 1,8-cineole, camphor, borneol, α-terpineol, and β-caryophyllene (Takayama et al. 2016; Borges et al. 2018; Mohammed et al. 2020). Lavender, on the other hand, contains linalool, 1,8-cineole, camphor, and linalyl acetate (Héral et al. 2021). The presence of secondary metabolites can be modulated by environmental conditions, both abiotic and biotic, to which the plant is exposed (Verma et al. 2010; Verma et al. 2012; Mahmoud et al., 2018; Aqeel et al. 2022). Sound vibrations also appear to have an effect in this regard, stimulating the plant's defense response and triggering a cascade of response signals at the molecular, phytochemical, and ultimately physiological levels, often resulting in a modulated production of secondary metabolites (Azgomi et al. 2023).

In recent years, various mechanosensory channels have been identified in the plasma membrane of plant cells. Their function seems to be invoked not only in touch perception but also in sound perception (Nejat et al. 2017, Cheung et al, 2020, Lamers et al. 2020). Sound waves, mechanically impacting plants, can induce a response to environmental stimuli such as wind, rain, or touch, modifying gene expression or hormonal signaling at the molecular level, cytological changes such as stomatal opening, and ultimately influencing physiological changes such as germination, growth, and flowering (Bochu et al. 2004; Ye et al., 2004; Ozkurt et al., 2018; Kim et al. 2020; Razavizadeh et al. 2021; Azgomi et al. 2023).

In **tables 2** and **3**, the compounds identified by GC/MS analyses in rosemary and lavender are listed, showcasing modulation, either increase or decrease, compared to plants not exposed to Vedic chant music.

Table 2: VOCs identified through gas chromatographic analysis of the essential oil extracted from rosemary plants. The 'x' in the columns indicates the condition in which the respective compound was detected. In blue are highlighted the compounds that increased in the treated group compared to the control, in red are the compounds that decrease in the treated group, while in black are the compounds that remained unchanged between control and treated groups.

Rt (Ritention Time)	COMPOUND	Control	Treated
6,87	α-Pinene	X	X
7,3	Sabinene	X	X
8,1	1-Octen-3-ol	X	X
9,39	p-Cymene	X	X
9,48/11,28	Terpinyl acetate	X	X
9,5	Eucalyptol (1,8 cinecole)	X	X
10	α-Ocimene	X	X
10,44	γ-Terpinene	X	X
10,6	α-terpienol	X	X
11,23	Dihydrocarvyl acetate	X	X
11,24	Terpinolene	X	X

11,47	1-O-menthen-8-ol	X	X
11,62	Linalool	X	X
11,7	Chrysanthenone	X	X
11,7	Linalyl acetate	X	X
11,98	Fenchol	X	X
12,7	Pinocarveol	X	X
12,8	Verbenol	X	X
12,91	2-Bornanone (D-camphor)	X	X
13,2	Isobornyl acetate	X	X
13,4	Pinocarvone	X	X
13,56	Borneol	X	X
13,78	3-Pinanone	X	X
13,89	Terpinen 4-ol	X	X
14,1	p-Cymene-8-ol	X	X
14,2	α-Terpineol	X	X
14,5	Myrtenol	X	X
15	Verbenone	X	X
15	cis-Carveol	X	X
16,86	Bornyl acetate	X	X
18,3	Piperitenone	X	X
20,43	β-Caryophyllene	X	X
21,2	Geranyl acetone	X	X
21,28	α-Humulene	X	X
21,66	Germacrene-D	X	X
22,58	β Bisabolene	X	X
23,69	caryophyllene oxide	X	X
26,62	α bisabolol	X	X

Table 3: VOCs identified through gas chromatographic analysis of the essential oil extracted from lavender plants. The 'x' in the columns indicates the condition in which the respective compound was detected. In blue are highlighted the compounds that increased in the treated group compared to the control, in red are the compounds that decrease in the treated group, while in black are the compounds that remained unchanged between control and treated groups.

Rt (Ritention Time)	COMPOUND	Control	Treated
7,28	Camphene	X	X
6,87	α-Pinene	X	X
7,3	Sabinene	X	X
7,95	β-Phellandrene	X	
8	β-Pinene	X	X
8,37	3-Octanone	X	
8,44	β-Myrcene	X	X
8,79	β-Thujene	X	
8,84	α-Phellandrene	X	X
9	3-Carene	X	X
9,19	α-Terpinene	X	X
9,49	D-Limonene	X	X
9,5	Eucalyptol (1,8 cinecole)	X	X
9,78	trans-β-Ocimene	X	X
10,44	γ-Terpinene	X	X
11,24	Terpinolene	X	X
11,62	Linalool	X	X
12,26	α-Terpineol	X	X
12,91	2-Bornanone (D-Camphor)	X	X
13,56	endo-borneol	X	X
13,57	Geraniol	X	X
13,89	Terpinen 4-ol	X	X
14,27	Hexyl butyrate	X	X
15,88	D-Carvone	X	X
16	Linalyl acetate	X	X
16,86	Bornyl acetate	X	X
17	Lavandulyl acetate	X	X

18,49	**Neryl acetate**	X	X
19	**Geranyl acetate**	X	X
20,3	**cis e trans α-Bergamotene**	X	X
20,43	**Caryophyllene**	X	X
20,9	**cis-α-Bisabolene**	X	X
21,16	**cis-β-Farnesene**	X	X
21,28	**Humulene**	X	X
21,66	**Germacrene D**	X	X
23,69	**Caryophyllene oxide**	X	X
26,62	**α bisabolol**	X	X

Some terpenic compounds such as endo-borneol, eucalyptol, linalool, caryophyllene, and bisabolol, which are among the fundamental constituents of the essential oils common to both investigated plants, increased in response to sound treatment. Plants, being sessile organisms, communicate with each other and the external world through volatile compounds. They can use these compounds to defend against herbivores (Oztekin et al. 2014) or attract predators or parasitoids natural to the herbivore itself (Arimura et al., 2005). Terpenoids such as β-ocimene and caryophyllene, which we found increased in our study, are known to be used in attracting entomopathogenic nematodes (Rasmann et al., 2005), parasitoid wasps (Tamiru et al., 2011), or predatory mites (Arimura et al., 2005), as if some of the frequencies present in the Vedic chants administered could be related to the presence of such pathogens.

Others, such as the monoterpenes α-pinene, D-limonene (attractive to pollinators), and camphor (natural insecticide) and eucalyptol, showed variations in their relative quantity, whether increased or decreased, independently, in both rosemary and lavender (Table 1 and 2).

In general, the results regarding the effect of music are consistent with those obtained by Cai et al. (2014), who examined the effects of music on essential oil production in aromatic plants, highlighting an increase in the synthesis of volatile compounds. They also align with results regarding the effect on growth and leaf pigments obtained by Ekici et al. (2007), Azgomi et al. 2021, Wang and Xiao 2023, who studied the effects of music on chlorophyll content and other pigments in plants, demonstrating a positive impact on photosynthesis.

These convergent results support the idea that music may have a significant impact on the physiological responses of plants, paving the way for further research to better understand the underlying mechanisms of this phenomenon.

2.4. Main Conclusions

Although this study adds to previous ones in highlighting the potentially positive effects of music on one or more plant characteristics, the mechanism by which this occurs is not yet clearly understood. This research, showcasing the numerous implications, even from an agronomic perspective, suggests further investigations to understand the specific mechanisms behind the observed differences and how music influences plant biology.

2.5. References

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3. New Perspectives on Monitoring Psychophysiological Wellbeing Effects after Inhaling Essential Oils

The effects of *Shinrin-yoku*, or "Forest Bathing", on human wellbeing have been traditionally acknowledged in Japan. A possible factor contributing to this effect is olfactory stimulation, as plants naturally emit essential oils that appear to have both positive physiological and psychological effects (**Par. 1.3.2**). In this context, the psychophysiological effects of olfactory stimulation by essential oils have been investigated in a controlled laboratory environment using a novel, non-invasive approach: VibraImage technology. VibraImage system is based on automated biometrics and computer vision, and is capable of analysing head microvibration of the subjects and transformation of microvibration to behavioral and psychophysiological parameters. VibraImage measurements were performed on the subjects before and after inhaling essential oils, and resulted in a statistically significant increase in positive behavioral parameters and a decrease in negative ones, leading to an improved overall wellbeing status.

3.1. State of the art

According to the Constitution of the World Health Organization, "health is a state of complete physical, mental and social well-being and not the mere absence of disease or infirmity". This definition underlines the importance of the overall wellbeing of people, which can be fostered in countless ways. However, due to population growth, urbanization and other factors, many people tend to live in areas with limited access to Nature. Despite this tendency, there is evidence that contact with Nature can be beneficial (Doimo et al. 2020). A recent literature review shows that being in contact with nature positively correlates with physical and mental health, and in turn fosters pro-environmental behaviour and values (Barrgan-Jason et al. 2022).

One of the ways for people to be in contact with Nature is *Shinrin-yoku*, also known as "Forest Bathing", a traditional Japanese practice that involves visiting a forest environment and experiencing the surroundings with all five senses. The benefits of Forest Bathing range from physiological responses like lower cortisol levels, pulse rate and blood pressure, to psychological effects like lower tension, anger and fatigue (Park et al. 2010).

The scientific analysis of every factor determining the Forest Bathing effect is complex, because the forest environment offers a wide variety of stimuli, e.g. the landscape view, the forest sounds, the volatile compounds in the air, the effect of physical activities like hiking, and more. However, examining each factor is important to better understand Forest Bathing as a whole. In this context we focus on the effects of the olfactory stimulus. The olfactory stimulus is known to affect both physiological and psychological parameters, such as nervous system activity, stress biomarkers, mood and behaviour (Angelucci et al. 2014; Herz et al. 2009).

Forest air contains plant-made essential oils (EO) as a mixture of VOCs, among which α-pinene, β-pinene, camphene, 3-carene and limonene are some of the main components (Lee at al. 2015). Essential oils can also be extracted in a laboratory with a good approximation of the mixture of essences that can be found in a certain forest or area. Essential oils were used to mimic the effect of olfactory stimulation in controlled laboratory conditions.

EO inhalation was examined in a sample of young men and women using VibraImage technology (Minkin, 2017; Minkin and Nikolaenko, 2017) to monitor variations in positive and negative behavior parameters such as Balance, Charm, Energy, Self-regulation, Stress, Tension, Aggression, and Suspect. These parameters were measured based on the analysis of muscle microvibration (Rohracher and Inanaga, 1969) and vestibulo-emotional reflex (Minkin and Nikolaenko, 2008). The chosen technique was based on its ability to output global well-being parameters, and a correlation has previously been identified between VibraImage and a traditional technique like EEG in response to olfactory stimulation (Kim and Kim, 2015)."

3.2. Materials and Methods

Eleven subjects were recruited (5 males and 6 females), with ages ranging from 21 to 25 (average age: 23). The study setup involved two separate rooms, and every test subject followed the same research procedure:

1. Sit in room No. 1 to measure head microvibration (1 min) before inhaling EO, using VibraImage technology. The movements were measured using a camera (Microsoft LiveCam Cinema with 640x480 resolution) placed on a tripod and linked to a laptop computer with the VibraMed10 acquisition software. The measured parameters were Aggression, Stress, Tension, Suspect (T1-T4), Balance, Charm, Energy, Self-regulation (T5-T8), Inhibition and Neuroticism (T9-T10). To maximize the reliability of VibraImage measurements, we placed a blank screen behind the subjects and required them to wear a white lab coat.

2. Move to room No. 2 to inhale EO (1 min) in a contained environment, with no external interference.
3. Sit back to room No. 1 to measure head microvibration (1 min) after inhaling EO. The experimental setup and the measured parameters were the same as before. Meanwhile, room No. 2 was ventilated for 5 min.

The EO were provided by the Italian company Fitomedical s.r.l. (Binasco (MI), IT). We used a mixture of oils called "Miscela di essenze – Estate" ("Summer Blend").

Data analysis was performed by open access VibraStat software from Elsys Corp, St. Petersburg, Russia (https://psymaker.com/downloads/tTestVibraStat.zip). It has been applied t-Student on Mt calculation by Excel function from VibraImage instant values, that means 300 values every VibraImage parameter each 60 seconds of individual test. This Mt calculation has great accuracy.

3.3. Results and discussion

According to the documentation provided by Fitomedical s.r.l., among the main components of the Summer Blend there can be found α- and β-pinene, myrcene, limonene, linalyl acetate, linalool, δ-3-carene, α-terpinene, jasmone, and cis- and trans- ocimene. These components present in the EO mixture have been proven to be responsible for the positive effects on physical and/or psychological level of the EO. For example, the terpenoids α- and β-pinene, and carene, that can be found in pine or Cupressus EO, are known to have sleep-enhancing and anxiolytic effect (Satou et al. 2014; Yang et al. 2016). Limonene in citrus enhances memory, attention and cognitive performance (Akpinar, 2005), moreover, limonene and linalool in lavender or jasmine have anxiolytic effects reducing stress levels and providing a feeling of relaxation (Carvalho-Freitas and Costa, 2002; Linck et al. 2009; Souto-Maior et al. 2011). Linalyl acetate, another main component with linalool in lavender, show a prolonged sleep-time, even in mice previously treated to be overreactive with caffein (Buchbauer et al. 1991) (**Par. 1.3.2**).

Inhalation of Summer Blend EO showed positive psychological effects, increasing the positive parameters and decreasing the negative ones (**Table 4**). All 10 behavioral parameters measured by VibraImage technology returned p-value < 0.01 by Student statistics.

	Negative behavioral parameters				Positive behavioral parameters				Physiological Parameters	
Abbr	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
M1	38,72	28,80	24,663	30,81	66,15	66,80	23,12	66,493	21,89	30,003
SD1	5,693	3,834	3,470	2,432	4,439	11,015	5,438	6,560	1,800	6,013
M2	36,28	27,44	22,732	29,024	70,30	69,254	24,15	69,771	20,695	30,152
SD2	6,417	5,647	7,306	3,081	6,039	12,798	6,750	8,216	2,219	8,359
M2-M1	-2,442	-1,357	-1,931	-1,792	4,144	2,450	1,034	3,279	-1,202	0,150
P	0,0000	0,0000	0,0000	0,0000	0,0000	0,000	0,0032	0,0000	0,0000	0,0090

Table 4: Monitoring Psychophysiological Wellbeing Results before (M1, SD1) and after (M2, SD2) inhaling Essential Oils. M= mean; SD= Standard Deviation.

Inhaling essential oils from a natural forest setting might have even superior effects on an individual's physical and mental wellbeing. This notion has been supported by a number of studies. A recent review over 20 comparative studies conducted by Rajoo and colleagues (2020), examining the physiological and psychosocial effects of forest therapy on various indicators, including blood pressure, heart rate, hormone levels, anxiety and depression symptoms, confirmed these positive effects, particularly on anxiety and depression symptoms. It must be taken in consideration however the fact that all these studies should be deeper investigated to confirm the monitored effects of forest therapy. The subjects monitored during the tests should be evaluated also later in time and not only after the forest session, in order to confirm the gained beneficial effects in the long term. Also, a multidisciplinary approach involving plant physiology, medicine, and psychology could help in understanding the relation between Forest Bathing and humans. In this view, using essential oils to simulate forest air VOCs in a controlled laboratory condition is a first step in a larger investigation.

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4. Impact of Urban Vegetation on Volatile Organic Compounds (VOCs) Variability Across Diverse Areas of Reggio Emilia, Italy

The Po Valley is one of the European regions most severely affected by air pollution. Within the spectrum of airborne molecules, Volatile Organic Compounds (VOCs) represent a significant component, derived from both natural processes and anthropogenic sources (**Par. 1.1**). All VOCs influence air quality, as they are precursors to ozone (O_3), secondary organic aerosol (SOA), and particulate matter (PM) (**Par 1.4**). While naturally occurring VOCs contribute to the formation of air pollutants, they also have beneficial effects on human health (**Par. 1.3**). Furthermore, vegetation plays a fundamental role in air purification and improvement of air quality both directly, through the metabolic processes of leaves, and indirectly, through physical mechanisms.

This study aims to evaluate the qualitative and quantitative fluctuations of VOCs in different zones within the city of Reggio Emilia (Italy), characterized by varying percentages of vegetation cover and proximity to high-traffic roads. The collected data suggest that air quality may be influenced by the spatial distribution and type of urban area, with urban parks and green zones showing lower concentrations of total VOCs (TVOCs) compared to areas with less vegetation cover. These observations can contribute to formulating strategies to improve air quality in urban areas and emphasize the importance of vegetation in an urban context.

4.1. State of the art

The geographical area known as the Po Valley, located in northern Italy, is characterized by a high population density and intense industrial and agricultural activities. These factors, associated with particular meteorological conditions which can favor the accumulation of polluting substances in the atmosphere, have contributed to making the Po Valley one of the most polluted areas in Europe. Previous studies, such as those conducted by Bigi et al. (2012), Pernigotti et al. (2012), and Thunis et al. (2009), have documented the serious air pollution situation in this area. The most widespread and worrying air pollutants include particulate matter (PM 2.5 and PM 10), tropospheric ozone (O_3), nitrogen oxides (NO_x), sulfur dioxide (SO_2), and Volatile Organic Compounds (VOCs). As demonstrated by some researchers (Bigi et al. 2012; 2016; Martilli et al. 2002; Masiol. 2014; Raffaelli et al. 2020), these pollutants have significant impacts on human health and the environment, making it essential to understand the sources and dynamics of these substances.

VOCs are a class of molecules containing carbon-hydrogen covalent bonds, often with functional groups that determine their chemical-physical behavior and reactivity. They are characterized by molecular weight generally less than 300 Da and high ability to evaporate easily at room temperature (with vapor pressure ≥ 0.01 kPa at 20 °C) and even though they have different structures they share these key characteristics (Dudareva et al. 2006; Baldwin 2010; Li et al. 2021).
VOCs can originate from natural sources (isoprene, terpenes, oxygenated compounds such as alcohols, aldehydes, and ketones) or human activities (such as aromatic hydrocarbons) (Dörter et al., 2020; Saarikoski et al., 2023).
Several factors influence the complex variability of VOCs in the air, including human activities, meteorological conditions, area topography, and seasonality (Wolkoff 1998; Song et al. 2019, Harrison et al. 2021; Li et al. 2021). Areas with limited air circulation are prone to accumulate higher amounts of air pollutants (Vogel 1995; Hang et al. 2022). Seasonality, on the other hand, influences the biological activity of plants. During warmer months, plants may increase VOC emissions, as already noted by Meneguzzo et al. (2019) and Antonelli et al. (2020).
These compounds, with their structures and chemical reactivity, can play a key role in the formation of air pollutants, such as ozone (O3), secondary organic aerosol (SOA), and particulate matter (PM), through photochemical oxidation processes (Zhan et al. 2021). VOCs such as benzene, formaldehyde, toluene, and xylene have demonstrated adverse effects on respiratory, cardiovascular, nervous, and digestive systems (Zhou et al. 2023).
However, not all VOCs are harmful to human health. Some VOCs emitted by plants, such as limonene and pinene, are associated with beneficial effects on human health, showing antioxidant, anti-inflammatory, analgesic, anxiolytic, and antidepressant activities (Antonelli et al. 2020). Others, emitted by typical Mediterranean vegetation, stimulate the immune system, enhancing the activity of natural killer (NK) cells (Li, 2010), and reducing airway inflammation (Kim et al., 2020).
The presence of vegetation in urban areas can significantly contribute to air quality, both through direct pollutant absorption and as a physical barrier that influences their dispersion (Grzędzicka, 2019). Pollutant concentrations can be reduced by up to 85% with the presence of trees near pollution sources due to their ability to reduce wind speed based on the height and porosity of the canopy (Hong et al. 2017). Vegetation can contribute to local air cooling and reduce atmospheric pollutant concentrations, as demonstrated by Novak et al. (2014), or can trap atmospheric pollutants on leaf surfaces and release them with rain, depositing them on the ground or absorbing them through stomata (Smith, 1990; Novak et al., 2006; Novak et al., 2014).
This research aims to highlight a possible correlation between the role of trees and vegetation in air quality in different areas of the city of Reggio Emilia. The town is located in a highly polluted area

like the Po Valley, and the study takes into account the contributions of factors such as VOC emissions, meteorological conditions, and vegetation cover.

4.2. Materials and Methods

4.2.1. Location of study areas

Figure 19 shows the location of the eight studied areas of the city of Reggio Emilia, Emilia Romagna region, Italy. In particular, three urban parks were chosen (Parco del Popolo - S1; Parco delle Caprette - S2; Parco Alcide Cervi - S3); two areas with a good percentage of vegetation cover but very close to key points of city traffic (S4 very close to the A1 motorway and S5 very close to the Reggio Emilia Central Station), a square in the historic center of the city in a Traffic Zone Limited (Piazza Fontanesi - S6) and two areas with reduced vegetation cover and high traffic (S7 and S8).
Table 5 provides the geographic coordinates of the eight zones under study and their elevation above sea level.

Figure 19: location of the 8 studied areas of the city of Reggio Emilia, Emilia Romagna region, Italy (https://earth.google.com/).

AREA	Latitude	Longitude	Elevation ASL
S1	44°42'05"N	10°37'48"E	53,58 m
S2	44°40'49"N	10°37'24"E	75,44 m
S3	44°41'40"N	10°37'39"E	57,36 m
S4	44°43'22"N	10°37'59"E	39,06 m
S5	44°41'44"N	10°39'11"E	47,75 m
S6	44°41'40"N	10°37'52"E	56,57 m
S7	44°41'58"N	10°38'39"E	48,64 m
S8	44°42'45"N	10°36'28"E	44,98 m

Table 5: geographic coordinates of the eight zones under study and their elevation above sea level (https://earth.google.com/).

4.2.2. Vegetation cover

In **Table 6** is reported the percentage of vegetation coverage in square meters (m2), calculated over an area of 1000 m2 using the Java-based imaging program ImageJ (Schneider et al. 2012).

For this research were selected three urban parks based on demonstrating the highest percentage of vegetation coverage. In descending order, these parks are Parco del Popolo (S1) with a coverage of 6851.1 m2, Parco delle Caprette (S2) with 6255.3 m2, and Parco Alcide Cervi (S3) with 5768.2 m2. These areas share some plant species, including the Cedar of Lebanon, Atlas Cedar, and broad-leaved trees such as poplar, lime, and ash.

Subsequently, in descending order of vegetation coverage, S4 is the next zone with a coverage of 5203.4 m2, characterized by white and black poplars. Following that is zone S5 with 4820.6 m2 of coverage, and S6 (Piazza Fontanesi) with 3395.2 m2, which is exclusively dominated by lime trees.

Two areas with a lower percentage of vegetation coverage and high traffic were also analyzed, namely S7 with 1363.6 m2 and S8 with 1264.3 m2. In these zones, vegetation mainly consists of broad-leaved trees and is devoid of conifers.

AREA	VEGETATION COVER (m^2)	TREE SPECIES
S1 (Parco del Popolo, RE, IT)	6851,1	*Aesculus hippocastanum L.* *Carpinus betulus L.* *Cedrus deodora* *Cedrus libani* *Fraxinus excelsior L.* *Picea abies L.* *Pinus nigra* *Platanus occidentalis L.* *Styphnolobium japonicum L.*
S2 (Parco delle Capette, RE, IT)	6255,3	*Acer campestre* *Aliantus altissima Mill.* *Carpinus betulus L.* *Cedrus deodora* *Cedrus libani* *Celtis australis L.* *Morus alba L.* *Populus alba L.* *Quercus robur L.* *Robinia pseudoacacia* *Tilia cordata Mill.*
S3 (Parco Alcide Cervi, RE, IT)	5768,2	*Acer negundo L.* *Acer pseudoplatanus L.* *Cedrus deodora* *Fraxinus excelsior L.* *Juglans nigra L.* *Magnolia grandiflora L.* *Morus nigra L.* *Populus alba L.* *Prunus laurocerasus L.* *Quercus robur L.* *Taxus baccata L.* *Tilia cordata Mill.*
S4	5203,4	*Populus alba L.* *Populus nigra L.*
S5	4820,6	*Quercus robur L.* *Carpinus betulus L.* *Fraxinus excelsior L.* *Acer campestre* *Prunus avium L.* *Juglans regia L.* *Populus nigra L.* *Prunus spinosa L*
S6 (Piazza Fontanesi, RE, IT)	3395,2	*Tilia cordata Mill.*
S7	1363,6	*Acer campestre L.* *Cenchrus setaceus* *Platanus occidentalis* L. *Populus alba L.* *Populus nigra L.* *Pyrus cordata Desv.* *Salvia yangii B.T. Drew*
S8	1264,3	*Celtis australis* L. *Populus alba* L. *Robinia pseudoacacia* L.

Table 6: percentage of vegetation coverage in m2 calculated over an area of 1000 m2 (S = study site) and tree species present at the sampling sites. (Areas are listed in descending order of vegetation coverage).

4.2.3. Sampling

The surveys were conducted in the autumn season of 2022 (September-November) at fifteen-day intervals. Table 7 presents the meteorological data for each of the sampling days (Source: weather data recorded by the regional monitoring network RIRER managed by Arpae-Simc).

	Daily average air temperature at 2 meters above ground level (°C)	Daily average relative humidity at 2 meters above ground level (%)	Instantaneous atmospheric pressure at station level at 2 meters above ground at 11:00 AM (Pa)	Daily average scalar wind speed at 10 meters above ground level (m/s)	Daily prevailing wind direction at 10 meters above ground level (Degree True)	Daily cumulative precipitation (Kg/m**2) (equivalent to mm)
09/09/2022	22.90	67	100350.0	1.4	180	0.2
23/09/2022	16.66	48	101230.0	0.9	45	0.0
07/10/2022	19.05	68	101700.0	1.2	225	0.0
21/10/2022	15.82	90	101080.0	1.0	90	0.0
04/04/2022	14.40	66	99600.0	1.9	270	3.8
18/04/2022	12.01	82	99300.0	1.4	270	0.0

Table 7: weather data recorded by the regional monitoring network RIRER managed by Arpae-Simc (https://simc.arpae.it/dext3r/).

The quantitative analysis was conducted using the TIGER LT instrument (Ion Science-Italia, BO, IT). The chemical characterization of VOCs was performed using GC-MS (Agilent Technologies, Inc) on carboxen/polydimethylsiloxane (Supelco, Bellefonte, PA; Carboxen/PDMS, coating thickness of 75 µm) SPME fibers. The air collected at the sampling sites was stored in specific Tedlar Bags (CEL Scientific) designated for this purpose and analyzed using the Tiger LT detector.

VOC present in the air sample were detected by GC–MS using the solid-phase microextraction (SPME) sampling technique. Volatile compounds occurring in the air collected in specific Tedlar Bags were analyzed using a 1 cm needle containing a fiber coated with 75 µm Carboxen/polydimethylsiloxane bonded to a flexible fused silica core (Supelco, Sigma–Aldrich Co, USA). The needle was inserted into the bag through the septum and the fiber was exposed to volatiles for 10 minutes at room temperature. Afterward, the needle was inserted into the injector port and the fiber was exposed for 5 min. GC–MS analyses were performed on a Agilent (USA) 7890A- MSD 5977B gas chromatograph–mass spectrometer equipped with a HP-5 column (25 m x 0.2 mm, 0.5 µm film thickness) coated with (5%)-diphenyl-(95%)-dimethylpolysiloxane copolymer. VOC were identified by comparing their respective mass fragmentation patterns (EI, 70 eV) with the database library NIST11 (MS Library Software Varian, USA). Temperature program: 40°C, hold for 4 min, 10°C/min to 260°C, hold for 4 min, Detector 270 °C, Injector 270 °C.

4.3. Results

Figure 20 illustrates the trend of VOC measurements in ppb for each of the areas considered in this study. The x-axis represents the number of measurements taken with the Tiger over time (10 minutes of monitoring), and the y-axis represents VOC levels (ppb). Each color represents a sampling day (blue: September 9; orange: September 23; gray: October 7; yellow: October 21; light blue: November 4; green: November 18).

Below is a summary of the variations in VOC levels in different areas:

- In **S1**, levels fluctuate between 0 and 50 ppb in both September and October, while increasing significantly in November, reaching 200-300 ppb.
- In **S2**, VOC levels vary between 0 and 100 ppb on all sampling days.
- In **S3**, VOC variations range between 0 and 50/70 ppb.
- In **S4**, significant oscillations are observed, with levels exceeding 1000 ppb on the two days in October (gray and yellow) and on November 18 (green).
- In **S5**, fluctuations remain below 100 ppb, with peaks at 220 ppb on October 7 and 390 ppb on November 4. October 21 and November 18 show similar patterns.
- In **S6**, VOC levels exceed 100 ppb only briefly on November 18.
- In **S7**, oscillations range between 0-100 ppb, with a peak of 200 ppb on November 18.
- In **S8**, levels fluctuate between 100 and 200 ppb, with notably high peaks (1000 ppb) on November 4.

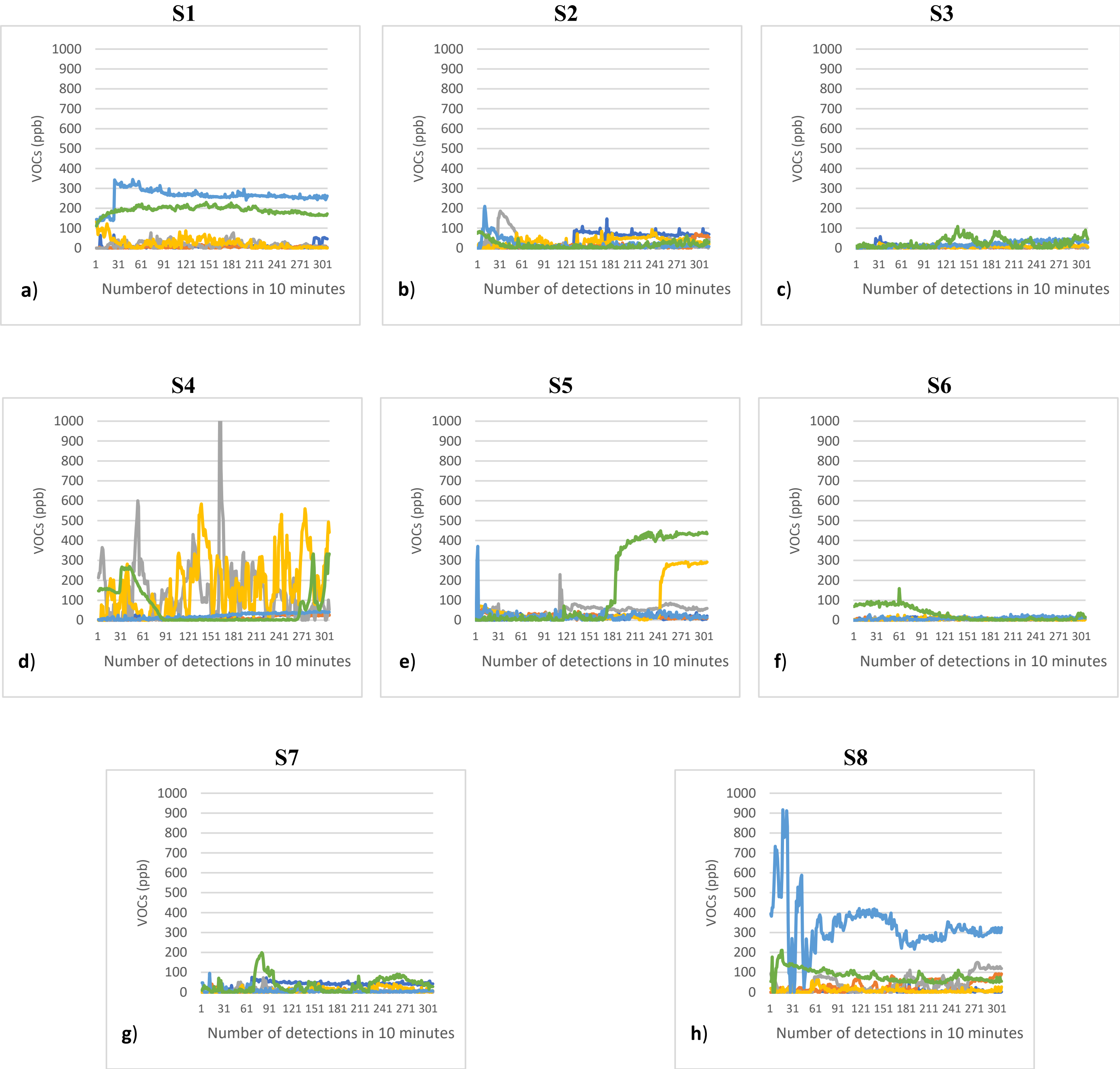


Figure 20: Graphs indicating the variations of VOCs over time for the specified areas. The x-axis represents the number of measurements taken with the Tiger over time (10 minutes of monitoring), and the y-axis represents VOC levels (ppb).

Figure 21 shows VOC concentrations in the 8 urban areas of the city of Reggio Emilia (Italy) recorded in September 2022. On the first day of sampling, September 9th (**Figure 21a**), there was notable variability in VOC concentrations among the monitored zones, with a significant difference between the averages of S2 and S7. S2 showed an average concentration of 57 ppb, while S7 had an average of 45 ppb.

From the analysis of the chemical composition of VOCs on September 9th (**Figure 21b**), it is noted that in the three urban parks (S1, S2, and S3), over 70% of VOCs consist of natural molecules like isoprene, monoterpenes, sesquiterpenes, acids, aldehydes, ketones, esters, and alcohols. In contrast, in other areas, the percentage of artificial molecules , like hydrocarbons, has significantly increased. On September 23rd (**Figure 21c**), there was a significant decrease in VOC concentrations in S2, with an average of 35 ppb. Despite this reduction, S2 remains significantly different from other zones, except for S8, which has a VOC average value similar to S2 (39 ppb) and is significant compared to several other zones.

During this sampling, the qualitative analysis (**Figure 21d**) showed a lower presence of hydrocarbons in urban parks (S1, S2, and S3) and a significant increase in areas with less vegetation cover (S4-S8).

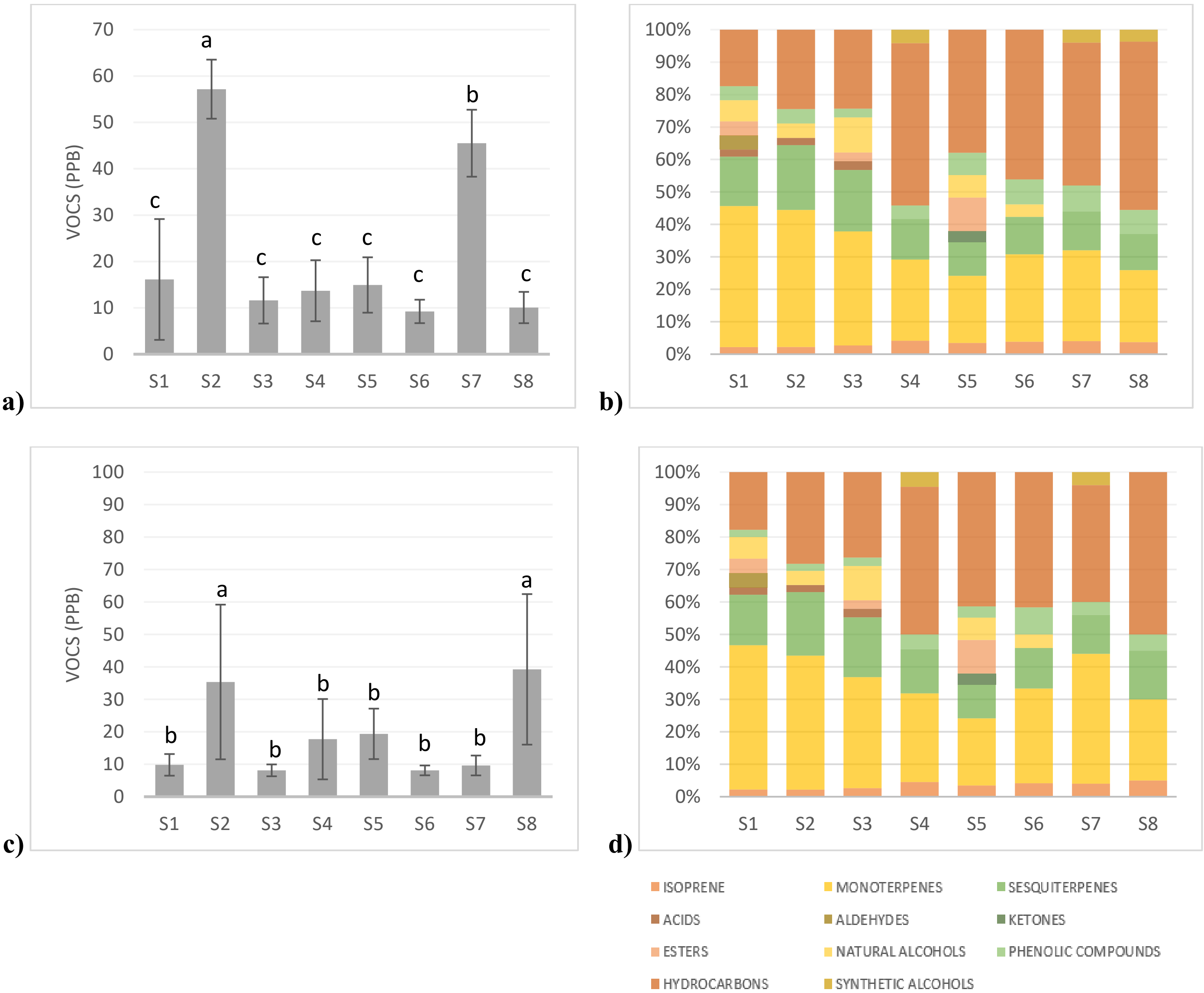
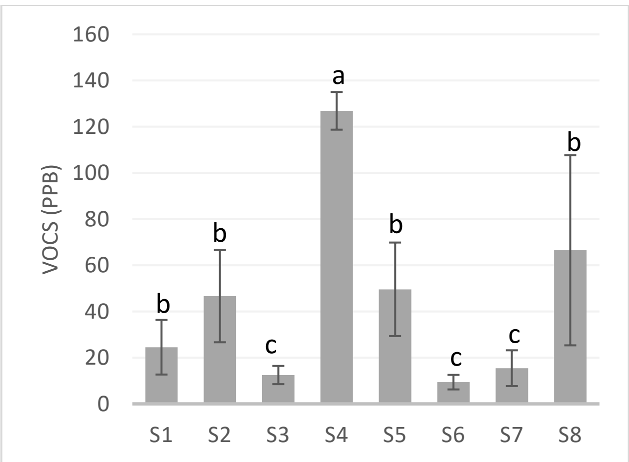


Figure 21: VOCs concentrations in the 8 urban areas of the city of Reggio Emilia (IT) recorded in September 2022. a and c) histograms of the quantitative averages of measured VOCs in ppb, detected with the Tiger on September 9th (a) and September 23rd (c). b) and d) graphs representing the percentage of different classes of molecules detected in GC/MS on September 9th (b) and September 23rd (d).

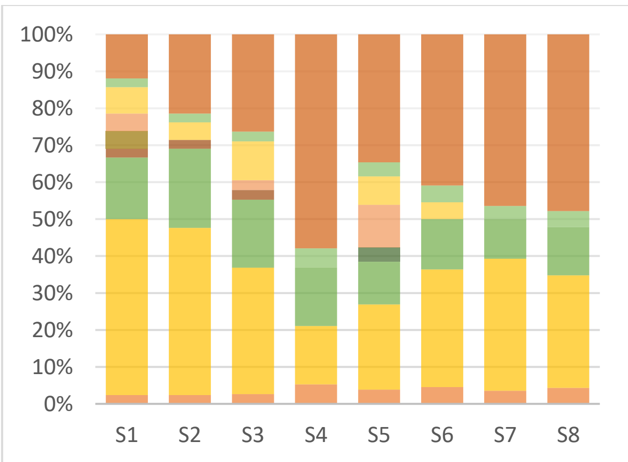
The analysis of the data collected in October (**Figure 22**) provides additional details about the dynamics of VOC concentrations. **Figures 22a** and **22c** highlight an increasing trend in VOC concentrations compared to September monitoring.

At the beginning of October (October 7th), the average VOC concentrations were higher than in the two previous monitoring periods. In particular, in S4, an average value of 126 ppb was observed, which was statistically higher compared to all other zones (**Figure 22a**). This result is relevant, considering that 57% of this concentration comes from hydrocarbons, suggesting a notable contribution of anthropogenic compounds. In line with previous days, urban parks, especially S1 (Parco del Popolo), had lower concentrations of hydrocarbons, with 50% of VOCs being monoterpenes and only 11% being hydrocarbons (**Figure 22b**).

At the end of October (day 21), a trend similar to the beginning of the month is observed, with a significantly higher average VOC concentration in S4, reaching 191 ppb (**Figure 22c**). In this case as well, 50% of this concentration comprises hydrocarbons, confirming the substantial contribution of anthropogenic compounds in this area. While significant differences in total VOC concentrations were observed for the other zones, these concentrations never exceeded 100 ppb (**Figure 22d**).



a)



b)

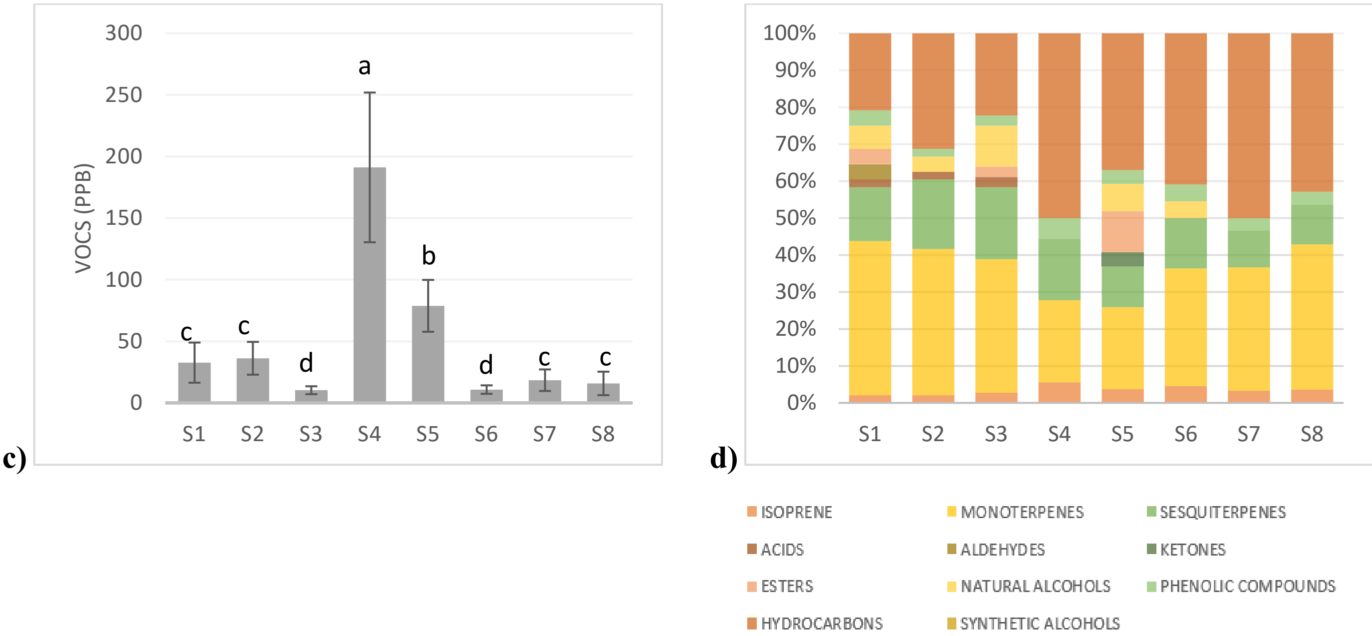


Figure 22: VOCs concentrations in the 8 urban areas of the city of Reggio Emilia (IT) recorded in October 2022. a and c) histograms of the quantitative averages of measured VOCs in ppb, detected with the Tiger on October 7th (a) and October 21st (c). b) and d) graphs representing the percentage of different classes of molecules detected in GC/MS on October 7th (b) and October 21st (d).

In **Figure 23**, the data related to samplings conducted in November are reported. The concentrations detected at the beginning of November (November 4th) and at the end (November 27th) differ significantly from the data in September (**Figure 21**) and October **(Figure 22**).

At the beginning of the month, a notable variation in VOC concentrations is observed among the monitored urban zones. The highest concentrations were observed in S1 with 260 ppb and in S8 with 329 ppb (**Figure 23a**). Qualitative analysis of VOCs reveals significant differences in chemical composition between the two zones. In S1, 80% of the molecules are of natural origin, while in S8, 50% of the molecules are anthropogenic, including hydrocarbons and synthetic alcohols (**Figure 23b**).

At the end of November (November 27th), total VOC concentrations are even higher than in the previous monitoring periods. In particular, in S5, a VOC concentration of 279 ppb is observed, which is statistically significant compared to all other zones. Also, in S1 (192 ppb) and S4 (140 ppb), VOCs are elevated and statistically significant compared to other average values. These results confirm the importance of vegetation in reducing TVOC concentrations, with S5 and S1 showing lower concentrations compared to other zones (**Figure 23c**).

The qualitative trend of VOCs (**Figure 23b** and **23d)** appears to be similar to what was observed in September **(Figure 21**) and October (**Figure 22**). Urban parks (S1, S2, and S3) continue to show low levels of hydrocarbons, especially S2, where hydrocarbons constitute only 8% of the detected molecules. On the other hand, in areas with less tree coverage (S4-S8), an increase in the presence of hydrocarbons is observed (**Figure 23d**).

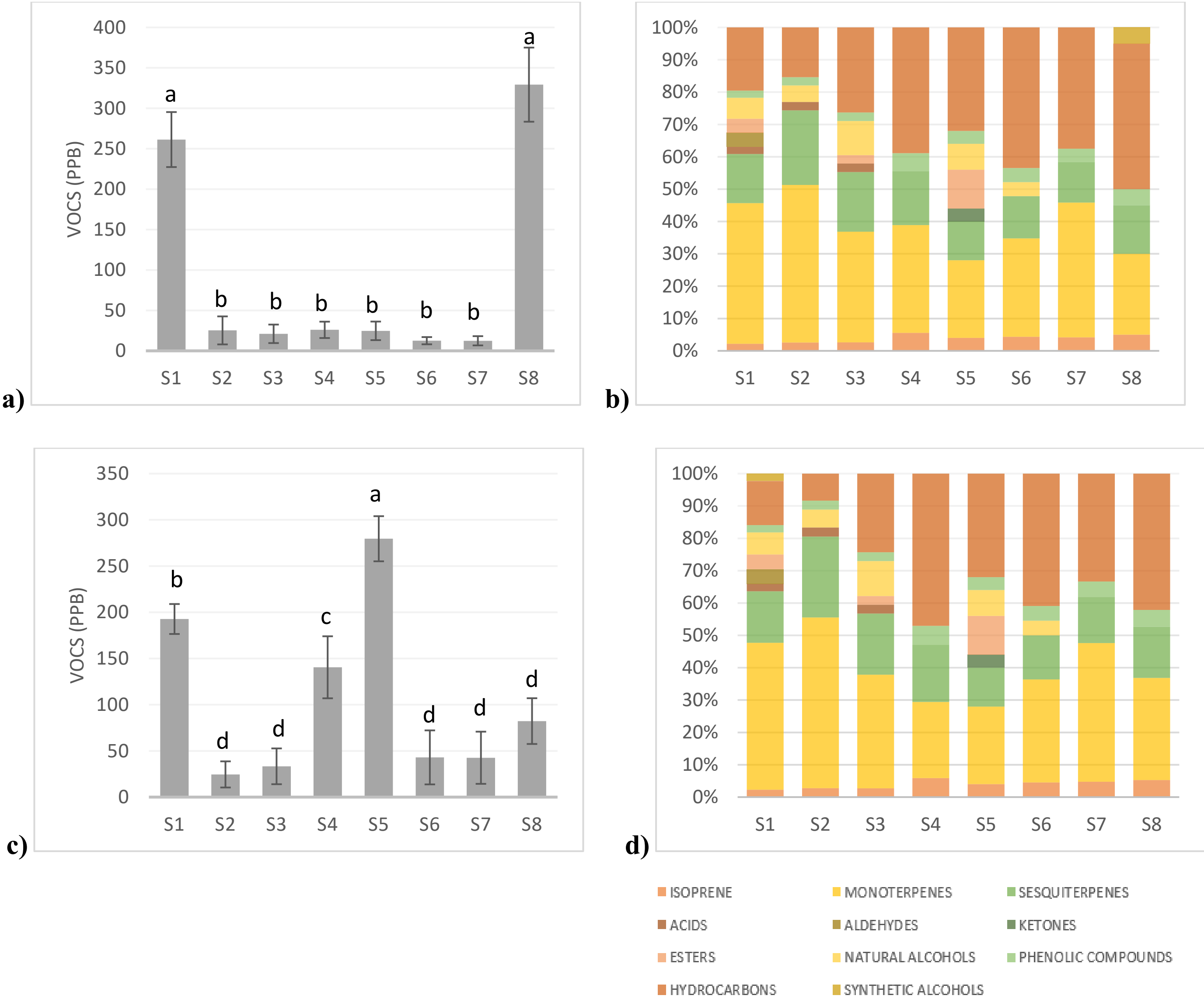


Figure 23: VOCs concentrations in the 8 urban areas of the city of Reggio Emilia (IT) recorded in November 2022. a and c) histograms of the quantitative averages of measured VOCs in ppb, detected with the Tiger on November 4th (a) and November 27th (c). b) and d) graphs representing the percentage of different classes of molecules detected in GC/MS on November 4th (b) and November 27th (d).

The analysis of Volatile Organic Compounds (VOCs) in Reggio Emilia's urban areas reveals distinct patterns across zones. Notably, urban parks consistently exhibit lower percentages of anthropogenic molecules, underscoring the role of vegetation in reducing pollutants. The data from October and November demonstrate increasing VOC concentrations, with vegetation-rich areas maintaining lower levels compared to less vegetated zones. The findings highlight the dynamic interplay between vegetation, urban characteristics, and VOC composition.

4.4. Discussion

The correlation between vegetation coverage, volatile organic compounds (VOCs), and atmospheric conditions in urban air quality is a topic of growing interest in scientific literature (Cai et al. 2021; Wang et al. 2021; Duan et al. 2023). This topic is particularly relevant in a global context where an increasing number of people live in urban areas, and air pollution poses a significant threat to public health and the environment. In this study, eight different zones in the city of Reggio Emilia, located in the Emilia Romagna region, Italy, were considered to attempt to extrapolate elements for assessing the complex interaction between vegetation coverage, VOC emissions, and local atmospheric dynamics. The selection of study zones was guided by various considerations, including vegetation coverage, proximity to critical points of city traffic, and geographical characteristics. The eight zones considered are as follows:

- S1 - Parco del Popolo
- S2 - Parco delle Caprette
- S3 - Parco Alcide Cervi
- S4 – Area near the A1 motorway
- S5 – Area near the Central Station of Reggio Emilia
- S6 - Piazza Fontanesi, in the historical center, in a Limited Traffic Zone
- S7 – Area with reduced vegetation coverage and high traffic
- S8 – Area with reduced vegetation coverage and high traffic

The selection of these zones reflects the goal of investigating a diverse range of environmental conditions and vegetation coverage, allowing for a more comprehensive analysis of the effects of these variables on air VOC concentrations. Before delving into data analysis, it is crucial to carefully consider the concept of "vegetation coverage." Often, this coverage is measured in terms of the land area occupied by plants or trees. However, this definition may not fully account for the importance of plant species diversity and health. The ability to absorb atmospheric pollutants can vary significantly depending on these factors. For instance, plants like greater duckweed (*Spirodela polyrhiza*) and *Blumea malcolmii* can break down complex organic pollutants into simpler molecules through the action of hydrolytic enzymes and metabolites (Kagalkar et al. 2011; Kristanti et al. 2012; Singh et al. 2019). Regarding particulate matter (PM), some studies show that among different types of forests, coniferous forests tend to have a higher capacity for PM collection compared to broad-leaved forests (Zhang et al. 2017). This ability is linked to the intricate leaf structures of conifers (Freer-Smith et al. 2005; Fares et al. 2020). Additionally, certain woody species like *Populus nigra* L. and *Camellia sasanqua* absorb and effective disperse ozone and organic pollutants, such as methyl ethyl ketone, primarily through stomatal action (Omasa et al. 200). Furthermore, it is crucial to assess the quality of green area management, including factors like irrigation and maintenance, as these can significantly influence the effectiveness of vegetation in reducing atmospheric pollutants (Escobedo et al. 2011). Large, healthy trees, with diameters exceeding 77 cm, remove approximately 70 times more air pollution per year (1.4 kg/year) compared to small trees with diameters less than 8 cm (0.02 kg/year) (Novak 2002). In a study by Endreny et al. (2017) the benefits of tree canopy cover for reducing air pollution and carbon emissions in London, UK, where highlighted, emphasizing the importance of nature conservation in cities, which can contribute to human well-being.

The collected data reveal significant variations in VOC levels among the monitored zones. In urban parks (S1, S2, and S3), VOC concentrations remain relatively contained during September (**Figure 21**) and October (**Figure 22**), with a predominance of molecules of natural origin. However, November (**Figure 23**) shows a notable increase in VOC concentrations in some zones, particularly in S1 and S8. This variation. underscores the importance of vegetation coverage in mitigating VOC concentrations, highlighting a direct correlation between vegetation presence and detected pollutant levels. A crucial aspect to consider is the chemical composition of VOCs. Throughout the study, it has become evident that in urban parks, the majority of VOCs consist of molecules of natural origin, such as isoprene, monoterpenes, and other biogenic compounds. In contrast, in urban areas with limited vegetation coverage, the percentage of anthropogenic molecules, such as hydrocarbons, significantly increases. These results suggest that the type of urban area plays a key role in determining the chemical composition of VOCs, with a higher presence of anthropogenic compounds in less green areas.

The data analysis of October **(Figure 22**) highlighted a general increase in VOC concentrations compared to September (**Figure 21**), with S4 showing the highest concentrations, primarily due to hydrocarbons. At the end of October, a similar trend occurred, confirming the significant contribution of anthropogenic compounds in this zone. It is important to note that, despite the variations, VOC concentrations in all zones remain relatively contained, except for S4.

The analysis of the data collected in November (**Figure 23**) provides further food for thought. At the beginning of the month, a notable variation in VOC concentrations is observed among the different monitored zones (Figure 5a and 5b). The highest concentrations were observed in S1 and S8, corresponding to the zones with the highest and lowest vegetation coverage, respectively. At the end of November, VOC concentrations are even higher, confirming the importance of vegetation in mitigating such concentrations. In terms of chemical composition, the qualitative trend of VOCs appears to be similar to what was observed in September (**Figure 21**) and October (**Figure 22**). Urban parks (S1, S2, and S3) continue to show low levels of hydrocarbons, while in urban zones with less tree coverage, an increase in the presence of hydrocarbons is observed. The November results confirm the importance of vegetation in mitigating VOC concentrations and suggest a direct correlation between vegetation coverage and VOC levels. Areas with greater vegetation coverage show lower concentrations, while zones with reduced vegetation coverage highlight higher levels, especially of anthropogenic molecules. These results underscore the crucial role of vegetation as an effective strategy in managing air quality in urban areas, with significant benefits for the environment and public health.

4.4.1. Relationship between anthropogenic molecules and percentage of vegetation coverage

The relationship between the percentages of anthropogenic molecules present in different study zones and the percentage of vegetation coverage is a key element in understanding how vegetation influences the chemical composition of urban air. The analysis of this relationship, as shown in **Figure 24**, reveals a series of important trends that repeat on all sampling days.

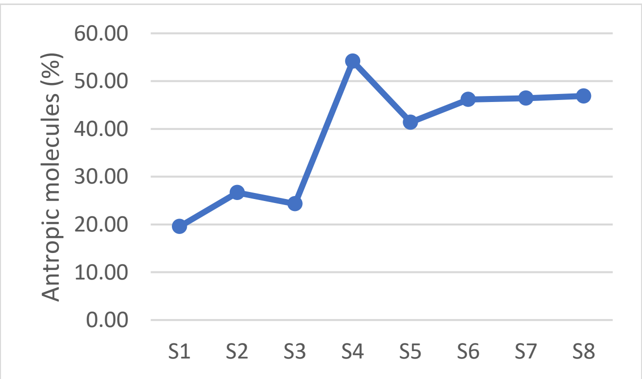
In particular, urban parks (S1, S2, and S3) with higher vegetation coverage exhibit relatively low percentages of anthropogenic molecules, never exceeding 30% of the molecules present. These parks represent areas where vegetation significantly contributes to reducing the presence of anthropogenic compounds, such as hydrocarbons.

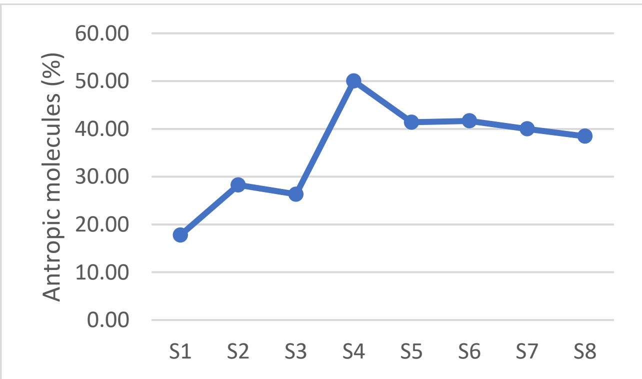
Conversely, in urban areas with less abundant vegetation coverage, the percentage of anthropogenic molecules, especially hydrocarbons, tends to increase. This is particularly evident in zone S4, where the highest concentrations of anthropogenic molecules are observed (54% on day 1, 50% on day 2, 50% on day 3, and 42% on day 6). These data indicate that the decrease in vegetation coverage correlates positively with the increase in anthropogenic molecules, reflecting the influence of human activities and the lack of vegetation in raising VOC concentrations.

Furthermore, interesting observations are made regarding zones S7 (day 4) and S6 (day 5), which also show high percentages of anthropogenic molecules (50% in S7 and 43% in S6). These results suggest that, even though these are not urban parks, the presence of significant vegetation coverage can contribute to limiting the presence of anthropogenic molecules, although not to the level of greener parks.

This analysis reinforces the importance of vegetation in urban areas in mitigating the presence of anthropogenic molecules, especially hydrocarbons. It also reinforces the idea that the presence of vegetation in urban areas can be an effective strategy in managing air quality and reducing pollutant concentrations. In recent years, phytoremediation has garnered immense interest from researchers, not only for soil reclamation but also for the removal of various types of atmospheric pollutants such as PM, VOCs, inorganic pollutants (CO2, SO2, NO2, O3), and heavy metals. This system, in addition to being economically advantageous, also enhances the aesthetic value of cities (Morikawa et al. 2003; Lee et al. 2020). In particular, the absorption of VOCs occurs through leaf stomata, as well as through the cuticle, and with their diffusion in intercellular spaces. They react with the water film covering the inner surfaces of the leaves, forming acids that will be transported and stored in various plant organs through the phloem (Pandey et al. 2019; Lee et al. 2020).

It should be noted that, despite recent studies highlighting the involvement of VOCs released by plants in the formation of atmospheric pollutants, the benefits and ecosystem services offered by urban greenery far outweigh the drawbacks. These benefits include the removal of atmospheric pollutants and carbon sequestration, noise reduction, ecosystem services related to microclimates, and benefits for human health, such as stress reduction, mood enhancement, and the reduction of respiratory-related issues (Roy et al. 2012). Additionally, the use of phytoremediation, a system that utilizes plants for soil reclamation and the removal of atmospheric pollutants such as PM, VOCs, inorganic pollutants, and heavy metals, offers an effective and economical solution for urban environmental management (Novak et al. 2014; Endreny et al. 2017).





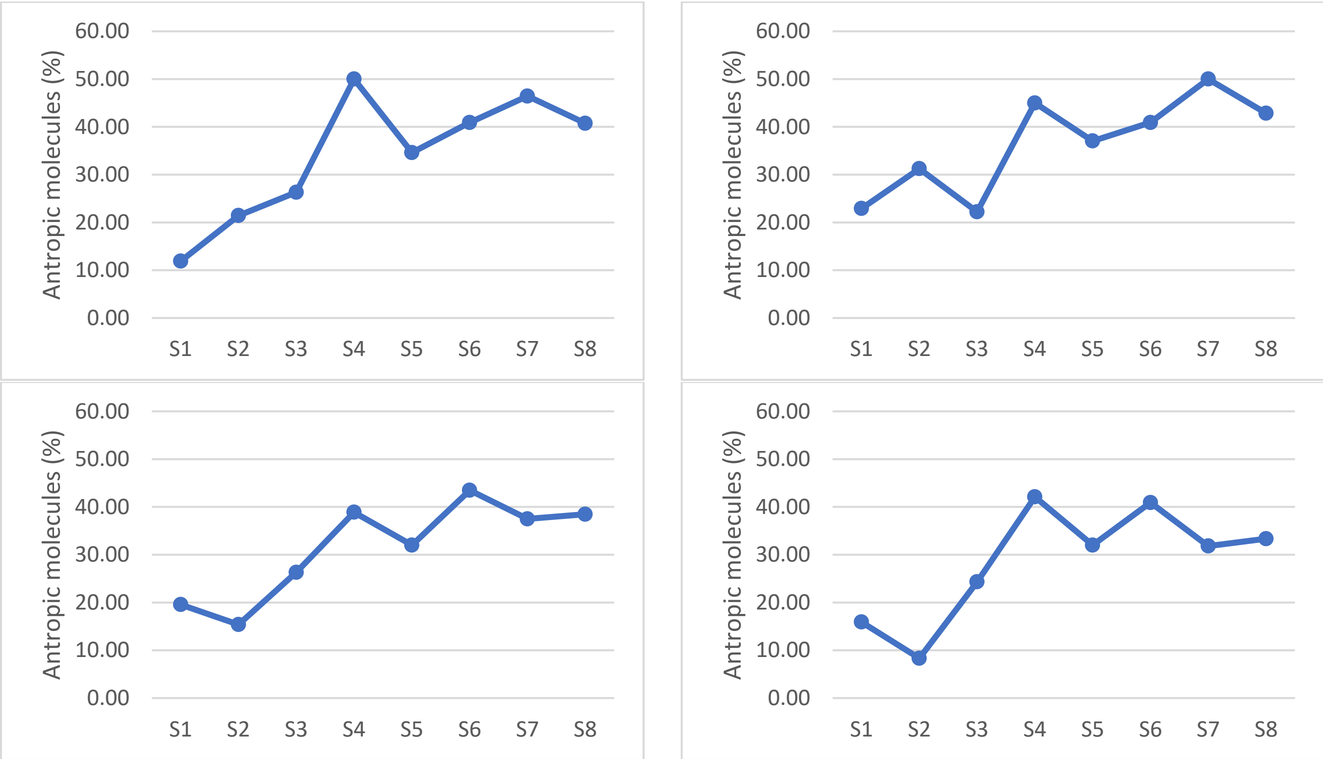


Figure 24: percentage of anthropogenic molecules (on the ordinate axis) present in each studied area in relation to the percentage of vegetative cover (areas ordered decreasingly from left to right). a)Day 1; b) Day 2; c) Day 3; d) Day 4; e) Day 5; f) Day 6.

4.5. Main conclusions

This study provides a comprehensive overview of the correlation between vegetative cover, concentrations of volatile organic compounds (VOCs), and atmospheric conditions in urban air. The results indicate that vegetation plays a crucial role in reducing concentrations of air pollutants, particularly anthropogenic VOCs. This underscores the need to consider vegetation as an essential component in urban planning and air quality management. The presence of green areas in cities not only contributes to air purification but also provides a range of benefits for the environment, human health, and the overall well-being of urban communities. Future research should continue to explore this complex relationship between vegetation and air pollution to develop sustainable management strategies for the cities of the future.

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5. General Conclusions

In plants, Volatile Organic Compounds (VOCs) play various important roles in interactions with the environment and organisms inhabiting it, including pollinators, herbivores, and both beneficial and pathogenic microorganisms. Specifically, VOCs are involved in plant communication, acting as a kind of "chemical language" enabling them to coordinate responses to environmental stimuli and stressful situations.

Studying this form of communication is therefore essential to understand the dynamics of plant communities and symbiotic relationships between plants and other organisms. From an applied perspective, understanding communication through VOCs has significant practical implications. In agriculture, for example, it could enable the development of more effective strategies for crop protection, reducing the need for harmful chemical pesticides. Furthermore, exploring this intricate chemical language could pave the way for selecting plants that are more resilient to adverse environmental conditions.

Part of this research work has been focused on the field of Plant Acoustics. The importance of studying the effects of sound on plants lies both in basic research, to better delineate the dynamics of bioacoustic interactions in the plant kingdom, and in applied research, for instance, in optimizing agricultural techniques and promoting plant growth through targeted sound stimuli. A specific experimental focus has been dedicated to studying variations in the production of secondary metabolites, particularly changes in VOC emissions, and various physiological parameters in response to acoustic stimuli. Through quantitative and qualitative analyses, a shift in the relative abundances of VOCs in essential oils in aromatic plants has been highlighted, suggesting a communication mechanism through these compounds.

The large quantity of volatile compounds emitted by plants in an urban park or forest can be a source of well-being for humans, thanks to their proven positive effects on physical and mental health. Particularly in the context of Shinrin-yoku or "Forest Bathing," psychophysiological responses to olfactory stimulation induced by essential oils, a natural source of VOCs, have been evaluated. The obtained results have shown a significant increase in positive behavioral parameters and a simultaneous decrease in negative ones, contributing to an overall improvement in the well-being of the subjects.

Studying these effects is of great importance for several reasons. Firstly, understanding how natural odors influence human health can pave the way for innovative and non-pharmacological therapeutic approaches to manage conditions such as anxiety and stress. Additionally, this research provides a scientific basis to support the design of urban environments that integrate natural elements, thereby contributing to improving the quality of life in cities. Understanding the types and effects of breathable VOCs in urban areas is essential for evaluating and mitigating the negative impacts on daily breathable air. The relevance of studying VOCs in urban settings is underscored by the fact that many of these substances can contribute to the formation of air pollutants, such as tropospheric ozone and fine particles, known for their harmful effects on respiratory and cardiovascular health.

In this context, a possible correlation between the role of trees and vegetation in air quality in different areas of the city of Reggio Emilia, located in the highly polluted Po Valley, was studied. Factors such as VOC emissions, meteorological conditions, and the extent of vegetation cover were taken into account. Spatial analysis of qualitative and quantitative variations in VOCs highlighted that areas with greater vegetation cover, such as urban parks, have lower total concentrations of VOCs compared to those with less vegetation. This type of study could provide the basis for developing sustainable strategies aimed at preserving both the environment and citizens' health.

In conclusion, the study of communication in the plant kingdom through volatile organic compounds is crucial for expanding our understanding of the plant world and opens the door to new perspectives for environmental and agricultural management. This research not only enriches our scientific knowledge but also offers concrete opportunities to develop practices and technologies that promote sustainability and harmonious coexistence among plants and the other organisms inhabiting the Earth.