The effect of changes to the method of estimating the pollen count from aerobiological samples

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Pollen data have been recorded at Novi Sad in Serbia since 2000. The adopted method of producing pollen counts has been the use of five longitudinal transects that examine 19.64% of total sample surface. However, counting five transects is time consuming and so the main objective of this study is to investigate whether reducing the number to three or even two transects would have a significant effect on daily average and bi-hourly pollen concentrations, as well as the main characteristics of the pollen season and long-term trends. This study has shown that there is a loss of accuracy in daily average and bi-hourly pollen concentrations (an increase in % ERROR) as the sub-sampling area is reduced from five to three or two longitudinal transects. However, this loss of accuracy does not impact on the main characteristics of the season or long-term trends. As a result, this study can be used to justify changing the sub-sampling method used at Novi Sad from five to three longitudinal transects. The use of two longitudinal transects has been ruled out because, although quicker, the counts produced: (a) had the greatest amount of % ERROR, (b) altered the amount of influence of the independent variable on the dependent variable (the slope in regression analysis) and (c) the total sampled surface (7.86%) was less than the minimum requirement recommended by the European Aerobiology Society working group on Quality Control (at least 10% of total slide area).

Introduction

Aerobiologists often monitor and report quantities of airborne pollen for use by allergy sufferers so that they can plan their medication and activities in advance, medical professionals who plan treatment and schedule clinical trials and those who produce and stock health care products.[1] The Hirst type volumetric spore trap[2] is currently the most commonly used method of sampling airborne pollen. The samples collected using this method are analysed by trained palynologists using light microscopes.

Many consumers of aerobiological data require the product to be supplied to them in almost real time. As a result, aerobiologists must decide upon which counting method to use and the main objective is to achieve the maximum amount of precision for the minimum amount of work. Comtois *et al.*[3] suggested that there will always be imprecision linked with the airborne pollen count unless aerobiologists count the whole slide, and there is always a trade off between precision and the amount of time required to produce the daily pollen count.

There are three sub-sampling methods that analyse a fraction of a 24 hour slide: the random field method,[4] the longitudinal (horizontal) transects method,[5,6] and the latitudinal (vertical) transects method.[7,8] The most commonly used of these sub-sampling methods are the longitudinal and latitudinal transect methods. This is because the random field method, although probably the least time consuming, does not allow for the estimation of valuable short term (hourly or bi-hourly) concentrations.[3,9] As a result, the random field method has not been considered in this paper. All three of these sub-sampling procedures significantly reduce the time spent on analysis, compared with examining the whole slide, especially during periods such as spring when the spectrum of monitored airborne pollen types is high.

All of these sub-sampling methods produce the daily average pollen count, that can be expressed as pollen concentration as grains per m^3 of air. It is a product of the total area of the slide, the area of slide sampled and volume of air sampled.[8] Several authors evaluated the differences between sub-sampling

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Environmental impact

Daily and bi-hourly pollen concentrations are an important source of information for allergy sufferers so that they can plan their medication and activities in advance, medical professionals who plan treatment and schedule clinical trials and those who produce and stock health care products. Many consumers of aerobiological data require the product to be supplied to them in almost real time. As a result, aerobiologists must decide upon which counting method to use and the main objective is to achieve the maximum amount of precision for the minimum amount of work.

methods[3,10,11] and concluded that, although each method has its advantages and disadvantages, all proposed methods enable the estimation of the whole biological population of a certain volume of air. However these authors did not examine the effect of different sub-sampling methods on bi-hourly pollen counts or characteristics of the pollen season.

Daily average pollen counts have been recorded at Novi Sad in Serbia since 2000. The adopted counting method has been the use of five longitudinal transects. The five transect method was originally selected because Comtois *et al.*[3] showed that it produced optimal results, *i.e.* the least amount of error for the smallest number of transects.[3] However, counting five transects is time consuming. The main objective of this study is therefore to investigate whether reducing the number to three or even two transects would have a significant effect on the counts. The specific aims of the study are: (1) to determine whether a significant difference exists between daily average pollen concentrations of selected taxa recorded along five, three and two longitudinal transects during the Main Pollen Season (MPS) in 2009; (2) to determine whether significant differences exist between bi-hourly pollen concentrations of selected taxa recorded along five, three and two longitudinal transects during the MPS in 2009; (3) to examine whether changes to sub-sampling method in 2009 influence characteristics of the season such as the start, peak day, duration and end of the season as well as the annual sum of daily average pollen concentrations (Seasonal Pollen Index—SPI); and (4) to test whether trends in the most important season characteristics change if pollen concentrations produced using a different sub-sampling method in 2009 are introduced to the whole dataset (whether changing the method in 2009 affects trends over the full 2000–2009 period).

Materials and methods

Airborne pollen samples

Daily average and bi-hourly airborne pollen samples were collected using a 7 day volumetric spore trap of the Hirst design[2] situated in Novi Sad, Serbia (45°15′00″N, 19°51′00″, altitude 85 m) from 2000–2009. The trap is situated on the roof of the Faculty of Sciences (height above ground level 15 m). Air is sucked into the trap at a rate of 10 l min^{-1} through a 2 mm × 14 mm orifice. Behind the orifice the air flows over a rotating drum that moves past the inlet at 2 mm h^{-1} and is covered with an adhesive coated, transparent plastic tape. Particles in the air impact on the tape to give a time related sample.[12] Following its removal from the trap, the tape is divided into segments corresponding to the 24 h period (48 mm in length).[13] Each segment is mounted between a glass slide and cover slip using a mixture that contains gelatine, glycerine, phenol, distilled water and basic fuchsine.[14] Each 24 h segment is scanned by light microscopy.

Slide analysis

In Novi Sad, airborne pollen is sampled from February to November which corresponds to the active growing season and main flowering period of plants. During nine pollen seasons (2000–2008) pollen grains were identified and counted along five longitudinal transects using an Olympus BX-51 light microscope at ×400 magnification with a field of view of 0.55 mm. This corresponds to 19.64% of the sampled surface area. Cumulative pollen counts were recorded every 4 mm along the longitudinal transects. The corresponding counts from each of the five transects were added together to give bi-hourly values (4 mm = 2 h) that were later converted to pollen concentration as grains per m^3 of air. However, the data for individual longitudinal transects during the period 2000–2008 were not recorded. In 2009, pollen grains were again counted along five longitudinal transects, but the data were also recorded for: (1) three longitudinal transects corresponding to 11.79% of the total sampled area and (2) two longitudinal transects corresponding to 7.86% of the total sampled area. The distribution of longitudinal transects on the slide (Fig. 1) was chosen to eliminate potential overestimates that can arise from counting only the central regions of the slide where most of the pollen is deposited.[15]

The selection of pollen types for analysis (Table 1) aimed to include representatives of different size and different abundance, which follows the method of Comtois *et al.*[3] Besides pollen types of allergological importance (*e.g. Ambrosia*, *Betula* and Poaceae), pollen types with the combination of entomophilous and anemophilous pollination strategy (*i.e. Tilia*) have also been included in the analysis in order to represent airborne pollen which tends to cluster due to the presence of pollenkitt—a sticky substance on its surface.[16,17] In addition, airborne *Tilia* pollen could be considered both of allergological importance as it can cause allergy symptoms[18] and of economical importance because it describes the flowering season of important bee forage. Pollen grains of selected airborne pollen types were identified and counted under light microscopy at ×400 magnification. Bi-hourly and daily average pollen concentrations are expressed as grains per m^3. For example, in order to obtain daily average concentration the number of pollen grains counted in

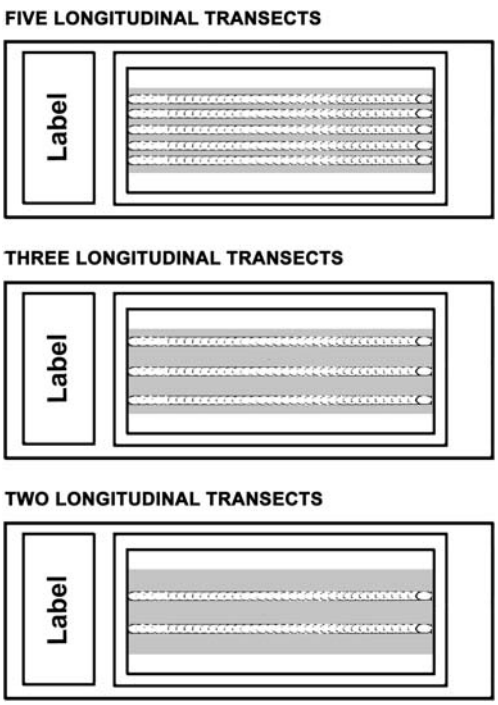

Fig. 1 Distribution of longitudinal transects along the slide in analysed sub-sampling methods.

Table 1 Analysed pollen types and their characteristics

Pollen type	Size (diameter range[a])	Pollination strategy	Abundance[b]	
			SPI	Contribution to total pollen catch
Ambrosia	Small (10–25 μm)	Anemophily	6478	14.08%
Betula	Small (10–25 μm)	Anemophily	3849	8.37%
Poaceae	Medium–large (26–100 μm)	Anemophily	1996	4.34%
Tilia	Medium (26–50 μm)	Anemophily–entomophily	208	0.45%

[a] PalDat—a palynological database: descriptions, illustrations, and identification, URL: http://www.paldat.org/. [b] Based on 10 year average.

a sub-sample are multiplied by a conversion factor (CF) resulting from the relationship between sub-sampled and total slide area[8] ($CF_{5transects} = 0.353$, $CF_{3transects} = 0.589$, $CF_{2transects} = 0.884$). The method of calculating the CF is described in literature.[8] In order to prevent potential human errors in the determination of pollen grains, all the slides have been counted by the same person.

Defining the limits of the pollen season

The start and end of the MPS were defined using the 98% method,[19] whereby the season starts when 1% of the total catch is achieved and ends when 99% is reached. This MPS is calculated in this way because it eliminates the long tails of low values at the start and the end of the seasons that may introduce bias to the results during statistical analysis. The 98% method was selected because it leaves the maximum number of data points in the analysis, and as a result it also includes the maximum amount of variation. This is because small amounts of pollen can cause the season to be extended. It is therefore more likely to show differences between the various sub-sampling methods (if any differences exist).

Statistical analysis

Based on Kolmogorov–Smirnov test, the analysis of MPS daily and bi-hourly pollen concentrations indicates the requirement of non-parametric tests as described by Sterling *et al.*[10] Spearman's correlation analysis was used to find out whether daily average and bi-hourly pollen concentrations recorded in 2009 by the three different sub-sampling methods were significantly related. Wilcoxon Signed Rank Test was used to find out whether there were significant differences in the magnitude of daily average and bi-hourly pollen concentrations recorded in 2009 using the three different sub-sampling methods.

The difference between daily and bi-hourly pollen concentrations recorded during the MPS in 2009 along five longitudinal transects and those recorded along three or two longitudinal transects has been expressed as percentage error (% ERROR) using the formula:

$\%\ \mathrm{ERROR} = (N_5 - N_x) \times 100/N_5$ where N_5 represents counts obtained by using five longitudinal transects method and N_x represents pollen concentration obtained by using either the three or two longitudinal transects method. Higher pollen concentrations compared to five longitudinal transects result in negative % ERROR while lower pollen concentrations result in positive % ERROR.

Simple linear regression analysis was used to determine whether changing the sub-sampling method in 2009 from 5 to 3 or 2 longitudinal transects influenced 10 year trends over the entire 2000–2009 period in pollen season characteristics (SPI, MPS start, maximum concentration), expressed as the slope of the regression, standard error of the slope (SE), coefficient of determination (R^2) and the level of significance (p). In simple linear regression analysis the R^2 value can be interpreted as the proportion of variance in the dependent variable that can be accounted for by the regression equation, and the slope describes the level of influence of the independent variable on the dependent variable. The calculations were carried out using statistical software SPSS 12.0.

Results

Comparison of daily average pollen concentrations obtained by different sub-sampling methods

The results of Spearman's correlation analysis show that daily average pollen concentrations for the selected taxa recorded during the MPS using the five longitudinal transects method were significantly related ($p < 0.01$) to the pollen concentrations recorded using three and two longitudinal transects (Table 2).

The results of Wilcoxon Signed Rank Test show that the change of sub-sampling method resulted in a significant difference ($p < 0.01$) between daily average Poaceae pollen concentrations recorded during MPS, but not for the other selected pollen types (Table 3).

The % ERROR between daily average pollen concentrations recorded using five longitudinal transects during the MPS and those recorded using three and two longitudinal transects shows that the greatest amount of % ERROR occurred for pollen concentrations derived from two longitudinal transects (Fig. 2 and 3). The greatest amount of % ERROR occurred for low

Table 2 Spearman's correlation coefficients between daily average pollen concentrations recorded during the 2009 MPS using 5, 3 and 2 longitudinal transects[a]

Pollen type	5 *vs.* 3 longitudinal transects	5 *vs.* 2 longitudinal transects
Ambrosia	0.991*	0.979*
Betula	0.961*	0.961*
Poaceae	0.960*	0.949*
Tilia	0.858*	0.899*

[a] *$p < 0.01$.

Table 3 *Z* and *p*-values (in brackets) obtained by Wilcoxon Signed Rank Test performed on daily pollen concentrations recorded during the 2009 MPS using five, three and two longitudinal transects[a]

Pollen type	5 *vs.* 3 longitudinal transects	5 *vs.* 2 longitudinal transects
Ambrosia	−0.360 (0.719[NS])	−0.261 (0.794[NS])
Betula	−0.217 (0.828[NS])	−0.020 (0.984[NS])
Poaceae	−3.086 (0.002*)	−3.769 (0.000*)
Tilia	−0.684 (0.494[NS])	−0.737 (0.461[NS])

[a] $*p < 0.01$ and [NS]non-significant.

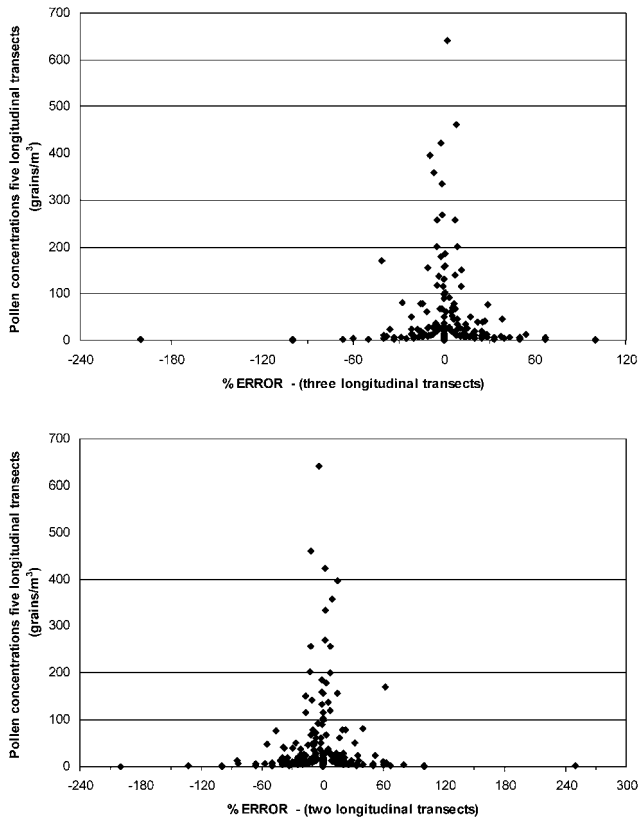

Fig. 2 Percentage error for daily average pollen concentrations recorded for three and two longitudinal transects. In these examples it is possible to see that the greatest number of pollen concentrations with error >30% were found at low values.

concentrations, and this is shown by the fact that *Ambrosia* pollen concentrations (which were generally of the highest magnitude) had the least amount of % ERROR (Fig. 3).

Comparison of bi-hourly pollen concentrations obtained by different sub-sampling methods

The results of Spearman's correlation analysis show that bi-hourly pollen concentrations for the selected taxa recorded during the MPS using the five longitudinal transects method were significantly related ($p < 0.01$) to the counts recorded using three and two longitudinal transects (Table 4).

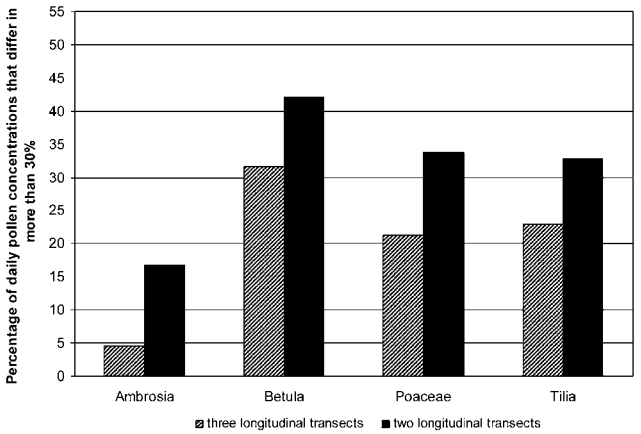

Fig. 3 Percentage of daily pollen concentrations with error greater than ±30% for all analysed pollen types.

Table 4 Spearman's correlation coefficients between bi-hourly pollen concentrations recorded during the 2009 MPS using five, three and two longitudinal transects[a]

Pollen type	5 *vs.* 3 longitudinal transects	5 *vs.* 2 longitudinal transects
Ambrosia	0.969*	0.917*
Betula	0.919*	0.842*
Poaceae	0.873*	0.822*
Tilia	0.876*	0.764*

[a] $*p < 0.01$.

The results of Wilcoxon Signed Rank Test show that changes in sub-sampling method resulted in a significant difference ($p < 0.01$) between bi-hourly Poaceae pollen concentrations recorded during the MPS, but not for any other pollen types (Table 5).

The % ERROR between bi-hourly pollen concentrations recorded using five longitudinal transects during the MPS and those recorded using three and two longitudinal transects shows that the greatest amount of % ERROR occurred for pollen concentrations derived from two longitudinal transects (Fig. 4 and 5). A greater percentage of bi-hourly than daily average pollen concentrations had % ERROR more than ±30%. This relates to the fact that the bi-hourly pollen concentrations contained more very low values, and so a difference of just a few grains per m^3 could result in % ERROR greater than ±30%.

Table 5 Z and *p*-values (in brackets) obtained by Wilcoxon Signed Rank Test performed on bi-hourly pollen concentrations recorded during the 2009 MPS using five, three and two longitudinal transects[a]

Pollen type	5 *vs.* 3 longitudinal transects	5 *vs.* 2 longitudinal transects
Ambrosia	−1.619 (0.106[NS])	−1.420 (0.156[NS])
Betula	−0.846 (0.397[NS])	−0.504 (0.615[NS])
Poaceae	−3.880 (0.000*)	−4.508 (0.000*)
Tilia	−0.387 (0.699[NS])	−0.693 (0.488[NS])

[a] $*p < 0.01$ and [NS]non-significant.

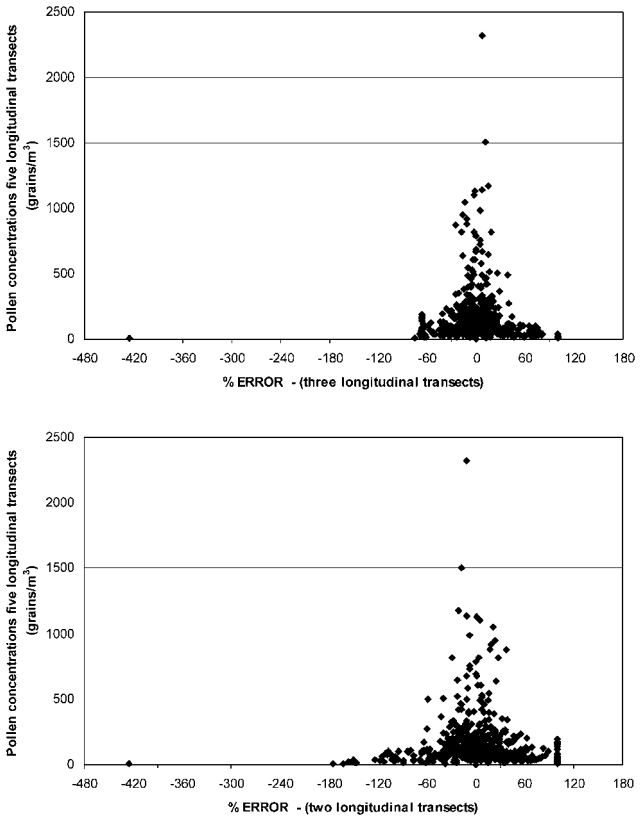

Fig. 4 Percentage error for bi-hourly pollen concentrations recorded for three and two longitudinal transects. In these examples it is possible to see that the greatest number of concentrations with error >30% were found at low values.

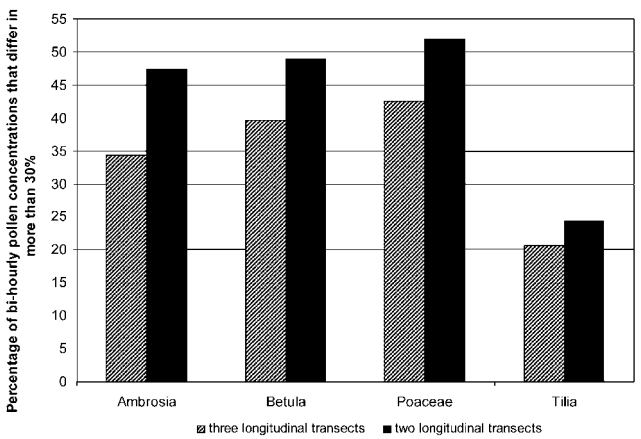

Fig. 5 Percentage of bi-hourly pollen concentrations with error greater than ±30% for all analysed pollen types.

Comparison of the main characteristics of the pollen season calculated using different sub-sampling methods

Changes in the sub-sampling procedure did not cause notable changes to the main characteristics of the pollen season described in Table 6, SPI, start, end and duration of the MPS, peak day, and the number of days > 0.

Table 6 The differences in the most important characters describing 2009 airborne pollen seasons if different sub-sampling methods are used (5 longitudinal transects = 19.64% total sample, 3 longitudinal transects = 11.79% total sample, 2 longitudinal transects = 7.86% total sample). MPS defined using 98% method[a]

Pollen type	5 longitudinal transects	3 longitudinal transects	2 longitudinal transects
Ambrosia			
SPI	5844	5967	5713
MPS start–end	213–275	213–274	212–277
Peak day	242	242	242
Number of days > 0	99	110	109
MPS duration	63	62	66
Betula			
SPI	2013	1996	2070
MPS start–end	93–129	93–130	94–130
Peak day	97	97	97
Number of days > 0	48	59	48
MPS duration	37	38	37
Poaceae			
SPI	1759	1671	1926
MPS start–end	112–269	111–270	111–270
Peak day	132	132	132
Number of days > 0	177	177	176
MPS duration	158	160	160
Tilia			
SPI	255	255	276
MPS start–end	147–203	144–206	143–203
Peak day	166	165	166
Number of days > 0	45	56	42
MPS duration	57	63	61

[a] Note: peak day and MPS start and end presented as number of days from 1st January.

Effect of changes in sub-sampling method on trends in pollen season characteristics over time

Reducing the number of longitudinal transects from five to three or two transects in 2009 did not make a notable difference to the regression coefficient of determination for the entire 2000–2009 period. However, there were several noteworthy changes to the slope. The largest changes in slope were for Poaceae and *Tilia* pollen concentrations recorded using two longitudinal transects (Table 7).

Discussion

Sources of error in aerobiological monitoring include random error, systematic bias and instrument reading error. The latter includes the human error during counting and identifying pollen grains and the fact that slide reading is also sampling and that the final concentration is only an approximation of the true value. There is always a general trend of increasing estimation error with decreasing percentage of the slide read and so the main disadvantage of sub-sampling is that only a small proportion of the daily sample is analysed.[3]

Obviously the most accurate method would be to count pollen on the entire surface of the 24 h sample. However from a routine pollen monitoring point of view, in the context of producing data for forecasting and informing the public about allergy risk, this would be unacceptably time-consuming. The main goal of this study was to find out whether a reduction in the number or transects (and subsequent reduction in sampled area) would

Table 7 10 year trends in certain characteristics of the pollen seasons of selected taxa; the annual sum of daily average pollen counts (SPI), start date of the main pollen season (MPS start), maximum daily pollen concentrations. Pollen during 2000–2008 were counted along five longitudinal transects, with the addition of the following: (A) 2009 counted using five longitudinal transects, (B) 2009 counted along three longitudinal transects, and (C) 2009 counted along two longitudinal transects

	SPI			MPS start			Maximum daily pollen concentration		
	A	B	C	A	B	C	A	B	C
Ambrosia									
Mean	6477.50	6489.80	6469.40	216.40	216.40	216.30	670.50	669.20	672.70
SD	2890.954	2888.219	2894.438	4.0006	4.0006	4.111	529.785	529.882	529.695
Slope	−121.994	−115.285	−129.139	0.279	0.279	0.224	−33.655	−34.364	−32.455
SE	334.824	334.799	334.900	0.457	0.475	0.473	60.711	60.672	60.781
R^2	0.016	0.015	0.018	0.044	0.044	0.027	0.037	0.039	0.034
p	0.725	0.739	0.710	0.559	0.559	0.648	0.594	0.587	0.608
Betula									
Mean	3849.20	3847.60	3855.00	81.50	81.50	81.60	664.10	660.40	669.70
SD	3123.399	3124.44	3119.66	10.201	10.201	10.330	480.635	482.511	478.326
Slope	482.121	481.248	485.285	0.055	0.055	0.109	66.067	64.048	69.121
SE	322.453	322.755	321.365	1.191	1.191	1.206	51.035	51.595	50.227
R^2	0.218	0.217	0.222	0.000	0.000	0.001	0.173	0.162	0.191
p	0.173	0.174	0.169	0.965	0.965	0.930	0.232	0.250	0.206
Poaceae									
Mean	1995.70	1988.10	2013.60	115.60	115.50	115.50	95.60	95.20	96.10
SD	505.318	510.024	498.656	5.967	6.042	6.042	31.718	32.086	31.324
Slope	8.212	4.067	17.976	−0.836	−0.891	−0.891	−0.606	−0.824	−0.333
SE	58.937	59.541	57.883	0.631	0.631	0.631	3.698	3.735	3.6565
R^2	0.002	0.001	0.012	0.180	0.199	0.199	0.003	0.006	0.001
p	0.893	0.947	0.764	0.222	0.196	0.196	0.874	0.831	0.930
Tilia									
Mean	208.20	208.20	210.30	142.60	142.30	142.20	29.30	28.30	30.90
SD	122.466	122.466	123.533	11.462	11.373	11.361	22.588	22.231	24.030
Slope	0.970	0.970	2.115	−0.436	−0.600	−0.655	0.176	−0.370	1.048
SE	14.297	14.297	14.406	1.330	1.311	1.306	2.637	2.593	2.781
R^2	0.001	0.001	0.003	0.013	0.026	0.030	0.001	0.003	0.017
p	0.948	0.948	0.887	0.751	0.659	0.630	0.948	0.890	0.716

make a difference to the quality of data produced, and to what degree could the sub-sampling area be decreased to make the job of pollen counting more efficient. The analyses of daily average pollen concentrations recorded using different sub-sampling techniques show that the results remain significantly related. There are, however, significant differences in the magnitude of daily average and bi-hourly concentrations of Poaceae pollen recorded using the different methods. Poaceae pollen is an important aeroallergen[20] and so any differences in concentration are important from an allergological point of view especially, if the aim of any study is to examine the relationship between exposure and symptoms.

Comtois *et al.*[3] stated that pollen concentrations can differ by some 30% by chance alone, which is why it was deemed important to examine the number of counts with % ERROR > 30% as this would indicate whether there had been a loss of accuracy by reducing sub-sampling area. In the case of Poaceae pollen it was found that about 20% of the counts produced using three transects exceeded % ERROR of 30%. This increase in % ERROR caused by the decrease in sampled area to 11.79% of the total sampled surface did not overly affect the quality of the data because there were no notable changes in the seasonal characteristics of the selected pollen types. For instance, the largest difference in the start of the Poaceae pollen season was one day. This is small compared to the sort of differences between observed and predicted start dates of the grass pollen season that can be produced by some forecast models (*e.g.* 11 days between observed and predicted values).[21] It is also important to keep homogeneity within datasets because pollen data are phenological observations and can be used to examine changes in the flowering phenology of plants (*e.g.* the start, duration and end of pollen seasons and magnitude of the SPI), especially in relation to climate and land use change.

The mode of pollination did not seem to affect the results of the analysis. *Tilia* pollen was the only insect pollinated plant examined. The correlation coefficients obtained for *Tilia* were slightly lower than the correlation coefficients obtained by the other pollen types (Tables 2 and 4), but this is more likely to be related to the small number of *Tilia* recorded rather than the mode of pollination.

The reliability of data is the prerequisite for successful monitoring. The importance of quality control in aerobiology has been stressed in several recent studies,[22] but such processes are time consuming as they require repeated slide analysis.[23] Reducing the area of the slide sampled might help to develop a sustainable procedure for the quality control of aerobiological data.

Although our results showed that changes of sub-sampling area could be considered acceptable in order to decrease the amount of time spent on analysis, aerobiologists should consider the recommendation proposed by the European Aerobiology Society working group on Quality Control that suggests as a rule of thumb at least 10% of total slide area should be analysed in order to consider sub-sampling as a representative. In this study,

the use of two longitudinal transects (corresponding to 7.86% of total sample surface) had the greatest amount of % ERROR. The change in sub-sampling area also affected the slope of the regression for several pollen types, which describes the level of influence of the independent variable on the dependent variable. This supports the view that 10% is the minimum area of the slide that should be examined.

Pollen counts are multiplied by a conversion factor (resulting from the relation between scanned and total slide area) in order to obtain a daily average pollen concentration (grains per m^3),[8] which is rounded to eliminate decimal points. Pollen counts performed on 3 or 2 longitudinal transects could lead to an over-estimation at lower concentrations because of the higher conversion factors involved (compared to counts obtained using 5 longitudinal transects). However, significant differences in pollen concentrations were only observed for Poaceae. Interestingly, the conversion factor used at Novi Sad for five longitudinal transects is quite low ($CF_{5transects} = 0.353$). A low conversion factor relates to the fact that a relatively large area of slide was examined (in this case 19.64% of total sample surface) and is caused by the model of the microscope and the size of the field of view. As a result of using the standard mathematical method of rounding numbers, it was usual to round single pollen grains down (from 0.353 grains per m^3 to 0). This resulted in the counts produced using five longitudinal transects to have fewer counts of 1 grain per m^3 than the counts produced using two or three longitudinal transects, and was particularly noticeable for *Ambrosia* pollen (Table 6). This was unexpected because a larger area of slide sampled should mean that more pollen grains are examined. The authors therefore stress the necessity of rounding very low counts (<1 grain per m^3) up. Note that this did not affect the characteristics of the season produced using alternative counting methods.

Analysis of ten years worth of data would have strengthened the study and given more weight to the analysis. Unfortunately, although bi-hourly and daily average pollen concentrations have been recorded at Novi Sad since 2000, the original data showing the number of grains per transect were not kept. This shows the importance of keeping long-term records of all aspects of pollen counting.

Conclusion

This study has shown that there is a loss of accuracy in daily average and bi-hourly pollen concentrations (an increase in % ERROR) as the sub-sampling area is reduced from five to three or two longitudinal transects. This follows the results presented by Comtois *et al.*[3] However, this loss of accuracy does not impact on the main characteristics of the season or long-term trends. As a result, this study can be used to justify changing the sub-sampling method used at Novi Sad from five to three longitudinal transects. The use of two longitudinal transects has been ruled out because, although quicker, the counts produced had the greatest amount of % ERROR, altered the amount of influence of the independent variable on the dependent variable (the slope in regression analysis) and the total sampled surface (7.86%) was less than the minimum requirement recommended by the European Aerobiology Society working group on Quality Control.

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