Evaluation of the Index of Atmospheric Purity in an American tropical valley through the sampling of corticulous lichens in different phorophyte species

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ABSTRACT
Lichens have been widely used worldwide as bioindicators of air quality and also integrated into national programs and protocols in several countries. The Index of Atmospheric Purity (IAP) has been a commonly used tool for the diagnosis of air quality using corticulous lichens. However, its implementation is recommended when phorophytes with similar characteristics of the bark are used. Therefore, many studies are restricted to the use of a single supporting phorophyte. This criterion makes it difficult to implement this index in areas with limited number of individuals and species of phorophytes. This research evaluates the functionality of this index without differentiating among carrier phorophytes for the classification of areas with differences in air quality. For this, the lichens were mapped and collected, along with bark samples to determine the pH bark of phorophytes close to air quality monitoring stations located in Medellín City.

A total of 148 lichen species were identified, belonging to 26 families and 52 genera. In addition, indicators such as thallus heterogeneity, richness (with correlation coefficients $r = 0.52$ with no phorophyte differentiation and for the tree species $F. uhdei$ and $T. rosea$, $r = 0.578$ and $r = 0.777$; respectively) and total coverage of corticulous lichens ($r = 0.76$) vary in response to air pollution levels, no matter if their evaluation is carried out on a single phorophyte or in a diversity of phorophytes.

This research also revealed that the evaluation of IAP in the biomonitoring zones is functional, resulting in positive correlations between the IAP and the air quality classification when this index is evaluated without differentiation of phorophytes ($r = 0.78$) and for $T. rosea$ ($r = 0.94$) and $F. uhdei$ ($r = 0.99$). In this sense, the uniformity criterion in the selection of the phorophyte use for the application of this index can be complemented taking into account the possibility of using a heterogeneous sample of phorophytes.

1. Introduction

Lichens are considered air pollution indicators, due to their anatomical, morphological, and physiological characteristics (Cleavitt et al., 2015; McCune, 2000; Will-Wolf et al., 2015). These organisms obtain nutrients directly from the atmosphere (Conti and Cecchetti, 2001), since the absence of cuticle facilitates the absorption of elements present in the air (lichens have no selectivity). In addition, given their bioaccumulative nature (no elimination mechanisms), these organisms incorporate elements into their intracellular sites (Falla et al., 2000). These characteristics make lichens susceptible to the presence of toxic or harmful substances (air pollutants).

Lichens are increasingly used as biomonitors, since they have advantages over conventional monitoring systems, which are expensive, require maintenance, and their transportation can prevent their implementation in difficult access areas (Agarwal, 2005). Additionally, conventional systems are limited to certain chemical compounds or pollutants and have no intrinsic relationship with the biological effect of such substances (Agarwal, 2005; Rodrigo et al., 1999). In contrast, lichens are widely distributed worldwide and may reflect the influence of environmental factors on living beings (Barreno and Pérez-Ortega, 2003). Moreover, the implementation techniques for using lichens as bioindicators are considered simple and not very expensive (Monge-Nájera et al., 2002) and it is stated that lichens establish limits difficult to

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Lichens have been used with great success in biomonitoring programs throughout the world (Anze et al., 2007; Van Herk et al., 2002; Monge-Nájera et al., 2002; USDA, 2008) and also, have been integrated into national protocols in countries such as England, Germany, the United States, and the Netherlands (Käffer et al., 2011). In this sense, different methodologies have been used for the diagnosis of air quality using lichens as bioindicators, such as Index of Atmospheric Purity (IAP) proposed by LeBlanc and De Sloover (1970) which has been widely used to make local assessments of air quality in countries such as Germany, Italy, Spain, France, and Slovenia, among others (Bartholomé et al., 1994; Cepeda and García, 1998; Gombert et al., 2004, 2006; Loppi et al., 1998; Monaci et al., 1997; Nimis et al., 2000; Poličnik et al., 2008; Ulshöfer and Ronser, 2001).

In temperate countries, it has been well demonstrated that environmental factors and air pollution directly influence the diversity and distribution of lichens (Hawsworth et al., 2005). However, in tropical countries there is limited information regarding the diversity of lichens and air quality (Attanayaka and Wijeyaratne, 2013). Despite this, some studies in the tropics have applied methodologies to bioindicate air quality through the use of lichens. In this regard, Vareschi and Moreno (1973) were the first to use lichens as bioindicators in Venezuela. The authors mapped the areas where lichens were present and categorized these locations based on the presence or absence of the organisms. Furthermore, Cohn-Berger and Quezada (2016) categorized pollution levels in 32 locations within the metropolitan corridor of Guatemala City, using the IAP, the coverage found for each species, and the Environmental Classification Factor. In Colombia, Jaramillo and Botero (2010) assessed the use of lichens as air pollution bioindicators in two areas in the Aburrá Valley in Antioquia, with two different levels of atmospheric pollution. Researchers proposed one tree species (Praxinus chinesis Roxb.) and two lichen species (Canoparmelia sp. and Parmotrema aureosinensis (Zahlbr.) Hale.) as appropriate species to carry out studies on air quality, by using lichens as bioindicators in the Aburra Valley area.

In some studies, the IAP is suggested as a good tool for the categorization of air pollution zones (Cohn-Berger and Quezada, 2016; Jaramillo and Botero, 2010; Lijteroff et al., 2009). However, the use of this index requires as a fundamental premise that the phorophyte species have similar physical and chemical bark properties (LeBlanc and De Sloover, 1970). Therefore, in many studies it has been chosen a single supporting phorophyte common to all areas, where sampling is carried out, in order to avoid substrate variables such as pH and roughness of the phorophyte have an influence on the lichen growth (Lijteroff et al., 2009).

In some scenarios, the use of a single carrier phorophyte may hamper the implementation of lichens as bioindicators with IAP, since cities are currently undergoing rapid spatial and demographic growth, which entails logging, timber transportation, and tree planting (Alvarez del Castillo and Agredo Cardona, 2013), causing tree species to be different, both in taxa and in longevity. Additionally, in the tropics there is a large diversity of tree species (FAO, 2018), so guaranteeing uniformity of bearing phorophytes represents a difficult challenge. The purpose of this research was to assess the behavior of the Index of Atmospheric Purity (IAP) in five areas with pollution differences in Medellín City, without differentiating among carrier phorophytes, to determine the functionality of this index under different conditions of phorophyte diversity.

2. Materials and methods

2.1. Study area

Medellín is located in the central mountain range of the Colombian Andes at 1450 m above sea level, in a deep and narrow valley (1000 m and 10 km on average), known as Aburra Valley (AV). Medellín city has a mild-dry climate, with annual average temperature values of 22.5 °C, precipitation 1685 mm and relative humidity of 67% (IDEM, 2018a). The prevailing direction of the winds in the axis of the valley is given in the north-south direction (Correia et al., 2009; IDEAM, 2018b). Medellín has an urban woodland of around 500,000 trees in green public spaces, distributed among about 550 species (del Medio and de Medellín, 2011). Most tree species are located in linear transects consisting of platforms, road separators and corridors.

In recent years, pollution levels in the AV area have raised great concern and have gained importance for different actors (AMVA, 2007). The particulate matter classified as PM<sub>2.5</sub> is the pollutant that most deteriorates air quality in the metropolitan area of the Aburrá Valley (AMVA, 2016). It has been confirmed that increased concentrations of such pollutant mainly stem from mobile sources, contributing nearly 79% of this agent and its precursors (AMVA, 2016, AMVA, 2017).

2.2. Biomonitoring areas

For this study, five areas were strategically selected near air quality monitoring stations in Medellín city (see Table 1 and Fig. 1), which are currently operated by the Early Warning System (Sistema de Alerta Temprana – SIATA). These stations register significant gradients in terms of pollutant concentrations and physical and environmental conditions. In addition, these locations represent the topographic and altitudinal variability of the study area. The location of biomonitoring stations is presented in Table 1.

MANT zone is located in the downtown area of the city. This area is characterized by being a commercial and institutional sector with emissions mainly related to mobile sources. It has little vegetation, with trees located in isolation and that are part of the urban planning of the area. For its part, EXSA is characterized by having a high vehicular flow with roads where collective public, intermunicipal public service routes and vehicles belonging to urban centers converge. It has scarce vegetation located mainly on the sides of the roads and road separators. In general, this area presents similar conditions to those in MANT zone.

UNNV zone presents heterogeneous conditions by combining exposure to high vehicular traffic and large vegetation cover. MED UNNV station is located inside the National University of Colombia that is characterized by having great diversity and coverage of tree species. On the other hand, PJJC is located in a highly urbanized sector with mainly institutional and residential land uses. It has trees located in isolation in the most residential areas and located on roads and road separators. In contrast, LAYE area is located in a sector where vegetation predominates, as well as low emissions of industrial origin. Despite being directly exposed to vehicular emissions from automobile traffic in its access roads, it is an important contrast area since it is characterized by mostly forest land use.

2.3. Air quality

Worldwide, particulate matter is considered an object of study because it has negative effects on human health (WHO, 2016). In this sense, this pollutant has been widely used to establish zonal
classifications of air quality and is considered as an objective for the evaluation of air quality (WHO, 2013). In the same way this pollutant is used in the AV for the classification of the contamination degree (AMVA, 2016).

Based on studies in which concentrations of particulate matter have been related to the composition of corticolous lichen communities in different zones (Massimi et al., 2019; Varela et al., 2018), the study areas were categorized using time series analysis of PM$_{2.5}$ and PM$_{10}$ recorded by air quality monitoring stations for the period 2013–2016 (AMVA, 2018). In this regard, the behavior of PM$_{2.5}$ average monthly concentrations reported for MED_MANT, MED_UNNV, MED_PJIC, and MED_LAYE stations were analyzed. Since MED_EXSA station does not measure this pollutant, then a representativeness coefficient was determined (for each month), considering that MED_MANT and MED_EXSA stations are located in zones that present similar conditions in terms of location, land use, emission sources, and vegetation presence. This similarity was also verified by comparing the reports on the PM$_{10}$ concentrations for the two stations during the period October 2015–December 2016, using the Wilcoxon test and the two-sample Kolmogorov-Smirnov test.

The representativeness coefficient was calculated with Eq. (1): 

\[
C_1 = \frac{PM_{2.5}}{PM_{10}} \cdot \frac{C_1}{C_2} = \frac{PM_{2.5}}{PM_{10}}
\]

(1)

where: $C_1$ symbolizes the representativeness coefficient of PM$_{2.5}$ in relation to PM$_{10}$ concentrations for MED_MANT station and $C_2$ the representativeness coefficient of PM$_{2.5}$ in relation to PM$_{10}$ concentrations for MED_EXSA station. Once monthly coefficients were obtained, the values were averaged.

After establishing that MED_MANT and MED_EXSA were comparable, it was possible to affirm that $C_1 \approx C_2$; therefore, the following

Fig. 1. Location of air quality stations and the biomonitoring zones object of study. A. EXSA (R = 300 m), B. MANT (R = 300 m), C. UNNV (R = 500 m), D. PJIC (R = 300 m), and E. LAYE (R = 500 m). R (radius around air quality station).
The equation could be empirically established:

\[ PM_{2.5} = PM_{10} \times C \]  

(2)

The result of Eq. (2) represents an estimation of PM\(_{2.5}\) concentrations on the basis of PM\(_{10}\) concentrations reported in MED_EXSA station by the representativeness coefficient of PM\(_{2.5}\) calculated for MED_MANT station. The behavior of the estimated PM\(_{2.5}\) concentrations for MED_EXSA station was analyzed together with PM\(_{2.5}\) average monthly concentrations reported for the other stations.

2.4. Sampling

In each of the five biomonitoring areas, 28 to 30 phorophytes were sampled (without species differentiation) in a radius (R) no larger than 600 m around the station (See Fig. 1). For the selection of the phorophytes, Barreno and Pérez-Ortega (2003) methodology was implemented, in which individuals must have a diameter at breast level above 20 cm, and a trunk inclination angle less than 20° with respect to the vertical, they cannot belong to closed forest formations, and should have tree morphological characteristics that correspond to healthy individuals with no resprouting at the trunk base.

Each selected phorophyte was mapped using a 100 cm × 50 cm acetate sheet with the lower edge placed 50 cm from the ground, where the contour of each lichen species was drawn using markers of different colors depending on morphotype (Fig. 2).

To identify lichen morphotypes, samples of the mapped species were collected following the methodology proposed by Chaparro and Aguirre (2002). During collection, the healthiest specimens were selected, with evidence of propagules or reproductive structures. These samples were stored separately in paper bags to prevent rotting and labeled with their corresponding station code, phorophyte number, and morphotype mapping color. The obtained samples were identified with different taxonomic keys. Lichen collections were preserved at the Herbarium of Universidad de Antioquia (HUA).

For measuring bark pH, pieces as thin as possible were removed from the phorophyte surface using a knife (Kricke, 2002). These samples were stored in paper bags.

Determination of lichens cover and richness

The acetate films obtained were digitized on a 1:1 scale in the presence of a metric pattern, and stored in a TIF format without compression. Lichens coverage was quantified using a code developed for digital image analysis programmed with MATLAB 2016a. This code individually isolates each closed perimeter curve that represents the outline of a lichen, calculates the area inside, and grouping the areas according to the color code Fig. 2. Also, the number of lichens (richness) was calculated as the number of different color codes.

2.5. Measurement of bark pH

The quantification of bark pH was carried out following the methodology proposed by Kricke (2002). The collected samples were macerated until a fine powder was obtained. One gram of the sample was weighed and placed in test tubes with 10 mL of distilled water. Each test tube was shaken to obtain a homogeneous mixture. After four hours, the mixture was filtered, and the pH was measured with a multiparameter meter (ref. HACH-HQ40d).

2.6. Index of Atmospheric Purity

To associate the cover and richness values of lichen species found in each station with pollution levels, the Index of Atmospheric Purity was determined, as proposed by LeBlanc and De Sloover (1970) and modified by Rubiano (2002), according to the following Eq. (3)

\[ IAP_j = \frac{\sum_{i=1}^{n} Q_i \times f_i \times C_i}{n} \]

(3)

where \( IAP_j \) is the Index of Atmospheric Purity for \( j \) zone; \( n \) is the number of sampling units in \( j \) zone; and \( C_i \) is the relative cover of the species in \( j \) zone, expressed as the ratio between the value for total species cover in \( j \) zone and the maximum cover value for the \( i \) species reached in any zone. Additionally, \( f_i \) is the frequency of \( i \) species (number of phorophytes in \( j \) zone where \( i \) is present), and \( Q_i \) is the resistance factor of the species expressed by means of Eq. (4), where \( A_j \) is the number of species present in each zone (richness) where \( i \) is found, and \( E_i \) is the number of zones where \( i \) is found.

\[ Q_i = \frac{\sum_{j=1}^{A_i} (A_j - 1) / E_j}{A_j} \]

(4)

In order to determine the behavior of IAP, when this is calculated using different phorophyte species, ten random combinations of eight phorophytes were generated for each zone and the IAP was calculated for different combinations, obtaining ten different IAP calculations in each area. Since the same species of phorophytes were not found in all areas, the IAP was calculated for two of the common species that were present in at least three of the five zones under study.

2.7. Data analysis

Correlations between the zone classification, richness, cover lichens and the IAP calculated for the combinations with different phorophyte species and for two common phorophyte species were tested with Spearman’s rank correlation coefficient. On the other hand, Kruskal Wallis test was performed in order to determine differences between pH bark (of the different phorophyte species) and Q factor, in the zone classification.

A cluster analysis was applied in order to group the IAP calculated for the different combinations of phorophyte species and for two common species and the zone classification.

3. Results and discussion

3.1. Classification of biomonitoring areas based on air quality

Fig. 3 shows the behavior of the monthly average concentrations of PM\(_{10}\) and PM\(_{2.5}\) for MED_MANT station and PM\(_{10}\) for MED_EXSA station during the period October 2015–December 2016. The Wilcoxon test (\( p = 0.222 > 0.05 \)) determined that no statistically significant differences exist between the reports of the two stations. Furthermore, the Kolmogorov-Smirnov test comparing sample distributions determined that concentrations in both locations have the same distribution (\( p = 0.925 > 0.05 \)). Thus, the particulate matter concentrations in both stations are established as comparable. Therefore, Eq. (2) can be used to establish an average representativeness coefficient of PM\(_{2.5}\) in relation to PM\(_{10}\) concentrations for the MED_EXSA station, which in this case corresponds to 0.61.
Fig. 4 shows the behavior of PM$_{2.5}$ monthly concentrations reported by the Air Quality Monitoring Network in MED_MANT, MED_UNNV, MED_PJIC, and MED_LAYE stations and the estimates for MED_EXSA. A significant increase is noted in March 2016, when an environmental contingency was declared in the city of Medellín. In 2015, some concentration values were not reported for MED_MANT station. This omission occurred because it was not possible to collect 75% of the valid data necessary for the report. In relation to MED_PJIC station, PM$_{2.5}$ began to be monitored with an automated equipment on May 5, 2015. As such, it was not possible to validate the automated equipment series with the series obtained with a manual equipment for January and February. The summary reported for 2015 corresponds to the results obtained with the automated equipment. In addition, a report for MED_LAYE station is only available beginning in October 2015, since this station was added to the network on September 16 of that year.

MED_EXSA Station reported the highest concentrations, exceeding more than 51% of the time the concentrations reported for MED_MANT station, which exceeded 100% of the times the records for MED_UNNV station. Finally, MED_UNNV concentrations were higher than MED_PJIC concentrations 97% of the time.

Comparisons between reports for MED_PJIC and MED_LAYE stations could not be carried out because they did not have any reporting periods in common. However, considering the environmental conditions, land uses, and zone types it is possible to infer that PJIC zone has higher polluting emissions than LAYE.
Taking into account the previous analysis, the biomonitoring zones can be classified from 1 to 5, 1 being the most polluted station and 5 the least polluted station. Table 2 presents the classification corresponding to each zone:

### 3.2. Tree species

In this study, 21 tree species randomly distributed in the biomonitoring areas were sampled, totaling 147 phorophytes; the different phorophyte species found in the study areas are presented in Appendix A.

### 3.3. Lichen cover, richness, and diversity

In this research, a total of 148 lichen species were identified (Appendix B), belonging to 26 families, of which Parmeliaceae was the most represented family with 37 species, followed by Physciaceae with 30. The great richness of these families lends support to the results obtained by authors such as Käffer et al. (2011), who found dominance of the same families in similar studies. According to Divakar et al. (2006) and Nimis et al. (1990), the largest lichen family is Parmeliaceae, which includes taxa that are frequently used as bioindicators of atmospheric pollution, additionally showing a wide habitat range and geographical distribution. In addition, the studies conducted by Rindita et al. (2015) show that Physciaceae family is characterized by abundance in urban areas. Finally, Chaparro and Aguirre (2002) indicate that both families are the most representative families in terms of genus and species in Colombia.

It was possible to identify 52 genera, of which Parmotrema was the most representative genus with 18 species, followed by Lecanora and Physcia with 12 each one. Regarding species, Phaopophyscia chloantha (Ach.) Moberg was the most frequent, present on 50 of the 147 phorophytes assessed at four zones, followed by Candelaria concolor (Dicks.) Arnold, present on 45 phorophytes at all areas.

Of the total species found, 82 were exclusive LAYE. This finding can be explained as a result of the species adaptation to a better air quality. Eight lichen species were exclusive to UNNV, three to PJIC, and four to EXSA. Regarding morphological groups, 37.16% belonged to crustose species, 51.35% to foliose, 4.05% to gelatinous, 2.03% to fruticose, and 1.35% to squamulose.

LAYE zone had the largest number of species (118) followed by UNNV with 44. In contrast, MANT zone had the lowest number of species, 13. In terms of lichen cover per mapped unit, LAYE had the highest cover with 0.253 cm²/cm² of mapped phorophyte, followed by UNNV with 0.252 cm²/cm² of mapped phorophyte. Additionally, EXSA presented the smallest cover with 0.054 cm²/cm² of mapped phorophyte (see Fig. 5). These findings are in agreement with similar observations to those made by Conti and Cecchetti (2001), who reported correlations in lichen community compositions and atmospheric pollution levels. This result is further confirmed by Käffer et al. (2011), who found that environments with limited human intervention present a higher lichen diversity than highly urbanized areas.

The correlation coefficients (r) between zone classification and total richness (r = 0.83) and cover values per mapped area (r = 0.76) indicate that a strong correlation exists among these variables. Additionally, positive correlations were found, indicating that, in general, richness and cover values increase as the air quality classification also increases (Fig. 5); in other words, when atmospheric pollution diminishes. In agreement with these finding Conti and Cecchetti (2001) affirm that changes in the composition of lichens communities are related with air pollution levels, with a negative correlation between lichens diversity and air pollutants concentration.

The cover, richness, and diversity values for UNNV do not agree with how the zone was classified in terms of air quality. This difference may be because this zone is located within an area of big vegetation cover, as described in Table 1, which may contribute to the development of suitable conditions for establishing lichen communities. Barreno and Pérez-Ortega (2003) argue that stand structure is also a factor that should be considered, in relation to the different types of microenvironments that can be created and immediately detected by lichens.

In general, the highest number of thallus types in the biomonitoring zones (except for LAYE) corresponded to foliose species; according to Lücking (1997), these types of lichens are usually sensitive indicators of pollution levels, since they do not attach closely to substrate and are therefore highly dependent on the air. However, Nimis et al. (1990) affirm that, in some species, sensitivity does not depend on growth forms but on the buffering capacity of the thallus, the anatomy, the water retention capacity, and their detoxification mechanisms and that this sensitivity can be altered by different environmental conditions and distribution areas. On the other hand, the number of crustose species
increased as air quality improved, in this sense, the highest percentage of crustose lichen was found at LAYE; the analysis of the crustose species found at this particular zone revealed that they had developed reproductive structures. According to Jaramillo and Botero (2010), reproductive structures do not develop in environments where lichens are exposed to pollution and the presence of these structures has only been reported for relatively undisturbed environments, reason for which the presence of reproductive structures is considered as an indicator of good air quality. Additionally, the presence of fruticose lichens (Usnea and Ramalina) at LAYE (see Fig. 6 and Appendix B) agrees with the findings of Perlmutter et al. (2017), who noted that the presence of fruticose lichens is infrequent in areas exposed to high concentrations of air pollutants, due to the fact that fruticose lichens are considered “sensitive” to disturbances and atmospheric pollution (Cleavitt et al., 2009; Perlmutter et al., 2017).

Along the same lines, the largest diversity of thallus types were found in LAYE (6 types), followed by UNNV (5 types); while MANT exhibited the lowest diversity (1 type) (Fig. 6). These results show differences in lichen morphological composition for each area and confirms the fact that the lichen morphology observed in different areas changes according to environmental characteristics (pollution levels, microenvironment, macroenvironment, etc.; in this regard, areas with higher thallus heterogeneity were associated with lower pollution levels.

Richness in terms of air quality and phorophyte type

The correlation coefficient between the zone classifications and richness values with no phorophyte differentiation ($r = 0.52$) and for the tree species $F. uhdei$ ($r = 0.578$) and $T. rosea$ ($r = 0.777$) common in at least three zones, demonstrated a positive correlation higher than 0.5, thus indicating that, regardless of phorophyte species, richness increases as air quality improves Fig. 7. This finding suggests that there exists a wide range of adaptive responses of lichens to different substrate types and that air quality is a constraining factor for the host tree species selected for studying lichens (Jaramillo and Botero, 2010) and are in agreement with Cáceres et al. (2007) concluded that lichen community formation is weakly related to factors, such as substrate texture, phorophyte bark pH, and microclimate but is strongly related to stochastic effects of dispersal species. In this regard, similar results were also obtained by Soto et al. (2012).

3.4. Bark pH

Bark pH for phorophytes ranged between 4.0 and 8.3 pH units at the different zones. This parameter varies considerably for a single species, even more than one unit (Fig. 8). At the zone level, the pH values obtained for the different phorophytes varied in the following intervals: 4.4 to 7.4 at EXSA, 5.3 to 7.3 at MANT, 4.0 to 6.7 at UNNV, 4.6 to 8.3 at MED_PJIC, and 4.9 to 6.9 at LAYE.

In order to establish if there are differences in bark pH of the same phorophytes species in the biomonitoring areas, a Kruskall Wallis test was carried out for the most common tree species in the biomonitoring areas (present at least in three of the five zones), yielding the following values: $F. uhdei$ ($p = 0.4610$), $T. rosea$ ($p = 0.0004$), $S. campanulata$ ($p = 0.2061$), and $M. indica$ ($p = 0.3503$). The p values assessed for the different tree species determined that, in most species, no statistically significant differences were noted in terms of the pH values among areas, with the exception of the species $T. rosea$, where the p value was less than 0.05. Fig. 9 shows that pH values vary widely for each species.

Previous research studies, such as those conducted by Bates (1992) and Pereira et al. (2014) note that environmental factors and anthropogenic disturbance may drastically change bark pH in phorophytes. Furthermore, they say that pH fluctuations do not remain constant but instead changes over time with phorophytes age.

3.5. IAP

The IAP calculated from the results obtained for the combinations with no differentiation of phorophyte species shows a similar behavior with the IAP calculated for the two most common species ($F. uhdei$ and $T. rosea$) among the zones (Fig. 10). Positive correlations were obtained

![Fig. 6. Number of species by thallus type sampled in the five Biomonitoring zones.](image)

![Fig. 7. Lichen richness with no phorophyte differentiation, for two phorophyte species. A. No phorophyte differentiation, B. F. uhdei, and C. T. rosea.](image)
between the IAP and the air quality classification in each area, either
data set with no phorophyte differentiation in the different cycles
(r = 0.94) or the data obtained for tree species F. uhdei (r = 0.99) and
T. rosea (r = 0.98). In all cases, correlation coefficients higher than 0.9
were obtained. This finding indicates a strong relationship between the
variables.

The strong relationship between the air quality classification for
each zone and the IAP values calculated from the result obtained for the
random combinations with no phorophyte differentiation, as well as
differentiating between F. uhdei and T. rosea (Fig. 10), and their similar
behavior indicates that the IAP for Medellín may be assessed using
phorophytes of different taxa.

Cluster analysis (Fig. 11) indicates that there are 3 kind of groups
according to the calculated IAP for each zone. Clusters C1 and C2 are
similar when the distance increases, that is to say, these two groups
form the same cluster. On the other hand, the group C3 is clearly dif-
ferent from the other groups, as well as, that its conglomerate does not
group with the others.

Following with the analysis, the IAP calculated from different
combinations (see Fig. 12), in all areas, it was found that no matter the
combination of tree species, the IAP values show a similar behavior in
the different zone classifications. A positive and strong relationship was
found between zone classification and the IAP data set in all combi-
nations, the correlation coefficient r = 0.74, indicate that IAP increases
when the air quality improves. This finding indicates that the adjust-
ment suggested for this research related to the modification of the
methodology for the selection of the phorophytes still provides

Fig. 8. Variation of bark pH in different phorophytes species sampled in the five
biomonitoring zones.

Fig. 9. pH variation in the different tree species and for each monitoring station F. uhdei, b) T. rosea, c) S. campanulata, d) M. indica.

Fig. 10. IAP calculation for different stations (with different combination without differentiating phorophyte species and for two common phorophyte species): a) No
phorophyte differentiation, b) F. uhdei, and T. rosea.
functionality to indicate the pollution levels among areas.

Fig. 13 shows resistance factor values (Q) for the different lichen species. The corresponding factor related to the species were grouped, considering the presence of each species in the areas. Through the Kruskal-Wallis test, it was determined that no statistically significant differences exist (p = 0.00 < 0.05) between the distribution medians of factor Q in the biomonitoring zones. As shown in Fig. 13, the paired comparison shows that stations MANT and EXSA do not show significant differences. The same applies for PJIC and UNNV.

The IAP value calculated for UNNV, again, did not match with the air quality classification assigned to this zone (Fig. 12). However, the resistance factors estimated for lichen species found in that area are similar to those found for PJIC. This finding indicates that the species found in UNNV may be considered resistant. Additionally, the resistance factors values found in EXSA and MANT zones suggest that species (mostly foliaceous thallus) found in this zone are resistance as well. The opposite result occurred with the species found in LAYE, which showed the highest resistance factors, thus indicating that the species in this area are more sensitive in comparison with those found in the other studied areas (see Fig. 13).

4. Conclusions

In cities like Medellín, with marked gradients (> 10 µg/m³) or differences in the concentrations of air pollutants among the different zones and great diversity of tree species, the IAP implementation represents a difficult challenge. Therefore, the adjustment suggested by this study related to the modification of the methodology for the selection of the supporting phorophytes, is an important contribution for the application of this index in highly urbanized cities in which there is a limited number of individuals and species of phorophytes.

In this sense, the uniformity criterion in the selection of the phorophyte used for the application of the IAP can be complemented taking into account that the possibility of using a heterogeneous sample of the Phorophytes is included, since the results with both criteria are comparable or without significant differences. The results showed correlation values between the IAP and atmospheric pollution levels above 0.9, both when making the differentiation of phorophytes in the different cycles (r = 0.94) and considering the species F. uhdei (r = 0.98) and T. rosea (r = 0.99) in the study. That indicates that IAP for Medellín may be assessed using phorophytes of different taxa.

Indicators such as thallus heterogeneity, richness (with correlations coefficients r = 0.52 with no phorophyte differentiation and for the tree species F. uhdei and T. rosea, r = 0.578 and r = 0.777; respectively) and total coverage of corticulous lichens (r = 0.76) vary in response to air pollution levels, no matter if their evaluation is carried out on a single phorophyte or in a diversity of phorophytes. These results coincide with the scientific literature on this subject, given that a positive relationship was found among areas with better air quality and increased richness and lichens coverage; while an opposite condition is
observed in areas with high levels of contamination. This could be evidenced indirectly by using a single phorophyte or diversity of these in the areas of biomonitoring.

In this study, it was found that the pH of the supporting substrate presents significant variations (± at a unit of pH) for the same phorophyte species among the biomonitoring areas. Therefore, maintaining the criterion of a single tree species, as an object of study, does not guarantee homogeneity in the properties of the substrate.

Special conditions found in some biomonitoring areas (UNLV) highlight the importance of taking into account the interaction lichen has with other factors different from pollution, such as the structure of the forest mass, since this contributes to the establishment and adaptation of lichen species even with high presence of air pollutants.

This research allowed the identification of dominant species under conditions of environmental stress that can be classified as resistant species (*Phaeophyscia chloanta (Ach.) Moberg and Candelaria concolor (Dicks.) Arnold*). Likewise, species sensitive to environmental disturbances (*Candelaria fibrosa (Fr.) Müll. Arg*) were identified and are recognized as bioindicators of good air quality under conditions of the American tropics.

It would be important to validate or use the methodology for the selection of phorophytes described in this study in other latitudes than the tropics.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at [https://doi.org/10.1016/j.ecolind.2020.106355](https://doi.org/10.1016/j.ecolind.2020.106355).

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