# Quality control in bio-monitoring networks, Spanish Aerobiology Network

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a b s t r a c t

Several of the airborne biological particles, such as pollen grains and fungal spores, are known to generate human health problems including allergies and infections. A number of aerobiologists have focused their research on these airborne particles. The Spanish Aerobiology Network (REA) was set up in 1992, and since then dozens of research groups have worked on a range of related topics, including the standardization of study methods and the quality control of data generated by this network.

In 2010, the REA started work on an inter-laboratory survey for proficiency testing purposes. The main goal of the study reported in the present paper was to determine the performance of technicians in the REA network using an analytical method that could be implemented by other bio-monitoring networks worldwide. The results recorded by each technician were compared with the scores obtained for a bounded mean of all results. The performance of each technician was expressed in terms of the relative error made in counting each of several pollen types.

The method developed and implemented here proved appropriate for proficiency testing in interlaboratory studies involving bio-monitoring networks, and enabled the source of data quality problems to be pinpointed. The test revealed a variation coefficient of 10%. The relative error was significant for 3.5% of observations.

In overall terms, the REA staff performed well, in accordance with the REA Management and Quality Manual. These findings serve to guarantee the quality of the data obtained, which can reliably be used for research purposes and published in the media in order to help prevent pollen-related health problems.

## 1. Introduction

Aerobiology studies the passive transport of microorganisms and biological particulate matter through the air. It has been widely demonstrated that certain biological particles present in the atmosphere, such as pollen grains and fungal spores, give rise to human health problems including allergies and infections. Moreover, human activity, such as changes in land use or in atmospheric emissions, is changing the biological effects of these particles on human health (Foley et al., 2005; Bartra et al., 2007). A number of recent papers have sought to chart changes in the content and behavior of airborne biological particles due to global climate change (Breton et al., 2006; Zhong et al., 2006; Docampo et al., 2011; Zhang et al., 2012; Ziello et al., 2012). Monitoring of the atmosphere is of crucial importance in ensuring the availability of large data series that enable to study the changes caused by human activities over time, and a number of aerobiology networks have been set up with this purpose in mind. Since the concentration of airborne biological particles, such as pollen grains, is closely linked to the incidence of adverse health reactions, there is a clear need to obtain databases to develop forecasting models, likely to further the adoption of preventive measures. The reliability of the data in databases is necessary to ensure the robustness of results obtained from them (Ferretti, 2011). For this purpose aerobiology networks require a standardized methodology and also an effective internal quality control through the implementation of quality programs (Quevauviller et al., 1996; Araujo et al., 2010).

The Spanish Aerobiology Network (REA) was founded in 1992, and since then the outset has belonged to the European Aeroallergen Network/European Pollen Information (EAN/EPI). From the beginning, the main goal of this network has been to use a standardized methodology to generate information regarding airborne concentrations of biological particles – especially pollen grains and fungal spores – at a national level. Research in this field has focused on a range of methodological issues, including the effective comparison of data obtained at

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different altitudes (Galan et al., 1995; Alcazar et al., 1999a, 1999b; Velasco-Jiménez et al., 2012), the sampling media used (Tormo et al., 1996; Galán and Dominguez Vilches, 1997; Comtois et al., 1999; Carvalho et al., 2008), the counting method employed (Cariñanos et al., 2000; Sikoparija et al., 2011; Cotos-Yáñez et al., 2012), the quality control tools applied (Docampo et al., 2009), and the definition of the pollen season (Jato et al., 2006). This research and during our long time acquired experience, led to the publication of the REA Management and Quality Manual (Galán et al., 2007) (from now REA-MQM), taking into account the EAN/EPI minimum requirements in the Methodology for Routinely Performed Monitoring of Airborne Pollen (Jäger, 1995).

Regarding quality control, different papers have been focused on detecting the source of errors. In order to improve the error detection on aerobiology, management and resolution, it is necessary to recognize the various sources of error in aerobiological data. For this purpose, we have performed below a description of the possible sources of error in these data, summarized in Fig. 1. Comtois et al. (1999) provided a preliminary overview of the types of errors affecting aerobiological data; their classification was enlarged here via the inclusion of other error sources. As Fig. 1 shows, aerobiological data can contain both random errors (errors) and systematic errors (bias). *Bias* may be defined as a non-random error which is usually unidirectional (systematic). A number of authors have sought to detect the bias caused by the counting method used (Gottardini et al., 2009), but bias may also be linked to many other methodological issues (Comtois and Mandrioli, 1997; Galán and Dominguez Vilches, 1997; Alcazar et al., 1999b). By contrast, *random errors* are the representation of the uncertainties of the natural world. Two types of random errors can be distinguished: those that arise by chance because of the working protocol, and instrument errors attributable to human failures. Random errors occurring as a result of the *working protocol* have been analyzed by several researchers, who have focused on a comparison of different counting techniques (Käpylä and Penttintn, 1981; Tormo et al., 1996; Comtois et al., 1999; Cariñanos et al., 2000; Sikoparija et al., 2011; Cotos-Yáñez et al., 2012). *Instrumental errors* arise from human failures in carrying out aerobiological analysis. Instrumental errors can be in turn divided into two kinds: *technician errors*, committed by operators in *counting* and *identifying* pollen grains, and *mathematical errors* arising from the data-correction method used to estimate real airborne particle.

Few research papers have specifically addressed quality control in inter-sampler comparisons carried out for proficiency testing purposes, this being the only way to detect instrumental errors. Berti et al. (2009) analyzed the precision and accuracy of various technicians using concentrations found by experts as true sample values. Berti et al. (2009) analyzed the total concentration of pollen grains and the total number of taxa identified in samples. Nevertheless, the distinction between pollen types is essential for detecting the source of instrumental error and subsequently applying effective corrective measures. Another study, outlined by Pedersen and Moseholm (1993) also stratified total pollen grains by taxa, but sought to investigate the reproducibility of pollen counts; since its main aim was not to study proficiency, it did not focus on the source of instrumental errors.

The main aim of the study was to evaluate the quality of all data and to give an overall assessment of the Spanish Aerobiology Network. The second aim of the present study was to perform a proficiency test to assess the performance of technicians and lay the groundwork for future research. And the third objective was to identify the source of any errors identified; to this end, a procedure has been developed to identify the source of the instrumental error and, therefore, to improve plans to remedy that error. All these goals allow us facilitate corrective actions and increase the effectiveness of REA quality improvement plans.

## 2. Material and methods

The *Spanish Aerobiology Network* (REA) has been involved in an ongoing quality-control program (QC). Given that the performance of aerobiological technicians is a crucial element in ensuring data quality, in 2010 the REA embarked upon an interlaboratory proficiency-testing study. The University of Córdoba working group, as the REA Coordinator Center, is providing scientific support for the inter-laboratory study, which follows IUPAC (Thompson et al., 2006) and ISO recommendations in order to assess the performance of each technician (ISO 17043). Standards on chemical testing have been adapted to specific characteristics of aerobiological data. The performance of each staff member is expressed as the relative error in pollen counts and the overall quality will be indicated by the variation coefficient and the number of cases in which the relative error is higher than acceptable. The relative error and the variation coefficient are obtained from calculation of several summary statistics contained in ISO instructions (ISO 13528).

A first step was focused on checking which REA members were interested in participating in the external QC exercise. 25 technicians belonging to 17 different REA research groups: Badajoz, Bilbao, Cartagena, Córdoba, Granada, León, Madrid, Málaga, Mallorca, Orense, Oviedo, San Sebastián, Santiago, Sevilla, Toledo, Vitoria and Zaragoza, have been involved on this proposal. Trying to study the most representative pollen types, three slides, from winter, spring and summer, obtained by Hirst type sampler into different geographical areas, have been distributed to the 25 participants. All technicians were instructed to analyze the same pollen types: *Amaranthaceae*, *Alnus*, *Fraxinus*, *Cupressaceae*, *Pinus*, *Populus*, *Platanus*, *Betula*, *Quercus*, *Morus*, *Urtica*, *Olea*, *Poaceae*, *Castanea*, *Rumex*, *Plantago* and total pollen count, following REA-MQM: counting method consists of 4 continuous horizontal sweeps over the whole slide with a 40 ×10 lens. This gives a subsample accounting for 12-13% of the total surface, depending on the microscopic field size at that magnification, which may vary depending on the microscope model. A percentage over 10% of analyzed surface has been recommended by the EAN/EPI in recent minimum recommendations. Data were expressed as average daily pollen counts per cubic meter of air.

Each pollen type on each slide was analyzed separately. Statistically speaking, each slide was considered as a set of $P$ populations, where $P$ was the number of pollen types analyzed. These were "special populations" with size $N$ =1, standard deviation σ=0, in which the population mean (μ) was represented by the only value, that is the real

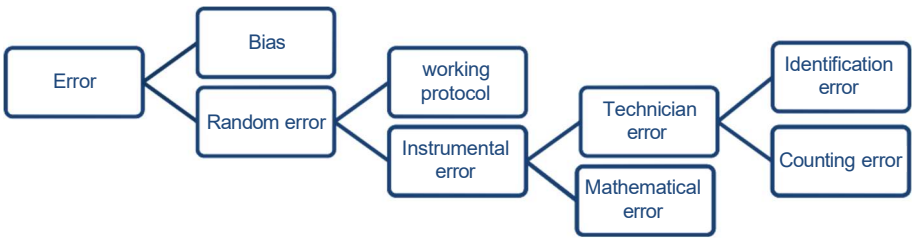


Fig. 1. Possible sources of error in aerobiological data.

concentration of pollen grains in the slide (unknown to us). A sampling on each population was carried out, being the size of each sample $n = 25$ elements, where each element represented the concentrations obtained by each technician staff.

To know the staff proficiency, we need to compare the counts of each staff with the unknown real value (μ). An approximation of this was used here as a reference for calculating the relative error committed by each technician. This parameter is commonly termed the "Assigned Value" ($X$). The Assigned Value depends directly on the sample mean ($\bar{X}$), but with several modifications to make it closer to the population mean. It is important to understand the differences between three concepts: population mean (μ), sample mean ($\bar{X}$) and Assigned Value ($X$).

To meet our three objectives (assessing the overall quality of the data, the individual staff proficiency and finding for the source of errors detected), we have followed four major steps: 1) analyze data normality and outliers; 2) calculate summary parameters; 3) calculate and evaluate errors; and 4) investigate the source of errors. The individual proficiency is evaluated in step 3 and causes of errors are analyzed in step 4. Finally, to evaluate the overall quality of the data using the variation coefficient (calculated in step 2) and the percentage of error (calculated in step 3). All this procedure allows an increase in the data quality of the network and makes useful recommendations for other bio-monitoring networks in the world.

### 2.1. Normality and outliers

#### 2.1.1. Normality

Results deriving from data whose distribution is non-normal should be viewed off course with caution, since many statistical procedures are only applicable to random samples from populations with a Gaussian distribution in order for results to be solid (Elveback et al., 1970; Limpert et al., 2008). Even the outcome of the simplest parameter "mean", which should be a good estimate of the true value, depends strongly on the type of data distribution. If any sample doesn't follow a normal distribution, we cannot estimate the population mean. In this case we will not use the involved pollen taxa given the impossibility of knowing the real concentration in the slide. The normality of data distribution has been checked using the Lilliefors test (Starink and Visser, 2010).

#### 2.1.2. Outliers

Statistical outliers may affect statistical parameters, and for that reason were eliminated here. Many standard outlier tests are not able to detect gross outliers, and a number of methods have been suggested for their detection (e.g. Dixon test, Grubb's test, and Huber's test). In the present study, the ISO recommended confidence levels were used, i.e. 95% for outliers classed as "stranglers" and 99% for those classed as "statistical outliers" (ISO 5725-2).

To calculate the Assigned Values for samples, z-scores were calculated for the central 95% of data, thus also eliminating outliers. The z-score of a raw score $\chi_i$ is given in formula (1), where $\bar{X}$ is the mean of the sample and $S$ is the standard deviation of the sample. Values above 1.96 or below −1.96 lay outside the central 95% and were not taken into account in calculating the Assigned Values.

$$Z = \frac{\chi_i - \bar{X}}{S} \tag{1}$$

To construct Assigned Values, only results with a z-score of less than 1.96 were taken into account and an outlier should also meet the following condition: $\chi_i - \bar{X} > |10|$, where $\chi_i$ is a raw score and $\bar{X}$ is the mean of the sample. This was because z-score values are strongly influenced by the sample mean: if the sample mean is very low, the z-score cannot identify the true outliers. Moreover, samples with a low mean are unlikely to fit into a normal distribution (Altman and Bland, 1995).

### 2.2. Summary parameters

Summary statistical parameters were calculated after rejection of non-valid results, and constructed for each pollen type: one summary parameter summarized the central value of data (*mean*), and another summarized data dispersion (*standard deviation*), confidence intervals and variation coefficients. Summary statistics were obtained following ISO recommendations (ISO 13528).

#### 2.2.1. Assigned Value

"Assigned Value" ($X$) for each population was calculated using the average of the data contained within the central 95% of values for the sample. The sample was bounded by calculating z-scores as indicate d in Section 2.1.2. $X$ is an approximation of the population mean (μ) that is also the true value of pollen concentration.

#### 2.2.2. Standard deviation for proficiency

The standard deviation ($S$) of the data with a 95% probability of belonging to the population was used as a summary parameter for data dispersion. This parameter was termed "standard deviation for proficiency" ($S'$). The procedure outlined in Section 2.1.2 was followed to delimit the sample.

#### 2.2.3. Confidence limits

Depending on the size of a given sample, the sample mean ($\bar{X}$) tended to equal the population mean (μ) as $n$ increased. The population mean fulfills the condition shown in formula (2) with 95% probability.

$$\mu \in \bar{X} - \frac{1.96 \times S}{\sqrt{n}}, \bar{X} + \frac{1.96 \times S}{\sqrt{n}} \tag{2}$$

Based on this property of the normal distribution, confidence limits ($CL$) were established using formula (3), where $X$ is the Assigned Value, $S'$ is the standard deviation for proficiency and $n$ is the size of the sample. This confidence limit refers to the values that should be considered as true, since there is an acceptable error that must be assumed. Consequently, population mean (μ), the true value, lies between the upper limit (UL) and the lower limit (LL) with 95% probability (Abraira, 2002a, 2002b)

$$CL = X \quad \frac{1.96 \times S'}{\sqrt{n}}. \tag{3}$$

#### 2.2.4. Coefficient of variation

The variation coefficient (VC) of each sample is given by formula (4), where $X$ is the Assigned Value and $S'$ is the standard deviation for proficiency.

$$VC = \frac{S'}{X} \times 100 \tag{4}$$

We can be sure that the Assigned Value agrees with the true value if an acceptable CV exists. The following selection criteria were used in determining the acceptable VC:

- Only pollen types whose Assigned Value ($X$) was over 10 were taken into account, because the VC is strongly influenced by low means.
- VC=30 was deemed unacceptably high when referring to pollen types with an $X$ value of between 10 and 25.
- VC=20 was deemed unacceptably high when referring to pollen types with an $X$ value of between 25 and 100.

- VC=15 was deemed unacceptably high when referring to pollen types with an $X$ value of between 100 and 500.
- VC=10 was deemed unacceptably high when referring to pollen types with an $X$ value above 500.

### 2.3. Errors

#### 2.3.1. Absolute errors

Absolute errors (AE) were calculated as the element of the sample ($\chi_i$) less than the "Assigned Value" ($X$) as shown in formula (5).

$$AE = \chi_i - X \tag{5}$$

#### 2.3.2. Relative errors

The relative error (RE) for each pollen type provided a summary of the performance of each technician. Relative errors were obtained using formula (6), where $\chi_i$ is the value recorded by each technician, $X$ is the Assigned Value and $CL$ is the confidence limit value nearest to $\chi_i$. Pollen data have a range of uncertainty below which no error is admissible; for that reason, the RE was calculated using confidence limits rather than the central reference value. RE was considered equal to zero when $\chi_i$ values lay between the two confidence limits.

$$RE = \frac{\chi_i - CL}{X} \times 100 \tag{6}$$

RE>|20%| was considered a significant error. Error was only considered significant when also: AE>|10|. The percentage of elements in which a significant error occurs is defined as percentage of error.

### 2.4. Analysis of sources of error

The main objective of this study is to determine the performance of staff and data quality. But the ultimate goal of assessing staff performance is to ensure that quality requirements are met, and that – should signs of poor quality be detected in the data – the problem is solved quickly and efficiently. It was essential to identify the source of error in order to address those quality issues detected. This required a number of different procedures.

All errors detected here were instrumental errors: as shown in Fig. 1, instrumental errors can be classified as mathematical errors, identification errors and counting errors. The first step was to check for mathematical errors, before going on to analyze the source of other relative errors.

#### 3.1.3. Mathematical errors

To detect mathematical errors, relative errors were classified on the basis of total pollen-grain counts. Data were plotted as a 3D matrix plot to facilitate visual detection of the presence of mathematical error, which is detected if the same error is constantly observed for all pollen types.

The presence of mathematical error was confirmed by multivariate principal component analysis (PCA). The variables considered were the pollen concentrations determined on each sample. Each element of the sample (each technician staff) was considered as a case in PCA.

#### 2.4.2. Identification errors and count errors

Pollen identification errors were detected as an inverse relationship between absolute errors committed in different pollen types by the same technician. That is, the presence of absolute error with magnitude=z committed by a technician staff in a pollen taxon and the presence of an absolute error of magnitude=−z made by the same technician in other pollen taxon of the same slide. Errors that are not identified as mathematical errors or as misidentifications are designated as counting errors, by discarding.

## 3. Results and discussion

### 3.1. Summary parameters

#### 3.1.1. Assigned Value and standard deviation for proficiency

Several options were analyzed as potential summary parameters of the central reference value, including median, mean, and semi-interquartile range; the mean of the bounded sample was finally selected as the most appropriate option. This has been termed the "Assigned Value" ($X$). Of the range of options available as potential summary parameters for data dispersion, the standard deviation of the bounded sample was selected as the most appropriate option. This has been termed: "standard deviation for proficiency" ($S'$).

#### 3.1.2. Confidence limits and variation coefficients

Confidence limits were calculated in order to draw more solid conclusions. Results have been expressed in Table 1. As can be seen, the larger the standard deviation value, the greater the assumed uncertainty about the true value.

VC also is shown in Table 2. Calculation of the VC for each pollen type was essential, since a very high variation coefficient implies a high variability in counts; what we would not ensure that the Assigned Value is a true representation of the true value, therefore this taxon is not to be used to assess the individual quality of technician staff. It would therefore indicate that the overall quality of the data on this taxon is not good. High VC indicates marked uncertainty in results, which may be due to one of two factors: either staff has failed to understand the test instructions, or there is a problem with the identification of a certain pollen type. The most appropriate solution in these cases would be to take into account only the results obtained by leading experts when constructing Assigned Values to assess individual proficiency or, where there is no expert committee, not to use these cases to assess individual proficiency given the impossibility of knowing the true values of the population. The criterion for setting the acceptable VC threshold to consider the type of pollen for proficiency testing depends on the purpose of the study and on the type of data involved; the VC threshold must be set by expert consensus.

VC is greatly influenced by low sample means; the validity of the VC threshold depends on the sample mean ($\bar{X}$), a dependence which – according to Käpylä and Penttintn (1981) – can be expressed as shown in formula (7).

$$VC = \frac{100}{\bar{X}} \tag{7}$$

Table 1
Assigned Value ($X$) ± standard deviation for proficiency ($S'$).

| Sample | A | B | C |
|---|---|---|---|
| *Alnus* | 0±0 | 0±0 | 0±0 |
| *Amaranth.* | 0±0 | 0±0 | 0±0 |
| *Betula* | 0±0 | 96±9 | 1±1 |
| *Castanea* | 0±0 | 0±0 | 0±0 |
| *Cupressus* | 156±19 | 23±4 | 0±0 |
| *Fraxinus* | 1±1 | 16±8 | 0±0 |
| *Morus* | 0±0 | 2±2 | 1±1 |
| *Olea* | 0±0 | 0±0 | 3±2 |
| *Pinus* | 1±1 | 167±20 | 7±2 |
| *Plantago* | 0±0 | 1±1 | 18±4 |
| *Platanus* | 0±0 | 142±18 | 114±12 |
| *Poaceae* | 1±1 | 7±3 | 11±1 |
| *Populus* | 86±14 | 43±8 | 0±0 |
| *Quercus* | 0±0 | 96±16 | 91±3 |
| *Rumex* | 0±0 | 0±0 | 24±3 |
| *Urtica* | 18±9 | 7±3 | 4±2 |
| Total | 273±32 | 618±54 | 277±28 |

Table 2
Confidence limits and variation coefficients (VC). Upper limit (UP), lower limit (LL), slide A (A), slide B (B) and slide C (C). Variation coefficients=* when $X$ b 10.

| Sample | A | | | B | | | C | | |
|---|---|---|---|---|---|---|---|---|---|
| | UL | LL | VC | UL | LL | VC | UL | LL | VC |
| *Alnus* | 0 | 0 | * | 1 | 0 | * | 0 | 0 | * |
| *Amaranth.* | 0 | 0 | * | 0 | 0 | * | 0 | 0 | * |
| *Betula* | 0 | 0 | * | 0 | 0 | * | 1 | 1 | * |
| *Castanea* | 0 | 0 | * | 100 | 93 | 10 | 0 | 0 | * |
| *Cupressus* | 163 | 148 | 12 | 25 | 21 | 19 | 0 | 0 | * |
| *Fraxinus* | 2 | 1 | * | 20 | 13 | 50 | 0 | 0 | * |
| *Morus* | 0 | 0 | * | 3 | 2 | * | 1 | 1 | * |
| *Olea* | 0 | 0 | * | 0 | 0 | * | 3 | 2 | * |
| *Pinus* | 1 | 1 | * | 175 | 159 | 12 | 8 | 6 | * |
| *Plantago* | 0 | 0 | * | 1 | 0 | * | 19 | 16 | 21 |
| *Platanus* | 0 | 0 | * | 149 | 135 | 13 | 119 | 109 | 11 |
| *Poaceae* | 1 | 0 | * | 8 | 6 | * | 11 | 10 | 13 |
| *Populus* | 91 | 80 | 17 | 47 | 40 | 19 | 0 | 0 | * |
| *Quercus* | 0 | 0 | * | 102 | 90 | 16 | 95 | 86 | 12 |
| *Rumex* | 0 | 0 | * | 0 | 0 | * | 25 | 23 | 12 |
| *Urtica* | 21 | 14 | 49 | 8 | 5 | * | 5 | 4 | * |
| Total | 285 | 260 | 12 | 639 | 597 | 9 | 288 | 266 | 10 |

The relationship between the VC and the sample mean ($\bar{X}$) for the results obtained here is shown in formula (8) and plotted in Fig. 2.

$$VC = 102.7 * \bar{X}^{-0.45} \quad (8)$$

As Table 2 shows, the VCs were generally acceptable, the only exceptions being those obtained for *Urtica* pollen in slide A and for *Fraxinus* pollen in slide B. Although one can hazard a guess at the cause of these high VCs, these pollen types should clearly not be taken into account for the proficiency test: external factors influenced *Fraxinus* pollen counts in sample B, while the high VC for the *Urtica* pollen count may be due to a misunderstanding of the technical instructions; in some cases all *Urtica* pollen types were taken into account, while in other cases only *Urtica membranacea* pollen or *Urtica/Parietaria* pollen was used.

The VC of samples with an AV greater than 20 was 13%, while the VC of data with an AV greater than 150 was 10%. These data confirm generally good performance by technicians.

### 3.2. Errors

Like the CV, error is strongly influenced by the sample mean, as noted by a number of authors (Comtois et al., 1999; Sikoparija et al., 2011). Errors were considered significant when RE>|20%| and AE>|10|. However, the acceptable threshold for RE should be taken by consensus among experts. Comtois et al. (1999), in a survey of the performance of aerobiology experts, highlighted the uncertainty regarding the error to be considered acceptable.

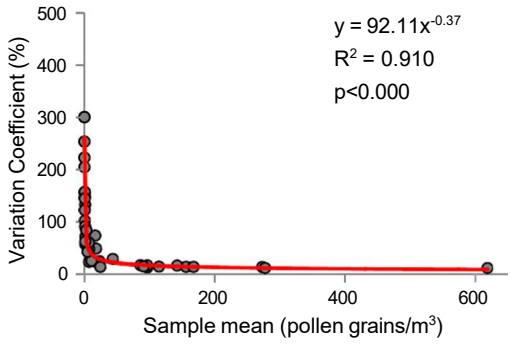


Fig. 2. Variation coefficients vs. sample mean.

Here, technicians made significant relative errors in only 3.5% of elements, thus confirming good overall performance.

### 3.3. Detection of error sources

As indicated earlier, this type of testing detects instrumental errors (Fig. 1); these may be technician errors (in pollen recognition or counting) or mathematical errors. Statistical testing can forcefully assert the presence of an error, and can even quantify its magnitude, as shown here. However, statistical tests cannot be used to detect the source of error. Below have been identified the source of error. This lay the basis for future specific remedies in order to enhance data quality. In case of finding any general problem in the quality of data, all members of the study must be informed so they can remedy it. Also, a report must be sent individually to each participant indicating the cause of their mistakes. Appropriate corrective measures must be implemented to solve the problem.

#### 3.3.1. Mathematical errors

In some cases, a mathematical error was apparent in the results: technicians recorded very low counts for all pollen types, suggesting an error in applying microscope correction factors. In such cases, results contained an internal bias. Mathematical error in the results obtained by four technicians (9, 12, 15 and 17), all of whom recorded very low mean concentrations for all taxa, is apparent in the diagram in Fig. 3.

The internal bias shown in Fig. 3 was confirmed by multivariate principal component analysis (PCA), Fig. 4, on the assumption that any technician displaying a repetitive pattern on all counts should be clearly reflected.

#### 3.3.2. Identification errors and counting errors

Only one significant identification error was found between *Populus* and *Platanus* taxa, and there were no counting errors. Certain statistical parameters for sample A as analyzed by several technicians (only *Populus* and *Platanus* pollen types) are shown in Table 3. Technician 22 displayed high relative errors in both pollen types: an absolute overcount error of 47 for *Platanus* pollen and an absolute undercount error of 40 for *Populus* pollen. This fact suggests an identification error between *Platanus* and *Populus* pollen grains.

## 4. Conclusions

In general, data quality is good. The average VC was 13% in samples where $X$ >20; however, the VC is strongly dependent on the sample mean, and using only samples with $X$ >150, the VC was 10%. Two pollen types showed unacceptable VC. Significant relative errors were found

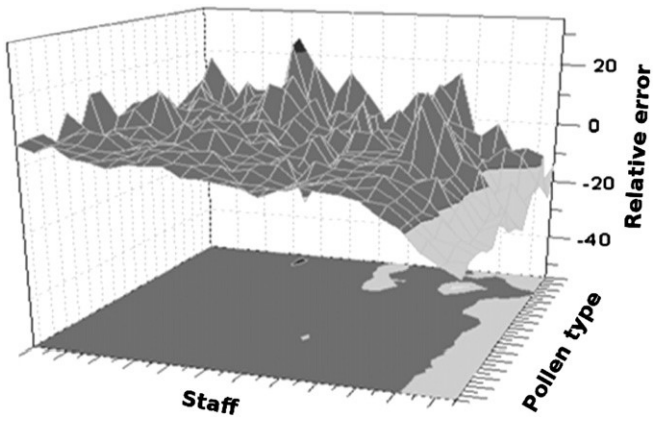


Fig. 3. Absolute errors matrix 3D plot.

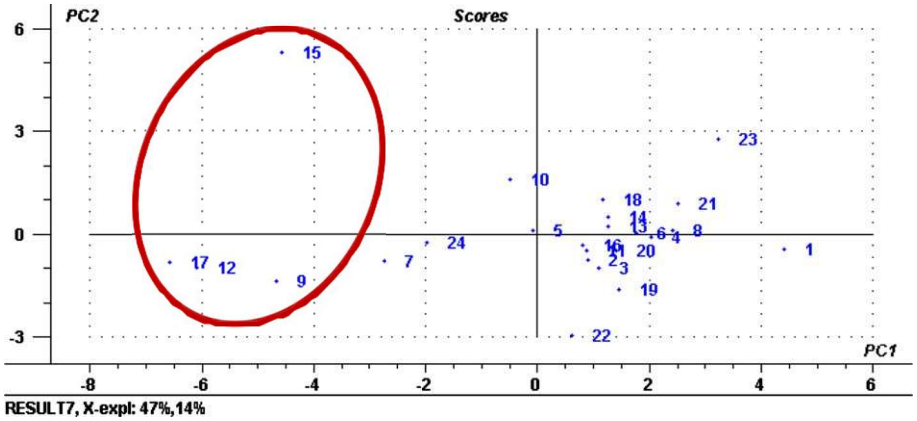


Fig. 4. PCA of all pollen concentrations.

Table 3
Some summary parameters of several staffs. RE (relative error), AE (absolute error).

| Staff | RE | RE | AE | AE | Count | Count |
|---|---|---|---|---|---|---|
| | *Platanus* | *Populus* | *Platanus* | *Populus* | *Platanus* | *Populus* |
| 23 | 0 | 5 | 0 | 2 | 149 | 49 |
| 5 | 0 | 9 | 0 | 4 | 137 | 51 |
| 3 | 0 | 3 | 0 | 1 | 146 | 48 |
| 25 | −2 | 0 | −3 | 0 | 132 | 41 |
| 22 | 33 | −100 | 47 | −40 | 196 | 0 |
| 7 | −1 | −9 | −2 | −4 | 133 | 36 |
| 1 | −4 | 0 | −5 | 0 | 130 | 44 |
| 16 | −1 | 1 | −1 | 0 | 134 | 47 |
| 2 | 0 | −2 | 0 | −1 | 136 | 39 |

only for 3.5% of observations. Errors have been detected in some counts of 5 staffs. The procedures tested here for identifying error sources have enabled the appropriate corrective measures to be implemented, and the problem has been solved.

Our results marked a new step forward in quality control programs, thanks to the development and implementation of a new, effective error-correction method as part of a QC program to be used in proficiency testing in aerobiology. This exercise has also served to confirm the good overall performance of REA technician staff.

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