Lichen in Manor Woods

A local study of air quality and lichen health

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Abstract

There have been rising concerns of degrading air quality in the local community (Bristol, United Kingdom). We have examined lichen's utility as a bioindicator to assess air quality and the effects of pollutants in an urban environment. Lichen has been proved to be a useful, cost-effective and plentiful resource in assessing air quality and identifying areas of concern. This paper aims to investigate lichen variability within Manor Woods, the distribution of heavy metal pollution within Manor Woods and the impact of heavy metal pollution on lichen physiology. To do this, microwave digestion was undertaken to identify heavy metals across the 27 samples. Absorbance was then measured using 435nm and 415nm wavelengths in a spectrophotometer in order to determine the ratio of healthy chlorophyll and degraded chlorophyll. Due to the small sample area of Manor Woods and small distance between the three transects, it was difficult to determine anything conclusive due to the lack of statistical significance between variables. There was a lack of variation in optical density ratios among the three transects. Heavy metals were assessed by individual concentrations of metals against the tested variables. Regression analysis of heavy metal concentration and altitude showed a statistically significant negative relationship between iron (Fe) concentration and altitude. Limitations such as limited sample size, limited environment data collection, and lack of molecular techniques has raised questions as to whether species identification may prove to be a more effective method of investigating air pollution. Therefore, this report into lichen health in Manor Woods has presented insights into the complexity of environmental factors as well as lichen physiology and distribution.

1. Introduction

1.1 Background

The World Health Organization (2000) state there are both natural and anthropogenic sources of air pollution. Natural air pollution originates from a range of sources such as plants, forest fires, geothermal sources, radiological decomposition and water and land emissions. Anthropogenic air pollution sources include the increasing use of fossil fuels for energy, and the expansion in the manufacture and use of chemicals. The impact of air pollution on humans is significant as it can have direct consequences on health and the environment. With the recent population expansion in Bristol of 10.6% in the last decade, and a projected future growth rising to 15% by 2043 (Bristol City Council, 2023), there is increasing concerns of air quality in Bristol's urban environment. This is due to the unplanned developments of the buildings, and a lack of consideration regarding air flow and the impact on the dispersion and self-cleaning ability of the atmosphere (Sushi Kumar, 2020). This concern is evidently relevant to our study site with a recent report by the Bristol City Council showing that nitrogen dioxide levels have exceeded the annual objective of $40 \mu g/m^3$ in the years 2010-2022 (Figure 1).

Nitrogen dioxide emissions have become a main pollutant of concern in Bristol with consistent exceedance of the annual objectives for NO_2 (Figure 1). Furthermore, a report conducted in 2017 concluded that 300 deaths in Bristol can be correlated to the exposure to air pollutants (Bristol City Council, 2023). This concept is developed further in Section 2.1. With air quality an existing concern in Bristol, Manor Woods offers a suitable location for a local study of air quality and lichen health.

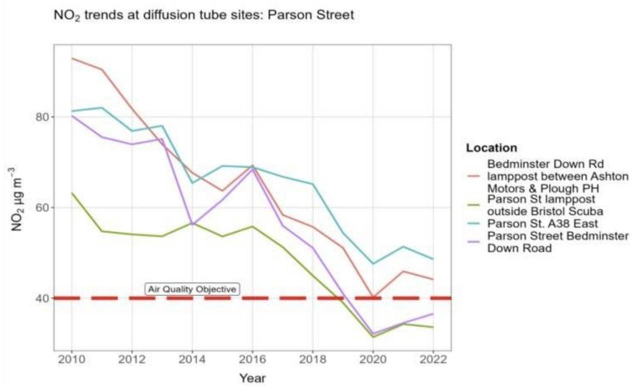

Figure 1: Trends in Nitrogen dioxide around parson street (Bristol City Council, 2023)

1.2 Usefulness of lichen

Lichen serves as an incredibly useful bioindicator as a result their properties as long living, ectohydric organisms, indicating that they transport water on their external surface and have a limited control on water and gas exchange (Bačkor, M. and Loppi, S, 2009). These properties relate to their sensitivity to air quality changes by enabling lichen to accumulate heavy metals, resulting in chlorophyll degradation (Yang, J., Oh, S. O., & Hur, J. S., 2023). Additionally, lichen is inexpensive and naturally abundant and therefore even more applicable to our study. Lichen is incredibly useful in gauging the effects of global warming, contamination, shifts in land use patterns, and various phylogenetic stressors on habitat, thus providing a plentiful tool to acquire analytical samples (Thakur et al, 2023). Therefore, using lichen will allow for the collection of a vast number of samples from Manor Woods because of its suitable environment for the growth of lichen exposed to properties we intend on analysing.

1.3 Study location

Manor Woods Valley is a nature reserve located in Bedminster, southwest Bristol that boasts a varied environment such as wildflower meadows, woodlands and an abundance of wildlife which runs alongside the Malago River. Manor Woods has been shaped by a constantly evolving history of land use change, with significant changes occurring after World War Two. After the Second World War the surrounding area changed from predominantly agricultural land to urban housing, accompanied by brick and tile work industry at the northeast of Manor Woods (Manor Woods Valley, 2021). The varied environments located within Manor Woods offer habitats alongside foraging and breeding opportunities for a host of species and therefore plays a critical role in maintaining local biodiversity. Manor Woods also falls in close proximity to the A3029, a heavily-used link between Bristol and the west and south. As a result, the location of Manor Woods, facing increasing levels of air pollutants, offers a relevant study site to investigate the impact of decreasing air quality on local ecosystems.

1.4 Motivation for study

Our study aims to reveal the ecological significance of lichen within an urban–impacted ecosystem, notably their role as bioindicators and contributors to environmental health. We seek to address both the scientific and conservation-oriented questions with relevance for both the local community and broader ecological understanding of the nature reserve. Understanding the diversity and distribution of lichen species within Manor Woods provides insight into the overall biodiversity and ecological dynamics of the environment. This means we can investigate the aims of our study by being able to identify lichen variability and the distribution of heavy metal pollution within Manor Woods and access the impact of heavy metal pollution on lichen physiology. To achieve these aims we produced three different hypotheses and associated research questions:

1. Lichen bioaccumulation of heavy metal will increase with proximity to the road A3029 due to this road having a high amount of traffic on it. This will see if lichen is accumulating pollutants from these emissions of vehicles.
    - How is heavy metal concentration influenced by proximity to the A3029?
        - How does this impact lichen physiology
2. Heavy metal bioaccumulation collects within the valley at lower altitudes. Here we are trying to see if altitude has any influence on trapping pollutants in the nature reserve therefore increasing bioaccumulation in the lichens.
    - How is heavy metal concentration influenced by altitude?
        - How does this impact lichen physiology?
3. Chlorophyll degradation increases with higher heavy metal bioaccumulation. By this we are trying to identify whether the land use change surrounding the nature reserve and the industry in northeast end are influencing lichen health.
    - How does lichen species vary across Manor Woods?
    - How does optical density vary across Manor Woods?

Using these hypotheses, we can test to see whether Manor Woods is suffering from poor air quality due to these factors by analysing the lichen health.

2. Literature review

2.1 Air quality in Bristol

The Bristol City Council annual air quality report of 2023 showed that NO_2 measurements in 2022 saw a reduction of 0.7% when compared to 2021 concentrations, with a reduction of 14.2% seen since the last measurements taken before Covid in 2019 (Bristol Council Annual Air Quality Report, 2023). However, this reduction falls short of the 24% national average reductions in NO_2 roadside locations since 2019. There was also an observed increase in particulate matter at these locations, however these measurements did not exceed targets.

Although the air quality status report did not take measurements within Manor Woods Valley specifically, there were fourteen locations measured north of the woods, in close proximity to the A3029 (Figure 2). Five out of fourteen of these measurements in 2022 exceeded the national objective of less than $40 \mu g/m^3$ of NO_2. These observations proved useful for developing our hypothesis that lichen samples collected closest to the road will have higher concentrations of heavy metals.

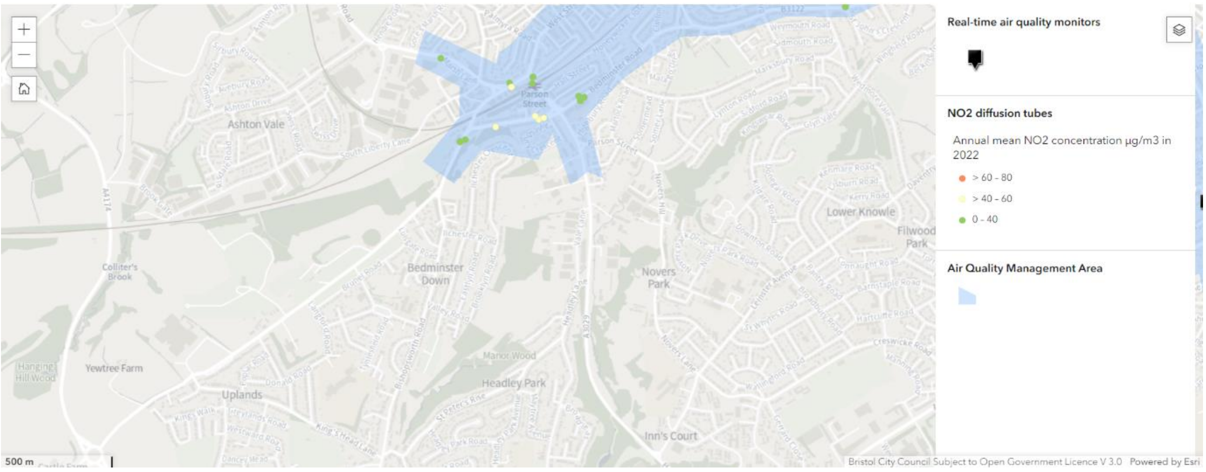

Figure 2: Locations of NO_2 air pollution measurements taken around Manor Woods Valley in 2022 (Bristol City Council 2023)

The study site experiences prevailing south-westerly winds, and secondary south-easterly winds, in the Bedminster area (Southwest Environmental, 2017), suggesting that perhaps the emissions from road traffic and industry at the top of Manor Woods may not have as much of a significant impact. Manor Woods falls just south of the lower boundary of the Air Quality Management Area (AMQA) in Bristol (Figure 3). AMQA identifies areas of poor exhaust dispersion from major roads and junctions. Manor Woods' proximity to this boundary may be indicative of potential issues with air pollution. When considering projected growth in Bristol, heavy metal pollution in Manor Woods is likely to become an increasingly pressing concern.

Figure 3: Air Quality Management Area boundary within Bristol's wards (Bristol City Council 2023)

The Air Quality Report (2023) states that over 80% of NO_2 pollution in the areas that exceed targets, including those surrounding Manor Woods, comes from local traffic emissions. If the same is true for other heavy metals, we expect to find a higher concentration of heavy metals in the lichen samples taken from the areas closest to the roads and manufacturing works. These observations aided in designing our hypothesis regarding heavy metal concentration and proximity to the A3029.

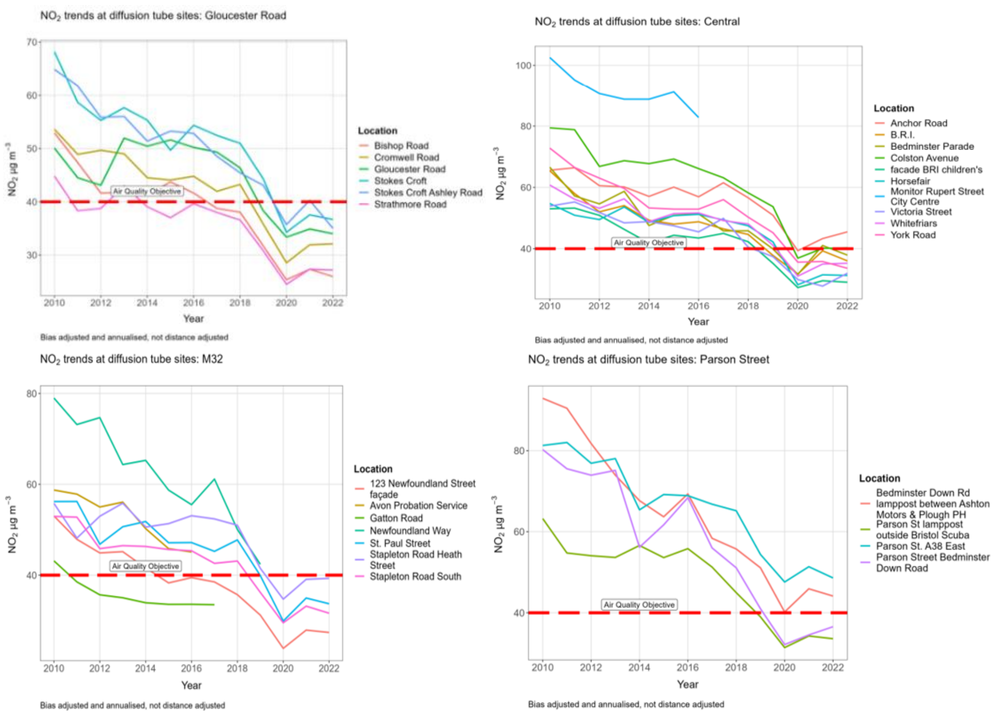

Figure 4: NO_2 trends across Bristol between 2010-2022 (Bristol Council Annual Air Quality Status Report, 2023)

The Bristol Air Quality Report also took measurements of emissions in many other areas of Bristol, which we used to compare to the emissions in our area of interest. Figure 4 shows that in many of the areas across Bristol, NO_2 measurements are below the objective of 40µg/m^3. There is an exception in the measurements taken in Central Bristol, with Anchor Road being marginally over the objective in 2022. However, the trends in Bedminster show that measurements have consistently exceeded the objective since 2010, only falling close to 40µg/m^3 in the year 2020, likely due to the pandemic.

These rates of emissions justify our site as a particular area of interest, especially considering the site's importance as a public space and nature reserve.

2.2 Previous Studies

A study by Yang et al. in 2023 on the response of lichen to heavy metal pollution was used to aid our study. Lichen are extremely sensitive to heavy metal pollution, which makes them useful in monitoring the presence of pollutants in an area. Yang et al. used the relationship between heavy metals and chlorophyll degradation for their analysis, proving useful since chlorophyll degradation was key component of our lab methodology. The metals Cu, Fe, Mn, and Zn were used in this study, as well as in our own laboratory work. These lichen samples were taken from the Gangwon Province in South Korea as well as from Jeju Island off the southern tip of the country.

There are, however, substantial differences between the Gangwon Province and Jeju Island and Manor Woods, notably the scale of the study. Yang et al's study spanned over 300 miles compared to this study which will cover approximately 0.7 miles. We expect this will result in smaller differences in heavy metal concentrations observed as well as a lower likelihood of statistical significance in analysis.

A study by Chetia et al. (2021) found that lichen samples that were taken from the area close to the refinery and roadside were found to have much higher values of heavy metal concentration. This assisted our investigation since one of the sample locations in Manor Woods Valley was from an area near manufacturing and tileworks. Therefore, if all other factors between the study carried out by Chetia et al. and our own remained constant, we could expect to see variations in heavy metal concentration in our own lichen samples based on their distance from the tileworks.

3. Methodology

3.1 Field methods

Three transects were established, aiming to collect lichen from the Old Manor Wood, Pond Wood, New Manor Wood, Allotment Wood, and the New Plantation, shown in figure 5. Transect A, at a bearing of 160 degrees found lichen from Old Manor Wood and Allotment Wood. Transect B, at a bearing of 140 degrees focused on New Manor Wood, either side of the path. Transect C, at a bearing of 160 degrees went through the New Plantation. Each transect collected 9 samples, which were approximately 15m apart. However, the distribution varied due to availability of lichen.

Figure 5 - Manor Woods valley with different woodlands and plotted transects (Manor Woods Valley Group, 2020)

At each site, lichen was either scraped off twigs and trees using a scalpel or collected off the floor and placed into a sample bag. The samples were then all placed in the fridge at approximately 3 ° C.

3.2 Lab methods

3.21 Lichen identification

In the lab, the samples were identified using the FSC 'Key to lichens on twigs' identification sheet (2013). This method is very subjective. Any remaining twigs were also removed before using the samples.

3.22 Test for heavy metals

Before testing the lichen for heavy metals, the samples were weighed, dried, weighed again and the loss of mass calculated.

3.221 Microwave digest

A study by Tuncel et al (2003) found microwave digestion to give the best results of over ten elements compared to other digestion methods, when compared to certified reference materials. This validated the decision to use microwave digestion to identify heavy metals in the lichen. The paper concluded that the use of a microwave digestion system is advisable when optimal analyte recovery and duration of digestion is important. Microwave digestion is used since it is a rapid and efficient way to decompose a sample prior to identification of trace methods (Robache et al, 2000; Sandroni et al, 2003).

Microwave digestion was used to identify heavy metals in our lichen samples. The samples were first ground and weighed, then reagents were added. The reagents used were nitric acid, hydrochloric acid, and hydrogen peroxide. Other studies additionally used hydrofluoric acid (Tuncel et al, 2003). The samples were then placed in the microwave. Once the programme was finished, the samples and three blanks were left overnight to completely cool. They were then filtered in preparation for analysis by ICP-OES or ICP-MS to identify the heavy metals.

Ali et al (2014) recommend using ICP-OES (Inductively Couples Plasma Optical Emission Spectrometry) as it is suitable for detecting heavy metals due to its high sample throughput capacity and high accuracy. This paper compared this technique with UV-Vis spectrophotometer on detecting heavy metals in soil, concluding that ICP-OES was more suitable.

After initial analysis by ICP-OES, negligible concentrations of cadmium, cobalt, nickel, and lead were found at a resolution of 0.1ppb. To provide a more complete data set, four more heavy metals with the highest concentrations present in samples were chosen: aluminium, calcium, magnesium, and manganese.

3.222 Metal identification

Given the lab capacity restraints, this study was limited to analysis of 7 heavy metals: Cd, Zn, Pb, Cu, Fe, Cr, and Ni. Under ideal situations a multitude of elements would be analysed to provide a comprehensive and detailed understanding of heavy metal bioaccumulation in lichen; similar studies analysed up to 42 different elements (Wu et al, 2020). A number of studies focusing on bioavailability of heavy metals informed the selection of metals, focusing on street dust and road traffic sources such as brake lining and tires (Charlesworth et al., 2003; Chetia et al., 2021; Hjortenkrans, 2008; Suryawanshi et al., 2016; Wu et al., 2020). These were used in conjunction with Puckett's (1976) study on the relative toxicities of heavy metals on photosynthesis. Cd, Zn, Pb, Cu, Fe, Cr, Ni were found to be significant elements both in bioavailability and in effect, notably Cu, Pb, Cd and Ni on photosynthesis toxicity.

3.23 Chlorophyll Degradation

Chlorophyll degradation was chosen as a focus of this study as it is directly impacted by heavy metal bioaccumulation in lichen, as demonstrated by Puckett (1976). Therefore, exploration into lichen chlorophyll degradation supports both the presence of heavy metals and understanding the impact of heavy metals on lichen physiology. Identifying the extent of chlorophyll degradation was chosen over chlorophyll concentrations in lichen as it is a direct and comparable measure of the dynamic response of lichen in the presence of heavy metals. The method used in this study is based on the paper by Ronen and Galun (1984) suggesting that 435nm and 415nm wavelengths are reliable parameters for determining the ratio of healthy chlorophyll and degraded chlorophyll with little interference from other pigments. Yang et al (2023) used this method as an optimal technique to perform a more comprehensive study with similar aims to this study.

For each sample, 50mg of ground lichen was covered by 10mL of alkaline acetone and wrapped in aluminium foil to keep dark throughout the research. Each sample was then vortexed for 2 minutes and sonicated in a sonicator bath for 20 minutes before placed in a spark free refrigerator for a 72-hour extraction period. Before measuring, the samples were once again vortexed and sonicated for 2 and 20 minutes respectively and placed in a centrifuge at 1200 rpm for 5 minutes. Absorbance was then measured using spectrophotometry for 415nm and 435nm wavelengths. To ensure sources of error were reduced, 3 blanks were also taken, and the mean absorbance of these blanks were taken off the sample absorbance data.
The extent of chlorophyll degradation was determined by establishing the ratio, R, of the optical density (OD) where $R = OD_{435}/OD_{415}$. This ratio acts as a relative measure of chlorophyll degradation by identifying the shifts in the absorption spectrum of chlorophyll when its structure and composition change as it degrades. A high ratio signifies a low degree of chlorophyll degradation as there is a relatively significantly higher absorbance of healthy chlorophyll particles (OD_{435}) compared to the absorbance of degraded chlorophyll (OD_{415}). 435nm and 415nm wavelengths were chosen based on Ronen and Galun's (1984) paper that suggests these wavelengths as reliable parameters with little interference from other pigments. They also stated that the OD ratio in a healthy lichen sample was found to be 1.40, with pollution reducing the value of the ratio, and this was helpful to our study because it gave a baseline for expected OD values in our study.

3.3 Statistical Methods

A combination of software tools was utilised, RStudio and Excel employed for all plots and graphs as well as statistical analyses. Additionally, mapping was carried out using Digimap. We assessed the normality of all variables, and for those found to deviate from normal distribution, a logarithmic transformation was applied to achieve normality. Consequently, all variables were successfully normalised except for manganese concentration, which exhibited persistent non-normality. Therefore, manganese concentration was excluded from analysis. Having confirmed the normal distribution of variables, parametric tests were deemed appropriate for subsequent analyses, ensuring reliable and robust interpretations of the data.

4. Results

4.1 Chlorophyll and optical density

Once the chlorophyll data from the lab was received, we used the Optical Density ratio (OD ratio) to assess its relationship with our chosen variables. A higher OD ratio indicates lower chlorophyll degradation, and therefore a higher presence of chlorophyll in the sample. The mean optical density ratios for transects A, B and C were 1.49, 1.19 and 1.09 respectively. Transect A exhibited a slightly higher mean optical density compared to the baseline value of 1.4 for healthy chlorophyll (Ronen and Galun, 1984), while transects B and C showed lower mean optical density ratios.

4.11 Optical density ratio distribution within Manor Woods (between transects)

Variations in optical density of samples were initially explored across the three transects using a boxplot (Figure 6). Upon visual inspection no substantial difference in optical density ratios was revealed, three outliers were identified and after consideration, removed to maintain data integrity and accuracy. We then conducted a more rigorous statistical examination using Welch's ANOVA to account for unequal sample sizes. No statistically significant difference was observed (F = 1.6734, p = 0.2264).

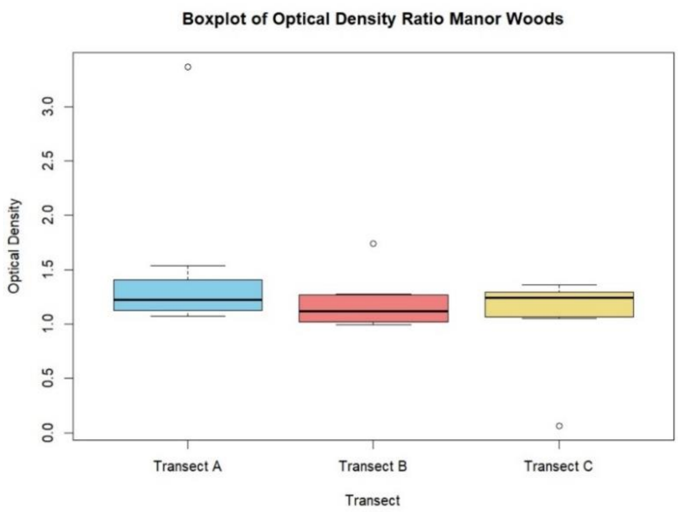

Figure 6 - Boxplot of Optical Density ratio across the three transects

4.12 Optical density ratio and altitude

We assessed the potential relationship between altitude and OD ratio using a linear regression model, revealing a slight negative difference relationship between OD ratio and altitude (estimate = –0.00121). However, there was no statistical significance in this relationship ($p = 0.663$)

4.13 Optical density ratio and distance from A3029

A second linear regression model was used to assess the relationship between OD ratio and distance from the A3029. This yielded similar results (estimate = –0.000121, $p = 0.553$), showing a lack of evidence to reject the null.

4.14 Optical density ratio against distance from closest edge of Manor Woods

A third linear regression model was created to assess the relationship between OD ratio and distance to the closest edge of Manor Woods. The model revealed a very slight negative relationship between the two variables but again, this relationship was not significant ($p = 0.616$).

4.15 Optical density ratio against woodland type

An ANOVA test was used to assess the impact of woodland type on OD ratio. The ANOVA results revealed that the variation in OD ratio among different types of woodland was not statistically significant ($F = 1.82$, $p = 0.167$).

4.2 Heavy metals

Heavy metal concentration data from the lab were next to be analysed. Due to the complexities of collating the different metals into one set of values, we instead assessed the concentrations of individual metals against our chosen variables. This would also allow us to identify which metals interact with altitude, leading to more useful results.

4.21 Individual heavy metal concentration against altitude

The following table shows the results of linear regression models for each heavy metal present in the lichen against altitude.

Table 1 – Linear regression of heavy metals against altitude

Metal	**Estimate (4 d.p)**	**p-value (3 d.p)**
Fe	-0.0202	0.039
Al	-0.0233	0.137
Ca	0.0219	0.224
Cu	0.0227	0.032
Mg	0.0127	0.067
Zn	-0.0046	0.597

Iron and copper are the only two metals that returned a significant relationship with altitude ($p = 0.039$ and 0.032, respectively). The relationship between iron and altitude showed that concentration decreases with an increase in altitude (estimate = –0.0202), with the opposite true for copper (estimate = 0.0227).

4.22 Individual heavy metal concentration against distance from A3029

Table 2 displays the statistical results from the linear regression models for each metal against distance from the A3029, as done in the previous section.

Table 2 – Linear regression of heavy metals against distance from A3029

Metal	Estimate (4 d.p)	p-value (3 d.p)
Fe	-0.0015	0.037
Al	-0.0007	0.580
Ca	0.0017	0.186
Cu	0.0018	0.023
Mg	0.0006	0.210
Zn	-0.0003	0.599

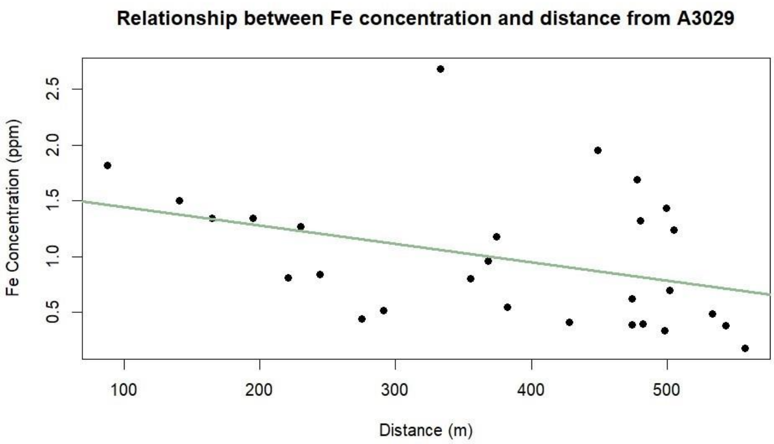

Figure 7 - Linear regression of iron (Fe) against distance from A3029

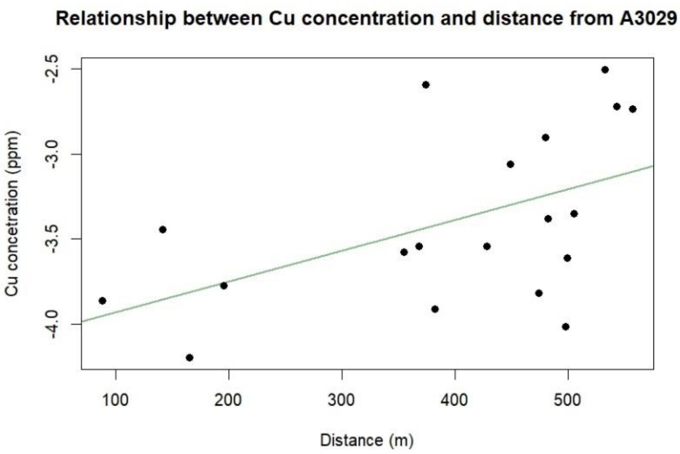

Figure 8 - Linear regression of copper (Cu) against distance from A3029

Iron once again showed a statistically significant relationship (p = 0.037), as did Copper (p = 0.023). Iron concentration was shown to decrease with increasing distance from the A3029 (estimate = -0.0015), as showing in figure 7, whilst copper concentration increased with distance (estimate = 0.0018).

4.23 Individual heavy metal concentration against distance from edge of Manor Woods

Table 3 - displays the statistical results from the linear regression models for each metal against distance from the closest edge of Manor Woods, as done in the previous section.

Metal	Estimate (4 d.p)	p-value (3 d.p)
Fe	0.0016	0.529
Al	0.0032	0.429
Ca	-0.0023	0.617
Cu	-0.0063	0.051
Mg	-0.0060	0.025
Zn	0.0002	0.933

Copper and Magnesium both presented statistically significant relationships, with both metal concentrations decreasing with increasing distance from the edge of Manor Woods (estimate = -0.0063, -0.0060, p = 0.051, 0.025, respectfully).

4.3 Lichen species distribution

The below pie chart depicts the number of lichen species that were identified as being present in each transect. For example, the orange segment represents the five lichen species that were found only in transect A. The even distribution across different transect combinations suggests there was not enough of a pattern in lichen species distribution to be worthy of note for this study, although it would be worth noting for future study how this could vary across a larger area.

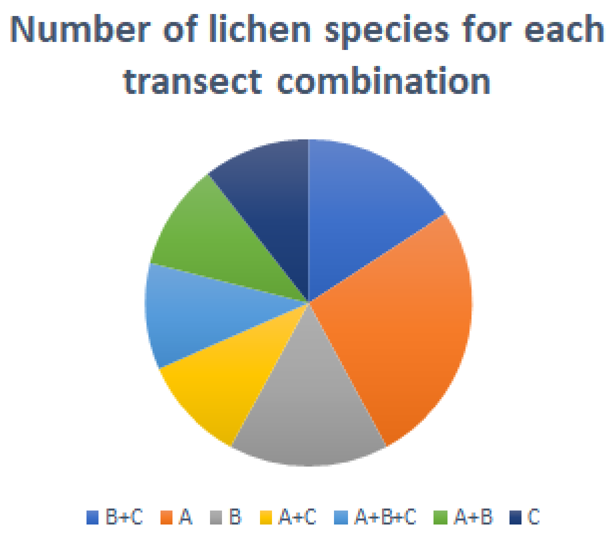

Figure 9 - Pie chart showing the distribution of different lichen species across the different transects

5. Discussion

5.1 How do lichen species vary across Manor Woods?

Overall, there was a significant range in lichen species across the three transects, where 20 different species were identified. The most predominant specie found was *xanthoria polycarpa*, which according to the FSC identification sheet (2013) is both nitrogen and acid tolerant, making it suitable for polluted areas. A study by Stringer et al (1974), based on understanding species diversity of lichens for urban and suburban areas, found *xanthoria polycarpa* to form in more polluted areas compared to other species.

Notably, the second most predominant species, *xanthoria parientina*, is identified as metal tolerant with little to no physiological consequences attributed to heavy metal bioaccumulation (Parviainen et. al., 2019).

5.2 How does optical density vary across Manor Woods?

The variation in OD ratio of lichen samples across Manor Woods was unanticipated. Our initial hypothesis had posited that transect C, situated in close proximity to industrial activity such as brickworks, would exhibit a lower OD ratio, reflecting potential pollution impacts. While the exploration of variation in OD ratio through boxplots did not reveal any substantial variation across the transects, they did provide insight in terms of identifying outliers. The elevated values could be attributed to favourable microclimates, or specific types of lichen thriving in these areas. In fact, studies have found that different species exhibit different trends despite being found in close proximity to one another. Renhorn et al., (1997) found that the biomass in *Platismatia glauca* (6.2% in 16 months) was 41% higher than *Lobaria pulmonaria* (4.4%), suggesting that there is an intricate interplay of environmental factors such as moisture levels or sunlight exposure creates microclimates favouring each species' specific requirements. Furthermore, they found that light intensity was 4.3 times higher at the edge of the site than the centre (Renhorn et al., 1997), providing more insight into the variation in climatic conditions that can occur over a study site.

In contrast, the lower outlier in transect C may be indicative of unique environmental stressors associated with close proximity to industrial activities. Some pollutants are known to damage lichen physiology, notably altering the concentration of assimilation pigments (Bačkor et al., 2009), therefore resulting in a lower OD ratio. Yet, given the already small-scale nature of the study, it is less probable that specific microclimate variations within each transect could account for such pronounced differences in OD ratios. Additionally, the substantial discrepancy, nearly four times higher than other values in the case of transect A indicates the possibility of data processing errors or anomalies during measurement could be the cause of these outliers. Similarly, the presence of a single outlier in each transect, rather than a consistent trend across multiple data points, suggests these anomalies are more likely attributable to errors in data collection and processing, rather than reflecting genuine ecological variations within each transect. This is supplemented by the fact that all three blanks undertaken in analysis returned optical densities ranging from 0.87-1.25. So, further investigation and methodological validation through repeated collection and analysis is imperative to discover the true nature of these outliers. As previously mentioned, these outliers were removed from the following data analysis to maintain data integrity and accuracy.

5.3 How is heavy metal concentration influenced by altitude?

- ### How does this impact on lichen physiology?

Our investigation into the relationship between altitude and OD ratio was prompted by our project partner's hypothesis that pollutants tend to accumulate at lower altitudes, particularly in mist-laden areas. However, subsequent analysis proved that the relationship was not statistically significant, meaning the null hypothesis was accepted. This contradicts our initial hypothesis that lichen would have a lower OD ratio at lower altitudes and would suggest that pollution is not hanging in lower altitudes, evidenced further by the similarity of OD ratio in our samples compared to the baseline value for healthy chlorophyll. However, regression analysis of the relationship between heavy metal concentration and altitude revealed that there is a statistically significant negative relationship between iron (Fe) concentration and altitude. This led us to consider whether lichen species in Manor Woods are particularly adapted to the air pollution in the local area, since iron concentrations were higher at lower altitudes, but optical density did not reflect the degradation in chlorophyll we would expect to observe as a result. It has been found that photobionts (the algal component of lichen) adapt under heavy-metal stress condition (Rola et al., 2019). This is an incredibly viable explanation for the observed relationship between OD ratio and altitude within the context of urban environment, since lichen particularly sensitive to pollution simply would not be able to survive under such conditions and therefore would not be found at the study site. This raises the question of whether species identification might be a more effective method for evaluating air pollution conditions.

5.4 How is heavy-metal concentration influenced by proximity to the A3029?

- ### How does this impact on lichen physiology?

The linear regression analyses conducted between individual metal concentrations and distance from the A3029 revealed a notable discrepancy in the results. While we observed a statistically significant negative relationship, as anticipated, for iron concentrations, this pattern was not consistently observed for other metals. This inconsistency suggests potential complexities in the factors influencing metal distribution within the study area. One plausible and probable explanation for the observed variation is the presence of additional sources of metals beyond vehicular emissions along the roadside gradient. Industrial activities, natural geological processes, and other anthropogenic sources (Bradl, 2005) may contribute to the overall metal concentrations in the vicinity, thereby masking the expected relationship between distance from the road and metal concentration for certain elements. This is also why we believe the linear regression conducted against optical density and distance from the A3029 yielded an insignificant result.

5.5 How is heavy-metal concentration influenced by proximity to the edge of Manor Woods?

- ### How does this impact on lichen physiology?

In an attempt to reflect the complexity of sources of air pollution in close proximity to Manor Woods, we undertook additional regression analysis on heavy metal concentration and the distance to the closest edge of Manor Woods (and therefore residential, industrial, and other anthropogenic emission sources). With both copper (Cu) and magnesium (Mg) displaying significant negative relationships, we can conclude that distance from the nearest edge of

Manor Woods is a better method for predicting heavy-metal concentration than distance from the A3029, yet it failed to provide a consistent trend across different heavy-metals. It is for this reason that natural sources of variation in heavy-metal concentration that had previously been overlooked, were considered. Interestingly, Georgopoulos et al (2001) found that the presence of copper in the environment comes from a wide variety of sources including wind-blown dust, whilst Wilkinson et al (1990) discuss how Magnesium concentration is altered by several different meteorological factors as well as by differing supplies of mineral nutrients. In addition, heat transferred to the atmosphere from Earth's surface results in convection, and with that turbulent mixing within the boundary layer of the atmosphere (Holzworth, 1967). Considering the abundance of both natural and anthropogenic sources of heavy metals, as well as the constant mixing of air parcels, the insignificance of the majority of our results is somewhat expected as a result of the intense complexity of pollutant sources and processes (such as wind) that may mislead observations. Again, it is because of this complexity that we believe the linear regression against optical density and distance from the nearest edge of Manor Woods yielded an insignificant result.

5.6 Limitations and future work

5.61 Limited sample size

A significant constraint in our study was the small dataset, which may limit the generalisability of our findings. Expanding the number of sampling sites and conducting replicate sampling is imperative to overcome this limitation. This approach would allow for capturing spatial variability effectively and ensuring sample sizes are sufficiently large for reliable statistical analysis. Moreover, it would enable the detection of more subtle patterns or trends in lichen communities that may have been previously overlooked.

5.62 Limited environmental data collection

Our study was constrained by the narrow scope of environmental data. Broadening the scope of environmental data collection to include variables such as microclimate parameters (e.g., substrate characteristics) is essential. This expansion would provide a more comprehensive understanding of factors influencing lichen physiology and distribution.

5.63 Taxonomic knowledge constraints

Prior to sample collection, a substantial limitation was the lack of comprehensive taxonomic knowledge of lichen species. Enhancing this is crucial to mitigate the risk of missing relevant samples due to lack of understanding surrounding the diverse forms of lichen. This would have also helped to collect a smaller diversity of species to compare chlorophyll more accurately. Restricting to the collection of one species of lichen was nearly impossible.

5.64 Lack of molecular techniques

Another constraint was the lack of molecular techniques such as DNA barcoding, which could significantly enhance the accuracy of species classification. Studies like Kelly et al., (2011) have demonstrated the efficacy of DNA barcoding in identifying lichen samples accurately. Integrating molecular techniques into our methodology would improve species identification and contribute to a more robust dataset. This is particularly important considering the relevance of lichen species being a potential factor in determining lichen physiology and air quality previously discussed.

5.65 Moss as a bioindicator

Sujetovienė and Galinytė (2016) argue that moss proves to be more effective for biomonitoring compared to lichen due to the observed physiological differences in response. Moss was found to experience irreversible ultrastructural changes, while lichen maintained its life and metabolism despite developing stress marks (Sujetovienė and Galinytė, 2016). As well as this, moss showed more significant damage in urban areas compared to lichen under different pollution scenarios, suggesting moss is more greatly affected by air pollution and may therefore be a better indicator. This is a particularly interesting concept for future works, perhaps in providing a comparative study assessing the utility of lichen and moss as bioindicators.

6. Conclusion

In conclusion, the investigation into the variation of OD ratio of lichen samples across Manor Woods has revealed intriguing insights into the complex interplay of environmental factors and lichen physiology and distribution. Our initial hypothesis regarding the impact of industrial activity on OD ratio was not fully supported, as evidenced by the absence of significant variation among transects and the identification of outliers. While the outliers identified could reflect unique environmental conditions, they are more likely attributed to error.

Contrary to our hypothesis regarding altitude's impact on OD ratio, subsequent analysis revealed a lack of statistical significance. However, regression analysis of heavy metal concentration and altitude unveiled a significant negative relationship with iron concentration, suggesting lichen adaptation to local pollution conditions. This revealed that perhaps lichen species would be a better indicator of heavy-metal pollution. Although not an explicitly quantitative measure, and therefore arguably less conclusive than assessing OD ratios, species identification can certainly provide valuable insights, comparisons and conclusions about general air quality and heavy-metal accumulation.

Further regression analysis between individual metal concentrations and distance from the A3029 highlighted complexities in metal distribution within the study area, potentially influenced by various anthropogenic and natural sources. Similarly, regression analysis on heavy metal concentration and distance from the closest edge of Manor Woods underscored the influence of diverse pollution sources, with copper and magnesium displaying significant negative relationships.

The insignificance of some results may be attributed to the intense complexity of pollutant sources and atmospheric processes. Our findings reflect the intricate interplay of environmental factors influencing lichen physiology and distribution.

Overall, our study contributes to a deeper understanding of the complexities of air pollution and its impact on ecosystems. Further research is warranted to explore the role of species identification in assessing air pollution conditions and to reveal the intricacies of air pollution and related processes.

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7. Appendix

Site Information

Site number	11
Latitude	51.42100143
Longitude	-2.61351247
Height	35.5m
X co-ordinate	357437
Y co-ordinate	169291
Location grid reference	ST 5743 6929

Lichen Identification

	Transect A	Transect B	Transect C
Sample 1	scoliciosporum chlorococcum hypotrachyna revoluta	xanthoria parietina parmelina pastillifera usnea subfloridana	xanthoria polycarpa Flavoparmelia caperata hypotrachyna laevigata Parmelia saxatilis hypotrachyna revoluta
Sample 2	xanthoria polycarpa hypotrachyna revoluta	platismatia glauca xanthoria parietina	xanthoria polycarpa Flavoparmelia caperata ramalina fastigiata hypotrachyna laevigata parmotrema perlatum
Sample 3	parmelina pastillifera xanthoria parietina platismatia glauca	physica tenella xanthoria parietina	xanthoria polycarpa Parmelia saxatilis
Sample 4	xanthoria polycarpa physica tenella	ramalina farinacea parmotrema perlatum Lecanora conizaeoides	xanthoria polycarpa Parmelia saxatilis hypotrachyna revoluta
Sample 5	physica tenella xanthoria parietina	hypotrachyna laevigata parmelina pastillifera	parmotrema perlatum
Sample 6	xanthoria parietina physica tenella	xanthoria parietina ramalina farinacea parmelina pastillifera	ramalina farinacea
Sample 7	fuscidea lightfootii	evernia prunastri	ramalina farinacea parmelina pastillifera
Sample 8	desmococcus spp	parmotrema perlatum ramalina farinacea	xanthoria parietina xanthoria polycarpa evernia prunastri
Sample 9	desmococcus spp fuscidea lightfootii pertusaria leioplaca	ramalina farinacea Punctelia subrudecta physica tenella	xanthoria parietina xanthoria polycarpa parmelina pastillifera

Lichen weights

Transect A

Sample	Boat weight (g)	Total wet weight (g)	Wet lichen weight (g)	Total dry weight (g)	Dry weight (g)	Loss (g)
1	1.0030	1.6401	0.6371	1.4507	0.4477	1.1924
2	1.0061	1.5442	0.5381	1.3705	0.3644	1.1798
3	1.0270	1.2191	0.1921	1.1973	0.1703	1.0488
4	1.0117	1.2982	0.2865	1.2478	0.2361	1.0621
5	1.0286	1.3830	0.3544	1.2987	0.2701	1.1129
6	1.0127	1.1975	0.1848	1.1689	0.1562	1.0413
7	1.0272	1.6530	0.6258	1.15529	0.12809	1.52491
8	1.0159	1.4290	0.4131	1.3608	0.3449	1.0841
9	1.0143	1.4922	0.4779	1.3392	0.3249	1.1673

Transect B

Sample	Boat weight (g)	Total wet weight (g)	Wet lichen weight (g)	Total dry weight (g)	Dry weight (g)	Loss (g)
1	1.0094	1.3267	0.3173	1.2142	0.2048	1.1219
2	1.0137	1.43	0.4163	1.3294	0.3157	1.1143
3	1.015	1.6815	0.6665	1.3533	0.3383	1.3432
4	1.0198	1.6439	0.6241	1.4463	0.4265	1.2174
5	1.0242	1.2929	0.2687	1.2121	0.1879	1.105
6	1.026	1.2879	0.2619	1.2409	0.2149	1.073
7	1.0279	1.8143	0.7864	1.6525	0.6246	1.1897
8a	1.0274	1.4509	0.4235	1.4015	0.3741	1.0768
8b	1.0182	1.1358	0.1176	1.1121	0.0939	1.0419
9	0.9998	1.7792	0.7794	1.5165	0.5167	1.2625

Transect C

Sample	Boat weight (g)	Total wet weight (g)	Wet lichen weight (g)	Total dry weight (g)	Dry weight (g)	Loss (g)
1	1.0154	1.5466	0.5312	1.4885	0.4731	1.0735
2	1.0206	1.2667	0.2461	1.2371	0.2165	1.0502
3	1.0246	1.3613	0.3367	1.3186	0.294	1.0673
4	1.013	1.5381	0.5251	1.4689	0.4559	1.0822
5	1.0036	1.6188	0.6152	1.5294	0.5258	1.093
6	1.0032	2.168	1.1648	1.9954	0.9922	1.1758
7	1.003	1.1898	0.1868	1.1662	0.1632	1.0266
8	1.0199	1.3829	0.363	1.3466	0.3267	1.0562
9	1.0157	1.4938	0.4781	1.4281	0.4124	1.0814

Optical density results

Transect A

Sample	415 nm	435nm	Ratio of optical density
1	0.096	0.386	4.020833
2	0.416	0.518	1.245192
3	0.439	0.491	1.118451
4	0.627	0.766	1.221691
5	0.449	0.579	1.289532
7	0.239	0.257	1.075314
8	0.485	0.553	1.140206
9	0.244	0.388	1.590164

Transect B

Sample	415 nm	435nm	Ratio of optical density
1	1.005	1.766	1.757214
2	0.176	0.199	1.130682
3	0.629	0.805	1.279809
4	0.408	0.416	1.019608
5	0.823	1.016	1.234508
6	0.786	1.008	1.282443
7	0.365	0.371	1.016438
8	0.135	0.133	0.985185
9	0.478	0.508	1.062762

Transect C

Sample	415 nm	435nm	Ratio of optical density
1	0.658	0.905	1.37538
2	0.876	1.092	1.246575
3	0.991	1.289	1.300706
4	0.636	0.669	1.051887
5	0.181	0.193	1.066298
6	0.261	0.311	1.191571
7	0.845	0.027	0.031953
8	0.536	0.694	1.294776
9	0.982	1.294	1.317719

Location Data

Eastings and Northings

Sample	A_ Easting	A_ Northing	A_ Altitude	B_ Easting	B_ Northing	B_ Altitude	C_ Easting	C_ Northing	C_ Altitude
1	357604	169322	43.4	357735	169422	46.5	357980	169778	19.3
2	357596	169347	43	357711	169445	39.1	357929	169787	18.5
3	357591	169370	34.2	357696	169461	34.5	357904	169780	19.2
4	357574	169385	33.2	357689	169474	26.2	357878	169815	20
5	357568	169408	27.3	357681	169483	26.6	357854	169830	21.5
6	357654	169443	29.5	357633	169538	26.1	357844	169833	22.3
7	357530	169483	44	357613	169562	30	357830	169832	24
8	357517	169509	47.8	357588	169584	49.9	357797	169826	29.8
9	357506	169511	49.1	357556	169596	48.2	357784	169846	32.5

The distance from the A3029

Sample	A_distance	B_distance	C_distance
1	478	333	88
2	480	355	141
3	482	368	165
4	498	374	195
5	499	382	221
6	502	428	230
7	533	449	244
8	543	474	275
9	557	505	291

The shortest distance from the edge of Manor Woods

Sample	A_sides_distance	B_sides_distance	C_sides_distance
1	6	9	39
2	30	41	93
3	55	62	114
4	74	78	150
5	98	87	154
6	103	102	143
7	52	72	131
8	25	40	100
9	15	7	82

Heavy metals raw data

Empty cells occur where the concentration of metal did not meet the threshold of 0.01ppb

	Fe	Fe	Al	Al	Ca	Ca	Cu	Cu	Mg	Mg	Mn	Mn	Zn	Zn
Sample Name	Average ppm Conc	Average ppm SD	Average ppm Conc	Average ppm SD	Average ppm Conc	Average ppm SD	Average ppb Conc	Average ppb SD	Average ppm Conc	Average ppm SD	Average ppb Conc	Average ppb SD	Average ppb Conc	Average ppb SD
G11-1	1.69	0.02	1.28	0.02	12.85	0.18	178.04	5.66	2.02	0.04	5.25	0.09	381.46	7.13
G11-2	1.32	0.01	0.84	0.01	6.38	0.01	55.05	1.98	1.85	0.01	3.06	0.06	55.51	0.99
G11-3	0.4	0.01	0.34	0.01	12.19	0.07	34.44	1.08	1.17	0.01	7.36	0.08	59.07	2.17
G11-4	0.34	0.01	0.24	0	7.56	0.09	18.2	0.76	0.95	0.02	1.92	0.03	25.93	1.43
G11-5	1.43	0.01	3.58	0.03	2.47	0.03	27.29	1.31	1.19	0.02	5.89	0.07	96.26	1.08
G11-6	0.7	0	0.61	0.01	5.43	0.07	82.49	3.54	1.34	0.02	2.28	0.04	111	1.44
G11-7	0.49	0.01	0.44	0.01	24.38	0.27	66.14	1.93	1.52	0.03	1.67	0.07	124.5	2.08
G11-8	0.38	0.01	0.35	0.01	20.07	0.29	65.39	0.9	3.03	0.03	5.54	0.07	53.02	0.99
G11-9	0.18	0	0.14	0	21.91	0.24	46.89	1.26	4.7	0.05	5.34	0.06	44.07	1.3
G11-10	2.68	0.04	2.8	0.05	1.81	0.04	30.41	2.18	1.39	0.03	6.87	0.12	42.62	1.14
G11-11	0.8	0.01	0.55	0.01	9.5	0.13	28.11	1.43	1.06	0.02	2.61	0.06	51.58	1.22
G11-12	0.96	0.01	2.49	0.03	5.08	0.07	29.16	3.21	1.22	0.02	4.42	0.09	47.59	0.88
G11-13	1.18	0.02	0.91	0.01	41.88	0.49	74.69	2.24	1.13	0.02	2.45	0.04	64.94	2.08
G11-14	0.55	0.01	0.38	0.01	3.04	0.03	19.53	1.06	1.04	0.01	1.71	0.03	40.22	1.29
G11-15	0.41	0.01	3.58	0.06	4.14	0.07	28.63	1.51	0.99	0.02	4.54	0.06	65.2	1.1
G11-16	1.95	0.03	2.01	0.03	10.32	0.14	47.1	2.71	2.39	0.03	7.41	0.15	50.85	0.72
G11-17	0.39	0.01	0.31	0	4.47	0.06			1.1	0.02	1.67	0.03	46.55	1.06
G11-18	0.62	0.01	0.59	0.01	4.72	0.03	22.43	1.52	1.27	0.01	2.33	0.05	37.48	0.93
G11-19	1.24	0.02	1.01	0.02	10.03	0.13	35.02	0.68	2.11	0.03	4.23	0.06	70.92	1.91
G11-20	1.82	0.02	1.37	0.02	2.21	0.03	20.66	2	1.46	0.02	3.75	0.08	99	0.93
G11-21	1.5	0.02	1.05	0.01	5.65	0.07	31.77	2.31	1.2	0.01	3.47	0.05	87.68	0.48
G11-22	1.34	0.01	0.93	0.01	1.47	0.01	15.45	1.37	1.72	0.01	3.08	0.03	54.18	0.89
G11-23	1.34	0.01	0.98	0.01	20.15	0.35	23.43	1.51	1.61	0.01	3.83	0.03	131.68	0.91
G11-24	0.81	0.01	0.75	0.01	5.2	0.07			0.65	0.01	2.1	0.03	49.55	1.23
G11-25	1.27	0.02	0.74	0.01	19.04	0.19			1.11	0.01	2.43	0.05	61.16	1.41
G11-26	0.84	0.01	0.78	0.01	24.21	0.23	26.45	0.59	0.86	0	2.39	0.03	73.05	0.49
G11-27	0.44	0.01	0.27	0.01	9.7	0.14			1.51	0.02	2.62	0.05	54.05	1.32
G11-28	0.52	0.01	0.37	0.01	1.92	0.04			0.88	0.01	1.58	0.03	31.93	1.26

Heavy metals vs altitude regressions

Metal	Estimate	p-value
Fe	-0.020217	0.0387
Al	-0.02334	0.137
Ca	0.02188	0.224
Cu	0.022746	0.0323
Mg	0.012658	0.0673
Mn	Could not be done since not normally distributed	
Zn	-0.004625	0.597

Metals vs the distance from the closest edge of Manor Woods

Metal	Estimate	p-value
Fe	0.001634	0.529
Al	0.003205	0.429
Ca	-0.00231	0.617
Cu	-0.006275	0.0506
Mg	-0.006028	0.0246
Zn	0.0001872	0.933

OD ratio vs the distance from the closest edge of Manor Woods

Estimate	p-value
-0.00035	0.616

Metals vs the distance from the A3029

Metal	Estimate	p-value
Fe	-0.0014985	0.0371
Al (log)	-0.0006541	0.58
Ca (log)	0.001743	0.1862
Cu (log)	0.0018122	0.0233
Mg (log)	0.0006518	0.21
Zn (log)	-0.000338	0.599

Variables Shapiro-Wilks tests

Variable	p-value	W Statistic
Altitude	0.05566	0.91635
Distance from A3029	0.92326	0.07828
Distance from edge of Manor Woods	0.5638	0.96467
Optical Density	0.2011	0.94228
Al (log)	0.2911	0.94989
Ca (log)	0.9165	0.98062
Cu	0.279	0.94136
Fe	0.05299	0.91534
Mg	0.7739	0.97358
Mn	Couldn't be normally distributed – the closest value we obtained was p=0.00153 with the inverse function	
Zn	0.5816	0.96545