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NOTES AND COMMENTS

Volumetric expression of palynological spectra for nutritional studies

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When pollen is studied from nutritional point of view, it would be desirable to correct its raw palynological spectrum in order to express the mass contribution of each pollen type. Different approaches applied for this correction through volumetric coefficients are discussed, and a simple and reliable procedure is proposed.

Keywords: pollen; palynological analysis; volume

Nutritional aspects of pollen are often studied because of its role in animal and human diet. Some nutritional traits of a pollen sample (e.g. protein content, lack of essential amino acids) can be predicted based on its palynological spectrum. Moreover, the knowledge of the palynological composition of the provisions collected by pollinivorous organisms allows to detect their trophic niches, and consequently to protect their nurse plant species and habitats.

To express the contribution of alimentary matter provided by each palynological type in a sample, the weight is the most appropriate measure. This approach is applicable for samples of pollen loads, where each pollen pellet is monofloral: the pellets are grouped according to their floral origin, and the groups are weighed. On the contrary, in case of an indistinguishable matrix, such as nest provisions (e.g. beebread for *Apis mellifera*) or the gut's content, only a "raw" palynological spectrum (pollen grains' count) can be obtained from the analysis. The mass of protoplasm in pollen grains can significantly vary, according to the grain's dimension and shape, which depend on plant species. It would be thus desirable to correct the raw spectrum by multiplying the relative abundance of each palynological type by its pollen grain's average weight.

Since at present, data regarding pollen grain's weight are available only for a few species, in many recent researches (e.g. Ferreira & Absy, 2015; Frias, Barbosa, & Lourenço, 2016; Hilgert-Moreira, Nascher, Callegari-Jacques, & Blochtein, 2014; Lucia, Telleria, Ramello, & Abrahamovich, 2017), the raw palynological spectrum is still used to express the composition of pollen samples. Requier et al. (2015) note the importance of the correction of the raw palynological spectrum and propose the pollen grain's diameter as the correcting factor. We actually consider this approach inadequate. In fact, the pollen grain's weight is not correlated to its linear dimension, but rather to the volume (Odoux et al., 2012), and thus the latter should be used as the correction factor, as suggested by Da Silveira (1991) and O'Rourke and Buchmann (1991).

Comparing the same palynological composition, expressed as the simple count (raw spectrum) and through the volumetric correction, relevant differences are often observed and the abundance ranking of palynological types may even be revolved, like in the two real examples reported in Table 1 (see *Castanea*, *Magnolia* in sample 2 and *Malva* f. in sample 1). Similar conclusions were driven by Buchmann and O'Rourke (1991) based on their example.

With this study, we discuss the different approaches applied for the correction of raw palynological spectra through volumetric coefficients, and we propose a simple and reliable procedure for this purpose.

In order to estimate the volume of a pollen grain, its dimensions are visually assessed, and the best fitting regular solid between ellipsoid, including sphere, and triangular prism is usually identified for calculation (Da Silveira, 1991; O'Rourke & Buchmann, 1991). We suggest simplifying the procedure by adopting the ellipsoid as the unique regular solid to represent pollen grains. In fact, given the same three dimensions, the volumes of an ellipsoid and a triangular prism differ for only 5%. This difference is negligible, considering the intrinsic low accuracy of the volumetric correction method due to several unavoidable factors (intraspecific variability of pollen grain's dimensions; interspecific variability of grain's specific weight, as well as of exine and intine thickness with the consequent different space left for protoplasm; different ratio of viable pollen grains).

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Table 1. Examples of palynological spectra expressed according to two different approaches: the raw result of the palynological analysis (count) and the result corrected by volumetric coefficients (volume).

	Sample 1		Sample 2	
Palynological type	Count (%)	Volume (%)	Count (%)	Volume (%)
Castanea	–	–	82	13
Cichorium f.	33	33	–	–
Clematis	33	11	–	–
Lagerstroemia	5	11	–	–
Magnolia $> 60 \mu$m	–	–	4	73
Malva f.	1	28	–	–
Plantago	12	5	–	–
Rubus f.	6	3	7	6
Verbascum f.	–	–	5	6
Others	11	9	2	2

Note: The data refer to two pollen samples collected in a monitoring campaign in 2014–2015 in the Ligurian region, Italy.

In order to calculate the grain's volume (assumed its ellipsoidal shape), the length of the three axes (a, b, and c) should be measured and processed through the following formula: $V = a*b*c*\Pi/6$. Brian (1951) uses the spherical grain's model, with the radius calculated as the average of the assessed length and breadth, but his procedure overestimates the volume in case of non-spherical pollen grains. For example, the volume of a prolate ellipsoidal pollen grain of *Lathyrus pratensis*, calculated according to our and Brian's procedure, results of 13600 and 16200 μm^3, respectively (being overestimated by 19% in the latter case). Even though this low level of precision may be sufficient for certain purposes, it can be easily improved by considering the assessed data separately. Contrarily to the 1950s, nowadays the computing is the less demanding part of work. We also point out that if instead of the grain's volume, we are interested in the proportions between pollen types in a sample (e.g. palynological spectrum), the coefficient $a*b*c$ is sufficient.

When autonomous measurement is not possible, we propose to base the calculations on the data available in one of the open access pollen databases. One of the most extensive databases for the purpose is Ponet (http://ponetweb.ages.at/pls/pollen/pollen_suche), elaborated and managed by the Austrian Agency for Health and Food Safety (AGES). Pollen grains of many botanical species are described and the average values of four dimensions (spines excluded) are reported. The first two dimensions correspond to the length (*Laenge*) and the width (*Breite*) measured in the most frequent position the grains assume on microscope slide (*Hauptlage*). The other two dimensions are the length and the width assessed in the secondary position (*Seitenlage*). For the calculation of the estimated pollen grain's volume, we select 3 of the 4 available measures: the length and width of the position where the highest value was recorded (laying grain), and the smallest measure of the other position. Sometimes, in the Ponet and other databases (e.g. Ricciardelli D'Albore, 1998), the two measures of only one position are reported. In these cases, the third lacking dimension is obtained in the following way: accordingly to whether the grain is "prolate" or "oblate", respectively the shortest or the longest measure is used twice.

Summarizing we point out that the raw palynological spectrum does not reflect appropriately the pollen sample's composition in terms of mass. We therefore strongly encourage to apply volumetric coefficients in the studies where the nutritional facts are important. We also believe that the procedure we propose in the present study can facilitate the application of the correction.

Disclosure statement

No potential conflict of interest was reported by the authors.

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