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Wildflower pollen quality in roadside habitats, with particular emphasis on *Hedera helix* (ivy).

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Abstract

The quality of air is of more significance today than ever before. Human population growth and pollutants emitted through fossil fuel consumption put pressures on air quality. It is widely acknowledged that atmospheric pollutants negatively impact human health; the same is also true for the health of wildlife and plants exposed to these pollutants (Brunekreef & Holgate, 2002; EEA, 2019; Schiavoni, *et al*., 2017). Pollen and seed formation of flowering plants have been affected when grown in areas of poor air quality (Azzazy, 2016; Sénéchal, *et al*., 2015). The purpose of this research was to assess pollen of *Hedera helix* (ivy), a native Irish wildflower (Devlin, 2019), growing in roadside habitats exposed to vehicular pollutants. Samples were collected in Sligo and Dublin; the anthers were removed from the flowers and dried at room temperature for 24 hours. The pollen was shaken from the dried anthers and mounted on to slides. Pollen grains were then observed under a compound light microscope and comparison microscope. Shape and size of the pollen grains were quantified. The findings were compared to typical shapes and sizes of *H. helix* pollen grains, found in literature and online pollen databases (Halbritter, 2016; PalDat, 2015). The results obtained from the study were examined in conjunction with air quality monitoring data from relevant local authorities and data from traffic count databases, to estimate the impacts of pollutants on pollen. Due to limited data available from the local authorities, no definite relationship between atmospheric pollutants and pollen grain shapes and sizes was established in this study. Proxy data was, however, used as representative of the pollen sample sites, based on road density, traffic flow and nearby green space. Despite being preliminary work, the findings of this study showed variations exist between samples collected at different sites, either due to pollutant exposure or other environmental factors. This study also highlighted the current air quality monitoring situation in the Dublin and Sligo areas where the samples were collected.

Keywords: air quality, wildflower, pollen, ivy, pollution

1. Introduction

Human population increases across the globe are putting pressures on the quality of the earth's resources, this includes air quality. Land use for living spaces and in which to farm livestock and grow crops can contribute to reduced air quality, as vegetation is often removed in order to achieve these practices (United Nations, 2019; Vanbergen, 2018). Additionally, industrial activities and transport, which emit atmospheric pollutants, negatively impact the quality of the air. Not only does human health suffer from poor air quality; plant, mammal and invertebrate species also suffer (Brunekreef & Holgate, 2002; Sénéchal, *et al*., 2015).

The impact of poor air quality is of interest to this research; in particular, the impact upon wildflower species growing in roadside habitats, where they would be exposed to atmospheric pollutants emitted by vehicles. The objective of this research was to investigate the effects of atmospheric pollutants on wildflower pollen grains, using traffic density and flow to estimate pollutant levels. This was achieved by i) carrying out a literature review to determine pollen structure, sources of pollution, and methods of pollen collection, storage and analysis; ii) collecting pollen from wildflowers growing in roadside habitats; iii) identifying pollutants using local authority monitoring data, or determining traffic volumes using traffic count data; iv) analysing collected pollen shape and size and comparing these findings to those in literature.

The native Irish wildflower species *Hedera helix* (ivy) was selected for this research. *H. helix* is a valuable wildflower species, prevalent throughout the island of Ireland (Akeryod, 2008; Branson, 2011; BSBI, 2019; Devlin, 2011). It plays an important role in trapping particulate matter, and numerous bird and insect species rely on this plant as a source of food and shelter throughout the year (RHS, 2020). These factors influenced the choice to focus on *H. helix* for this research. In addition, *H. helix* flowers later in the year, compared to other wildflower species (Devlin, 2019), and was the only available source of pollen at the time of year this research was taking place; late autumn-early winter.

Anthers of the plant were collected from various roadside habitats surrounding the Institute of Technology Sligo (IT Sligo) campus and also in Dublin. Plants growing in these habitats were identified, samples taken and brought back to the lab for visual analysis of the pollen grains, using a compound light microscope and a comparison microscope, in order to assess the pollen grain size and shape. An evaluation of the health of the pollen grains could be made based on comparison with the typical size and shape of *H. helix* pollen grains found in databases (Halbritter, 2016; PalDat, 2015). Data obtained from both Sligo County Council (SCC) and Dublin City Council (DCC) was used in conjunction with the pollen analysis findings, in order to establish a relationship between pollen grain shape and size and the pollutants present at time of collection.

2. Materials and Methods

Pollen Collection

Flowers of *H. helix* were collected from plants growing in roadside habitats. The locations for sample collection were based around the IT Sligo campus and in Dublin. The *H. helix* plants were identified along roadsides and the flowers were removed using secateurs. Gloves were worn while removing the flowers, to avoid skin irritations. Five flowerheads were removed from each site and placed in a labelled envelope, detailing plant name, collection location and date. The envelopes were sealed to avoid contamination with other pollen species and brought back to the lab for drying.

Dying Samples

Anthers were removed from all collected *H. helix* flowers, using tweezers. Once removed, the anthers were placed on a sheet of cardboard to dry out at room temperature (approx. 20°C) for 24 h before being observed under microscope. The samples were dried to assist with storage and to avoid unwanted deterioration of the pollen grains due to moisture and fungal growth. Dried samples were placed in labelled air-tight containers, detailing plant name, collection location, date collected and date dried.

Sample preparation
Dried anthers were held over a glass slide using a tweezers, and the dried pollen was shaken onto the glass slide. A cover slip was place over the pollen sample for the dry mounted samples. For the samples mounted in glycerine, one drop of glycerine was added before placing the cover slip over the pollen samples. The addition of glycerine to the samples hydrated the pollen grains, to aid observation.

Microscopy
Microscopy was used to visually assess the *H. helix* pollen samples. Samples, mounted in glycerine, were observed using a compound light microscope and a comparison microscope. Photographs of the pollen samples were taken, through the eye piece of the Nikon Alphaphot-2 YS2 compound light microscope, using the camera feature of a Samsung J6 smartphone. Images were generated using Leica Application Suite (LAS) software associated with the Leica DM2000R comparison microscope. This software was also used to take measurements of the pollen grains, in order to determine the average pollen grain length and the average pollen grain shape (using the ratio between the polar and equatorial axes of the pollen grain).

Calculating average pollen grain size and shape
The website www.paldat.org (PalDat, 2015) was used, along with notes on pollen shape (Biology Discussion, 2010) to gain an understanding of the typical pollen grain shape and size of *H. helix* pollen grains. Using this information as a guideline, the collected *H. helix* pollen grains were measured using the LAS software to determine whether they fell into the typical size and shape range of *H. helix*. If the pollen samples were within the typical ranges, it was assumed that the pollen came from a healthy plant, unaffected by atmospheric pollution; whereas if the pollen samples were outside the typical range, it was assumed that this atypical size or shape was a result of atmospheric pollutant exposure. The pollen grain size is the length (μm) of the polar axis of the pollen grain. While the pollen grain shape is the ratio between the polar axis length and the equatorial axis length.

3. Results

The average pollen grain size (μm) was taken for ten pollen grains from each sample site location: N16 IT Sligo Campus (Sligo); N16 Yeats Village (Sligo); Clarion Road (Sligo); Sligo Hospital (Sligo) and Walkinstown Road (Dublin) (see *Figure 1*). Each sample was dried at room temperature after collection and rehydrated with glycerine prior to being viewed under the microscope. It is important to note that the glycerine did not fully rehydrate the pollen grains, therefore they will be considered dry for the interpretation of the results.
The average pollen grain size for each pollen sample site is medium, as the average for each of the five sample sites falls within the 25 - 49 μm range, shown in *Figure 1*. The pollen from the N16 Yeats Village (Sligo) site had the smallest average pollen grain size of 33.87 μm. It also had the smallest variation between samples within the site - 8.23 μm, with the smallest N16 Yeats Village (Sligo) pollen grain measured at 29.03 μm, and the largest N16 Yeats Village (Sligo) pollen grain measured at 37.26 μm. The pollen from the Walkinstown Road (Dublin) site had the largest average pollen grain size of 41.81 μm, 7.94 μm larger than N16 Yeats Village. The pollen from the Walkinstown Road (Dublin) site also had the largest variation between samples within the site - 17.38 μm, with the smallest Walkinstown Road (Dublin) pollen grain measured at 32.22 μm, and the largest Walkinstown Road (Dublin) pollen grain measured at 49.60 μm. *Figures 2* and *3* show examples of pollen from N16 Yeats Village (Sligo) and Walkinstown Road (Dublin), respectively, at 400 x magnification under the comparison microscope.

Figure 1 shows a comparison of sizes between the ten pollen grains measured from each sample site, ranging from the smallest (1) to the largest (10). This graph shows the variation of size within each sample site, as discussed above. The pollen samples from the N16 Yeats Village (Sligo) site, indicated by the blue bars, have a relatively consistent size and there are no extreme variations of size. In contrast, the pollen samples from the Walkinstown Road (Dublin) site, indicated by the dark blue bars, have a greater variation of pollen grain size within the sample. Despite the variations in pollen grain size for each sample site, the pollen grains from all sites fit into the medium pollen grain size.

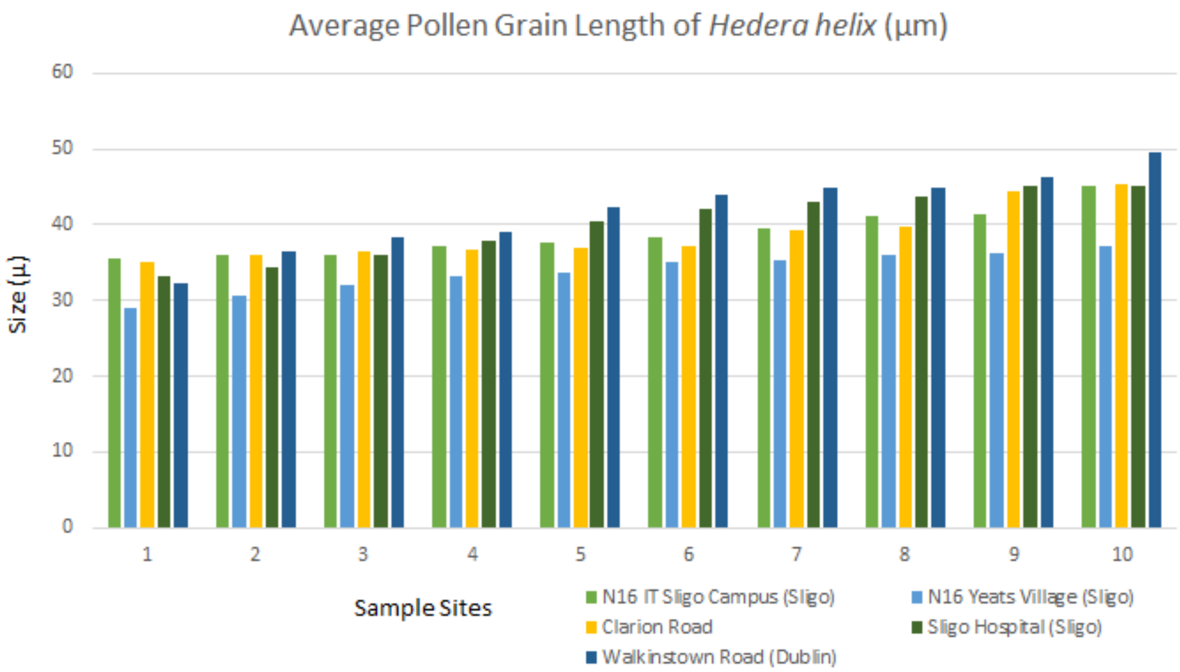

Figure 1: Comparison of the pollen grain size (µm) of collected *Hedera helix* pollen samples. From smallest (1) to largest (10), showing N16 IT Sligo Campus, Sligo (green); N16 Yeats Village, Sligo (blue); Clarion Road, Sligo (yellow); Sligo Hospital, Sligo (dark green) and Walkinstown Road, Dublin (dark blue).

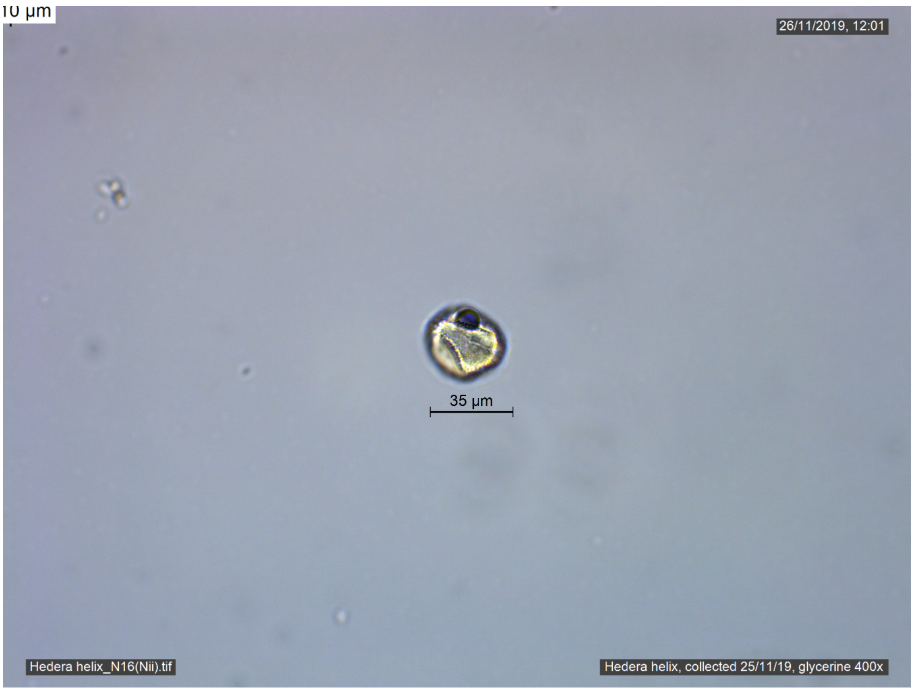

Figure *2***:** *H. helix* pollen grains collected at N16 Yeats Village (Sligo) site. One pollen grain treated with glycerine for rehydration (centre), with an air bubble (black spot). Sample viewed at magnification 400x, using Leica DM2000R comparison microscope and LAS software to generate image. Scale bar at 35µm for reference. Image generated on 26/11/19 (top right), one day after sample collection (bottom right).

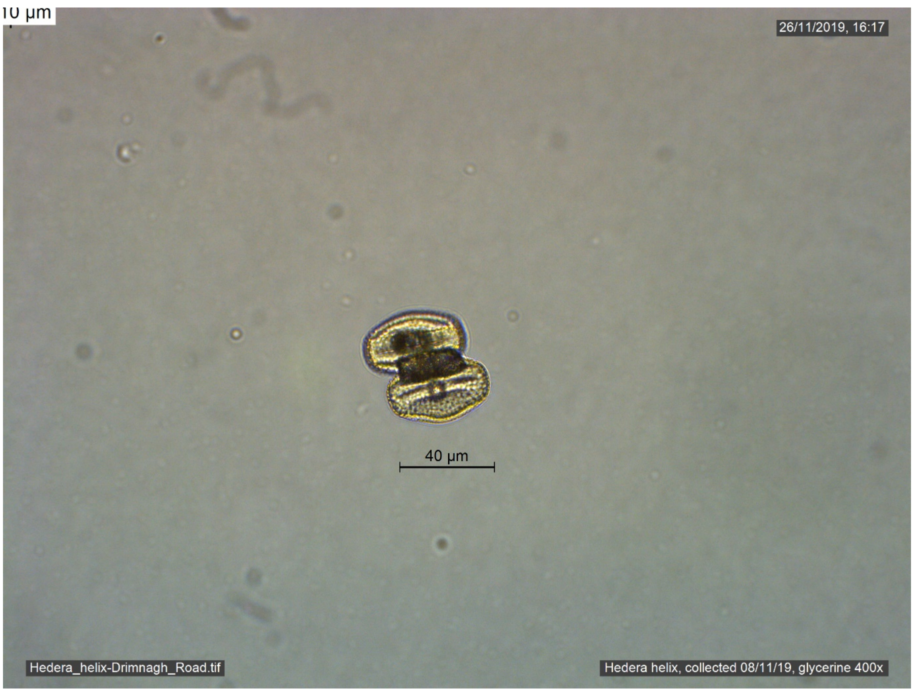

Figure 3: Pollen grains collected at Walkinstown Road (Dublin) site. Two pollen grains, with air bubble between (black spot), treated with glycerine for rehydration (*centre*). Sample viewed at magnification 400 x, using Leica DMR2000 comparison microscope and LAS software to generate image. Scale bar at 40 µm for reference. Image generated on 26/11/19 (*top right*), twenty-two days after sample collection (*bottom right*).

The average pollen grain shape, determined by the ratio between the polar axis and equatorial axis (Biology Discussion, 2010; Halbritter, 2016; PalDat, 2015), was measured for ten pollen grains from each sample site location: N16 IT Sligo Campus (Sligo); N16 Yeats Village (Sligo); Clarion Road (Sligo); Sligo Hospital (Sligo) and Walkinstown Road (Dublin; see *Table 1*).Each sample was dried at room temperature after collection and rehydrated with glycerine prior to being viewed under the microscope. Note that the glycerine did not fully rehydrate the pollen grains, therefore they will be considered dry for the interpretation of the results.

Table 1: Average pollen grain shape of *H. helix* pollen samples. Pollen grain shape is determined based on a ratio between the lengths of the polar (length) axis and equatorial (width) axis. A ratio of 1.00, where the polar axis and equatorial axis are of the same length, would mean a spheroidal shaped pollen grain, while a ratio of 1.34 – 2.00, where the polar axis is greater than the equatorial axis, would mean an oblong (prolate) shaped pollen grain. The data in the table below shows pollen grain ratios of ten pollen grains for each sample site, showing the average shape for each site in bold.

	N16 IT Sligo (Sligo) Collected 25/11/19	**N16 Yeats Village (Sligo)** Collected 25/11/19	**Clarion Road (Sligo)** Collected 18/11/19	**Sligo Hospital (Sligo)** Collected 11/11/19	**Walkinstown Road (Dublin)** Collected 08/11/19
Shape Ratio	1.05	1.04	1.22	1.45	1.33
	1.14	1.14	1.23	1.46	1.36
	1.26	1.18	1.27	1.49	1.50
	1.26	1.25	1.33	1.54	1.56
	1.31	1.30	1.38	1.54	1.58
	1.31	1.30	1.45	1.55	1.58
	1.37	1.32	1.50	1.55	1.62
	1.45	1.33	1.53	1.56	1.70
	1.50	1.33	1.55	1.61	1.72
	1.55	1.34	1.68	1.62	1.81
Average Ratio	1.32	1.25	1.41	1.54	1.58
Shape	Subprolate	Subprolate	Prolate	Prolate	Prolate

Under the Access to Information on the Environment Regulation (2003/4/EC; EU, 2003), raw data for NO_x levels were obtained for Ballyfermot, Davitt Road and St. Johns Road West air monitoring stations for October 2019 from DCC, which coincides with the time of pollen sample collection in Walkinstown Road (Dublin) site. The NO_x data proved useful and interpretation provided information representative of the Walkinstown Road (Dublin) sample site, using Davitt Road as a proxy, see *Discussion*. The daily (24 hours) NO_x averages were calculated from the raw data, in accordance with the Ambient Air Quality Directive (2008/50/EU; EU, 2008). Davitt Road recorded the second highest level of NO_x, this was significantly lower than the levels recorded at St. Johns Road West. Levels ranged from 16.5 $\mu g/m^3$ to 135.9 $\mu g/m^3$. The NO_x limit value of 200 $\mu g/m^3$ was not exceeded at the Davitt Road station during the October 2019 (see *Figure 4*). Ballyfermot recorded the lowest NO_x levels of the three stations, with levels ranging from 9.6 $\mu g/m^3$ to 90.1 $\mu g/m^3$. Similar to the Davitt Road station, the NO_x limit value of 200 $\mu g/m^3$ was not exceeded at the Ballyfermot station during October 2019 (see *Figure 4*). The Walkinstown Road (Dublin) pollen sample collection site is most analogous to the Ballyfermot and Davitt Road sites, based on traffic volumes, see *Discussion*.

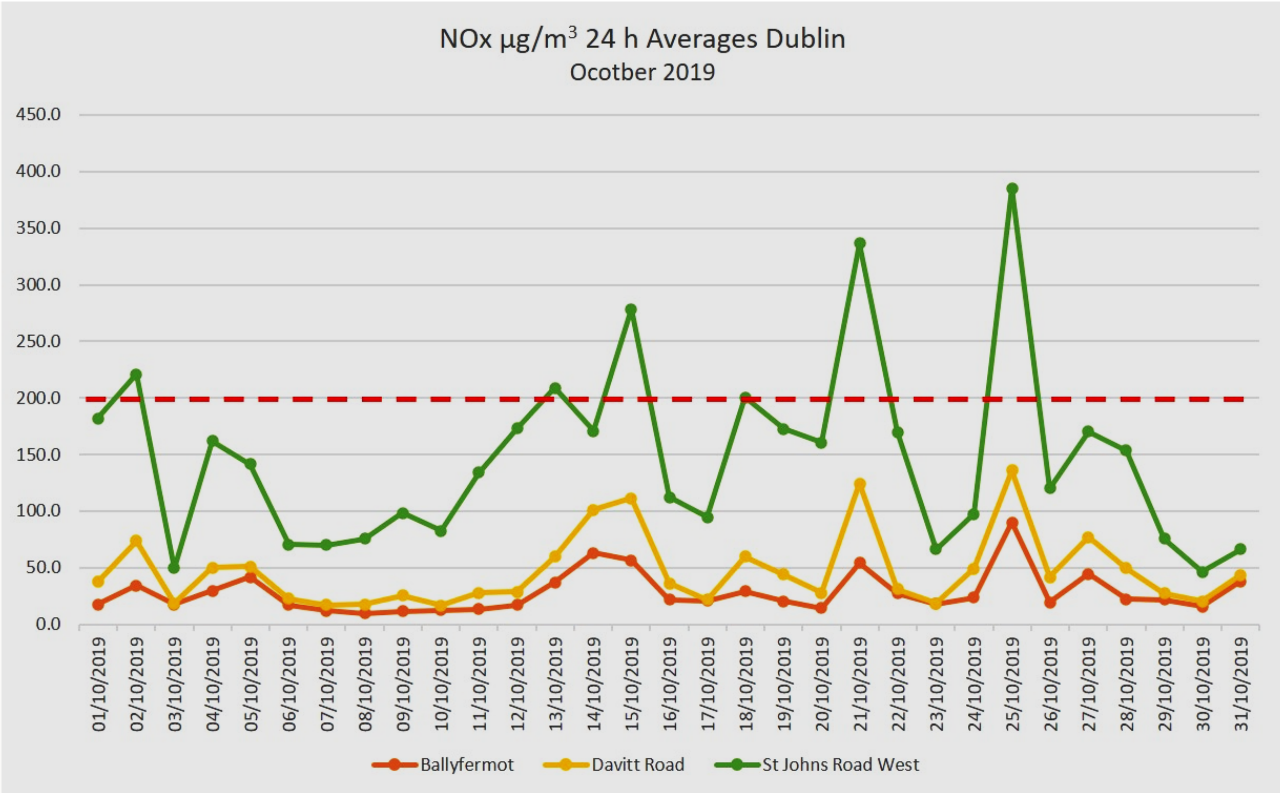

Figure 4: NO_x ($\mu m/m^3$) 24 h averages for three air quality monitoring stations in Dublin: Ballyfermot, Dublin 10 (orange); Davitt Road, Dublin 12 (yellow); St. Johns Road West, Dublin 8 (green). The red dashed line indicates the NO_x limit value of 200 $\mu m/m^3$ set out in the Ambient Air Quality Directive (2008/50/EU), averaging period is 24 h. Raw data provided by DCC.

4. Discussion

The choice of *H. helix* pollen as the focus of this study gave a good insight to the effect of atmospheric pollutants on wildflowers growing in the winter months. This provided pollen for the study until early November, when most other wildflower species would have completed their flowering for the year. Studying a broader range of wildflower plant species would provide a better understanding of how air quality affects pollen, and whether these effects differ between species. Time of year and wildflower availability would need to be considered. Ideally, running the research between Spring and Autumn would provide a good cross section of the effects of air quality of different pollen species.

The average pollen grain sizes and shapes were determined using standard classifications from databases (Halbritter, 2016; PalDat 2015). All the pollen grains were studied at varying periods after collection, ranging from one day to twenty-two days. This impacted on the shapes of the pollen due to varying levels of moisture loss between sample sites. Had it been clearly stated in literature, it would have been known it is best to assess the pollen while at its freshest, either on the day of collection or the following day, in order to accurately calculate the pollen grain shape and size. It is unclear whether the twenty-two-day period between collection of the Walkinstown Road (Dublin) pollen is the reason for this sample site having the longest pollen grains. If this research was to be carried out again, the pollen grains would not be dried, and only fresh pollen would be studied, in order to avoid further loss while in storage. Glycerine was not effective in rehydrating the dried pollen grains, which was noticeable when the pollen grain shapes were being calculated for the five sample sites. It was found that the freshest pollen grains, collected from the N16 IT Sligo and N16 Yeats Village sites, had average shapes (subprolate) that were closer to that of the typical fresh pollen grain shape which is spheroidal (Halbritter, 2016). The other three sample sites: Clarion Road (Sligo); Sligo Hospital (Sligo) and Walkinstown Road (Dublin), all had an average pollen grain size of prolate. This shape conforms to the typical dry pollen grain shape of *H. helix* as detailed by Halbritter (2016). Perhaps the use of glycerine for rehydration of dried pollen grains is species dependent. The use of a scanning electron microscope (SEM) would provide more detailed images and could be used to characterise the chemical composition of the pollen grains and identify any pollutants present on the samples.

SCC, DCC and the Environmental Protection Agency (EPA) were contacted in order to access air quality data. Air quality from the time the pollen samples were collected would assist in interpreting the results obtained, to see if there was a relationship with pollen grain size or shape and levels of atmospheric pollutants present in the air. Regarding Sligo, no air quality monitoring was in operation at the time for Sligo town. Historic data exists up to 2003, when monitoring took place from a mobile unit located in the grounds of Sligo General Hospital (EPA, 2003). This monitoring ceased due to the levels of pollutants being under the threshold limit values set out by the Air Quality Framework Directive 96/62/EC (European Union, 1996). A new air quality monitoring unit has been installed in Sligo Town by SCC, in conjunction with the EPA, and was expected to be online by mid-2020. No data exists for the period between 2003 and 2020. The location was chosen to be representative of roadside air quality. Once set up, the new air quality monitoring unit will measure levels of PM_{10}, $PM_{2.5}$ and NO_x. O_3 levels may be measured in the future. SCC are also considering the installation of an air quality monitoring unit in a residential area of the town, but it is not yet confirmed whether this will happen this year. Despite this lack of current air quality data for Sligo, traffic data was examined, in order to estimate the pollutants present in the sample areas based in traffic volumes.

Through correspondence with DCC, NO_x data for October 2019 was obtained for three Dublin air quality monitoring sites: Ballyfermot; Davitt Road and St. Johns Road West. Daily averages ($\mu m/m^3$) were interpreted and plotted in graph form (*Figure 4*). The Ambient Air Quality Directive (2008/50/EU) sets limit values for various pollutants, for NO_x the limit value is an average of 200 $\mu m/m^3$ over a 24 h period. The pollutant levels for the three sites were looked at in conjunction with the results obtained from the pollen analysis of the Walkinstrown Road pollen sample, to determine whether the air quality had an impact in the pollen size or shape. This data was of interest, as the *H. helix* pollen samples were collected the following month. The NO_x levels at both the Ballyfermot and Davitt Road sites were significantly below the limit value of 200 $\mu m/m^3$. While the St. Johns Road West site exceeded the limit value six times during October 2019. The Ballyfermot and Davitt Road sites would be more akin to the Walkinstown Road (Dublin) sample site, compared to the St. Johns Road West site. Therefore, it can be estimated that the levels of NO_x the Walkinstown Road (Dublin) pollen was exposed to would have been low. The sample site Walkinstown Road (Dublin) is a national secondary road (R819) comprising of an asphalt surface and is 1,105m in length. As there was no air quality data available for this area, the air quality monitoring station located at Davitt Road was used as a proxy. Similar to Walkinstown Road, Davitt Road is a national secondary road (R812) comprising of an asphalt surface and is 1,492m in length (DCC, 2016). These two roads were deemed similar in terms of traffic counts, road length, proximity of sample site from the road and surrounding green space. According to traffic counts obtained from IDASO, 3,830 cars travelled southbound on the R812, while 4,399 cars travelled northbound during the same period (IDASO, 2021). Considering that the NO_x limit value was not exceeded at the Davitt Road air monitoring sites, it can be assumed that the NO_x limit value was also not exceeded at the Walkinstown Road sample site, as the Davitt Road air quality results are being considered as proxy for the Walkinstown Road site. According to the Traffic Counter Data, available from Transport Infrastructure Ireland (TII), the monthly average traffic counts on the N16 in Sligo during October 2019 were an average of 382.46 cars on weekdays and an average of 232.88 cars on weekends. The peak traffic times were 8:00 and 17:00 on weekdays, and 11:00 and 15:00 on weekends (TII, 2019). Considering that the traffic counts for Sligo were significantly lower than those recorded at Walkinstown Road, Dublin, it could also be assumed that the NO_x limit values were not exceeded at N16 IT Sligo and N16 Yeats Village either. It could be assumed, by using the Davitt Road air quality results as proxy data, that the *H. hedera* pollen samples collected for the purpose of this research were no exposed to levels of NO_x greater than 200 $\mu m/m^3$. Despite this, the pollen grains analysed in this research may have been exposed to other atmospheric pollutants such as particulate matter.

The unprecedented global outbreak of COVID-19 in early 2020 had a huge impact to society across the globe. Signs of infection include respiratory symptoms, fever, cough and breathing difficulties. Severe cases of infection can lead to pneumonia, acute respiratory syndrome, kidney failure and even death (HSE, 2020; WHO, 2020). As of 26th March 2020, COVID-19 was identified in 200 countries, the number of global confirmed cases stood at 465,915. The highest number of cases being recorded in China and Italy. The number of global confirmed deaths stood at 21,031 (WHO, 2020). In Ireland, as of 26th March 2020, the number of confirmed cases stood at 1,819 with 19 confirmed deaths (Department of Health, 2020). These figures are expected to rise rapidly by April 2020, both nationally and internationally. Not to ignore the devastation that this outbreak has caused to many people around the world, one benefit has emerged, although it seems insignificant during such a difficult time - considerable decreases in levels of atmospheric pollutants have been observed in cities and countries across the world since the outbreak of COVID-19. Preliminary studies in New York have shown a reduction of CO levels by almost 50%, a similar drop in CO_2 levels was also observed (BBC News, 2020). The environmental impact of COVID-19 was clearly seen in China, with maps detailing a sharp contrast in levels of NO_2 just before the outbreaks and during the outbreaks. A 25% decrease in energy use and emissions was seen during a two-week period in China, related to reduced car journeys and industrial activity (BBC News, 2020). This indicates that the decrease in global industrial activity and transport due to the COVID-19 pandemic, resulted in associated emissions also decreasing. Other emissions are likely to follow this trend, due to measures implemented by governments across the world to curtail the spread of the virus. Significant reductions of global emissions for the year are likely to be seen, if the pandemic continues over the next few months. The biggest impact on air quality will come after the pandemic, when governments attempt to revive their economies (BBC News, 2020). It would be a good opportunity to renovate buildings and to install heat pumps and electrical charging points to avoid the emissions levels rapidly increasing once the pandemic lessens (BBC News, 2020). Perhaps the impact that the COVID-19 pandemic has had on global air quality will highlight the need for actions that are urgently required to reduce atmospheric pollutants across the world.

5. Conclusions

According to literature, air quality does affect the quality/viability of pollen. Poor air quality can affect the pollen grain by damaging the pollen grain coat, allowing pathogens to enter and for deterioration of the male gamete. It is unclear whether the pollen grains collected for this study were affected by the air quality in any of the sample site locations, as the air quality data either does not exist for the selected sample sites or is not easily accessible to the public. Were this information available, a conclusion as to whether the air quality influenced the average pollen grain length and shape could have been determined. With regards to the methods of pollen analysis, no clear standardised methods exist. This impacted on the decision to dry the pollen grains and rehydrate them at a later date with glycerine. If it had been known that the glycerine would not fully rehydrate the dried pollen grains, all samples would have been looked at on the same day as collection in order to view them fresh.

6. Future Work

While no definitive results were obtained in relation to the effect of atmospheric pollutants in Sligo town or Walkinstown, Dublin on the collected *Hedera helix* pollen grains, it was found that it is an area where more work is required. Pollen analysis is a useful tool for assessing the impact of atmospheric pollutants on plant health. While conventional assessment of air quality with monitoring stations and mobile units is the most accurate way to understand the pollutant levels in a particular area, the impact of pollutants on pollen grains must be acknowledged in order to avoid negative effects to biodiversity and crops grown for food supply.

Lichen surveys of Sligo and Dublin may prove useful to assess the air quality in these areas, where no air quality monitoring data exists, for any future studies that may be carried out concerning the effect of atmospheric pollutants on pollen grains. Additionally, further research into traffic volumes prior to collection of pollen samples may also prove beneficial to future studies. This would allow a focus to be placed on roads with known heavy and light traffic volumes. Samples sites could then be based on locations due to their known traffic volumes. In turn, known areas of high levels of vehicle emitted atmospheric pollutants could be compared to known areas of lower levels. This would need to be carried out at a time of year when pollen sources are abundant.

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