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Article

Exposure to Forest Air Monoterpenes and Pulmonary Function Tests in Adolescents With Asthma: A Cohort Study

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Abstract: Increasing evidence supports the direct healing effects of forests, partly attributed to the exposure to plant-emitted monoterpenes available in the forest atmosphere. The potential benefits on respiratory functions from inhaling monoterpenes have gained attention, especially due to the global rise in respiratory diseases. This study involved 42 asthmatic adolescents attending a summer rehabilitation camp at an Altitude Pediatric Asthma Center within a densely forested area in the Eastern Italian Alps. Volatile organic compound measurements indicated a pristine atmosphere, enabling modeling of continuous hourly monoterpenes concentration. Monoterpene concentration exposure and total inhaled dose were assessed over a 14-day stay, during which spirometry, lung oscillometry, and fractional exhaled nitric oxide were measured. Statistically significant correlations were observed between modifications in lung function parameters among asthmatic adolescents and monoterpenes exposure. These findings suggest a potential localized airway effect specific to monoterpenes. This pilot cohort study might pave the way for further investigations into the therapeutic effects of forest monoterpenes on lung function tests, asthma, and the broader healing potential of forest environments.

Keywords: asthma; forest atmosphere; monoterpenes; respiratory functions; volatile organic compounds

1. Introduction

The exposure to forest environments has been associated with distinct and significant benefits for human health and well-being, encompassing both psychological and physiological aspects [1,2]. Among the short-term effects linked to forest exposure, evidence has been gathered regarding immunological parameters [3], and inflammation-related factors [4,5]. Additionally, meta-analyses have reported positive impacts of interventions based in forest settings on the cardiovascular system [6].

Among various contributing factors, the effects of monoterpenes (MTs) emitted by plants and forest soil and available in the forest atmosphere have been attributed a noteworthy role in enhancing the psycho-physiological health [7–11]. Presently, our understanding of the health properties of MTs largely derives from in vitro studies, animal models, or indoor/laboratory research, often with limited sample sizes and concentrations much higher than those typically found in the forest atmosphere [12]. Recently, a few studies have explored the significant short-term psycho-physiological effects of individual exposure to plant-emitted MTs, and, in particular, a recent study identified a specific, significant, and dose-dependent effect of exposure to MTs in actual forest environments on the reduction of anxiety symptoms, based on a representative cohort of hundreds of participants in standardized forest therapy sessions [13]. In the latter study, it was suggested, in line with widely accepted evidence, that the improvement in anxiety symptoms might be associated to enhanced cardiovascular health. These findings confirmed preclinical evidence from animal studies, which indicated an anxiolytic activity associated with the monoterpene α -pinene [14–17].

The upper respiratory tract serves as the entry point and main pathway for inhaled MTs during immersion in the forest atmosphere. It has been hypothesized that MTs enter the bloodstream through the nose and lung mucosa, affecting olfactory receptors and crossing the blood-brain barrier [18]. However, studies examining the pharmacokinetics and detailed mechanisms of inhaled MTs' action are scarce [19].

Potential benefits of MTs for respiratory functions have gained increased attention, especially considering the widespread prevalence of respiratory diseases [2]. Among these diseases, asthma affects more than 300 million individuals worldwide [20], and remains the most common chronic disease in childhood, showing an increasing trend in the recent decades. Asthma is a complex condition resulting from the interplay of genetic, environmental, and lifestyle factors contributing to its development and severity. Exposure to air pollutants (CO, NO₂, O₃, SO₂, ultrafine particulate matter, and volatile organic pollutants) can exacerbate respiratory health issues [21–24]. Exposure to outdoor air pollution was shown to potentially worsen pre-existing asthma, and numerous studies indicated its role in the onset of asthma exacerbations [25].

The anti-inflammatory activity of a few MTs has been established [5,26,27], and positive effects of certain MTs on asthma symptoms have been demonstrated in both animal models and preclinical studies [28–30]. Oral administration of eucalyptol (1,8-cineole) for 6 months has been associated with significant improvements in lung function, asthma symptoms and quality of life in asthmatic patients, as compared to control groups [31].

The beneficial effects of staying in mountainous areas on asthma symptoms are well-documented, and Alpine Altitude Climate Treatment (AACT) has been utilized for over a century for the treatment of asthma [32]. A recent study reaffirmed the positive impact of staying in a mountain environment on respiratory functions in asthmatic children, assessed through spirometry and oscillometer tests [33]. While the underlying mechanisms remain partially understood, air characteristics related to mountain altitude (such as air density, temperature, and relative humidity), as well as reduced exposure to allergens and air pollutants, have been proposed as key factors contributing to the benefits associated with the altitude environment. Factors related to the forest environment, directly linked to forest coverage, may also play a role in positive outcomes for asthma patients. Nevertheless, conclusive associations between improvements in pulmonary function and asthma symptoms, and specific features of the mountain and forest environments, have yet to be thoroughly investigated.

The objective of this study is to investigate whether the inhalation of MTs available in the atmosphere of a mountain forest can positively impact lung function in asthmatic adolescents during a stay in an altitude rehabilitation center.

2. Materials and Methods

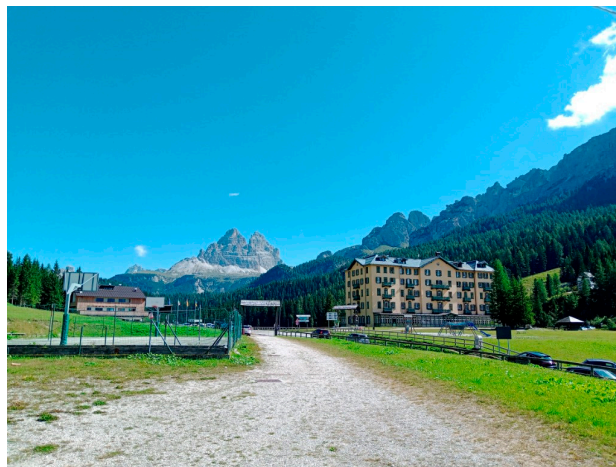
2.1. Study Area

The experimental site was the Pio XII Institute, an Altitude Pediatric Asthma Center, located in northeastern Italy, in a densely forested area in the Eastern Italian Alps, at about 1,800 m a.s.l., around the natural alpine Lake Misurina (46°34'41"N; 12°15'08"E).

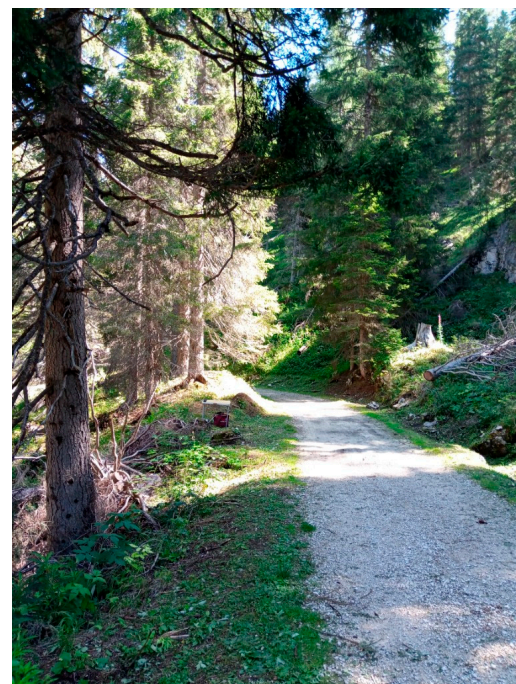
Lake Misurina, located in a protected area, precisely a Site of European Community Importance (SIC), denoted as SIC IT3230019, lies in a very scenic valley, surrounded by dolomitic peaks exceeding 3,000 m a.s.l. Fed by streams, the lake is surrounded by the arboreal vegetation of the alpine forest, which, in the eastern area, reaches its banks. Dominant species are spruce (*Picea abies*), larch (*Larix decidua*), and stone pine (*Pinus cembra*), with scattered specimens of silver fir (*Abies alba*), and a few specimens of mountain pines (*Pinus mugus*).

The main forest area is located to the east of the lake, extends in height up to more than 2,000 m a.s.l., while it continues seamlessly down the valley for few tenths of km, often changing composition and merging with the biogenetic nature reserve of Somadida, and further down in large areas of cultivated forest. Locally, it is a relatively young forest, with maximum diameter measured by the authors across 50 trees belonging to the three dominant species not exceeding 55 cm. The local forest stand shows a random arrangement of the trees, suggesting a natural evolution.

Figure 1 shows the landscape with the Pio XII Institute in the foreground and the coniferous forest in the background, as well as the location of the air quantities measurement site inside the forest.



(a)



(b)

Figure 1. Forest landscapes around Lake Misurina: (a) Landscape with the Pio XII Institute in the foreground and the coniferous forest in the background; (b) Location of the atmospheric parameters measurement site in the forest. Photos: F. Meneguzzo.

2.2. Design of the Study, Cohort, and Respiratory Measurements

A cohort of 42 asthmatic adolescents (mean age 15+/-2yo, 12 females) admitted to the Pio XII Institute to participate to an intensive rehabilitation camp, were included in this research. The patients were consecutively recruited in the period from July to September 2022. All subjects were

born and lived at sea level in urban areas. The basic information on patients’ characteristics is reported in Table 1.

Table 1. Main characteristics of the patients.

Variable	Mean	SE Mean	StDev
Age	14.6	0.31	2.01
Height	165.9	1.70	11.02
Weight	59.9	2.13	13.83
Zscore	0.3	0.15	1.00
BMI	21.6	0.57	3.72
IBW	52.5	1.45	9.41

Respiratory functions for all patients were assessed upon admission to the Institute (T0) and after a 14-day stay (T1). All evaluations were conducted at the same time of day to mitigate circadian effects. Pre-study therapy remained unchanged throughout the study period. Following medical protocols, spirometry, lung oscillometry, and fractional exhaled nitric oxide (FeNO) measurements were performed to monitor changes in respiratory function, encompassing both central and peripheral airways, as well as airway inflammation. All tests adhered to international guidelines [34,35], and were conducted using Master-screen IOS/SentrySuite® (VyAire Medical, Chicago, IL, USA), and Analyzer CLD 88 sp (Eco Medics, Duernten, Switzerland).

Regarding spirometric values, forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), maximum mid-expiratory flow (FEF25%–75%, the mean flow between 25% and 75% of forced expiration), and FEV1/FVC ratio, were considered for analysis. For data derived from impulse oscillometry (IOS), parameters including respiratory system resistance at 5Hz (R5) and 20Hz (R20), the difference between R5 and R20 (R5–R20), reactance at 5Hz (X5), resonant frequency of reactance (Fres), and the area under the reactance curve between Fres and 5Hz (AX) were considered. Fractional exhaled nitric oxide (FeNO) was utilized to gauge airway inflammation [36].

The study received approval from the Ethics Committee of the Province of Treviso and Belluno (May 26, 2022, n. 1174). Written informed consent was obtained from parents or legal representatives.

2.3. Measurement of Volatile Organic Compounds

The total and individual concentrations of the volatile organic compounds (VOCs) were measured in the forested area frequented by children for the ordinary outdoor activities. Within about 1,000 meters around the VOCs measurement point, forest trees are dominated by spruce, followed by larch, with few specimens of stone pine.

Biogenic volatile organic compounds (BVOCs), in which MTs represented the biologically active compounds, and anthropogenic volatile organic compounds (AVOCs), including mono-aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene isomers (BTEX), were quantified via air samplings. Each sampling extended for one hour and was conducted in triplicate at various times of the day, resulting in a total of 74 hourly measurements spanning from early July to late September 2022. Specifically, using portable pumps (PocketPump, SKC Inc., PA, USA), air was directed into adsorption traps at a consistent flow rate of 200 ml/min, with each measurement involving 12 liters of air. These traps, composed of inert metal tubes (8 cm × 0.3 cm i.d.) filled with Tenax TA and Carbograph 1TD (350 mg; 35/60 and 40/60 mesh, respectively, Markes International, Ltd., Llantrisant, UK), were stored at -20°C until analysis. BVOCs, extracted from the traps via thermal desorption (unity series 2, Markes International, Sacramento, CA, USA), were injected into a 60 m capillary column (HP-1, 0.25 mm I.D.) coated internally with a 0.25 µm film of polymethylsiloxane (J&W Scientific USA, Agilent Technologies, Palo Alto, CA, USA). A 7890A gas chromatograph facilitated BVOC separation, while eluted compounds were detected with a 5975C mass spectrometer (GC–MS, Agilent Technologies, Wilmington, USA). The oven temperature held at 40 °C for 10 minutes before progressively increasing to 280 °C in increments of 5 °C/min. Helium flow

rate into the column was set at 1.0 mL/min. In full-scan (SCAN) mode, sample ionization occurred at 70 eV. The mass range analyzed spanned from 30 to 350 m/z.

For BVOC identification, retention times and mass spectra were gathered and cross-referenced with the NIST 11 library, using Agilent MassHunter Qualitative Analysis software. Following identification, compounds were quantified through external standard calibration, utilizing a gas cylinder with predetermined concentrations of distinct BVOCs (isoprene and α -pinene; manufacturer: Apel-Riemer Environmental Inc., Broomfield, CO, USA). Relative response factors were computed for all components, and interpolation techniques were applied for a subset of compounds. Compound concentrations at experimental sites were calculated in $\mu\text{g}/\text{m}^3$ as the mean and standard error of the three replicates [37–39]. A total of 30 VOCs were detected, encompassing BVOCs (including MTs), and AVOCs (including BTEX).

2.4. Reconstruction of a Continuous Series of Hourly MTs Concentration

Based on the 74 hourly measurements collected from early July to late September, 2022, a continuous series of hourly average MTs concentration data was reconstructed. Due to dominance of coniferous trees, the MTs emission factor from plants was attributed only to pool emission, neglecting the *de novo* synthesized MTs, thus considered dependent only on the temperature [40], using the approach of Guenther et al. (1993) [41], as per equation (1):

$$\text{MTER} = \text{MTER}_s \cdot \exp [\beta(T-T_s)], \quad (1)$$

where MTER is the MTs emission rate at the leaf temperature $T(\text{K})$, MTER_s is the MTs emission rate at the standard leaf temperature $T_s(\text{K})$ of 303.15 K, and $\beta(\text{K}^{-1})$ is an empirical coefficient depending on specific MTs, defined at the level of 0.1 K^{-1} for the emission of total MTs [40,41]. Due to the limited period of measures and the dominant evergreen coniferous plants, no seasonal emission factors were considered. In equation (1), T was assumed as the air temperature [40].

Although no comprehensive models were available for the reconstruction of MTs concentration in the forest atmosphere, despite early attempts [42], previous results showed a strong dependence of atmospheric MTs concentration on MTER and the time of the day, the latter in turn connected to surface atmospheric stability, such as in [43,44]. Hourly series of atmospheric MTs concentrations were reconstructed by means of a simple model based on MTER and the surface atmospheric stability, in particular performing separate linear regressions of observed concentrations against MTER for each atmospheric surface stability classes. The reason is that, once emitted, MTs diffuse horizontally and dilute vertically in different ways according to the surface atmospheric stability conditions.

Atmospheric surface stability was reconstructed based on the Pasquill-Turner atmospheric surface stability classes [45]. Such classification was performed based on hourly weather data available from a public source [46]: in daytime, hourly average solar direct radiation and 10-meters wind speed, and in nighttime, 10-meters wind speed.

2.5. Individual Exposure to MTs

The exposure to MTs for each patient was evaluated by considering the average daily concentrations of MTs (ADMTc) during outdoor activity hours. To determine the total inhaled dose (TID), parameters such as tidal volume (V_t), which refers to the volume of gas inhaled or exhaled in a single breath, and minute ventilation (V_e), representing the average volume of gas entering or exiting the lungs per minute, were estimated. Subsequently, the total volume of air breathed during outdoor activities over the course of 14 days was calculated. This value was then multiplied by the ADMTc to derive the TID. Various approaches were employed to estimate V_e and V_t , utilizing factors like heart rate, respiratory rate, age, sex, height, forced vital capacity, FEV1, BMI, and ideal body weight [47–51].

2.6. Statistical Analysis

Statistical analysis was conducted using Microsoft® Office Excel® and R Statistical Software version 4.4.1 [52]. Generalized linear models (GLM) were employed to assess the correlation between exposure to total monoterpenes as the dependent variable and lung function parameters as outcomes. These models were adjusted for sex, age, BMI, and therapy to account for potential confounding factors. Two exposure metrics were utilized to enhance sensitivity: the average daily monoterpenes concentration (ADMTc) and the total inhaled dose (TID) over the 14-day period considering outdoor hours. To compute the total inhaled dose, minute ventilation (V_e) was estimated using body weight, height, and age. Multiple models of minute ventilation estimation were applied, as explained above, to ensure robustness in the analysis.

3. Results

3.1. Characterization of the Forest Atmosphere

Figure 2 shows the distribution of measured BTEX by concentration classes in the forest atmosphere around Misurina Lake, compared with the same distribution based on data collected during the years 2021 and 2022 at 33 sites distributed throughout Italy at mountains sites. The concentrations of volatile pollutants at Misurina Lake fell into the lowest classes, with 90% of levels lower than 50 ng/m^3 , while more than 65% of concentration levels across the mountain sites in Italy fell in classes above 100 ng/m^3 . Furthermore, the main BTEX detected were benzene and toluene, with ethylbenzene, and xylene isomers present only in traces. Overall, the forest atmosphere around Misurina Lake can be considered particularly pristine, with respect to volatile pollutants of both vehicular (traffic) and industrial origin. These characteristics help to avoid a possible confounding factor due to the action of the pollutants in worsening or eliciting asthma symptoms, affecting outcomes and measured parameters.

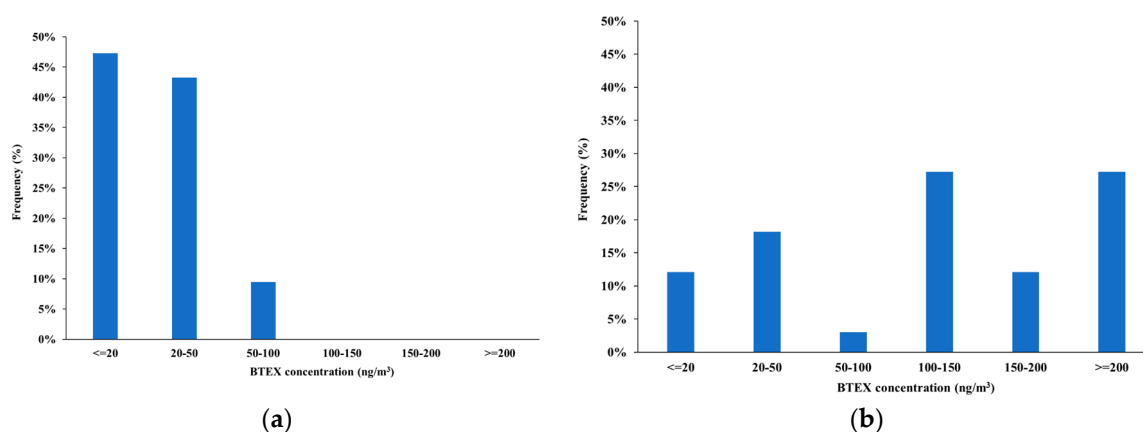


Figure 2. Distribution of BTEX by atmospheric concentration classes: (a) At Misurina Lake; (b) At 33 mountain sites in Italy (2021-2022).

Figure 3 shows the distribution of MTs by concentration classes, compared with the same distribution based on data collected between 2021 and 2022 at 33 mountain sites in Italy, previously presented more synthetically [13]. The distribution of total MTs concentration at Misurina Lake was characterized by generally lower levels than at the other mountain sites. In particular, very few cases were observed with total MTs concentration levels higher than 100 ng/m^3 .

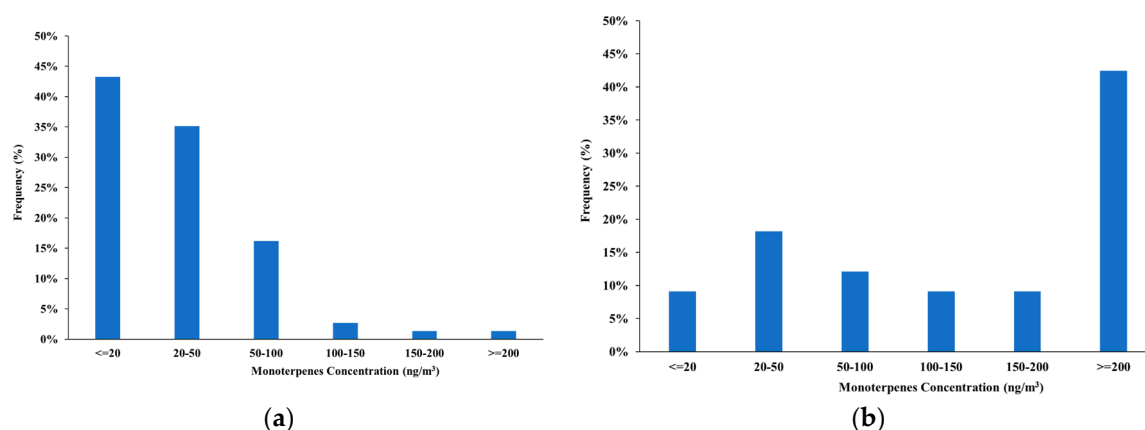


Figure 3. Distribution of MTs by atmospheric concentration classes: (a) At Misurina Lake; (b) At 33 mountain sites in Italy (2021-2022).

Figure 4 shows the relative frequency of the measured concentrations of specific MTs with regard to the total MTs concentration. α -pinene accounts for about 53% of total observed MTs concentrations, as well as it explains about 91% of the variance of total observed MTs concentrations (not shown), which agrees with the dominance of coniferous trees [44]. Sabinene, mostly emitted by beech tree [44], is the second dominant MT, with about 16%.

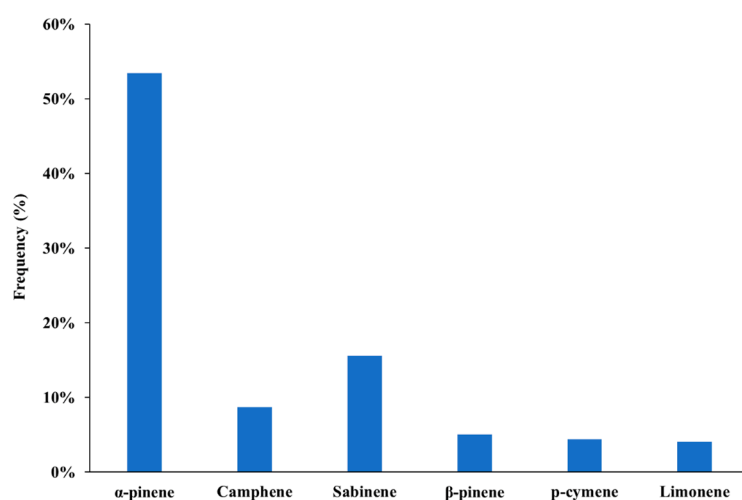


Figure 4. Relative frequency of the measured concentrations of specific MTs at Misurina Lake.

Figure 5 shows the hourly MTs concentrations, computed according to the simple model described in Section 2.4, against the measured ones. The agreement was fairly good, despite a small pool of overestimated levels in the lower concentration range, corresponding to higher sabinene emissions, which behave differently from conifer-emitted MTs, in particular showing a strong dependence on photosynthetically active solar radiation [44], and a moderate underestimation in the higher concentration range. The explained variance was 80.3%.

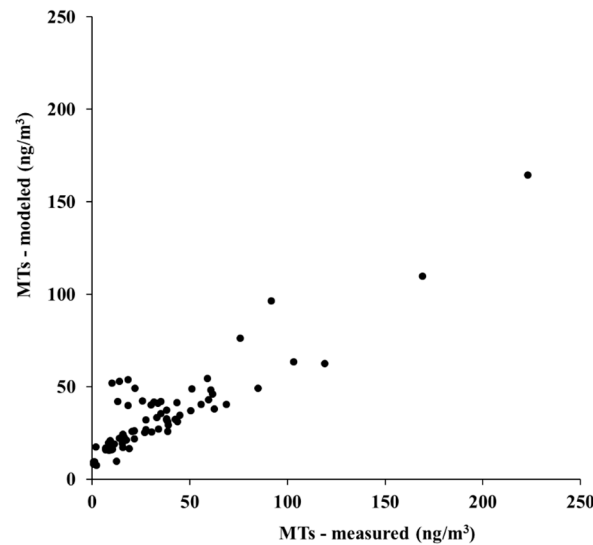


Figure 5. Modeled vs measured MTs concentration.

Finally, Figure 6 shows the continuous series of model-reconstructed hourly MTs concentration since 1 July 2022. Apparently, MTs concentration was generally higher in July (up to hour 744), and lower in September (since hour 1489), which agrees with previous findings in a Mediterranean holm oak forest [53], and in a central European conifer forest [44], highlighting a remarkable variability.

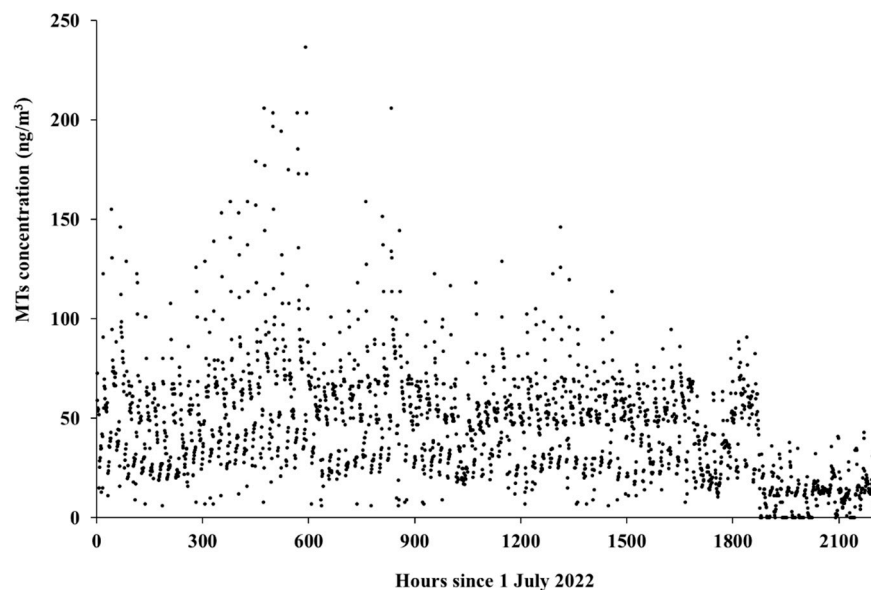


Figure 6. Continuous hourly series of model-reconstructed MTs concentration.

3.2. Association of MTs Exposure with Pulmonary Functions

The average daily monoterpenes concentration (ADMTc) ranged from 38 to 62 ng/m³, while the total inhaled dose (TID) over 14 days ranged from 0.67 to 0.84 µg. Table 2 synthesizes the GLM results for both tested exposures (ADMTc and TID), showing pulmonary outcomes for which a statistically significant (or near-to-significant) association was found.

Table 2. Outcomes for which a statistically significant association of pulmonary functions with MTs exposure was found.

	Outcome	Estimate ^a	std. error	t	p value
ADMTc exposure	FEV1/FVC%	302	147	2.05	0.05
	FEF25/75%	13	558	2.36	0.02
	R5	-5.76	2.21	-2.61	0.01
	R5%	-1038	532	-1.95	0.05
	AX	-28.6	11.7	-2.45	0.02
TID exposure	FEV1/FVC%	10.6	4.9	2.2	0.04
	FEF25/75%	46.6	19.6	2.4	0.02
	R5	-0.27	0.07	-3.78	<0.001
	R5%	-70	34	-2.1	0.04
	R20	-0.14	0.06	-2.32	0.03
	R20%	-39	19	-2.1	0.04
	X5	0.07	0.03	2.4	0.02
	AX	-1.38	0.4	-3.65	<0.001
	R5R20	-0.12	0.04	-2.94	0.006

^a The estimates refer to the change in the outcome for 1 µg/m3 increase in ADMTc or 1 µg increase in TID exposure.

The outcomes derived from TID exposure corroborate the findings obtained from ADMTc exposure. Additionally, associations with further pulmonary oscillometry parameters were identified. No significant associations were observed between the evaluated exposures and FeNO, FEV1, FVC, blood pressure, or peripheral saturation. Importantly, in the supplementary robustness tests employing various minute ventilation estimation techniques, significant associations were observed with FEV1 at the 14-day mark. However, the more conservative model was retained.

4. Discussion

In this study, we demonstrated a statistically significant association between certain lung function parameters and total MTs exposure in asthmatic adolescents during a 14-day stay at the Pio XII Institute in Misurina, situated near an alpine forest and a natural lake. Notably, we observed meaningful associations between the 14-day TID and the enhancement of impulse oscillometry (IOS) parameters related to small airway patency. These findings are in line with previous research indicating improved lung function following periods spent in comparable high-altitude mountain regions [32,33,54,55]. To the best of our knowledge, only one study had previously shown IOS improvement; other research typically focused solely on spirometric parameters, typically FEV1, FVC, and FEF25-75, for assessing lung function in asthmatic children in mountain environments.

Enhancements in airway resistance and reactance hold significant importance, as peripheral airway impairment is recognized to precede asthma exacerbations and predict their likelihood [56,57]. The positive clinical and functional changes observed in asthmatic participants during altitude stays have been attributed primarily to allergen avoidance, especially with regard to dust mites and pollen. At higher elevations, the pollen season is shorter, and Dermatophagoides struggle to thrive above 1500-1800 m a.s.l. due to lower humidity and temperatures.

While many of the asthmatic participants had allergies to pollen and Dermatophagoides, clinical and functional improvements were noted in non-allergic individuals as well. This suggests that aside from allergen reduction, other factors play a pivotal role in alleviating the inflammation underlying asthma. A potential positive impact of reduced pollution has been proposed, considering the known connection between environmental pollution and lung function, particularly in asthmatic children [58].

Our data additionally reveal consistently low atmospheric BTEX concentrations throughout the study period, thereby sidestepping potential confounding due to pollutant impact on asthma

symptoms. These insights partly elucidate the improvements, aligning with the non-pharmacological approach of asthma trigger avoidance, which involves irritants or inflammation exacerbators in the airways [59]. This study thus contributes novel insights by revealing a plausible underlying mechanism tied to atmospheric MTs concentrations.

While this study offers a pioneering insight into MTs' distinct impact on asthmatic adolescents' lung functions, it bears limitations. The relatively small sample size and the single-center nature in the first place. Expanding this investigation to other altitude-based asthma rehabilitation centers could bolster findings. Another limitation pertains to the accuracy of modeling a continuous hourly MTs concentration series based on discrete measurements. Finally, the pharmacokinetics and pharmacodynamic healing mechanisms of MTs inhaled in forest atmospheres remain still to be clarified, both for short-term exposures as seen in Donelli et al. 2023 [13], and for more extended durations, as observed in this study.

5. Conclusions

This pilot study has substantiated that exposure to plant-emitted MTs in the forest atmosphere significantly enhances lung function among asthmatic adolescents, potentially via localized airway effects. This therapeutic influence gains further credence from the more robust and substantial associations observed with the total dose of inhaled MTs compared to the simple exposure to their air concentration. Nonetheless, further research is warranted to validate these findings and unravel the mechanisms underpinning the therapeutic impact of forest MTs on asthma symptoms.

This study contributes additional evidence to the increasingly explored direct healing potential of forest environments. Additionally, the relatively modest average MTs concentrations imply the efficacy of prolonged exposure to the forest atmosphere, expanding on the dose-dependent effect noted in brief exposures to different MTs concentrations in relation to anxiety symptoms [13]. Such insights may guide the planning of forest immersion therapeutic interventions even during less favorable seasons like spring, fall, or winter.

Within the context of forest medicine, this research, grounded in a forest-centric approach, seeks to deepen the comprehension of forest environments' therapeutic role, a domain that still remains to be explored [60]. In addition to evaluating the health benefits arising from exposure to forest ecosystems, this study also concentrated on scrutinizing the environmental attributes and components associated with these health benefits. This twofold approach aims to advance the adoption of standardized guidelines for public health interventions and enhance forest management practices.

Author Contributions: Conceptualization, Davide Donelli, Giovanni Margheritini, Francesco Meneguzzo and Annalisa Cogo; Data curation, Rita Baraldi, Anna Corli, Francesco Meneguzzo, Luisa Neri and Annalisa Cogo; Formal analysis, Davide Donelli; Investigation, Rita Baraldi, Anna Corli, Federica Gardini, Giovanni Margheritini, Luisa Neri and Giorgio Piacentini; Methodology, Davide Donelli, Francesco Meneguzzo and Davide Lazzeroni; Project administration, Franco Finelli, Giovanni Margheritini, Federica Zabini and Annalisa Cogo; Resources, Giovanni Margheritini, Federica Zabini and Annalisa Cogo; Software, Davide Donelli; Supervision, Giovanni Margheritini, Francesco Meneguzzo, Diego Ardissino, Federica Zabini and Annalisa Cogo; Validation, Michele Antonelli, Franco Finelli, Federica Gardini, Davide Lazzeroni, Diego Ardissino and Giorgio Piacentini; Visualization, Davide Donelli and Francesco Meneguzzo; Writing – original draft, Davide Donelli, Michele Antonelli, Rita Baraldi, Francesco Meneguzzo, Luisa Neri, Federica Zabini and Annalisa Cogo. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was designed and conducted in compliance with the Declaration of Helsinki and its subsequent modifications. The study received approval from the Ethics Committee of the Province of Treviso and Belluno (May 26, 2022, n. 1174). All participants voluntarily provided their informed consent prior to enrollment and were free to withdraw from the study at any time. Written informed consent was obtained from parents or legal representatives, all the information was strictly handled according to the EU privacy regulation ("The General Data Protection Regulation" – GDPR 2016/679).

Data Availability Statement: All data are available by contacting the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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