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Intercomparison of Indoor and Outdoor Pollen Concentrations in Rural and Suburban Research Workplaces

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Abstract: Pollen exposure in occupational settings involves different categories of workers. In this paper the effects of diurnal pollen variations have been evaluated in two sites characterized by different vegetation and urbanization: the suburban site of Tor Vergata (TV) and the rural site of Monte Porzio Catone (MPC). Aerobiological and meteorological monitoring was performed in the two sites during the winter of 2017. The data analysis focuses on the comparison between pollen concentrations observed in relation to meteorological variables. In general, it can be stated that the indoor and outdoor dynamics for MPC and TV are different, with the outdoor concentration of pollen for MPC always higher than for TV, in accordance with significant presence of vegetation. The high nocturnal peaks detected in MPC and completely absent in TV could be caused by the presence of particular conditions of stagnation combined with greater emissions from the pollen sources. Furthermore the higher I/O ratio observed during the working hours in TV compared to MPC could be ascribed to the workers' behavior. Exposure to pollen can be responsible for several health effects and the knowledge of its level can be useful to improve the evaluation and management of this biological risk.

Keywords: pollen; aerobiology; allergens; indoor; outdoor; occupational health; daily patterns; hourly peaks; meteorological parameters; monitoring

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1. Introduction

Pollen is one of the main components of bioaerosol. As the microgametophyte of spermatophytes, its role in the life cycle of seed plants (both wild and cultivated) is crucial, and its presence in the air is ubiquitous. From a biological, ecological and agricultural point of view, pollen is indispensable. However, from a human health perspective, pollen can be seen as an aerobiological pollutant mainly responsible for respiratory and allergic diseases [1–6] and has been recently associated with different health effects. Lung function, seasonality of flu-like illnesses, SARS-CoV-2 infection rates, and occurrence of several cancers have been put related to airborne pollen variations [7–11].

Environmental exposure is a critical aspect and several studies evidenced that different outdoor, indoor, rural, and urban environments may contribute to determine or exacerbate the health effects [12–14]. In this regard a key role should be attributed to occupational settings that may increase pre-existing sensitization and/or respiratory pathologies due to other biological, chemical and physical agents mainly present in workplaces. With respect to other agents, biological agents have the peculiarity that exposure may occur both in domestic [15,16] and work environments [17,18].

Aerobiological particles responsible for occupational allergic diseases (e.g., asthma and rhinitis) show a variation in their allergenicity, concentrations, dispersal and transport, in relation to several factors such as meteorological conditions and in particular air temperature, relative humidity, rainfall, wind speed, climate change and other factors including atmospheric pollutants, characteristics of the vegetation site, seasonality, and buildings and their occupants [19–27].

Many epidemiological and research studies in the literature have focused on potential interactions between chemical pollutants (e.g., ozone, carbon monoxide, nitrogen dioxide, sulfur dioxide), particulate matter including PM_{10}, $PM_{2,5}$ and airborne particles, showing the relevant effects of air pollutants on bioaerosol properties [13,14,28–31].

In this regard, important alterations have been reported in the biological and reproduction functions of pollen (e.g., decrease in pollen germination and survival) and modifications of pollen surface characteristics, and in addition pollutants seem to act as adjuvants to pollen allergens enhancing their potential health risks [13,14,28–30]. Prominent studies have shown that air pollutants might adhere on pollen surface damaging pollen cell membranes, with a significant number of allergens released into the environment [14,32,33]. Furthermore, some researchers use the neologism "polluen" to indicate a specific atmospheric material made up of allergens and pollutants, gases, and particulates of many sizes and shapes carried on pollen or fungal spores that act as carriers of allergens, facilitating their diffusion [28,29].

In addition to air pollutants and meteorological factors, several papers have evidenced the effect of urbanization on plant reproduction (e.g., earlier pollen flowering), pollen abundance (e.g., overexpression of allergenic proteins), pollen dispersion, people's exposure to allergens, and severity of allergic diseases/symptoms. Furthermore, associations between urbanization and the rising prevalence of allergic epidemics in urban areas compared to those in rural environments have been studied [13,14,34,35]. However, the pollen count pattern in urban and rural areas is also linked to many critical aspects such as size of vegetation areas, distance to pollen source in relation to pollen transport and dispersal, and presence of ornamental vegetation. In this respect, ornamental trees used in urban environments in relation to the concept of green spaces and urban greening to reduce pollution levels could on the contrary produce other sources of allergenic pollen types (Oleaceae, *Platanus*, Poaceae, Cupressaceae, Betulaceae) as well as natural vegetation, and cause adverse effects on people's health such as pollinosis. The knowledge of pollen distribution can be helpful in reducing pollen risk exposure in order to increase quality of life in urban environments [36–38].

Different trends of pollen linked to year [39,40], seasonality [41–43], daily [44–46], intradiurnal [40,47–52] or hourly variations have been reported [27,53,54], and have to be taken into account for the further distinction between urban or non-urban environments [25,26,52,55–58]. In this regard, urban and non-urban components around occupational settings add complexity. and the evaluation of environmental exposure in assessing health effects should consider the peculiarity of exposure to sources.

Occupational environments can be responsible for allergies in terms of developing and/or exacerbation in the context of public health due to their high prevalence and their economic costs, concerning workdays lost and decreases in productivity [59,60].

Pollen is a critical source of exposure in living and work environments, indoor and outdoor, urban and non-urban occupational settings, involving numerous categories of workers, e.g., farmers, gardeners, foresters, office workers [15–19,61–65].

Many papers have shown that indoor workplaces are also directly influenced by the outdoor environment. Pollen grains and, as a rule, air pollutants, can enter a building by infiltration, through open windows and doors and unfiltered ventilation systems, and can be transported on the feet and bodies of occupants [19,66–68].

The aim of the study is to evaluate the effects of diurnal pollen variation in two occupational settings with different vegetation and urbanization characteristics in relation to meteorological variables, working days (WDs), non-working days (NWDs), working hours (WHs), and non-working hours (WHs), considering the indoor and outdoor ratios.

2. Materials and Methods

2.1. Study Sites and Vegetation

The study was conducted inside and outside the Research Centre building of the National Workers' Compensation Authority (INAIL) in Monte Porzio Catone (MPC) as a rural area, and inside and outside the University of Tor Vergata (TV) in Rome as a suburban area. Monte Porzio Catone is a hilly area located in southeast Rome, in the Castelli Romani regional park at ~300 m a.s.l. (41°49′19.5″ N, 12°42′24.1″ E); Tor Vergata is located in a south-easterly neighborhood of Rome at about 80 m a.s.l. (41°51′13.5″ N, 12°36′14.2″ E); the two sites are located in the Mediterranean biogeographical region. The climate of Rome is broadly sub-Mediterranean, with mild winters and hot summers (three/four months of aridity). The average annual temperature is 15 °C and average annual rainfall is 839 mm [69,70]. The vegetation is complex and varied, characterized by flora of high biodiversity and many taxa of high conservation value. The dominant types are sub-Mediterranean deciduous and evergreen mixed forests with prevalence of oak woods with *Quercus cerris*, *Q. frainetto*, *Quercus ilex*, *Q. robur*, *Q. dalechampii* and *Q. pubescens*, other common woody species such as *A. monspessulanum*, *Acer campestre*, *Fraxinus ornus*, *Corylus avellana*, *Crataegus monogyna*, *Viburnum tinus*, *Pistacia lentiscus* and trees belonging to the *Robinia*, *Ulmus*, and *Ailanthus* genera [69–71]. In the rural area (MPC) cultivates areas are most common including species such as *Olea europaea*, *Vitis vinifera*, *Corylus avellana* and chestnut woods (*Castanea sativa* Miller) fields, continuous prairies, hedges, many forests with oak trees, coniferous trees, vineyards, olive groves, and vegetable gardens. The area is surrounded by Cupressaceae, Pinaceae and Oleaceae. Herbaceous plants such as Urticaceae, Plantaginaceae and Poaceae are also present (http://websit.cittametropolitanaroma.it/DescriviMappa.aspx?i=7 accessed on 12 January 2021). The greenery of the suburban area (TV) is composed of pastures, abandoned fields, uncultivated land, urbanized and degraded areas, artificial surfaces, numerous native species due to human impact that are well adapted to human presence, and non-native species of uncertain origin [71].

Data regarding the vegetation maps around the sampling area were retrieved from the website http://websit.cittametropolitanaroma.it/DescriviMappa.aspx?i=7 (accessed on 12 January 2021) while data on buildings were obtained from https://dati.lazio.it/it (accessed on 22 April 2021). The retrieved data were processed using QGIS 3.16.1, obtaining measures of the areas covered by vegetation, artificial surfaces and buildings. The area measures were used to calculate the plan-area density (λp) of buildings and the Vegetation Index (Vi). The λp indicator (i.e., the fraction of area), defined as the ratio between the built area and the total area, is used to identify the presence of buildings whereas, regarding the green areas, the Vegetation index (Vi), defined as the ratio between the overall vegetated area and the total area, is considered.

2.2. Aerobiological Monitoring

Aerobiological monitoring was performed during winter 2017 (from 2 to 21 February 2017), collecting aerobiological particles in accordance with the UNI 11108/2004 and following UNI CEN/TS 16868:2015, using 7-day volumetric samplers, Lanzoni VPPS 2000 (Bologna, Italy), Hirst-type [72]. In the rural area (MPC) one sampler was placed outside the Research Center of INAIL, Monte Porzio Catone, at 1.10 m above ground level, another one inside the building at 0.6 m above ground level.

In the suburban area (TV) one sampler was located on the roof of the Biology Department building of the University Tor Vergata, Rome, at about 12 m above the ground level, and another one inside a room of the same building at 1 m above ground level.

The sampler consists of different components: a single-stage impactor of particles, a suction pump, an intake orifice, a rotating drum, and a directional wing. The air is captured by a vacuum pump to assure a constant flow of 10 L/min corresponding to the human mean breath rate, through an orifice of known dimension (2 × 14 mm) oriented continually against the wind due to a directional wing and positioned at a height of at least 1 m. The air flow is directed onto a surface consisting of a transparent plastic Melinex® tape properly equipped, coated with a 2% silicon solution as trapping surface. The trapping surface moves at a speed of 2 mm/h on a cylindrical rotating drum that makes a complete round in a week [19,73,74].

At the end of the exposure the sampling surface (sticky tape) is cut into segments of 48 mm representing daily samplings and prepared to be mounted on microscope slides. The tape is treated with glycerin jelly in order to adhere perfectly to the slides and stained with basic fuchsine solution which colors only pollen grains. Lastly the slides are observed via a light microscope with a magnification of 400×.

According to the Italian standard methodology the minimum number of horizontal sweeps must correspond to an area close to 20% of the impacted surface and the sweeps spaced 2 mm from each other, as well as from the edge of the sampling surface [73]. The pollen counts are expressed as number of particles/m^3 of air [19,73,75], pollen concentrations are provided with a 30-min time resolution and, in the analysis, 2-h and 6-h averages are calculated and considered.

2.3. Meteorological Monitoring

Meteorological data were provided by the University of Rome "Tor Vergata" and collected simultaneously with the aerobiological measurements, during winter 2017 (from 2 to 21 February 2017), by a meteorological station located on the roof of the Experimental Ecology and Aquaculture Laboratory (LESA), 2.18 Km from the TV pollen trap. The monitoring was performed using a CAE SPM 20 station (Bologna, Italy) connected to sensors for air temperature, humidity, wind speed and direction (at 10 m above ground level) and rain (at 2 m above ground level). Data for air temperature (°C), relative humidity (%), wind speed and direction (m/s and deg), precipitation (mm) and solar radiation (W/m^2) were recorded with a 30-min time resolution and, regarding the pollen analysis, the 2-and 6-h averages were subsequently considered. Preliminarily, the daily evolution of the main meteorological variables, also in relation to the vegetation maps, allow better characterization of the possible pollen transport/dilution conditions during the study period. The arithmetical means and the standard deviations of the meteorological variables over the entire period were calculated to complete the site description. Daily evolution of the meteorological variables is also presented and scatter plot between wind speed and direction highlights the main sectors of provenience related to the intensity of wind. It must be kept in mind that, for the MPC site, meteorological measurements are not available but, considering the short distance (7.12 km) and the objectives of the analysis presented in this study, we can consider the meteorological condition as homogeneous between the two sites.

2.4. Data Analysis

Data analysis focuses on the comparison between pollen concentrations observed in two sites, relatively close to each other but characterized by profoundly different land use (see Section 2.1); the analysis aims also to highlight the differences that can occur during the working days (WDs) and the non-working days (NWDs), working hours (WHs) and the non-working hours (NWHs), in each monitoring site in order to observe possible and different behavior patterns in relation to specific conditions, regarding also the exposure of workers [19].

In brief, three topics are the main components of the data analysis and the results will be presented as follows:

1. outdoor and indoor pollen characterization in WDs and NWDs, WHs and NWHs in the two sites;
2. the peak analysis, where the maximum of the outdoor pollen concentration for each day of measure is selected and related to the meteorological conditions;
3. finally, the indoor and outdoor Ratios (I/O) are calculated and compared for the two sites.

As well as described above for the meteorological data, pollen concentrations are provided with a 30-min time resolution and, in the analysis, 2-h and 6-h averages are calculated and considered.

Pollen data were extrapolated onto spreadsheets as previously reported [19,76].

3. Results and Discussion

3.1. Average Pollen Values for TV and MPC

Starting from the observations collected every 30-min, average values for indoor and outdoor pollen concentrations were calculated for the considered study period. Mean values for the two sites and their standard deviations are reported in Table 1.

Table 1. Average pollen concentration, for the indoor and the outdoor components in the two study sites (TV and MPC). Mean values and standard deviation are calculated over the study period from observations collected at 30-min time resolutions.

	TV		MPC	
	Indoor	**Outdoor**	**Indoor**	**Outdoor**
Pollen (particles/m^3)	0.14 ± 0.53	3.19 ± 4.69	0.10 ± 0.49	5.91 ± 9.30

As shown in Table 1, the two sites exhibit different pollen concentrations; regarding the outdoor component, MPC is characterized by higher values of about 46% more compared to TV.

The observed values of the outdoor pollen concentration compared to the indoor are higher in both sites (about 59 times for MPC and 22 times for TV).

In general, it can be stated that the indoor and outdoor dynamics for MPC and TV are different.

3.2. Land Use Characterization of TV and MPC

In order to interpret the observed pollen behavior, the buildings and vegetation distribution of an area of about 12 km^2 around the two sites are taken into account and shown in Figures 1 and 2.

The area of interest has been classified into two categories (green areas and artificial surfaces), grouping the different types of vegetation into the green area category and non-vegetated areas into artificial surfaces. Green areas are the main potential sources of pollen; however, no consideration has been made regarding the type of plants or pollen species.

The pollen impact measured at the two sites depends, firstly, on transport related to the wind direction but also on the distance between the site and the possible source of pollen. For this reason, data have been considered as a function of different classes of distance, reported in Table 2.

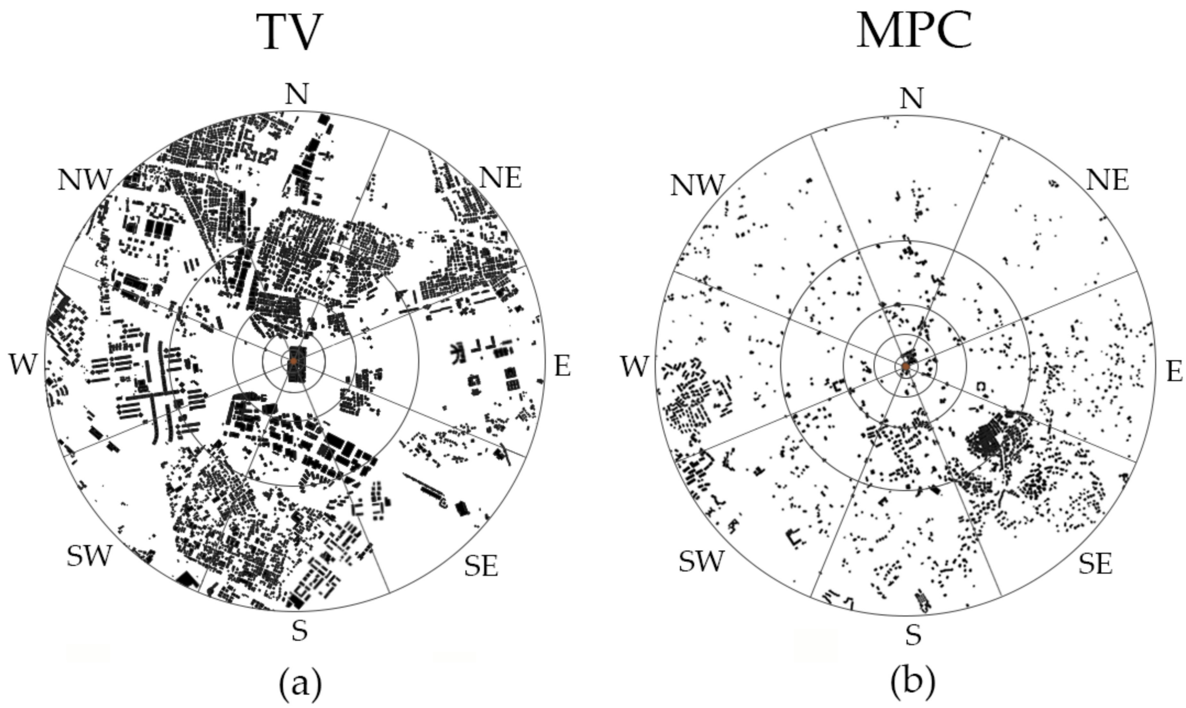

Figure 1. Maps of the buildings (in black) present in a 2 km radius around the pollen samplers in TV (**a**) and MPC (**b**). In the maps, the concentric circumferences of 1 km, 0.5 km, 0.25 km and 0.1 km radius and the octants corresponding to cardinal directions are also shown.

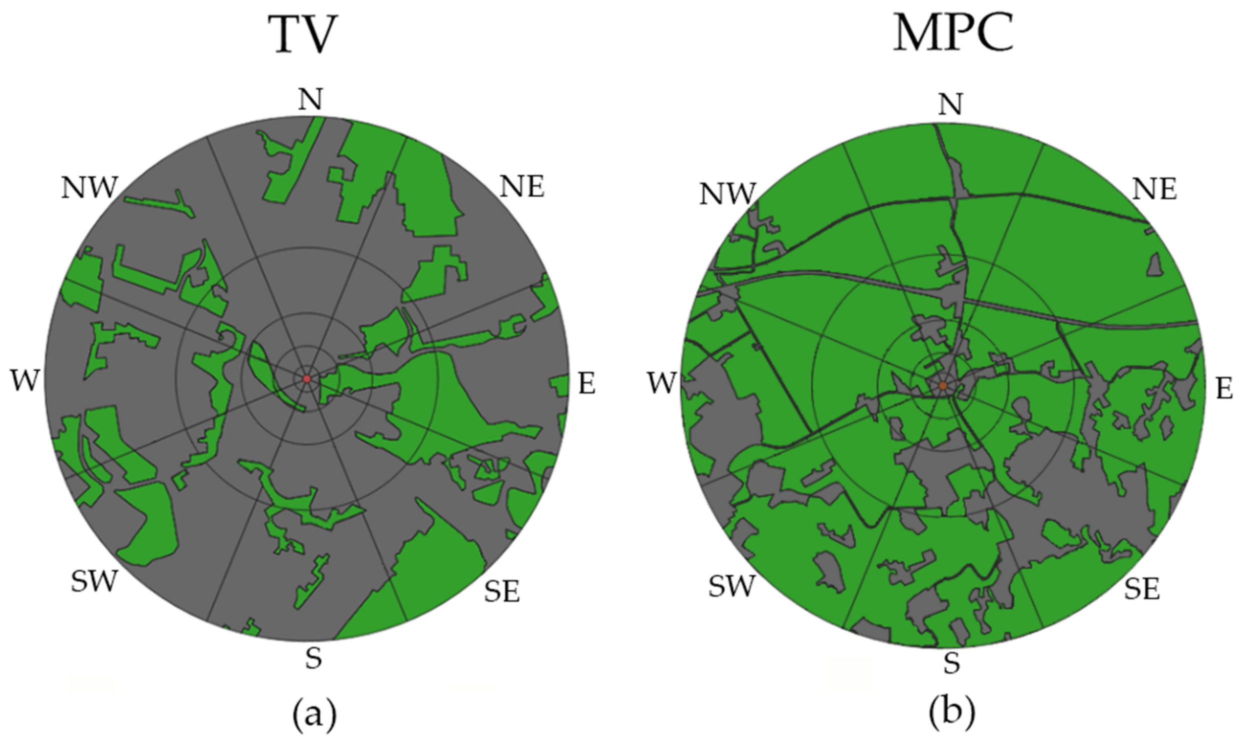

Figure 2. Maps of the artificial surfaces (in grey) and green areas (in green) present in a 2 km radius around the pollen samplers in TV (**a**) and MPC (**b**). In the maps, the concentric circumferences of 1 km, 0.5 km, 0.25 km and 0.1 km radius and the octants corresponding to cardinal directions are also shown.

Table 2. Distance classes and the relative maximum and minimum distance from the center of the annular area covered.

	Dmin (m)	**Dmax (m)**
D100	0	100
D250	100	250
D500	250	500
D1000	500	1000
D2000	1000	2000

In this way, it is possible to identify, at different distances (up to a maximum of 2 km), the characteristics of the terrain in the vicinity of the two examined sites. Eight sectors (octants) are considered for the wind direction.

Figure 1 shows the built-up area for TV and MPC, whereas Figure 2 reports the green areas. In both figures the angular sectors representing the wind direction, as in Table 2, are highlighted.

The average values of λp (calculated from Table 3, as function of the distance and the sector of provenance) for TV and MPC are shown in Figure 3.

Table 3. Values of λ_p as function of the distance and the sector of provenance for TV and MPC.

	TV (λ_p)					**MPC (λ_p)**				
	D100	**D250**	**D500**	**D1000**	**D2000**	**D100**	**D250**	**D500**	**D1000**	**D2000**
N	0.60	0.17	0.26	0.26	0.16	0.31	0.05	0.05	0.03	0.01
NE	0.61	0.15	0.13	0.14	0.14	0.71	0.06	0.04	0.01	0
E	0.58	0	0.01	0.04	0.06	0	0.09	0	0.02	0.03
SE	0.65	0.13	0.02	0.15	0.05	0.05	0.03	0.02	0.16	0.09
S	0.64	0.18	0.12	0.24	0.14	0.08	0.03	0.06	0.08	0.03
SW	0.38	0.01	0.20	0.17	0.11	0.06	0.01	0.03	0.03	0.04
W	0.13	0	0	0.09	0.14	0	0	0.02	0.02	0.05
NW	0.18	0.03	0.18	0.19	0.20	0	0	0	0.01	0.01
Average	0.47	0.08	0.11	0.16	0.12	0.15	0.03	0.02	0.04	0.03

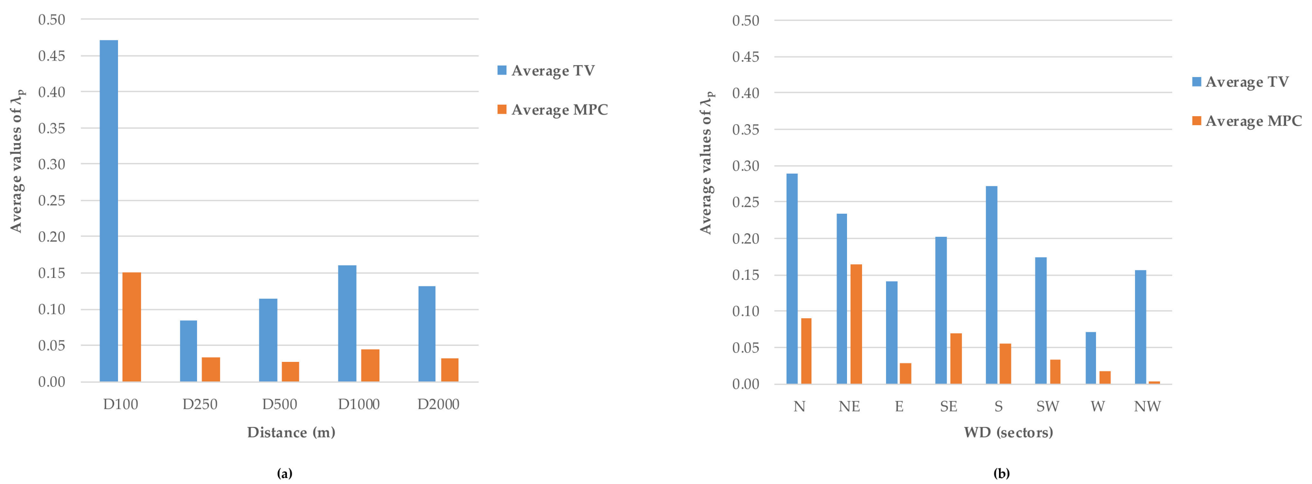

Figure 3. The average values of λ_p as function of the distance (**a**) and the wind direction (**b**), for TV and MPC.

Both sites are characterized by unbuilt areas (except for TV in the first 100 m, where λp = 0.47), but TV is characterized by a higher presence of buildings compared to MPC (λp = 0.19 and λp = 0.06, respectively).

Table 4 reports the Vi as function of the distance and the wind direction for TV and MPC.

Table 4. Values of V_i as function of the distance and the sector of provenance for TV and MPC.

	TV (V_i)					MPC (V_i)				
	D100	**D250**	**D500**	**D1000**	**D2000**	**D100**	**D250**	**D500**	**D1000**	**D2000**
N	0	0	0	0	30.55	0	40.47	55.02	72.07	95.52
NE	0	0.13	8.25	26.95	31.9	0	63.05	77.38	93.31	97.21
E	8.24	77.55	60.15	64.79	31.32	0	29.83	81.84	84.57	82.12
SE	0	56.32	1.77	28.24	46.52	8.42	76.19	94.53	45.93	45.33
S	0	15.32	0.22	14.05	19.95	28.7	100	59.38	42.29	76.87
SW	0	38.6	1.32	15.84	25.79	0.85	92.13	87.72	92.1	75.42
W	0	11.66	43.08	15.86	22.89	0	73.15	54.6	93.53	68.27
NW	0	0	17.84	16.41	19.32	0	89.29	93.66	96.63	91.42
Average	1.03	24.95	16.58	22.77	28.53	4.75	70.51	79.27	77.55	79.02

It is evident that MPC has a significant vegetation presence (Vi = 62.2) compared to TV (Vi = 18.7).

However, the vegetation index is small in the areas close to the two sites (first 100 m) but grows with the distance (Figure 4a), assuming on average values of 23.2 and 76.6, between 250 and 2000 m for TV and MPC, respectively.

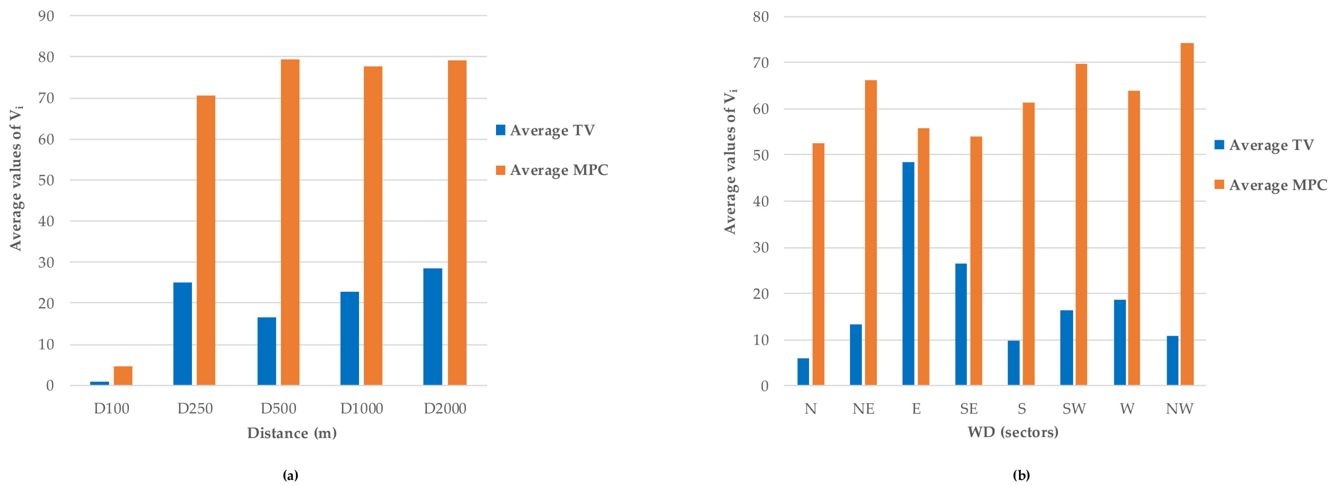

Figure 4. The average values of V_i as function of distance (**a**) and the wind direction (**b**), for TV and MPC.

Considering the wind direction, the vegetation index, for MPC, does not vary considerably whereas, for TV, Vi remains low, showing values comparable to MPC when the wind comes from the east (Figure 4a,b).

3.3. Study of Meteorological Variables and Pollen Concentrations

TV and MPC terrains are profoundly different and this aspect influences the possible source of pollen with respect to the wind direction.

To better evaluate this aspect, considering the meteorological condition as homogeneous between the two sites as indicated in Section 3.3, meteorological variables measured on TV were related to the outdoor pollen concentration observed in the two sites.

Table 5 shows the mean values of the meteorological variables observed during the measurement campaign.

Table 5. Mean values of the meteorological variables during the measurement campaign as function of the sector of provenience (wind direction).

	N	NE	E	SE	S	SW	W	NW
WD	142.3	45.2	93.6	132.2	180.3	224.0	264.2	317.7
WS	1.5	1.1	1.9	1.2	1.4	0.8	0.7	0.8
T	10.8	9.5	7.8	7.6	10.9	10.4	11.9	11.9
Rh	68.3	78.4	83.5	87.1	84.3	84.7	67.8	61.3
RS	251.6	171.2	44.6	56.7	70.0	82.1	195.4	329.2
Rain	0	0	0	0.1	0.1	0.2	0	0

Wind speed values are on average higher for the sector between North–South (WS = 1.5 m/s) while lower values are observed from West (WS = 0.8 m/s). Data for solar radiation show higher values for directions from SW–W–NW. During the night (not shown in the table), the mean temperature and wind speed are lower (Tnight = 6.8° and WS = 1.02 m/s) than those observed during the day (Tday = 11.6° and WS = 1.33 m/s), corresponding to a mean relative humidity of 92.5% and 72.5%, respectively.

Considering the pollen at TV and MPC, Figure 5 shows the observed values as function of the wind direction measured at TV.

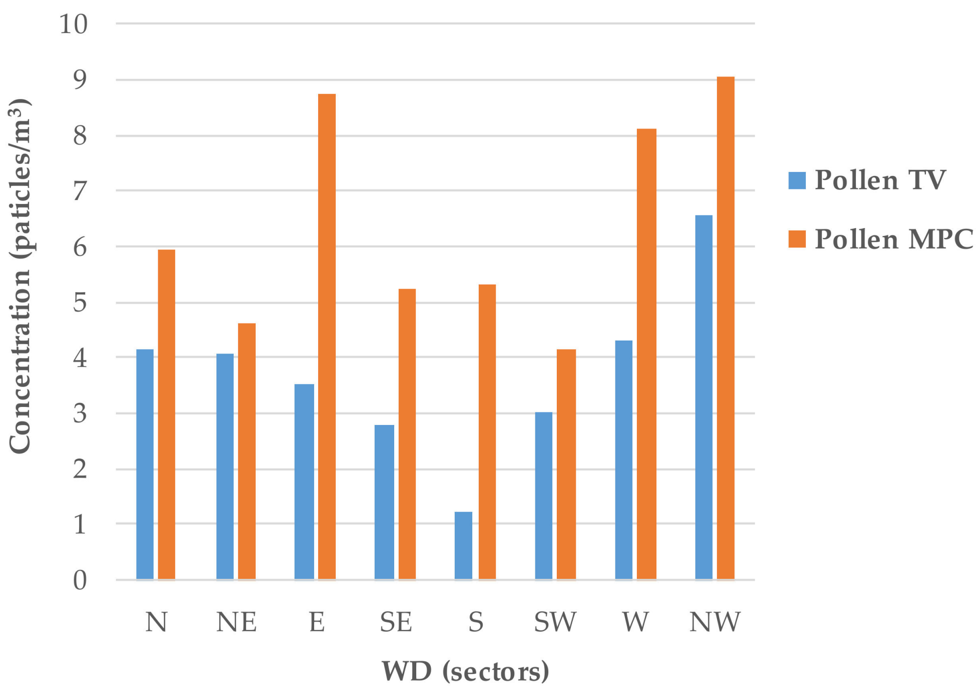

Figure 5. Pollen concentration at TV and MPC as function of measured wind direction.

It should be emphasized that while for TV, meteorological variables and pollen concentration are measured on the same site, for MPC we assume that the wind regime is similar to that observed in TV; our assumption is supported by the evidence that the terrain between the two sites is generally free from obstacles, as shown in Table 3.

Figure 5 shows that, for TV, the minimum pollen concentration corresponds to the South direction (P (TV) = 1.24 particles/m^3), while the maximum of transport occurs for winds from NW (P (TV) = 6.57 particles/m^3). For MPC, the observed values are, on average, higher and less dependent on the wind direction in accordance with the vegetation index (Table 4), which shows higher values for MPC, independently of the wind direction (see Figure 4).

3.4. Analysis of Typical Days NWDS and WDS for Indoor and Outdoor Pollen Concentrations

Aiming to evaluate occupational exposure, the hourly average values are considered in the analysis of a typical day, taking into account separately the working days and the non-working days. Figure 6a,b shows the outdoor concentrations for the MPC and TV. The outdoor concentrations for MPC are always higher than for TV, about 79% and 27% more during the working days and the non-working days, respectively; the difference between the pollen concentration in the two sites is probably due to the influence of the Vi that characterizes MPC and TV, but also to the high agricultural vocation of the MPC area, where the average pollen values reach the maximum during the holidays (P_{NWDs} (MPC) = 6.5 particles/m^3), definitely higher than that observed at TV (1.1 particles/m^3) for the same period.

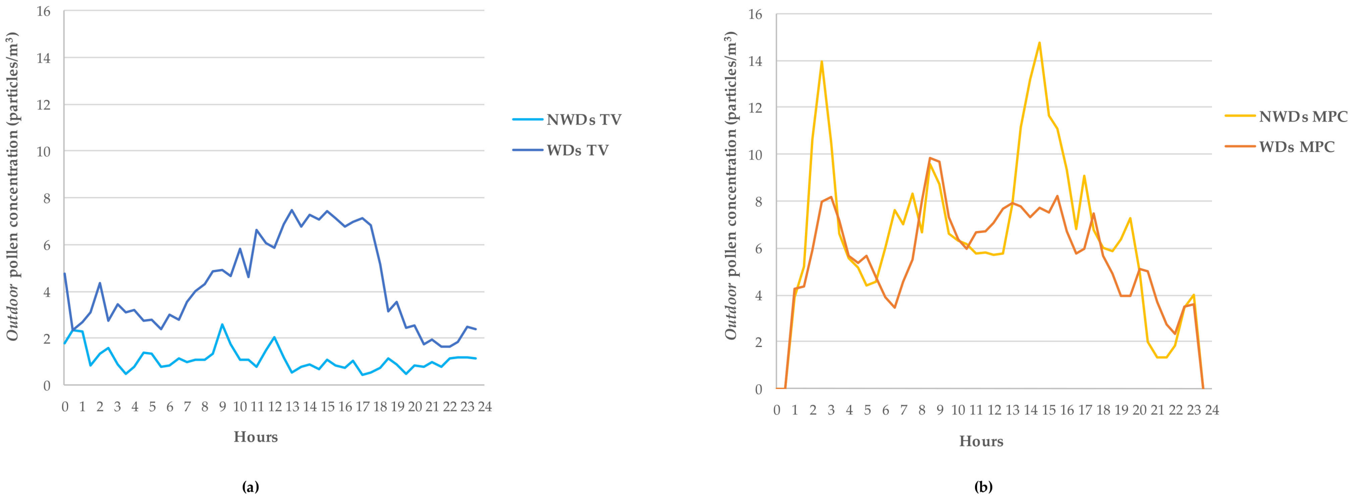

Figure 6. Daily trend of the outdoor pollen concentration for TV (**a**) and MPC (**b**) in NWDs and WDs.

Figure 7 shows the values for indoor pollution. No substantial differences are observed between MPC and TV during non-working days contrary to what was observed during working days. The average values in TV are slightly higher than in MPC (0.20 particles/m^3 versus 0.13 particles/m^3) but the most relevant aspect, however, is the hourly distribution of indoor pollen that is observed between MPC and TV during working days. During the first part of the morning (from 8:00 a.m. to 14:00 p.m.), very low values are observed in MPC (Pindoor (MPC) = 0.10 particles/m^3) compared to TV (Pindoor (TV) = 0.38 particles/m^3), while in the second part of the day (from 14:00 p.m. to 20:00 p.m.), the values of MPC are slightly higher than those observed in TV 0.37 particles/m^3 versus 0.27 particles/m^3).

A first consideration that can be made regards the habits of workers (their presence and behaviors) that influence the indoor pollen concentration during the day. This aspect will be discussed in Section 3.6.

3.5. Peaks Analysis: Comparison between Outdoor Pollen in the Two Monitoring Sites

3.5.1. Two Hour Resolution Analysis

The purpose of the peak analysis is to identify the outdoor events characterized by the maximum concentration of pollen, also considering when during the day these events occur. In general, peaks can be studied at the maximum resolution (in our case 30 min); however, this temporal resolution is too high, giving rise to multiple maxima of equal intensity; thus, the 2-h and 6-h averages were considered. In the first case, this permits evaluation of the impact of pollen following the evolution during the day, whereas, in the second case, it studies which time slot of the day (morning, afternoon, evening or night) is characterized by the presence of the maximum.

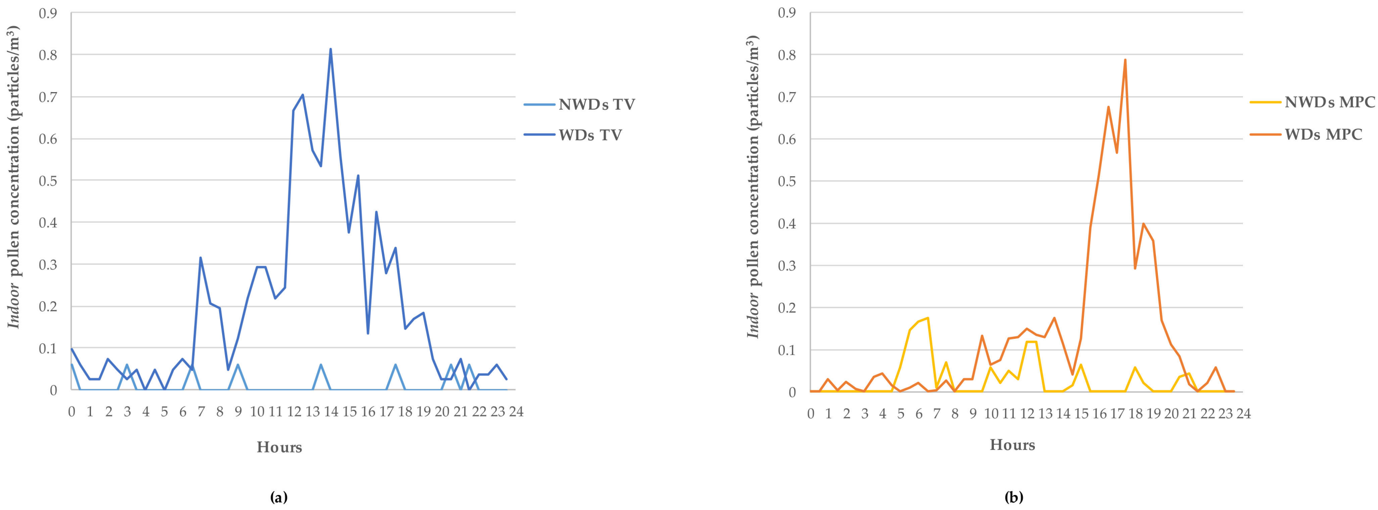

Figure 7. Daily trend of the indoor pollen concentration for TV (**a**) and MPC (**b**) in NWDs and WDs.

As also highlighted earlier, MPC presents higher values of the average maximum concentration (18.1 particles/m^3), compared to TV (9.3 particles/m^3).

Figure 8 shows a very high nocturnal peak and anomalous MPC (25.9 particles/m^3), completely absent in TV. Conversely, during the working hours (from 9:00 to 19:00 p.m.), the two sites show a very similar situation, with maximum equal to 19.0 particles/m^3 at MPC and 10.6 particles/m^3 at TV.

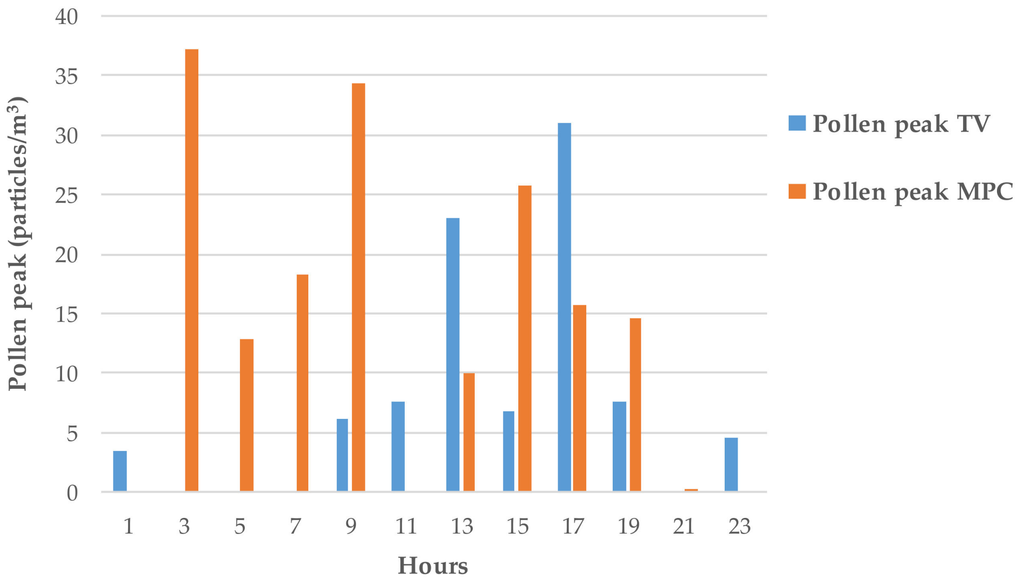

Figure 8. Mean pollen peak during the day in TV and MPC.

Considering the time of the day when peaks occur, during working activities the most critical time range is from 1:00 to 5:00 p.m. for both MPC and TV (Figure 8).

This result shows how the two sites respond differently to the solicitation related to the emission sources and to the presence of turbulence.

As reported by other papers, the peaks can occur at all hours of day and their variations depend on a complex effect of meteorological factors and distance from the pollen source [50,56]. Analysis of two hours peak resolution have shown differences in relation to areas of sampling due to pollen transport dynamics, with the highest pollen concentrations during daylight hours and maximum intradiurnal values recorded usually between 12 and

14 a.m. and early afternoon [25,26,48,51,53]. Furthermore, several authors have shown that pollen concentrations are higher in rural than in urban areas [36].

3.5.2. Six Hours Resolution Analysis

Pollen concentration maxima are also considered for the following time ranges of the day (6-h averaged data).

Figure 9 clearly shows how MPC is affected by pollen peaks during the night (Pmax (MPC) = 17.3 particles/m^3) and in the morning (Pmax (MPC) = 14.9 particles/m^3), while for TV peaks occur in the afternoon, with concentration value of 12.0 particles/m^3, very close to the value registered in MPC (10.4 particles/m^3).

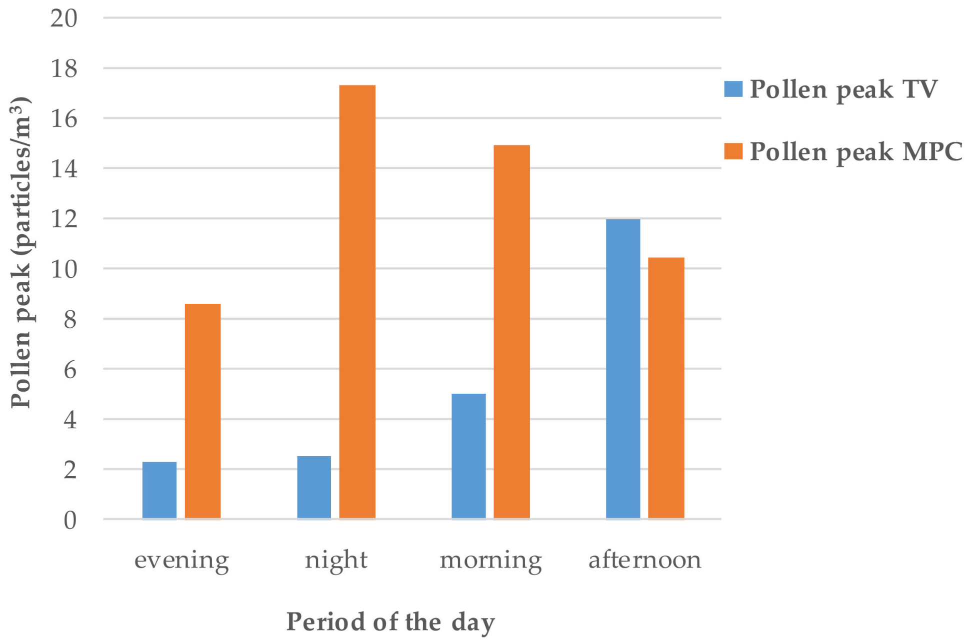

Figure 9. Mean pollen peak during the daily time ranges (evening, night, morning and afternoon) in TV and MPC.

As stated before, in the literature, the daily pattern distribution of pollen has been investigated and several authors have highlighted the presence of high values of pollen in the morning (06:00–12:00 a.m.) and in the afternoon (12:00–18:00 p.m.) [40,47,48].

3.6. Evaluation of I/O Ratios for TV and MPC

The I/O ratio indicates the presence of the indoor pollen we can expect in relation to the outdoor pollution. This relationship is based on the assumption of an instantaneous correspondence between external and internal pollution, but this is, especially for a short time range, not always true; thus, to obtain more reliable results, data averages (2-h, 6-h and 24-h averages) are considered.

In Figure 10, the evolution during the day of the mean I/O ratio for the two sites is shown. During the night and in the early morning (from 7:00 p.m. to 9:00 a.m.), TV and MPC exhibit similar values, slightly higher for TV (0.06 and 0.02, respectively). Instead, during the working hours (from 11:00 a.m. to 5:00 p.m.), values observed for TV and MPC are very different. TV is characterized by higher values of the ratio (mean I/O TV = 0.40) compared to MPC (mean I/O MPC = 0.03). As reported in the previous section, it should be kept in mind that, at MPC, the outdoor pollen concentrations are higher than those observed at TV.

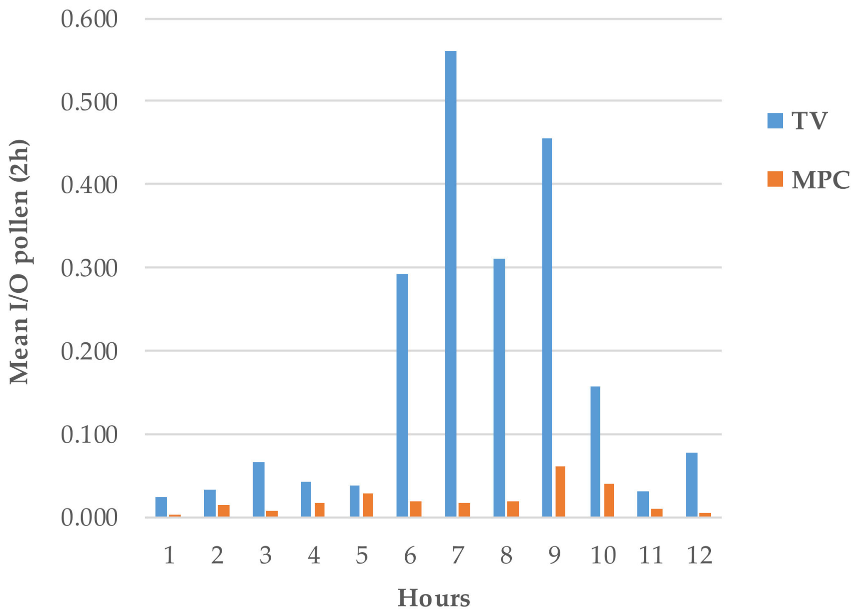

Figure 10. Daily evolution of the mean I/O ratio (two hours average) in TV and MPC.

In this regard many studies have calculated the I/O ratio in different environments and conditions in relation to pollen types and seasons, such as *Betula* pollen from February to April (I/O = 0.01–0.22 and I/O 0.02–0.88) [66,68], cedar pollen in early spring (I/O = 0.02–0.19) [77], and Poaceae, *Quercus*, *Olea*, *Plantago*, *Cupressus* from April to March (I/O = 0.075 and 0.917) [78].

To evaluate the differences observed in the I/O ratios for the two sites, worker habits, as highlighted in Table 6; have been taken into account; data on these habits are obtained by means of a survey form [19,20] filled in by workers, reporting any changes in occupant numbers present in the room and the indication of open windows or doors (in minutes). Data are then averaged.

Table 6. Mean worker behavior (number of workers, opening time of doors and windows) for TV and MPC in two different time ranges (night and early morning, afternoon).

	Occupants		Opening Door		Opening Window	
	TV	**MPC**	**TV**	**MPC**	**TV**	**MPC**
19:00–09:00	0.03	0.03	0.15	2.81	0.00	1.11
11:00–17:00	0.48	0.39	1.18	33.16	4.99	12.24

While the conditions, comparing the two sites, do not vary with time during the evening and night, during the working hours MPC habits are profoundly different; doors and windows in MPC are often open, in comparison with TV (33.16% versus 1.18% for the door and 12.24% versus 4.99% for windows) showing that ventilation conditions are fundamental for the determination of the I/O ratio and that differences between the two sites are principally ascribable to habits rather than to the outdoor pollen concentrations.

In Figure 11 the I/O ratios for the different time range of the day (the 6-h averages) are shown; data are profoundly different in the afternoon while the two sites exhibit similar values for the rest of the day. If we consider the daily mean, values remain very close (I/O TV_{24h} = 0.09 and I/O MPC_{24h} = 0.02) and the slight difference could be attributable to the different habits and behaviors inside the rooms.

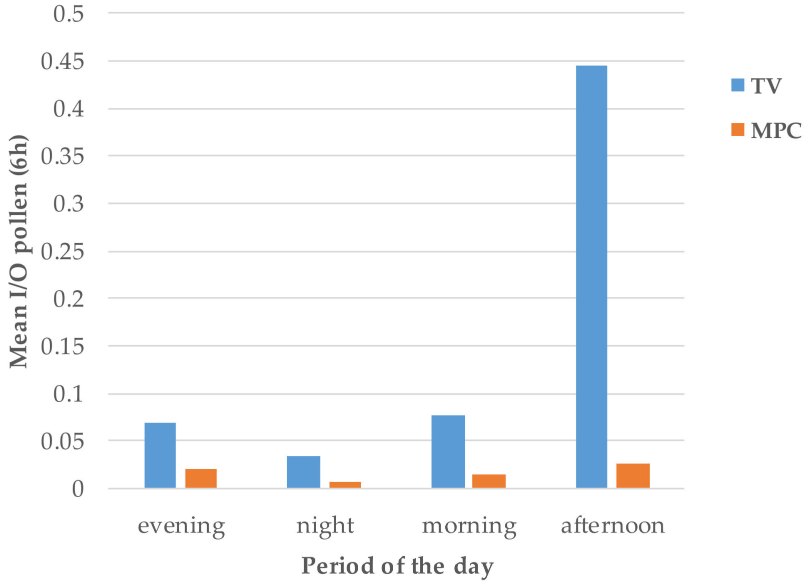

Figure 11. Mean I/O ratio (6-h average) during the daily time ranges (evening, night, morning and afternoon) in TV and MPC.

4. Conclusions

Bioaerosol exposure derived by natural and anthropogenic sources has assumed greater importance in recent years [5]. Exposure to pollen biocomponents can be responsible for several health effects and knowledge of their levels, interactions with other components, modification in relationship with the presence and absence of occupants, as well as their diffusion in several environments can be useful in improving the evaluation and management of this biological risk.

The concern in urban environments with respect to rural ones is peculiar causing different health consequences, also due to interactions with pollutants of different origins, and with urban greening that can be associated with increased exposure to pesticides and allergenic pollen, although this evidence is weaker [79,80]. On the other hand, health benefits due to the presence of urban green spaces and infrastructures have been reported, including the attenuation of indoor temperatures and heat islands, the improving of air quality, the reduction of temperature and air pollution [81] and a lack of vegetation associated with a higher risk of heat-related mortality [82]. In addition, Sustainable Development Goal 3 of the 2030 Agenda for Sustainable Development is to "ensure healthy lives and promoting well-being for all at all ages". Moreover, another target is to reduce the number of deaths and illnesses from hazardous chemicals and pollution (https://sdgs.un.org/topics/health-and-population accessed on 3 June 2021), and goal 11 aims to "Make cities and human settlements inclusive, safe, resilient and sustainable" (https://sdgs.un.org/goals/goal11 accessed on 3 June 2021).

It is essential to improve pollen monitoring techniques and to implement automatic methods, favoring the use of personal pollen samplers and the application of molecular methodologies [83], and taking into account the role of occupants as a potential cause of indoor pollen level variation, as highlighted in previous studies [19,66,67] and in the current study, conducted in two different occupational settings. The important role of human behavior in pollen diffusion and/or retention has also been evidenced by experimental studies, where a mean of 0.93% of the initial pollen load was retained after a single wash of hands and traces of several species were found after numerous hand-wash cycles [84]. Moreover, the knowledge of pollen hourly peaks creates the opportunity to plan appropriate preventive measures and recommendations, addressed mainly to the sensitization of workers [85].

We suggest that different items should be considered in order to evaluate health effects derived by pollen exposure, such as urbanization and, conversely, vegetation areas [37,38,86], implementing predictive models in order to reduce pollen exposure and understand the complex interactions between allergens and other particles, in order to investigate the genesis of allergy and develop new preventive and therapeutic strategies [6,87], in relationship also to death due to respiratory and cardiovascular diseases [88] and to health costs [59].

The promotion of synergic and integrated approaches is needed [22], coupling data derived by individual experimental and epidemiological studies in order to optimize preventive strategies considering the most peculiar variables belonging to several occupational settings.

The knowledge of the spatial and temporal dynamics of pollen intradiurnal variations is indispensable to plan in advance and implement effective prevention strategies [26,27,35,49,53]. In this respect it is fundamental to increase the awareness of workers regarding the risk derived from exposure to allergens and to define action mechanisms, control and corrective measures such as limitation of outdoor activities, reducing the time of exposure to allergenic pollen in the peak periods, and the taking of biological vaccines and drugs before the pollen season begins. Furthermore it is important to promote the use of new techniques (i.e., GPS, remote sensing technology) to identify areas free of allergens and progressively remove allergenic taxa of ornamental plants to prevent high pollen concentrations in urban environments [27,36,38,61].

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