

# ENSINO, SAÚDE E AMBIENTE

## Lichen Assessment for Biomonitoring the Environmental Impact of the Distance from a Road on an Atlantic Forest Fragment in Barueri, São Paulo State, Brazil

*Avaliação de Líquens para Biomonitoramento de Impacto Ambiental da Distância de uma Rodovia sobre um Fragmento de Mata Atlântica em Barueri, Estado de São Paulo, Brasil*

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### Keywords:

Lichens; bioindicators; Atlantic Forest; Pollution; Tropical Forests; Morphofunctional Groups; Rapid Biodiversity Assessment; Traffic; Air Quality; Health.

### Palavras-chave:

Líquens; bioindicadores; Mata Atlântica; Poluição; Florestas Tropicais; Grupos morfofuncionais; Avaliação Rápida de Biodiversidade; Tráfego; Qualidade do Ar, Saúde.

**ABSTRACT:** This study assesses the impact of traffic on Castelo Branco Road on the epiphytic lichens in an Atlantic Forest park in Barueri municipality, Brazil. The investigation evaluates how distance to road influences the diversity and trunk coverage of selected sensitive lichen morphofunctional groups. The sampling and measurement were based on the European Union protocol for lichen biomonitoring, adapted for tropical forests. There was a significant positive log-linear relationship ( $p$ -value = 2.5%) between the distance from the road and lichen diversity, although the  $R^2$  (0.12) of the respective regression was low, which is probably due to the high tree biodiversity in the Atlantic Forest, which creates diversified bark suitable for lichens. Maps indicate that the effect of road proximity on lichen diversity and coverage is more evident in the first 100 m. Most of the park area contains morphofunctional groups that appeared to be potential indicators of good environmental air quality.

**RESUMO:** Este artigo avalia o impacto do tráfego da Rodovia Castelo Branco sobre líquens epifíticos em um Parque de Mata Atlântica no Município de Barueri, no Brasil. A investigação avalia como a distância à rodovia influencia a diversidade e a cobertura de tronco de grupos morfofuncionais selecionados de líquens sensíveis. A amostragem e as mensurações foram baseadas no protocolo da União Europeia para biomonitoramento com líquens, adaptado para florestas tropicais. Houve uma relação positiva log-linear positiva (valor- $p$  = 2,5%) entre a distância à rodovia e a diversidade de líquens, apesar que o  $R^2$  (0,12) da respectiva regressão foi baixo, provavelmente devido à alta biodiversidade de árvores da Mata Atlântica, que cria um ambiente de aptidões de ambientes para os líquens. Mapas indicaram que o efeito da proximidade da rodovia sobre a diversidade e cobertura de líquens é mais evidente nos primeiros 100 metros. A maior parte da área do parque contém grupos morfofuncionais que aparentam ser potenciais indicadores de boa qualidade do ar.

## 1. INTRODUCTION

Lichens are very sensitive to air pollution and have long been used as bioindicator species (NIMIS et al., 2002). SO<sub>2</sub> has been described as the main pollutant that affects lichens (NASH III, 2008), although damage from nitrogen, ozone, and heavy metals (deposited with particulate matter) also causes adverse effects (CONTI and CECCHETTI, 2001). Biomonitoring with lichens can be utilized to collect spatialized information on continuous pollution effects with better trade-offs between area size, time and costs than those for conventional methods, such as air gauge stations (GIORDANI and BRUNIALTI, 2015).

### 1.1. Environmental impacts of roads on lichens

This study focuses on the environmental impacts on lichens from nearby roadways. Brawn and Ogden III (1977) stated that the intensity of bus traffic in Nova Scotia, Canada, had decreased lichen biodiversity and abundance. Coffey and Fahrig (2012) also found the same effects of air pollution on epiphytic lichen biodiversity and coverage from roads in Ottawa, Canada, that extended up to 300 m. However, the authors also warned that the environmental impact related to the distance from roads was caused by more than simply environmental pollution because the impacts of the roads on lichens via both changes in air humidity (1 km gradient due to the lack of evapotranspiration over paved areas) and the blocking of lichen recolonization sources were even more intense than the impact of air pollution. Therefore, Coffey and Fahrig (2012) advised that a “distance to roads” variable should be used as a general environmental impact factor in lichen biomonitoring studies and not used only as a pollution proxy.

Bedell-Stiles (2004) found that lichen richness near a road in Cañitas, Costa Rica, was lower than that in an adjacent primary tropical forest. However, the lichen richness on isolated trees in pastures around this forest was even higher due to abundant sunlight and less competition from mosses on the trunks. However, Irving (2008) found no relationship between lichen diversity and distance to roads in Ontario, Canada, while Tulumello (2010) found an inverse relationship in the same municipality. Both studies suggested that these apparently unexpected results could be due to confounding environmental variables, such as canopy coverage and moss coverage on trunks, which both increase from the boundary of the forest (near the road) to the interior of the forest.

However, because these two studies did not specifically control for these variables, it is possible that the different influences in each study could be why one study found no relationship and the other found an inverse relationship. In this context, this study attempted to control for these two confounding variables as much as possible during lichen data collection and modelling.

Viana (2010) monitored lichens in Atlantic Forest fragments in an urban park in Brazil (same phytophysiology as this case study and in a similar neighborhood context) and concluded that the proximity to roads and wind direction were complementary factors that controlled the deposition of particulate matter and heavy metals on lichens in different parts of the park. Viana (2010) emphasized how wind roses for the collection period are relevant for lichen biomonitoring surveys, and these will also be evaluated in this study.

Therefore, in this study, the distance to roads was interpreted as a general proxy for environmental impact on lichens, including air pollutants (e.g., particulate matter, heavy metals, and Sulphur), air humidity and recolonization barriers. The biomonitoring methodology also took special care regarding the aforementioned confounding environmental variables of canopy coverage, moss coverage and wind direction.

## **1.2. Protocols for biomonitoring air pollution with lichens**

The European Union developed a standard protocol for biomonitoring air pollution with lichens (CEN, 2014; STOFER et al., 2016). The European standard (CEN, 2014) proposes to sample trees from species or groups that have similar bark properties because distinct lichen species are specialized to different bark characteristics (LAMIT et al., 2015). Previous studies have indicated that lichen abundance and biodiversity are higher on rough/fibrous barks than smooth barks because they can provide more moisture and nutrients for the fungus symbiont in urban areas (LAMIT et al., 2015) and the Atlantic Forest (CÁCERES et al., 2007), which matches the interfacing environments of the current study.

However, because there are few experts capable of recognizing lichens species, Giordani et al. (2009), Casanovas et al. (2014) and Aragón et al. (2016) proposed rapid biodiversity assessment methods, in which morphologic groups of epiphytic lichens serve as surrogates to evaluate species biodiversity. Giordani et al. (2009) argued that this methodological option is useful for areas where systematic lichen taxonomy is still not

well known, such as in the Atlantic tropical forest region, which is analyzed in this case study.

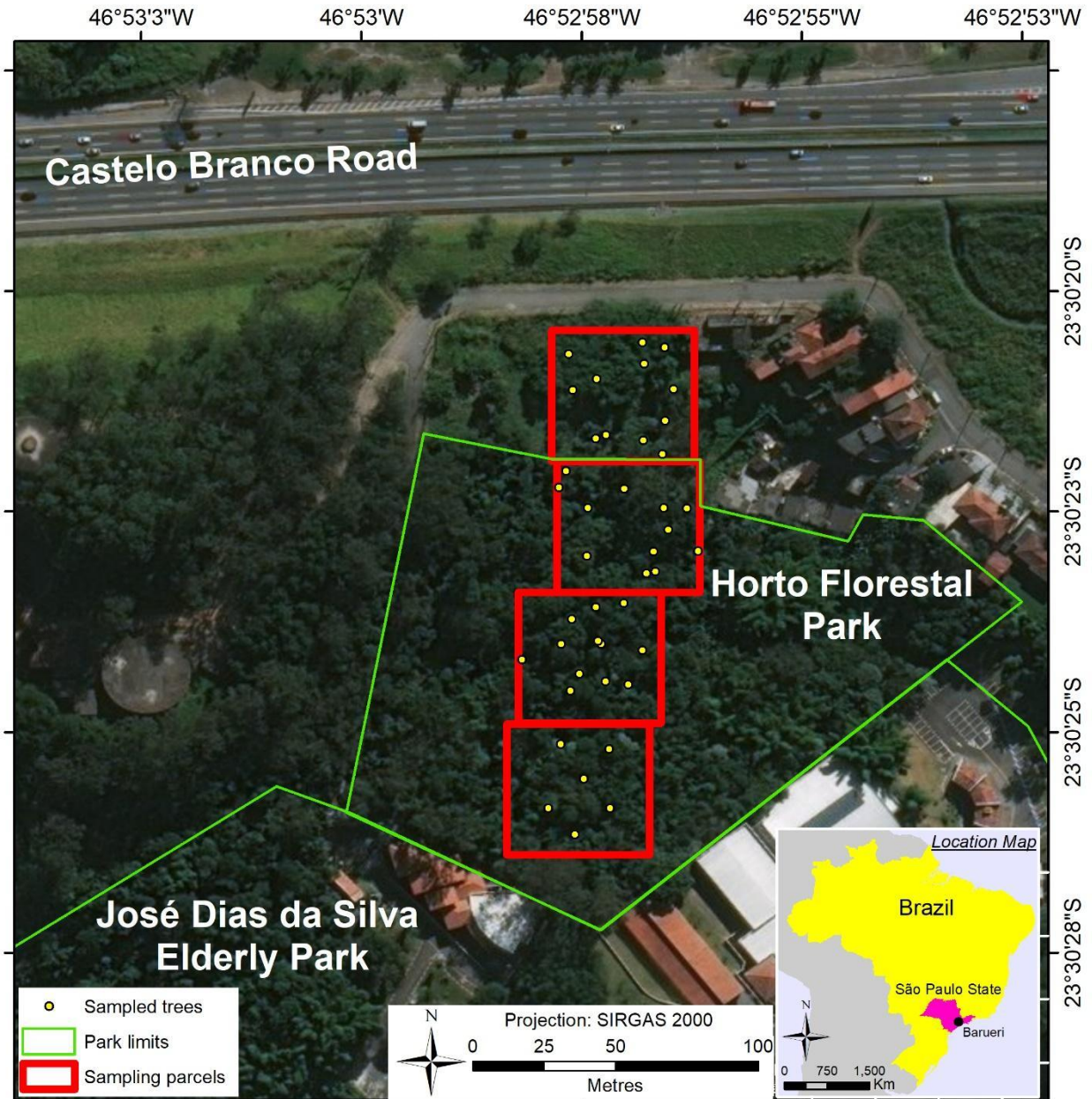
### 1.3. Objectives

The main objective of this research is to evaluate how the morphofunctional richness of epiphytic lichens (evaluated through the Lichen Diversity Index – LDV) and the trunk coverage percentage can be used to monitor the spatial distribution of the environmental impact from a road with heavy traffic (Rodovia Castelo Branco) on an Atlantic Forest fragment in Barueri municipality, Brazil (Figure 1). In general, this study aims to evaluate an adaptation of the European protocol (CEN, 2014) to tropical forests. Regarding this adaptation, it aims to contribute to the development and evaluation of simpler, low-cost and rapid protocols for biomonitoring with lichens. Specifically, regression models based on field data are tested, evaluating the relationship of a possible independent variable, the distance from the road (proxy for the general impact from the road), on the two potential dependent variables regarding lichen richness and coverage.

## 2. METHODS

### 2.1. Characterization of the study area

The study area (Figure 1) comprises an Atlantic Forest fragment of 2.23 ha. In 2018, the municipal government of Barueri decided to build a public park called “Horto Florestal” in this area with an interpretative trail aimed at environmental education excursions. The Horto Florestal lies between Castelo Branco Road and another park called “Parque da Maturidade José Dias da Silva” (José Dias da Silva Elderly Park) (Figure 1), which is restricted to only elderly people. Although walking in parks is beneficial for the health of elderly people and for children who participate in the excursions, pollution from the road may also be a risk for developing respiratory problems. The focus in the selected parks is socially justified because it will be possible to analyze the spatial patterns of lichens as bioindicators of air pollution and then prescribe the safest areas for walking to decrease the risk of respiratory health problems.



**Figure 1** – Study area and location of the sampled trees

**Source:** the authors

Castelo Branco Road is a large highway that connects the metropolitan region of São Paulo with the municipalities of Sorocaba, Santana do Parnaíba and Barueri. This is the main passageway through which agricultural and industrial products are exchanged between these municipalities (SILVEIRA, 2010), resulting in heavy diesel truck traffic. Pendulum migration (traveling from home to work between these cities or travel to weekend country houses) is also an important traffic source (FREY, 2010).

Compared with the 300 m gradient found by Coffey and Fahrige (2012), the

study area lies between 50 and 450 m from the main road and represents a potential pollution gradient. Duarte and Pasqual (2000) found high abundances of heavy metals (Cd, Pb, Ni and Zn) in soils, plants and human hair tissues in the areas adjacent to Castello Branco Road, resulting in concerns of contamination from the heavy traffic on this road in the surrounding neighborhoods.

## 2.2. Monitoring protocol framework

The European standard (CEN, 2014; STOFER et al., 2016) for biomonitoring with lichens was preferred over the American standard (US FOREST SERVICE, 2011) in this study because it can give results for each tree rather than each sampling area. Furthermore, the European standard provides quantitative results at a higher resolution of lichen frequency (1 to 20 quadrats for each lichen type) instead of the 4 abundance classes of the American Standard. Therefore, it is more suitable for statistical techniques, such as regression models. Moreover, the European standard accounts for microlichens (crustose, squamulose and leprose), being broader in this aspect than just the macrolichens (foliose and fruticose) accounted by the American standard. Nevertheless, the results of the European and American standards have been shown to exhibit high correlation and allow for the same interpretative patterns (MATOS et al., 2017).

## 2.3. Variables and hypotheses

Each tree was an experimental unit for the multiple regression model. For each tree, lichen measurements were collected, and the distance to the main road was measured. The underlying idea was to test whether lichens could be useful bioindicators to measure the environmental impact of the nearby road on the park.

The relationships between these variables were tested to build a multiple regression model where the independent variables were the indicators of pollution (distance from the road) and the dependent variable was an index related to lichens. One possible dependent variable is the lichen diversity value (LDV) (Equation 1), which was adopted as a standard in Europe (CEN, 2014) and is the sum of the frequency of each type of lichen in 20 quadrats on a trunk. As an alternative dependent variable, the lichen coverage percentage in the quadrats was also evaluated because it has been reported to be a useful index for monitoring pollution in urban areas (COFFEY and FAHRIG, 2012).

Cen (2014) also suggested that indicators using only select lichen groups that seem to be more sensitive to pollution instead of all lichens would be more efficient for biomonitoring purposes. In this context, the frequency and the coverage percentage of each lichen group were measured; then, the best combinations for modelling were tested. The tested hypotheses are described in Table 1.

**Equation 1** - Lichen Diversity Value (CEN, 2014)

$$LDV_t = SF_{Nt} + SF_{Et} + SF_{Wt} + SF_{St}$$

where:

SF is the sum of the frequency of each lichen type in each quadrat of one aspect of tree t;

N, E, S, and W are North, East, South and West.

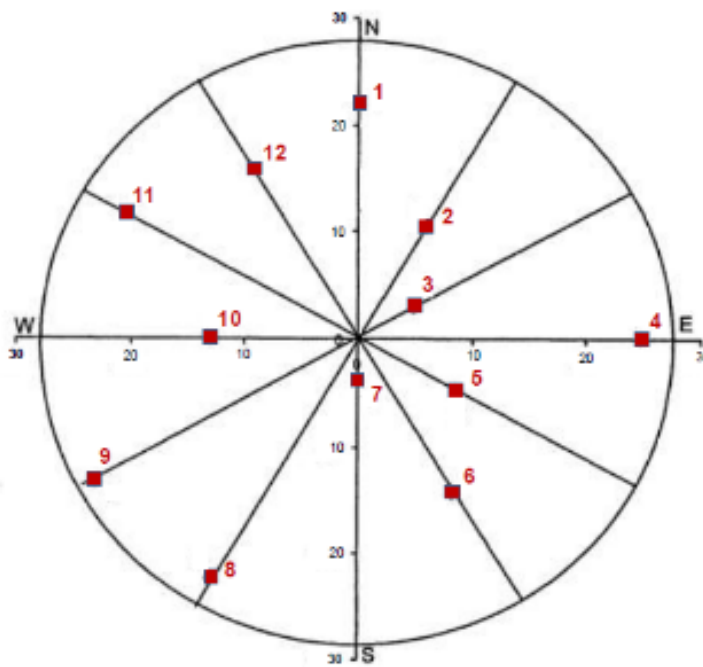
**Table 1** – Null and alternative hypotheses

		<b>Independent variable</b>
		<b>Distance from the main road</b>
<b>P o s s i b l e p e n d e n t v a r i a b l e s</b>	<b>Percent lichen cover</b>	H <sub>0</sub> There is no relationship between the distance from the main road and lichen coverage percentage
		H <sub>A</sub> There is a relationship between the distance from the main road and lichen coverage percentage
	<b>Lichen morphofunctional diversity (through LDV)</b>	H <sub>0</sub> There is no relationship between the distance from the main road and lichen morphofunctional diversity (LDV)
		H <sub>A</sub> There is a relationship between the distance from the main road and lichen morphofunctional diversity (LDV)

**Source:** the authors

## 2.4. Experimental design

According to the sampling design prescribed for the European standard (STOFER et al., 2016), the study area was divided into 4 parcels of 0.25 hectares (50 m X 50 m). Within the 3 parcels nearest the road, 12 trees were selected in each parcel using a radial scheme of 12 equally interspersed radii from the parcel center (Figure 2). Only 6 trees were measured in the fourth parcel due to time constraints. The 4 parcels (Figure 1), totaling 42 trees, were selected at different distances from the main road (50 to 200 m) to evaluate the contrasting effects of pollution on lichen richness and abundance within a multiple regression model.



**Figure 2** – Radial scheme for sampling in each parcel. The red squares are trees suitable for sampling.

**Source:** Stofer et al., 2016

Based on Asta et al. (2002), Cen (2014) and Stofer et al. (2016), the variability in the confounding biotic factors was controlled by selecting the trees with the following characteristics: minimum trunk circumference at breast height (circumference >40 cm at 150 cm height), low trunk inclination (<20°), low bark decortication (<20%), and under closed forest canopy (canopy coverage >50%). The percentage of decortication was measured in the same quadrats used for the lichen assessment. Trunk inclination was measured with an inclinometer. Canopy coverage was calculated as the average of three



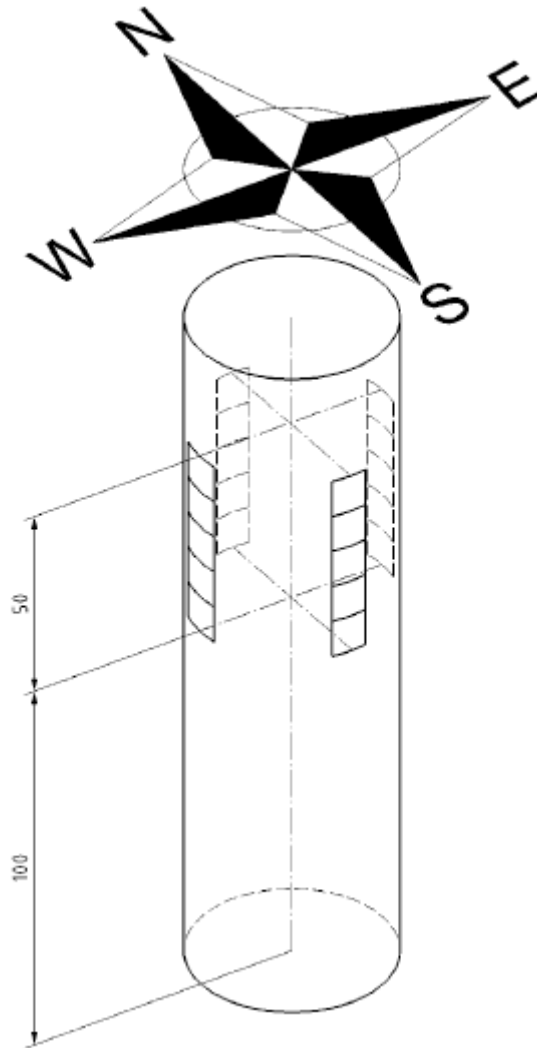
180° fish-eye lens images for each tree, analyzed using GLAMA (Gap Light Analysis Mobile App) software (TICHÝ, 2015), as proposed by Tichý (2016). The percentage of the trunk covered by mosses was also assessed within the quadrats, which is a proxy for both air humidity and possible competition with lichens for space on the trunk. The wind direction during the data collection period was analyzed through wind roses generated from the Meteoblue (2018) portal.

Although the European standard (CEN, 2014) proposes to sample trees species that have similar bark properties, this study required some adaptation in this context because there are no similar studies regarding bark properties for the Atlantic Forest. Moreover, the biodiversity of trees in the Atlantic Forest is much higher than that in European temperate and boreal forests, thus identifying and sampling species would not only be technically complex but also possibly result in very few individuals selected for sampling. As an adaptation, the sampling strategy selected trees with similar bark texture. One weakness of this adaptation is that trees with barks exhibiting distinct pH levels may be sampled, which is one of the characteristics that affect lichen species composition (CEN, 2014). Another adaptation to the European standard is that because almost all trees of the study area are fully covered by mosses (although lichens still grow over the mosses), there was no constraint on trunk moss coverage for the sampling design.

The lichen measurements followed the European standard (CEN, 2014) using a grid of five vertical quadrats of 10 cm x 10 cm each, and the lichen richness and coverage were measured in each quadrat. The grids were placed between 1-1.5 m above the ground on the trunk, and this operation was repeated in the four cardinal directions (north, south, east, west) (Figure 3). Following the guidance of Cen (2014) and Stofer et al. (2016), in cases where the coverage disturbance was greater than 20% (decortication, seepage marks, intersection with branches, etc.) within the grids, the cardinal direction of the grid on the trunk was shifted up to 20°, first clockwise and then counterclockwise.

An additional adaptation to the European standard (CEN, 2014) was the use of morphological lichen groups as surrogates for lichen species diversity in rapid biodiversity assessments as proposed by Giordani et al. (2009), Casanovas et al. (2014) and Aragón et al. (2016). The lichens were grouped based on the visual characteristics of the thallus (crustose, leprose, foliose, and fruticose) and color following the systematic approach of morphofunctional grouping proposed by Giordani et al. (2009). The lichen color was identified in the field with a printed reference chart in RGBa

(Red-Green-Blue-alfa) additive scale (Grassmann, 1853). The thallus groups were classified with the aid of the lichen morphology guides from Spielman and Marcelli (2006) and Marques (2008). The fieldwork was carried out on 23 April 2018.



**Figure 3** – Position of quadrats for measuring lichen richness and abundance

**Source:** Cen, 2014

## 2.5. Statistical and spatial analyses

The variables were also mapped to analyze their spatial patterns. In sequence, scatterplot diagrams were built to graphically evaluate the possible linear or nonlinear relationships between the variables. Subsequently, correlation tests were used to evaluate the relationship between plausible independent and dependent variables as well as the confounding environmental variables. Finally, a linear regression model was built to determine the significant relationships. The results of the model were interpreted in in the

context of the literature.

## 2.6. Environmental education

The research project also involved activities with the environmental education group for the elderly people of José Dias da Silva Park. The interaction methods were previously approved by the Park Manager, which represents the Social Assistance Secretary of Barueri. The elders were informed about the project and 21 agreed participate, while approximately 20 others also agreed to join as listeners in some of the activities. The interaction consisted of a preliminary talk and field visit before the main fieldwork and a second visit to discuss the results of the research.

## 3. RESULTS

### 3.1. Field data

Eleven morphofunctional groups were identified (Table 2) and classified as opportunists (concentrated near the road), tolerant (widespread) and sensitive (concentrated far from the road). Pictures of each group are provided in the Appendix. Figures 4 and 5 present maps of the LDV and coverage (for selected sensitive bioindicator groups). These maps show sharp reductions in both variables along the first 100 m from the road.

Figures 6 and 7 show wind roses covering the data collection period and for the 5-year average, respectively. A northerly wind prevailed (i.e., from the road to the study area) 37.26% of the time during the collection period and 42,81% of the time over the last 5 years. During the collection period, the northerly winds were often stronger the southerly winds.

**Table 2** – Morphofunctional groups, respective characteristics and spatial patterns.

Spatial patterns	Thallus	Lichen color	Additional characteristic
Opportunists (concentrated near the road)	Crustose		-
	Crustose		-
Tolerant (widespread)	Crustose		-
	Leprose		-
	Foliose		-
	Crustose		White border
Sensitive (concentrated far from the road)	Leprose		-
	Fruticose		-
	Foliose		-

Crustose		Pink border
Leprose		-

Source: the authors

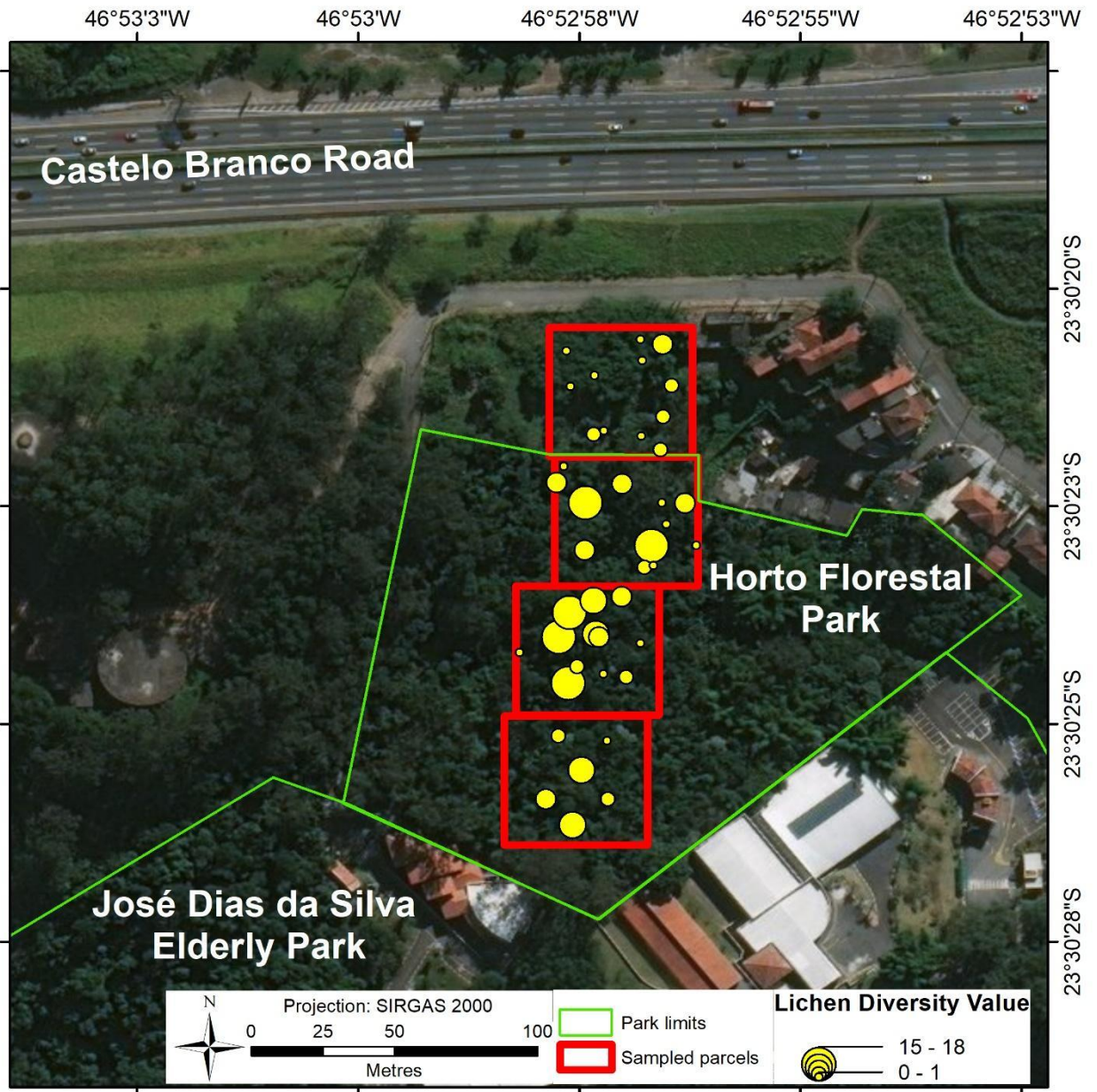


Figure 4 – Map of the LDV for selected sensitive morphofunctional indicator groups

Source: the authors

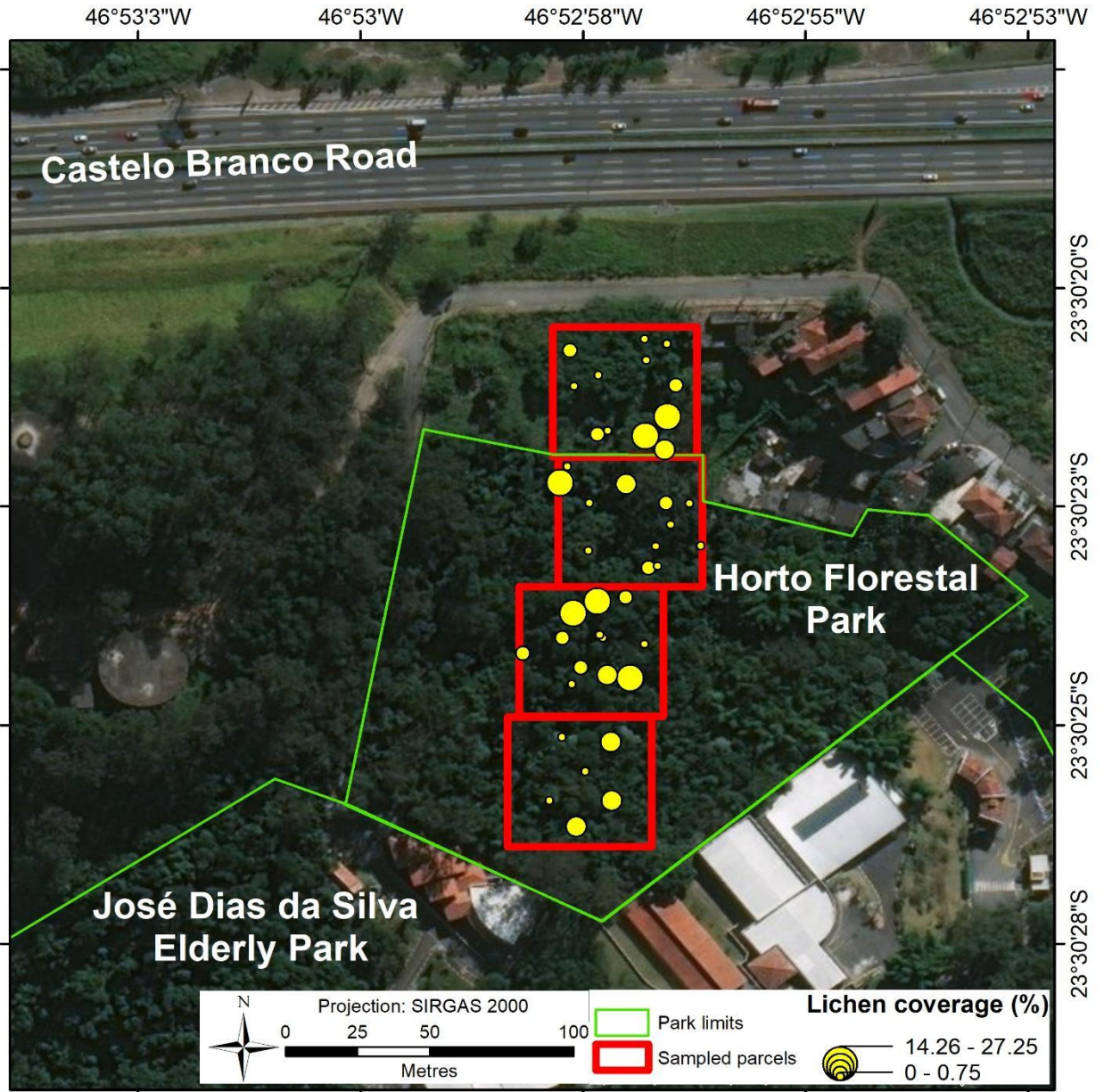


Figure 5 – Map of lichen coverage (%) for selected morphofunctional indicator groups

Source: the authors

Wind Rose at Castelo Branco Road, northern side of the Study Area

Data from 23 April to 30 May 2018

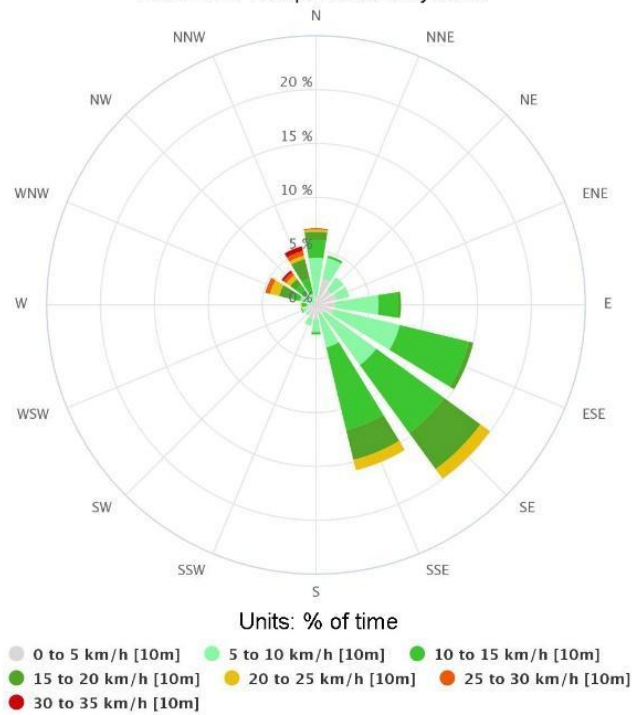


Figure 6 – Wind rose at Castelo Branco Road covering the data collection period. Adapted from Meteoblue (2018).

Source: the authors

Wind Rose at Castelo Branco Road, northern side of the Study Area

Data from 30 May 2013 to 30 May 2018

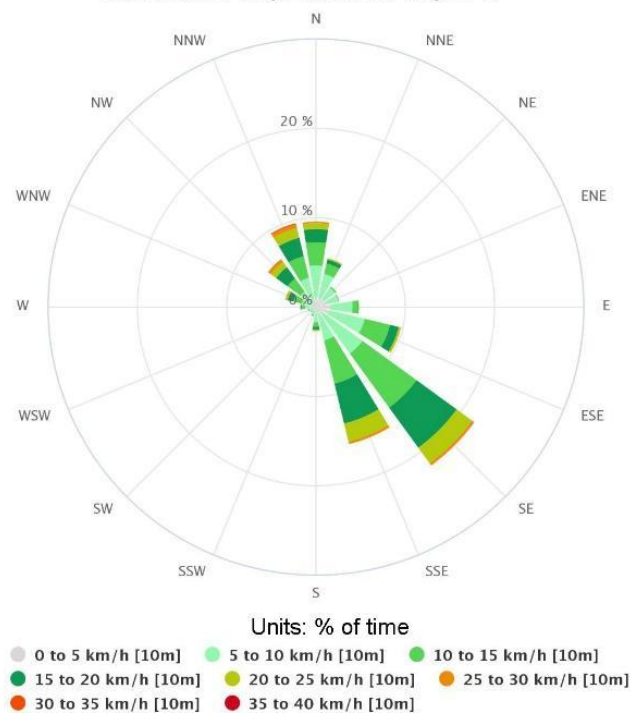


Figure 7 – Wind rose at Castelo Branco Road covering the 5 years preceding the data collection period. Adapted from Meteoblue (2018).

Source: the authors

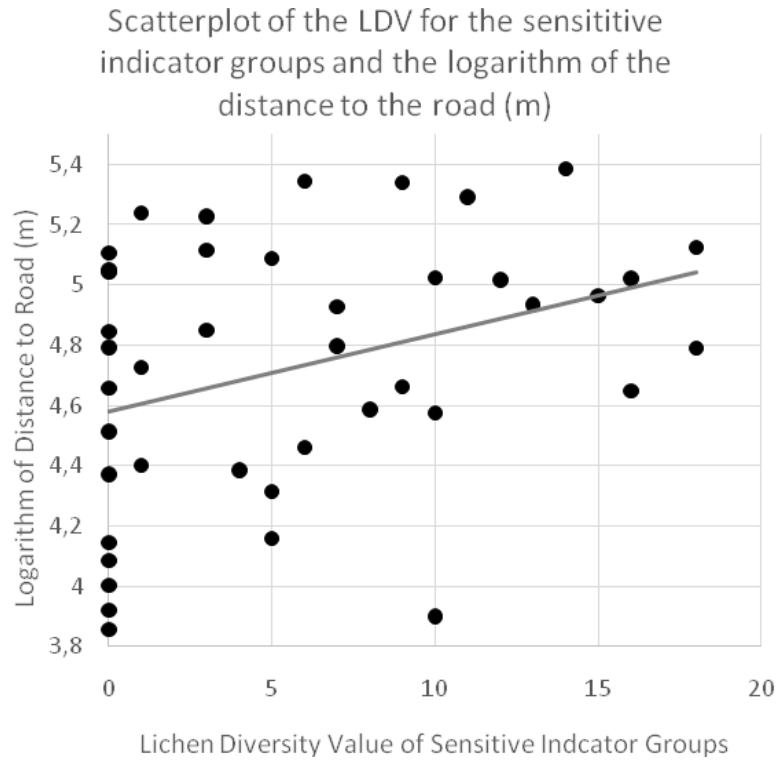
### 3.2. Statistical analysis results

Table 3 presents the variable correlation matrix, and Figures 8 to 10 present scatterplots of the variable pairs with significant correlations ( $p < 0.05$ ) as well as the linear trend for each pairwise linear regression. Logarithmic transformation of the distance from the road resulted in the best correlation with the potential dependent variables. The significant positive correlation with the logarithm of the distance to the road indicates that the impact of the road on the lichens is strong at shorter distances and decreases logarithmically with distance. Despite not being significantly correlated with the dependent variables, the moss trunk coverage (%) was highly and significantly ( $p \leq 0.01$ ) positively correlated with the logarithm of the distance to the road.

**Table 3** – Correlation test between dependent and both independent and confounding variables. The blue text indicates the results that are statistically significant ( $p < 0.05$ ).

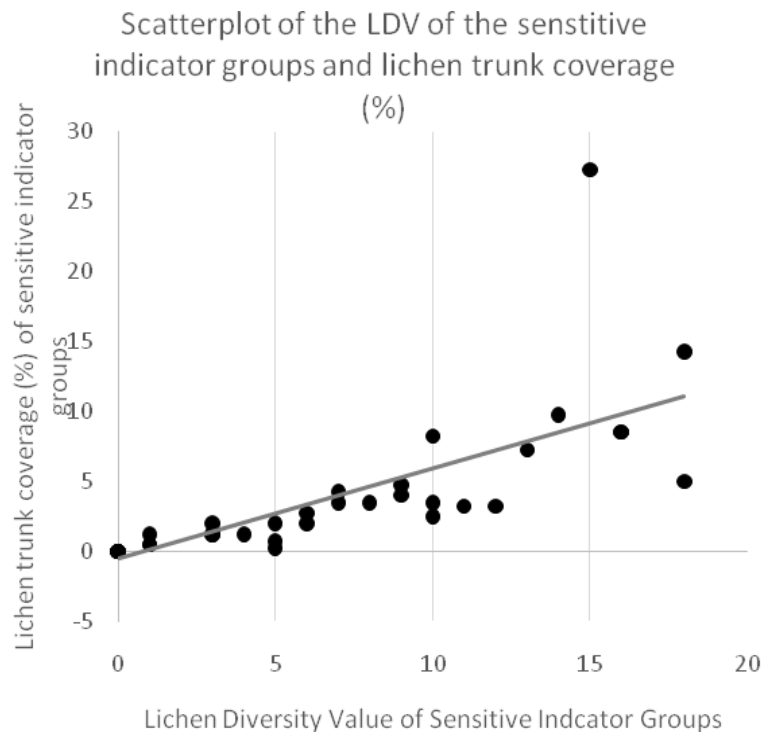
	Lichen diversity (LDV) of the indicator groups	Lichen trunk coverage (%) of the indicator groups	Logarithm of the distance to the road (m)	Canopy coverage (%)	Trunk circumference (cm) at 1.5 m height	Bark damage (%)	Moss trunk coverage (%)
Lichen diversity of the indicator groups	1	<b><math>r=0.75</math> <math>p \leq 0.01</math></b>	<b><math>r=0.35</math> <math>p=0.025</math></b>	$r=0.026$ $p=0.87$	$r=-0.15$ $p=0.35$	$r=0.15$ $p=0.34$	$r=0.14$ $p=0.37$
Lichen trunk coverage (%) of the indicator groups		1	$r=0.30$ $p=0.054$	$r=-0.029$ $p=0.85$	$r=-0.20$ $p=0.20$	$r=0.089$ $p=0.58$	$r=0.12$ $p=0.45$
Logarithm of the distance to the road (m)			1	$r=0.22$ $p=0.16$	$r=0.097$ $p=0.54$	$r=-0.15$ $p=0.35$	<b><math>r=0.42</math> <math>p \leq 0.01</math></b>

Source: the authors



**Figure 8** – Scatterplot of the LDV of the sensitive indicator groups and the logarithm of the distance to the road; the linear regression trend line is also shown

**Source:** the authors



**Figure 9** - Scatterplot of the LDV and lichen trunk coverage (%) of the sensitive indicator groups; the linear

regression trend line is also shown



Source: the authors

Scatterplot of the moss trunk coverage (%) and the logarithm of the distance to the road (m)

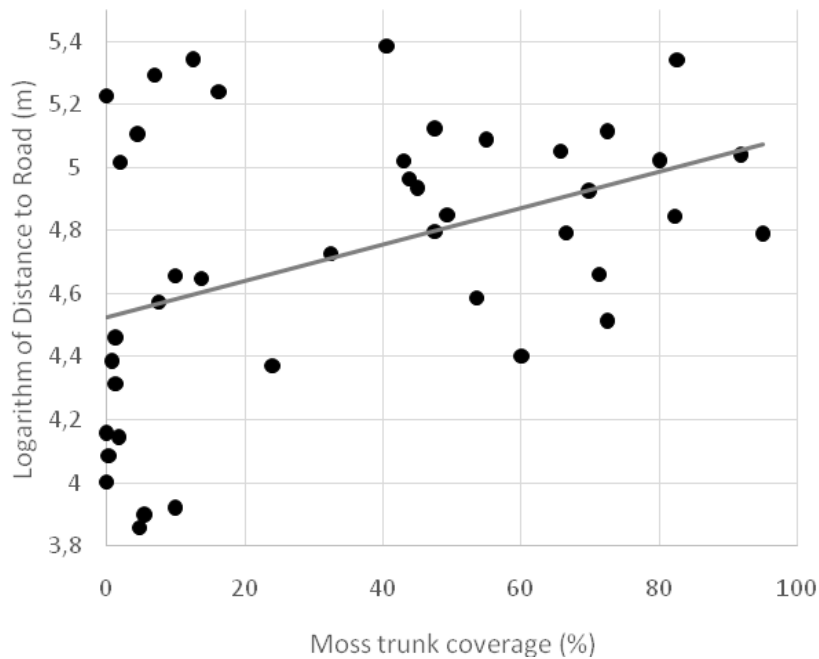


Figure 10 - Scatterplot of moss trunk coverage (%) and the logarithm of the distance to the road; the linear regression trend line is also shown

Source: the authors

Tables 4 and 5 present the results of the tested regression models. Only the regression of the logarithm of distance to the road on the LDV was significant. Regarding the regression models, it was possible to reject the null hypothesis that the logarithm of the distance to the road does not influence the LDV of the indicator morphofunctional lichen groups (greater than 95% confidence) and therefore accept the alternative hypothesis (Table 1). When the logarithm of the distance (m) to the road is increased by one unit, the LDV increases by 2.332 units according to the regression. However, the logarithm of the distance to the road explains only 11.97% of the variance in the LDV. It was not possible to reject the null hypotheses regarding the absence of an effect of the logarithm of the distance to the road on lichen coverage (95% confidence).

Table 4 – Significance (p-value) and determination coefficients ( $R^2$  and adjusted  $R^2$ ) of the regression models

Equation	p-value	$R^2$	Adjusted
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			R <sup>2</sup>
LDV= $\beta_1+(\beta_2*\text{Logarithm of Distance})$	0.0248	0.1197	0.0977
“Lichen Coverage”= $\beta_1+(\beta_2*\text{Logarithm of Distance})$	0.0540	0.0897	0.0670

Obs: for (p)\*<5%.

Source: the authors

Table 5 – Coefficients of the significant (p-value≤0.05) regression model

Equation	Coefficients	
	$\beta_1$	$\beta_2$
LDV= $\beta_1+(\beta_2*\text{Logarithm of Distance})$	-1.708	2.332*

Obs: for (p)\*<5%.

Source: the authors

## 4. DISCUSSION

### 4.1. Spatial patterns and relationships between the distance to the road and lichens

Nieboer and Richardson (1981) found that the concentration of contaminants on lichens is often negatively correlated with the logarithm of the distance to pollution sources, which is compatible with the underlying hypothesis of contamination from road traffic in the current study. In most of the area inside Horto Florestal (except the first 100 m from the road), the sensitive indicator lichen groups are already present. Dymytrova (2009) found a similar pattern, with pollution-sensitive lichen species remaining in the inner cores of urban park forests in Kiev, Ukraine. Although the study area of Dymytrova (2009) had different forest phytophysionomies (temperate broad-leaved and forested steppe areas), both cases show how urban parks may be important to preserve lichen biodiversity.

The impact of the road on José Dias da Silva Elderly Park, which is further from the road than Horto Florestal, may be comparatively lower in terms of the LDV, as a possible extrapolation of the regression model. Therefore, considering that the regression is based on the logarithm of the distance, the older people may be much less exposed to the environmental impacts of the road than the excursionists at Horto Florestal.

Some of the main potential causes of the unexplained variability in the LDV in the regression model using the logarithm of the distance to the road, as emphasized by Cen (2014) and Stofer et al. (2016), are the influence of tree species and their respective bark pH on lichen suitability, which were not controlled in this experiment. Cáceres et al. (2007) also reported a high stochastic variance in lichen occurrence models in the

Atlantic Forest (same vegetation type as in this case study) due to the diverse bark properties resulting from the high tree biodiversity of this biome. In this context, the high biodiversity of tropical forests and their understudied influence on bark suitability for lichens are a challenge for adapting the European Union protocol for biomonitoring air pollution with lichens in these areas.

The low and nonsignificant correlation ( $p\text{-value} \geq 0.05$ ) between the LDV and the confounding environmental variables (canopy cover, trunk circumference, bark damage and mosses coverage) may be partially because of the deliberate standardization effort in this experiment, although they may still cause noise in the model. The positive correlation between the moss trunk coverage (%) and the logarithm of the distance to the road is coherent with the results of Coffey and Fahrig (2012) regarding the impact of roads on decreasing relative air humidity in the vicinity. Moreover, the mosses could compete for space with the lichens, and the humidity could also decrease particulate matter because dry air can support a higher dust concentration (GUPTA et al., 2006; HAGA and NAKABEPPU, 2016). The humidity gradient also provides distinct suitability for different lichen species (SEAWARD, 2008).

The p-value of the regression between the logarithm of the distance to the road and lichen coverage was 0.054, indicating that a larger sample size might reach a significant result with 95% confidence. For example, previous studies that found a correlation between lichen coverage with the distance to roads analyzed 272 trees (DYMYTROVA, 2009) and 420 trees (COFFEY and FAHRIG, 2012), and both studies also found a correlation between the distance to the road and lichen diversity. Nevertheless, the R<sup>2</sup> value of the regression between the logarithm of the distance to the road and lichen coverage was lower than that in the regression based on the LDV, which appears to be a better indicator in this case, even considering the high correlation (0.75,  $p < 0.01$ ) between lichen coverage and the LDV.

#### **4.2. Limitations and possibilities for further research**

Previous rapid lichen morphofunctional biodiversity assessments focused on the structural disturbance of vegetation, such as successional stage or border effects (ARAGÓN et al., 2016; BENÍTEZ et al., 2018), and not on air pollution, as evaluated in this study. Nevertheless, the distinct characteristics of the opportunist, tolerant and sensitive morphofunctional groups in the current study show patterns similar to those of

previous studies, such as the predominance of crustose lichens as opportunists as well as sensitive groups with the predominance of darker and bluer lichens (i.e., indicating an increased presence of cyanophyte algae) and more complex structures (foliose, fruticose, and 2nd border color). Giordani et al. (2012) proposed that crustose lichens would have a competitive advantage in polluted environments because they would have less surface exposed to the atmosphere than other lichens with more complex thallus forms. Cyanolichens have been reported to be very sensitive to air pollution, especially heavy metal deposition (HAUCK et al., 2006). Hauck and Wirth (2010) found that lichen species adapted to shady environments, such as those in the interior of forest fragments, are less tolerant to bark eutrophication caused by nitrogen air pollution. Cyanolichens are typically found in shady environments (ARAGÓN et al., 2016), possibly sharing similar nitrogen pollution sensitivity. However, the current study does not provide elements to differentiate if the spatial patterns in the study area occur because of the air pollution impact from the road or because of other processes, such as the border effects of humidity, light and wind. Further studies with more detailed analyses of air pollutant contamination on lichens could focus on distinguishing these characteristics of morphofunctional groups to evaluate the effectiveness of this biomonitoring approach.

The experimental design of the radial parcel sampling scheme contributed to assuring that the experimental units (the trees) were interspersed from each other, combining a systematic and randomized approach that would avoid pseudoreplication problems within the studied area, as suggested by Hurlbert (1984). However, although the sampling design helps to assure the validity of the conclusions within the study area, the restricted area makes it more difficult to extrapolate to other areas and situations. Generally, the spatial pattern of decreasing diversity of sensitive lichen species (or functional groups) near roads in urban environments was also found in many other studies, such as Brawn and Ogden III (1977), Giordani (2007), Dymytrova (2009), Tulumello (2010) and Cofey and Fahrig (2012). Nevertheless, all these studies were conducted in areas of temperate forest species in Europe and Canada, unlike the current case study.

Moreover, the strength, scale and spatial fading of the environmental impact on lichens, affecting the coefficient of the regressions based on the distance from the road, may depend on specific site characteristics, as demonstrated in comparative studies focused on traffic volume (IRVING, 2008; DYMYTROVA, 2009), balance between gasoline/diesel vehicles (BRAWN and OGDEN III, 1977), wind direction and strength

(VIANA, 2010), and lichen recolonization sources (COFEY and FAHRIG, 2012). In Brazil, the environmental restrictions on vehicle emissions are less strict than those in Europe and Canada (MILLER and FAÇANHA, 2016), increasing the potential for pollution contamination. Castelo Branco Road is an intermunicipal road with heavy diesel truck and bus traffic (SILVEIRA, 2010; FREY, 2010), and emissions from diesel vehicles under Brazilian regulations contain more particulate matter, heavy metals and Sulphur than those from gasoline and ethanol vehicles (SILVA, 2007). However, the dense intertwinement of lianas, branches, canopy, subcanopy, trunks and spider webs in the Atlantic Forest may hamper the wind circulation in tropical forests (LOVEJOY et al., 1986) more than that in temperate forests, decreasing the diffusion of pollution, especially particulate matter.

#### 4.3. Environmental education

The first visit to the environmental education group consisted of an open talk about the research topics, such as lichens, forests, air pollution and health, followed by a field visit to analyze lichens on some of the trees in the park. During the open talk, many elders (52%) reported respiratory health problems, although their general impression was that the air within the park was cleaner than in their own houses (which usually face the streets). In average, they spend 4 days every week in the park, during daytime, which possibly makes the park a good sheltering place with clean air, especially for the elders with respiratory problems. For the elders, it was relevant to clarify that the lichens were not “diseases” on the tree trunks but actually could be indicators of good environmental health. During the last visit to present the research results, it was important to discuss the role of forest trees in environmental services, including the wind barrier effect for better air quality in the core area of the parks.

#### 5. CONCLUSIONS

There was a significant positive correlation between the logarithm of the distance from the road and the LDV. However, the respective regression had a low  $R^2$  value, which is possibly due to the diversity of environmental conditions, especially bark pH. The first 100 m from Castello Branco Road presents a distinct composition of morphofunctional lichen groups. The relative absence of some morphofunctional groups near the road, especially the darker/bluer groups and those with more complex thalli, was

a possible indication of their sensitivity to environmental impacts. Assuming these lichen groups are indicators of sensitivity to environmental impacts, they could be used as bioindicators of environmental quality. In this respect, the greater part of Horto Florestal would probably have good environmental quality. Assuming this environmental impact gradient, the adjacent José Dias da Silva Elderly Park would also have even better protection from the environmental impacts from the road.

The adaptation of the European protocol (CEN, 2014) to tropical forests shows many challenges. This study indicates that the lack of taxonomic knowledge on lichen diversity may be partially addressed by rapid assessments based on morphofunctional groups. However, the lack of studies on species bark pH and high tree biodiversity may result in large variations in the biomonitoring data.

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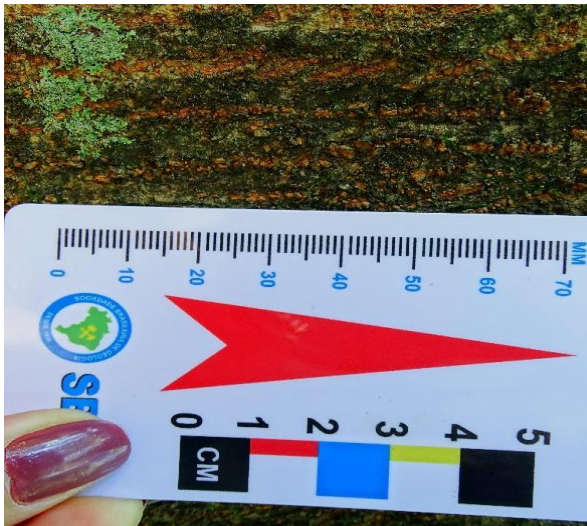
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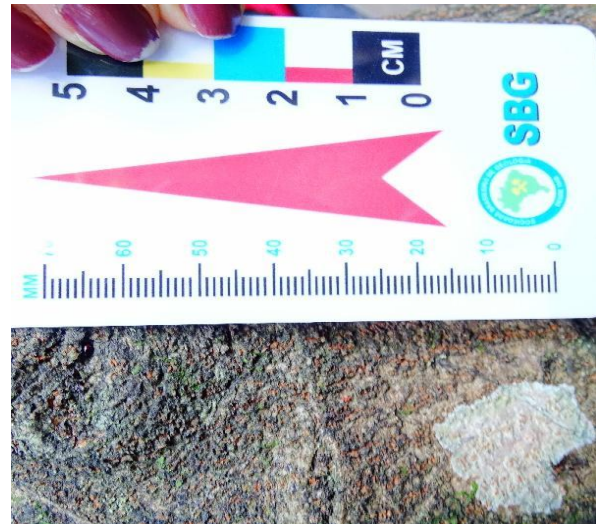
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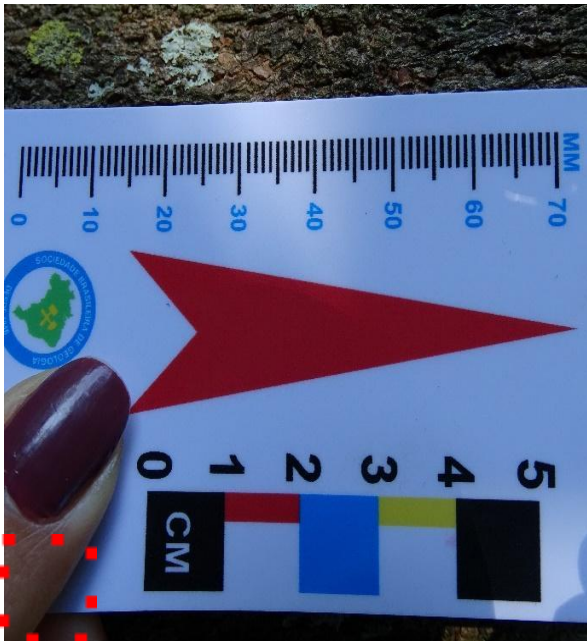
## APPENDIX



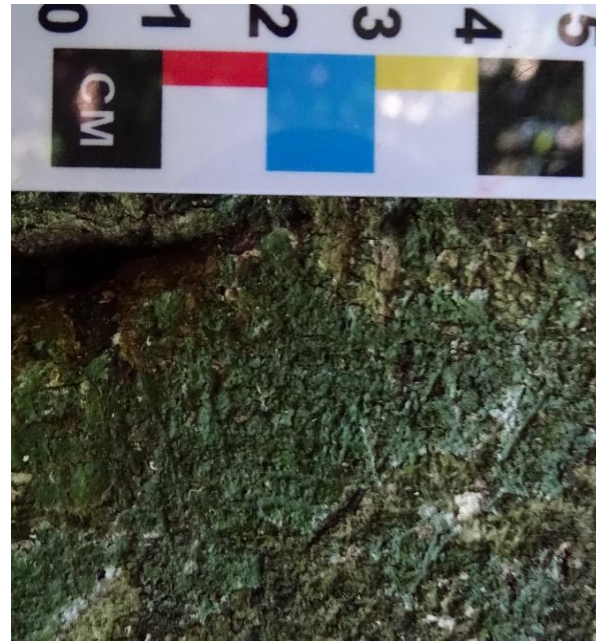
**Figure 11** – Example from morphofunctional lichen group 1  
**Source:** the authors



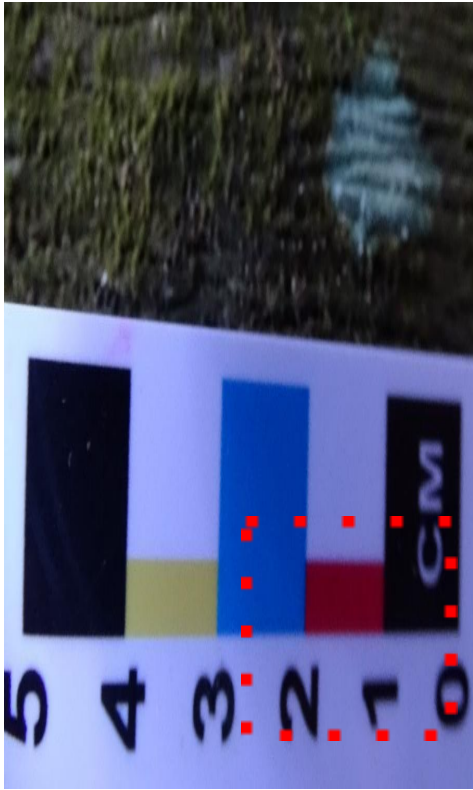
**Figure 12** – Example from morphofunctional lichen group 2  
**Source:** the authors



**Figure 13** – Example from morphofunctional lichen group 3  
**Source:** the authors



**Figure 14** – Example from morphofunctional lichen group 4  
**Source:** the authors



**Figure 15** – Example from morphofunctional lichen group 5  
**Source:** the authors



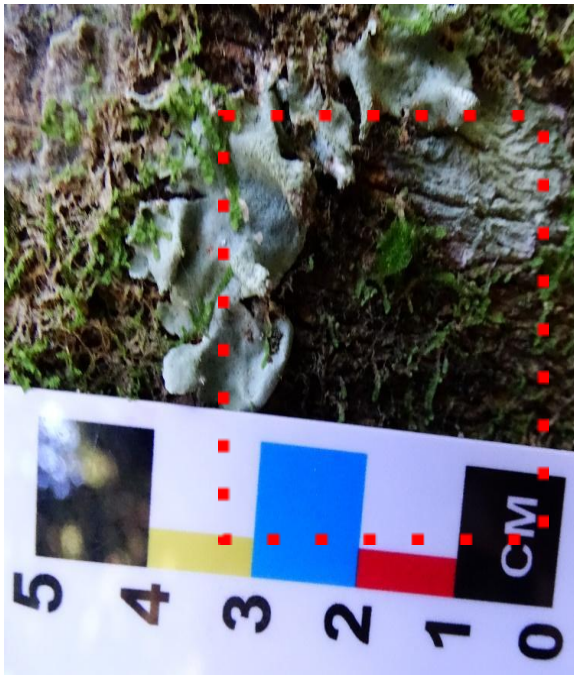
**Figure 16** – Example from morphofunctional lichen group 6  
**Source:** the authors



**Figure 17** – Example from morphofunctional lichen group 7  
**Source:** the authors



**Figure 18** – Example from morphofunctional lichen group 8  
**Source:** the authors



**Figure 19** – Example from morphofunctional lichen group 9  
**Source:** the authors



**Figure 20** – Example from morphofunctional lichen group 10  
**Source:** the authors



**Figure 21** – Example from morphofunctional lichen group 11  
**Source:** the authors

Table 6a - Database

Tree number	LD V	Lichen coverage (%)	Log of distance (m)	Canopy coverage (%)	Trunk circumference (cm)	Bark damage (%)	Moss coverage (%)
1	0	0	3.86	71.01	75	0	4.75
2	0	0	3.92	62.71	60	0	10
3	0	0	4.09	66.29	63	0	0.25
4	0	0	4.00	76.12	68	13.75	0
5	0	0	4.15	68.79	50	0	1.75
6	0	0	4.37	77.29	76	0	24
7	4	1.25	4.39	60.83	66	3.25	0.75
8	1	1.25	4.40	70.21	92	2.5	60
9	6	2	4.46	61.04	68	0	1.25
10	5	0.25	4.32	67.98	67	0	1.25
11	5	0.75	4.16	56.64	85	0	0
12	10	3.5	3.90	71.58	44	0	5.5
13	10	8.25	4.57	79.01	50	2.5	7.5
14	0	0	4.51	82.03	143	0	72.5
15	16	8.5	4.65	75.90	49	32.5	13.75
16	8	3.5	4.59	77.63	66	0	53.5
17	18	5	4.79	73.06	84	0	95
18	3	1.25	4.85	75.19	120	0	49.25
19	0	0	4.85	74.54	92	0.5	82.25
20	7	3.5	4.80	80.17	90	0	47.5
21	0	0	4.79	82.47	56	0	66.5
22	9	4	4.66	78.56	64	0.25	71.25
23	0	0	4.66	82.83	151	6.25	10
24	1	0.5	4.73	78.21	84	0	32.5
25	7	4.25	4.93	73.73	88	0	69.75
26	13	7.25	4.93	72.84	86	0	45
27	16	8.5	5.02	76.58	140	0	43
28	0	0	5.05	67.14	53	0	65.75
29	5	2	5.09	70.54	50	0	55
30	10	2.5	5.02	72.81	67	0	80
31	3	2	5.11	67.70	69	0	72.5
32	0	0	5.04	69.11	66	0	91.75
33	18	14.25	5.12	77.40	73	0	47.5
34	0	0	5.11	74.93	91	0	4.5
35	15	27.25	4.96	64.55	40	0	43.75
36	12	3.25	5.02	70.99	44	0	2
37	3	1.25	5.23	71.76	65	2.5	0
38	11	3.25	5.29	69.84	63	0	7
39	6	2.75	5.34	74.79	100	0	12.5
40	14	9.75	5.38	69.86	64	0	40.5
41	9	4.75	5.34	73.00	83	0	82.5
42	1	0.5	5.24	75.05	69	0	16.25

Source: the authors

## **ABOUT THE AUTHORS**

**Caroline Diogo Ishikawa**, joined the Federal University of ABC in 2017 in the Bachelor of Science and Humanities course, volunteered to participate in the PDPD Project (Researching Since Day One) between 2017 and 2018 and collaborated in the present study during this period. She graduated in the Bachelor of Science and Humanities in Dec / 2020 and is expecting to graduate in the Bachelor of Economics in Sep / 2021, in addition to this, her courses had high affinity with the course of Bachelor of Spatial Planning.

**Vitor Vieira Vasconcelos**, Assistant Professor at the Federal University of ABC. Post-doctorate at the Stockholm Environment Institute. He holds a PhD in Natural Sciences with a concentration in Environmental Geology and Natural Resources Conservation from the Federal University of Ouro Preto, with a sandwich doctorate in Water Resources Engineering at the University of Chulalongkorn (Thailand). His academic formation includes: Master in Geography, Specialist in Soils and Environment, Graduate degree in Geography, Bachelor in Environmental Science, Bachelor in Philosophy, Technician in Environment and Technician in Industrial Informatics. He was Carolina Diogo Ishikawa's advisor in the present study.

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