UNIVERSIDAD REGIONAL AMAZÓNICA IKIAM

FACULTAD DE CIENCIAS DE LA TIERRA Y AGUA

CARRERA DE GEOCIENCIAS

DETERMINACIÓN DEL SESGO PRODUCIDO POR LA DISPERSIÓN DE GRANOS DE POLEN DURANTE EL PROCESO DE MONTAJE DE MUESTRAS.

Proyecto de investigación previo a la obtención del Título de:

Ingeniero en Geociencias

Autor: Olga Nagelly Silva Icaza

Tena- Ecuador

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Tutor: Ph.D. Bryan Guido Valencia

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2025

Carrera Geociencias

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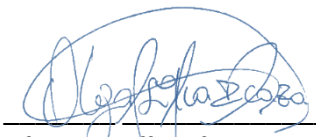

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Agradecimientos

El presente trabajo investigativo fue realizado gracias a **Dios** que me dio el entendimiento para escoger mi tema y quien me dirigió en este arduo camino donde me encontré con muchas personas maravillosas que de alguno u otra manera simbolizaron algo para la realización y culminación de mi tesis.

Quiero expresar mi más sincero agradecimiento a mi Tutor **Ph.D. Brayan Valencia**, cuya guía experta ha sido un faro en este camino. Sus conocimientos, paciencia, motivación constante y su capacidad para inspirarme me han permitido superar los desafíos que se presentaron a lo largo del proceso. Su compromiso con la excelencia y su dedicación a la investigación han dejado una huella imborrable en mi formación académica.

Agradezco de manera especial a **Andrea Salgado**, cuyo apoyo técnico ha sido inigualable. Su disposición para ayudarme en cualquier momento, así como su conocimiento y experiencia, han sido cruciales para el desarrollo de este trabajo. No solo me brindo asistencia técnica, sino que también me ofreció palabras de aliento en los momentos más difíciles.

A mi familia, que ha sido mi pilar incondicional les agradezco por su amor, compresión y apoyo emocional. Gracias por estar siempre a mi lado, por creer en mí, por entender las largas horas que dedique a este proyecto. Su confianza en mis capacidades me ha motivado a seguir adelante y a dar lo mejor de mí.

A mis amigos, quienes han sido un gran apoyo durante este proceso, les agradezco por su compañía, por las charlas que me ayudaron a despejar la mente y por su paciencia al escucharme hablar sobre mis avances y desafíos. Su amistad ha sido un regalo invaluable en este viaje.

Finalmente, quiero extender mi más sincero agradecimiento a todos aquellos que, de alguna manera, contribuyeron a la realización de este trabajo. A los colegas que compartieron sus ideas y conocimientos, a los profesores que me guiaron en mi formación y a todos los que con su apoyo y colaboración hicieron posible que este proyecto se materializara.

Dedicatoria

Dedico esta tesis con todo mi corazón a mis padres, quienes han sido mi fuente inagotable de amor y apoyo incondicional en mi vida. Su sacrificio, dedicación y fe en mi han sido el motor que ha impulsado a alcanzar mis metas. Gracias por enseñarme el valor del esfuerzo y la perseverancia, y por estar siempre a mi lado, animándome a seguir adelante incluso en los momentos más desafiantes.

A mi familia, que ha creído en mi desde el primer día y ha estado presente en cada paso de este camino. Su aliento constante y su confianza en mis capacidades me han dado la fuerza necesaria para enfrentar los obstáculos y seguir adelante. Cada palabra de aliento, cada gesto de cariño y cada momento compartido han sido fundamental en mis desarrollo personal y académico.

A mis amigos, quienes han sido un pilar esencial en mi vida. Gracias por estar siempre ahí, brindado me animo y alegría en los momentos de duda y celebrando mis logros como si fueran propios. Su compañía ha hecho que este viaje sea mucho más llevadero y significativo, y cada risa compartida ha sido un recordatorio de la importancia de la amistad en este proceso.

A todos aquellos que, con su sabiduría y paciencia, me han inspirado a ser mejor. Cada consejo, cada enseñanza y cada momento de reflexión han dejado una huella en mi vida y han contribuido a mi crecimiento personal y académico. Ustedes han sido faros de luz en mi camino, y por ello, les estoy profundamente agradecida.

Este trabajo está dedicado a ustedes, quienes han sido mi roca en este viaje. Sin su amor, apoyo y guía, este logro no habría sido posible. Gracias por ser parte de mi vida y por acompañarme en esta aventura. Su influencia perdurara en cada página de esta tesis y en cada paso que dé en el futuro.

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RESUMEN

El método de colocación de polen para su conteo es una técnica utilizada en la construcción de modelos paleoclimáticos y paleoambientales. Este proceso incluye la extracción y preparación del polen para su análisis microscópico, proporcionando información acerca de la composición de individuos vegetales en la zona a través del tiempo. Para evaluar la precisión del método, se aplicó un análisis estadístico basado en la geoestadística, empleando el análisis de patrón de puntos y la prueba de Chi-Cuadrado (x^2) para determinar posibles diferencias en la distribución de granos de polen de diferentes tamaños. Los datos se organizaron en una cuadricula de 10 segmentos y los diámetros de los granos de polen se recategorizaron en quintiles. Los resultados indican que no hay diferencias estadísticamente significativas en la distribución de granos de polen pequeños, medianos y grandes al usar el método estándar de montaje. Sin embargo, se sugiere la necesidad de una revisión metodológica más profunda para minimizar posibles sesgos.

Palabras claves: Método de colocación de polen, Análisis de patrón de puntos, Distribución espacial.

SUMMARY

The method of placing pollen for counting is a technique used in the construction of paleoclimatic and paleoenvironmental models. This process includes the extraction and preparation of pollen for microscopic analysis, providing information about the composition of plant individuals in the area through time. To evaluate the accuracy of the method, a statistical analysis based on geostatistics was applied, using point pattern analysis and the Chi-square test (x^2) to determine possible differences in the distribution of pollen grains of different sizes. The data were organized into a 10-segment grid and pollen grain diameters were recategorized into quintiles. The results indicate that there are no statistically significant differences in the distribution of small, medium and large pollen grains when using the standard mounting method. However, the need for further methodological review is suggested to minimize possible biases.

Keywords: Pollen placement method, point pattern analysis, Spatial distribution.

Journal to be submitted: PALAEO: Paleogeography, Paleoclimatology, Paleoecology

Link of the journal: https://www.sciencedirect.com/journal/palaeogeography-palaeoclimatology-palaeoecology

Determination of bias produced by the dispersion of pollen grain during the sample mounting process.

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1. INTRODUCTION

Mounting pollen for counting is a fundamental technique in the field of geosciences, especially when it comes to reconstructing paleoclimatic and paleoenvironmental models (Bennett, 1990). This method allows us to analyze and quantify pollen grains that have been deposited in geological sediments over time, giving us a glimpse into the past to understand what vegetation was like in a given area (Stockmarr, 1971).

To carry out this technique, sediment cores are extracted from strategic locations where records have been preserved continuously (Traverse, 2007). Then, through a chemical process, the pollen is prepared and concentrated, always taking care to preserve the shape and structure of the grains. Finally, they are placed on a thin slide for observation under a microscope (Holt & Bennett, 2014).

The method of mounting pollen on the slide was developed by Gunnar Erdtman in 1943 and remains a widely used technique today. This procedure involves placing a drop of the aqueous solution containing the pollen grains in the center of the slide, forming a meniscus, and then carefully covering it with a glass coverslip. This approach not only preserves the samples but also facilitates their long-term study, making it a standard in the preparation of pollen samples for microscopic observation (Erdtman et al., 1961; Faegri & Iversen, 1989).

This method is particularly useful because it preserves the morphological characteristics of the pollen grains, which is essential for their identification and analysis. Additionally, its simplicity and effectiveness have made it a reliable technique. (Riding, 2021)

Counting and identifying pollen grains not only helps us reconstruct what past environments and climates were like (Scott, 1991), but it also gives us clues about how plant species were distributed throughout geological eras. Additionally, this information is key to detecting climatic changes, such as variations in temperature, humidity, or even seasonal patterns (Birks & Birks, 2000).

In this work, we use a statistical tool called geostatistics, which focuses on analyzing data with a spatial component. One of its most useful techniques is point pattern analysis (Clements, A. C. A., & Pfeiffer, D. U., 2008). This technique allows us to study how certain natural phenomena or geographic variables are distributed within a specific area

(Anselin, 1995). It is especially useful in fields like geography, ecology, where the location of data is crucial for understanding patterns and trends (Bailey, T. C., & Gatrell, A. C., 1995).

When we apply point pattern analysis to pollen grains observed under a microscope, we can determine if there are differences in their distribution based on their size. (Johnson & Kuby, 2015).

Developing a methodology to study these differences is essential to ensure that palynological studies are accurate and reliable, especially when it comes to reconstructing past climatic and environmental models. The sample preparation processes, although seemingly simple, can have a significant impact on how we interpret the data. Therefore, it is crucial to understand and control these factors to obtain robust and reproducible results in research that relies on the accurate identification of plant species through their pollen grains.

In this study, we focus on analyzing how pollen grains are distributed on a slide and whether their size influences this distribution. Additionally, we evaluate whether the standard method of mounting pollen samples introduces any bias, starting from the hypothesis that there are no significant differences between small and large grains.

2. METHODOLOGY

In this study, the focus was on preparing pollen samples, mounting them on slides, and analyzing them under a microscope. The goal was to evaluate whether the mounting method produces different distribution patterns for small pollen grains compared to larger ones. The analysis was structured into three main phases, considering data categorization and visualizations to interpret the spatial distribution of pollen grains. It's important to note that this analysis was conducted with only one sample, so any conclusions drawn should be approached with caution, as the limited sample size may not be representative of reality.

The coordinates (X, Y) obtained from counting the pollen grains on the slide underwent a transformation and scaling process to standardize the coordinates. This step was taken

to facilitate visual identification, resulting in the variables X_scaled and Y_scaled. The formula used was:

$$x' = \frac{x - \mu}{\sigma}$$

Where:

$$x' : New\ value$$
$$x : It\ is\ the\ original\ value$$
$$\mu : Average\ of\ the\ data$$
$$\sigma : Standard\ deviation\ of\ the\ data$$

The data was then categorized into a grid divided into 10 equal parts. It was observed that increasing the number of grids beyond 10 artificially inflates the Chi-square statistic (X^2), which could lead to incorrect conclusions. The Chi-square statistic is used to evaluate the relationship between categorical variables. The general formula for calculating the Chi-square value is:

$$X^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

Where:

$$O_i = Observed\ value$$

$$E_i = Expected\ value$$

The grid division allowed for an analysis of the observed and expected proportions in the spatial distribution of pollen grains on the slide.

To analyze how particle size affects the results, the pollen grain diameter was recategorized into three groups, **Small particles:** (< 23.1 µm), **Medium particles:** (23.1 µm), **Large particles:** (> 23.1 µm), using the 33%, 66%, and 100% values as reference points. These values represent the midpoints of the data, meaning that half of the

diameter measurements fall below these points and the other half above. **Figure 2 and Figure 3: Graphs C and D** illustrate this distribution.

This classification allows for a more detailed and segmented comparison between the distributions of different particle sizes, facilitating the analysis of how particle size might influence the study's results (Riding, 2021).

An analysis was conducted to calculate the proportions of points in each segment, referring to the number of pollen grains present in each grid of the analyzed slide. This calculation aimed to evaluate whether the observed differences in pollen grain distributions were significant compared to the expected distributions. In other words, the goal was to determine if the distribution of small grains is similar to that of large grains.

The analysis used the Chi-square statistic (χ^2), which in this case yielded an overall value of 0.20. This result suggests that, in general, there is homogeneity in the distributions of pollen grains, implying no significant differences between the distributions of small and large grains (Anselin, 1995).

Data visualization was carried out using heat maps and distribution graphs, which helped in interpreting the patterns of pollen dispersion on the slide (Bailey & Gatrell, 1995). The results were assessed for statistical significance, enabling us to ascertain if the mounting process has a differential impact on the distribution of pollen grains according to their size.

3. RESULTS

This chapter presents the results obtained from the analysis of pollen grain distribution on the slide, focusing on how particle size influences their spatial behavior. Using statistical techniques such as Chi-square ($\chi 2$) and visualization tools like heat maps and scaled graphs, key patterns in pollen grain distribution have been identified. The results reveal that, although there are no significant differences at a global level, there are subtle variations in specific areas that may be related to factors such as size, weight, and

intermolecular forces. These findings provide a solid foundation for better understanding the dynamics of pollen grain dispersion and their implications in paleoenvironmental and climatic studies.

Figure 1: Graph A and Figure 2: Graph C: Graphical representations showing the scaled points, meaning that each point in these graphs has been adjusted in size to reflect different categories. In particular, **Graph C** focuses on clearly distinguishing these size categories, allowing observers to better understand how the data are grouped or distributed based on their size (Bailey & Gatrell, 1995).

Figure 1: Graph B and Figure 3: Graph D: Use a technique known as heat maps, useful for visualizing densities and proportions of data in different segments. Heat maps use colors to represent data intensity, facilitating the identification of patterns and differences in data distribution (Getis & Ord, 1992).

Figure 4: Graph E: Graphical representation showing the values of (χ^2), a statistical measure used to evaluate the relationship between different variables, in this case, pollen size and position. This graph helps identify the cells that contribute most to the overall (χ^2) value, allowing observers to understand which elements are most relevant in the dataset (Clements & Pfeiffer, 2008).

Most relevant cells:

X Bin=6, Y Bin=5 with a value of 0.037.

X Bin=6, Y Bin=9 with a value of 0.038.

X Bin=8, Y Bin=9 with a value of 0.018.

X Bin=6, Y Bin=9 with a value of 0.015.

In **Figure 4: Graph E**, the distribution of (χ^2) values in each grid cell is presented. This graph highlights the cells that show the greatest differences compared to what was expected. This visual approach is very useful, as it allows for the identification of specific patterns within the different categories of pollen grains. This can provide valuable information about their distribution and behavior in the studied environment (Riding, 2021).

In **Figure 1: Graph A**, a uniform distribution of the points in the scaled space can be observed. This means that the points are evenly distributed and show no obvious patterns of accumulation or clustering. In other words, there are no areas where the points are more concentrated than in others, suggesting that there are no factors

influencing the grouping of these points in space (Holt & Bennett, 2014). On the other hand, when analyzing the representation shown in **Figure 2: Graph C**, which has been segmented according to the recategorized diameter, some subtle differences in the way the particles are dispersed can be noted. In particular, it is observed that the smaller particles, those with a diameter less than 23.1 µm, are more evenly distributed compared to the larger particles, which have a diameter greater than 23.1 µm (Stockmarr, 1971).

In **Figure 1: Graph B**, a heat map illustrates the density of particles in different cells within a matrix. In the map, the cells that show a higher density of particles are marked with intense red colors. As we move from the center of the matrix towards the edges, the particle density decreases, and the distribution becomes more homogeneous. This means that, in the peripheral areas, the particles are more dispersed and are not clustered as much as in the center (Traverse, 2007).

When looking at **Figure 3: Graph D**, which segments the particles according to their diameter, differences in the distribution of large and small particles can be noted. Particles with a diameter greater than 23.1 µm tend to concentrate in specific cells of the map. This suggests that there are certain areas where these larger particles are more prevalent. In contrast, the smaller particles are more evenly distributed throughout the matrix, indicating that they are not clustered in particular zones but are more dispersed throughout the space (Birks & Birks, 2000).

This distribution pattern reinforces the hypothesis that the physical characteristics of pollen, such as its size and weight, influence its spatial behavior. That is, large particles, due to their greater weight, may be affected by different physical forces that lead them to accumulate in certain areas, while smaller particles, being lighter, can move and disperse more easily, resulting in a more uniform distribution. Understanding these dynamics is crucial to study how particles behave in the environment and how they interact with each other, which can have important implications in fields such as ecology and biology (Scott, 1991).

The statistical analysis performed resulted in an overall (χ^2) value of **0.20**. This value is important because it helps us understand whether there are significant differences between the particle distributions we expected to observe and those we actually measured in the different intervals. In this case, such a low value of (χ^2) indicates that,

overall, there are no statistically significant differences between the expected and observed distributions. This suggests that, although the diameter of the particles may influence their behavior, the dispersion patterns remain relatively homogeneous in a broader context (Anselin, 1995).

However, upon closer examination of the data, as illustrated in **Figure 4: Graph E**, some specific cells present more notable differences in their (χ^2) values. In these cells, the values reach peaks close to **0.063**, indicating that, in those particular cases, the observed distributions deviate more than expected. These localized differences are significant and may be related to several factors. For example, it could be the result of intermolecular forces that promote particle agglomeration, meaning that certain particles tend to coalesce and form clusters rather than being uniformly distributed. In addition, these differences could also be linked to faster settling processes that affect larger particles (Riding, 2021).

In summary, although the overall analysis suggests a homogeneous distribution of particles, the variations observed in particular cells indicate that there are additional factors on the slide that may influence how particles are distributed in space. Understanding these particle dynamics and the processes that affect them is essential for correctly interpreting the results (Holt & Bennett, 2014).

The decision to divide the coordinates into 10 equal segments was essential to accurately capture the differences in data distribution without giving rise to statistical artifacts that could distort the results. In this context, it was decided to maintain three categories for classifying pollen sizes (large, medium, and small). This choice is based on the selection of groups with the highest difference in size and an intermediate size.

By introducing more splits into the analysis, the expected frequencies would be distributed across a larger number of categories, which would increase the likelihood of obtaining very low or even zero Chi-square values (χ^2). This is problematic because a low Chi-square value may indicate that there are no significant differences between categories, when in fact there could be important variations that are simply not being adequately captured. On the other hand, choosing to have fewer intervals, while it could improve the resolution of the analysis and make it easier to identify relevant patterns, could also artificially increase the value of χ^2 due to the presence of empty cells or cells

with extreme values, which would compromise the validity of the analysis (Anselin, 1995).

Brownian motion is a physical phenomenon that describes the random motion of particles suspended in a fluid, resulting from collisions with molecules in the surrounding medium (Einstein, 1905). This phenomenon is fundamental to understanding how particles, such as pollen grains, are distributed in liquid or gaseous media. In the context of this study, Brownian motion could explain why smaller particles tend to distribute more uniformly, while larger particles, due to their greater mass, are less affected by this motion and tend to accumulate in specific areas.

Table 1:Description of the variable used.

Variables	Descripción	Tipo de Variable
X	Coordinates on the X axis	Continua
X_scaled	Coordinates on the X axis scale	Continua
X_bin	Refactored Interval to which the coordinate. (Indicate the row number on the X-axis)	Categórica [Tabla 2]
Y	Coordinates on the Y axis	Continua
Y_scaled	Coordinates on the Y axis scale	Continua
Y_bin	Refactored Interval to which the coordinate. (Indicate the row number on the Y-axis)	Categórica [Tabla 2]
N	Number of suspended pollen particles.	Discreta
Diámetro	Diameter of the pollen particles	Continua
Diámetro Refactorizado	Refactored pollen diameter considering the quintiles of distribution del 0-0.5 y 0.5 -1.	Factor [G-P]

Table 2:Scaled intervals used

Intervalo	X_scaled	Y_scaled	X_Bin
2-3	(0.018, 0.035)	(0.013, 0.026)	2
3-4	(0.035, 0.053)	(0.026, 0.039)	3
4-5	(0.053, 0.071)	(0.039, 0.052)	4
5-6	(0.071, 0.089)	(0.052, 0.065)	5
6-7	(0.089, 0.106)	(0.065, 0.079)	6
7-8	(0.106, 0.124)	(0.079, 0.092)	7
8-9	(0.124, 0.142)	(0.092, 0.105)	8
9-10	(0.142, 0.160)	(0.105, 0.118)	9

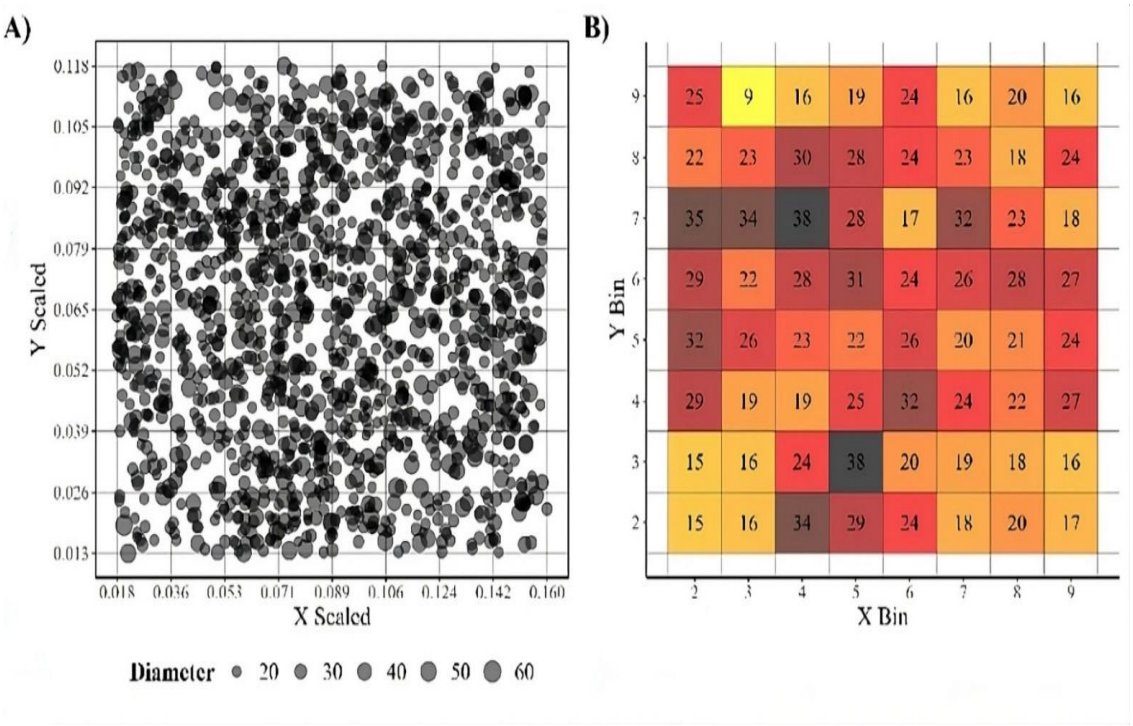


Figure 1: Exploratory analysis.

This Figure is subdivided into **graphs A and B** showing the distribution of pollen grains on a sheet, segmented into a 10x10 grid. The dots represent pollen grains, and their size and color indicate different diameter categories (small, medium and large). Areas with a higher density of dots (in intense red tones) indicate a higher concentration of pollen grains, while lighter areas (in yellow and green tones) show a more dispersed distribution. This visualization makes it possible to identify patterns in the distribution of pollen grains according to their size and position on the laminate.

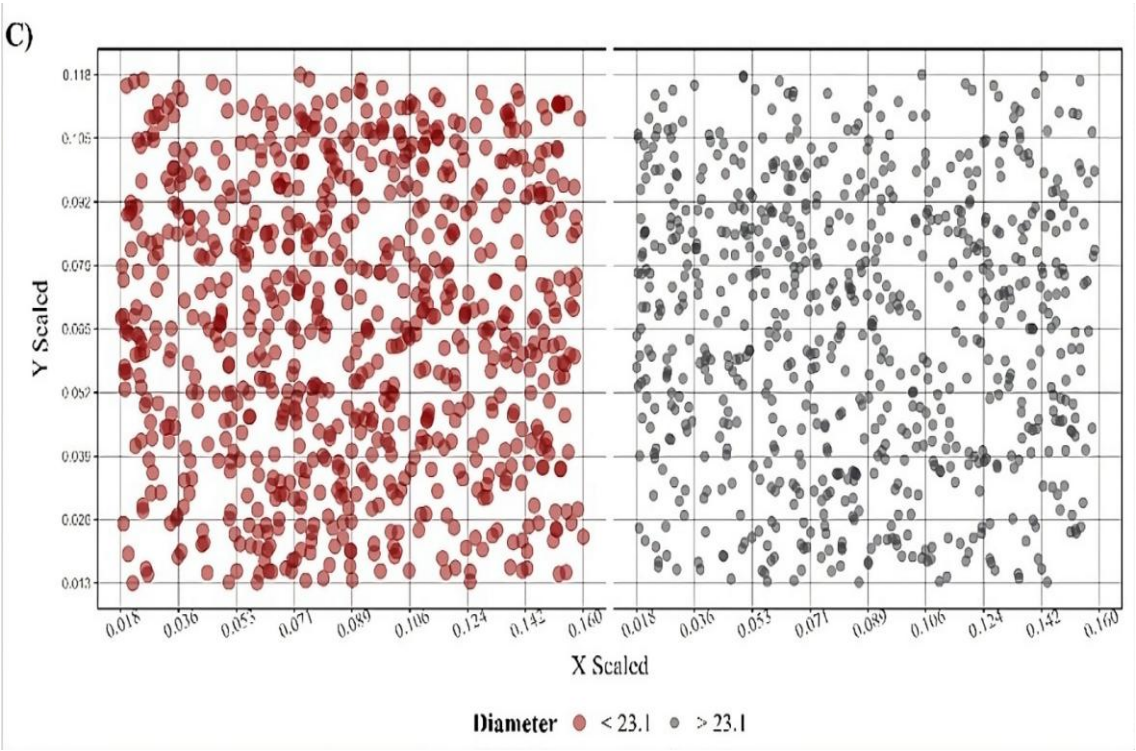


Figure 2: Exploratory analysis.

This **graph C** is a heat map showing the distribution of pollen grains on a sheet, segmented according to their diameter. The X axis represents the scaled coordinates on the sheet, while the Y axis shows the diameter of the pollen grains. The red dots show pollen grains with a diameter greater than 23 µm, while the gray dots show pollen grains with a diameter less than 23 µm. This visualization makes it possible to identify patterns in the distribution of pollen grains according to their size and position on the laminate.

D)

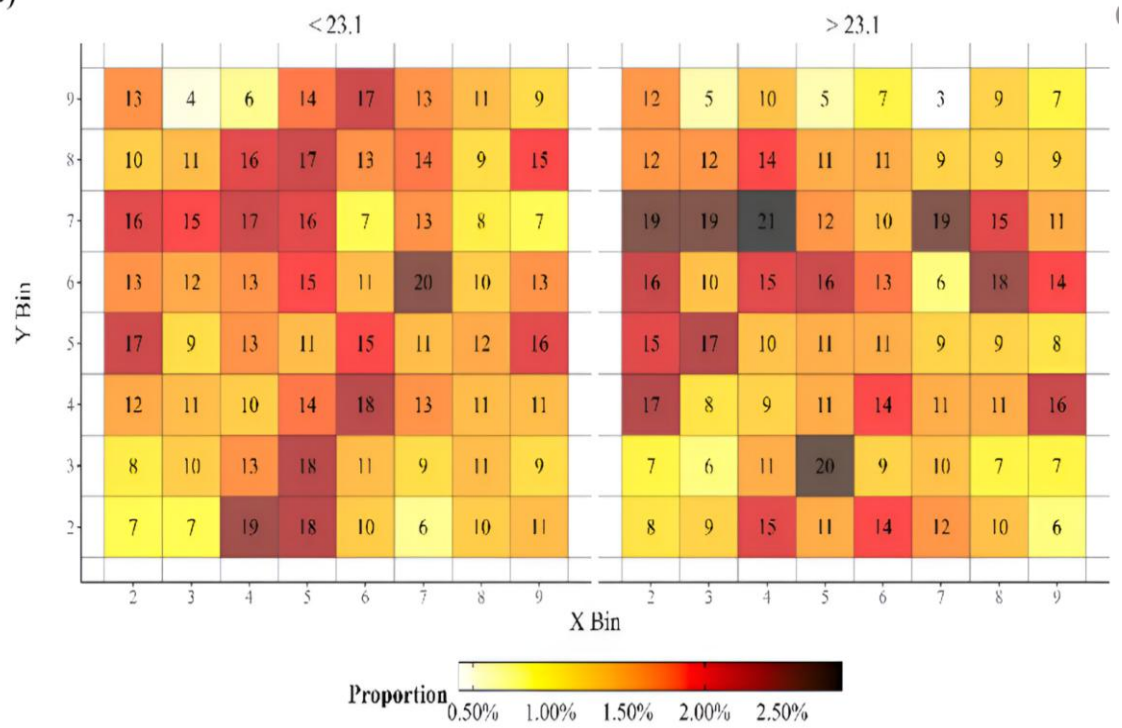


Figure 3:Exploratory analysis.
This in **graph D** showing the distribution of pollen grains on a leaf, segmented into a 10x10 grid. Areas with a higher density of dots (in intense red tones) indicate a higher concentration of pollen grains, while lighter areas (in yellow and green tones) show a more dispersed distribution.

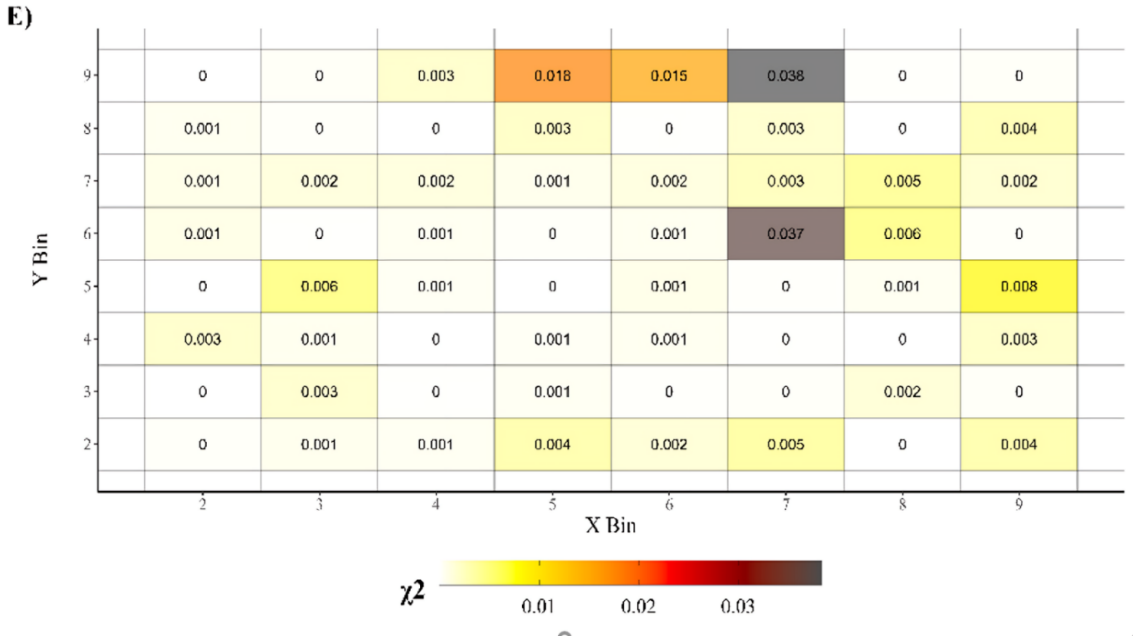


Figure 4:Exploratory analysis.

This **graph E**, some specific cells show more notable differences in their (χ^2) values. In these cells, the values reach peaks close to **0.063**, indicating that, in those particular cases, the observed distributions deviate more than expected.

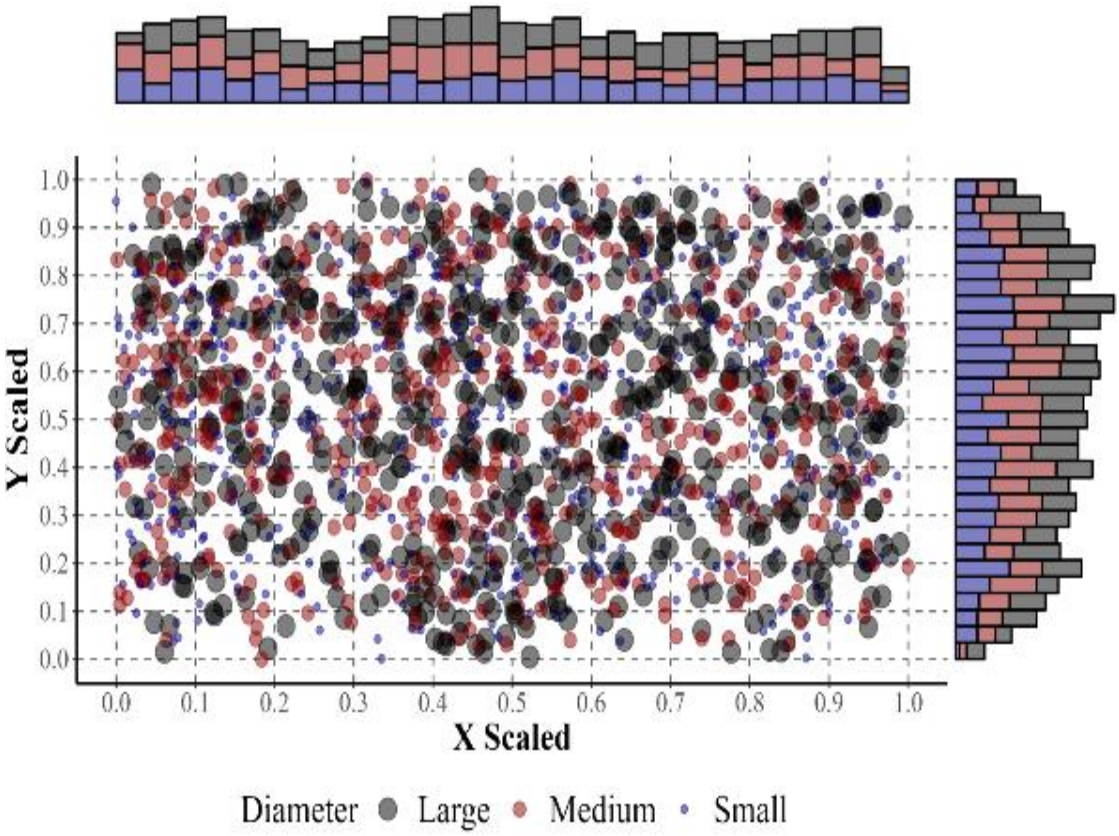


Figure 5: Dispersion of reclassified pollen grains by size and associate frequency plots on the axes.

Figure 5 shows the dispersion of pollen particles in each Interval of the scaled axes.

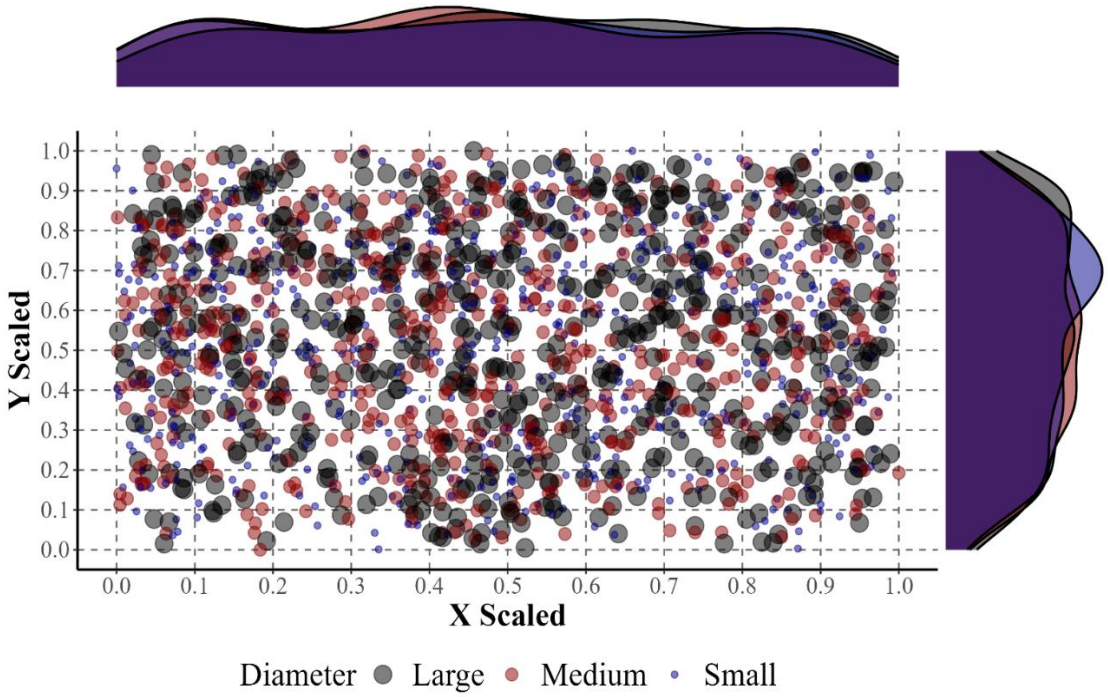
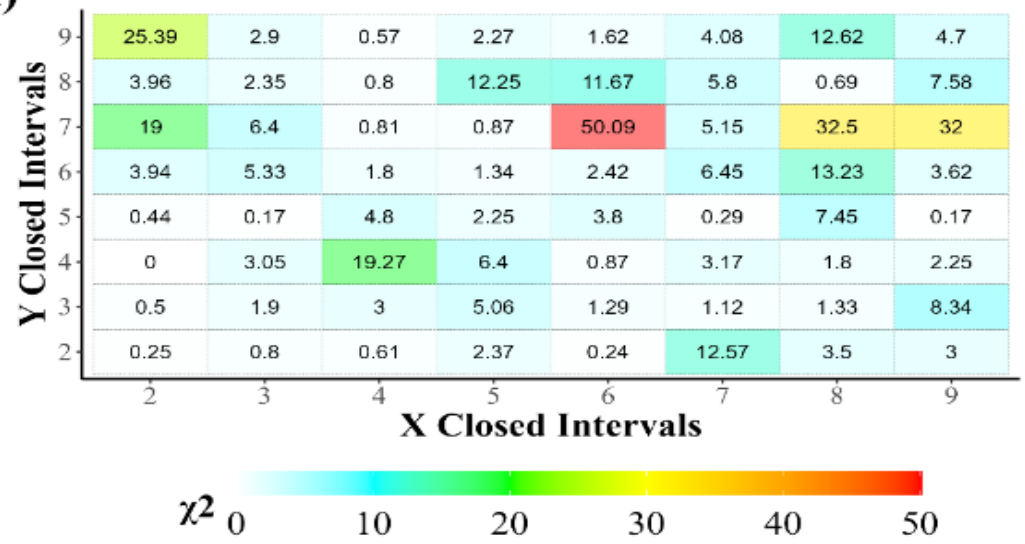


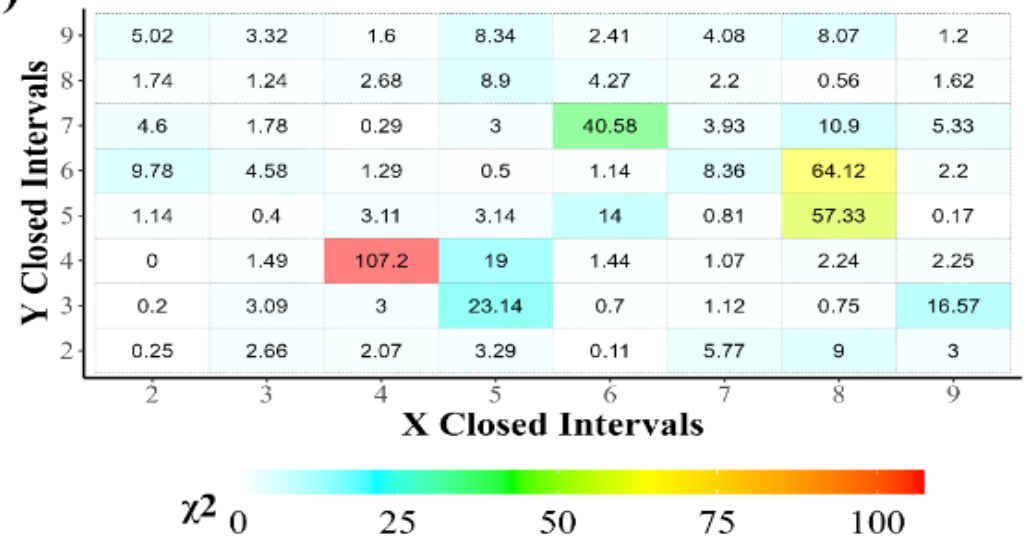
Figure 6:Represents density Curves.

Figure 6 This graph represents density curves, which reflect frequency in fixed intervals. It is essentially counting how many particles are in each interval on the Y and X axes.

A)


Y Closed Intervals
9 25.39 2.9 0.57 2.27 1.62 4.08 12.62 4.7
8 3.96 2.35 0.8 12.25 11.67 5.8 0.69 7.58
7 19 6.4 0.81 0.87 50.09 5.15 32.5 32
6 3.94 5.33 1.8 1.34 2.42 6.45 13.23 3.62
5 0.44 0.17 4.8 2.25 3.8 0.29 7.45 0.17
4 0 3.05 19.27 6.4 0.87 3.17 1.8 2.25
3 0.5 1.9 3 5.06 1.29 1.12 1.33 8.34
2 0.25 0.8 0.61 2.37 0.24 12.57 3.5 3
2 3 4 5 6 7 8 9
X Closed Intervals
χ2 0 10 20 30 40 50

B)


Y Closed Intervals
9 5.02 3.32 1.6 8.34 2.41 4.08 8.07 1.2
8 1.74 1.24 2.68 8.9 4.27 2.2 0.56 1.62
7 4.6 1.78 0.29 3 40.58 3.93 10.9 5.33
6 9.78 4.58 1.29 0.5 1.14 8.36 64.12 2.2
5 1.14 0.4 3.11 3.14 14 0.81 57.33 0.17
4 0 1.49 107.2 19 1.44 1.07 2.24 2.25
3 0.2 3.09 3 23.14 0.7 1.12 0.75 16.57
2 0.25 2.66 2.07 3.29 0.11 5.77 9 3
2 3 4 5 6 7 8 9
X Closed Intervals
χ2 0 25 50 75 100

C)

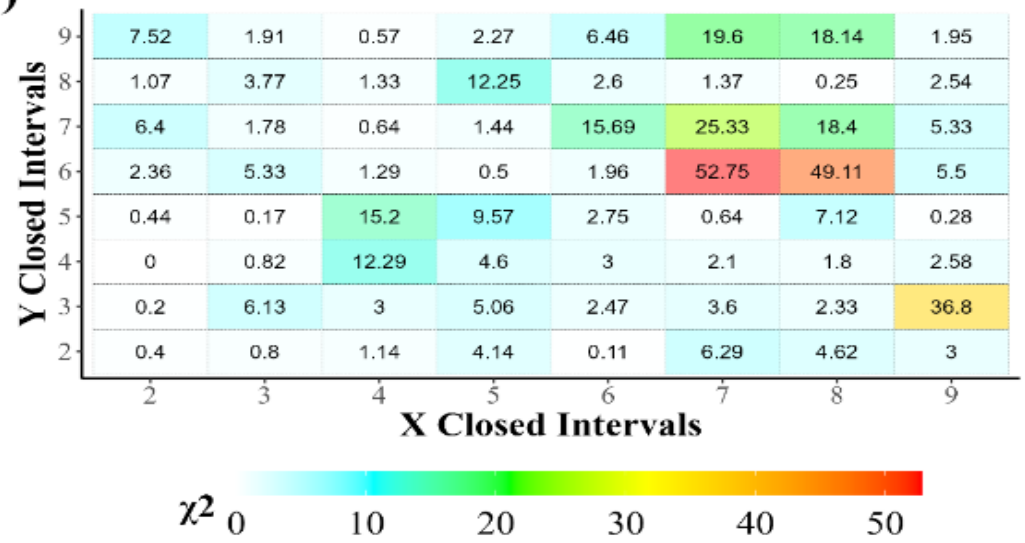

Y Closed Intervals
9 7.52 1.91 0.57 2.27 6.46 19.6 18.14 1.95
8 1.07 3.77 1.33 12.25 2.6 1.37 0.25 2.54
7 6.4 1.78 0.64 1.44 15.69 25.33 18.4 5.33
6 2.36 5.33 1.29 0.5 1.96 52.75 49.11 5.5
5 0.44 0.17 15.2 9.57 2.75 0.64 7.12 0.28
4 0 0.82 12.29 4.6 3 2.1 1.8 2.58
3 0.2 6.13 3 5.06 2.47 3.6 2.33 36.8
2 0.4 0.8 1.14 4.14 0.11 6.29 4.62 3
2 3 4 5 6 7 8 9
X Closed Intervals
χ2 0 10 20 30 40 50

Figure 7:Values of χ2 by cells.
Figure 7 shows the χ2 values corresponding to each cell for three different scenarios. No specific pattern is observed in the differences, which is consistent with what is expected under the fulfillment of the null hypothesis**. Graph A** uses the small group as the exacted value. **Graph B** uses Medium, and **Graph C** uses Large.

4. DISCUSSION

The analysis of pollen grain distribution on the slide has provided valuable insights into how particle size influences their spatial behavior. The results obtained through Chi-square (χ^2) analysis and data visualization using heat maps and scaled graphs suggest that, overall, there are no significant differences between the distribution of small and large grains. However, upon closer examination of the data, subtle patterns emerge that may be related to the physical characteristics of pollen grains, such as their size and weight.

First, the overall (χ^2) value of 0.20 indicates that, in general terms, the distributions of pollen grains are homogeneous. This suggests that the mounting method used does not introduce significant bias in the distribution of grains based on their size. However, when analyzing specific cells in **Figure 4: Graph E**, higher (χ^2) values (close to 0.063) are observed, indicating that in certain areas of the slide, the observed distributions differ from what was expected. These differences could be related to factors such as intermolecular forces that promote particle agglomeration or faster sedimentation processes that affect larger particles (Riding, 2021).

Regarding spatial distribution, **Figure 1: Graph A and Figure 2: Graph C**: show that smaller pollen grains (<23.1 μm) tend to distribute more uniformly compared to larger grains (>23.1 μm). This pattern can be explained by the phenomenon of Brownian motion, which describes the random movement of particles suspended in a fluid due to collisions with surrounding molecules (Einstein, 1905). Smaller particles, being lighter, are more susceptible to this motion, resulting in a more homogeneous distribution. On the other hand, larger particles, due to their greater mass, are less affected by Brownian motion and tend to accumulate in specific areas, as seen in **Figure 1: Graph B and Figure 3: Graph D**.

The heat maps (Graphs B and D) reveal that larger particles tend to concentrate in certain areas of the slide, while smaller particles are more dispersed. This behavior could be influenced by factors such as gravity, the viscosity of the mounting medium, and intermolecular forces. For example, larger particles might sediment more quickly due to their greater weight, which would explain their accumulation in specific areas. In contrast, smaller particles, being lighter, remain suspended for longer and distribute more uniformly (Holt & Bennett, 2014).

Additionally, the decision to divide the slide into 10 equal segments was crucial for capturing differences in data distribution without introducing statistical artifacts. A higher number of divisions could have artificially increased the (χ^2) value, while a lower number might have hidden important variations in pollen grain distribution (Anselin, 1995). This methodological choice allowed for the identification of specific patterns in grain distribution, which is essential for future studies seeking to understand how the physical characteristics of particles influence their behavior in different environments.

In summary, the results of this study suggest that, although there are no significant differences in the overall distribution of pollen grains based on size, there are subtle patterns that may be related to physical and chemical factors. These findings have important implications for fields such as paleoclimatology, where the distribution of particles like pollen can provide valuable information about past climates and environments (Birks & Birks, 2000). Future research could explore how other factors, such as the viscosity of the mounting medium or humidity, affect pollen grain distribution.

5. CONCLUSION

The study of pollen grain distribution on the slide has provided a deeper understanding of how particle size influences their spatial behavior. Through statistical analysis and data visualization, it has been demonstrated that, although there are no significant differences in the overall distribution of pollen grains based on size, there are subtle patterns suggesting that the physical characteristics of particles, such as their size and

weight, play an important role in their dispersion. These findings are not only relevant to the field of palynology but also have important implications for disciplines such as ecology, biology, and paleoclimatology.

First, the Chi-square (χ^2) analysis yielded an overall value of 0.20, indicating that, in general terms, the distributions of pollen grains are homogeneous. This suggests that the mounting method used does not introduce significant bias in the distribution of grains based on their size. However, upon closer examination of the data, higher (χ^2) values were observed in certain cells (close to 0.063), indicating that in specific areas of the slide, the observed distributions differ from what was expected. These differences could be related to factors such as intermolecular forces that promote particle agglomeration or faster sedimentation processes that affect larger particles (Riding, 2021).

Regarding spatial distribution, the results showed that smaller pollen grains (<23.1 µm) tend to distribute more uniformly compared to larger grains (>23.1 µm). This pattern can be explained by the phenomenon of Brownian motion, which describes the random movement of particles suspended in a fluid due to collisions with surrounding molecules (Einstein, 1905). Smaller particles, being lighter, are more susceptible to this motion, resulting in a more homogeneous distribution. On the other hand, larger particles, due to their greater mass, are less affected by Brownian motion and tend to accumulate in specific areas, as observed in the heat maps.

The heat maps revealed that larger particles tend to concentrate in certain areas of the slide, while smaller particles are more dispersed. This behavior could be influenced by factors such as gravity, the viscosity of the mounting medium, and intermolecular forces. For example, larger particles might sediment more quickly due to their greater weight, which would explain their accumulation in specific areas. In contrast, smaller particles, being lighter, remain suspended for longer and distribute more uniformly (Holt & Bennett, 2014).

The decision to divide the slide into 10 equal segments was crucial for capturing differences in data distribution without introducing statistical artifacts. A higher number of divisions could have artificially increased the (χ^2) value, while a lower number might have hidden important variations in pollen grain distribution (Anselin, 1995). This methodological choice allowed for the identification of specific patterns in grain

distribution, which is essential for future studies seeking to understand how the physical characteristics of particles influence their behavior in different environments.

In summary, the results of this study suggest that, although there are no significant differences in the overall distribution of pollen grains based on size, there are subtle patterns that may be related to physical and chemical factors. These findings have important implications for fields such as ecology, biology, and paleoclimatology, where the distribution of particles like pollen can provide valuable information about past climates and environments (Birks & Birks, 2000). Future research could explore how other factors, such as the viscosity of the mounting medium or humidity, affect pollen grain distribution.

Furthermore, this study highlights the importance of using advanced statistical and visual tools, such as Chi-square analysis and heat maps, to better understand particle distribution patterns in palynological studies. These tools not only allow for the identification of subtle differences in the data but also facilitate the interpretation of results in a broader context.

In conclusion, this work contributes to a better understanding of how the physical characteristics of pollen grains influence their spatial distribution. Although the results do not show significant differences at a global level, the patterns observed in specific areas suggest that factors such as size, weight, and intermolecular forces play an important role in particle dispersion. These findings open new avenues for research to explore how other environmental and methodological factors may affect pollen grain distribution, which could have important implications for the reconstruction of paleoenvironments and paleoclimates.

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