

Comparison between the counting methods used by two aerobiology networks in southern Europe (Spain and Italy)

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Abstract Aerobiology studies the biological particles suspended in the atmosphere in order to know what types are present in the air and how their concentrations vary spatially or seasonally. The measurement of airborne pollen concentration requires a standardized methodology. Normally, the analysis of the slides obtained after samplings is carried out according to national network procedures. Different networks use different slide counting methods (longitudinal sweeps, transverse sweeps or random fields), and a different number of lines/fields are routinely read. In this study, we compared two slide counting methods adopted by two monitoring centres belonging to different aerobiology networks that operate in southern Europe, the University of Malaga (Spanish Aerobiology Network, the REA) and the University of Roma Tor Vergata (Rete Italiana di Monitoraggio in Aerobiologia, the RIMA). For that, the same samples were counted following the two methodologies consisting on the reading of four (REA) and six (RIMA), respectively, longitudinal sweeps at a magnification of 400X (REA). Statistical analysis was performed in

order to search the degree of association between the two series of data obtained, as well as whether or not there were significant differences between them. The results showed highly significant correlation and regression coefficients. On the other hand, Wilcoxon tests, in general, did not showed significant differences between the series for the total pollen as well as for the major pollen types. This confirms that the two methods can be used indistinctly although it would be convenient for the different networks to standardize and unify methodologies in order to follow the same operating procedures.

Keywords Aerobiological monitoring · Counting methods · Pollen counts · Statistical analysis

1 Introduction

Aerobiology is the science that studies the biological particles present in the air, their atmospheric dispersion and their impact on both environment and organisms. Moreover, the main purpose for aerobiology is to measure and report the quantities of airborne pollen present in the atmosphere. Currently, many aerobiological monitoring networks are using Hirst-type volumetric pollen traps (Hirst 1952). Despite this, the different networks adopt different protocols to estimate the atmospheric pollen concentration. So, the slide reading methodology (longitudinal or transverse

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sweeps, or random fields) and the number of lines/fields counted can differ from one to another. The International Association for Aerobiology suggests following a standardized methodology based on the minimum requirements: reading of 3 longitudinal sweeps at a magnification of 400X or, at least, 12 vertical sweeps per slide (Jäger et al. 1995). In fact, the measurement of the pollen atmospheric concentrations is just an approximation of the real value (Comtois et al. 1999), and it may be subject to diverse errors during the different operational phases (Mandrioli 2000), such as errors due to the instruments, to preparation of samples, errors carried out during the identification of particles, or in the calculation of the atmospheric concentration.

In this study, we compare the results obtained by using two different counting methods, adopted by two monitoring centres, belonging to different aerobiology networks that operate in southern Europe, the University of Malaga (Spanish Aerobiology Network, the REA) and the University of Roma Tor Vergata (Rete Italiana di Monitoraggio in Aerobiologia, the RIMA).

The purpose of this study is to search whether or not the different methods of counting, with different sampling surfaces observed, can be cause of significant differences in the results obtained, once all of the other factors have been controlled by using standard procedures.

2 Materials and methods

The study was carried out at the laboratory of the Department of Biology of the University of Roma *Tor Vergata*, Italy. The samples were obtained by using a Hirst volumetric pollen trap (Hirst 1952), model 2000 VPPS Lanzoni, placed on the roof of the Faculty of Science at a height of 15 m above ground level.

A total of 40 slides, corresponding to 40 sampling days of the year 2015, were examined. The slides used were randomly selected among the four seasons, in periods of 10 consecutive days: winter (February 04–13), spring (April 02–11), summer (September 07–16) and autumn (October 26–November 04). The slides were observed under a light microscope Nikon Eclipse 200 at a magnification of X400. Pollen types were identified following the recent works published

in Italy (Travaglini et al. 2014), and the counts were expressed as number of pollen grains/m³ of air (Mandrioli 1996).

Pollen counting was made by using two different methods. The first one was followed by the Spanish Aerobiology Network (REA) (Galán et al. 2007), while the second one was adopted by the Rete Italiana di Monitoraggio in Aerobiologia, RIMA, (Mandrioli 1994; UNI 2004; Travaglini et al. 2009).

The Spanish Aerobiology Network (REA) requires that the area analysed represents at least 10 % of the whole slide (according to European Aeroallergen Network rules), the counting method consisting in reading 4 continuous horizontal sweeps per slide at a magnification of 40×. The 4 horizontal sweeps are marked by the technician with dots and distributed in the slide, with at least 1 mm of distance from the edge of the impacted surface (Galán et al. 2007).

However, the Italian standard methodology requires that the minimum number of horizontal sweeps must correspond to an area close to 20 % of the impacted surface (UNI 2004). In this case, the method consists in the reading of 6 horizontal sweeps per slide at a magnification of 400×, the sweeps spaced 2 mm from each other as well as from the edge of the sampling surface (UNI 2004; Travaglini et al. 2009).

For the statistical analysis, daily mean pollen concentrations of each taxon were used, as well as the total pollen counts for the entire period (40 days). Only pollen types observed during the two counting method, with daily mean pollen concentrations ≥ 1 grain/m³, would be considered.

Data normality was evaluated by the Shapiro–Wilk test (W). Since the data did not adjust to normal distributions, nonparametric statistic tests were applied. To evaluate the relationships between the results obtained by the two counting methods, Spearman's correlation coefficients were calculated, while Wilcoxon test was used in order to search whether or not differences observed were significant. Additionally, regression analyses were also carried out. The statistical software package used was IBM SPSS 21.0.

3 Results

During this study, the most abundant pollen types found in the atmosphere of Rome for the days studied were

Corylus, *Urticaceae* and, especially, *Cupressaceae* in winter. In spring, many pollen types entered to form part of the atmospheric spectrum, especially those coming from arboreal species, such as *Betulaceae*, *Cupressaceae*, *Fraxinus*, *Morus*, *Pinus*, *Platanus*, *Populus* and *Quercus*. However, *Urticaceae* was the type with a highest amount of pollen during the days selected for the study. In summer, the pollen types from herbaceous plants are the most abundant, mainly *Urticaceae* and *Amaranthaceae*. In autumn, the concentrations are very low, with only 3 pollen types exceeding the sum of 10 pollen grains/m³ during the period studied: *Urticaceae*, *Mercurialis* and *Amaranthaceae* (Fig. 1).

In Fig. 1 is shown how pollen counts for the different taxa, according to the different counting methods, yielded similar results. The same occurred when all the daily pollen counts were taken as a whole (Fig. 2). In Table 1, the results of the statistical

analysis are given. Spearman's correlation tests carried out between the daily pollen means values of the two series (REA and RIMA) for the four seasons showed positive and highly significant coefficients in all cases. A similar behaviour presented the results of the regression analysis, with high coefficients and positive slopes.

Regarding Wilcoxon tests, significant differences can be observed for the data grouped by seasons, specifically in spring and autumn. Additionally, we applied the same test to the data obtained for the different pollen types when they appeared in two or more seasons, and therefore, we had 20 or more data for running the statistical analysis (Table 2). In this case, only for *Cupressaceae* and *Urticaceae*, the most abundant pollen types, as well as for *Pinus* and *Plantago*, we did not obtain significant differences, at

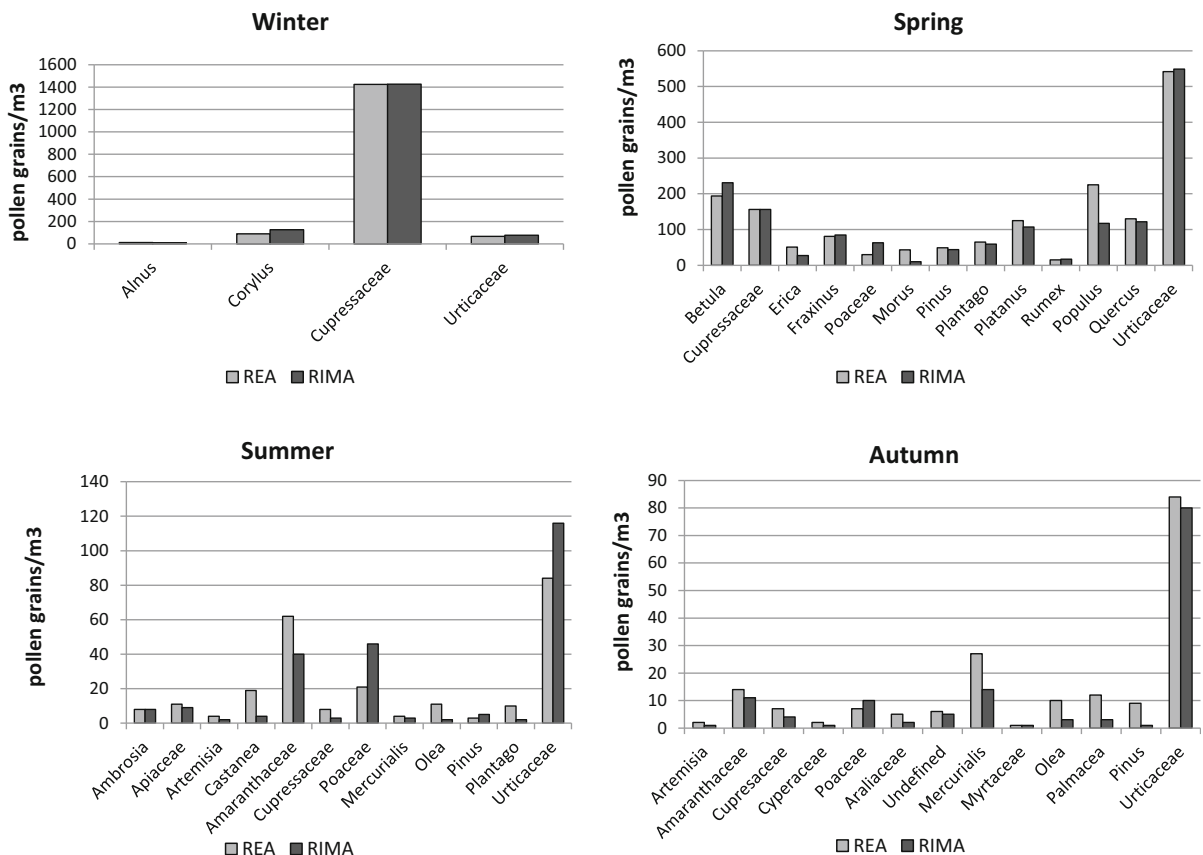


Fig. 1 Levels reached by the pollen types recorded in the days of study for the four seasons of the year, using the methodology proposed by the two aerobiology networks (REA and RIMA)

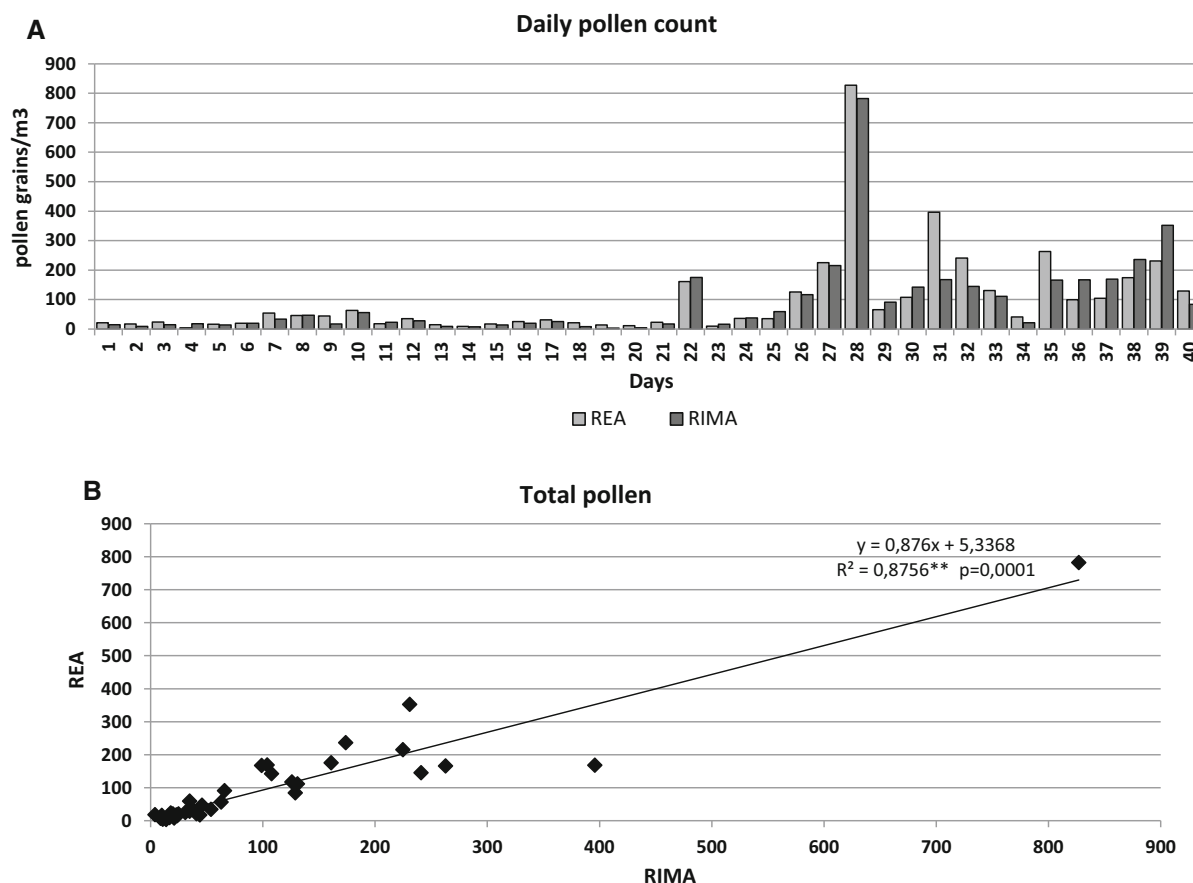


Fig. 2 Comparison of daily total pollen between REA and RIMA methodologies during the 4 studied periods of 10 days. **a** Daily mean concentrations **b** regression line

Table 1 Results of the statistical analysis carried out between the pollen concentrations estimated by the two counting methods for the different seasons, as well as the total period

REA vs RIMA	Spearman's test		Regression analysis			Wilcoxon's test	
	<i>r</i>	<i>p</i>	<i>a</i>	<i>b</i>	<i>R</i> ²	<i>Z</i>	<i>p</i>
Winter (<i>n</i> = 50)	0.919	0	1.000	6.893	0.876	−0.969	0.332
Spring (<i>n</i> = 140)	0.728	0	0.760	4.131	0.587	−2.214	0.027
Summer (<i>n</i> = 120)	0.609	0	0.867	0.229	0.649	−1.314	0.189
Autumn (<i>n</i> = 140)	0.665	0	0.930	−0.294	0.771	−4.519	0.000
Total (<i>n</i> = 450)	0.773	0.000	1.031	−0.018	0.960	−0.847	0.397

a significance level of 95 % ($P \leq 0.05$). Nor we found significant differences regarding total pollen.

4 Discussion

Partial reading is a method followed by all the aerobiology networks for estimating the amounts of pollen grains contained in the whole sample, by means of a

correction factor whose value is determined by the surface observed. This procedure allows the aerobiologists to save time and, therefore, release their results in a time record. The problem is to establish the extension of the surface to observe, a balance between observed and estimated data. Obviously, the larger the surface observed, the smaller the error made. This error depends also on the density of the sample (Gottardini et al. 2009; Micheli et al. 2009): low pollen concentration

Table 2 Results of statistical analysis carried out between the series obtained for the different pollen types, as well as the total period

Pollen types	Spearman's test		Wilcoxon's test	
	<i>r</i>	<i>p</i>	<i>Z</i>	<i>p</i>
Amaranthaceae (<i>n</i> = 20)	0.658	0.002	−2.193	0.028
<i>Artemisia</i> (<i>n</i> = 20)	0.675	0.001	−2.388	0.017
Cupressaceae (<i>n</i> = 40)	0.958	0.000	−0.24	0.81
<i>Mercurialis</i> (<i>n</i> = 20)	0.675	0.001	−2.388	0.017
<i>Olea</i> (<i>n</i> = 20)	0.454	0.045	−3.358	0.001
<i>Pinus</i> (<i>n</i> = 30)	0.543	0.002	−1.271	0.204
<i>Plantago</i> (<i>n</i> = 20)	0.669	0.001	−1.175	0.24
Poaceae (<i>n</i> = 30)	0.539	0.002	−3.001	0.003
Urticaceae (<i>n</i> = 40)	0.847	0.000	−0.792	0.428
Total (<i>n</i> = 240)	0.794	0.000	−0.819	0.413

determines a greater error (Gottardini et al. 2009; Onorari et al. 2007; Cariñanos et al. 2000; K  pyla and Penttinen 1981).

In Italy, some authors state that readings of 20 % of the total sampling surface widely ensure significant measurements (Felicori et al. 2012), this being the methodology followed by the RIMA. Nevertheless, other authors estimate a degree of uncertainty, with an error rate ranging from 7 to 52 % (Gottardini et al. 2009). On the other hand, the Spanish aerobiology network, the REA, considers that by reading a 12 % of the total sample the error is assumable, with a variation coefficient around 10 % (Gal  n et al. 2007; Oteros et al. 2013). Anyway, bias not only arises from the counting methods used. In fact, it can be linked with other methodological issues, such as operational and technician errors or those due to a bad identification or, even, to random distribution of the pollen grains (Comtois and Mandrioli 1997; Comtois et al. 1999; Gal  n and Dom  nguez Vilchez 1997).

The methodologies used by both networks, the Spanish and the Italian, for counting the pollen grains present a high degree of similarity, and they are coherent with the minimum recommendations proposed by the European Aerobiology Society: at least 10 % of the slide should be read (Gal  n et al. 2014).

In general, we have observed a high degree of similarity between the two series of data studied, with Spearman's correlation coefficients highly significant and regression coefficients with positive slope and also significant values for all the taxa, as well as for the

seasonal periods (Tables 1 and 2). However, these results were not always followed by the Wilcoxon tests and, although they showed no significant differences for total pollen and for the more abundant taxa (*Cupressaceae* and *Urticaceae*), there were other minority pollen types for which significant differences appeared. They were pollen types that hardly reached values of 50 as sum of the daily mean concentrations for the period considered. In fact, 11 of 14 taxa considered showed lower values than 15 grains/m³. Regarding this, K  pyla and Penttinen (1981) considered that the amount of particles observed in longitudinal sweeps should be about 50 or more for reliable estimation of the short-term concentrations.

If we compare the results obtained by using the two methods, reading 4 or 6 sweeps, we can observe how there is an increased number of cases in which we obtained higher values by reading 4 sweeps (REA method). This could be due to the correction factor, which is greater the smaller the number of sweeps (Figs. 1, 2).

In our opinion, we can conclude that the results obtained are quite similar and both methods can be used indistinctly. Accuracy is important but also is important to release the results as soon as possible, and the difference in the time spent in counting the pollen content in the samples by reading 4 or 6 sweeps can be considerable. On the other hand, due to the number of national networks of aerobiology is growing, it is necessary to standardize the techniques of sampling and analysis of biological particles in order to apply the same methodology and the same operating procedures (Thibaudon et al. 2014). In fact, an agreement about the methodology in use is especially important for ensuring the quality and comparability of the results obtained by the different aerobiology stations (Zieger 2007).

References

- Cari  n  s, P., Emberlin, J., Gal  n, C., & Dominguez-Vilches, E. (2000). Comparison of two pollen counting methods of slides from a hisrt type volumetric trap. *Aerobiologia*, 16, 339–346.
- Comtois, P., Alc  zar, P., & Neron, D. (1999). Pollen count statistics and its relevance to precision. *Aerobiologia*, 15, 19–28.
- Comtois, P., & Mandrioli, P. (1997). Pollen capture media: a comparative study. *Aerobiologia*, 13, 149–154.

- Felicori, M., Bordignon, G., & Billi, B. M. (2012). Migliora la qualità del monitoraggio aerobiologico. *Ecoscienza*, 2, 76–77.
- Galán, C., Cariñanos, P., Alcázar, P., & Domínguez-Vilches, E. (2007). *Spanish Aerobiology Network (REA): Management and quality manual*. Córdoba: Servicio de publicaciones, Universidad de Córdoba.
- Galán, C., & Domínguez-Vilches, E. (1997). The capture media in aerobiological sampling. *Aerobiologia*, 13, 155–160.
- Galán, C., Smith, M., Thibaudon, M., Frenguelli, G., Oteros, J., Gehrig, R., et al. (2014). Pollen monitoring: minimum requirements and reproducibility of analysis. *Aerobiologia*, 30, 385–395.
- Gottardini, E., Cristofolini, F., Cristofori, A., Vannini, A., & Ferretti, M. (2009). Sampling bias and sampling errors in pollen counting in aerobiological monitoring in Italy. *Journal of Environmental Monitoring*, 11, 751–755.
- Hirst, J. M. (1952). An automatic volumetric spore trap. *Annals of Applied Biology*, 39, 257–265.
- Jäger, S., Mandrioli, P., Spijksma, F., Emberlin, J., Hjelmroos, M., Rantio-Lehtimäki, A., et al. (1995). News. *Aerobiologia*, 11, 69–70.
- Käpylä, M., & Penttinen, A. (1981). An evaluation of the microscopical counting methods of the tape in Hirst-Burkard pollen and spore trap. *Grana*, 20, 131–141.
- Mandrioli, P. (1994). Metodica di campionamento e conteggio dei granuli pollinici e delle spore fungine aerodisperse. Monitoraggio aerobiologico in Emilia Romagna. *Collana Prevenzione nei luoghi di vita e di lavoro. Contributi (Biblioteca Antonio Panizzi)*, 30, 9–19.
- Mandrioli, P. (1996). Il monitoraggio aerobiologico: linee guida per una standardizzazione. *Atti del 15° Congresso Nazionale AIDII*, Collana della Fondazione Maugeri, I Documenti n.6, pp 30–41.
- Mandrioli, P. (2000). Metodo di campionamento e conteggio dei granuli pollinici e delle spore fungine aerodisperse. Progetto di norma proposto alla Commissione Ambiente UNI dal Dr. Paolo Mandrioli codice progetto ufficiale U53000810. *Consiglio Nazionale delle Ricerche—Istituto di Scienze dell'Atmosfera e dell'Oceano*.
- Micheli, V., Brighetti, M. A., Capucci, E., & Travaglini, A. (2009). Osservazioni sulla superficie minima di lettura nel campionamento aerobiologico. *A.I.A. Atti del XII Congresso Nazionale "Salute dell'ambiente salute dell'uomo"*. Firenze 7-9 maggio 2009.
- Onorari, M., Domeneghetti, M. P., & Begliomini, V. (2007). Metodo UNI 11108:2004—Percorso per l'accreditamento. *Gea*, 3, 33–34.
- Oteros, J., Galán, C., Alcázar, P., & Domínguez-Vilches, E. (2013). Quality control in bio-monitoring networks, Spanish Aerobiology Network. *Science of the Total Environment*, 443, 559–565.
- Thibaudon, M., Monnier, S., & Berger, U. (2014). Standardization of Hirst method for airborne pollen and fungal spores measurements. "Meeting of the European Aeroallergen Network and the European Aerobiology Society". Vienna; p. 47.
- Travaglini, A., Albertini, R., & Zieger, E. (2009). Manuale di gestione e qualità della Rete Italiana di Monitoraggio in Aerobiologia R.I.M.A.[®]. Tipografia LEGO, Bologna, p.147.
- Travaglini, A., Brighetti, M.A., Arsieni, A., & Vinciguerra, F. (2014). Atlante del polline delle principali specie allergiche d'Italia. Il raggio verde edizioni. ISBN: 978-88-89663-79-0.
- UNI Ente Nazionale Italiano di Unificazione. (2004). Qualità dell'Aria metodo di campionamento e Conteggio dei granuli pollinici e delle spore fungine aerodisperse. *Classificazione ICS*, 13(040), 20.
- Zieger, E. (2007). Linee guida e accreditamento in Aerobiologia. *Gea*, 3(1), 32–33.