THE BIODIVERSITY OF EPIPHYTIC LICHENS IN PRETORIA (SOUTH AFRICA) AND ITS VALUE FOR ECOLOGICAL INDICATION

by

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DEDICATION

To my mother (Selinah Maphangwa), Azwifaneli, Mpho and Mashudu
who always believed in me.

DECLARATION

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The biodiversity of epiphytic lichens in Pretoria (South Africa) and its value for ecological indication

I declare that the above thesis is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

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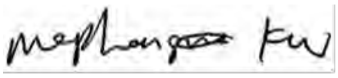
Signature: 

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ABSTRACT

The purpose of this study was to assess the diversity of epiphytic lichens growing on *Acacia karroo*, *A. caffra* and *Jacaranda mimosifolia* trees in Pretoria, and to investigate the influence of air pollution, land use, altitude and climate on lichen diversity. Lichen diversity was first studied at 12 sampling sites under different land use types (high traffic areas, residential areas and industrial areas) and in protected areas (open-air museums and nature reserves). The "European guidelines for monitoring lichen diversity as an indicator of environmental stress" were then tested in 29 sites using the same tree species and under two main land use types ("Industrial areas and busy roads" and "Parks and nature reserves"). Lichen Diversity Values (LDVs) were calculated for 164 trees. Correlations of LDVs and single lichen species with environmental parameters were studied by descriptive statistics, univariate analysis, Principal Component Analysis (PCA) and Generalized Linear Models (GLM). A naturality/alteration interpretative scale based on the percentile deviation of LDVs from natural conditions was developed for the first time in South Africa. Altogether 25 taxa, predominantly foliose and subtropical to tropical species, were recorded and are reported with their ecology and distribution. An identification key was developed for easy identification of species in the field. The highest lichen diversity was found in protected areas. The LDVs of *Jacaranda* are lower than values for both *Acacia* species. "Parks and nature reserves" have significantly higher LDVs than "industrial areas and busy roads", as demonstrated by the PCA. The GLM models were significant for LDV and some lichen species. Sampling sites in industrial areas and the proximity of busy roads are negatively related to LDV and with the frequency of many lichen species. Higher atmospheric concentrations of NOx were negatively related to LDV and to the frequency of the species *Candelaria concolor*, *Lepraria* spp. and *Pyxine cocoes*. *Culbersonia nubila* and *Lepraria* spp. were respectively positively and negatively correlated with atmospheric SO_2. The intensity of land use appears to have a negative impact on lichen diversity. In conclusion, lichens respond well to human disturbances in Pretoria and can be used as bioindicators of naturality/alteration. The European standardised monitoring method can be applied to estimate the degree of environmental alteration in South Africa, by adopting a stratified random sampling and a more flexible strategy for tree selection.

Keywords: lichen, biodiversity, distribution, monitoring, disturbance, Pretoria, South Africa

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CHAPTER 1: INTRODUCTION

1.1 Introduction and background

Lichens are not single organisms but a symbiosis among fungal partners (mycobiont) and photosynthetic partners (photobionts), which are green algae or cyanobacteria, with each partner contributing in various ways to the symbiosis (Nash, 2008; Atala *et al*., 2015). Although the symbiosis among mycobionts and phobionts is well acknowledged, it is less well known that the lichens actually host other types of organisms such as yeasts and other fungi, virus, bacteria as well as small animals, and can be therefore regarded as a sort of microhabitat (Grube *et al*., 2015; Zedda and Rambold, 2015; Cernava *et al*., 2016; Spribille *et al*., 2016; Muggia and Grube, 2018). Spribille *et al*. (2016) recently discovered that basidiomycete yeasts growing in the cortex of ascomycete macrolichens can play an important function in the symbiosis and may influence lichen traits.

The main advantage of the symbiosis for the photobionts is that the fungal hyphae protect them from intense insolation and drying out, and absorb mineral nutrients from the substratum and the atmosphere to share with the photobionts. The photobionts synthesise organic compounds by photosynthesis and cyanobiontal photobionts are even able to fix nitrogen compounds (Hale, 1983; Purvis, 2000; Nash, 2008).

Lichens are perennials and maintain a uniform morphology over time (Nash, 2008). They develop gradually and rely heavily on the environment for long-term nutrition (Hale, 1983; Asplund and Wardle, 2017).

Lichens are traditionally classified into three main growth forms, with many intermediate forms. The main forms are the crustose, foliose and fruticose (Hale, 1983; Nash, 2008; Asplund and Wardle, 2017). These forms, which are in no sense natural divisions, are at best points on a scale of continuous differentiation from primitive to highly structured thalli. Each form has different arrangements of cortical, algal and medullary tissues as well as different modes of attachment to the substrate, and each form represents an ecological adaptation to environmental conditions (Hale, 1983).

Lichens colonise tree bark, wood, rocks, man-made substrate and soil and are found in all terrestrial ecosystems, where they cover between 6-8% of the earth’s land surface (Nimis *et al*., 2002; Nash, 2008; Purvis and Pawlik-Skowrońska, 2008; Colesie *et al*., 2014; Bajpai *et al*., 2016; Asplund and Wardle, 2017). Lichens can live in a variety of habitats and can survive extreme environmental conditions (heat or cold), for instance deserts, cold regions, tropical rain forests, natural and managed environments (Nimis *et al*., 2002; Purvis, 2014; Zedda and Rambold, 2015; Bajpai *et al*., 2016).

Ecological factors such as climate, topography and geology as well as the prevailing land use, the extent of pollution (Hauck *et al*., 2013), the age and type of trees, and bark pH influence the occurrence of lichens (Nascimbene *et al*., 2013; Nelson *et al*., 2015). Other factors include bark properties and light availability (Mulligan, 2009). James *et al*. (1977), as cited by Mulligan (2009), noted humidity of the environment, age of the bark surface, inclination of tree, degree of bark leaching by rain, degree of impregnation of bark with organic nutrients, air pollution, soil pollution by agricultural chemicals, pH of the bark surface and basic nutrient status of bark as factors that can affect lichen distribution.

The growth of lichen species on given tree species is dependent on bark, crown features and tree structure (Cáceres *et al*., 2007; Frati *et al*., 2008; Mežaka *et al*., 2012; Ódor *et al*., 2013; Frisch *et al*., 2015).

Lichens were perceived as potential indicators of air pollution as early as the 1860s in Europe where a progressive loss of lichens in major cities was noticed (Hale, 1967). Since then, lichens have played important roles as bioindicators of sulphur dioxide (SO_2) air pollution throughout the world (Nimis *et al*., 2002; Tiwari, 2008; Gibson *et al*., 2013; Tregidgo *et al*., 2013). Furthermore, lichens accumulate large amounts of elements, especially heavy metals, from wet and dry deposition sources (Nash, 1996; Hussan *et al*., 2013; Purvis, 2014). Accumulation mostly exceeds their physiological requirements (Nash, 2008). The analysis of elements in epiphytic lichens provides information on the possible sources of pollution (Budka *et al*., 2002; Nash, 2008; Maphangwa *et al*., 2012a; Monaci *et al*., 2012; Loppi, 2014). For this reason, lichens can be used as bioaccumulators of heavy metals such as mercury (Grangeon *et al*., 2012) and lead (Sujetoviene and Sliumpaite, 2013) and for biomonitoring environmental pollution (Puckett, 1988; Garty, 2001; Kularatne and de Freitas, 2013). Many species, such as the epiphytic, foliose lichen *Flavoparmelia caperata* (L.) Hale have been widely utilised to assess atmospheric depositions and air quality (Loppi *et al*., 1998).

Epiphytic lichen diversity has also been effectively used for evaluating other effects of anthropogenic disturbances, not only atmospheric pollution. Changes in lichen diversity can, for instance, indicate the intensity of land use and habitat fragmentation in urbanised and rural environments, especially in woodlands (Budka *et al*., 2002; Nimis *et al*., 2002; Ohnuki *et al*., 2002; Ng *et al*., 2005; Werth *et al*., 2006; Brunialti *et al*., 2012; Benítez *et al*., 2012; 2018;

Benítez, 2016). This is possible because many species of epiphytic lichens are very sensitive to even minor environmental changes, while others are more resistant (Nimis *et al*., 2002; Zedda, 2002; Brodekovà *et al*., 2006; Nash, 2008; Danesh *et al*., 2013).

Lichens are more sensitive to pollution than plants because they lack cuticles and stomata and have no roots. For this reason, they absorb much of their raw materials and also pollutants directly from the air and moisture around them without restrictions (Nash, 2008; Sett and Kundu, 2016; Asplund and Wardle, 2017). Lichens are furthermore perennial and grow slowly (Zvěřina *et al*., 2018). Pollutant uptake by lichens is considerably higher than by vascular plants (Nash, 2008).

Lichen functional traits such as growth forms (foliose, fruticose and crustose), reproductive strategy (sexual, asexual), photobiont type (green algal or cyanobacterial), production of secondary metabolites such as photoprotective, antioxidant compounds, thickness of thallus layers, substrate preference and geographical distribution are dependent on environmental factors, especially air and substrate humidity, sun radiation, rainfall, temperature, light and land use intensity. These ecological factors determine lichen distribution and can be detected through analyses of functional types or morphotypes (Diaz and Cabido, 2001; Zedda *et al*., 2011a; Benítez *et al*., 2012; Ellis, 2012; Giordani *et al*., 2012; Pinho *et al*., 2012; Gauslaa, 2014; Atala *et al*., 2015; Nelson *et al.*, 2015; López *et al.,* 2016; Rubio-Salcedo *et al*., 2017).

Since various lichen species have a wide scope of ideal conditions concerning humidity, temperature, substrate quality and stability, and nutrients needs, certain traits such as the ones listed above, are related to various environmental factors (Weber *et al*., 2010; Marini *et al*., 2011).

1.2 Problem statement

South Africa is a well-known biodiversity hotspot for phanerogams (Myers *et al*., 2000; Mittermeier *et al*., 2004). However, lichen diversity still remains relatively underexplored (Crous *et al*., 2006, Maphangwa *et al*., 2012b, Mayrhofer *et al*., 2014). One problem is due to the fact that herbarium vouchers of South African lichens are very scattered throughout numerous herbaria in South Africa, Europe and North America and are therefore difficult to check (Fryday, 2015). This hinders further floristic investigation of South Africa's lichen diversity (Maphangwa *et al*., 2018).

As illustrated above, lichens are excellent bioindicators of environmental pollution. In particular, epiphytic species growing on the bark of trees and bushes, are regularly utilised in assessing air pollution in urbanised areas (Budka *et al*., 2002, 2004; Ohnuki *et al*., 2002) and rural environments (Ng *et al*., 2005). As fruticose lichens are the most sensitive to air pollution, they are the first group to vanish from polluted sites, followed by foliose lichens. In contrast, crustose lichens are generally the most resistant to air pollution (Boonpragob, 2003; Díaz-Escandón *et al*., 2016). One example is the lichen *Lecanora conizaeoides* Nyl. ex Cromb., which grows typically in SO_2-polluted areas (Boonpragob, 2003).

Epiphytic lichens have been used as ecological indicators for several decades (Brunialti *et al*., 2012). Most investigations have, however, been restricted to Europe and the US (Asta *et al*., 2002a, 2002b; VDI-Richtlinien, 2005; Thormann, 2006; Geiser and Neitlich, 2007; Munzi *et al*., 2007; Hauck *et al*., 2013). Some studies have been carried out in Asian countries, for example, lichens were utilised as indicators of air quality in Northern Thailand (Budka *et al*., 2002). Also Wolseley and Aguirre-Hudson (1997) have used epiphytic lichen communities as bioindicators of environmental change in Thailand. Lichen communities were valuable indicators of succession stages in tropical rainforests (Koch *et al*., 2013). In Venezuelan tropical lowland rainforest, Komposch and Hafellner (2000) assessed the diversity and distribution of lichen in relation to changing conditions.

The indicator value of lichen diversity in urban environments of South Africa has been poorly investigated, with limited studies focusing on lichens as bioaccumulators of heavy metals in the Pretoria area of Gauteng (Forbes *et al*., 2009; Olowoyo *et al*., 2010; Trüe *et al*., 2012; Panichev *et al*., 2019). The diversity of epiphytic lichens and their ecology in relation to urban pollution or to other types of human disturbances in the city of Pretoria has not been fully explored, although lichens are important bioindicators of air quality and environmental disturbance.

Only a few studies on epiphytic lichens or on lichens as bioindicators have been carried out in Africa and in Southern Africa (Aptroot, 2001; Crous *et al*., 2006; Swinscow and Krog, 1988; Schultz *et al*., 2009; Zedda and Rambold, 2004; Zedda *et al*., 2009, 2010a, 2010b; Boamponsem *et al*., 2010). While other studies have been carried out in desert and semi-desert habitats of South Africa (Zedda and Rambold, 2004, 2009; Zedda *et al*., 2010a, b), this is the first study on the biodiversity of lichens and its value for ecological indication in a South African *urban* environment.

The purpose of this study was to assess the diversity of epiphytic lichens on selected trees in Pretoria, and to investigate to what extent lichen diversity is influenced by the phorophyte type on which lichens are growing, by human activities, especially by air pollution and land use, or by other environmental factors such as altitude and climate. The study aimed also to explore the potential for using lichens as bioindicators of air pollution in the city.

1.3 The need for this research

Different authors have published numerous taxonomical revisions, ecological and floristic studies on lichens from South Africa (for example, Almborn, 1966; Hale, 1984; Brusse, 1986; Zedda and Rambold, 2009). These studies focused, however, mainly on saxicolous (i.e. Kärnefelt, 1988; Matzer and Mayrhofer, 1996) and terricolous lichens (i.e. Zedda and Rambold, 2004), and on given regions of South Africa, such as the Cape region, while epiphytic lichens and the Pretoria area have been less explored (Maphangwa *et al*., 2018). Lichen diversity and ecology studies in South Africa are detailed in section 2.2. In order to improve knowledge on lichen diversity, especially on epiphytic lichens in different environments of South Africa, floristic and ecological studies remain necessary.

Urbanisation has been attributed to rampant environmental pollution and urban transportation is among the major cause of atmospheric pollution. The City of Tshwane (Pretoria) is affected by different kinds of environmental pollutants. Among the air pollutants are particulates (ash and aerosols), sulphur dioxide (SO_2), sulphur oxides (SO_x), oxides of nitrogen (NO_x), carbon monoxide (CO), carbon dioxide (CO_2), volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), methane (CH_4), ammonia (NH_3), hydrogen chloride (HCl), hydrogen sulphide (H_2S), ozone (O_3), lead (Pb) and other secondary pollutants and numerous trace elements (Liebenberg-Enslin and Petzer, 2005). Organic compounds released into the air include benzene, PCBs and dioxins and furans (Liebenberg-Enslin and Petzer, 2005; Forbes *et al*., 2009; Olowoyo *et al*., 2011). The sources of these organic pollutants are power generation (Rooiwal and Pretoria West power stations), industrial areas (ceramic, cement, iron and steel), transport (diesel and petrol vehicle emissions) and household fuel combustion. The City of Tshwane has seven air quality monitoring stations to check if the pollution is within set standards (Liebenberg-Enslin and Petzer, 2005; Olowoyo *et al*., 2011, South African Air Quality Information Systems (SAAQIS), 2018 http://www.saaqis.org.za/), but none of the monitoring systems use bioindicators.

This study not only assesses lichen diversity, but also analyses lichen occurrence in relationship to changing ecological conditions. Understanding the reaction of lichens to the investigated environmental parameters and to air pollution would facilitate estimation of air quality or of naturality/alteration conditions on a wider area so as to ascertain the possibility of using lichens as bioindicators in environmental monitoring, thus limiting costs associated with setting and sustaining monitoring stations. Biomonitoring also allows detecting the synergistic effects of the different pollutants on living organisms, which cannot be detected by monitoring stations.

The present study focused on the biodiversity of epiphytic lichens in Pretoria and its value for ecological indication. It represents a first assessment of the biodiversity of epiphytic lichens in an urban environment in South Africa. To allow the elaboration of a monitoring methodology, the floristic evaluation was restricted to the three most widespread tree species: the native *Acacia caffra* (Thunb.) Willd and *A. karroo* Hayne and the exotic *Jacaranda mimosifolia* D.Don. The lichen biota of these phorophytes was investigated for the first time. Comparisons have been made between the different trees. The research further provides new scientific information on the diversity of epiphytic lichens occurring in Pretoria in relation to different levels of environmental disturbance, due to air pollution (industry, traffic), plantations of exotic plants and habitat fragmentation. This study is important for the town of Pretoria, as it develops a methodology to estimate air quality and other disturbances (environmental naturality/alteration) in a cheaper and more reliable way than by using monitoring stations. A long-term monitoring approach using lichens as bioindicators could be established on the basis of the present research work, and could be extended to other southern African towns as well. This study adds information about the importance of lichens and their conservation, and supports building expertise on these organisms in South Africa, where lichen experts are rare.

1.4 Research questions

- What is the richness, distribution and composition of lichen diversity in Pretoria on selected tree species (*Acacia caffra*, *Acacia karroo* and *Jacaranda mimosifolia*)?
- How do lichen communities vary across the selected tree species, considering taxonomy as well as native vs exotic species?
- How do lichen species respond to environmental pollution and disturbances?
- To what extent is lichen diversity related to air quality in the town?
- Which lichen species are more sensitive and related to the given environmental conditions?
- What is the indicator value of the different species occurring on trees?
- Can lichens be used reliably for biomonitoring to estimate air quality and the level of human disturbance (naturality/alteration) in Pretoria?

1.5 Research objectives

The objectives of the research were:

- To assess the diversity of lichens growing on selected trees (epiphytic) in the urban environment of Pretoria.
- To analyse the ecological conditions under which given lichen species occur.
- To evaluate the diversity of lichens in relationship to tree species (two native *Acacia caffra*, *A*. *karroo* and one exotic, *Jacaranda mimosofolia*) and human disturbance, with special reference to air quality (comparison of areas with different degrees of pollution).
- To analyse lichen communities (spatial changes in species composition, diversity and frequency) in relation to changing environmental factors (human disturbance).
- To identify the indicator value of lichen species growing on trees and develop a long-term monitoring approach for detecting air quality changes and/or environmental naturality/alteration using lichens as biodindicators.

1.6 Ethical considerations

The study required collection and monitoring of lichens in nature reserves, open-air museums and public areas around the City of Tshwane. Ethical considerations to conduct research were followed. Permission to conduct the study and to collect lichen samples from different trees was obtained from the City of Tshwane, Voortrekker Monument and Pionier Museum through an official letter before the study commenced (see Appendices 1, 2 and 3). The aim and purpose of the study were included in the permission letter. Ethical clearance for this study was obtained by the researcher from University of South Africa, College of Agriculture and Environmental Science (Ref: 2014/CAES/157) (see Appendix 4).

1.7 Study limitations

This study focused on epiphytic lichens growing on only three different tree species. The identification of lichens was difficult at the beginning of the research as identification keys and reference material on epiphytic lichens is very limited for the region. This was also why the study was limited to the three trees and did not consider the entire epiphytic diversity of the investigated area.

Though the study intended to cover the wider Tshwane area, it was difficult to collect and monitor lichens in more protected areas such as Onderstepoort Nature Reserve, Pretoria Metal Pressing and Pretoria Zoological Gardens. The researcher tried several times unsuccessfully to obtain permission to access and collect data. Some residential areas could not be visited due to safety concerns. Some of the trees in the study were not suitable for lichen monitoring as they did not meet the selection criteria of international guidelines. Certain trees were not sufficiently straight or were affected by termite mounds (see Appendix 5). Some trees were too thin (less than 60 cm trunk diameter) whereas other were too large (more than 1 m in diameter). In addition, people often pin advertisements and notices on *Jacaranda* trees, which makes these not suitable for monitoring (see Appendix 6). This was observed mostly in the city centre, Brooklyn and Hatfield. In the rural sites like Hermanstad, Saulsville and Suiderberg, trees had been often cut down for firewood.

For this study, each site was only monitored once. Monitoring was only spatial and not repeated in different years (long-term monitoring) for the following reasons. The first part of the study was dedicated to the collection and identification of species (assessment of lichen diversity in the study area), a work that had never been done before. This investigation took more than one year of my PhD-period. During the second and third year the monitoring was started and methodology from Europe tested, with a focus on spatial monitoring (investigation of different sites and environments). However, the examined trunks were accurately and durably marked for long-term monitoring purposes, so that this can be carried out in the future.

In protected areas like Rietvlei Nature Reserve and Voortrekker Monument Nature Reserve, there were signs that certain wild animals were eating lichens. Some animals also use *Acacias* to scratch their bodies as observed in the field. Often *Acacias* were multi-stemmed and not wide enough for monitoring. All these factors made many trees unsuitable for monitoring in the protected areas. In the Akasia site, vegetation had recently been burnt, so the trees here were unsuitable for monitoring. The European guidelines for choosing plots and trees to monitor lichen diversity, according to Asta *et al*. (2002a, 2002b) could therefore not always be applied.

The pollution and climate data for this study was obtained from the South African Air Quality Information System (SAAQIS). However, only data from five monitoring stations, relatively close to the sampling areas, were used as the datasets for pollution and climate were not complete for the other stations. Data for benzene and xylene could not be considered in the elaborations because it was not complete.

1.8 Chapter breakdown

This thesis comprises five chapters. The first chapter includes an introduction, describes the significance of the research as well as the study objectives, limitations of the study and includes a chapter breakdown. The second chapter presents a literature review, while Chapter

Three contains a description of the study sites and the materials and methods of the research (site selection, lichen collection, identification, and monitoring and data analysis). The fourth chapter describes results and is split into two sections. The first section reports on the general diversity of epiphytic lichens found in Pretoria and includes an annotated list of all species with information on their distribution and ecology worldwide recorded at the study sites. The second part of Chapter Four reports on monitoring and elaborates on statistics. The fifth chapter presents a discussion, recommendations and conclusions. References and appendices are included at the end of the thesis.

CHAPTER 2: LITERATURE REVIEW

2.1 Lichen morphology

The mycobiont is reponsible for the appearance of a lichen thallus. In a few cases, the photobiont determines the pattern of the entire thallus (Nash, 2008). As reported in section 1.1, lichens generally have three principal growth habits: these are the crustose, foliose and fruticose types, with several intermediate forms (Hale, 1983; Nash, 2008).

Crustose lichens expose a limited surface, are firmly attached to the substrate (often bark of trees and shrubs, wood, rock, soil, and tree leaves in moist forests) with their lower surface (Figure 2.1), and may not be detached from it without decimation (Nash, 2008; Asplund and Wardle, 2017). Water uptake and loss is limited fundamentally to the upper, uncovered surface (Nash, 2008). These features enable crustose lichens to tolerate the environmental conditions of extreme habitats such as bare and exposed rock surfaces or soil in dry zones (Zedda and Rambold, 2011). Sub-types of crustose lichens include: powdery, endolithic, endophloeodic, squamulose, peltate, pulvinate, lobate, effigurate and subfruticose crusts. The thallus association of crustose lichens is either homoiomerous or heteromerous (Nash, 2008). In the homoiomerous thallus, the mycobionts and photobionts are evenly distributed, whereas in a heteromerous thallus they are well structured and distributed in well-defined thallus layers (Nash, 2008).

Figure 2.1. Crustose lichen: *Caloplaca haematodes* (A. Massal.) Zahlbr. (image by Felix Schumm, from http://dryades.units.it/italic, CC BY-SA4.0)

Foliose lichens are leaf-like horizontally and are only partly connected to the substrate (Figure 2.2). These thalli are either homoiomerous (i.e. gelatinous lichens) or heteromerous. Usually they have a dorsiventral structure with different upper and lower surfaces (Nash, 2008). Frequently, the thallus is partitioned into lobes, which show different degrees of expanding branching. Occasionally the lobes overlap like tiles, and the lower side can frequently have fixing rhizinae (Hale, 1983; Asplund and Wardle, 2017). Common foliose genera are *Anaptychia, Cetraria, Heterodermia*, *Parmelia* s.l., *Physcia* and *Xanthoria*. This well-established form has given rise to an extraordinary range of thallus sizes and diversity (Hale, 1983; Nash, 2008).

Figure 2.2. Foliose lichen: *Parmotrema austrosinense* (Zahlbr.) Hale (image by Felix Schumm, from http://dryades.units.it/italic, CC BY-SA4.0)

Fruticose lichens stand out from the substrate and are attached to it with only a very small part of the thallus (Figure 2.3). They may present hair-like, strap-shaped or shrubby ramifications, and can be flat or cylindrical. They can have dorsiventrally arranged (e.g. *Evernia prunastri* (L.) Ach.) or radial symmetric thalli (e.g. *Usnea* species). The branching pattern of lobes varies considerably among different systematic groups and also within a single genus. There are considerable variations in size with some *Usnea* species growing to a few metres while certain minute species measure only 1 or 2 mm. Fruticose lichens are found in different climates, ranging from desert to wet rainforest and on different kinds of substrates, but all require high levels of air humidity or fog (Hale, 1983; Nash, 2008).

Figure 2.3. Fruticose lichen: *Evernia prunastri* (L.) Ach. (image by Simonetta Peruzzi, from http://dryades.units.it/italic, CC BY-SA4.0)

2.2 Lichen diversity and ecology in South Africa

Most relevant among the existing taxonomic and floristic studies concerning South African lichens are those of Massalongo (1861), Crombie (1876a; 1876b), Van der Byl (1931), Doidge (1950), Almborn (1966, 1987, 1988), Hale (1971, 1984, 1990), Brusse (1984, 1985, 1986, 1988, 1994), Kärnefelt (1986, 1987, 1988), Thomas and Bhat (1994, 1995, 1996), Jürgens and Niebel-Lohmann (1995), Elix (1999, 2002, 2003), Zedda and Rambold (2004, 2009), Schultz *et al*. (2009), Wirth (2010), Zedda *et al*. (2009, 2010a, 2010b, 2011b, 2011c), Ahti *et al*. (2016), Wirth and Sipman (2018), Wirth *et al*. (2018) and Maphangwa *et al*. (2018).

The first checklist of South African lichens was compiled by Doidge (1950) and supplemented by Almborn (1988), Feuerer and Zedda (2001), Fryday (2015), Feuerer (2016) and Ahti *et al*. (2016). Online versions of the checklists of Fryday (2015) and Feuerer (2016) are available and updated periodically. However, checklists are still provisional and far from being complete (Feuerer and Zedda, 2001; Maphangwa *et al*., 2018).

The preliminary version of a checklist of the lichens and lichenicolous fungi of South Africa compiled by Feuerer (2016)[1] lists 1 728 lichen taxa, which comprises 9.2% of the total global

[1] The website http://www.lichens.uni-hamburg.de/lichens/africa/south-africa_checklists_switch.htm was last visited on 22 October 2018. At present (September 2019), it is not available and it is not clear when it will be online again.

list of 18 882 lichen species (Feuerer, 2009[2]; Feuerer and Hawksworth, 2007). The Pretoria Centralised Information System database (PRECIS) registers 1 460 quarter degree square distribution records for 412 lichen species, comprising 105 genera in South Africa (Maphangwa, 2010). Lichen species in South Africa are estimated to be about 2 000 (Crous *et al*., 2006). The diversity of soil lichens in South Africa, covering different biomes of the Western and Northern Cape, is of 73 taxa (Zedda *et al*., 2010a, 2010b), most of which are not yet included in Feuerer's checklist (2009).

Concerning ecology, physio-ecological works have been carried out in northern areas of South Africa, the Karoo and other areas by Wessels and van Vuuren (1986), Wessels and Schoeman (1988), Wessels and Wessels (1991), Wessels and Kappen (1994). Maphangwa *et al*. (2012b) examined the effects on lichens of raised temperatures and reduced amounts of rainfall, resembling impending climate scenarios that would harmfully influence the photosynthesis of sensitive lichen species in the arid southern African Succulent Karoo Biome. These authors concluded that climate change will cause lichen death. Maphangwa *et al*. (2012a) examined the interception and evaporation of fog, dew and water vapour by soils and lichens in a coastal desert of South Africa and investigated the chemical analysis. These authors found that atmospheric water vapour and fog plays a crucial role in driving lichen photosynthesis and distribution in a coastal desert. Maphangwa *et al*. (2014) tested the deadly photosynthetic temperature edges of lichens in a southern African arid region. It was found that only atypical conditions of lichen exposure in a hydrated state to extreme temperatures at midday with high levels of solar irradiances during summer could affect photosynthetic thresholds in sensitive lichen species (Maphangwa *et al*., 2014). Musil *et al*. (2010) examined the reaction of dwarf succulent plants, lichens and soils to experimental climate warming in an arid South African ecosystem and found that lichens are more sensitive indicators of climate change than other plants. Mukherjee *et al*. (2010) investigated the potential importance of lichens in enriching spider diversity and richness on Robben Island and reported that habitat structure and epiphytic lichen abundance may be reasons for the greater number of spiders on the island.

Lalley and Viles (2005, 2006) assessed the diversity and the vehicle track disturbances of soil-growing lichens in the fog of the Namib Desert (which extends from South Africa, Namibia, and into Angola) and found that global climate change can cause significant changes in the composition of lichen communities. Büdel *et al*. (2010) investigated the ecophysiology of biological soil crusts including lichens from the arid and semi-arid regions of South Western

[2] The website http://www.lichens.uni-hamburg.de/lichens/africa/south-africa_checklists_switch.htm was last visited on 22 October 2018. At present (September 2019), it is not available and it is not clear when it will be online again.

Africa. These authors found the biological soil crusts among the most diverse worldwide. Weber *et al*. (2010) used remote sensing data to map the biological soil crust in the succulent Karoo and found many biological soil crusts. Pfiz *et al*. (2010) assessed the fluctuating patterns of lichen growth form distributions in central Namib within the lichen field and reported wind erosion as an important functional parameter of the lichen species. Wirth *et al*. (2010) investigated the distribution of lichens along an ocean-inland gradient in the fog zone of the Central Namib, where 42 lichen species were reported.

Recently, lichen diversity and its changes due to land use and climate change have been investigated at the South African BIOTA biodiversity observatories (www.biota-africa.org) and lists of species together with ecological analyses, concerning mainly soil lichens and lichens on pebbles, were reported by Zedda and Rambold (2004, 2009) and Zedda *et al*. (2010a, 2010b, 2011a, 2011b, 2011c). These authors reported lichens as a valuable bioindicator for climate and soil change. Data on these collections (1 930 records) are now available through the GBIF online database (http://data.gbif.org/datasets/resource/12005/). Descriptive data on South African lichens were included within the BIOTA-projekt in the online LIAS light database, from which identification keys can be automatically generated (Rambold *et al*., 2001 onwards; 2014).

Trüe *et al*. (2012) determined mercury levels in epiphytic lichens and tree bark in the cities of Pretoria and Witbank. Tree bark had lower concentrations of mercury compared to lichens from both cities. Monna *et al*. (2006) differentiated the various sources of lead from epiphytic lichens in and around Johannesburg and it was found that leaded antiknock compounds added to gasoline were the main source of lead around Johannesburg. Forbes *et al*. (2009) used the lichen *Parmotrema austrosinense* (Zahlbr.) Hale as a bioindicator of air quality to determine the effects of the phasing out of leaded petrol and the simultaneous introduction of manganese anti-knock additives to fuel in South Africa. Specimens were collected from the Pretoria central business district (CBD), as well as from three sites to the east of Pretoria: the National Botanical Gardens, the CSIR campus and the suburb of Lynnwood. The highest concentrations of lead were found in the CBD.

Panichev and McCrindle (2004) assessed the levels of metals in topsoil, dust, leaves, grass, lichens and bark from the Kruger National Park utilising electro thermal atomic absorption techniques. Lichens and bark were found to be more sensitive to air pollution than grasses and trees leaves. Olowoyo *et al*. (2011) used *Parmelia sulcata* Taylor to evaluate the possible sources of trace elements in the Tshwane metropolis, with the objective of assessing the capacity of this lichen species to screen air contaminations in a polluted environment. It was observed that *P. sulcata* can be used as bioindicator of air pollution because higher concentrations were reported in the polluted sites. Panichev *et al*. (2019) used *Parmelia caperata* (current name: *Flavoparmelia caperata* (L.) Hale) as bioindicators for mercury in South African provinces. It was shown that *P. caperata* can be used to assess mercury and the highest concentration was found in lichens from Secunda (Mpumalanga province).

Despite these previous works, many regions of southern Africa remain underexplored in terms of lichens. Numerous taxa have not yet been described and taxonomical groups need to be reviewed. Identification keys are missing for several families and genera. More floristic and ecological investigations are necessary to better understand the influence of anthropogenic impacts on these sensitive organisms, which could be used more often as bioindicators in southern Africa (Crous *et al*., 2006; Maphangwa, 2010).

2.3 Ecological roles of lichens

Lichens support numerous ecosystem services, for example soil richness, nutrient cycling, and soil development, nitrogen and carbon fixing (De Bello *et al*., 2010; Zedda and Rambold, 2015). Additionally, they bolster the diversity of various organisms through the provision of nutrients, habitat, shelter, camouflage and nesting material, among others (Zedda and Rambold, 2015). Lichens are principal colonisers and on rock surfaces and they are involved in physical and chemical weathering of rocks, playing a leading role in soil formation and nutrient enrichment in an ecosystem (Nash, 2008; Zedda and Rambold, 2015; Giordani *et al*., 2016). The physical impacts are reflected by the mechanical disturbance of rocks brought about by hyphal infiltration, development and compression of lichen thallus, swelling activity of the natural and inorganic salts originating from lichen action (Chen *et al*., 2000). Chemical breakdown occurs when oxalic acids are secreted and react with mineral components of rocks to form various metal oxalates and acidic polysaccharides that dissolve the cementing material in sandstones releasing the quartz crystals (Nash, 2008). Organic matter produced by lichen thalli contributes to improving soil even in arid to semi-arid ecosystems (Chen *et al*., 2000; Zedda and Rambold, 2009; Maphangwa, 2010; Jackson, 2015). Lichen also promotes vegetation succession as the soil cover by terricolous lichens can influence vascular plant germination (Zedda and Rambold, 2015; Gypser *et al*., 2016).

Lichens accumulate and store a pool of nutrients through wet and dry atmospheric deposition (Pike, 1978; Loppi and Pirintsos, 2003; Nash, 2008; Maphangwa, 2010; Asplund and Wardle, 2017; Gupta *et al*., 2017). They do not have cuticles and stomata making them very effective at absorbing nutrients (Nieboer *et al.*, 1978; Asplund and Wardle, 2017). Fruticose lichens such as *Teloschistes capensis* (L.f.) Müll. Arg. are particularly effective at capturing both dew and fog, which often contain more nutrients than rain (Nash, 2008; Maphangwa *et al*., 2012a).

Some lichens play an important role in ecosystems by fixing atmospheric nitrogen (cyanolichens) and other elements into nutrient poor soils and by contributing to nutrient cycling (Nieboer *et al.*, 1978; Knops and Nash, 1996; Longton, 1997; Asplund and Wardle, 2017). The fixed nitrogen by lichen even facilitates the colonisation and growth of vascular plants (Asplund and Wardle, 2017). The captured nutrients reach other ecosystem components through leaching, litter fall, decomposition and consumption by animals or by bacterial incorporation (Pike, 1978; Asplund and Wardle, 2017). Lichens are also important carbon sinks and therefore play a role in mitigating atmospheric greenhouse effects (Maphangwa, 2010; Nash, 2008; Palmqvist *et al*., 2008; Ding *et al*., 2013; de Guevara *et al*., 2014).

Lichens are an important source of food for animals worldwide (Zedda and Rambold, 2015). Monkeys, snails, bacteria, protozoa, nematodes, reindeer, caribou and rodents consume lichens as source of food (Pekkarinen *et al*., 2015; Zhao *et al*., 2015; Asplund and Wardle, 2017). Monkeys prefer fruticose lichens such as *Usnea longissima* Ach. (current name: *Dolichousnea longissima* (Ach.) Articus) and *Bryoria* species (Grueter *et al*., 2012). In China, some monkeys such as *Rhinopithecus roxellana* Milne-Edwards eat lichens as their fundamental source of nourishment (Yiming, 2006; Liu *et al*., 2013). Sheep in Libyan deserts graze extensively on the lichen *Lecanora esculenta* (Pall.) Eversm. (current name: *Circinaria esculenta* (Pall.) Sohrabi) (Hale, 1983). *Cladonia rangiferina* (L.) Weber ex F.H. Wigg. are significant winter forage for caribou and reindeer in northern ecosystems (Seaward, 2008; Athukorala and Piercey-Normore, 2014).

Flying squirrels feed on fruticose lichens of the genus *Bryoria*. For example, Northern flying squirrels consume *Bryoria fremontii* (Tuck.) Brodo and D. Hawksw. (Hayward and Rosentreter, 1994; Seaward, 2008). Different groups of terrestrial invertebrates feed on lichens, in particular arthropods such as mites and bark lice (Mukherjee *et al*., 2010; Asplund and Wardle, 2017) and snails (Baur *et al*., 1995; Asplund and Wardle, 2017).

In addition, birds use lichen material to build their nests (Richardson and Young, 1977). *Ploceus olivaceiceps* Reichenow and *Carduelis spinus* (Linnaeus) construct their nests fully from *Usnea* species (Purvis, 2000). Hummingbirds in Colombia and in Arizona protect their nests with the foliose *Parmotrema reticulatum* (Taylor) M. Choisy (Hale, 1983). Bowerbirds use lichens for decorating their nests (Seaward, 2008). Rufous-bellied bush tyrant birds build their nest using *Unsea* lichens in Peru and western Bolivia (Kingwell and Londoño, 2015).

Some spiders on Robben Island (South Africa) are reportedly dependent on lichens as their primary habitat and the giant crab spider uses lichens of the genus *Usnea* (Mukherjee *et al*., 2010) for their nests. Lichens also give shelter to other animals, such as water bears, mites and lizards (Mukherjee *et al*., 2010). Some invertebrates (peppered moth) and vertebrates (amphibians) animals are able to camouflage themselves using lichens (Purvis, 2000; Sewards, 2008; Mukherjee *et al*., 2010).

Lichens are sensitive biological indicators of environmental change (details are provided in section 2.4). Human beings are at present the biggest threats for lichen, devastating their habitats worldwide through deforestation, agricultural practices, urbanisation, pollution and habitat degradation, fragmentation and overuse of natural resources and biological invasion (Gradstein, 2008; Hulme *et al*., 2008; Seaward, 2008; Ardelean *et al*., 2015; Zedda and Rambold, 2015; Boch *et al*., 2016; Zarabska-Bożejewicz and Kujawa, 2018).

2.4 Lichen as a bioindicator of human disturbances

Lichens have been acknowledged as indicators of environmental quality, especially air quality, since 1866 by Nylander (1866), as cited by Olowoyo *et al*. (2010), and have been used as such worldwide (Aptroot and van Herk, 2007; Dyer and Letourneau, 2007; Pinho *et al*., 2012; Li *et al*., 2013). The impact of different types of human disturbances on lichens and their role as bioindicators is discussed below.

2.4.1 Air pollution

Lichens are recognised as excellent indicators of environmental stress caused by various pollutants (Lupšina, 1992; Budka *et al*., 2004; Lisowska, 2011; Sett and Kundu, 2016; Giordani and Malaspina, 2017). Epiphytic lichens are, in particular, used for assessing air quality in urbanised and industrial areas (Budka *et al*., 2004; Giordani, 2007). They show differing sensitivities to air pollution (Branquinho *et al*., 1999), with sensitive species disappearing with even low levels of pollution (Hawksworth and Rose, 1970; Nash, 1976; Eversman, 1978). More tolerant species include *Candelaria concolor* (Dicks.) Arnold (tolerant to NO_x), *Hypogymnia physodes* (L.) Nyl. (fairly tolerant to SO_2), *Lecanora conizaeoides* Nyl. ex Cromb. (very tolerant to SO_2) and *Physcia tenella* (Scop.) DC. (tolerant to NO_x) (Conti and Cecchetti, 2001; Sujetovienė, 2010; Sett and Kundu, 2016; Manninen, 2018). Other species such as *Lobaria pulmonaria* (L.) Hoffm. or *Usnea* spp. are very sensitive to SO_2 (Conti and Cecchetti, 2001; Sett and Kundu, 2016). Isocrono *et al*. (2007) reported on changes in lichen diversity in Italy between 1960 and 1996 due to changes in environmental conditions especially pollution.

Apart from SO_2, a variety of other elements and chemical compounds in the atmosphere affect lichen development and spreading. These comprise nitrous oxides, fluoride, and other secondary pollutants formed through chemical reactions in the atmosphere, for example, ozone, peroxy-acetyl nitrate, sulphuric and nitric acids (Tiwari, 2008). Thus lichens have been used as indicators of a range of pollutants. The influence of different pollutants on lichens is discussed below.

Information on air pollution and pollution sources in South Africa and in Pretoria are reported in Chapter 1, section 1.3. Data on air pollutants and their concentration in Pretoria are reported in Chapter 4, section 4.4.1.

2.4.1.1 Pollution by sulphur dioxide (SO_2) and lichen diversity

Sulphur oxides, especially sulphur dioxide (**SO_2**), affect lichen communities (Hawksworth and Rose, 1970; Aarrestad and Aamlid, 1999; Pescott *et al*., 2015). SO_2 pollution is the primary cause of death of lichens in most urban and industrial areas (Gilbert, 1970; Nimis *et al*., 2002; Tiwari, 2008). The main human source of SO_2 pollution is due to burning of fossil fuels such as coal, oil and natural gas, i.e. from thermoelectric power plants, road traffic and heating systems. Additionally, smaller sources of SO_2 are released from industrial processes (Giordani *et al*., 2002; Tiwari, 2008). This kind of pollution was most serious in Europe and in North America until the nineties but has been declining in the last few decades. However, SO_2 remains a significant pollutant in developing countries such as China and India. Lichens respond to sulphur dioxide pollution with different sensitivities as confirmed by studies carried out throughout the world (Beekley and Hoffman, 1981; Aarrestad and Aamlid, 1999; Giordani *et al*., 2002; Wolseley, 2002; Nash, 2008). Nimis *et al*. (1996) and Giordani *et al*. (2002) reported that SO_2 was the main pollutant affecting lichen biodiversity in Italy for the period from 1996 to 1999. Pollution from vehicle exhausts and SO_2 clearly damaged the lichen *Ramalina menziesii* Taylor in Los Angeles (Riddell *et al*., 2008).

High levels of SO_2 pollution causes lichen death in most urban and industrial areas and the consequent reduction of lichen cover ("lichen deserts"). For instance, in England and Wales it was observed that at SO_2 levels of about 125 $\mu g/m^3$ most lichens disappeared from trees and only few very tolerant species such as *Lecanora conizaeoides* still occured (Aarrestad and Aamlid, 1999; Gilbert, 1970; Nimis *et al*., 2002; Tiwari, 2008). In northeastern South Dakota

(United State) photosynthesis and respiration of the lichens *Physconia grisea* (Lam.) Poelt and *Physcia stellaris* (L.) Nyl. decreased significantly after fumigation with 2.5 ppm of SO_2 (Beekley and Hoffman, 1981). After the decline in SO_2 since the beginning of this century, due to changes in legislation regarding pollution (Larsen *et al*., 2007), lichens have been recolonising urban and industrial areas across Europe (Kandler, 1987; Seaward and Letrouit-Galinou, 1991).

In London, fruticose and foliose lichens such as *E. prunastri, Ramalina farinacea* (L.) Ach. and *Parmelia caperata*, which are among the most sensitive to SO_2 pollution, are now found in central parks (Hawksworth and McManus, 1989). The reduction of SO_2 pollution in London has led in the last years to re-establishment of sensitive lichens such as *Ramalina farinacea* and *Parmelia caperata* (Hawksworth and McManus, 1989). Lisowska (2011) has also reported the same trend in Poland. In Finland, the reduction of SO_2 levels led to the decline of SO_2-resistent lichen species *L. conizaeoides* (Manninen, 2018). This was also noticed in other European countries and in North America (Beekley and Hoffman, 1981; Seaward and Letrouit-Galinou, 1991; Aarrestad and Aamlid, 1999; Wolseley, 2002; Nash, 2008). In Seoul (Korea), the SO_2 concentration declined from 0.094 ppm in the 1980s to 0.04 ppm post-1992 leading to the re-colonisation of lichens (Ahn *et al*., 2011). There is no longer a lichen desert in Rome (Italy) as a result of CO, NO_x and SO_2 pollution reduction (Munzi *et al*., 2007). In Sudbury (Canada), *Evernia mesomorpha* Nyl. and *Usnea hirta* (L.) Weber ex F.H. Wigg., known to be very sensitive to pollution, are now found in areas where they were not previously found as a result of the reduction in SO_2 pollution (Gunn *et al*., 1995).

2.4.1.2 Pollution by nitrogen compounds and lichen diversity

Nitrogen oxides (**NO_x, NO** and **NO_2**) are the main causes of atmospheric nitrogen deposition (Truscott *et al*., 2005). NO_x are among the most significant pollutants at present in urban areas worldwide (Davies *et al*., 2007; Ahn *et al*., 2011). They are produced by coal burning and by automobile emissions. NO_x resulting from domestic heating and traffic emissions are also responsible for changes in lichen diversity worldwide (Fuentes and Rowe, 1998; Purvis *et al*., 2001; Loppi and Corsini, 2003). NO_x have been reported to reduce chlorophyll concentrations in lichens, even at a concentration of only 4 ppm NO_2 (Nash, 1976; Davies *et al*., 2007; Tiwari, 2008). In the lichens *Lecanora chrysoleuca* (Sm.) Ach. (current name: *Rhizoplaca chrysoleuca* (Sm.) Zopf), *Usnea cavernosa* Tuck. and *Parmelia praesignis* Nyl., 4 ppm nitrogen dioxide resulted in significant chlorophyll reductions (Nash, 1976). NO_x was considered as the factor that prevented the growth of lichen *Parmelia saxatilis* (L.) Ach. next to busy roads (Batty *et al*., 2003) and caused poor health of *Parmelia sulcata* Taylor in London (Purvis *et al*., 2003). Large amounts of NO_x hinder photosynthesis of *Flavoparmelia caperata* (Tretiach *et al*., 2007). In the Netherlands and in other European countries, SO_2 reduction led to an increased abundance of nitrophilous lichens belonging to the Xanthorion association (van Dobben and ter Braak, 1998), which are tolerant of nitrogen pollution. An example of a more tolerant species is *Phaeophyscia orbicularis* (Neck.) Moberg recorded this species, for example, in an area with high peaks of NO_x in London (Larsen *et al*., 2007).

Lichens have been furthermore utilised to monitor ammonia deposition (**NH_3**) in rural areas (Fangmeier *et al*., 1994; van Dobben and ter Braak, 1998; Wolseley *et al*., 2006; Jovan and Carlberg, 2007; Sparrius, 2007; Nash, 2008; Paoli *et al*., 2014; Paoli *et al*., 2015a). Paoli *et al*. (2015a) used the lichens *Flavoparmelia caperata* and *Xanthoria parietina* (L.) Th. Fr., as indicators of ammonia concentration in the environment. These lichens were exposed to NH_3 for eight weeks and for *F. caperata,* both the photobiont and the mycobiont were affected, while in *X. parietina,* only the photobiont was altered. In *F. caperata* there was decrease in chlorophyll content, photosynthesis performance and alteration of the secondary metabolite usnic acid, whereas in *X. parietina* there was only alteration of photosynthesis. In the Netherlands, the decrease of ammonia in polluted areas was followed by a quick increase in nitrogen-sensitive species such as *Bryoria fuscescens* (Gyeln.) Brodo and D. Hawksw. and *E. prunastri* (Sparrius, 2007).

2.4.1.3 Pollution by particulate matter (PM_{10}, $PM_{2.5}$) and lichen diversity

Particulate matter (PM) is a combination of dense particles suspended in the air, which differ in size and originate from different anthropogenic sources (Degtjarenko, 2018). These particles can be both fine and coarse. Coarse PM ($PM_{10,}$ particulate matter 10 micrometers or less in diameter) is primary in nature and originates from point sources such as abrasion and combustion and crushing processes, soil disturbances, desiccation of marine aerosol, rock quarrying and limestone quarry road surfaces (Zaharopoulou *et al*., 1993; Grantz *et al*., 2003; Degtjarenko, 2018). Fine PM ($PM_{2.5,}$ particulate matter 2.5 micrometers or less in diameter) is secondary in nature and contains condensates of volatile organic compounds, volatilised metals and products of incomplete combustion (Grantz *et al*., 2003). PM is one of the most dangerous group of pollutants, exceeding the acceptable levels in many countries worldwide (Degtjarenko, 2018; Varela *et al*., 2018; Rola and Osyczka, 2019). The $PM_{2.5}$ concentration of more than 60 $\mu g.m^{-3}$ affect lichen abundance negatively in Chile (Varela *et al*., 2018).

Despite the increase in PMs in many urban areas worldwide, there are few studies investigating their effects on lichens (Varela *et al*., 2018). In Thessaloniki (Greece), limestone dust caused reduction in chlorophyll content of foliose lichen *Physcia adscendens* H. Olivier (Zaharopoulou *et al*., 1993). Lichen growth forms and reproductive strategies, as well as species richness and abundance have been used as bioindicators of PM in Chile and Estonia (Degtjarenko, 2018; Varela *et al*., 2018). Crustose lichens with sexual reproduction can be utilised as indicators of alkaline dust pollution, as they are more resistant to this kind of pollution than foliose narrow-lobed and fruticose species, due to their smaller surface of thalli exposed to the dust particles (Giordani *et al*., 2012; Degtjarenko, 2018; Varela *et al*., 2018). Lichen abundance, not only lichen presence, can be used as an ecological indicator of $PM_{2.5}$ concentrations in urban environments (Varela *et al*., 2018).

2.4.1.4 Pollution by heavy metals and lichens as biomonitors

Lichens are also used as bioindicators of heavy metals (Agnan *et al*., 2017), for instance **lead,** in different countries including Italy, South Africa, Sri Lanka and in the Antarctica (Conti and Cecchetti, 2001; Forbes *et al*., 2009; Gunathilaka *et al*., 2011; Zvěřina *et al*., 2018) and of **mercury** (Bargagli, 2016; Vasilevich and Vasilevich, 2018; Zvěřina *et al*., 2018). In Pretoria, the lead concentrations in lichen *Parmotrema austrosinense* (Zahlbr.) Hale found in the central business district (CBD) (181.1 ± 98.0 $\mu g.g^{-1}$) were significantly higher than in those growing outside of the CBD area (41.5 ± 36.4 $\mu g.g^{-1}$) (Forbes *et al*., 2009). There was a significant difference in accumulation of **mercury** by lichens of different genera in northwest of Russia (Vasilevich and Vasilevich, 2018). The average content was 113.4 ±22.6 µg/kg for the genus *Usnea* and 192.0±40.0 µg/kg for *Bryoria* (Vasilevich and Vasilevich, 2018).

Hypogymnia physodes was utilised as a bioindicator of mercury and methyl mercury in Slovenia (Lupšina *et al*., 1992) and *Usnea antarctica* Du Rietz in Antarctica (Zvěřina *et al*., 2014; 2018). The highest concentration of mercury (115-188 $\mu g.g^{-1}$) was found next to the smelting house in *H*. *physodes* whereas the lowest concentration was found at the control site in a mountain area (0.4 $\mu g.g^{-1}$) (Lupšina *et al*., 1992). López Berdonces *et al*. (2017) used the lichen genera *Ramalina* and *Xanthoria* as biomonitors of mercury in Spain. The highest concentration was found next to the emitting source of mercury (110 $ng.m^{-3}$) and the lowest far away from sources (20-40 $ng.m^{-3}$) (López Berdonces *et al*., 2017). Panichev *et al*. (2019) used *P*. *caperata* as a bioindicator for mercury concentration in South African provinces. The highest concentration was found in Secunda (Mpumalanga Province) which has many power stations and the lowest was recorded in Limpopo Province, which has fewer power stations (Panichev *et al*., 2019). This shows that lichens can be used as indicators of mercury.

Lichens were also used as indicators of nitrogen pollution and **cadmium** in California (Sierra Nevada) and Antarctica (Jovan and Carlberg, 2007; Zvěřina *et al*., 2018). The concentration of cadium was 0.04 mg.kg^{-1} for lichen *Usnea antarctica* Du Rietz in James Ross Island, Antarctica (Zvěřina *et al*., 2018). The lichen *Ramalina fastigiata* (Pers.) Ach. was used as a biomonitor of copper dust emissions from coal mines in Portugal and the highest concentration was found next to the mine (Branquinho *et al*., 1999).

Lichens have furthermore been used as biomonitors of **uranium** in many countries (Beckett *et al*., 1982; Garty, 2001; Di Lella *et al*., 2003; Loppi *et al*., 2003; Rosamilia *et al*., 2004; Golubev *et al*., 2005; Boryło *et al*., 2017). They accumulate and retain **uranium** for numerous decades even after the source is no longer in existence (Fahselt *et al*., 1995). Sources of uranium includes milling of uranium, mining, ammunition repository and yellow cake production (Beckett *et al*., 1982; Di Lella *et al*., 2003; Loppi *et al*., 2003; Jeran *et al*., 2005). The elemental contents of lichens decrease with increasing distance from the emission source as indicated by Beckett *et al*. (1982) in Ontario (Canada) for the lichen *Cladonia rangiferina* (L.) Weber ex F.H. Wigg. *Hypogymnia physodes* was also used as biomonitor of uranium in Russia by Golubev *et al*. (2005). These authors reported high concentrations of uranium (1.45 mg kg^{-1}) next to the contaminated site and low concentrations (0.106 mg.kg^{-1}) in the clean site. Boryło *et al*. (2017) reported the highest concentration of uranium next to the Orle settlements belonging to Sobieszewo Island in Poland. These authors reported the highest concentration of uranium in the crustose lichen *Lepraria incana* (L.) Ach., followed by the fruticose lichen *Evernia prunastri*, while the smallest concentration was observed in *Platismatia glauca* (L.) W.L. Culb. and C.F. Culb. Jeran *et al*. (2005) also observed the highest concentration of uranium of transplanted *Hypogymnia physodes* next to an emission source in Slovenia compared to in areas far away from this source.

In Yugoslavia, Di Lella *et al*. (2003) found the highest concentration of uranium in lichen *Physcia adscendens* (4.26 µg.g^{-1}) and *Phaeophyscia orbicularis* (2.15 µg.g^{-1}) followed by *Physcia biziana* (A. Massal.) Zahlbr. (1.44 µg.g^{-1}) and the lowest concentration in *Xanthoria parietina* (0.11 µg.g^{-1}). In the Balkan area, Loppi *et al*. (2003) evaluated the contribution of the conflict of 1999 to the environmental levels of uranium by means of lichens, but they did not find high concentrations related to depleted uranium. Lichen species such as *P. sulcata* and *P*. *saxatilis* were also used as biomonitors of depleted uranium dusts in Bosnia-Herzegovina (Rosamilia *et al*., 2004). Lichen are also used to monitor Perfluorinated contaminants (PFCs). Lichen species *Usnea aurantiaciparvula* A. Gerlach and P. Clerc was used as indicator of PFCs in Antarctic Peninsula – 60% of PFCs were detected in this lichen species (Alava *et al*., 2015).

2.4.1.5 Methods used to detect and monitor air pollution

Different methods are used to monitor pollution. Commonly used worldwide are scales and maps of air quality. In the past, scales had been based on the calculation of an Index of Atmospheric Purity (I.A.P.), which considered the number of lichen species and their frequency-abundance, sometimes also their degree of toxiphoby (Nimis *et al*., 2002). They evolved later in scales of air quality based on a calculation of Lichen Diversity Values (LDV) (Asta *et al*., 2002a; VDI-Richtlinien, 2005). A different sampling design may be more or less appropriate in different ecological situations (Leedy and Ormrod, 2016). The LDV of a sample unit is a measurable estimator of the environmental conditions in that unit. The initial phase in calculating the LDV of a sampling unit is to sum the frequencies of all lichen species found on selected trees at different orientations (N, S, W and E). Since considerable variances in lichen development might be normal on various sides of the trunks, the frequencies must be summed independently for every aspect (Asta *et al*., 2002a, b). The sums of lichen frequencies at each aspect of one tree were used to calculate mean values. Later, average values were calculated for all the trees within a sampling unit (plot). The LDV of an area was obtained by adding the average sum of LDVs resulting from all sampling units of that area (Asta *et al*., 2002a, b) (see Appendix 7).

During recent years, these guidelines have been further modified in European countries by considering the occurrence of eutrophic species as well as nitrogen-tolerant species which have become more frequent in urban areas because of increasing nitrogen pollution (Kirschbaum and Wirth, 2010). Different authors (i.e. Asta *et al*., 2002b) introduced scales of "naturality/alteration" based on LDVs and this method is also applied in the present study. There are several applications and further developments of such scales in Italy by Loppi *et al*. (2002), Giordani *et al*. (2002), Brunialti *et al*. (2008), Cocozza *et al*. (2016), Cecconi *et al*. (2019). The following publications were also consulted to design the monitoring approach for the Pretoria area: Larsen *et al*., 2007; Jovan, 2008; Paoli *et al*., 2015b. These authors indicated that all aspects of the trees must be monitored (N, S, E and W) and the trees must be straight and not damaged. Lichen diversity values are generally lower in urban areas than rural areas due to vehicular traffic (Loppi *et al*., 2002; Frati and Brunialti, 2006). For example, Giordani *et al*. (2002) found very high alteration (low lichen diversity value) in areas characterised by high pollution such as urban and industrial districts of the towns Genova and Savona in Italy. Isocrono *et al*. (2007) reported highest lichen diversity value in the areas characterised by lower vehicular traffic and industries in Turin (Italy). The lowest lichen diversity value was limited to the area characterised by industries and municipal rubbish dump (Isocrono *et al*., 2007) (see Appendix 7).

2.5 Lichens' response to global warming

Lichens are among the most sensitive organisms to global warming (Insarov and Insarova, 2002; Aptroot and Van Herk, 2007; Giordani *et al*., 2014; Root *et al*., 2014; Aptroot *et al*., 2015; Nascimbene and Marini, 2015; Zedda and Rambold, 2015; Allen and Lendemer, 2016; Bajpai *et al*., 2016; Nascimbene *et al*., 2016) due to their particular physiological and ecological characteristics (Matos *et al*., 2015). Specifically, epiphytic lichens are climate-sensitive and may give early warning signs of climate change (Nash and Olafsen, 1995; Insarov and Schroeter, 2002; Ellis *et al*., 2007; Nascimbene and Marini, 2015). Lichen thallus size and growth-form, secondary metabolites and photobiont type are lichen characters known to be related to climatic conditions and to respond to climate change. For instance, green-algae lichens show less vulnerability to climate change than cyanolichens (Rubio-Salcedo *et al*., 2017). Crustose lichens are more tolerant than foliose and fruticose species to increasing temperature in arid regions of the world as well as in temperate and Mediterranean ones (Maphangwa *et al*., 2014; Nascimbene and Marini, 2015).

Lichen growth is especially dependent on climate (temperature and precipitation), which means that a minor change in the climate may change the lichen community structure of a given site (Aptroot and Van Herk, 2007; Sancho *et al*., 2007). Global warming is the main cause of fast changes in the epiphytic lichen flora in some parts of Europe (Van Herk *et al*., 2002; Aptroot *et al*., 2015). Changes in temperature and humidity can severely affect the structure of epiphytic communities and may cause local extinction of several species in Spain (Aragón *et al*., 2012). Climate change poses a substantial risk especially to montane lichens and in cold environments, where future decline of lichens is expected (Allen and Lendemer, 2016). For instance, *Lobaria* species, which require temperate-humid conditions, are facing a high extinction risk in Italy because of climate change (Nascimbene *et al*., 2016). They are among the most vulnerable lichens in Europe (Nascimbene *et al*., 2013; Otalora *et al*., 2015). Søchting (2004) reported the first signs of global warming effects on lichen distribution in Denmark. Soil lichens are threatened by global change in natural alpine environments, especially on moss-dominated soils, where there is a decline of mosses and lichens and replacement by vascular plants (Bueno de Mesquita *et al*., 2017).

Soil lichens have declined in response to climate warming in lower-altitude sub-arctic and mid-arctic ecosystems as well (Cornelissen *et al*., 2001). Warming experiments resulted in the decrease of lichen cover in the tundra biome (Walker *et al*., 2006).

Climate change effects could lead to the extinction of rare and endemic terricolous lichen vegetation in the arid Succulent Karoo Biome and in the Desert Biome (Alexander Bay, Namib) in southern Africa (Zedda and Rambold, 2009; Maphangwa *et al*., 2012b; 2014). This will have possibly significant consequences for such biomes, which host a huge variety of lichen taxa with high endemism (Zedda and Rambold, 2009; Zedda *et al.* 2011b, 2011c).

Increased temperatures influence lichens negatively in two ways: through direct effects on metabolic rates, and by increasing evaporation in tropical lowlands (Zotz and Bader, 2009). Slight increments in temperature might make tropical lowlands not habitable to lichens (Zotz and Bader, 2009).

The diversity of epiphytic species is however increasing in other regions such as in the Netherlands because of the colonisation of trees by Mediterranean lichen species as a result of global warming in Western Europe (Aptroot and Van Herk, 2007). In Denmark and the Netherlands, growth rates of *F. caperata*, a drought resistant, warm-temperate lichen, have increased over the last 100 years (Van Herk and Aptroot, 1996, 2004; Van Herk *et al*., 2002). This appears to be linked to the increase of temperatures during the last 20 years (Van Herk and Aptroot, 1996, 2004; Søchting, 2004). Other warm-temperate species such as *Candelariella reflexa* (Nyl.) Lettau, *Lecidella flavosorediata* (Vězda) Hertel and Leuckert, *Parmelia borreri* Turner and *P. soredians* Nyl. have increased in frequency in the Netherlands as well, while species characteristics of colder environments have either declined or become extinct (Van Herk *et al*., 2002; Hauck, 2009). Species most rapidly increasing contain *Trentepohlia* as phycobiont, which is often associated with warm-humid environmental conditions (Aptroot and Van Herk, 2007) because of the increase of temperature.

P. borreri has also increased its frequency in the Netherlands (Spier and Van Herk, 1997) and *Physcia tribacioides* Nyl. and *Heterodermia japonica* (M. Satô) Swinscow and Krog (current name: *Heterodermia obscurata* (Nyl.) Trevis.) have been found here for the first time (Wolfskeel and Van Herk, 2000 as cited by Aptroot and Van Herk, 2007).

Climate change might also have positive effects on some Mediterranean species such as *Buellia cedricola* Werner (current name: *Diplotomma cedricola* (Werner) Etayo) and *Cladonia mediterranea* P.A. Duvign. and Abbayes, as well as in *Pyrrhospora lusitanica* (Räsänen) Hafellner, *Solenopsora holophaea* (Mont.) Samp. and *Waynea adscendens* V.J. Rico with a Mediterranean Atlantic distribution and preferring higher temperature and low precipitation (Rubio-Salcedo *et al*., 2017). This is because in the Mediterranean, temperature is expected to increase and precipitation to decrease in future (Rubio-Salcedo *et al*., 2017).

2.6 Other kinds of human-driven ecosystem disturbance on lichens

Forest disturbances and **deforestation** are major human activities affecting lichen diversity and composition on trees. This can lead to the disappearance of epiphytic lichens, especially the most sensitive ones (Seaward, 2008; Boch *et al*., 2016), such as foliose and pendulous forms as well as cyanolichens as their habitat is affected (Seaward, 2008). In the tropical montane rainforests, deforestation causes significant loss in the species diversity of epiphytic macrolichens and lichen total richness (Gradstein, 2008; Benítez *et al*., 2012, 2015). Lichens are also used as indicators of forest disturbance in Ethiopia, Uganda and Kenya (Yeshitela, 2008).

When land use is intense due to **agricultural practice or overgrazing**, lichen diversity is reportedly low as compared to areas with lower land use intensity (Gilbert, 1980; Ruoss, 1999; Giordani *et al*., 2010; Boch *et al*., 2016). Overgrazing by small stock is likely to be the responsible factor for the relative low species diversity in some rangeland areas of South Africa, where climatic conditions are potentially feasible for lichen growth (Zedda and Rambold, 2010a). Overgrazing by sheep and goats causes trampling impacts on lichen crusts (Weber *et al*., 2010; Zedda and Rambold, 2010a; Zedda *et al.,* 2011b). The future of soil-inhabiting fruticose lichens, which are dominant in arctic and sub-arctic landscapes, is uncertain due to **commercial exploitation of oil, minerals and timber** (Seaward, 2008).

Biological invasions by alien plant species are viewed as another main environmental problem (Osyczka, 2010; Hulme *et al*., 2008; Zedda *et al*., 2010c; Cogoni *et al*., 2011). In USA, invasive plants frequently occupied former biologically crusted interspaces (Belnap, 2001). Under such conditions, biological soil crusts including lichens have lower diversity and cover. In the Mediterranean areas, lichen and bryophyte covers on sand dunes along the coast decrease significantly or these organisms tend to vanish by an increasing cover of alien plants such as *Carpobrotus* spp. (Zedda *et al*., 2010c; Cogoni *et al*., 2011). The thick deposits of litter produced by some exotic trees rot slowly and may promote acidic and dry top soil conditions, which are not favourable for the growth of numerous bryophyte and lichen species (Babu and Kandasamy 1997; Zedda *et al*., 2010c).

Lichen diversity is also affected by **human trampling, off-road driving, fire and mining activities** (Zedda *et al*., 2010c; Cogoni *et al*., 2011) in different parts of the world. In Northern Sweden, it has been shown that trampling decreases species richness of lichens (Jägerbrand and Alatalo, 2017). In Namibia, open cast mining and extensive off-road driving affect lichen distribution (Lalley and Vleis, 2005). High fire intensities in savanna cause changes in woody vegetation composition and structure (Trollope and Trollope, 1997; Smit *et al*., 2010). It led, for instance, to the reduction of woody vegetation in Kruger National Park (Smit *et al*., 2010). This may affect also the diversity of epiphytic lichens in affected areas as the trees will be reduced or destroyed and lichens burnt. In the eastern Iberian Peninsula, fire was found to affect the diversity of lichens on holm oak trees (Garrido-Benavent *et al*., 2015). Previous lichen richness had still not recovered 20 years after fires (Garrido-Benavent *et al*., 2015). Fire appears also to disturb lichen growth in the Cape region of South Africa (Zedda and Rambold, 2010b).

2.7 Economic importance of lichens

Lichens are important natural resources which have been utilised for various purposes for a long time all over the world by people. They are used for medication, nourishment, grain, aroma, fuel, flavours, dyes and other purposes (Llano, 1948; Malhotra *et al*., 2007; Nash, 2008; Basile *et al*., 2015; Crawford, 2015; Vitalini *et al*., 2015; Zedda and Rambold, 2015; Xu *et al*., 2016; Devkota *et al*., 2017; Poulin, 2017).

In South Africa (Cape area), species of the family *Parmeliaceae* are used for the treatment of back torment and kidney trouble and against other diseases (Van Wyk *et al*., 2008; De Beer and Van Wyk, 2011). They are also used for anointing babies (Van Wyk *et al*., 2008; De Beer and Van Wyk, 2011). The Khoisan utilised lichens for treating general torments, wounds and bladder sicknesses (De Beer and Van Wyk, 2011). Additionally, they utilised them for sore throats and oral thrush in new-born children, abdominal pain and kidney sicknesses (Van Wyk and Gericke, 2000). The Xhosa people use the lichen *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale to treat syphilis eruptions, both known and suspected snakebites (Crawford, 2015). The Xhosas in the Eastern Cape also used lichen *Usnea filipendula* Stirt. for the treatment of mammary infections in cattle (Afolayan *et al*., 2002).

Considering the lack of comprehensive information on lichen diversity studies in urban environments with a considerable number of anthropogenic activities, there is a need to undertake studies on determining lichen diversity and their environmental relevance. As evidenced from a plethora of evidence on the potential applications of lichens in environmental/ air pollution bioindication, there is a need to explain the biodiversity of lichens in Pretoria in relation to possible use as indicator of pollution and climate change.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

This study was carried out in Pretoria and its metropolis (Figure 3.1A). Pretoria lies in the northern part of Gauteng Province (25°44′46 S; 28°11′1 E) and is located around 55 km north-northeast of Johannesburg in the north-east of South Africa. It is divided into three sections: west, east and north, with respect to the central business district. Pretoria is located inside the City of Tshwane Metropolitan Municipality. It is known as the ‘Jacaranda City’ (see Appendix 8) because of the large number of *Jacaranda* trees growing in its streets, parks and gardens (Henderson, 1990; Joseph, 2006; Coetzee *et al*., 2015). Pretoria is located in a transitional belt between the level of the Highveld towards the south and the lower-lying Bushveld towards the north.

The town has a population of around 2.9 million (Statistics South Africa, 2011). It has a dry, sunny climate, except for incidental late evening storms between October to April. Temperatures are usually fairly mild because of the city's high elevation (1,271 m a.s.l.) with the normal maximum daytime temperature of 21.5°C in January, dropping to a normal limit of 11°C in July (Olowoyo *et al*., 2010). Snow is very uncommon and the mean annual rainfall is 784 mm. June to August are the coldest months (Olowoyo *et al*., 2013).

All study sites fall within the Savannah biome with the exception of one located in the Grassland biome (see section 3.2. below). The Savannah biome, the most far-reaching biome in Africa, covers 32.8% of South Africa (399 600 km^2). Savannah occupies northern Gauteng with more isolated occurrences in the south of this province (Mucina and Rutherford, 2006). Much of the Savannah prospered during early geographical periods under hot, wet climatic conditions (Mucina and Rutherford, 2006). The Grassland biome covers a large part of central South Africa and has a high frequency of lightning flashes making the incidence of fire relatively high (Mucina and Rutherford, 2006).

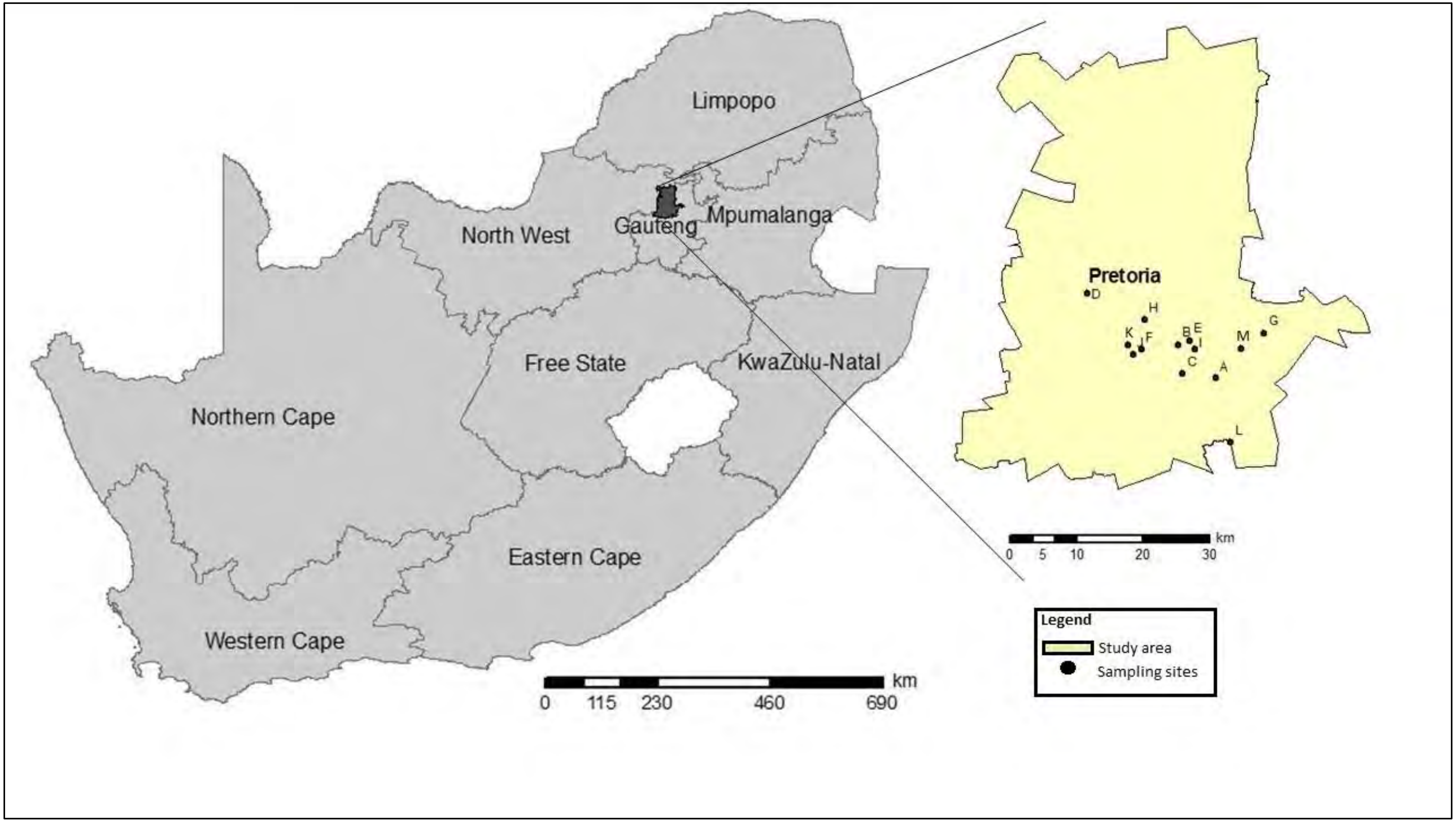

Figure 3.1A. Study area and sampling sites. Sampling sites are marked with alphabetical letters

3.2 Sampling sites

Sampling was carried out from 32 different sites. A first floristic survey was carried out at twelve sampling sites. Further sites were later selected for monitoring according to a stratified sampling method (see 3.7, 3.8). The first sites were identified with the help of a local map and field surveys were carried out in July 2016 to March 2018. The sites were selected so as to be representative of different kinds of land use activities in Pretoria, e.g. high traffic areas, residential areas, industrial areas (metal pressings), open-air museums and nature reserves. The study sites where lichens were collected for a first floristic survey are listed below and their distribution is shown on the map in Figure 3.1A. Some were visited again for lichen monitoring and further sites were identified for monitoring (see 3.7). Each visited site is defined by a progressive code (e.g. S01). When the same locality was visited several times, different codes were assigned to the different sampling/monitoring sites. Study sites are described in the sections that follow and section 3.7 covers the monitoring sites (see also Figure 3.1B).

3.2.1 (A) Waterkloof

This is a residential area situated in the East of Pretoria around 10 km from the city centre. The site falls within the Savannah biome. Sampling was done at Rigel Avenue (around 25°47"44 S; 28°14'36 E, elevation 1546 m a.s.l.) from three trees. There are many *Jacaranda mimosifolia* trees planted along the streets. Traffic is heavy only along the main avenues and roads, but lower compared to the city centre. Visited on 18 July 2016. Code **S07**.

3.2.2 (B) Pretoria central business district

This site is located in the city centre and falls within the Savannah biome. Collections were done at Nana Sita Street (around 25°74'50'' S; 28°19'25'' E, elevation 1354 m a.s.l.) from one tree. This is a high traffic area frequented by heavy trucks, buses, small cars and taxis. The street is lined with *J. mimosifolia* trees. Visited on 19 July 2016. Code **S016**.

3.2.3 (C) Groenkloof Nature Reserve

The nature reserve is located adjacent to Fountains Valley (Christina De Wit Ave), about 5 km south of the city centre. The reserve is about 600 ha in size and falls within the Savannah biome. In terms of vegetation, there is semi-open thicket but this is dominated by woody species such as *Acacia karroo, A. caffra, Commelina erecta* L. and *Drimia multisetosa* (Baker) Jessop (Marais, 2004). Collections were made in several spots (around 25°47'22'' S; 28°11'54'' E, elevation 1390 m a.s.l.) from seven trees. Visited on 19 July 2016. Code **S05**.

3.2.4 (D) Magalies

This is a mountainous area approximately 15 km NW of the city centre. It falls within the Savannah biome. The sampling site was situated along the Hornsnek Road (around 25°68’12'' S; 28°06’82'' E, elevation 1453 m a.s.l.) and collection was done from two trees. The area was characterised by the presence of *A. caffra* and other *Acacia* species and grasses. Visited on 19 July 2016. Code **S014**.

3.2.5 (E) Arcadia

This area is situated around the Union Buildings. It falls within the Savannah biome. The collecting site was located at Stanza Bopape Street from one tree (around 25°44’41'' S; 28°12’30'' E, elevation 1332 m a.s.l.) next to the Union Buildings. Traffic is very heavy. The street is lined up with many *J. mimosifolia* trees, on the bark of which citizens often pin notices and advertisements. Visited on 20 July 2016. Code **S010**.

3.2.6 (F and J) Pretoria West

This area is situated approximately 7-8 km west of the city centre and falls within the Savannah biome. Two sites were investigated here. The first was along Quagga Road (F) (around 25°45’24'' S; 28°08’32'' E, elevation 1340 m a.s.l.), near a coal-fired power plant belonging to the City of Tshwane, a road busy with cars and trucks and lined with old *J. mimosifolia* trees. Collection was done from three trees. Visited on 13 September 2017. Code **S030.**

The second site was in the Pretoria West industrial area at Staal Road (J) (around 25°45’50'' S; 28°07’52'' E, elevation 1360 m a.s.l.) from one tree, opposite the metal pressings. *J. mimosifolia* trees are found along the street. There are also other small industries and traffic is heavy. Visited on 04 February 2017. Code **S02**.

3.2.7 (G) Pionier Museum

This area is approximately 10 km east of the city centre and falls within the Savannah biome. The collecting site was situated at Keuning Drive (around 25°44’07'' S; 28°18’36'' E, elevation 1315 m a.s.l.) from seven trees. The vegetation here includes *A. karroo, A. caffra* and other indigenous trees. Visited on 14 November 2016. Code **S04**.

3.2.8 (H) Hercules

This is a residential area located north-west of the city centre. The site falls within the Savannah biome. Collections were made at Van Der Hoff Road (25°43’03'' S; 28°08’25'' E, elevation 1293 m a.s.l.), at a site dominated by *A. karroo* and *J. mimosifolia* trees. Car traffic is very heavy. Collection was done from four trees. There is also coal burning by households in winter. Visited on 18 November 2016. Code **S03**.

3.2.9 (I) Sunnyside

This area is situated less than 3 km from the city centre and falls within the Savannah biome. The collecting site was located in Jorissen Street (around 25°45’27'' S; 28°12’56'' E, elevation 1343 m a.s.l.). Collection was done from six trees. This is a busy street with heavy traffic, lined with *J. mimosifolia* trees. Visited on 03 February 2017. Code **S031**.

3.2.10 (K) Lotus Gardens

This residential area is situated approximately 10 km from the city centre and falls within the Savannah biome. The collecting site was located at WF Nkomo Street (around 25°75’90'' S; 28°08’68'' E, elevation 1389 m a.s.l.). Collection was done from four trees. The area has heavy traffic. Residents also burn coal for heating their homes during winter months. Vegetation found in this site includes planted *J. mimosifolia* and other woody trees, as well as different grasses. Visited on 04 February 2017. Code **S032**.

3.2.11 (L) Rietvlei Nature Reserve

This reserve is located along the R21 highway, 18 km SSE of the centre of Pretoria and 38 km north of OR Tambo International Airport. The area is approximately 3 800 ha and falls within the Grassland biome. Tree vegetation includes *Acacia caffra, A. decurrens* Willd.*, A. karroo, Euclea crispa* (Thunb.) Gürke. Lichen collections were made at one site close to Rietvlei Dam (around 25°53’47'' S; 28°15’50'' E, elevation 1529 m a.s.l.). Collection was done from five trees. Visited on 23 July and 17 February 2017. Code **S09**.

3.2.12 (M) SANBI

This is a Botanical Garden situated in the east of Pretoria around 10 km from the city centre along Cussonia Avenue. It falls within the Savannah biome (around 25°44’12'' S; 28°16’17'' E, elevation 1356 m a.s.l.). Collection was done from one tree. Car traffic is very low inside the garden but high in the areas surrounding the garden. Visited 08 April 2018. Code **S06**.

3.3 Phorophytes

All available epiphytic lichen species were collected from selected native and exotic trees. Sampling was focused on the following three tree species:

3.3.1 *Jacaranda mimosifolia* D.Don

This species is commonly known as *jacaranda* and belongs to the family *Bignoniaceae* (Walker, 1986; Henderson, 1990). *Jacaranda* is a fast-growing tree, 15 to 22 m tall, with a fairly dark green, rounded and spreading crown. The young bark is smooth and pale grey, but with age it becomes rougher and darker brownish-grey (Henderson, 1990). Jacaranda is indigenous to North-Western Argentina (Coetzee *et al*., 2015), where it is found mostly on river banks in warm temperate sub-humid conditions. The species is cultivated for ornamental reasons (particularly on streets which become carpeted with its purple flowers during October and November), shade and timber (Van Wyk and Van Wyk, 1997; Henderson, 2007). This beautiful tree, however, is invasive and presents potential risk for indigenous vegetation in the subtropical regions of the country, and specifically in Mpumalanga and Lowveld (Henderson, 1990). According to Department of Agriculture (1985), *Jacaranda* is an invader plant (Category 3 of CARA List) which may no longer be propagated or sold, but its existing plants do not need to be removed.

3.3.2 *Acacia karroo* Hayne (current name: *Vachellia karroo* (Hayne) Banfi and Glasso

This is a shrub to medium-sized tree, variable in shape but typically with a somewhat rounded spreading crown (Van Wyk and Van Wyk, 1997; Boon, 2010) and is usually single stemmed with pendulous branches. Its bark is dark, coarse, fissured, reddish in cracks when old, while young stems are red-brown. *A. karroo* is a heterogeneous species best split into a number of distinct entities (Van Wyk and Van Wyk, 1997; Boon, 2010). This is one of the most widespread trees in Africa occurring in many different habitats (Palgrave, 1977; Boon, 2010), in particular in bushveld, grassland and in coastal dune forest. It ranges from the South Western Cape northwards into Angola, Botswana, Namibia, Swaziland, Zambia and Zimbabwe (Pooley, 1993; O'Connor, 1995). The wood is used for building and furniture and the tree is valued for shade, shelter and firewood. It has many medicinal uses from poultices for wounds to gargles for sore throats (Palgrave, 1977; Pooley, 1993; Boon, 2010).

3.3.3 *Acacia caffra* (Thunb.) Willd. (current name: *Senegalia caffra* (Thunb.) P.J.H.Hurter and Mabb.)

This is a shrub to medium-sized deciduous tree up to 12 m in height, often with a twisted trunk and rather thin spreading somewhat rounded crown and drooping foliage. It is single or multi-stemmed (Palgrave, 1977; Pooley, 1993; Boon, 2010). Its bark is dark brown to black and rough, sometimes fissured and horizontally cracked forming squares. *A. caffra* is one of the least thorny *Acacia* species (Palgrave, 1977; Boon, 2010). It occurs in bushveld, grassland and coastal shrub, often also on rocky ridges up to 1700 m altitude. It is found in South Africa, Botswana, south Mozambique and Zimbabwe (Boon, 2010). Bark, leaves and roots of the plant are used for medicinal purposes (Palgrave, 1977; Van Wyk and Van Wyk, 1997). It can be easily distinguished from *A. karroo* because of its narrow, straight, brown seed pods, which are narrow, flat and crescent shaped in *A. karroo*.

These tree species were selected for the investigation as they are widespread across the town. *Jacaranda* trees are planted and widely distributed in Pretoria along most roads and avenues. *Acacia karroo* and *A. caffra* are part of the natural savannah vegetation and are widespread in urbanised areas where they are able to survive (Alexander Heunis; personal communication, 2016). These trees were simple to identify and were mostly easily accessible for sampling (Walker, 1986).

3.4 Floristic survey: Sampling of lichen species and identification methods

Different epiphytic lichen species were collected mainly from tree trunks with the aid of a knife as well as with a chisel and hammer for woodworking. The samples were placed in paper bags, on which sampling information was indicated (locality, coordinates, tree species, land use, date, and operators). Collected specimens were transported to the laboratory for preparation and identification.

After identification, the specimens were sealed in paper envelopes and labels were prepared with the taxon name, the collection site, elevation and coordinates, the collector's name, the date of collection, the date of identification, and the name of the identifying person. Identification was made using the LIASLight online interactive identification keys (http://www.lias.net) and the following publications: Swinscow and Krog (1988), Brodo *et al*. (2001), Nash *et al*. (2002, 2004) and Sipman (2003). Further publications with identification keys were used for given genera or families, i.e. for the family *Physciaceae* (Moberg, 2004), for the genera *Chrysothrix* (Elix and Kantvilas, 2007; Elix 2009) and *Rinodina* (Matzer and Mayrhofer, 1996; Mayrhofer *et al*., 2007, 2014).

Taxonomical nomenclature follows the databases:

- "LIASnames" (www.lias.net)
- "Index Fungorum" (www.indexfungorum.org/) and
- "Species Fungorum" (http://www.speciesfungorum.org/Names/Names.asp).

Identification was performed at the University of South Africa (UNISA), the Botanical Garden and Botanical Museum Berlin and in Bonn (Germany) in 2016 and 2017. The identification of some specimens of *Culbersonia nubila* was confirmed by DNA analsyis (Aptroot *et al*., 2019).

Some specimens were compared with herbarium specimens stored at the South African National Biodiversity Institute (SANBI) in Pretoria (South Africa) (February 2017) and at the Botanical Garden and Botanical Museum Berlin (Germany) (in March-April 2017).

Lichen traits were observed with a binocular microscope capable of magnification up to 40×. Microscopic features (thallus, apothecia and asci anatomy or spores of some species) were observed by cutting thin vertical slices of lichen thalli or of fruiting bodies with a razor blade. Sections were placed in a drop of water or on potassium hydroxide on a microscope slide and examined with a microscope having 10×, 40× and 100× objectives (the last one with oil immersion), following the methodology described by Purvis *et al.* (1992) and Nash *et al*. (2002).

Reagent tests of thalli were performed during microscopic examination to detect the presence of lichen secondary metabolites through colour reaction of thalli or fruiting bodies. The following reagents were used: pure bleach (sodium hypochlorite solution) (C), 10% potassium hydroxide solution (K), paraphenylenediamine (Pd), and Lugol's iodine solution for studying asci. Pictures of some of the species were taken with an Olympus DP72 digital color camera for microscopes, with 2×, 7× and 10× magnification. The Olympus SZX7 stereomicroscope and an Olympus BX50 compound microscope with interference contrast, connected to a Nikon Coolpix digital camera, were also used to identify some species.

Thin Layer Chromatography (TLC) was carried out on some of the collected lichens, following the methodology described by Orange *et al*. (2001). TLC is important for the identification of lichens. Thin vertical slices of dry lichen material were cut with a razor blade and placed in numbered plastic phials into which cold acetone was added to extract lichen substances. A glass capillary tube was used to transfer the acetone extracts from each phial to corresponding numbered points on the TLC plates. Three applications per spot were used on three different plates. Acetone extracts from voucher specimens of *Evernia divaricata* (L.) Ach. and *Cladonia impexa* Harm. were used as references for usnic, divaricatic, perlatolic acids and other lichen substances. The prepared plates from each application were placed into three developing chambers each containing a different solvent (A, B and C). The solvents used were toluene-dioxan-acetic acid (A), hexane-diethyl ether-formic acid (B) and toluene-acetic acid (C) (see Appendix 9A, B and C). The plates were removed from the developing chambers when the solvent reached the terminating front line and dried with a hairdryer.

Water from the tap was brushed over the three plates to mark fatty acids. A 10% solution of sulphuric acid was also brushed over the three plates to mark more spots. All spots were marked with a dotted outline. The plates were subsequently transferred to a pre-heated oven at 110ºC for a few minutes for colour development. The plates were then allowed to cool before being transferred to the lab to check the spots under UV light. The coloured spots on the dried plates were examined under UV-B (254 nm) and UV-A (365 nm) light. All spots illuminated under UV light were marked by circling spots with an unbroken outline and also with the first letter of the name of the seen colour. The occurring secondary metabolites were finally identified on the basis of their retention factor and colour of spots.

Voucher specimens have been deposited in the Horticulture Centre Herbarium of the University of South Africa. Duplicates of the specimens will also be deposited in the Herbarium of Pretoria (PRE) at the South African National Biodiversity Institute (SANBI). A list of the identified species was reported in a Microsoft Excel table. Information on species (description, ecology, distribution worldwide, in Africa and in Southern Africa, was gathered from the literature and reported in the same table or in a text file. Graphs were elaborated using Microsoft Excel for the frequency of occurrence of the different lichen species, world distribution and diversity of species occurrence. An identification key, based on the collected lichen descriptions, was prepared with the aim of facilitating monitoring and the identification of species in the field for this study, but also for future investigations. Descriptions and the key are going to be published in *Bothalia* (Maphangwa *et al*., in preparation).

3.5 Molecular analyses of specimens of the genus *Culbersonia*

To detect the phylogenetic position of *Culbersonia nubila* (Moberg) Essl., which had not been studied before, molecular analyses were carried out. Analyses consisted of DNA extraction, amplification and sequencing according to a methodology described in detail by Aptroot *et al*. (2019). Total DNA was extracted from dry specimens employing a modified protocol based on Murray and Thompson (1980). PCR amplification was performed with the primers ITS1F and ITS4 (White *et al*., 1990; Gardes and Bruns, 1993) for the rDNA internal transcribed spacer 1, 5.8S and internal transcribed spacer 2 (collectively referred to as ITS), and LR0R and LR5 (Vilgalys and Hester, 1990; Cubeta *et al*., 1991) were used to amplify the 28S rDNA of the nuclear ribosomal repeat. PCR reactions were performed with a programme consisting of a hot start at 95°C for 5 min, followed by 35 cycles at 94°C, 54°C and 72°C (45, 30 and 45 s respectively) with a final 72°C step for 10 minutes. PCR products were checked in 1% agarose gels and positive reactions were sequenced with one of the PCR primers. BLAST (Altschul *et al*., 1997) of 5.8S-ITS2 and 28S rDNA sequences was used to select the most closely related taxa; 5.8S-ITS2 and 28S rDNA were the only regions amplified from the samples. Sequences were first aligned in MEGA 5.0 (Tamura *et al*., 2011) with the ClustalW application and then corrected manually. Ambiguous regions were not removed from the alignment. ITS1 was excluded because insertions/deletions make alignment difficult.

3.6 Lichen monitoring: Sampling design

Lichen spatial monitoring was carried out following the European guidelines for mapping lichen diversity as an indicator of environmental stress (Asta *et al*., 2002b; Van Haluwyn and Van Herk, 2002; VDI-Richtlinien, 2005; EN 16413, 2014; Giordani and Brunialti, 2015) described in Chapter Two, section 2.4.1.5. The following publications were also consulted to design the monitoring approach for the Pretoria area: Saipunkaew *et al*., 2005, 2007; Geiser and Neitlich, 2007; Larsen *et al*., 2007; Jovan, 2008; Poličnik *et al*., 2008; Samsudi *et al*., 2012; Mulligan, 2009; Paoli *et al*., 2015b. The European sampling design recommends the use of a grid for the selection of sampling units. Grid density and the number of sampling units can be variable for different geographical scales and type of study. It depends also on the presence of perturbation. Grid density can vary from 0.25 km x 0.25 km to 12 km x 12 km. Sampling tactics, concerning the size of the sampling units, the quantity of trees to be sampled and their selection within the sampling units, is also indicated in the European guidelines (Asta *et al*., 2002a, b). The quantity of trees per sampling unit relies on its size, on the within-unit data variability and on the accessibility of appropriate trees in small areas. The recommended minimum number of trees to be investigated for sampling units of 0.25 km x 0.25 km is 3-4, in larger units of 1 km x 1 km, 6-12 trees (EN 16413, 2014) (see Appendix 7).

This design of the present study had to be slightly modified and adapted to different South African environmental conditions, especially concerning the selection of suitable trees at monitored sites. For this investigation a “stratified random sampling” design was preferred to a random/raster (grid) method for selecting of sampling units, as most sites were heterogeneous and characterised by different land use types. This design consists of separating habitats into non-overlapping strata and choosing independent simple random sampling from each of these strata (Manly, 2001; Leedy and Ormrod, 2016). Moreover, some parts of the town are not easily accessible (i.e. private areas, unsafe townships) to allow a faster investigation.

Within this study, smaller sampling units were chosen (100 m × 100 m) and here at least two trees were sampled. In each area with different ecological and land use conditions, 2 to 4 sampling units were selected, according to the area’s size. These are shown in Figures 3.2 and 3.3. At least two trees were chosen randomly and monitored per plot (or sampling unit). In many cases, there were not enough trees or these where damaged, so only two trees could be considered. The monitoring was exclusively spatial monitoring carried out in 2017 and 2018. It was not repeated during different periods at the same site. Surveys were carried out as reported under Chapter 3.8.

3.7 Lichen monitoring: Monitored sites

Most of the sites where lichens had been collected for a floristic survey, were revisited for carrying out monitoring of lichen diversity. All these sites are already described in section 3.2 and thus only information relative to monitoring is reported below. These areas were:

- Pretoria central business district. Monitoring was conducted on 20 May 2018 from six trees. Code **S016**.
- SANBI. Monitoring was conducted on 8 April 2018 from five trees. Code **S06**.
- Pretoria West. Three sites were monitored here. The first site was situated around industrial area at Staal Road. Monitoring was conducted on 26 August 2017 from two trees. Code **S02**. Second site was at WF Nkomo Street (around 25°45’03'' S; 28°07’29'' E, elevation 1344 m a.s.l). There is heavy traffic throughout the area, which is situated 5 km west of the city centre. Monitoring was conducted on 26 August 2017 from seven trees. Code **S01**. The third site was Es'kia Mphahlele Drive, which is 2 km from city centre (around 25°45’03'' S; 28°07’29'' E, elevation 1344 m a.s.l). There is heavy traffic all around the area. Trees were scattered and not straight. Citizens also pin notices and advertisements on trees. Visited on 22 July 2018. Code **S026**. Monitoring was conducted from five trees.
- Waterkloof. Monitoring was conducted on 24 April 2018 (around 25°48’24'' S; 28°14’53'' E, elevation 1535 m a.s.l.) from four trees. Code **S07**.
- Groenkloof Nature Reserve. Monitoring was conducted on 21 March 2018 from nine trees. Code **S05**.
- Magalies. Monitoring was conducted on the 19 May 2018 from four trees. Code **S014**.
- Arcadia. Monitoring was conducted on (around 25°44’24'' S; 28°13’02'' E, elevation 1393 m a.s.l.) on 13 May 2018 from six trees. Code **S010**.
- Pionier Museum. Monitoring was conducted on 18 March 2018 from six trees. Code **S04**.
- Hercules. Monitoring was conducted on 27 August 2017 from three trees. Code **S03**.
- Rietvlei Nature Reserve, Monitoring was conducted on 28 April 2018 from four trees. Code **S09**.

The following sites were investigated additionally according to the monitoring approach described in sections 3.6 and 3.8.

3.7.1 Muckleneuk

This is a residential area situated about 3 km from the city centre. Monitoring was conducted along Celliers Street (around 25°45’42'' S; 28°12’11'' E, elevation 1358 m a.s.l.) from four trees. The area is lined with *J. mimosifolia* trees that are big and mostly not straight. Termite mounds also disturb trees. Visited on 24 April 2018. Code **S08**.

3.7.2 Queenswood

This is a residential area situated in the north-east of Pretoria around 9 km from the city centre. Monitoring was done at CR Swart Drive (around 25°42’52'' S; 28°15’28'' E, elevation 1300 m a.s.l.) from five trees. The area has many *J. mimosifolia* trees. Traffic volume is very high. Some trees have termite mounds. Visited on 13 May 2018. Code **S011**.

3.7.3 Akasia

This is a residential area situated in the north-west of Pretoria around 11 km from the city centre. Monitoring was done at Brits Road (around 25°40’36'' S; 28°06’58'' E, elevation 1304 m a.s.l.) from nine trees. The area has high traffic and is characterised by the presence of many *Acacia* trees, which are mostly multi-stemmed and not straight. Some have recently been cut. Visited on 18 May 2018. Code **S012**.

3.7.4 Mayville

This is a park situated in the north-east of Pretoria, 9 km from city centre along Es'kia Mphahlele Drive (around 25°41’49'' S; 28°11’06'' E, elevation 1233 m a.s.l.) from five trees. It has many very old and not straight *Acacia* trees, some of which have been recently cut. Traffic by vehicles is very high. Visited on 18 May 2018. Code **S013**.

3.7.5 Hermanstad

This is an industrial area (cement industry) situated around 7 km north-west from the city centre. The survey of lichen diversity was done at Moot Street (around 25°42’59'' S; 28°09’58'' E, elevation 1262 m a.s.l.) from four trees. The area has several *Acacia* trees, but many are either very small in diameter or have been damaged by termite mounds. Furthermore, people often fell the trees. Traffic is very high. Visited on 20 May 2018. Code **S015**.

3.7.6 Kameeldrift

This is an open area situated in the east of Pretoria around 20 km from the city centre. Monitoring was done along Sefako Makgatho Drive (around 25°41’06'' S; 28°17’00'' E, elevation 1262 m a.s.l.) from four trees. This is a rural area with high vehicle traffic. The area has numerous *Acacias* and is used for church services. Not all trees are suitable for monitoring as some are very young while others are not straight enough. Visited on 20 May 2018. Code **S017**.

3.7.7 Saulsville

This is a residential area situated in the west of Pretoria around 16 km from the city centre. Monitoring was carried out along the Masopha Street (around 25°45’48'' S; 28°03’27'' E, elevation 1389 m a.s.l.) from four trees. The area is rich in *Acacia* trees but some have termite mounds and others are not straight. It is used by people for church services and meetings. This is a rural area with high traffic. Visited on 10 June 2018. Code **S018**.

3.7.8 Philip Nel Park

This site is in a residential area in the north-west of Pretoria around 6 km from the city centre. Monitoring was carried out along the Transoranje Road (around 25°44’22'' S; 28°08’08'' E, elevation 1329 m a.s.l.) from five trees. The road is lined with *J. mimosifolia* trees, most of which have termite mounds and/or large trunks. People often damage tree trunks by pinning notices and advertisements onto them. Traffic is very high. Visited on 10 June 2018. Code **S019**.

3.7.9 Brooklyn

This is a residential area situated in the east of Pretoria around 8 km from the city centre. Monitoring was done along Roper Street (around 25°45’26'' S; 28°13’52'' E, elevation 1371 m a.s.l.) from eleven trees. The area has both *Jacaranda* and *Acacia* trees, but they are often disturbed by people pinning notices and advertisements onto their trunks which are also affected by termite mounds. Some *Acacia* trees are not straight. Traffic levels are high. Visited on 23 June 2018. Code **S020**.

3.7.10 Hatfield

This area is next to a shopping centre situated east of Pretoria around 8 km from the city centre. Monitoring was done at two sites. The first was along the Duxbury Road (around 25°45’21'' S; 28°14’23'' E, elevation 1369 m a.s.l.) from five trees. Visited on 23 June 2018. Code **S021**.

The second site was along Pretorius Street (around 25°44’39'' S; 28°14’32'' E, elevation 1356 m a.s.l.) from four trees. Visited on 7 July 2018. Both areas have *Jacaranda* trees, on which people often pin notices and advertisements. Some trees have large trunks; on others termite mounds are evident. Traffic levels are very high. Visited on 23 June 2018. Code **S022**.

3.7.11. Gezina

This is a residential area situated in the north-east of Pretoria around 6 km from the city centre. Monitoring was carried out along Rose Street (around 25°43’53'' S; 28°12’24'' E, elevation 1309 m a.s.l.) from four trees. The area has many *Jacaranda* trees, but several were not suitable for monitoring being too big, not straight or with termite mounds. Traffic levels are not very high. Visited on 7 July 2018. Code **S023**.

3.7.12 Voortrekker Monument Nature Reserve

This nature reserve is in the south of Pretoria around 7.8 km from city centre. The reserve is located along Eeufees Road and there are numerous *Acacia* trees. Monitoring surveys were carried out on sixteen trees (around 25°46’36'' S; 28°10’25'' E, elevation 1470 m a.s.l.) although some were either not old enough (low trunk diameter) or not straight. Some lichens had been eaten or damaged by wild animals that use acacias to scratch their bodies. Traffic levels inside the reserve are low. Visited on 8 July 2018. Code **S024**.

3.7.13 Suiderberg

This is a residential area situated in the north-east of Pretoria around 13 km from the city centre. Monitoring was done along Sarel Avenue (around 25°42’03'' S; 28°08’39'' E, elevation 1256 m a.s.l.) from four trees. Different *Acacias* are evident though some had termite mounds or were not straight. Traffic levels are not very high. The area is used to dump waste. Visited on 21 July 2018. Code **S025**.

3.7.14 The Willows 340-Jr

This is a residential area situated in the north-east of Pretoria around 23 km from the city centre. Monitoring was done along Solomon Mahlangu Drive (around 25°45’22'' S; 28°21’53'' E, elevation 1317 m a.s.l.) from four trees. The area has some *Acacias*, most of which are too old or not straight enough for monitoring. Traffic levels are very high. Visited on 18 August 2018. Code **S027**.

3.7.15 Mamelodi

This is a residential area situated in the north-east of Pretoria around 30 km from the city centre. Monitoring was done along Tsamaya Avenue (around 25°43'05" S; 28°20'48" E, elevation 1302 m a.s.l.), using *Jacaranda* trees, many of which have termite mounds and/or are damaged by pinned notices and advertisements. Traffic is very high. Visited on 18 July 2018. Monitoring was conducted from four trees. Code **S028**.

3.7.16 Koedoespoort 456-jr

This is a park situated in the east of Pretoria around 10 km from the city centre. Monitoring was done along the N4 Highway (around 25°44'34" S; 28°14'59" E, elevation 1335 m a.s.l.) from twelve trees. The area has many different *Acacias* trees, which are mostly very old and not straight or too small in diameter for monitoring. Traffic levels are very high. Visited on 19 August 2018. Code **S029**.

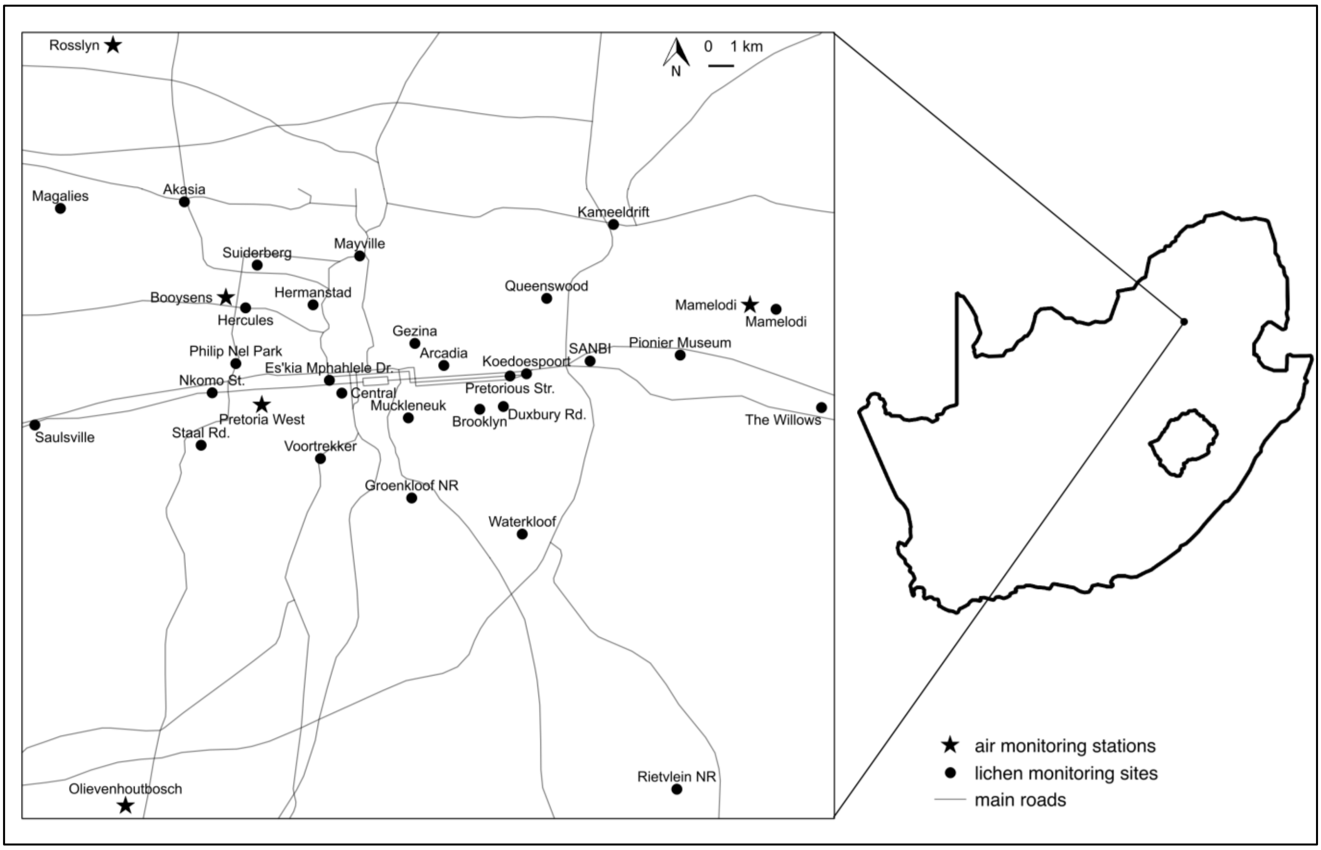

Figure 3.1B. The town of Pretoria showing distribution of the lichen monitoring sites

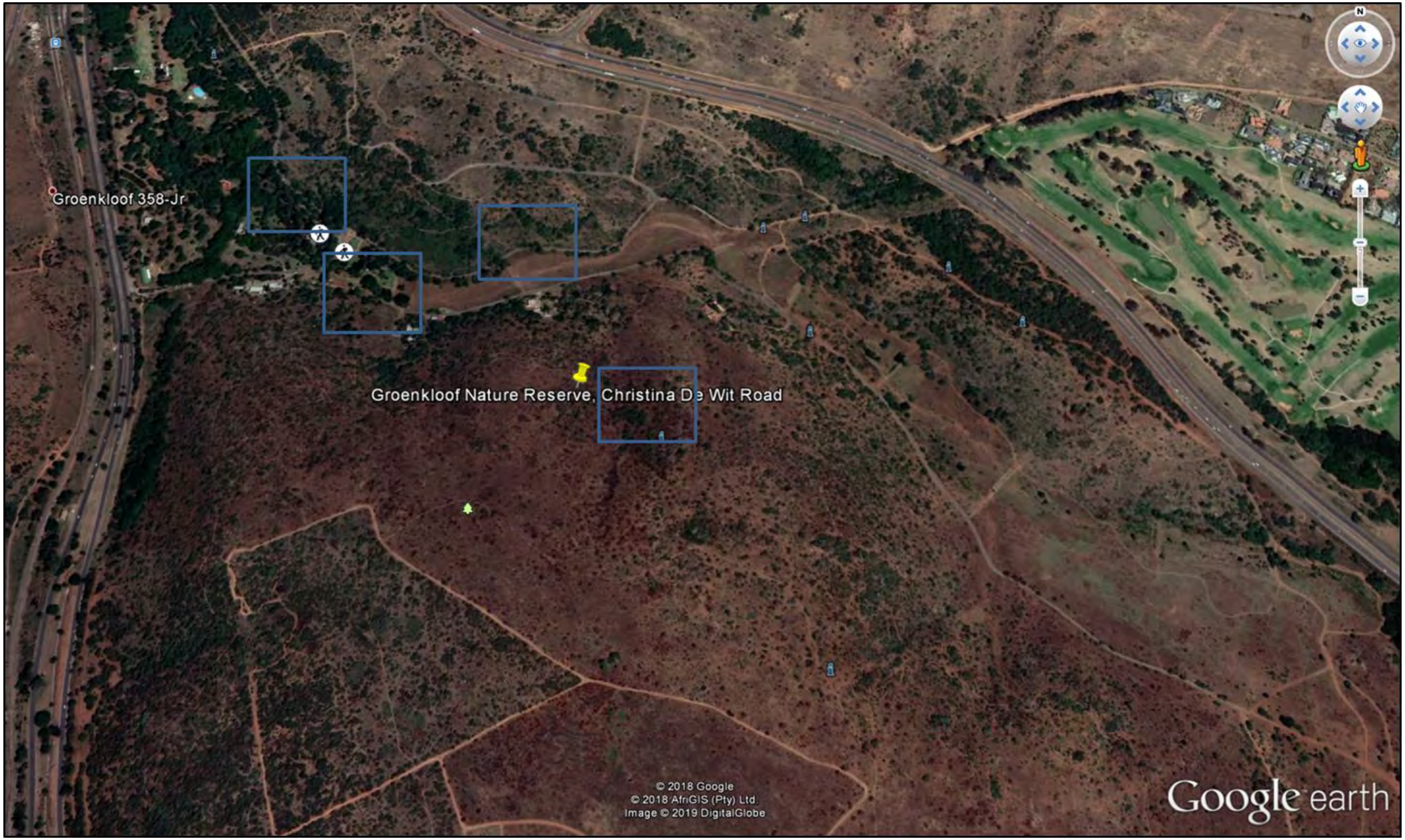

Figure 3.2. Groenkloof Nature Reserve with sampling points (Google Earth, 2019)

Figure 3.3. Voortrekker Nature Reserve with sampling points (Google Earth, 2019)

Figure 3.4. Monitoring grid attached to tree *J. mimosifolia*

3.8 Lichen monitoring: Survey of Lichen Diversity

The same tree species (*Jacaranda mimosifolia, Acacia karroo* and *A. caffra*) used for floristic surveys were used for the monitoring survey as well within the sampling units. Trees of the above mentioned species were selected according to the criteria suggested by the European guidelines (Asta *et al*., 2002a, 2002b). These include: free-standing trees, whose trunks must have an inclination lower than 10° from the vertical position, must receive direct solar radiation for at least part of the day and have a trunk circumference not less than 40 cm and not larger than 150 cm (Asta *et al*., 2002a, 2002b; Minganti *et al*., 2003; Brunialti *et al*., 2008). Trees of the same size must be used within a survey for monitoring. Injured trees are not appropriate for survey purposes and they were therefore not considered during this monitoring work. Trees evidently affected by actions such as liming, removal of the bark or of the lichens by humans or by grazing animals are additionally not appropriate and were excluded from survey as well as trees with termite nests (Asta *et al*., 2002a,b; Minganti *et al*., 2003). If it was not possible to place at least three ladders of the grid onto one tree, the tree was not surveyed. Parts of the tree with greater than 25% cover of bryophytes were not used (Asta *et al*., 2002a, 2002b).

A sampling grid composed of four ladders each with five quadrats sized 10 cm × 10 cm (Figure 3.4), was appended vertically to the trunk so that the lower edge of the ladder was 1 m above the highest point of the ground (Asta *et al*., 2002a, 2002b; Cristofolini *et al*., 2014). The four ladders of the sampling grid were placed to correspond to the four aspects (NSEW) of the tree trunk. The examined trunks were accurately and durably marked with numbers for long-term monitoring purposes with permanent marker (Asta *et al*., 2002a,2002b; Cristofolini *et al*., 2014) see Appendix 7.

All lichen species present within each quadrat portion were recorded using a survey form (see Appendix 10) together with their frequency occurrence in the five quadrats of each ladder. Even if more individuals of the same species occurred in one quadrat (10 cm × 10 cm square), only the frequency value “1” was given to that species for each square. For this reason, the value 5 can be the maximal value for each species in one 10 cm × 50 cm ladder. Lichens within the quadrat segments were not collected, but identified in the field. If there was a new species not previously collected during floristic investigation, a specimen was collected near the sampling grid but not inside the quadrats. This was done to allow future monitoring on the same plots. All the information regarding the areas where the trees were assessed was recorded on the survey form and later collected in Excel tables (Asta *et al*., 2002a, 2002b).

3.9 Calculation of the Lichen Diversity Value (LDV) for trees and sites and further data elaborations

For each tree, four totals of frequencies can be recorded at four different aspects (N, S, W and E) (Asta *et al*., 2002a). The sums of lichen frequencies at each aspect of one tree were used to calculate mean values. Later, average values were calculated for all the trees within a sampling unit (plot). The Lichen Diversity Value (LDV) of an area was obtained by adding the average sum of LDVs resulting from all sampling units of that area.

Lichen diversity values were related to the type of land use (categories), to tree species and to climatic and pollution data. Land use was categorised into two main classes. The first is "industrial areas and busy roads", which includes industrial, urban and rural areas and roads with high vehicle traffic, all characterised by higher levels of human disturbance. Here the felling of trees is common. The second land use category was "parks and nature reserves", which includes savannahs with low disturbance located in nature reserves, private gardens and more natural areas, with lower levels of car traffic, pollution levels and other kinds of man-made disturbance.

Seven pollutants were considered for elaborations, namely CO (ppb) measured with Carbon monoxide analyser, NO (ppb) measured with NO_x analyser, NO_2 (ppb) measured with NO_x analyser, NO_x (ppb) measured with No_x analyser, O_3 ($\mu g/m^3$) measured with Ozone analyser, PM_{10} measured with Particulate matter analysers and SO_2 (ppb) was measured with Sulphur dioxide analysers. Climatic variables considered were pressure PRES (kPa) measured with Atmospheric pressure Sensor, RAIN (mm) measured with Rain gauge and solar radiation (SOL) (W/m^2) was measured with Solar radiation Sensor. These information was obtained from the South African Air Quality Information System (SAAQIS). Correlation analyses with these parameters were tested. A naturality/alteration interpretative scale was developed for the first time for a town in South Africa. This was based on the percentile deviations from the maximum lichen diversity observed in the study area.

The influence of land use and tree species on LDV and on lichen species was studied by statistical analyses, carried out with the freeware software R (R Core Team 2017) and Quantum GIS 2.18.22. The following analyses were applied:

- ANOVA and Kruskal Wallis test, Wilcoxon test for paired data.
- Principal Component Analysis (PCA), used as explorative unsupervised multivariate analysis to study the relationships among the response variables (lichen diversity, lichen species) and the predictive variables (i.e., climate, pollution, photophyte, land use).

- A multiple factor analysis (MFA), on the whole dataset in order to test correlation between the same variable mentioned above.
- Generalised Linear Models, applied to fit the relationship between the same set of environmental predictors and the response variables.

This dataset was interpolated (Inverse Distance Weighting) to assign missing values to each of the 29 lichen monitoring stations. Results are presented graphically and in table format in the next chapter.

CHAPTER 4: RESULTS

4.1 List of recorded species

This study recorded 25 lichen taxa on the three selected trees. Altogether, 362 specimens of these species were collected and identified. The recorded lichens are listed below with notes on sampling sites, ecology and distribution of each species in the world and in Southern Africa. The pictures of all the lichens are in Appendix 11A, B, C and D. The results of Thin Layer Chromatography (TLC) are included for some species as the detection of given lichen substances by TLC is important for the identification of lichens (see Appendix 12).

Amandinea natalensis (Vain.) Marbach **–** Magalies, Hornsnek Road, on *A. caffra,* Maphangwa and Zedda KWM_0032 (PRE).
This subtropical crustose species has been reported only once in South Africa, in particular from Howick in KwaZulu-Natal (Marbach, 2000). Marbach (2000) provided a detailed description of this species, and Sipman (2003) included this in a global identification key of the genus *Amandinea*. Fryday (2015) also included it in the checklist of South African lichens. In Pretoria, it was found once during this study, in a mountain area, where traffic is very low.

Candelaria concolor (Dicks.) Arnold **–** Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karroo* and *A. caffra,* Maphangwa and Zedda KWM_0009; KWM_0012 (PRE). Magalies, Hornsnek Road, on *A. caffra,* Maphangwa and Zedda KWM_0031 (PRE). Hercules, Van Der Hoff Road, on *A. karroo* and *J. mimosifolia*, Maphangwa KWM_0039; KWM_0056 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0082 (PRE). Pretoria West, Staal Road, on *J. mimosifolia,* Maphangwa KWM_0115 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0119 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0140 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0148 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo* and *A. caffra,* Maphangwa KWM_0182; KWM_0197 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia,* Maphangwa and Zedda KWM_0207 (PRE). Central, Nana Sita Street, on *J. mimosifolia,* Maphangwa and Zedda KWM_0210 (PRE). Waterkloof, Rigel Avenue, on *J. mimosifolia,* Maphangwa and Zedda KWM_0218 (PRE).
This is a cosmopolitan species common on nutrient-rich substrates (Almborn, 1966; Brodo *et al*., 2001). It is common in tropical and temperate regions of the world and in East Africa at an altitude of 1000 to 2000 m a.s.l. (Swinscow and Krog, 1988). Almborn (1966) and Zedda *et al*. (2009) reported it on different trees in Namibia and Almborn (1966, 1988) and Fryday (2015) in South Africa as well. In Pretoria, it was widespread across all sampling sites.

Canoparmelia texana (Tuck.) Elix and Hale – Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa and Zedda KWM_0004 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra* and *A. karroo,* Maphangwa and Zedda KWM_0014, KWM_0023 (PRE). Hercules, Van Der Hoff Road, on *A. karroo*, Maphangwa KWM_0041 (PRE). Pionier Museum, Keuning Dr, on *A. karroo,* Maphangwa KWM_0086 (PRE). Lotus Gardens, WF Nkomo, on *J. mimosifolia*, Maphangwa KWM_0128 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0156 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo* and *A. caffra*, Maphangwa KWM_0184 (PRE).
This species (pantropical world distribution) is found in in Southern and East Africa (Swinscow and Krog, 1988) and in Madagascar (Aptroot, 2016), where it grows in dry, sun-exposed habitats on lowlands and coastal hills at up to 1000 m a.s.l., and. It has been reported in South Africa by Fryday (2015). In Pretoria, it is relatively common and was found in both human disturbed and undisturbed areas. Atranorin and divaricatic acid were found in the specimens examined by TLC in KWM_0041, KWM_0094, and KWM_0203.

Chrysothrix xanthina (Vain.) Kalb – Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra*, Maphangwa KWM_0232 (PRE).
The species is known across Africa, Asia, Macaronesia, Madagascar, New Zealand, Norfolk Island, North and South America (Laundon, 1981; Kalb, 2001; Elix and Kantvilas, 2007; Fryday, 2015). In Pretoria, it was found only once during this study in a nature reserve.

Culbersonia nubila (Moberg) Essl. – Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karroo* and *A. caffra*, Maphangwa and Zedda KWM_0011; KWM_0025 (PRE). Pionier Museum, Keuning Dr, on *A. caffra*, Maphangwa KWM_0103 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0138 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0150 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia,* Maphangwa and Zedda KWM_0205 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia,* Maphangwa and Zedda KWM_0218 (PRE).
This species is also known as *Pyxine nubila* Morberg. It has a scattered distribution in dry, subtropical areas of Africa, America, Australia and Eurasia, where it can occur on trees and rocks (Swinscow and Krog, 1988; Moberg, 2004, Obermayer *et al*., 2009). In Southern Africa, the species has been reported in South Africa (Eastern Cape, Free State, Mpumalanga and KwaZulu-Natal provinces) and Lesotho (Moberg, 2004). It has also been reported in Gauteng Province by Obermayer *et al*. (2009). In Pretoria, it is common and was found in the less disturbed areas during this study. No recognisable substances were found by TLC in

KWM_0103, KWM_0138 and KWM_0204. The phylogenetic position of this monotypic genus is explained under 4.2.

Dirinaria applanata (Fée) D.D. Awasthi – Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0078 (PRE). *D. applanata* is a common corticolous and saxicolous lichen widespread in all tropical regions worldwide (Nash *et al*., 2004). The species has been reported in Tanzania, Kenya, South Africa, Ethiopia and Uganda, where it occurs in natural woodlands, parks, avenues, and plantations, from sea level up to 2300 m a.s.l. (Swinscow and Krog, 1988; Fryday, 2015; Aptroot, 2016). In Pretoria, it was recorded only once, in a protected area.

Flavopunctelia flaventior (Stirt.) Hale – Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karroo* and *A. caffra,* Maphangwa and Zedda KWM_0008; KWM_0016 (PRE). Hercules, Van Der Hoff Road, on *A. karroo* and on *J. mimosifolia*, Maphangwa KWM_0044; KWM_0047 (PRE). Pionier Museum, Keuning Dr, on *A. karroo,* Maphangwa KWM_0069 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0117 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0155 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo* and *A. caffra*, Maphangwa KWM_0179; KWM_0200 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa and Zedda KWM_0217 (PRE).
This species is widespread in temperate as well as tropical regions of Africa, Europe, India, North and South America at moderate elevations (Swinscow and Krog, 1988; Nash *et al*., 2004; Fryday, 2015). It is common in East Africa (Swinscow and Krog, 1988; Killmann and Fischer, 2005) and Schultz *et al*. (2009) and Zedda *et al*. (2009) reported it in Namibia. In Pretoria it has been previously collected by Degelius in the Fountains Valley (UPS:BOT:L-053801). During this study, it was found at many of the research sites. The collected specimens had usnic and lecanoric acids as detected by TLC in KWM_0099, KWM_0122, and KWM_0186.

Flavopunctelia soredica (Nyl.) Hale – Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa and Zedda KWM_0002 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra* and *A. karroo*, Maphangwa and Zedda KWM_0007; KWM_0224 (PRE). Hercules, Van Der Hoff Road, on *A. karroo* and on *J. mimosifolia*, Maphangwa KWM_0048 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0095 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0130 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0146

(PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo*, Maphangwa KWM_0179 (PRE).

This species is prevalent in temperate areas of Asia, North and South America where it grows on bark and wood of different tree species (Nash *et al*., 2004). Fryday (2015) reported it in South Africa, while Brusse (1988) recorded it on *Jacaranda* trees in Windhoek (Namibia) as *Parmelia soredica* Nyl. Also Zedda *et al*. (2009) reported it as an epiphyte from Namibia. In Pretoria, it has the same distribution as *F. flaventior* (Maphangwa *et al*., 2018). The specimens investigated by TLC (KWM_0134, KWM_0189 and KWM_0224) were found to contain usnic and lecanoric acids as secondary metabolites.

Heterodermia speciosa (Wulfen) Trevis. – Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karroo* and *A. caffra*, Maphangwa and Zedda KWM_0008; KWM_0022 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0067 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0120 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0140 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0157 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo* and *A. caffra*, Maphangwa KWM_0175; KWM_0203 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia,* Maphangwa and Zedda KWM_0206 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa and Zedda KWM_0218 (PRE).

H. speciosa is widely distributed in subtropical to temperate areas of the world (Nash *et al*., 2002). It is common on sheltered tree trunks in natural and artificial habitats at 1100 to 3600 m altitude in East Africa (Swinscow and Krog, 1988), while in North America it grows on sunny, but moist rocks or on tree trunks in humid conditions (Nash *et al*., 2002). The species has been reported from East Africa, Madagascar and South Africa (Swinscow and Krog, 1988; Killmann and Fischer, 2005; Fryday, 2015; Aptroot, 2016). In Pretoria, it is common as an epiphyte and was found at several sites. TLC applied to specimens KWM_0079, KWM_0162, and KWM_0204 revealed the presence of atranorin and zeorin.

Hyperphyscia adglutinata (Flörke) H. Mayrhofer and Poelt – Hercules, Van Der Hoff Road, on *J. mimosifolia*, Maphangwa KWM_0045 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0066 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0124 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0139 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0149 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo*, Maphangwa KWM_0198 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia,* Maphangwa and Zedda KWM_0209 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa and Zedda KWM_0211 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra*, Maphangwa and Zedda KWM_0221 (PRE).

This species is widespread and common worldwide from tropical to temperate regions (Swinscow and Krog, 1988; Purvis *et al*., 1992; Zedda *et al*., 2009). In South Africa, it has a wide distribution and grows mainly on nutrient-rich or nutrient-enriched tree trunks, branches, twigs and rocks in open or partly shaded habitats, even in polluted areas (Swinscow and Krog, 1988; Moberg, 2004, Fryday, 2015). It is frequent and widespread in Pretoria.

Hyperphyscia granulata (Poelt) Moberg – Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_119 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa and Zedda KWM_0214 (PRE).

H. granulata is widespread in tropical-subtropical regions of East Africa, Madagascar, Southern Africa, South America and Asia (Swinscow and Krog, 1988; Moberg, 2004; Schultz *et al*., 2009; Zedda *et al*., 2009; Fryday, 2015; Aptroot, 2016). In South Africa, it has been collected in Gauteng, KwaZulu-Natal and Limpopo provinces (Moberg, 2004). It grows on trunks, branches, and twigs mixed with other *Hyperphyscia* species at 850 to 2470 m a.s.l. (Swinscow and Krog, 1988; Moberg, 2004). In Pretoria, it was only found in residential areas.

Hyperphyscia isidiata Moberg – Hercules, Van Der Hoff Road, on *A. karroo* and *J. mimosifolia*, Maphangwa KWM_0042; KWM_0051 (PRE).

The species is uncommon, but known worldwide from Angola, Australia, Costa Rica, South Africa and a few localities in Kenya (Swinscow and Krog, 1988; Moberg, 2004; Fryday, 2015). Moberg (2004) reported it from one locality in Pretoria (UPS:BOT:L-057759). It grows on tree trunks in open conditions associated with other species of *Hyperphyscia* at 800 to 1600 m a.s.l. (Swinscow and Krog, 1988; Moberg, 2004). In this study, it was found only once in a residential area with high traffic.

Hyperphyscia pandani (H. Magn.) Moberg – Hercules, Van Der Hoff Road, on *J. mimosifolia*, Maphangwa KWM_0052 (PRE). Magalies, Hornsnek Road, on *A. caffra*, Maphangwa and Zedda KWM_0062 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0119 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0144 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0165 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. caffra*, Maphangwa KWM_0199 (PRE).

The species is found in tropical to subtropical areas of America, Australia, East Africa, the Hawaiian Islands and South Africa (Swinscow and Krog, 1988; Moberg, 2004; Fryday, 2015). It grows on trunks, branches, and twigs of different tree species, often with other species of *Hyperphyscia* (Swinscow and Krog, 1988; Moberg, 2004). It appears to be widespread in

South Africa, where it occurs in the Eastern Cape, Gauteng, KwaZulu-Natal and Limpopo Provinces (Moberg, 2004). In Pretoria, it is common and was found in disturbed and protected areas.

Hyperphyscia pruinosa Moberg – Magalies, Hornsnek Road, on *A. caffra*, Maphangwa and Zedda KWM_0033 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_00087 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0160 (PRE). This species is found in Australia (Moberg, 1987), Lesotho, South Africa (Eastern and Northern Cape) (Moberg, 2004; Fryday 2015), Namibia (Zedda *et al*., 2009) and East Africa (Moberg, 2004; Swinscow and Krog, 1988). The species is uncommon and is found on old decorticated wood and on bark of trees and shrubs at 1500 to 2900 m a.s.l. in East Africa (Swinscow and Krog, 1988). In Pretoria, it was found in mountain, rural areas and once at downtown Sunnyside.

Lepraria spp. – Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra,* Maphangwa and Zedda KWM_0013 (PRE). Magalies, Hornsnek Road, on *A. caffra,* Maphangwa and Zedda KWM_0063 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0104 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0171 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo,* Maphangwa KWM_0188 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia,* Maphangwa and Zedda KWM_0213 (PRE).

Lepraria species are widespread worldwide, however the highest number of species is found in temperate areas (Saag *et al*., 2009). They look similar and are difficult to distinguish, without TLC analyses. The only traits enabling identification are differences in colour, thallus thickness, substrate and especially chemistry of secondary metabolites (Brodo *et al*., 2001). Saag *et al*. (2009) have reported few taxa from Africa so far. These include *Lepraria nigrocincta* Diedrich, Sérus and Aptroot, *L. pallida* Sipman, *L. rigidula* (de Lesd.) Tønsberg, *L. leuckertiana* (Zedda) L. Saag, *L. sipmaniana* (Kümmerl. and Leuckert) Kukwa, *L. umbricola* Tønsberg, *L. usnica* Sipman, *L. glaucella* Ach., *L. incana* (L.) Ach. and *L. yunnaniana* (Hue) Zahlbr. Most have been found in Northern or East Africa. *L. sipmaniana, L. usnica*, *L. glaucella* and *L. incana* have been recorded from Southern Africa as well (Fryday, 2015). The collected specimens from Pretoria could not be identified to species level within this study. Either important thallus traits or characteristic secondary metabolites could not be observed or detected (Maphangwa *et al*., 2018). The genus as a whole is widespread in Pretoria but the thalli are always poorly developed. TLC in KWM_0018, KWM_0061 and KWM_0104 detected atranorin and unidentified traces of other compounds.

Parmotrema austrosinense (Zahlbr.) Hale – Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karroo* and *A. caffra,* Maphangwa and Zedda KWM_0005; KWM_0019 (PRE). Hercules, Van Der Hoff Road, on *A. karroo* and *J. mimosifolia*, Maphangwa KWM_0040; KWM_0055 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0072 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0116 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0143 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0152 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo*, Maphangwa KWM_0177 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia,* Maphangwa and Zedda KWM_0207 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa and Zedda KWM_0216 (PRE).

This species is widespread in tropical and temperate regions (Swinscow and Krog, 1988; Brodo *et al*., 2001). It is known from Africa, Australia, North and South America, and Oceania (Nash *et al*., 2002; Kukwa *et al*., 2012). In Africa, it is relatively common and it has been recorded in East Africa, Madagascar, Namibia, and South Africa (Almborn, 1988; Swinscow and Krog, 1988; Thomas and Bhat, 1994; Killmann and Fischer, 2005; Forbes *et al*., 2009; Schultz *et al*., 2009; Zedda *et al*., 2009; Trüe *et al*., 2012; Fryday, 2015; Aptroot, 2016). In Pretoria, it was also very common at most sites. Atranorin and lecanoric acid were found by TLC in KWM_0117, KWM_0188 and KWM_0216.

Parmotrema reticulatum (Taylor) M. Choisy – Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0089 (PRE).

This species is widespread throughout the tropical and temperate regions of Africa, Australasia, Europe, India, North and South America, Oceania and southern Asia (Swinscow and Krog, 1988; Purvis *et al*., 1992; Nash *et al*., 2002; Aptroot and Feijen, 2002; Purvis *et al*., 1992; Nash *et al*., 2002). In Africa, the species was found in East Africa (Swinscow and Krog, 1988), Madagascar (Aptroot, 2016) and Southern Africa (Doidge, 1950; Almborn, 1988; Fryday, 2015). *P. reticulatum* is corticolous, saxicolous, and terricolous in a wide variety of natural and artificial, and more or less open habitats. In East Africa, it is common and widespread at 1000 to 3000 m a.s.l. (Swinscow and Krog, 1988). In Pretoria, it is infrequent and was found only once, in a protected area. Atranorin and salazinic acid were detected by TLC.

Physcia biziana (A. Massal.) Zahlbr. – Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0126 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0142 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. caffra*, Maphangwa KWM_0199 (PRE).

This is a Mediterranean to mild-temperate species (Nimis and Martellos, 2017), known from Africa, Australia, Europe, North and South America (Swinscow and Krog, 1988; Moberg, 2004). The species has been previously reported in Africa from East Africa (Swinscow and Krog, 1988), Namibia (Zedda *et al*., 2009), South Africa (Almborn, 1988; Moberg, 2004; Fryday, 2015), and Tunisia (Guttová *et al*., 2015). In East Africa, it is found at 1500 to 2100 m a.s.l. and is uncommon (Swinscow and Krog, 1988). In South Africa it is established in Mpumalanga province so far, where it grows on tree trunks and branches in open conditions (Moberg, 2004) and in the Botanical Garden of Pretoria (collected by Almborn, specimens in B and UPS:BOT:L-012954). During this investigation, it was found on roadside trees in streets with heavy traffic and once in a nature reserve in Pretoria.

Physcia erumpens Moberg – Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia,* Maphangwa and Zedda KWM_0003 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra,* Maphangwa and Zedda KWM_0021 (PRE).
This species is subtropical (Nimis and Martellos, 2017) and known from Europe, Asia, East Africa, Madagascar, North America and South Africa (Moberg, 2004; Aptroot, 2016). In South Africa, it has been reported in scattered localities in the Eastern and Western Cape, KwaZulu-Natal and Mpumalanga provinces (Moberg, 2004; Fryday, 2015). It grows on trees and rocks in more or less open conditions (Moberg, 2004). In Pretoria it was found only at two sites, on roadside trees in a green residential area and in a nature reserve.

Physcia poncinsii Hue – Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0104 (PRE).
P. poncinsii is found in tropical to subtropical regions of America, Australia, East Africa, Madagascar and Southern Africa (Nash *et al*., 2002; Moberg, 2004; Fryday, 2015; Aptroot, 2016). It is known in South Africa (Eastern and Western Cape, Mpumalanga and KwaZulu-Natal provinces) (Moberg, 2004) and Zedda *et al*., (2009) reported it on trees in Namibia. In southern Africa, it grows on tree trunk, wood and rocks in open conditions (Moberg, 2004). In Pretoria, it is uncommon and was found only once in a protected area.

Physcia tribacia (Ach.) Nyl. – Groenkloof Nature Reserve, Christina De Wit Ave, on *Acacia caffra,* Maphangwa and Zedda KWM_0016 (PRE). Hercules, Van Der Hoff Road, on *A. karroo* and *J. mimosifolia*, Maphangwa KWM_0043; KWM_0043 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0113 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo* and *A. caffra*, Maphangwa KWM_0188; KWM_0196 (PRE).
This species is widely distributed but not common in temperate regions (Swinscow and Krog 1988; Nash *et al*., 2002; Moberg, 2004). According to Nimis and Martellos (2017), it is a

Mediterranean to sub-tropical lichen, usually growing on rather exposed rocks and rarely on bark (Swinscow and Krog, 1988; Moberg, 2004). In Africa *P. tribacia* is known from East Africa, Lesotho and South Africa (Swinscow and Krog, 1988; Moberg, 2004; Fryday, 2015). In South Africa, it is well-known in the Eastern and Northern Cape, Free State, KwaZulu-Natal and Mpumalanga provinces (Moberg 2004). In Pretoria, it was found mainly in protected areas and once in a street with heavy traffic.

Physcia undulata Moberg – Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra*, Maphangwa and Zedda KWM_0027 (PRE).
It is known in Africa, Australia and South and Central America (Swinscow and Krog, 1988; Moberg, 2004) and has probably a tropical-subtropical distribution. In Africa, it has been reported in Kenya (Moberg, 1986), Namibia (Zedda *et al*., 2009), Lesotho and South Africa, in particular in the Eastern and Northern Cape and KwaZulu-Natal provinces (Moberg, 2004; Fryday, 2015). It grows on trunks and branches of solitary trees in open sites (Nash *et al*., 2002) at 500 to 3000 m a.s.l. (Swinscow and Krog, 1988). In Pretoria, it was found once in a protected area.

Pyxine cocoes (Sw.) Nyl. – Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karroo* and *A. caffra,* Maphangwa and Zedda KWM_0008; KWM_0017 (PRE). Magalies, Hornsnek Road, on *A. caffra,* Maphangwa and Zedda KWM_0030 (PRE). Hercules, Van Der Hoff Road, on *A. karroo* and *J. mimosifolia*, Maphangwa KWM_0039; KWM_0058 (PRE). Pionier Museum, Keuning Dr, on *A. karroo*, Maphangwa KWM_0068 (PRE). Lotus Gardens, Church Street, on *J. mimosifolia*, Maphangwa KWM_0121 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo* and *A. caffra*, Maphangwa KWM_0181; KWM_0196 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia,* Maphangwa and Zedda KWM_0220 (PRE).
This is a pantropical species with scattered records from the subtropics and Laurimacaronesia (Nash *et al*., 2002). Swinscow and Krog (1988) reported it as widespread in the tropics and sub-tropics. It is common and widespread in East Africa, where it occurs on bark and wood of trees and shrubs, sometimes on rocks in sunny or partial shaded conditions, from sea level up to about 2500 m a.s.l. It even thrives in artificial habitats (Swinscow and Krog, 1988). Zedda *et al*. (2009) and Schultz *et al*. (2009) reported it in Namibia and Aptroot (2016) in Madagascar. It seems to be rather common in South Africa, as it has been reported by Moberg (2004) and Fryday (2015) from Eastern and Northern Cape and Mpumalanga provinces as corticolous and saxicolous. In Pretoria, it was collected during the present study in nature reserves, rural areas and on avenue trees in residential areas. Lichexanthone and terpenoids were detected by TLC in KWM_0223.

Pyxine petricola Nyl. – Pretoria Botanical Garden, Cussonia Avenue on *Acacia caffra*, Maphangwa KWM_0233 (PRE). Pretoria Voortrekker Monument, on *Acacia karroo*, Maphangwa KWM_0234 (PRE).
This is a pantropical to nearly cosmopolitan species (Zedda *et al*., 2009) reported from tropical East Africa (Swinscow and Krog, 1988), Namibia (Schultz *et al*., 2009; Zedda *et al*., 2009) and Zimbabwe (Becker, 2002). It has also formerly been reported from South Africa (Doidge, 1950; Moberg, 2004; Fryday, 2015). In East Africa it is common on trees, shrubs, and rocks in exposed or partly shaded places in artificial as well as natural habitats up to 2000 m altitude (Swinscow and Krog, 1988). In Pretoria, it was only found in nature reserves.

Rinodina sp. – Rietvlei Nature Reserve, OR Tambo (R21), on *A. karroo,* Maphangwa KWM_0176 (PRE).
A recent revision of corticolous species of the genus *Rinodina* is available for Southern Africa (Mayrhofer *et al*., 2014). Four species are reported: *Rinodina albocincta* Zahlbr., *R. australiensis* Müll. Arg., *R. capensis* Hampe and *R. ficta* (Stizenb.) Zahlbr. *R. ficta* has been previously reported from Pretoria (Zwartdam) on bark of *Acacia*, Transvaal and Namibia, while the other listed species have been found mainly in the Cape regions and along the coast so far (Mayrhofer *et al*., 2014). Fryday (2015) lists further *Rinodina* spp. The collected specimen could not be identified to the species level due to a lack of well-developed ascospores, is most likely *R. ficta*. In Pretoria, it was found once in a nature reserve.

4.1.1 Additional species reported from Pretoria in the literature

The following specimens were reported from Pretoria by other authors but were not found during the present study.

Parmelia sulcata Taylor, reported around Garankuwa in Pretoria and Johannesburg by Olowoyo *et al*. (2011) and Monna *et al*. (2006). It is a cosmopolitan, pantemperate to southern boreal species (CNALH, 2017; Swinscow and Krog, 1988). It is mainly saxicolous in the lower alpine zone at 3500 to 4200 m a.s.l. and rare in East Africa (Swinscow and Krog, 1988).

Phaeophyscia adiastola (Essl.) Essl., reported in Fountains Valley in Pretoria on tree bark by Moberg (2004). It is also known to exist in Ethiopia, Kenya, Lesotho, Tanzania, Uganda, North America and Eastern Russia (Swinscow and Krog, 1988; Moberg, 2004). In South Africa, it was found in the Eastern Cape, Gauteng and Western Cape provinces as well (Moberg, 2004; Fryday, 2015). The species is corticolous, but occasionally also saxicolous, in well-lit sites at 900 to 3600 m a.s.l. (Swinscow and Krog, 1988).

Phaeophyscia orbicularis (Neck.) Moberg, reported by Moberg (2004) from the zoological garden of Pretoria. It is also known in the Eastern Cape, Gauteng, Lesotho and North West provinces in South Africa (Moberg, 2004; Fryday, 2015). It occurs worldwide in temperate regions of the northern hemisphere, where it is very common on tree trunks in open conditions (Moberg, 2004).

Rinodina ficta (Stizenb.) Zahlbr. reported in Pretoria by Mayrhofer *et al*. (2014) (Zwartdam, on bark of an *Acacia* tree) and in South Africa by Fryday (2015). It is also known in Namibia (Zedda *et al*., 2009, reported as *Rinodina* aff. *boleana*; Mayrhofer *et al*., 2014), Italy, New Zealand and USA (Giralt and Mayrhofer, 1995; Mayrhofer *et al*., 2007; Sheard *et al*., 2011). It occurs in parkland and woodlands, where it grows on bark (Giralt and Mayrhofer, 1991; Mayrhofer *et al*., 2014).

4.1.2 Frequency and phytogeography of the recorded lichens

Of the 25 taxa of epiphytic lichens recorded during this study in Pretoria, the most frequent species were *Candelaria concolor* (18.2% occurrence), *Parmotrema austrosinense* (12.2%), *Heterodermia speciosa* (11.9%), *Flavopunctelia flaventior* (8.3%), *Hyperphyscia adglutinata* (7.7%), *Pyxine cocoes* (7.2%), *F. soredica* and *Lepraria* spp. (6.4%), *Canoparmelia texana* (6.1%) and *Culbersonia nubila* (3.3%) (Figure 4.1). The percentage occurrences of *Hyperphyscia pandani* and *Physcia tribacia* were 3.0%. *Physcia biziana* occurrence was 1.4%, whereas the rest of the species had less than 1% occurrence; this means they were found only once. More than half of the species belong to the family Physciaceae.

As shown in Figure 4.2, the majority of the species recorded in this study are subtropical to tropical (31%), tropical (17%) and tropical-temperate (17%), making a total of 65%. The subtropical (9%) and subtropical-temperate (9%) taxa amount together to 18%. The cosmopolitan species are 9%, while the Mediterranean-subtropical (4%) and Mediterranean-mild temperate (4%) are 8% of the study samples.

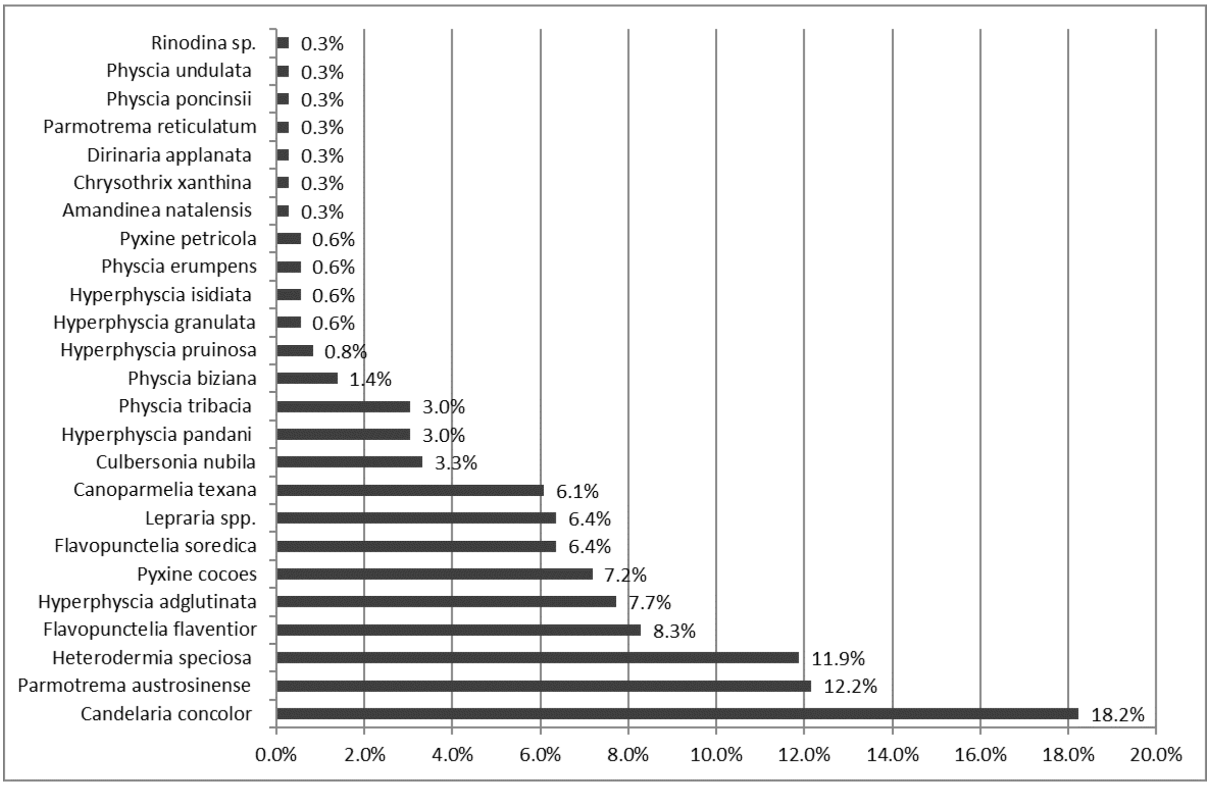

Figure 4.1. Frequency occurrence (%) of the different lichen species at the investigated sites (n=362; n= number of lichen specimen collected)

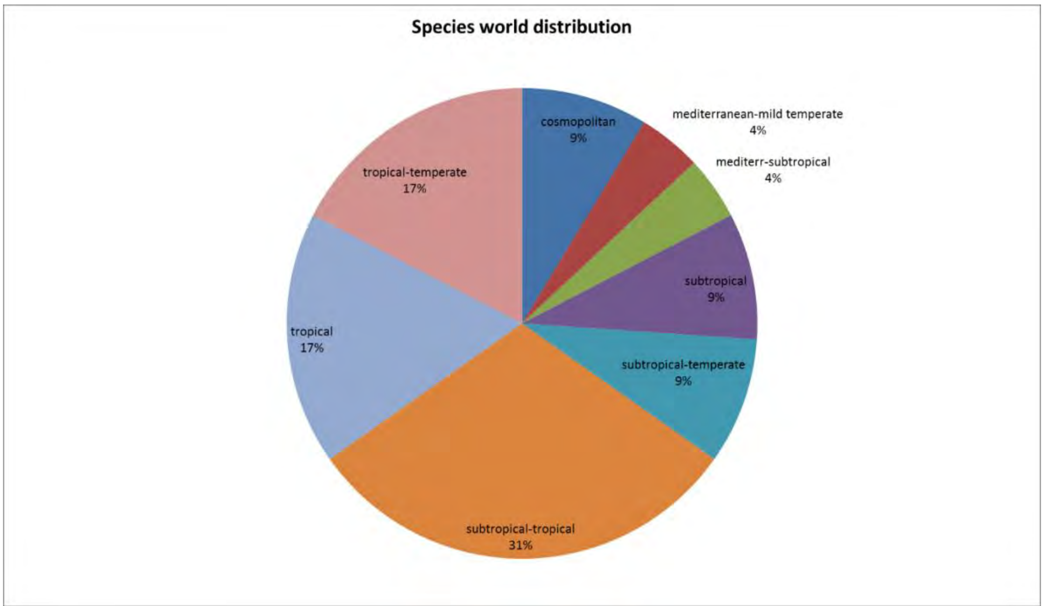

Figure 4.2. World distribution (phytogeography) of the collected lichen species in percentage occurrence (n=25; n = number of recorded species)

The lichen diversity (in terms of number of species on the three different phorophytes, *Jacaranda mimosifolia*, *Acacia caffra* and *A. karroo)* does not differ much (17, 18 and 19 taxa respectively) (Figure 4.3). However, cosmopolitan and more disturbance-tolerant species such as *Candelaria concolor* and *Hyperphyscia adglutinata* are more common on alien *Jacaranda* (blue bars). Most of the rare species collected in Pretoria such as *Chrysothrix xanthina*, *Dirinaria applanata*, *Parmotrema reticulatum* and *Rinodina* sp. were found growing exclusively on native *A*. *karroo*. *A. caffra* usually hosts more common and widespread species.

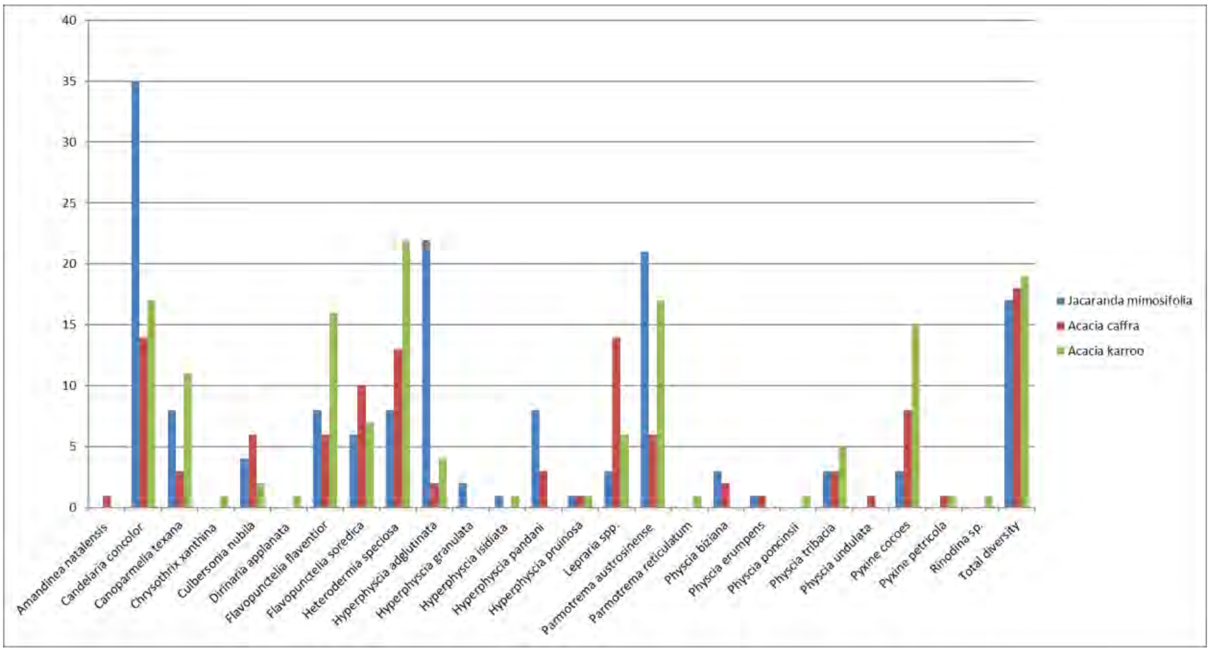

Figure 4.3. Differences in species distribution between the phorophytes and lichen diversity on each tree species

Most of the recorded species are foliose-narrow lobed (60%), followed by foliose-broad-lobed species (24%), while leprose (8%) and crustose (8%) lichens are rare (Figure 4.4). No fruticose lichens were found.

The greater diversity of specimens collected is found in protected sites such as Pionier Museum, Rietvlei Nature Reserve and Groenkloof Nature Reserve (Figure 4.5). Followed by Sunnyside (low traffic area), Lotus Gardens, Hercules, Waterkloof and Magalies. The sites with lower diversity are Pretoria central and Pretoria West (industrial area, Staal Road), hosting only the widespread *Candelaria concolor*. At SANBI and Voortrekker Monument (protected areas) a higher diversity of lichens was observed, but only *Pyxine petricola* was collected there, as no collecting permit was available during the first phase of the study.

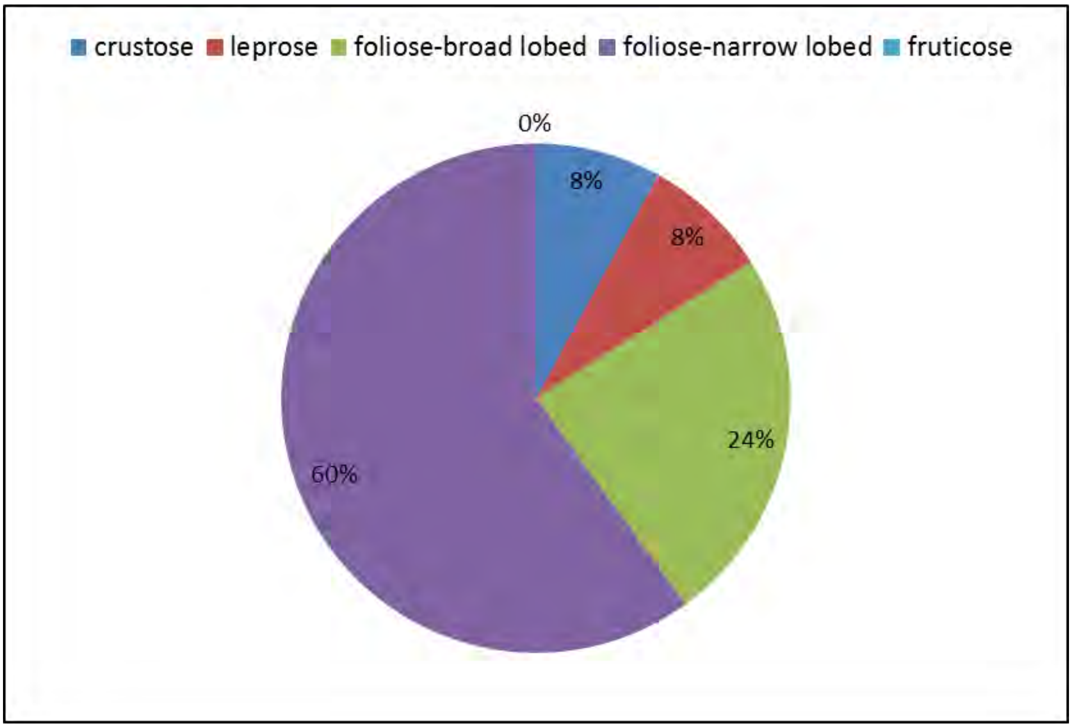

Figure 4.4. Percentage occurrence of growth forms of the recorded species

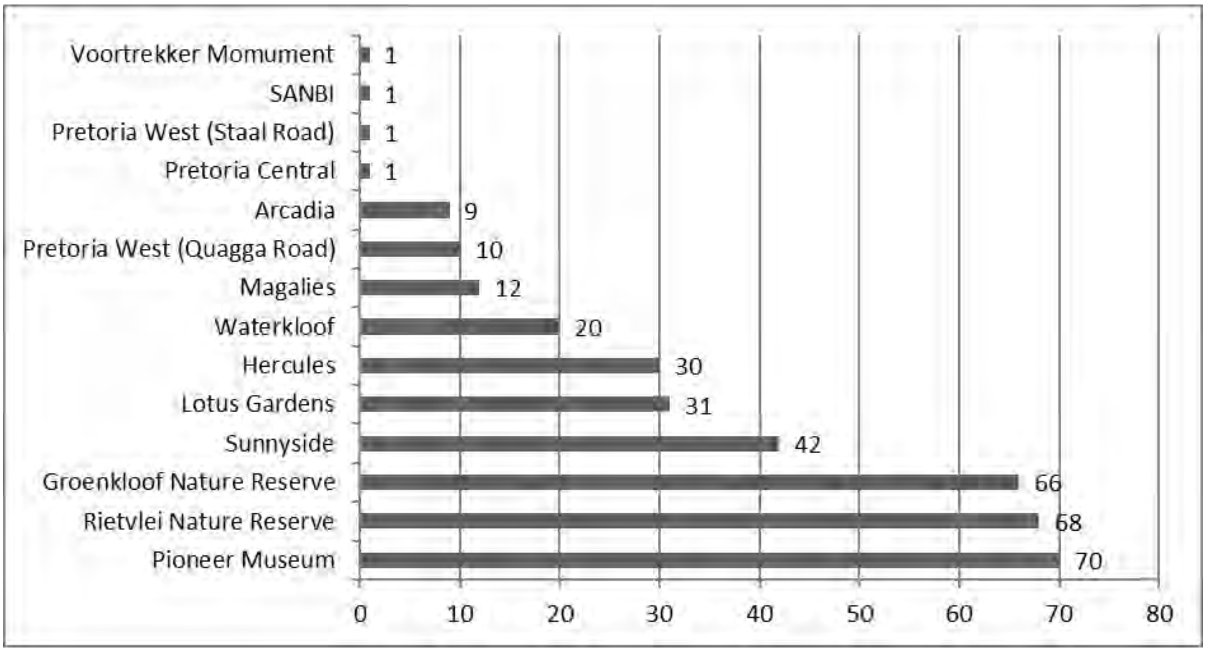

Figure 4.5. Number of specimens collected at each site

4.2 Simplified identification key to the most common species on trees

A preliminary simplified identification key to the most common species found on trees in the city was developed. This can be used in the field, e.g. during monitoring, or in the laboratory, even without an optical microscope. But the use of a good magnifying glass, binocular microscope and of a UV-lamp is recommended. The key and more detailed descriptions of species are going to be published in *Bothalia*.

1a Thallus crustose (crust-like or whole surface granular)…..……………….………………… **2**

2a Thallus leprose (surface entirely granular, without corticated thallus).………………... **3**

3a Thallus bright yellow to yellowish green …………………………*Chrysothrix xanthina*

3b Thallus whitish to blue-grey, rarely pale yellowish green, sterile ..……..….. *Lepraria*

2b Thallus not leprose (crust-like, ± corticated, at least in some parts) …………………. **4**

4a Thallus crustose, thin with small black, lecideine (without a corticated thalline margin) apothecia, 0.2-0.6 mm diam., superficial ..…………….*Amandinea natalensis*

4b Thallus crustose, thick with lecanorine (with a corticated thalline margin) …………………………………… …….. *Rinodina* sp.

1b Thallus foliose (leaf-like, with an upper and lower cortex ±easily separable from the substratum of growth)………………………………………………………………….…………..….**5**

5a Thallus small foliose (lobes less than 2 mm wide) ……………………………………… **6**

6a Thallus yellow, lobes branched and up to 0.5 mm wide; minutely granular isidia and soredia marginal and laminal present ..……………………………*Candelaria concolor*

6b Thallus white to pale grey, dark grey or brownish grey, with or without pruina…..…**7**

7a Tightly adnate, small foliose thalli, almost crustose in appearance, brownish grey to grey, lower surface pale to black, without (or with few) rhizines; Cortex PD-, K-, KC-, C-……………………………………………………………………………...**8**

8a Thallus with soralia ……………………………………………………………….. **9**

9a Thallus orbicular to irregular to 2 cm diameter, closely or loosely adnate; soralia laminal, maniculiform or capitate, near lobe apices with marginal granular soredia; medulla white …………………….. *Hyperphyscia adglutinata*

9b Thallus orbicular up to 3 cm in diameter, firmly adnate to substrate, upper surface usually paler at the lobe tips; soralia laminal, maculiform, capitate to crateriform, occasionally confluent and covering central parts of the thallus, often dark coloured or rusty red; medulla orange-red to red-brown (with skyrin) … …. *Hyperphyscia pandani*

9c Thallus usually orbicular to 2 cm in diameter firmly adnate to substrate, distinctly lobate at margins; thallus mostly with a white to blue-grey pruina;

soralia starting as marginal, delimited patches on inner lobes, developing into more or less confluent soredia-covered areas with granular to almost isidiate soredia, medulla white ………………………………... *Hyperphyscia pruinosa*

8b Thallus with isidia ……………………………………………………………. **10**

10a Thallus without pruina; isidia globular; medulla orange to red (skyrin) mainly in the lowest part ……………………..….. *Hyperphyscia granulata*

10b Thallus mostly with a whitish, thin pruina; isidia cylindrical, sometimes coralloid to 0.5 mm high, often crowded; medulla white ……………………………………………………..…*Hyperphyscia isidiata*

7b Lobes non-tightly adnate, and not crustose in appearance ……..……………. ..**11**

11a Thallus K+ yellow ………………..…………..……………………………. **12**

12a with apothecia; rhizines scattered, white to dark grey; medulla K- ………………………………………………………….……... *Physcia biziana*

12b without apothecia, with laminal soralia ……………………………...…. **13**

13a Soralia crateriform to almost capitate; thallus to 5 cm in diameter; lower surface white to weakly brownish grey, rhizines white to dark grey; medulla K+ yellow ……………………………..………….. *Physcia poncinsii*

13b Soralia crateriform,± capitate; thallus to 3 cm diameter, lower surface black except at tips, rhizines black; medulla K+ yellow ……………………………………………………….…..... *Physcia erumpens*

13c Soralia hemispherical, sometimes erose and crateriform; thallus to 6 cm diameter, white; lower surface dark grey to black without rhizines; medulla K- ……………………………………..…*Dirinaria applanata*

12c mostly without apothecia, with marginal soralia ……..……..………..…. **14**

14a Upper surface grey to dark grey and frosted; lobes loosely adnate to ascending to 2 mm wide, tips rounded ….……….………*Physcia undulata*

14b Upper surface whitish grey to dark-grey or cream coloured, glossy and epruinose or rarely pruinose, with darker margins; lobes to 1 mm wide …………………………………………………..…... *Physcia tribacia*

14c Upper surface cream-colored or brownish to bluish gray, ± shiny, the lobe-tips sometimes darkening, very rarely pruinose; lobes plane, not ascending; soredia gray to bluish gray, in labiate soralia on lateral lobes …………………………………………….………*Heterodermia speciosa*

11b Thallus K- ……………………………………………………………….. **15**

15a Thallus appressed but loosely adnate to 4 cm in diameter, lobes 1-3 mm wide, rounded and overlapping; upper surface gray, usually with bluish tint, evenly pruinose; soralia marginal first and then laminal; lower surface mostly pale, moderately rhizinate, the rhizines simple to irregularly furcate, pale; thallus UV- …………………………………………………*Culbersonia nubila*

15b Thallus closely attached to 10 cm in diameter, lobes to 1 mm wide; upper side white to yellowish grey with a distinct and patchy pruina; soralia marginal; lower surface dark, with black rhizines. Apothecia common to 1.5 mm in diameter without pruina; thallus UV+ yellow ………………… *Pyxine cocoes*

15c Thallus more or less closely appressed to 5 cm in diameter, lobes flat to 0.7-1 mm wide; upper side gray, greenish gray or whitish with pruina; pseudocyphellae present sparse, laminal and marginal, usually restricted to the peripheral parts of the lobes, sometimes reticulate; soralia absent; lower surface black, paler towards lobe tips, with black rhizines. Apothecia common to 1.5 mm in diameter without pruina; thallus UV+ yellow …. *Pyxine petricola*

5b Thallus broad foliose (lobes more than 2 mm wide) ……………………………………. **16**

16a Lobes usually more than 1 cm wide, with a distinct marginal zone without rhizines on the underside; thallus pale grey to grey ……………………………………………… **17**

17a Lobes 1-3 cm wide, rounded, without cilia; upper cortex weakly maculate; thallus C+ yellow, P-; underside black; soralia marginal and sub marginal; medulla K-, C+ red, P- ………………………………………………*Parmotrema austrosinense*

17b Lobes 0.5-1.5(2) cm wide, rounded to deeply incised, ciliate; upper cortex reticulately maculate and cracked; thallus C-, P+ yellow; soralia laminal or marginal, linear to orbicular/subcapitate; medulla K+ yellow turning red, C-, P+ orange ………………………………………………………………… *Parmotrema reticulatum*

16b Lobes usually less than 1 cm wide, without a distinct marginal zone without rhizines on the underside; thallus pale grey or yellowish-green ………………………. **18**

18a Thallus grey, closely adnate, lobes mostly 2-4 mm wide with a wrinkled surface and course, granular, laminal soredia mainly along the crests of the wrinkles, maniculae absent or very inconspicuous; lower surface dull reddish brown darkening to almost black in the centre of the thallus. Photobiont green. Medulla white. Chemistry: Soralia and medulla PD-, K-,C-, KC- or KC+ faint purple, UV+ bright blue-white (divaricatic acid)… ……………….....*Canoparmelia texana*

18b Thallus greenish yellow to yellow-green; lobes sometimes with white maculae; pseudocyphellae common; soredia in round, laminal soralia arising from pseudocyphellae, sometimes coalescing; medulla K-, C+ red, P- ………………………………………………………………… *Flavopunctelia flaventior*

18c Thallus pale green, greenish yellow to yellow-green; lobes frequently with white maculae; pseudocyphellae absent or rare; soredia mostly in marginal soralia; medulla K-, C+ red, P- ………………………………………. *Flavopunctelia soredica*

4.3 Phylogenetic position of *Culbersonia nubila*

The genus *Culbersonia* was hitherto thought to belong to the family *Physciaceae* Zahlbr. because its morphology resembles that of the genus *Physconia* Poelt (Esslinger, 2000). During this study and according to phylogenetic analyses of collected specimens, it has been demonstrated that it belongs to the family Caliciaceae (Aptroot *et al.*, 2019). For more information on methods and the sequencing results of *Culbersonia nubila*, see Appendix 13 (published paper).

4.4 Monitoring results

4.4.1 Descriptive statistics

Table 4.1 below reports on the descriptive statistics regarding the **air pollution and climate data** available from five measuring stations (Booysens, Mamelodi, Pretoria West, Oliovienhoutbosch and Rossylin Monitoring station) for eight years, from 2010 to 2017. These stations were relatively close to the sampling areas. The annual average values of the different parameters were used for elaboration.

Table 4.1. Descriptive statistics for the average annual values of air pollution and climate variables

Variables	Mean	SD	Median	Min	Max	CV	National limit
CO (Carbon monoxide)	0.84	0.25	0.62	0.62	1.19	30.13	10
NO (Nitric oxide)	19.91	2.21	21.81	16.93	21.81	11.08	50
NO_2 (Nitrogen dioxide)	17.02	7.74	11.65	11.65	28.96	45.46	40
NO_x (Nitrogen oxide)	36.97	3.27	35.41	33.18	41.9	8.85	50
O_3 (Ozone)	16.09	4.83	11.94	11.94	22.68	30.01	120
PM_{10} (Particulate matter)	**82.28**	35.19	64.78	43.6	135.62	42.76	40
SO_2 (Sulphur dioxide)	4.85	0.5	5.3	4.29	5.3	10.3	50
PRES (Atmospheric pressure)	387.25	201.29	208.7	208.7	657.79	51.98	
RAIN (Rainfall)	60.33	50.71	105.61	3.29	105.61	84.06	
SOL (Solar radiation)	130.19	90.35	51.14	51.14	266.1	69.4	

PM_{10} has a mean annual value of 82.28 μg/m^3, which is higher in comparison to national limits (40 μg/m^3). NO_x has mean annual value of 36.97 ppb and compared to national limits, it is low (50 ppb). NO is lower than national limits (50 ppb) with a mean annual value of 19.91 ppb. NO_2 has a mean value of 17.02 ppb and is lower than the limits reported for RSA (40 ppb). O_3 with mean 16.09 μg/m^3 is low compared to national limits (120 μg/m^3). SO_2 with mean value of 4.85 (ppb) is also low compared to national limits (50 ppb) as is CO (mean: 0.84 ppb) in comparison to national limits (10 ppb). The national limits are reported in https://www.gov.za/documents/national-environmental-management-air-quality-act-national-ambient-air-quality-standards (accessed 30 September 2019).

PRES has mean values of 387.25 (kPa), with Standard Deviation (SD): 201.29, min and max: 208.7 and 657.79 respectively; Coefficient of Variation (CV): 51.98. The mean value of SOL is 130.19 (W/m2); SD: 90.35; min and max: 51.14 and 266.1 respectively; CV: 69.4. RAIN shows (mean values of 60.33 mm/year; SD: 50.71; min and max: 3.29 and 105.61 respectively; CV: 84.06).

Table 4.2. shows the average monthly values of air pollution and climate data from the five monitoring stations. The variables RAIN and SOL were excluded from the subsequent analyses because the data set was not complete nor therefore reliable.

Table 4.2. Average monthly values of the selected variables in the five monitoring stations for the period 2010 to 2014 (5 years)

Monitoring station	**CO** (ppb)	**NO** (ppb)	**NO_2** (ppb)	**NO_x** (ppb)	**SO_2** (ppb)	**O_3** (ppb)	**PM_{10}** ($\mu g/m^3$)	**HUM** (%)	**PRES** (hPa)	**TEMP** (° C)	**WIND DIR** (°)	**WIND SPEED** (m/sec)
Booysens	1.113	7.364	7.739	16.48	3.249	16.8	37.08	60.47	834.4	19.45	153.7	1.311
Mamelodi	**1.687**	**34.36**	**69.41**	**78.3**	2.893	**24.46**	44.94	54.67	774.6	19.04	116.5	1.776
Oliovienhoutbosch	0.507	17.41	10.68	25.4	3.54	22.45	56.69	55.7	754.6	18.46	169.3	2.131
PTAWest	0.618	10.94	15.15	24.92	**5.343**	19.57	**104.1**	NA	781.6	17.28	183.5	2.414
Rosslyn	0.747	7.308	12.15	19.11	**5.394**	22.64	23.14	50.97	906.8	18.9	184.3	1.876

There is a difference in pollutant concentration between the monitoring stations. The highest values for pollutants, exceeding national limits, were found for PM_{10} in Pretoria West (PTA), Oliovienhoutbosch and Mamelodi. Booysens and Rosslyn have lower values than the national limit of 40 $\mu g/m^3$. For NO, the highest value was recorded at Mamelodi and Oliovienhoutbosch, whereas PTA West, Booysens and Rosslyn have much lower values. For NO_2 and NO_x, the highest value, exceeding national limits, was found at Mamelodi, while all other stations have much lower values. In Oliovienhoutbosch, PTAWest, Rosslyn and Booysens, much lower values were recorded for NO_2 and NO_x. In contrast to the concentration of the NO compounds, the highest values of SO_2 were recorded at Rosslyn and PTAWest; these are however below the national limits. O_3 has the highest value at Mamelodi similarly to NO-compounds, followed by Rosslyn, Oliovienhoutbosch, PTA West and Booysens. But these values are below the national limit of 120 $\mu g/m^3$. The highest value of CO was also observed at Mamelodi (but it does not exceed national limits) followed by Booysens, Rosslyn, PTAWest and Oliovienhoutbosch, which show much lower values.

There is a climatic difference between the stations. Humidity ranges from 54.65 to 60.47 (higher value in Booysens). Pressure ranges from 754.6 µg/m^3 to 906.8 µg/m^3 (higher value in Rosslyn). Temperature ranges from 17.28°C to 19.45°C (higher value in Booysens). Wind direction ranges from 116.5° to 184.3° (higher value in Rosslyn) between stations whereas wind speed ranges from 1.311 m/sec to 2.414 m/sec (higher value in PTAWest).

Descriptive statistics of the variables related to **site and tree features** (altitude, distance from emission sources and tree circumference) and to **lichen diversity** data (Lichen Diversity Value (LDV)), frequency occurrence of each lichen species) are reported in Table 4.3. One hundred and sixty-four trees, belonging to the investigated species (*A. caffra*: 24*, A. karroo*: 70*, J. mimosofolia*: 70) were sampled from 29 sites (2-16 trees per site), according to the methodology reported in Chapter 3.

Regarding lichen species, *Candelaria concolor* (mean: 16.51; SD: 5.45; median: 20; min and max: 0 and 20 respectively), *Hyperphyscia adglutinata* (mean: 8.12; SD: 6.33; median: 8; min and max: 0 and 20 respectively), *Lepraria* spp. (mean: 5.98; SD: 7.61; median: 1; min and max: 0 and 20 respectively) and *Parmotrema austrosinense* (mean: 6.73; SD: 6.2; median: 5; min and max: 0 and 20 respectively) have higher frequency occurrence as shown by mean and median values in contrast to other species. However, SD, the range between minimum and maximum values, is relatively high for some species as indicated in Table 4.2, this showing high variability among trees. Species with the lowest frequency occurrence are *Hyperphyscia granulata* (mean: 0.25; SD: 1.86; median: 0; min and max: 0 and 18 respectively), *Hyperphyscia pandani* (mean: 0.01; SD: 0.11; median: 0; min and max: 0 and 1 respectively), *Physcia undulata* (mean: 0.1; SD: 0.71; median: 0; min and max: 0 and 7 respectively) and *Pyxine petricola* (mean: 0.09; SD: 0.47; median: 0; min and max: 0 and 4 respectively). LDV is in average of 53.26 with an SD of 32.67 and CV of 61.35.

Concerning environmental and tree parameters, the mean altitude is 1357 m a.s.l. with an SD of 70.2 and a low CV. The mean distance from emission sources is 0.4 km, with an SD of 1.28 and high CV. Tree circumference has mean value of 87.34 cm with an SD of 16.13 and CV of 18.47.

Table 4.3. Descriptive statistics of the variables related to site, tree level and to lichen diversity data

	Variables	Mean	SD	Median	Min	Max	CV
Site and tree level data	Altitude	1357	70.2	1343.5	1233	1536	5.17
	Distance from emission sources	0.4	1.28	0.03	0	10.01	324.34
	Tree circumference	87.34	16.13	86	56	142	18.47
Lichen diversity data	LDV	53.26	32.67	49.5	3	140	61.35
	Candelaria concolor	16.51	5.45	20	0	20	33.03
	Canoparmelia texana	0.93	2.35	0	0	14	253.41
	Culbersonia nubila	1.63	3.05	0	0	17	186.75
	Flavopunctelia flaventior	1.8	3.46	0	0	15	192.6
	Flavopunctelia soredica	3.71	6	0	0	20	161.65
	Heterodermia speciosa	2.43	4.38	0	0	19	180.46
	Hyperphyscia adglutinata	8.12	6.33	8	0	20	77.98
	Hyperphyscia granulata	0.25	1.86	0	0	18	744.55
	Hyperphyscia pandani	0.01	0.11	0	0	1	902.76
	Lepraria spp.	5.98	7.61	1	0	20	127.18
	Parmotrema austrosinense	6.73	6.2	5	0	20	92.15
	Physcia tribacia	2.8	3.61	1	0	17	128.93
	Physcia undulata	0.1	0.71	0	0	7	728.95
	Pyxine cocoes	2.21	3.07	1	0	14	138.76
	Pyxine petricola	0.09	0.47	0	0	4	511.16

4.4.1.1 Comparison of habitat and land use types

Two classes of land use were distinguished for analysis: “Industrial areas and busy roads”, which includes industrial, urban and rural areas with higher levels of human disturbance due to traffic and pollution, and “Parks and nature reserves”, which includes savanna, private gardens and more natural areas or with less human disturbance. Table 4.4. reports the descriptive statistics of sites and of lichen variables under the two classes of land use. “Parks and nature reserves” show significantly higher Lichen Diversity Values (LDV) than “Industrial areas and busy roads”. This is also confirmed by the Wilcoxon test ($p<0.001$) as shown in Figure 4.6. In particular, mean LDVs are 40.44±23.37 and median LDVs 38.5 in “Industrial areas and busy roads”. In “Parks and nature reserves” mean LDVs are 88.2±28.77 and median values 83.5. However, the variability of LDVs among sites is quite high as shown by SD and Min-Max-values. In “Industrial areas and busy roads” LDVs range between 3 and 97, and in “Parks and nature reserves” between 43 and 140. All species are more frequently found in parks and reserves with the exception of *Hyperphyscia granulata* which is more frequently found in industrial areas and along busy roads.

Table 4.4. Descriptive statistics of the values of the variables in the two classes of land use (only species occurring at least more than 5% considered in the analysis)

Variables	Industrial areas and busy roads				Parks and nature reserves			
	Mean±SD	Median	Min-Max	CV	Mean±SD	Median	Min-Max	CV
Altitude	1333.46±56.06	1331	1233-1536	4.2	1421.2±64.97	1451	1305-1505	4.57
Distance.emission.sources	0.15±0.92	0.01	0-10.01	615.51	1.07±1.8	0.45	0.25-7.5	169.3
Tree.circumference	89.3±16.29	89.5	56-142	18.25	82±14.54	78	60-110	17.73
LDV	40.44±23.37	38.5	3-97	57.77	88.2±28.77	83.5	43-140	32.62
Candelaria.concolor	15.82±5.89	19	0-20	37.23	18.36±3.43	20	4-20	18.68
Canoparmelia.texana	0.25±0.76	0	0-5	303.48	2.77±3.81	1	0-14	137.58
Culbersonia.nubila	0.82±1.56	0	0-7	189.65	3.84±4.66	2	0-17	121.33
Flavopunctelia.flaventior	0.96±2.21	0	0-14	230.13	4.09±4.97	1.5	0-15	121.46
Flavopunctelia.soredica	1.57±3.21	0	0-17	205	9.57±7.75	9	0-20	81.03
Heterodermia.speciosa	0.77±1.81	0	0-10	235.97	6.95±5.91	6.5	0-19	85.04
Hyperphyscia.adglutinata	7.48±6.45	6.5	0-20	86.13	9.84±5.72	10.5	0-20	58.1
Hyperphyscia.granulata	0.34±2.17	0	0-18	635.48	0±0	0	0-0	NA
Hyperphyscia.pandani	0±0	0	0-0	NA	0.05±0.21	0	0-1	463.56
Lepraria spp.	3.12±5.4	0	0-20	173.27	13.8±7.3	17.5	0-20	52.91
Parmotrema.austrosinense	5.68±5.9	4	0-20	103.78	9.59±6.18	10	0-20	64.4
Physcia.tribacia	1.96±2.59	1	0-12	132.13	5.09±4.85	6	0-17	95.18
Physcia.undulata	0.08±0.54	0	0-5	716.09	0.16±1.06	0	0-7	663.32
Pyxine.cocoes	1.55±2.66	0	0-14	171.15	4.05±3.4	3.5	0-11	84.12
Pyxine.petricola	0.08±0.4	0	0-3	481.59	0.11±0.62	0	0-4	543.99

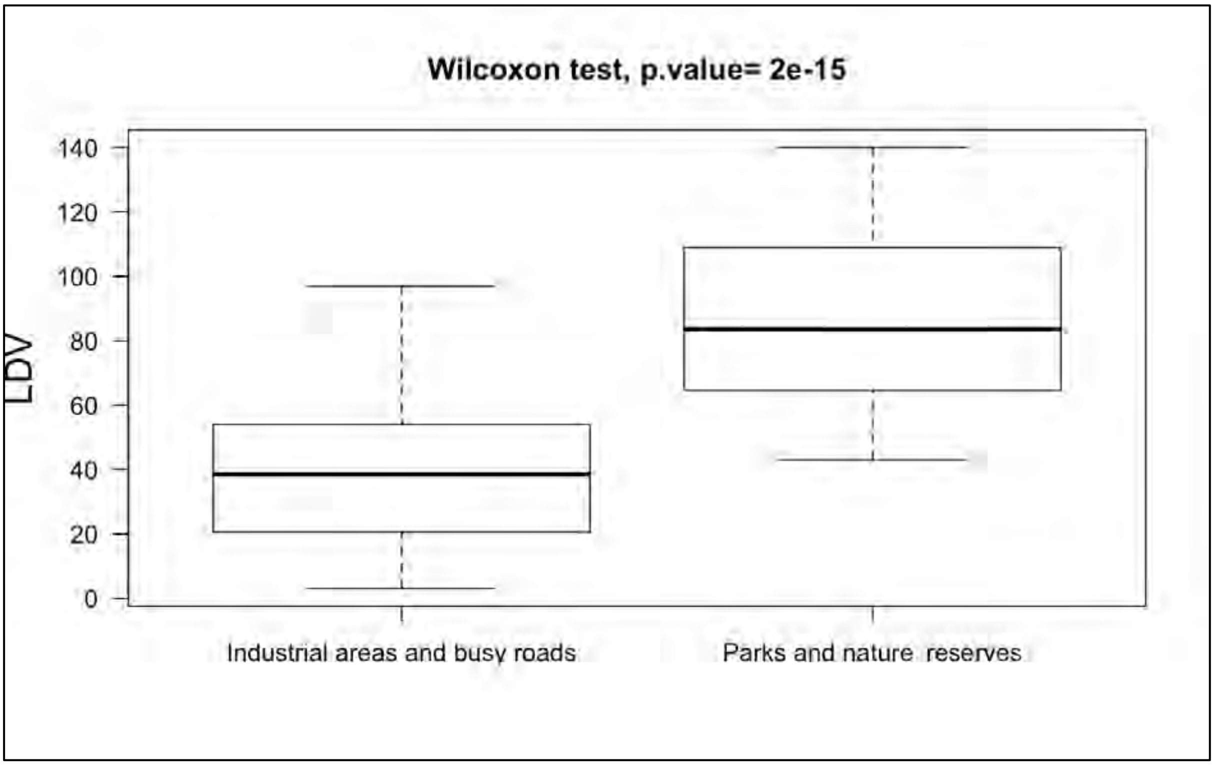

Figure 4.6. Wilcoxon test of LDV values in the two land use classes

4.4.1.2 Comparison of phorophytes

Table 4.5 reports the descriptive statistics of selected ecological variables concerning site and tree features (Altitude, Distance from emission sources, Tree circumference) and the most common lichen species for the three different phorophytes. Regarding species distribution on the tree species, the species less frequently found on exotic *Jacaranda* and more common on *Acacias* are: *Canoparmelia texana*, *Flavopunctelia flaventior*, *F. soredica*, *Heterodermia speciosa*, *Hyperphyscia adglutinata*, *Lepraria* spp., *Parmotrema austrosinense, Physcia tribacia*, *P. undulata* and *Pyxine cocoes*.

Candelaria concolor is only slightly more frequently found on *Acacias* than on *Jacaranda*. The frequency of *Hyperphyscia granulata* and *Pyxine petricola* is higher on *Jacaranda* in contrast to *A. karroo* and *A. caffra*. Between the two *Acacias* there are also some differences: *Culbersonia nubila, Flavopunctelia flaventior, F. soredica, Lepraria* spp., *Parmotrema austrosinense* and *Pyxine cocoes* more common on *A. karroo*. On the contrary, *Heterodermia speciosa, Hyperphyscia adglutinata, H. pandani, Physcia tribacia* and *P. undulata* are slightly more frequent on *A. caffra*.

The *Jacarandas* in this study were closer in distance from emission sources when compared to both *A. karroo* and *A. caffra.* This is because these trees were found mostly downtown and next to the industries. *A. caffra* and *A. karroo* have smaller average tree circumferences than *Jacarandas*. Both *Acacias* are also found at slightly higher altitudes in comparison to *Jacarandas*.

LDVs are significantly different on the three species as also confirmed by the Kruskal-Wallis test ($p<0.001$, Figure 4.7), especially on *Jacaranda* in comparison to the two *Acacias*. *Jacaranda* trees show the lowest average LDV (mean±SD: 34.39±19.85; median: 35.5) compared with *Acacia karroo* (mean±SD: 68.34±35.48; median: 67.5) and *A. caffra* (mean±SD: 64.29±26.52; median: 58). The Wilcoxon tests between plots with *Jacaranda* vs *A. karroo* and of *Jacaranda* vs *A. caffra* are very significant (see Table 4.6). The two species of *Acacia* show similar LDVs as confirmed also by the W and p-values of Wilcoxon test (see also Table 4.7 that shows the number of investigated trees for the different species). *A. karroo* has the highest average and median LDVs, but also the highest SD.

Table 4.5. Descriptive statistics of the values of the variables for the different tree substrates

Variables	*Acacia caffra*				*Acacia karroo*				*Jacaranda mimosifolis*			
	MEAN± SD	**MEDIAN**	**MIN- MAX**	**CV**	**MEAN± SD**	**MEDIAN**	**MIN- MAX**	**CV**	**MEAN± SD**	**MEDIAN**	**MIN- MAX**	**CV**
Altitude	1353.12±64.38	1346.5	1262-1456	4.76	1364.41±83.63	1342	1233-1505	6.13	1350.91±56.28	1344.5	1292-1536	4.17
Distance.emission.sources	0.63±0.28	0.6	0.3-1.2	43.88	0.56±1.53	0.1	0-7.5	274.19	0.15±1.2	0	0-10.01	777.29
Tree.circumference	80.21±15.94	76	56-107	19.87	82.87±14.81	79	60-130	17.87	94.26±14.97	92	60-142	15.88
LDV	64.29±26.52	58	8-131	41.25	68.34±35.48	67.5	5-140	51.92	34.39±19.85	35.5	3-87	57.72
Candelaria concolor	18.17±3.68	20	4-20	20.25	16.91±5.6	20	0-20	33.09	15.53±5.68	18	2-20	36.59
Canoparmelia texana	1.54±3.71	0	0-14	240.41	1.46±2.65	0	0-13	182.02	0.19±0.67	0	0-4	358.5
Culbersonia nubila	0.38±0.92	0	0-4	246.33	2.96±4.05	1	0-17	136.9	0.74±1.48	0	0-7	199.38
Flavopunctelia flaventior	1.42±3.61	0	0-15	254.86	3.16±4.04	2	0-15	127.91	0.57±2.06	0	0-14	360.71
Flavopunctelia soredica	3.17±4.04	1	0-12	127.57	6.5±7.37	2	0-20	113.35	1.11±3.25	0	0-17	291.72
Heterodermia speciosa	4.17±4.91	2.5	0-14	117.93	3.64±5.3	1	0-19	145.56	0.61±1.8	0	0-10	293.69
Hyperphyscia adglutinata	11.54±5.73	11.5	0-20	49.67	8.34±6.05	8	0-20	72.46	6.71±6.4	5	0-20	95.35
Hyperphyscia granulata	0±0	0	0-0	NA	0.27±2.15	0	0-18	793.21	0.31±1.88	0	0-15	597.21
Hyperphyscia pandani	0.04±0.2	0	0-1	489.9	0.01±0.12	0	0-1	836.66	0±0	0	0-0	NA
Lepraria spp.	7.17±7.53	5.5	0-20	105.12	9.9±8.6	11	0-20	86.84	1.66±2.97	0	0-13	179.39
Parmotrema austrosinense	6.29±6.36	5.5	0-20	101.12	9.23±5.97	10	0-19	64.73	4.39±5.45	2	0-20	124.36
Physcia tribacia	7±4.99	6.5	0-17	71.24	2.51±2.72	2	0-11	108.29	1.64±2.73	0	0-12	166.47
Physcia undulata	0.29±1.43	0	0-7	489.9	0.04±0.36	0	0-3	836.66	0.09±0.61	0	0-5	709.04
Pyxine cocoes	3.21±3.35	2.5	0-11	104.38	3.26±3.57	2	0-14	109.73	0.83±1.54	0	0-8	186.03
Pyxine petricola	0.04±0.2	0	0-1	489.9	0.09±0.5	0	0-4	587.31	0.11±0.5	0	0-3	435.41

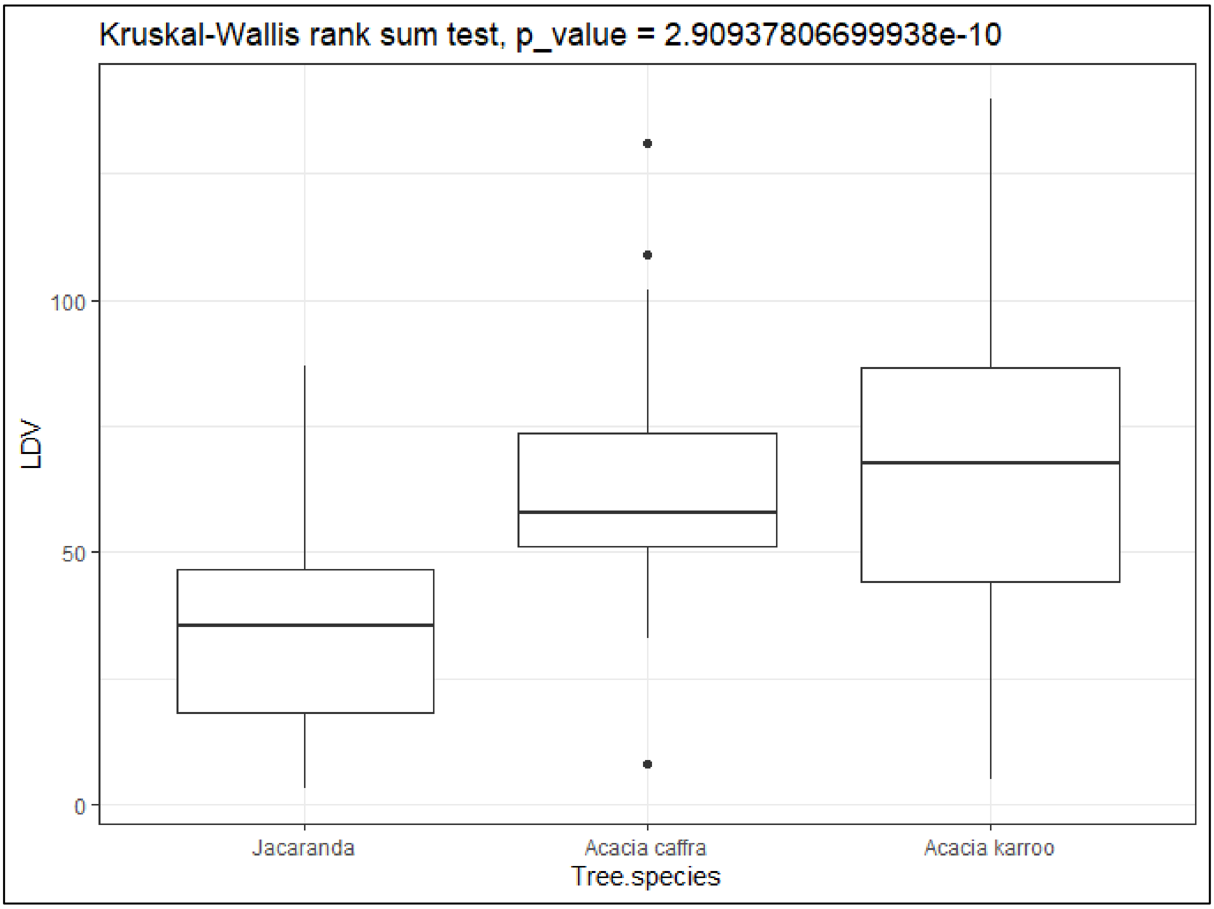

Figure 4.7. Distribution of LDV values for the three tree species

Table 4.6. Wilcoxon test between pairs of tree species

Acacia caffra - Acacia karroo:
Wilcoxon rank sum test with continuity correction
data: LDV by Tree.species W = 779.5, p-value = 0.6028 alternative hypothesis: true location shift is not equal to 0
Acacia karroo - Jacaranda
Wilcoxon rank sum test with continuity correction
data: LDV by Tree.species W = 3885, p-value = 2.242e-09 alternative hypothesis: true location shift is not equal to 0
Acacia caffra - Jacaranda
Wilcoxon rank sum test with continuity correction
data: LDV by Tree.species W = 1398, p-value = 1.328e-06 alternative hypothesis: true location shift is not equal to 0
LDV sampled in the three tree species are significantly different (Kruskal-Wallis test, p<0.001). The main differences are drived by the Jacaranda trees. The two species of Acacia show similar LDV.

Table 4.7 Descriptive statistics of LDV at tree and site level

Spatial level	N	Mean ± SD	Median	Min-Max
Tree total	164	53.3 ± 32.7	49	3-141
Acacia caffra	24	64.4 ± 26.5	58.5	8-132
Acacia karroo	70	68.3 ± 35.5	68.5	5-141
Jacaranda mimosifolia	70	34.5 ± 19.9	35	3-87
Site	29	46.7 ± 24.8	44.5	10.7-113.7

4.4.1.3 Comparison of sites

LDV values measured at each site are reported in Figure 4.8. The highest LDV values were observed in the protected areas such as Site 24 (Voortrekker Monument Nature Reserve), Site 6 (SANBI Botanical Garden), Site 5 (Groenkloof Nature Reserve), Site 9 (Rietvlei Nature Reserve) and Site 4 (Pionier Museum). Higher values were also found at site 25 (Suiderberg), which is characterised by low traffic. On the contrary, the sites with lower lichen diversity were Site 27 (The Willows 340-Jr,), Site 28 (Mamelodi), Site 22 (Hatfield) and Site 16 (Pretoria Central Business District, Nana Sita Street), all characterised by high traffic and tree disturbance.

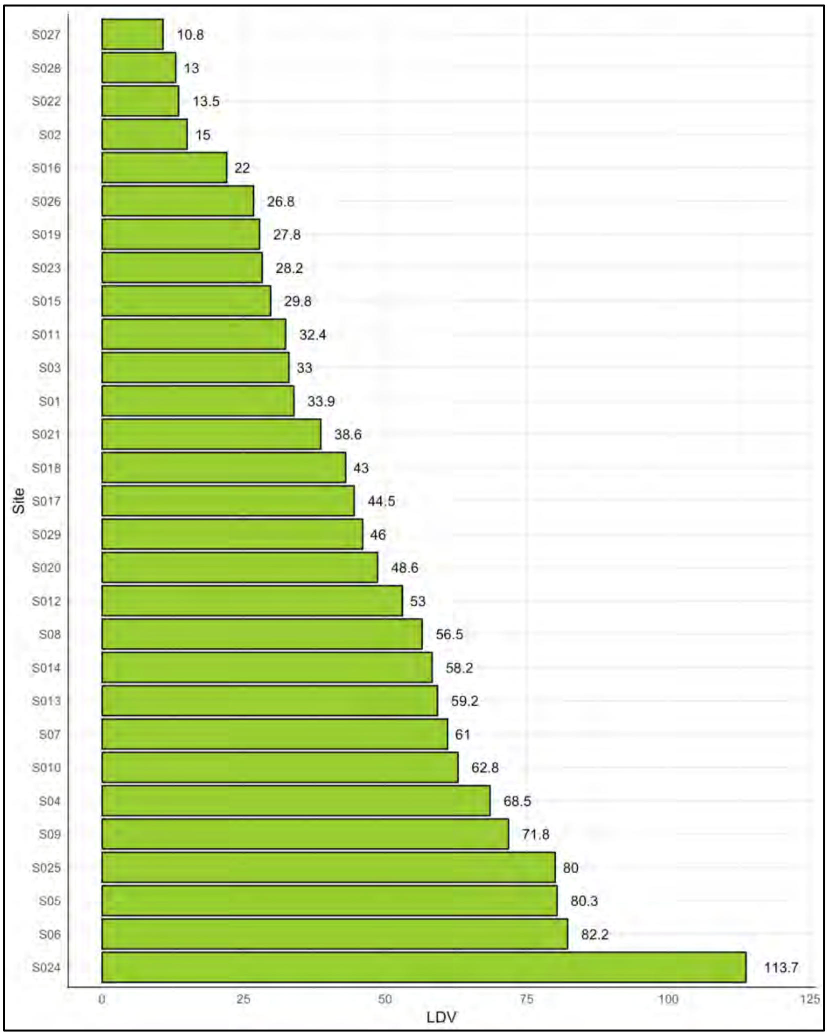

Figure 4.8. LDV values measured at each site (n: 29)

4.4.2 A naturality/alteration interpretative scale

A naturality/alteration interpretative scale was developed based on the percentile deviations from the maximum lichen diversity observed in the study area (Figure 4.9, Table 4.8). All examined sites were attributed to five naturality/alteration classes:

1) Lichen desert (LDV 0)
2) Alteration (LDV 1-35)
3) Semi-alteration (LDV 35-70)
4) Semi-naturality (LDV 70-100)
5) Naturality (LDV > 100)

Table 4.8. A naturality/alteration interpretative scale based on the percentile deviations

min	3
10° percentile	13
20° percentile	23
30° percentile	35
40° percentile	43
50° percentile	49.5
60° percentile	55
70° percentile	67.1
80° percentile	79.2
90° percentile	96.1
95° percentile	124.1
98° percentile	134.7
max	140

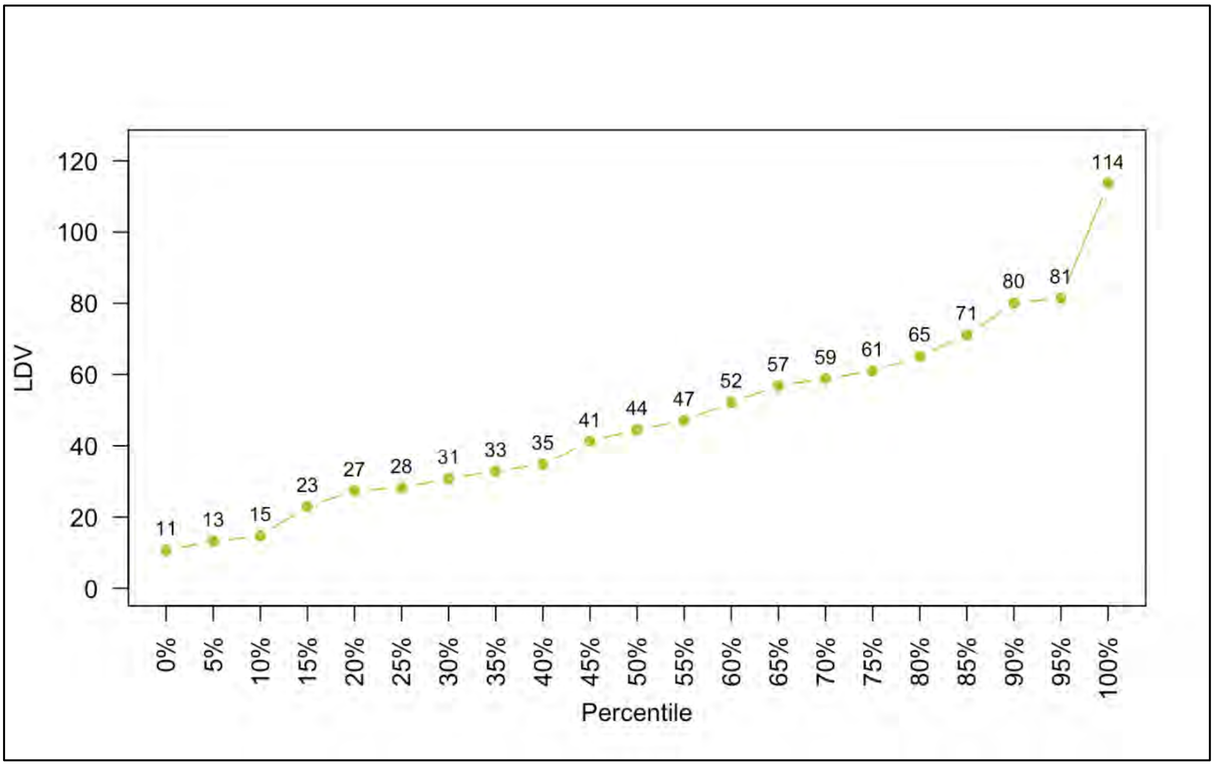

Figure 4.9. Percentile distribution of the LDV values detected at site level

The number of sites (n=29) within the five selected naturality/alteration classes is reported below in Figure 4.10. Most sites have LDVs between 1 and 70 (alteration and semialteration), while conditions of seminaturality (LDV 70-100) and of naturality (LDV>100) are poorly represented. A “lichen desert” condition was never found.

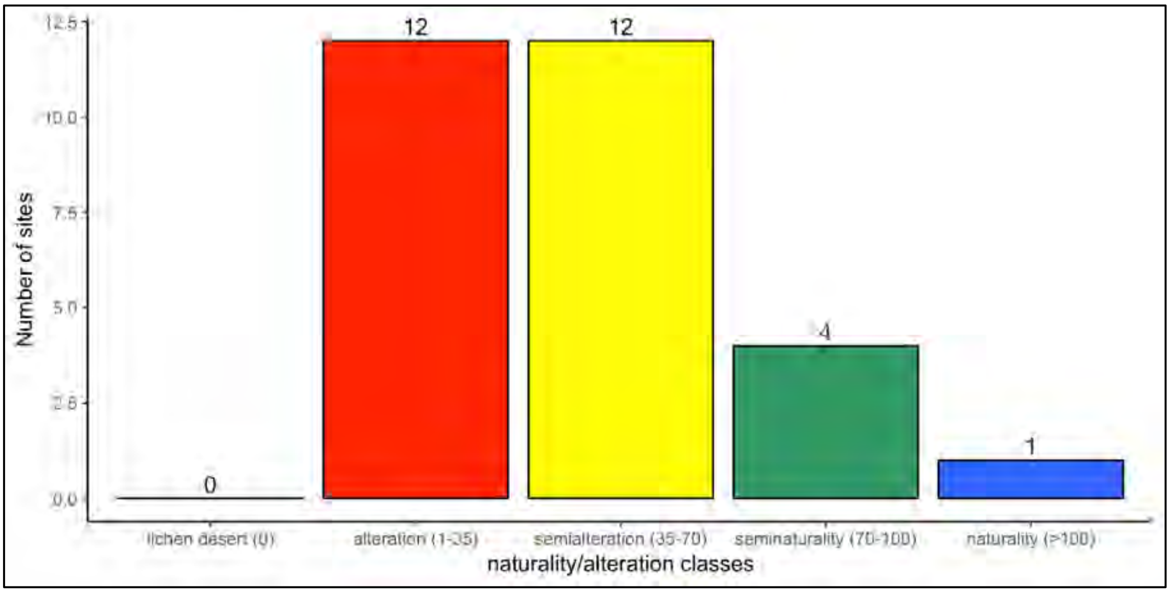

Figure 4.10. Distribution of LDV values at site level (n=29) categorised into five naturality/alteration classes

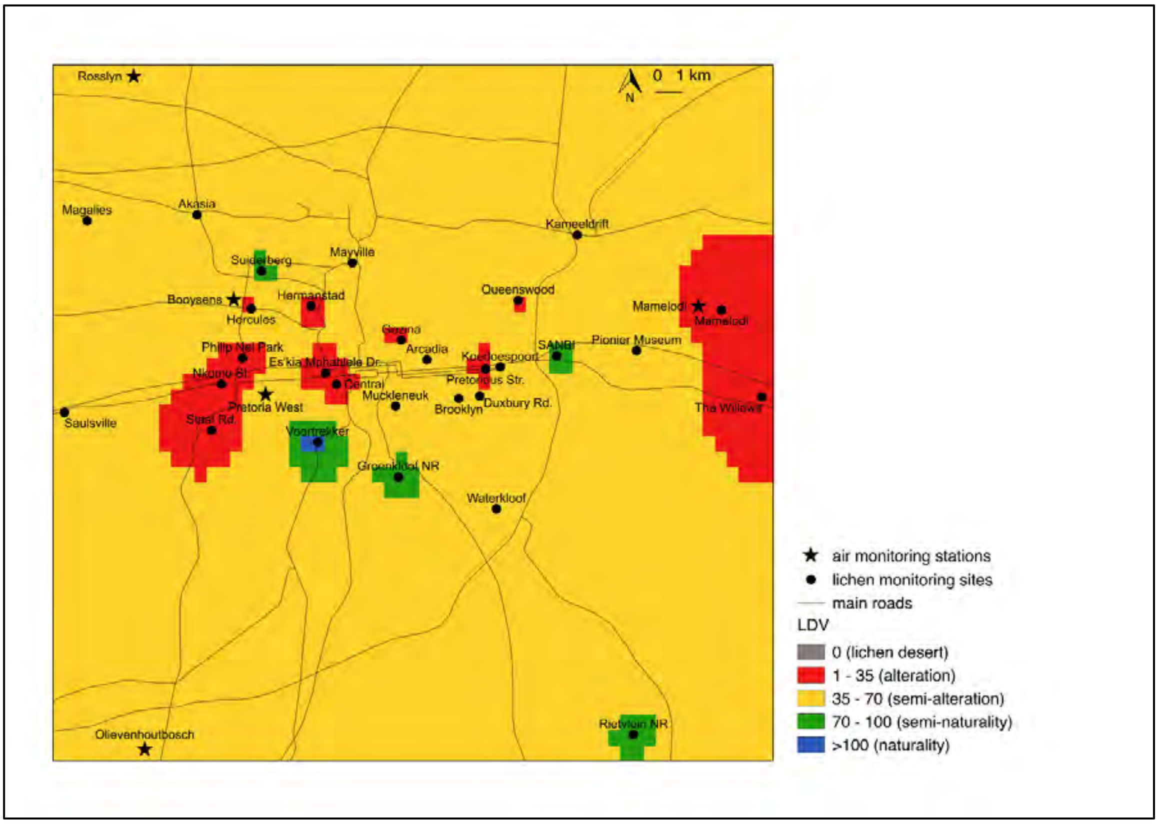

Figure 4.11. Interpolated LDV map of the study area with air monitoring stations, monitoring sites, main roads and five naturality/alteration classes

The alteration class (LDV 1-35) comprises industrial, urban and rural areas such as Mamelodi, Pretoria central business district, Pretoria west (Staal road) and Philip Nel among others. The semi-alteration class (LDV 35-70) comprises, for instance, the sites Magalies, Mayville, Waterkloof and Pionier Museum. To the semi-naturality class (LDV 70-100) belong parks and nature reserves, like Rietvlei Nature Reserve, Groenkloof Nature Reserve, SANBI and Suiderberg. Voortrekker Monument Nature Reserve represents the only naturality class (LDV > 100).

Most trees found in “Industrial areas and busy roads” belong to the class “semi-alteration” and “alteration” (50+56), only 14 to the “semi-naturality” class, while no tree from sites with this land use type occur under the class “naturality”. No trees belonging to the class “alteration” are found in “Parks and natural reserves”. Thirteen trees from these sites are found in the class “semi-alteration”, 16 in “semi-naturality and 15 in “naturality” (Table 4.9).

Table 4.9. Number of trees and Mean LDV under the 5 naturality/alteration classes and their distribution under the two land use categories

LDV	**Naturality/alteration classes**	**Mean LDV**	**N trees**	**Industrial areas and busy roads (n=120)**	**Parks and natural reserves (n=44)**
0	lichen desert	-	0	0	0
0-35	alteration	18.5	50	50	0
35-70	semi-alteration	50.8	69	56	13
70-100	semi-naturality	82.4	30	14	16
>100	naturality	122.6	15	0	15

As shown in Table 4.10, *Jacaranda mimosifolia* is exclusively found in “Industrial areas and busy roads” and is most frequent in the class “alteration”, but also common in the “semi-naturality” class. *Acacia caffra* trees are found both in “Industrial areas and busy roads“ and in “Parks and nature reserves“, but this tree species is more frequent in the last land use type and in the classes “semi-alteration” and “semi-naturality”. *A. karroo* also occurs under both land use types, but is more common in “Industrial areas and busy roads” rather than in “Parks and nature reserves”. It is also more common in the classes “semi-alteration” and “semi-naturality”. Considering all tree species, trees belonging to “Industrial areas and busy roads” occur more frequently in the classes “alteration” and “semi-alteration”, while the tree species found in “Parks and nature reserves” follow within the categories “semi-alteration” to “naturality”.

Table 4.10. Comparison of host tree species (phorophytes) with regard to their occurrence in the five naturality/alteration classes and within the two land use types

	Total	**Land use**	
		Industrial areas and busy roads	**Parks and nature reserves**
Jacaranda mimosifolia			
N of trees	70	70	0
Mean LDV	34.4	34.4	-
LDV, range min-max	3 - 87	3 - 87	-
Naturality/alteration classes (n trees, %)			
lichen desert	0 (0%)	0 (0%)	-
alteration	**35 (50%)**	**35 (50%)**	-
semi-alteration	4 (6%)	4 (6%)	-
semi-naturality	31 (44%)	31 (44%)	-
naturality	0 (0%)	0 (0%)	-
Acacia caffra			
N of trees	24	4	20
Mean LDV	64,3	29,7	71,2
LDV, range min-max	8 - 131	8 - 43	43 - 131
Naturality/alteration classes (n trees, %)			
lichen desert	0 (0%)	0 (0%)	0 (0%)
alteration	3 (13%)	**3 (75%)**	0 (0%)
semi-alteration	**12 (50%)**	1 (25%)	**11 (55%)**
semi-naturality	6 (25%)	0 (0%)	6 (30%)
naturality	3 (13%)	0 (0%)	3 (15%)
Acacia karroo			
N of trees	70	46	24
Mean LDV	68,3	50,6	102,4
LDV, range min-max	5 - 140	5 - 97	53 - 140
Naturality/alteration classes (n trees, %)			
lichen desert	0 (0%)	0 (0%)	0 (0%)
alteration	12 (17%)	12 (26%)	0 (0%)
semi-alteration	**26 (37%)**	**24 (52%)**	2 (8%)
semi-naturality	20 (29%)	10 (22%)	**10 (42%)**
naturality	12 (17%)	0 (0%)	12 (50%)
Total			
N of trees	164	120	44
Mean LDV	53,3	40,4	88,2
LDV, range min-max	3 - 140	3 - 97	43 - 140
Naturality/alteration classes (n trees, %)			
lichen desert	0 (0%)	0 (0%)	0 (0%)
alteration	50 (30%)	50 (42%)	0 (0%)
semi-alteration	**69 (42%)**	**56 (47%)**	13 (30%)
semi-naturality	30 (18%)	14 (12%)	**16 (36%)**
naturality	15 (9%)	0 (0%)	15 (34%)

4.4.3 Multivariate analysis

The results of a multivariate analysis (Principal Component Analysis, PCA) performed at tree level for different parameters are presented in Figures 4.12 to 4.15. Lichen species present in less than 5% of trees were excluded from the analysis. Axis 1 explains 50.29%, while Axis 2 explains 11.7% (Table 4.11).

Concerning single variables (Table 4.12), lichen diversity (LDV) correlates positively and significantly with Axis 1 (coefficient=0.992) but much less with Axis 2 (coefficient=0.083). The parameter “Number of species” correlates positively with Axis 1 (coefficient=0.883) but less with Axis 2 (coefficient=0.159). Also the occurrence of the following single species correlates positively with Axis 1: *Canoparmelia texana*, *Culbersonia nubila*, *Flavopunctelia flaventior*, *F. soredica*, *Heterodermia speciosa*, *Lepraria* spp., *Parmotrema austrosinense*, and *Pyxine cocoes*. *Canoparmelia texana*, *Culbersonia nubila, Flavopunctelia flaventior, F. soredica, Heterodermia speciosa* and *Lepraria* spp. are negatively related to Axis 2. Distance from emission sources" is positively related to Axis 1, while "Tree circumference" is negatively related. In contrast, *Candelaria concolor*, *Hyperphyscia adglutinata* and *Physcia tribacia* are more positively related to Axis 2 than to Axis 1. These relationships are charted in Figure 4.12.

Table 4.11. Eigen values of the PCA solution

	Dimensions												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Variance	6.537	1.524	0.988	0.805	0.678	0.58	0.506	0.444	0.328	0.293	0.173	0.142	0.002
% of variance	50.285	11.721	7.597	6.193	5.218	4.458	3.891	3.412	2.524	2.256	1.332	1.095	0.018
Cumulative % of variance	50.285	62.006	69.603	75.796	81.014	85.472	89.362	92.774	95.299	97.555	98.887	99.982	100

Table 4.12. Significant correlation coefficients of the variables with Axis 1 and 2 (p<0.001)

Variables	Axis 1	Axis 2
LDV	0.992	0.083
N of species	0.883	0.159
Flavopunctelia soredica	0.851	-0.317
Lepraria spp.	0.823	-0.134
Flavopunctelia flaventior	0.778	-0.092
Culbersonia nubila	0.762	-0.306
Parmotrema austrosinense	0.736	0.109
Heterodermia speciosa	0.684	-0.206
Canoparmelia texana	0.674	-0.358
Pyxine cocoes	0.570	0.282
Candelaria concolor	0.478	0.617
Hyperphyscia adglutinata	0.375	0.607
Physcia tribacia	0.159	0.509
Distance from emission sources	0.072	ns
Tree circumference	-0.235	ns

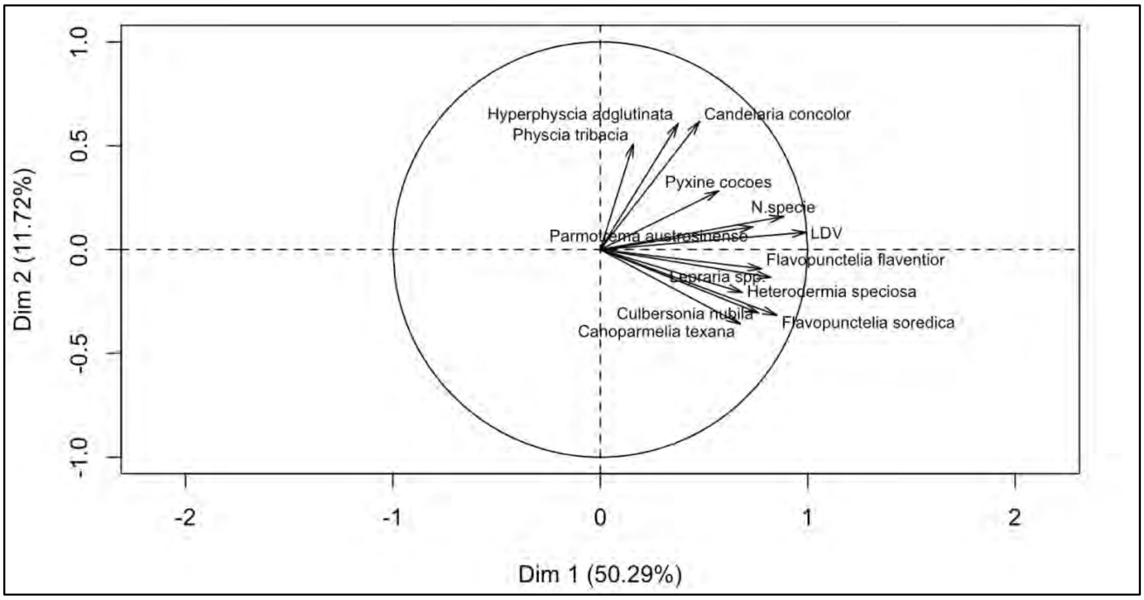

Figure 4.12. Loadings plot of the PCA ordination of lichen diversity parameters (LDV, N. of species, lichen taxa)

Figure 4.13. compares LDV and lichen species within the two land use types and shows significant differences between “Parks and natural reserves” and “Industrial areas and busy roads”. “Parks and natural reserves” are also far from emission sources and are therefore distinct from “Industrial areas and busy roads”, which are more directly impacted by emissions.

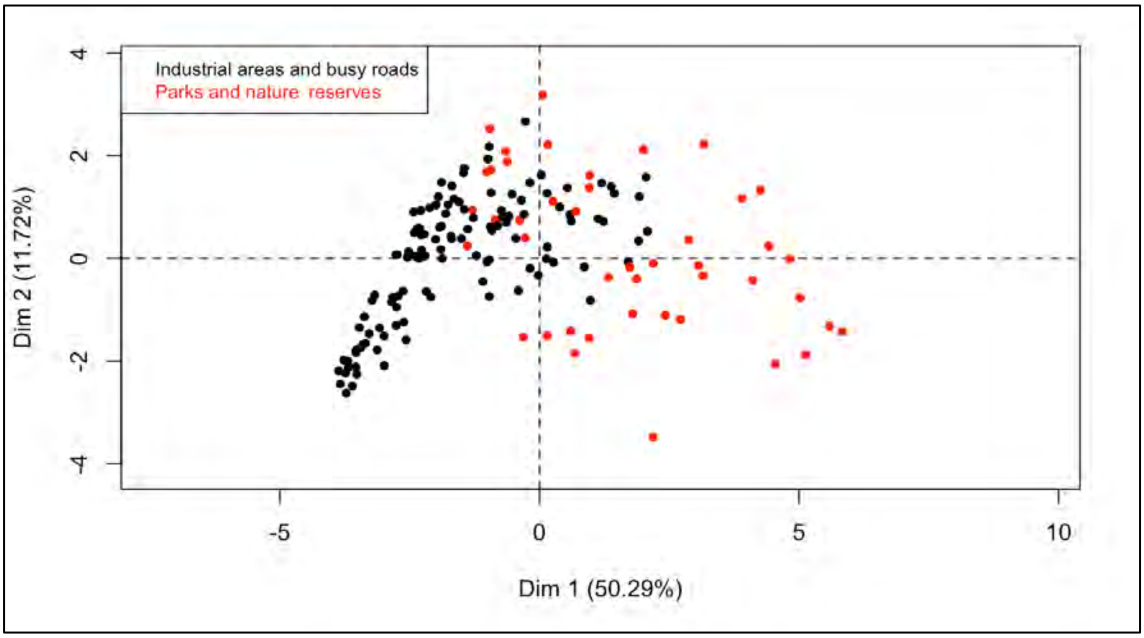

Figure 4.13. Score plot of the PCA ordination of LDV and lichen species on sampled trees within different land use types: “Industrial areas and busy roads” and “Parks and natural reserves”

Figure 4.14 below shows differences in LDV and lichen species composition among the tree species. In this aspect, *Jacaranda* trees are clearly distinguished from *Acacias*, with only some overlap with *A. karroo*, with *Jacaranda* generally closer to emission sources in contrast to the other two species.

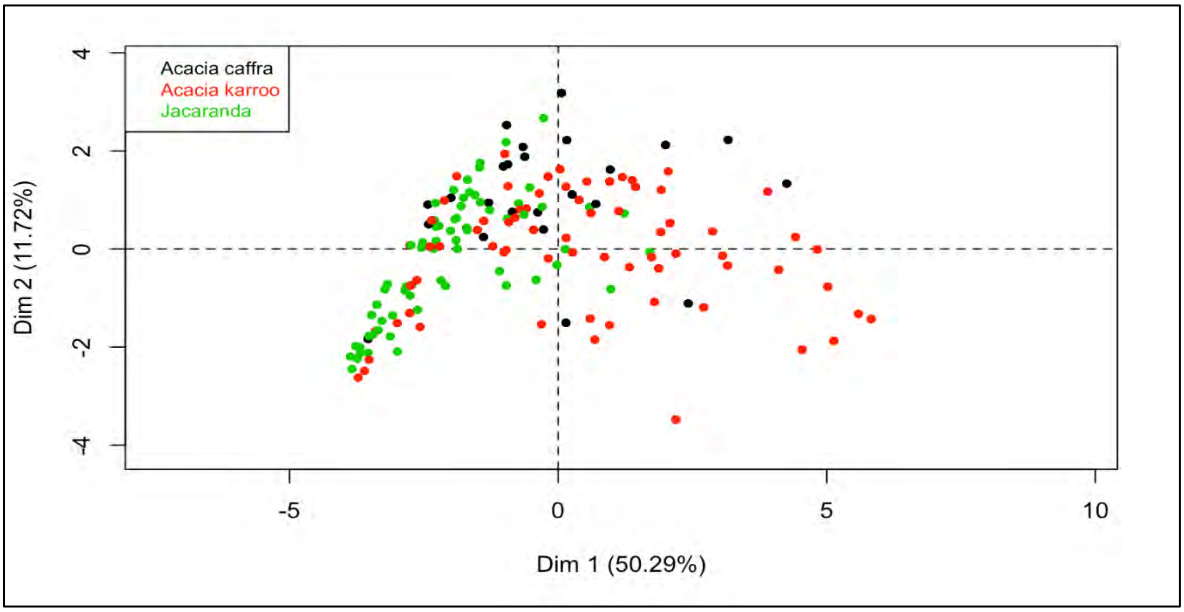

Figure 4.14. Score plot of the PCA ordination of LDV and lichen species on the selected tree substrates *Acacia caffra, A. karroo* and *Jacaranda mimosifolia*

Figure 4.15 shows which environmental variables correlate significantly with the two axes. Axis 1 has a positive relationship with the factor “Distance from emission sources” and a negative relationship with the parameter “Tree circumference”.

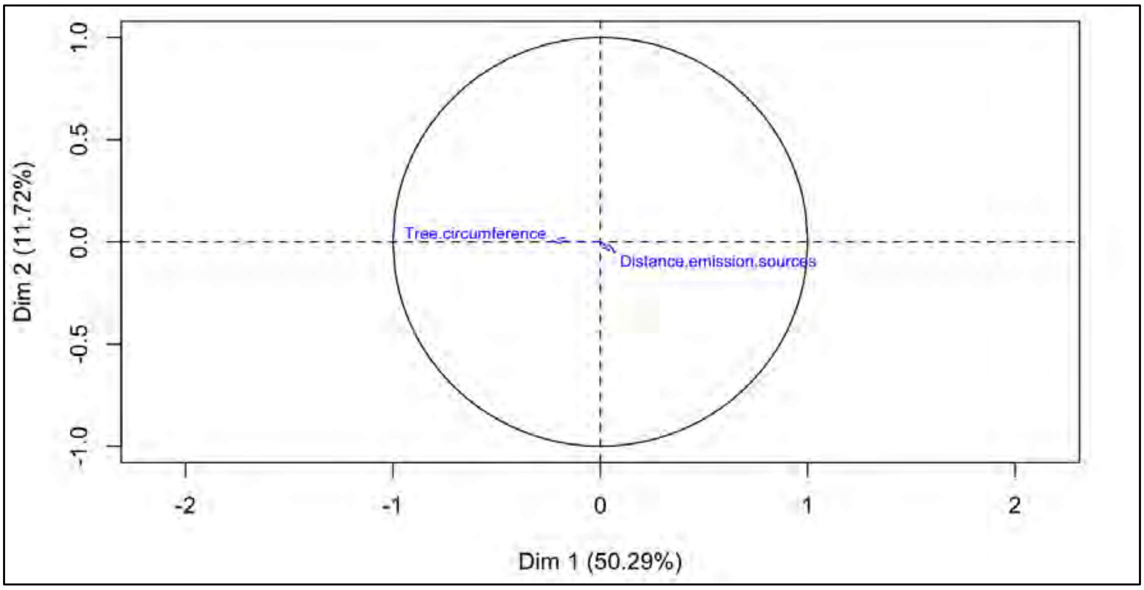

Figure 4.15. Loadings plot of the PCA ordination showing vectors with statistically significant relationship to the environmental variables “Distance from emission sources” and “Tree circumference”

4.4.4 Interpolation of air pollution and climate data

As air pollution and climate data were only available for five monitoring stations, an interpolation was carried out to obtain data for all 29 lichen sampling sites to test correlations with lichen diversity variables (see 4.4.5-4.4.7). Table 4.13 shows the interpolated values (IDW) of the air pollution, climate variables obtained with the elaboration and LDV at each site. “Industrial areas and busy roads” are coloured orange and sites from “Parks and nature reserves” are coloured green.

Table 4.13. Interpolated values (IDW) of the air pollution, climate variables and LDV obtained for the 29 lichen sampling sites

Site	ID	CO	NO	NO_2	NO_X	PRES	SO_2	TEMP	WIND dir	WIND Speed	PM_{10}	O_3	HUM	LDV
1	Pretoria West - Nkomo Street	0.73	10.3	13.8	23.4	794	4.86	17.8	177	2.16	87.72	19.1	59.5	33.9
2	Pretoria West - Staal Road	0.72	10.7	14.3	23.9	793	4.89	17.8	177	2.19	88.06	19.3	58.7	15
3	Hercules - Van Der Hoff	1.09	7.6	8.2	17.0	832	3.36	19.3	155	1.37	40.29	17.0	60.4	33
7	Waterkloof - Rigel Avenue	1.00	17.2	27.7	37.5	796	4.04	18.4	158	1.95	63.22	20.9	56.2	61
8	Muckleneuk - Celliers Street	0.84	12.3	17.9	27.5	799	4.50	18.1	169	2.04	75.12	19.6	57.6	56.5
10	Arcadia - Government Avenue	0.93	13.6	21.0	30.3	803	4.27	18.4	164	1.94	67.41	19.9	57.3	62.8
11	Queenswood - CR Swart Drive	1.22	21.4	39.0	48.2	797	3.70	18.7	145	1.85	54.83	21.7	55.8	32.4
12	Akasia - Brits Road	0.96	8.6	11.0	19.6	840	4.01	19.0	164	1.61	43.80	18.7	57.8	53
13	Mayville - Es'kia Mphahlele Drive	0.94	10.5	14.5	23.5	819	4.09	18.7	164	1.76	57.69	18.9	58.4	59.2
15	Hermanstad - Moot Street	0.93	9.4	12.0	21.1	816	4.06	18.7	165	1.74	60.31	18.3	59.6	29.8
16	Pretoria Central Business District - Skinner Street	0.75	10.9	14.9	24.5	795	4.80	17.8	175	2.15	85.38	19.3	58.6	22
17	Kameeldrift - Sefako Makgatho Drive	1.41	26.6	51.3	60.3	790	3.38	18.9	134	1.81	49.63	22.9	55.2	44.5
18	Saulsville - Masopha Street	0.83	10.8	13.5	23.2	812	4.30	18.5	169	1.90	62.23	19.5	57.4	43
19	Philip Nel Park - Transoranje Road	0.79	9.9	12.9	22.3	801	4.61	18.1	173	2.03	79.65	18.7	59.9	27.8
20	Brooklyn - Roper Street	0.98	15.5	25.0	34.4	800	4.17	18.4	160	1.95	65.65	20.4	56.7	46.8
21	Hatfield - Duxbury Road	1.04	17.0	28.4	37.8	799	4.05	18.5	157	1.92	62.94	20.7	56.4	36.8
22	Hatfield - Pretorious Street	1.07	17.7	30.2	39.6	799	3.98	18.5	155	1.90	61.07	20.9	56.3	13.5
23	Gezina - Rose Street	0.90	12.3	18.2	27.6	806	4.32	18.3	166	1.94	68.25	19.5	57.8	28.2
26	Pretoria West - Es'kia Mphahlele Drive	0.75	10.7	14.5	24.1	795	4.82	17.8	176	2.15	85.87	19.2	58.9	26.8
27	The Willows - Solomon Mahlangu Drive	1.57	31.4	62.3	71.3	780	3.08	19.0	123	1.80	47.01	23.9	54.8	10.8
28	Mamelodi - Tsamaya Avenue	1.68	34.2	69.0	77.9	775	2.90	19.0	117	1.78	45.06	24.4	54.7	13
29	Koedoespoort - N4 Highway	1.12	19.0	33.3	42.6	798	3.89	18.6	152	1.89	59.30	21.2	56.1	46
25	Suiderberg - Sarel Avenue	1.06	7.9	9.0	17.8	831	3.49	19.2	157	1.43	42.96	17.3	60.1	80
14	Magalies - Hornsnek Road	0.87	9.0	12.5	20.8	853	4.50	18.8	171	1.77	42.62	20.1	55.3	58.2
4	Pionier Museum - Keuning Drive	1.60	32.0	63.7	72.7	779	3.04	19.0	122	1.79	46.71	24.0	54.8	68.5
5	Groenkloof Nature Reserve	0.84	13.1	18.8	28.5	797	4.46	18.1	168	2.05	74.18	19.9	57.1	80.3
6	SANBI - Cussonia Avenue	1.34	24.9	47.2	56.3	790	3.49	18.8	138	1.84	52.76	22.5	55.4	82.2
9	Rietvlein Nature reserve - R21	1.00	18.6	28.9	39.1	792	3.86	18.6	155	1.94	57.88	21.5	55.8	71.8
24	Voortrekker Monument - Eeufees Road	0.71	11.1	15.2	24.9	791	4.93	17.7	177	2.22	89.90	19.5	58.2	113.7

Some of the obtained values are questionable as many sites with high traffic have low values of NO, NO_2 and NO_x. For example, Site 2 (Pretoria West - Staal Road) has lower values of these pollutants, while site 24 (Voortrekker Monument - Eeufees Road), in a more protected area with low traffic, has higher values for those pollutants. This is despite LDV being much higher in Voortrekker Monument compared to Pretoria West. The same results were observed at site 6 (SANBI), with levels of NO, NO_2 and NO_x very high compared to site 22 (Hatfield - Pretorious Street) which is characterised by high traffic but low values of these pollutants. As the table shows, the LDV was higher at SANBI compared to Hatfield. This shows that LDV is a more reliable indicator of good air quality compared to interpolated pollution data which does not give a true reflection of the degree of pollution in Pretoria, due to insufficient numbers of monitoring stations. Other interpolated pollution parameters also proved unreliable: SO_2, PM_{10} and O_3 were lowest at site 28 (Mamelodi - Tsamaya Avenue) which is however characterised by high traffic and low LDV, while the highest values were found at Voortrekker Monument, an area with the highest LDV.

Figures 4.16 and 4.17 show the interpolated maps for NO_x and SO_2 in the study area. They confirm that interpolated data does not give true reflections. NO_x is mainly concentrated in the eastern part of the study area according to the map, whereas SO_2 is concentrated in the western and northern sites of the study area. This is erroneous and can also be explained by the low number of monitoring stations in the municipality, which do not provide representative data for the entire area and all investigated sites.

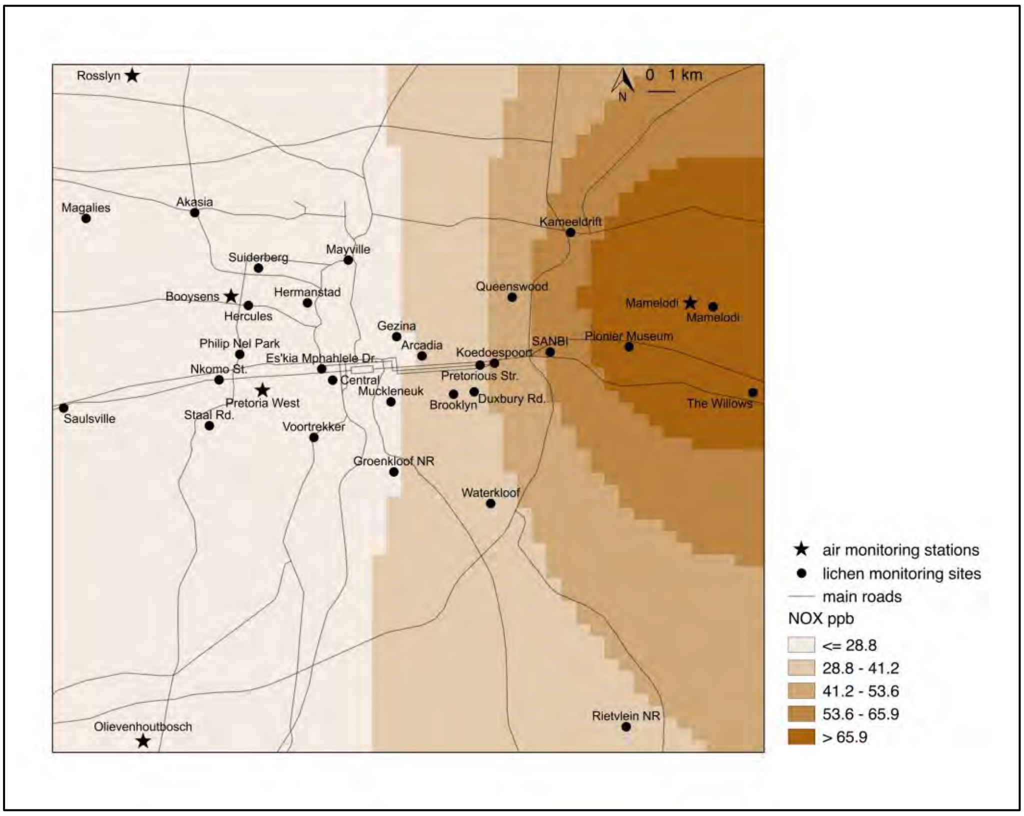

Figure 4.16. Interpolated map (IDW) of NO_x (ppb) concentrations in the study area

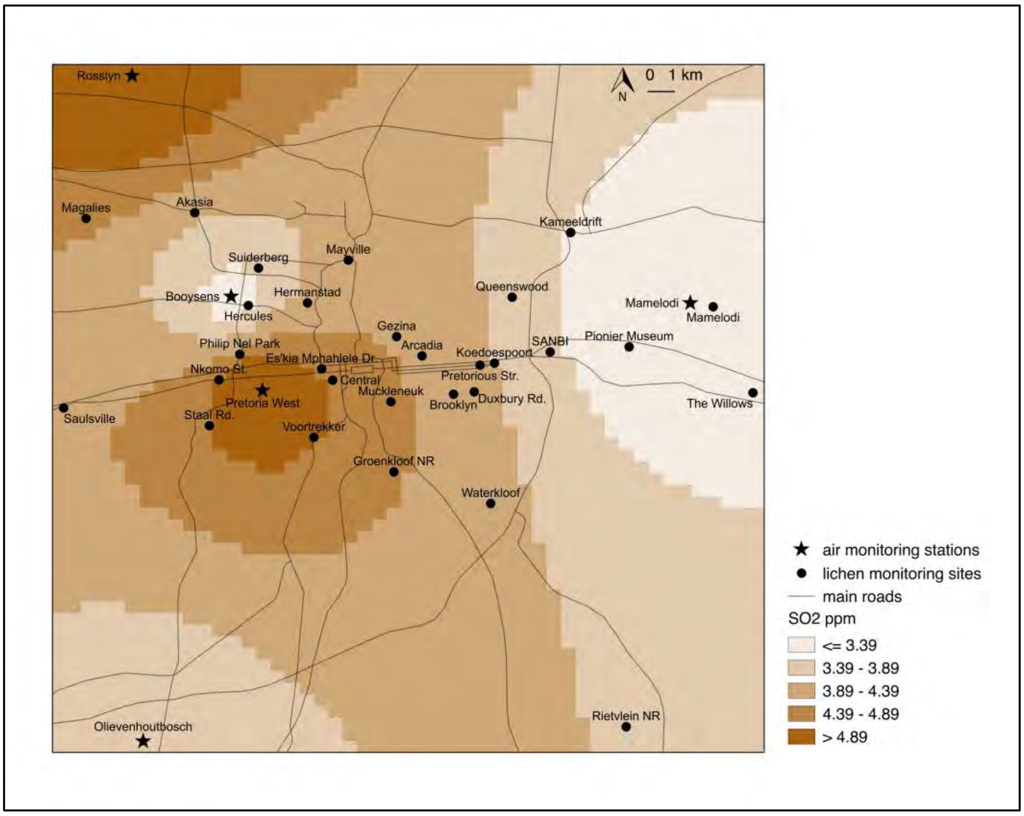

Figure 4.17. Interpolated map (IDW) of SO_2 (ppb) concentrations in the study area

4.4.5 Multiple Factor Analysis (MFA) with interpolated environmental data

A Multiple Factor Analysis (MFA) was carried out with the interpolated environmental data. Table 4.14 shows the correlation coefficients of the three groups of variables with the dimensions of the Multiple Factor Analysis (MFA). The first two dimensions explain respectively only 34.2% (Dim.1) and 18.4% (Dim.2) of the variance (see Figures 4.18 and 4.20). The climatic variables PRES, WIND, WINDS and HUM correlate positively with Dim.1, whereas TEMP correlates negatively. All variables, excepting WINDS, correlate negatively with Dim.2. PRES and TEMP correlate positively with Dim.3, WINDS negatively. Pollutants CO, NO, NO_2, NO_x and O_3 are negatively correlated with Dim.1, while SO_2 and PM_{10} are positively correlated. NO, NO_2, NO_x, PM_{10} and O_3 correlate positively with Dim. 2. Correlations with Dim. 3 are weaker and in most cases negative.

Lichen diversity (LDV) and a large group of lichen species (*Candelaria concolor*, *Canoparmelia texana*, *Flavopunctelia flaventior*, *F. soredica*, *Heterodermia speciosa, Parmotrema austrosinense* and *Lepraria* spp. are positively correlated with all three Dim. *Physcia tribacia* is negatively related to Dim.1, but positively to the other two Dim. *Hyperphyscia adglutinata* and *Pyxine cocoes* are significantly and positively correlated only with Dim.2 and Dim.3.

Table 4.14. Correlation coefficients of the variables with the three dimensions of the Multiple Factor Analysis. The highest correlations (>0.2) with the 3 dimensions are highlighted.

Groups	Variables	Dim.1	Dim.2	Dim.3
Climatic variables	PRES	**0,205**	**-0,774**	**0,517**
	TEMP	**-0,789**	**-0,400**	**0,454**
	WIND	**0,969**	-0,189	-0,062
	WINDS	**0,600**	**0,583**	**-0,521**
	HUM	**0,746**	**-0,429**	-0,018
Pollutants	CO	**-0,972**	0,149	0,084
	NO	**-0,859**	**0,483**	-0,152
	NO_2	**-0,870**	**0,460**	-0,140
	NO_X	**-0,862**	**0,474**	-0,150
	SO_2	**0,944**	0,060	**-0,234**
	PM_{10}	**0,795**	**0,376**	**-0,457**
	O_3	**-0,796**	**0,545**	-0,177
Lichen diversity variables	LDV	**0,391**	**0,579**	**0,703**
	Candelaria concolor	**0,284**	0,103	**0,518**
	Canoparmelia texana	**0,220**	**0,544**	**0,332**
	Culbersonia nubila	**0,459**	**0,502**	**0,269**
	Flavopunctelia flaventior	**0,278**	**0,534**	**0,465**
	Flavopunctelia soredica	**0,397**	**0,580**	**0,415**
	Heterodermia speciosa	**0,261**	**0,560**	**0,332**
	Hyperphyscia adglutinata	0,062	**0,202**	**0,335**
	Hyperphyscia granulata	0,022	-0,045	-0,037
	Hyperphyscia pandani	0,119	0,095	-0,020
	Lepraria spp.	**0,420**	**0,331**	**0,595**
	Parmotrema	**0,232**	**0,410**	**0,544**
	Physcia tribacia	**-0,209**	**0,245**	**0,321**
	Physcia undulata	-0,031	0,139	0,069
	Pyxine cocoes	0,104	**0,213**	**0,576**
	Pyxine petricola	0,081	0,121	0,009

As shown in Figures 4.18 and 4.19, the increasing gradient of lichen diversity (LDV and a large group of species) along positive values of Dim. 1 is positively related to climatic variables such as wind direction and speed, and humidity, and pollutants PM_{10} and SO_2. On the contrary, most of the other pollutants (NO, NO_2, NO_x, CO and O_3) show an increasing gradient for negative values of Dim.1. Almost no lichens relate to temperature (Figures 4.18 to 4.20).

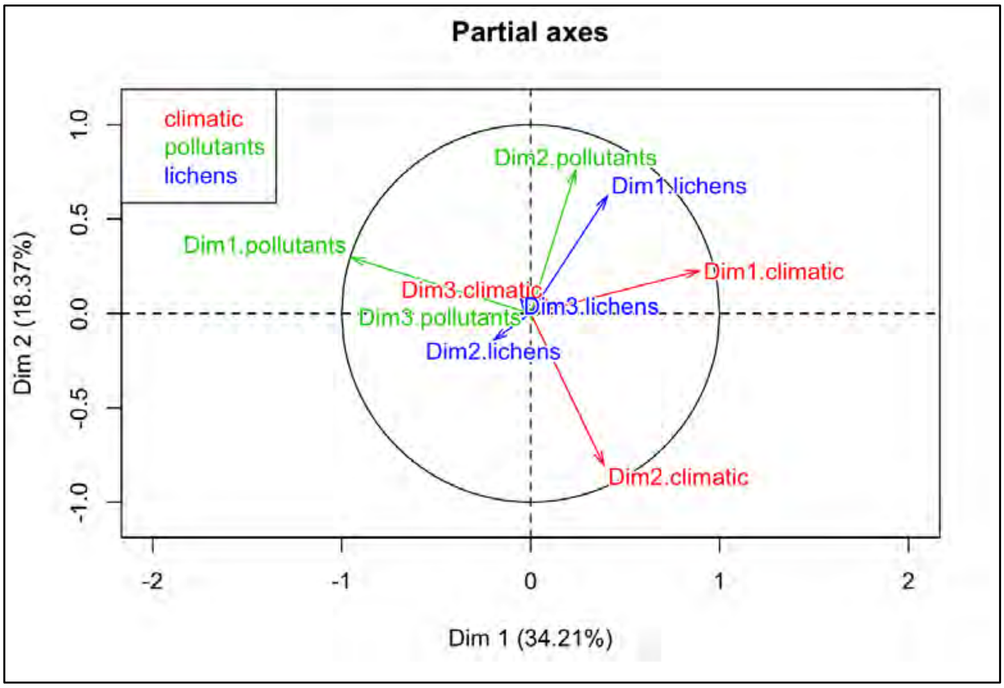

Figure 4.18. Loadings plot of the MFA ordination. Vectors show the axes of the single PCA performed on each of the three groups (climatic, pollutants and lichens)

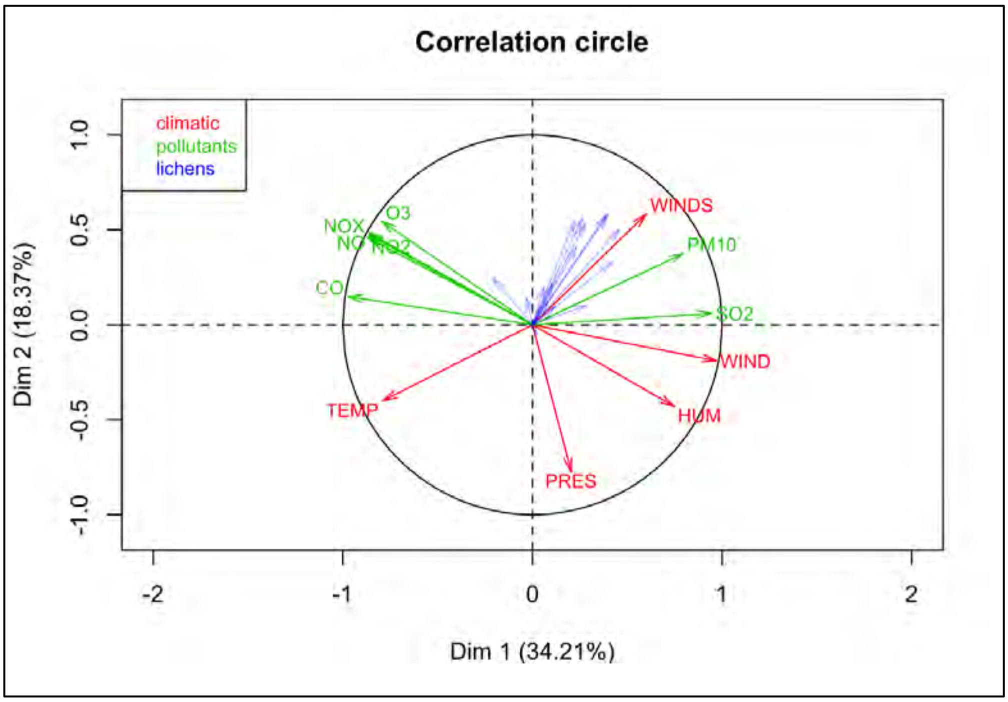

Figure 4.19. Loadings plot of the MFA ordination. Vectors show the correlation of environmental variables separated in the main three factors (climatic, pollutants and lichens) with the three dimensions of the MFA.

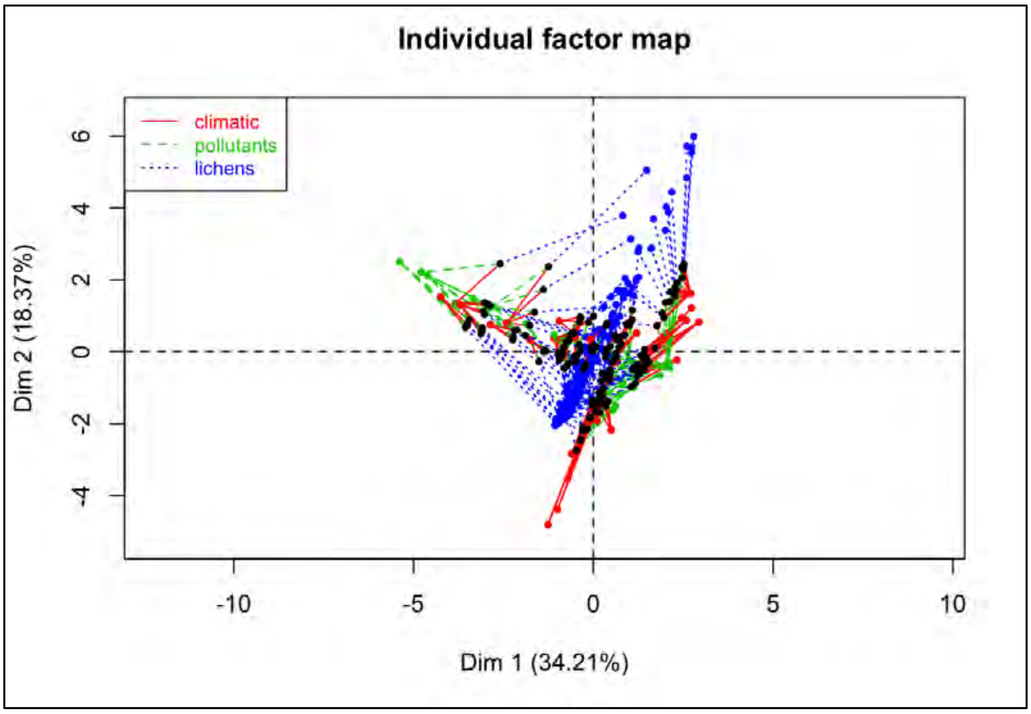

Figure 4.20. Score plot of the MFA ordination showing the distribution of the sampled trees and the partial contribution of the three environmental factors (climatic, pollutants and lichens) to the variance

4.4.6 Multivariate analysis (PCA) with interpolated environmental data

Table 4.15 shows the correlation coefficients of the variables with Axis 1 and 2. Axis 1 of the ordination explains 29.8% of variance and Axis 2 17.7%. Figures 4.21 to 4.25 report the PCA ordination results. Regarding site and tree parameters, only “Altitude” correlates significantly and positively with Axis 2. Other correlations were not significant. Pollutants CO, NO, NO_2, NO_x and O_3 are positively correlated with Axis 2, but in most cases correlations are not significant (only for O_3), while their negative relationships with Axis 1 are significant. SO_2 and PM_{10} are positively correlated with Axis 1. The negative correlations with Axis 2 are not significant. Significant correlations of the climatic variables with both axes are: WIND and HUM, which correlate positively with Axis 1 and negatively with Axis 2. TEMP correlates negatively with Axis 1. WINDS correlate positively with Axis 1 (Table 4.15).

Lichen diversity (LDV) and a numerous group of lichen species (*Canoparmelia texana*, *Culbersonia nubila, Flavopunctelia flaventior*, *F. soredica*, *Heterodermia speciosa, Lepraria* spp., and *Parmotrema austrosinense* are positively related to Axis 2 (Table 4.15).

Table 4.15. Correlation coefficients of the variables with Axis 1 and 2. Values >0.2 are highlighted.

	PC 1 (expl. var. 29.82%)	**PC 2 (expl. var. 17.67%)**
Altitude	0.158	**0.211**
Distance.emission.sources	0.011	0.088
Tree.circumference	-0.022	-0.086
CO	-0.311	0.116
NO	**-0.277**	0.197
NO_2	**-0.281**	0.190
NO_X	**-0.278**	0.194
PRES	0.075	**-0.207**
SO_2	**0.299**	-0.064
TEMP	**-0.245**	-0.031
WIND	**0.310**	-0.128
WINDS	0.185	0.099
PM_{10}	**0.245**	0.018
O_3	**-0.254**	**0.217**
HUM	**0.227**	**-0.210**
LDV	0.167	**0.328**
Candelaria concolor	0.114	0.111
Canoparmelia texana	0.096	**0.260**
Culbersonia nubila	0.171	**0.215**
Flavopunctelia flaventior	0.121	**0.277**
Flavopunctelia soredica	0.163	**0.290**
Heterodermia speciosa	0.111	**0.264**
Hyperphyscia adglutinata	0.034	0.124
Hyperphyscia granulata	0.002	-0.032
Hyperphyscia pandani	0.042	0.030
Lepraria spp.	0.171	**0.220**
Parmotrema austrosinense	0.103	**0.238**
Physcia tribacia	-0.053	0.162
Physcia undulata	-0.007	0.062
Pyxine cocoes	0.058	0.174
Pyxine petricola	0.028	0.042

Positive values of Axis 2 show therefore a clear increasing gradient of lichen diversity, from alteration to naturality classes (Figure 4.21). An increasing gradient is also present in relation to land use in “Parks and natural reserves” (Figure 4.22), where *Acacia* trees (both *A. karroo* and *A. caffra*) are mainly distributed (Figure 4.23). Lichen diversity is lower in “Industrial areas and busy roads’ and on *Jacaranda* trees, which are distributed in the lower parts of plots, having low or negative correlations with Axes 1 and 2 (Figure 4.22 and 4.23).

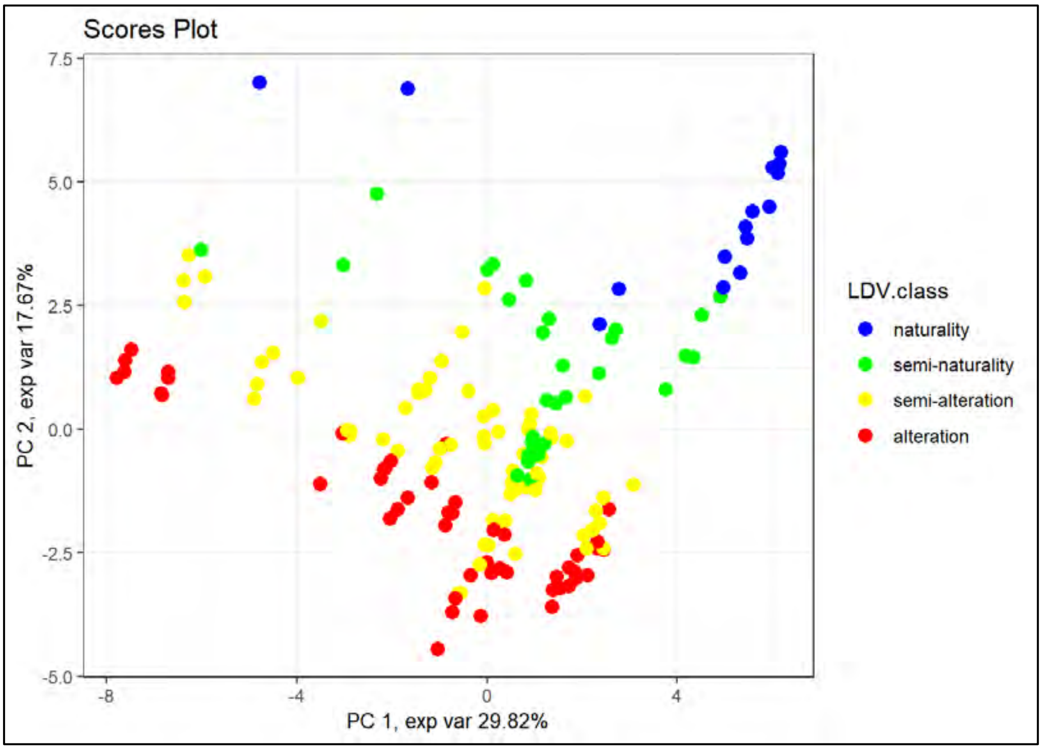

Figure 4.21. Score plot of the PCA ordination with tree substrates categorised by LDV classes

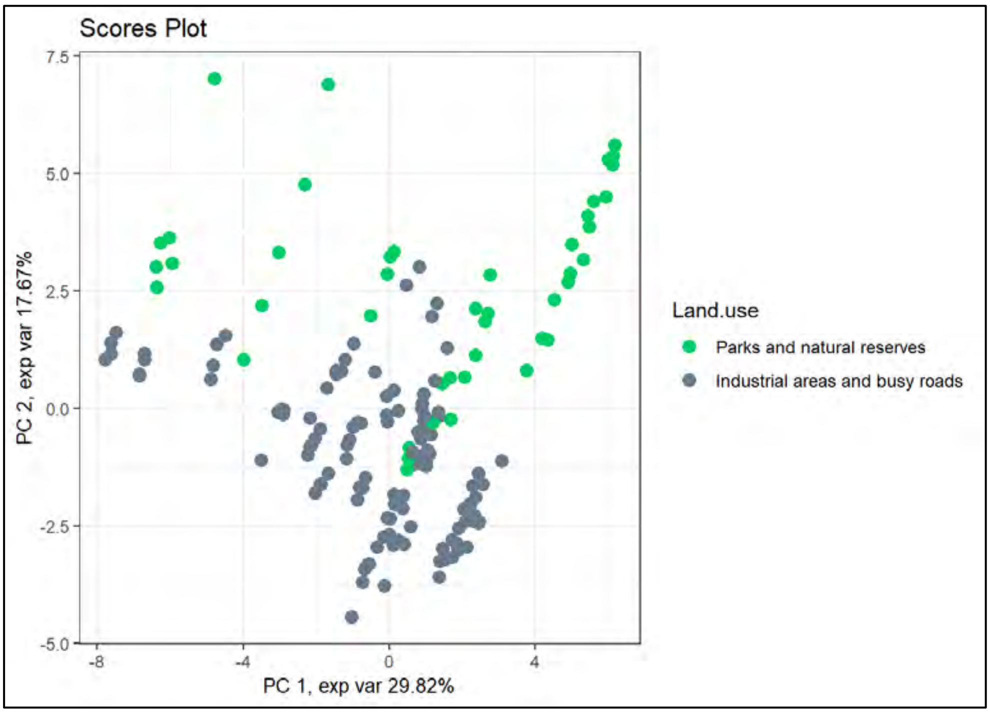

Figure 4.22. Score plot of the PCA ordination with sampled trees categorised by land use: industrial areas and busy roads, parks and natural reserves

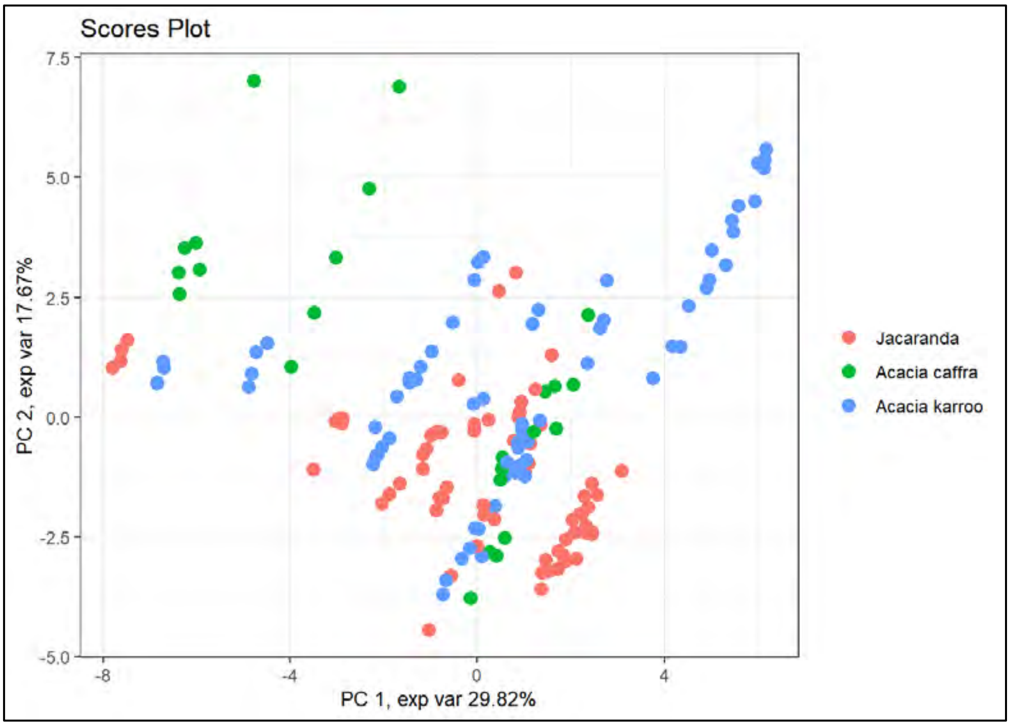

Figure 4.23. Score plot of the PCA ordination with tree substrates categorised by tree species (*Acacia caffra*, *A. karroo* and *Jacaranda mimosifolia*)

In addition, SO_2, wind, pressure and humidity are negatively correlated with Axis 2, even if with low correlation values (Figure 4.24). LDV and most of the lichen species such as *Candelaria concolor*, *Canoparmelia texana, Culbersonia nubila*, *Flavopunctelia flaventior, F. soredica, Heterodermia speciosa, Hyperphyscia adglutinata* and *Lepraria* spp. are distributed in the positive values of Axis 1, with an opposite trend with respect to the main atmospheric pollutants (O_3, NO, NO_2, NO_x and CO). These are distributed in the positive values of Axis 2, thus showing a clear negative influence of these variables on lichen diversity (Figure 4.24).

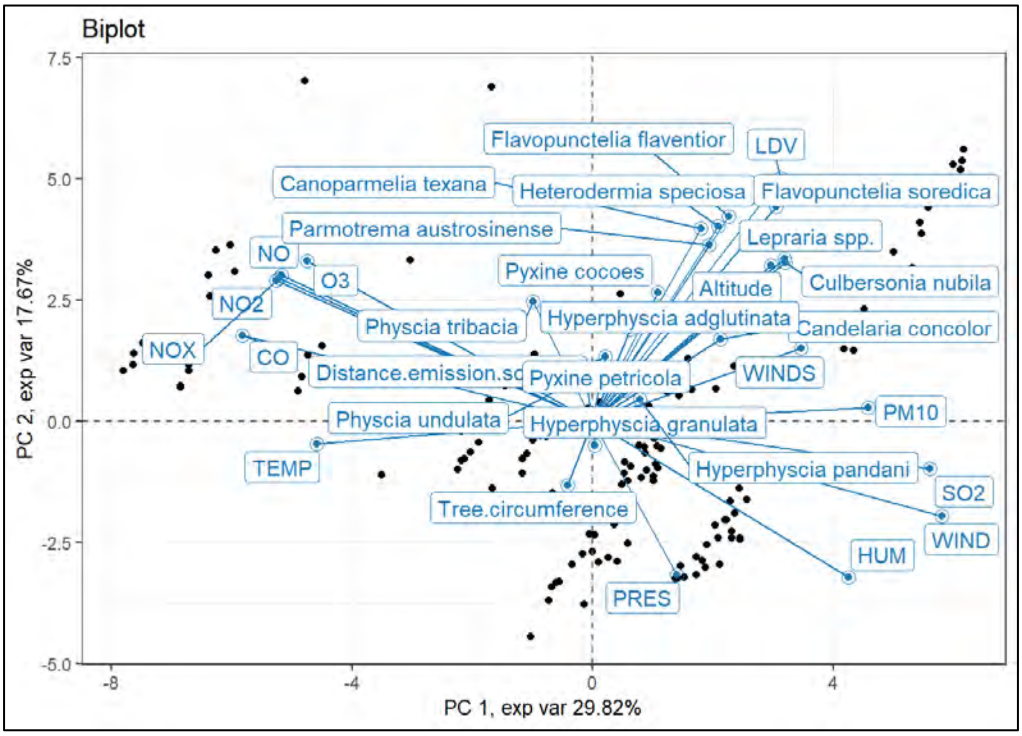

Figure 4.24. Loadings plot of the PCA ordination. Vectors show the correlation of environmental variables with the two axes.

The distribution of LDV in relation to land use and tree species is shown in Figure 4.25 below. Independently from the phorophyte, the lowest values were found in industrial areas and busy roads, and this is particularly evident for *Jacaranda* trees, that are mostly found under these conditions. The highest lichen diversity values where found in the parks and natural reserves where *Acacia caffra* and *A*. *karroo* are most frequently found.

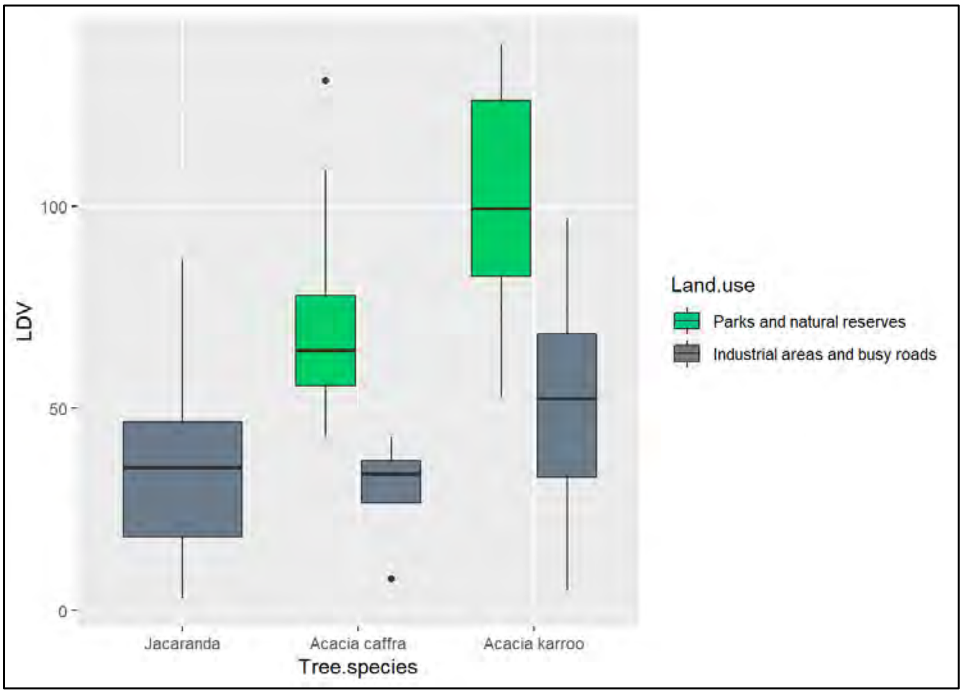

Figure 4.25. Distribution of LDV values in the species substrates in relation to the two land use categories. Boxplot: median, interquartile range, 1.5 interquartile range, outliers.

In order to explore in more detail the multivariate relationship between environmental variables and LDV of the tree species, and to discover any specific trends, PCA was also performed separately for each tree species. Figures 4.26 to 4.28 reported the PCA ordinations for *Jacaranda* trees, Figures 4.29 to 4.31 for *Acacia caffra*, and Figures 4.32 to 4.34 for *Acacia karroo*.

A general trend of lichen diversity is evident in relation to land use, with “industrial areas and busy roads” showing LDV values mainly included in the alteration classes, and, on the contrary, “parks and natural reserves” within naturality classes (Figures 4.26 to 4.27, 4.29 to 4.30 and 4.32 to 4.33). This trend is clearly related to the distribution of the atmospheric pollutants, which are negatively correlated with LDV values and with the frequency of lichen species (Figures 4.28, 4.31 and 4.34).

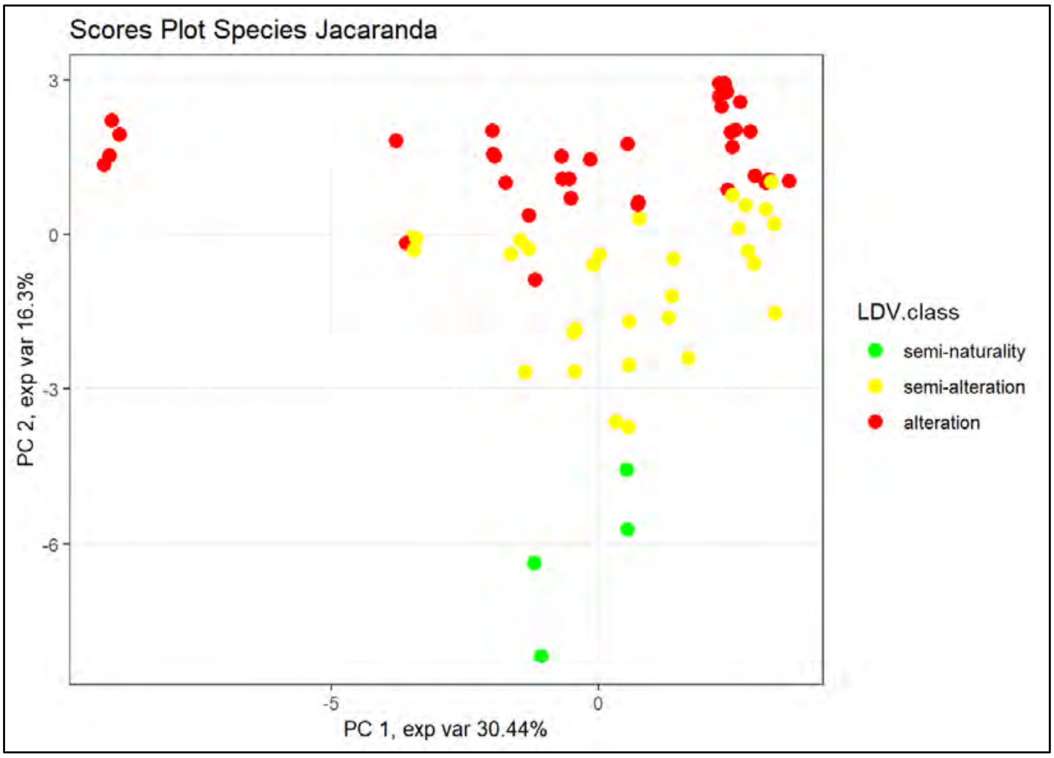

Figure 4.26. *Jacaranda* trees. Score plot of the PCA ordination with tree substrates categorised by LDV classes

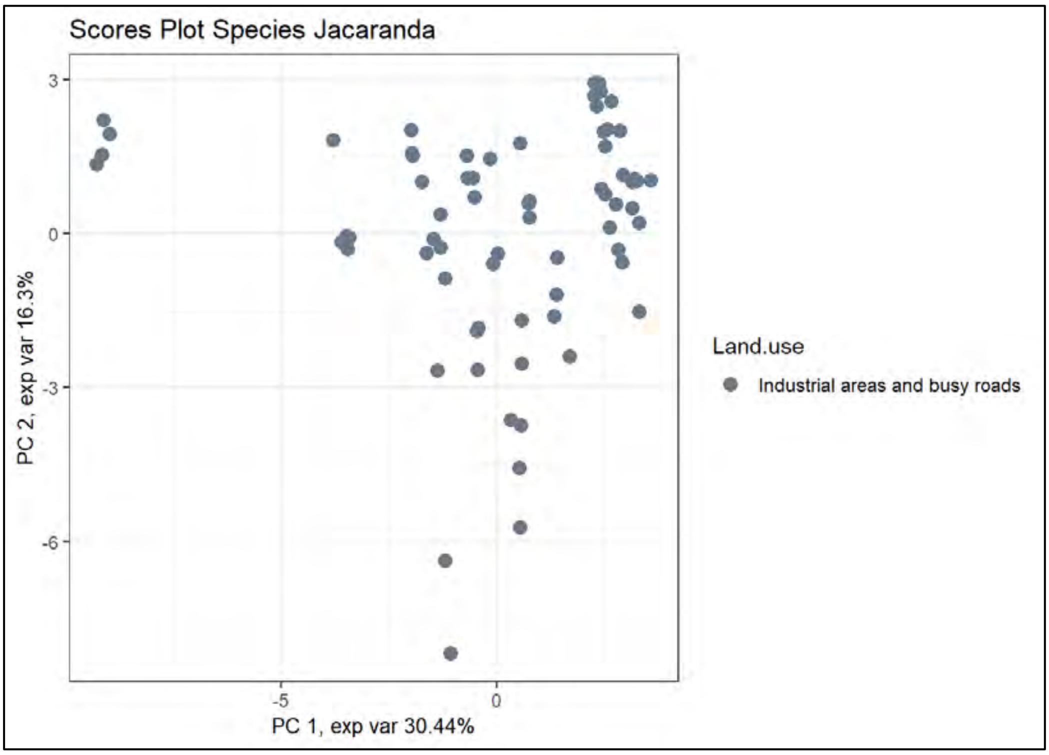

Figure 4.27. *Jacaranda* trees. Score plot of the PCA ordination with sampled trees categorised by land use: industrial areas and busy roads, parks and natural reserves

In terms of results for *Jacaranda mimosifolia*, pressure and altitude are negatively correlated with Axis 2, but positively with Axis 1, together with LDV and *Candelaria concolor*, *Canoparmelia texana, Culbersonia nubila, Hyperphyscia adglutinata, Parmotrema austrosinense,* and *Physcia tribacia*. Other species are negatively related to both axes. These are *Flavopunctelia flaventior*, *F. soredica*, *Heterodermia speciosa*, *Hyperphyscia granulata*, *H. pandani*, *Lepraria* spp., *Physcia undulata*, *Pyxine cocoes* and *P. petricola*. In contrast, the pollutants CO, NO, NO_2 and NO_x are negatively related to Axis 1 and slightly positively with Axis 2. PM_{10}, Humidity and SO_2 are positively related to both axes and show no positive correlations with lichen diversity (Figure 4.28).

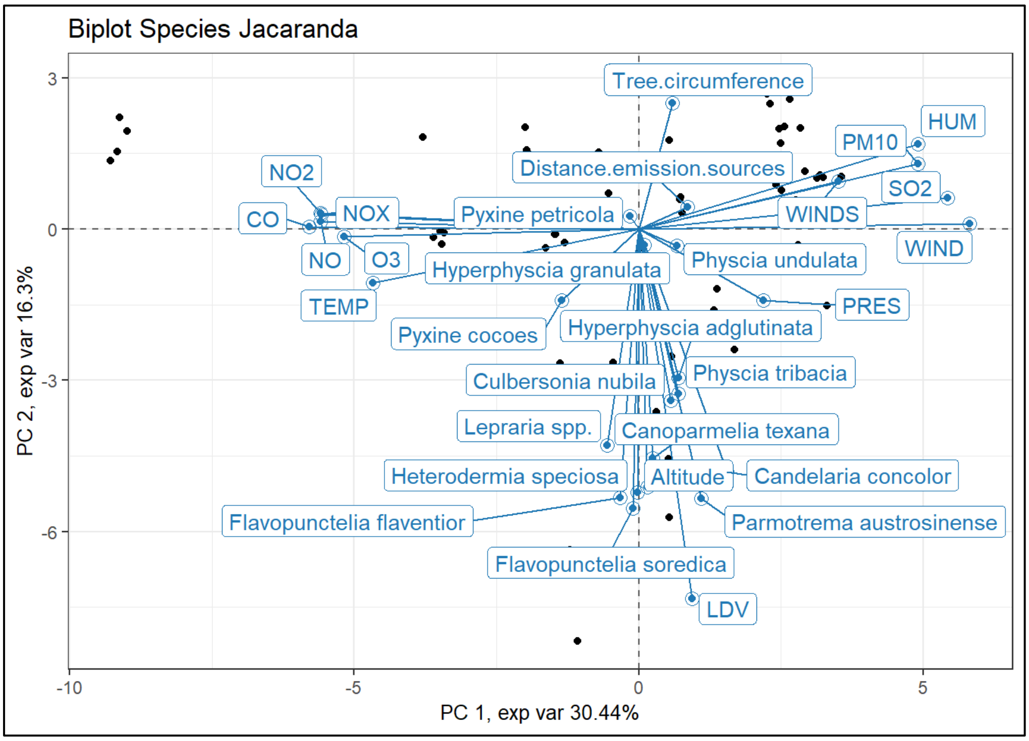

Figure 4.28. *Jacaranda* trees. Loadings plot of the PCA ordination. Vectors show the correlation of environmental variables with the two axes.

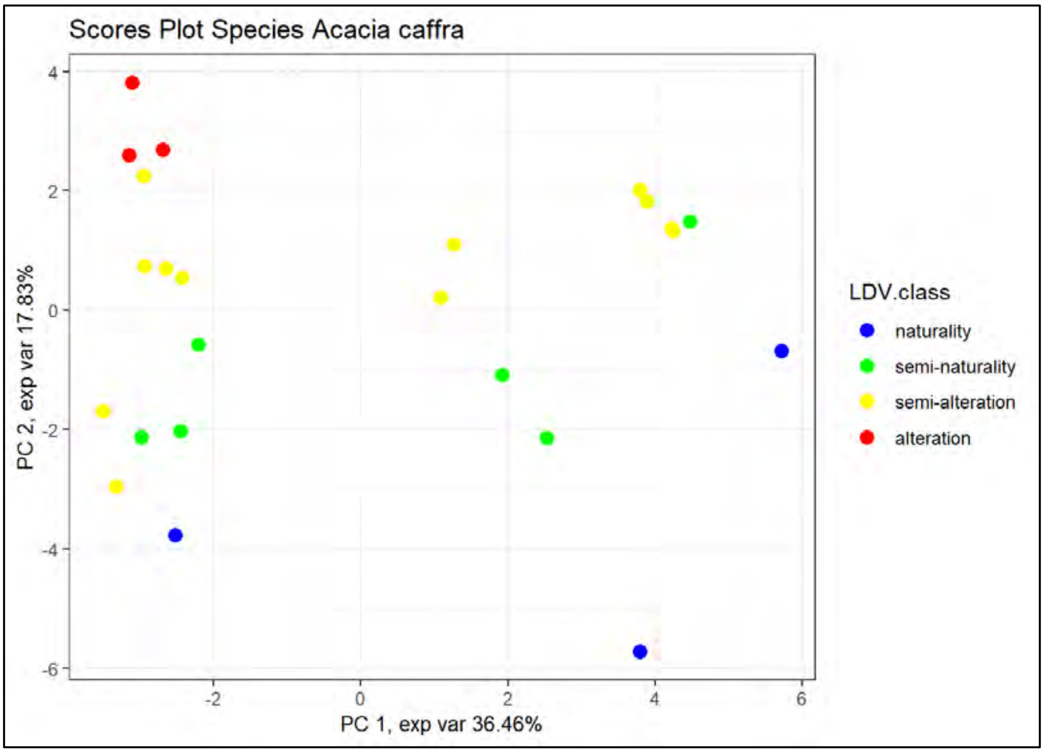

Figure 4.29. *Acacia caffra*. Score plot of the PCA ordination with tree substrates categorised by LDV classes

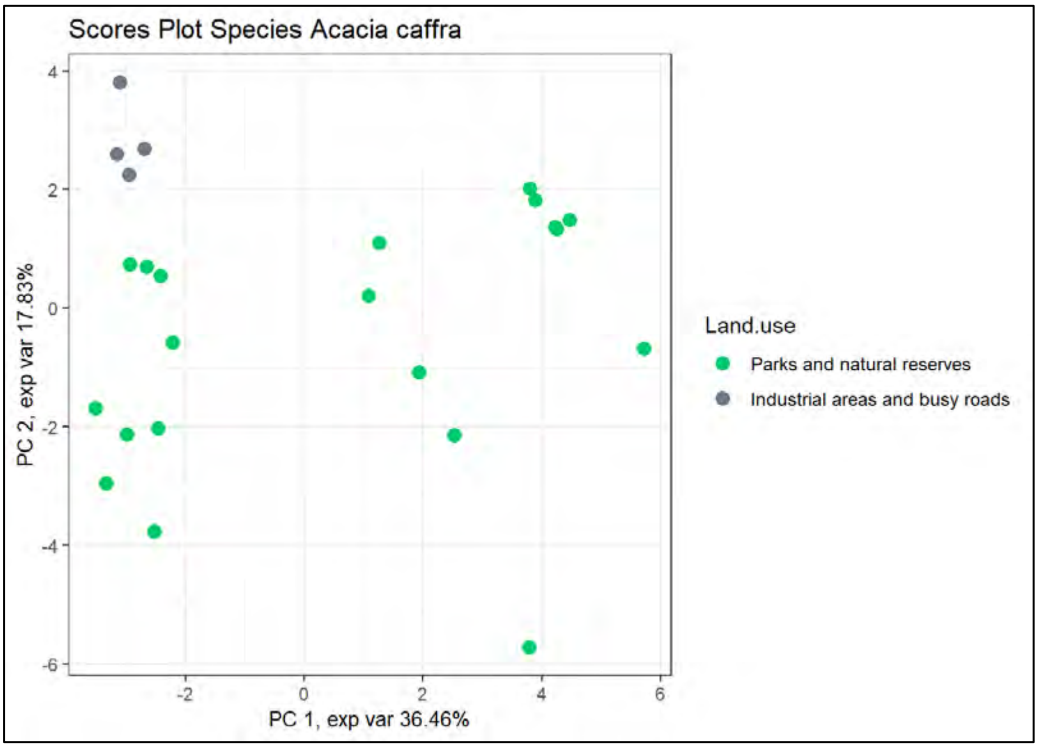

Figure 4.30. *Acacia caffra* trees. Score plot of the PCA ordination with sampled trees categorised by land use: industrial areas and busy roads, parks and natural reserves

In considering the dataset of *Acacia caffra*, LDV and the majority of lichens are positively related to Axis 1 and negatively to Axis 2. These species include *Canoparmelia taxana*, *Flavopunctelia flaventior*, *Heterodermia speciosa* and *Pyxine petricola*. SO_2, PM_{10}, Altitude, Winds and a few species such as *Candelaria concolor*, *Culbersonia nubila, Hyperphyscia pandani* and *Lepraria* spp. correlate negatively with Axis 1, whereas Humidity and Pressure correlate positively with Axis 2. The Distance from emission sources and Temperature positively correlated with both Axis (Figure 4.31).

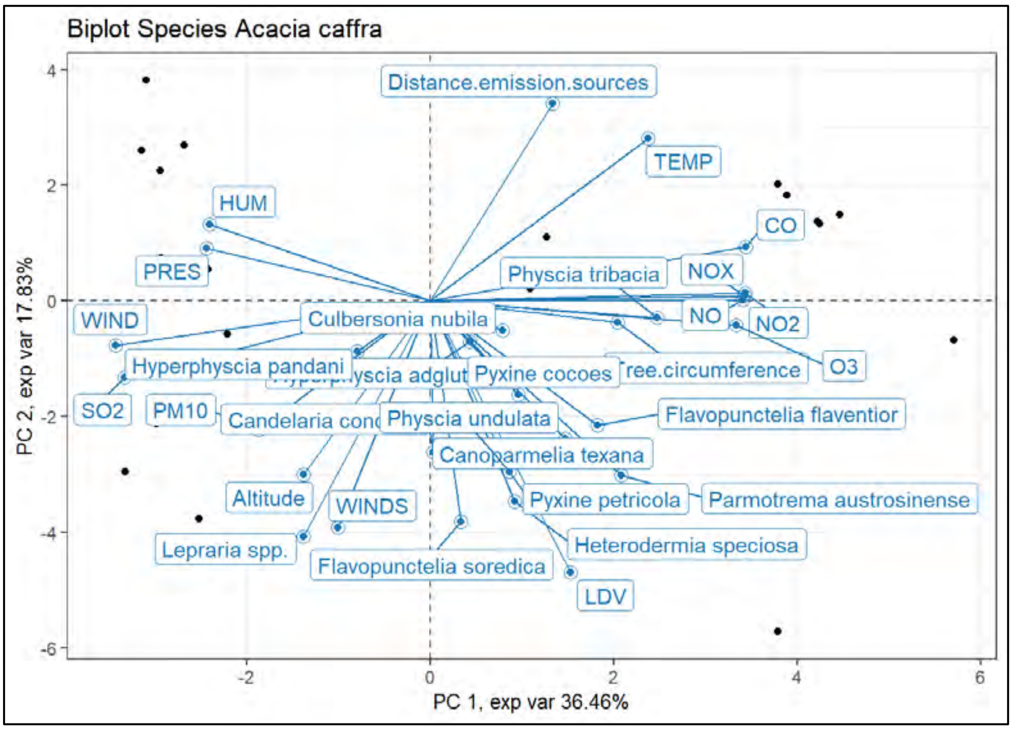

Figure 4.31. *Acacia caffra*. Loadings plot of the PCA ordination. Vectors show the correlation of environmental variables with the two axes.

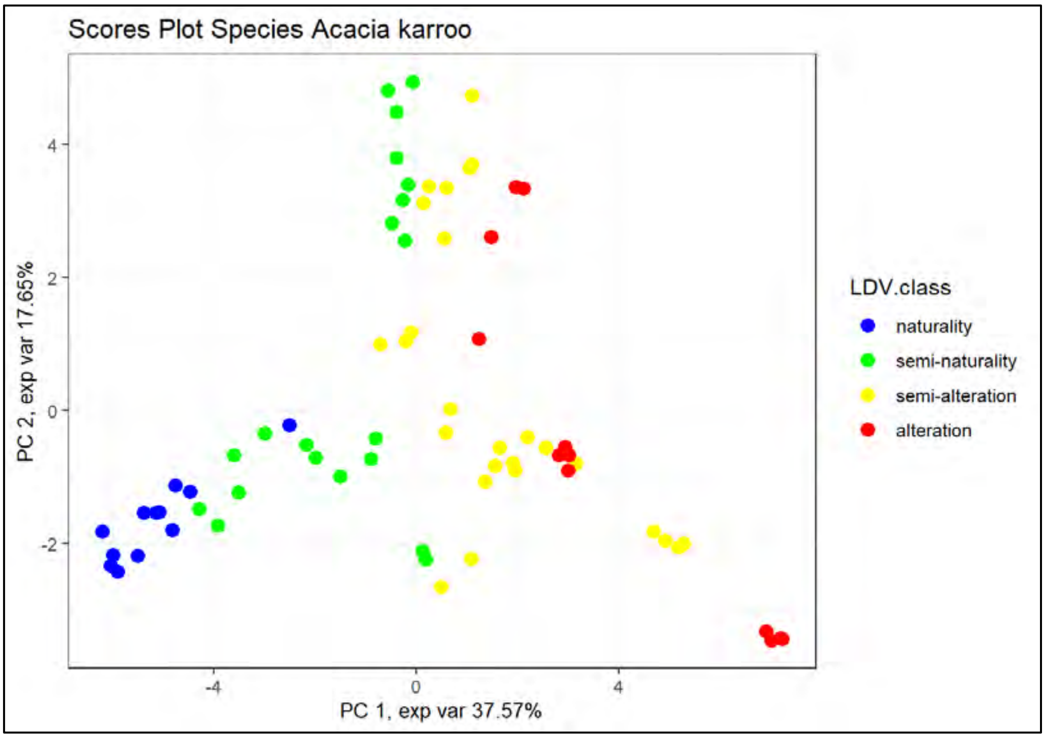

Figure 4.32. *Acacia karroo*. Score plot of the PCA ordination with tree substrates categorised by LDV classes

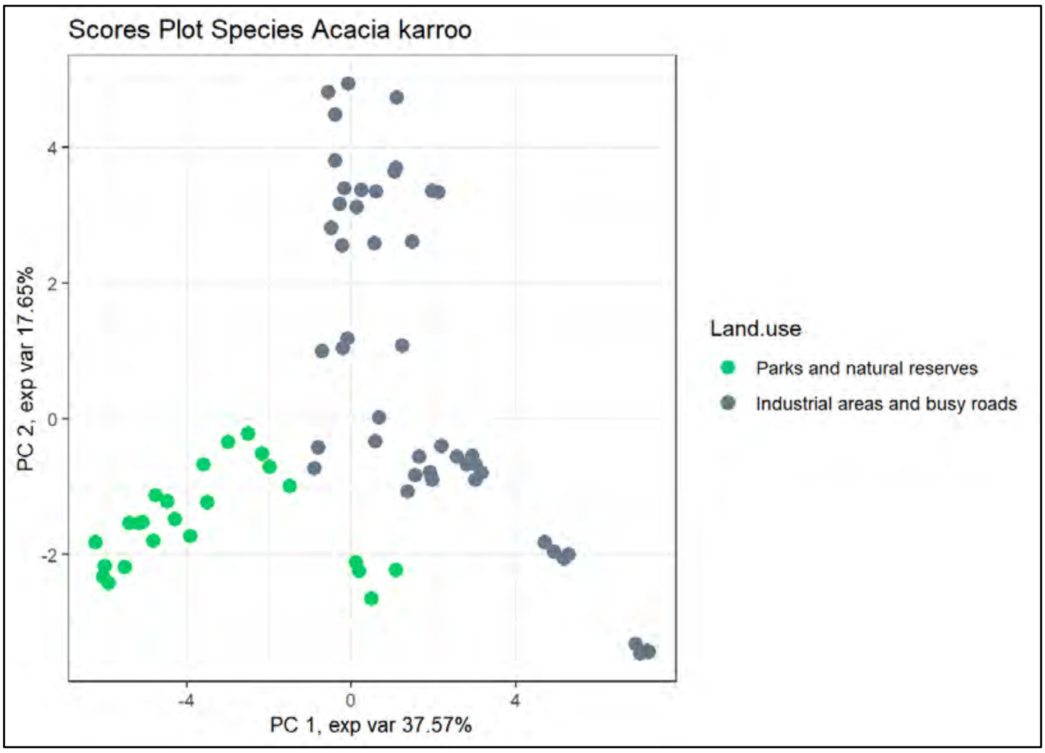

Figure 4.33. *Acacia karroo*. Score plot of the PCA ordination with sampled trees categorised by land use: industrial areas and busy roads, parks and natural reserves

In considering the dataset of *Acacia karroo*, CO, NO, NO_2 and NO_x and O_3 are positively correlated with Axis 1 while PM_{10} and SO_2 are negatively correlated with Axis 1 and Axis 2. Humidity and Wind are also negatively correlated with Axis 1, but positively with Axis 2. Pressure and Temperature are positively correlated with Axis 2. LDV and the majority of lichen species such as *Canoparmelia texana*, *Flavopunctelia flaventior* and *F. soredica* as well as the Distance from Emission sources and Altitude are negatively correlated with Axis 1 and Axis 2. Other species, such as *Pyxine cocoes, Lepraria* spp., but as well as Humidity, are negatively correlated with Axis 2 (Figure 4.34).

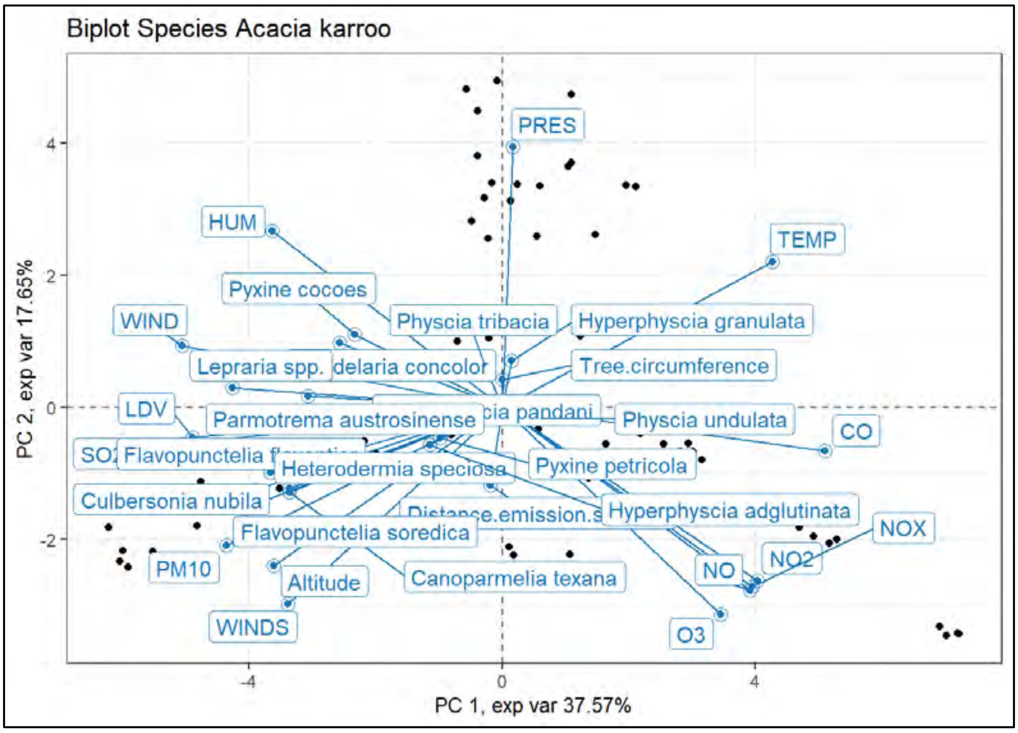

Figure 4.34. *Acacia karroo*. Loadings plot of the PCA ordination. Vectors show the correlation of environmental variables with the two axes.

4.4.7 Generalised linear regression models (GLM) with interpolated environmental data

Table 4.16 reports the results of the multiple linear regression models describing the effects of the selected environmental variables on LDV and species frequency (also see Appendix 14). The models were significant ($p<0.001$) for LDV and for 10 of the 15 lichen species, thus confirming the relationship with some variables as already shown with MFA and PCA.

Although the **altitude** range of the study area is rather small (min-max: 1233-1536 m), this variable is positively correlated with the frequency of *Flavopunctelia flaventior* ($p < 0.05$) and *F. soredica* ($p < 0.001$).

The **circumference of tree trunks** does not correlate significantly with lichen diversity, despite the high range of this variable on the sampled trees (median: 86 cm; min-max: 56-142 cm) see appendix 14.

Higher **land use** impact in industrial areas and at the proximity of busy roads is clearly negatively related to LDV and to the frequency of the following lichens: *Canoparmelia texana, Culbersonia nubila, Flavopunctelia flaventior* and *F. soredica, Heterodermia speciosa, Lepraria* spp. and *Pyxine cocoes.*

In terms of the tree species, ***Acacia karroo*** trees show a positive relationship with lichen diversity and an abundance of some species, such as *Culbersonia nubila*, *Pyxine cocoes*, *Flavopunctelia. flaventior*, *F. soredica*, *Lepraria* spp. and *Parmotrema austrosinense*.

Acacia caffra is not strongly related to the variable LDV, but it is positively related with an abundance of *H. adglutinata* and *P. tribacia*. On the contrary, it has a negative relationship with the frequency of *C. nubila*, *F. soredica* and *Lepraria*. Higher atmospheric concentrations of NO_x are negatively related to LDV and the frequency of *Candelaria concolor*, *Lepraria* spp. and *Pyxine cocoes*. The frequencies of *Culbersonia nubila* and *Lepraria* spp. are positively and negatively correlated with atmospheric SO_2 respectively. This pollutant does not show any significant effect on LDV. All other relationships are not significant.

Table 4.16. Multiple Linear Regression Models describing the effects of the environmental variables on LDV and species frequencies. Estimated values are reported together with statistically significant p values (* p<0.05; ** p<0.01; *** p<0.001). Last column shows the summary statistics of each model (156 df), with Multiple R2 (Mult R2), Adjusted R2 (Adj R2), F-statistic (on 7 and 156 df), p value.

	Intercept	**Altitude**	**Land use Industrial areas and busy roads**	**Tree species Acacia caffra**	**Tree species Acacia karroo**	**Tree circumference**	**NO_X**	**SO_2**	**Summary statistics of each model**
LDV	37.4	0.06	**-44.26*** **	-4.12	**17.61*** **	0.02	**-0.48***	-4.91	Mult R2: 0.565; Adj R2: 0.546 F: 29; ***p<0.001***
Candelaria concolor	12.8	0.01	-1.39	2.52	0.82	0.02	**-0.14** **	-1.68	Mult R2: 0.148; Adj R2: 0.109 F: 3.854; ***p<0.001***
Canoparmelia texana	-1.86	0.002	**-2.45*** **	-0.67	0.43	-0.001	0.01	0.33	Mult R2: 0.264; Adj R2: 0.231 F: 7.976; ***p<0.001***
Culbersonia nubila	2.16	-0.004	**-4.22*** **	**-3.30*** **	**1.05***	0.003	0.01	**1.65** **	Mult R2: 0.468; Adj R2: 0.444 F: 19.63; ***p<0.001***
Flavopuntelia flaventior	**-16.6***	**0.015** **	**-2.04***	-0.85	**1.73** **	0.008	-0.007	-0.35	Mult R2: 0.318; Adj R2: 0.287 F: 10.38; ***p<0.001***
Flavopuntelia soredica	**-25.3***	**0.03*** **	**-6.87*** **	**-3.81***	**2.38** **	-0.01	-0.05	-1.00	Mult R2: 0.608; Adj R2: 0.591 F: 34.63; ***p<0.001***
Heterodermia speciosa	9.70	-0.001	**-7.12*** **	-2.40	0.50	-0.01	-0.006	0.16	Mult R2: 0.439; Adj R2: 0.414 F: 17.45; ***p<0.001***
Hyperphyscia adglutinata	1.18	-0.002	0.65	**5.79***	2.04	-0.003	0.02	1.66	Mult R2: 0.073; Adj R2: 0.0315 F: 1.758; *p>0.05*
Hyperphyscia granulata	1.52	-0.004	0.11	0.14	0.28	0.02	0.005	0.42	Mult R2: 0.0395; Adj R2: -0.0036 F: 0.916; *p>0.05*
Hyperphyscia pandani	-0.09	0.000	-0.025	0.025	0.004	0.000	0.000	0.021	Mult R2: 0.0464; Adj R2: 0.00358 F: 1.084; *p>0.05*
Lepraria spp.	**33.7***	0.006	**-13.1*** **	**-4.94** **	**2.93** **	-0.019	**-0.24*** **	**-3.98** **	Mult R2: 0.650; Adj R2: 0.635 F: 41.42; ***p<0.001***
Parmotrema austrosinense	-5.83	0.010	-2.64	0.10	**3.96** **	0.02	-0.034	-0.47	Mult R2: 0.190; Adj R2: 0.154 F: 5.225; ***p<0.001***
Physcia tribacia	5.85	0.000	-1.70	**3.49** **	0.18	-0.001	0.01	-0.78	Mult R2: 0.282; Adj R2: 0.250 F: 8.766; ***p<0.001***
Physcia undulata	-1.18	0.000	0.08	0.32	0.04	0.004	0.005	0.12	Mult R2: 0.0236; Adj R2: -0.0202 F: 0.539; *p>0.05*
Pyxine cocoes	**15.6***	-0.004	**-3.08** **	-0.16	**1.30***	0.001	**-0.05***	-1.13	Mult R2: 0.221; Adj R2: 0.186 F: 6.333; ***p<0.001***
Pyxine petricola	-0.14	0.000	-0.07	-0.12	-0.04	0.000	0.002	0.07	Mult R2: 0.0112; Adj R2: -0.0332 F: 0.252; *p>0.05*

CHAPTER 5: DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

5.1 Discussion

5.1.1 Lichen taxa on the investigated trees under different land use types

Twenty-five taxa of epiphytic lichens were recorded during this study. Lichens were investigated only on three selected tree species and the trees provided comparison data for lichens on native and exotic phorophytes and for different land use conditions. Furthermore, an analysis of relationships among environmental factors, in particular land use, climate and pollution and lichens, was another important goal of the work.

The diversity of epiphytic lichens from Pretoria is clearly considerable as observed especially at the more natural sites (Botanical Garden, nature reserves) and further phorophytes and sites still need to be investigated to assess the full diversity of epiphytic lichens in the city. There are only very few studies concerning the diversity of epiphytic lichens in Africa and Southern Africa with which to compare the diversity in Pretoria. A comparison with the study carried out in Namibia by Zedda *et al*. (2009) shows that the recorded epiphytic lichen diversity of Pretoria is lower as these authors reported 37 taxa. However, the observations in Namibia were on different tree species, on a wider area and mainly outside towns. Aptroot (2001) reported 27 epiphytic lichens from Gambia. Frisch *et al*. (2015) recorded 191 epiphytic lichen species from 276 trees in Uganda (Bwindi National Park), but investigated different habitats in other climatic conditions.

Interestingly, most of the recorded species are subtropical to tropical or subtropical-/ tropical-temperate. A few are more widely spread and are present also in the Mediterranean area. An initial floristic survey of the area at the beginning of the project revealed that the entire lichen mycota collected from all tree parts (branches, twigs, trunks) across the city does not differ so much in terms of number of species on the tree phorophytes (17 species on *Jacaranda mimosifolia*, 18 on *Acacia caffra* and 19 on *A. karroo*). However, the composition of lichen communities is different as the exotic *Jacaranda* hosts more widely-found and more disturbance-tolerant species than the two *Acacias*.

A clear difference emerged moreover between the two identified land use types “Industrial areas and busy roads” and “Parks and nature reserves”. More protected sites such as Pionier

Museum, Rietvlei Nature Reserve and Groenkloof Nature Reserve have the highest lichen diversity (Maphangwa *et al*., 2018). Most of the rare species in this study such as *Chrysothrix xanthina*, *Dirinaria applanata*, *Parmotrema reticulatum*, *Physcia poncinsii*, *Pyxine petricola* and *Rinodina* sp. were found exclusively in protected areas. The sites with less human disturbance are predominantly colonised by subtropical to tropical species, such as *Hyperphyscia granulata*, *H. isidiata*, *H. pandani*, *H. pruinosa*, *Pyxine cocoes*, *Physcia poncinsii* and *P. undulata*.

The most disturbed sites, Pretoria central and Pretoria West (Staal Road), show the lowest diversity as only *Candelaria concolor* was observed there. The protected areas are characterised by low traffic whereas Pretoria central and Pretoria West are characterised by high traffic and the presence of industrial activities. Disturbed sites host more cosmopolitan species such as *Candelaria concolor* and *Hyperphyscia adglutinata,* which were mostly found on *Jacaranda*, as this is the dominant tree across the city, especially along avenues with high car traffic, and under disturbed conditions. These lichen species prefer nutrient-enriched and sun exposed barks (Almborn, 1966, Killmann and Fischer, 2005, Zedda *et al*., 2009). They are usually found in areas with high man-made disturbance such as urban areas (Güvenç and Öztürk, 2017).

Candelaria concolor appeared to be widespread in a study by Coffey and Fahrig (2012), assessing the effects of vehicle pollution in Canada. *C. concolor* flourishes under high nitrogen-contaminated air situations (Leith *et al*., 2005). *Canoparmelia texana*, *Flavopunctelia flaventior, F. soredica and Heterodermia speciosa* prefer sites with nitrogen deposition, but are sensitive to acid deposition, e.g. by SO_2 (United States Forest Service, 2019). Also *Parmotrema austrosinense* and *P. reticulatum* prefer sites with nitrogen deposition. The latter is also tolerant to acid deposition (United States Forest Service, 2019). *Pyxine cocoes* is tolerant to both nitrogen and SO_2 pollution (Abas and Awang, 2017; Abas *et al*., 2018). *Chrysothrix xanthina* prefers moderate pollution (Abas *et al*., 2018).

The results of this study show that many of the recorded species belong to the family Physciaceae. In particular, the genus *Hyperphyscia* is very species-rich. This is in agreement with the findings of Zedda *et al*. (2009), who found that the majority of epiphytic lichens in Namibia also belong to this family. Several species of the *Physciaceae* are known from other regions of the world to be tolerant to nitrogen pollution, while many crustose lichens are relatively sensitive.

The findings that fruticose lichens were absent, is also in agreement with Zedda *et al*. (2009), who did not record any fruticose lichens in the Savannah biome of Namibia, probably because air humidity is low in savannas and/or disturbance factors, such as fire and grazing, frequent. Foliose lichens were much more frequently found than crustose lichens both in Pretoria and in Namibia. It remains unclear if the scarcity of crustose lichen is affected by human influences, in particular by air pollution by nitrogen compounds, or only by, for example, the dry climate. Certainly the checklist of South Africa (Fryday, 2015) contains numerous epiphytic crustose lichens, which might potentially be present in Pretoria. This aspect needs to be better investigated.

5.1.2 The indicator value of lichen diversity

The results of the monitoring carried out using the grid on the tree trunk of 164 trees and at 29 sites also showed that the most frequent species are the cosmopolitans *Candelaria concolor* and *Hyperphyscia adglutinata*. Also common are *Parmotrema austrosinense* and *Lepraria* spp.

Descriptive statistics, univariate analysis as well as PCA confirmed that lichen diversity on tree trunk differs according to area and anthropogenic activities. Sites under the category "Parks and nature reserves" shows also in this case significantly higher **LDVs** than sites under the land use category "Industrial areas and busy roads". LDVs and species composition also vary among the photophytes. *Acacia karroo* and *A*. *caffra* have higher values in contrast to *Jacaranda mimosifolia*. Both are native species and more frequently found in the protected areas compared to *Jacaranda*, which is only found at disturbed sites.

According to the monitoring results, some species are more frequently found on *Jacaranda* like *Hyperphyscia granulata* and *Pyxine petricola*. Other lichens are more common or restricted to Acacias such as *Canoparmelia texana*, *Flavopunctelia flaventior*, *F. soredica*, *Heterodermia speciosa*, *Hyperphyscia adglutinata*, *Lepraria* spp., *Parmotrema austrosinense, Physcia tribacia*, *P. undulata* and *Pyxine cocoes*. Between the two *Acacias* there are also some differences, *Culbersonia nubila, Flavopunctelia flaventior, F. soredica, Lepraria* spp., *Parmotrema austrosinense* and *Pyxine cocoes are* more common on *A*. *karroo*. *Heterodermia speciosa, Hyperphyscia adglutinata, H. pandani, Physcia tribacia* and *P. undulata* are slightly more frequent on *A*. *caffra*.

Based on the results of the monitoring study, a **naturality/alteration interpretative scale** with five classes could be developed and this was done for the first time for an African urban environment. The scale was used to produce a map of naturality/alteration for the city.

Alteration classes have very low LDVs, such as in Mamelodi, Pretoria central business district and Pretoria west (Staal Road), whereas more natural and less impacted sites, like the Voortrekker Monument Nature Reserve, have higher levels of naturality and LDVs. Such scales have been developed and used for several years by different researchers worldwide to define the alteration degree of an environment (Giordani and Brunialti, 2015). Giordani *et al*. (2002) defined, for instance, naturality/alteration classes for the region of Liguria in Italy. These authors found very high alteration in areas characterised by high pollution such as urban and industrial districts (towns of Genova and Savona), with frequent lichen desert conditions. Isocrono *et al*. (2007) reported lichen desert in areas characterised by higher traffic and industries in Italy in contrast to natural areas. Frati and Brunialti (2006) found alteration classes near emission sources in Italy (Ancona), while naturality classes were found in areas characterised by less anthropogenic impacts.

In other regions, like Slovakia, Svoboda *et al*. (2010) found higher LDV in semi natural and natural sites characterised by low air pollution, and lowest values in areas with high pollution from various sources. The naturality/alteration scale of this study differs only slightly from the scale developed by Loppi *et al*. (2002) for Toscana and Liguria in Italy, wherein LDV = 0 represents a lichen desert condition and LDV > 75 represents a natural class. In this study, the lichen desert class was not found.

These scales can be useful to estimate the effects of air pollution on lichen diversity (Giordani *et al*., 2002), but also of the impact of land use in general in a cheap and reliable way (Asta *et al*., 2002b; Brunialti *et al*., 2008). They could be used in Southern Africa in areas where there are no monitoring stations, like in remote rural areas. The public authorities of the City of Tshwane and/or the governmental Department of Environmental Affairs, could apply these scales to extend similar monitoring to other parts of Pretoria and/or to other towns of South Africa. Practitioners and other stakeholders could also profit from using these scales for identifying areas with higher impact by land use.

According to PCA (Figure 4.12), there is a significant relationship among LDV, the number of species found on tree trunk, and species composition in Pretoria. The most important gradient is related to the **distance from emission source**. This is in agreement with numerous studies carried out worldwide on epiphytic lichens, for instance Li *et al*. (2013) found that anthropogenic disturbances severely affect the diversity of epiphytes lichens in the subtropical forests of southwest China, Käffer *et al*. (2011) in Brazil, Łubek *et al*. (2018) in Poland, Aragón *et al*. (2010) in the Mediterranean and Hauck *et al*. (2012) in the Mongolian Altai. Other examples have been reported in Chapter Two.

Although the parameter “land use type” showed a strong relationship with lichen diversity, the tested relationships among LDVs, single species and measured ecological parameters (climate, pollution) were not very strong in Pretoria. This is surely due to the fact that only climate and pollution data from five monitoring stations were available, with many gaps in the dataset. The data from these stations appears not to be representative for the entire town, while lichen parameters (LDV, N. of species and lichen species composition) respond better to changing environmental conditions, as demonstrated by the significant relationship with the parameter “distance from emission source” which was measured within the monitoring survey of the present study.

To fill the data gaps, an interpolation of air pollution and climate data was carried out for the 29 lichen sampling sites and these new datasets were then correlated with lichen parameters. The results were, however, also in this case unsatisfactory and not realistic and reliable for all sites. For instance, Pretoria West - Staal Road, characterised by higher traffic and industries, showed lower (interpolated) values of NO, NO_2 and NO_x pollutants, while the Voortrekker Monument Nature Reserve had higher values of these pollutants, although it was characterised by low traffic and human impacts, and higher LDV compared to Pretoria West. The same trend was also observed at Rietvlei Nature Reserve that has low traffic, but here the same interpolated values of the same pollutants were high compared to Pretoria central business district, which has higher traffic and much lower LVDs (Chapter 4.2, Table 4.13).

Despite the poor reliability of some of the interpolated data, the multivariate analyses and the generalised linear regression models (GLM) give an indication of significant relationships among some of the tested parameters, for instance the climatic parameters wind direction and speed, and also air humidity positively relate to lichen diversity.

The relationship of lichen diversity to **air humidity** is well-known from many studies and in agreement, for instance, with the findings of Nimis (1986), Loppi *et al*. (2002), Brunialti *et al*. (2008) and Giordani and Brunialti (2015) for Italy. Concerning Southern Africa, air humidity is considered one of the most important ecological factors influencing lichen diversity as demonstrated in the studies of Zedda and Rambold (2009), Maphangwa *et al*. (2012a, 2014) and Zedda *et al*. (2011a) for soil and stone lichens. In particular, the predominance of green algal photobionts and the occurrence of foliose lichens are demonstrative of raised air humidity in the desert biome, where green algal lichens can utilise tremendously small amounts of water and water vapour for photosynthetic activity (Zedda *et al*., 2011b). Also in this study, only green algal lichens were found and these were mostly foliose.

Altitude, which was measured in the field during monitoring work (not interpolated), was positively related to the occurrence of some lichen species such as *Flavopunctelia flaventior* and *F. soredica*. The finding that altitude has a positive influence on the frequency of epiphytic lichens is in accordance with the results of numerous authors, who investigated diversity changes along altitudinal gradients. For instance, Güvenç and Öztürk (2017) found altitude to be the main factor influencing lichen distribution in Turkey. Also in Mediterranean forests, altitude is one of the main factors affecting lichen distribution together with management intensity (Aragón *et al*., 2010). In Kenya, Kirika *et al*. (2018) reported altitude as the main factor influencing lichen assemblage, whereas Loppi *et al*. (1997) reported altitude as an important parameter in Tuscany (Italy). According to Zedda *et al*. (2011b), altitude does not influence soil lichen diversity directly in Namibia and in the western part of South Africa, but altitude may have an indirect positive effect on the occurrence of dewfall events, because of lower temperatures during the night. Dewfall is an important water source for lichens in semi-arid to arid regions of the world (Maphangwa *et al*., 2012a).

The factors most strongly related to lichen diversity and composition on the investigated trees and sites were, however, the "phorophyte" and "land use" type. Regarding the **phorophyte,** see what is reported on page 104. The dependence of lichen species on certain tree species is well documented by numerous studies carried out worldwide. This is mainly due to bark and crown features, as well as to ecological conditions of sites where trees grow (Cáceres *et al*., 2007; Frati *et al*., 2008; Mežaka *et al*., 2012; Trüe *et al*., 2012; Ódor *et al*., 2013; Frisch *et al*., 2015). The **circumference of trees** does not correlate significantly with lichen communities in the present study, despite the high range of this variable on the sampled trees.

Concerning **land use**, the negative correlation of sites with high traffic, in the proximity of busy roads and located in industrial areas with lichen parameters is clear, and confirmed by the different elaborations. The finding that epiphytic lichen diversity is higher in protected areas than in more disturbed urban areas is in agreement with the results of several authors, among others, Nimis *et al*. (2002), Bergamini *et al*. (2005), Stofer *et al*. (2006) and Aragón *et al*. (2010). The last authors found that when land use (forestry practice, agricultural and livestock use) increases, lichen species richness and the richness of functional groups tend to decrease in eight European countries (Switzerland, Ireland, United Kingdom, Finland, France, Portugal, Spain and Hungary) and in six different biogeographic zones (Bergamini *et al*., 2005; Aragón *et al*., 2010). Hauck *et al*. (2012) reported that epiphytic lichen diversity is affected by different land use activities in Asia, such as grazing and fuelwood collection (Mongolian Altai). In the Mediterranean area and in the USA, lichen diversity is also influenced by different land uses such as traffic and air quality (Washburn and Culley, 2006; Llop *et al*., 2012; Giordani and

Brunialti, 2015). McCune *et al*. (1997) also reported a higher diversity of lichens in remote areas and lower diversity in urban and industrial areas in the USA. Svoboda *et al*. (2010) reported similar results for Slovakia. According to Ahn *et al*. (2011), the diversity of lichens increases with increasing distance from the city centre in Seoul (Korea). In contrast with the findings of this study and most findings worldwide, Perlmutter (2010) did not find any correlation between LDV and traffic in North Carolina in the USA.

The correlations with interpolated data show that the pollutants **NO, NO_2, NO_x, CO** and **O_3** are negatively related to lichen diversity and to a group of species. The finding that N-compounds affect lichen diversity in this study is in agreement with the works of Giordani *et al*. (2002) and Giordani *et al*. (2013) carried out in Italy. NO_2 from traffic emissions affects lichen diversity in Spain (Fuentes and Rowe, 1998; Purvis *et al*., 2001) and in Italy (Lorenzini *et al*., 2003). Hawksworth and Rose (1970), Davies *et al*. (2007) and Larsen *et al*. (2007) reported that SO_2 and NO_x limit lichen diversity in England and Wales and in London, a large amounts of NO_x are found next to busy roads. *Parmelia saxatilis* (L.) Ach. is prevented from growing next to busy roads in London because of NO_x concentrations (Batty *et al*., 2003). NO_x is also reported to injure the lichens *Parmelia sulcata* Taylor and *Hypogymnia physodes* (L.) Nyl. in London (Purvis *et al*., 2003). Ahn *et al*. (2011) reported NO_x as the main pollutants affecting lichen diversity in Seoul in Korea. NO_x is one of the main pollutants in most urban areas today worldwide (Davies *et al*., 2007; Ahn *et al*., 2011). *Heterodermia speciosa* was used as an indicator of N-pollution in Sri Lanka and the highest pollution was found in the congested urban area in Colombo with industries (Gunathilaka *et al*., 2011). The lowest pollution was reported in Kurunegala city, which is less congested and has few industries (Gunathilaka *et al*., 2011).

The results of the present work in relation to pollution are in accordance with studies carried out by Liebenberg-Enslin and Petzer (2005) in the City of Tshwane. These authors reported that N-pollutants, which emanate from tyre burning, domestic burning and industries, are a priority, and that Pretoria West is the most affected area. Also in the present study, the lowest lichen diversity was observed in these areas, which have high concentrations of mercury as indicated by Trüe *et al*. (2012), who analysed thalli of *Parmotrema austrosinense.* Mercury concentrations were in contrast lower in Hatfield, which is a more natural area. The highest annual concentration of SO_2 was in Pretoria West (Wright *et al*., 2011), normally found in winter, where coal domestic burning is high. This is also in agreement with the observations of Forbes *et al*. (2009) and Olowoyo *et al*. (2010, 2011), who observed that sites situated away from Pretoria central such as Pionier Museum are characterised by low pollution compared to Pretoria central.

The coal-fired power stations of the energy sector are the biggest emission sources of NO_x and SO_2 in South Africa (Wright *et al*., 2011; Pretorius *et al*., 2015; Girmay and Chikobvu, 2017; Muyemeki *et al*., 2017). The power stations that are mostly owned by Eskom contribute enormously to energy supply in South Africa (Girmay and Chikobvu, 2017; Muyemeki *et al*., 2017). Nitrogen dioxide concentration in South Africa is a big concern, especially in Mpumalanga Province, which has many coal-fired power stations (Collett *et al*., 2010). Coal and fuel burning in South African townships such as Garankuwa, Mamelodi and Soshanguve are a main source of pollution as well (Wright *et al*., 2011). NO_x concentrations are higher in winter months (Broccardo *et al*., 2008; Collett *et al*., 2010; Naidoo *et al*., 2014). Pretorius *et al*. (2015) also reported vehicle emissions and industries as other sources of pollution in South Africa, where between 2006 and 2012, NO_X emission increased by 10% and by 2030 is projected to increase by 40%.

In this study, only the frequency of *Culbersonia nubila* was positively related to SO_2, while SO_2 was negatively related to *Lepraria* spp. There was no other significant relationship between SO_2 and further lichen species. **SO_2** is also often a main cause of lichen diversity decline in both urban and industrial areas, although pollution by SO_2 has become less severe during the last decades in many countries (Gilbert, 1970; Nimis, 1986; Nimis and Purvis, 2002; Nimis *et al*., 1996; Nimis *et al*., 2002; Giordani, 2007; Brunialti *et al*., 2008; Svoboda *et al*., 2010; Tiwari, 2008; Giordani and Brunialti, 2015).

Domestic coal burning, power stations, traffic emissions and industries are some of the main causes of SO_2 pollution in South Africa (Collett *et al*., 2010; Girmay and Chikobvu, 2017; Muyemeki *et al*., 2017; Sangeetha and Sivakumar, 2019). SO_2 is of great concern in South Africa as it poses threats to different organisms (Muyemeki *et al*., 2017). In Pretoria West, the main sources of pollution are industrial and residential areas (Sangeetha and Sivakumar, 2019) and SO_2 pollution is high next to power stations (Muyemeki *et al*., 2017). The SO_2 emission is projected to increase in South Africa, as coal is the main source of energy supply (Henneman *et al*., 2016); for this reason the monitoring of environmental impacts is very important. Pretorius *et al*. (2015) projected SO_2 emissions to increase by 38% under worst-case scenarios by the year 2030. The relationship between SO_2 and lichens therefore needs to be better analysed in future studies.

5.2 Contribution of the study

This study adds to new knowledge on epiphytic lichens in Pretoria and in South Africa. Lichen descriptions and information on species distribution and frequency in Pretoria, South Africa, Africa and worldwide, as well as information on ecology of each species are provided in this work and in the published papers (see Appendix 15) (Maphangwa *et al*., 2018). This study has developed a lichen identification key that can be used in Pretoria and in other cities of South Africa. This will encourage more lichen studies in urban areas of South Africa, which still remain unexplored (Crous *et al*., 2006; Maphangwa *et al*., 2012b) as many people in South Africa are not familiar with lichens and find their identification difficult (Mukherjee *et al*., 2010). Identification keys are missing for many areas of South Africa and taxonomic lichen groups.

This is the first study investigating epiphytic lichens and their importance as bioindicators in urban environments of Africa, and tests a methodology to monitor environmental impact and air quality using lichens, which was developed for other regions of the world. Relationships among lichen species and lichen diversity with the phorophyte type, the land use type and some climatic and pollution parameters could be demonstrated. This study also developed a naturality/alteration interpretative scale, which can be used to monitor environmental alteration in Pretoria and similar areas in South Africa. The use of such scales is a cheaper way of monitoring effects of land use and estimating air quality (Rindita *et al*., 2015) and could be applied all over South Africa.

Three more papers will be published soon from this study (see Appendix 16) and results have been presented at an international conference (see Appendix 17). The results will also be presented at an international conference in 2020 (see Appendix 17).

The identified material is available for future studies at SANBI (National Herbarium) and UNISA Horticulture Centre where the identified specimens will be stored. This contributes to the availability of identified specimens for further lichenological surveys in the country.

This study also revealed that the phylogenetic position of *Culbersonia* is in the *Caliciaceae* (as per paper published from this study in the Appendix, Aptroot *et al*., 2019). This has never been studied before, according to Lücking *et al*. (2016) and Aptroot *et al*. (2019).

5.3 Conclusion and recommendations

In conclusion, human disturbances and land-use in Pretoria appear to have a negative impact on lichen diversity, reducing species number and changing species composition, and influencing morphological and biogeographical groups. There is good evidence from the present study that epiphytic lichens are, in South Africa, suitable indicators of given climatic and air quality conditions as well as indicators of land use type, even if species composition is different in comparison to European towns.

The European standardised monitoring method for mapping lichen diversity as an indicator of environmental stress (Asta *et al*., 2002b; VDI-Richtlinien, 2005; EN 16413, 2014; Giordani and Brunialti, 2015) needs, however, to be adapted to South African conditions. The methodology can be more easily applied in South Africa, by adopting a stratified random sampling and a more flexible spatial selection of suitable trees. It would be interesting to extend such studies to more sites in Pretoria, also assessing the lichen diversity on other tree species. These sites should include more mountainous and protected areas such as nature reserves that are situated far from industries and pollution sources. Other cities could furthermore be assessed for comparison, for instance Johannesburg, which has similar climatic and altitudinal conditions as Pretoria. Future studies should use more sampling units so that more suitable trees can be found.

In future studies, the bark properties of trees need to be investigated, so that trees with similar bark properties such as structure, pH and nutrient content can be identified and used for monitoring in the future. In order to better define the indicator value of given lichen species, the different pollution and climate parameters should be measured at more sites and relationships further tested.

More training, identification facilities and financial support are needed in South Africa to facilitate further studies on the rich lichen diversity and for building new generations of lichen experts, who are presently missing in the country.

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APPENDICES

The sequence of appendices is according to order of citation in the chapters.

Appendix 1A: City of Tshwane

CITY OF TSHWANE
IGNITING EXCELLENCE

Environmental Management Services Department

Nr 11 Francis Baard Street I Pretoria I 0001
PO Box 1454 I Pretoria I 0001
Tel: 012 358 8871 I Fax: 012 358 8934
Email: livhuwanis@tshwane.gov.za I www.tshwane.gov.za

My ref: 4/7/4/6
Your ref:
Contact person: Celia Masilo
Division/Section/Unit: Environmental Management and Parks

Tel: 012 358 5766
Fax: 012 358 8934
Email: CeliaM@tshwane.gov.za

01 October 2014

Mr KW Maphangwa

University of South Africa
Environmental Science Department
Calabash Building
Block B, Room 129

RE: PERMISSION REQUEST

Dear Sir

Your letter dated 30 September 2014 refers:

Permission is granted to you to carry out a research study – the biodiversity of epiphytic lichens in Pretoria and its value for ecological indication in the Tshwane Nature Reserves, Parks and on trees along roads reserves within the City of Tshwane.

Yours faithfully

.... 2014/10/3 ....
LIVHUWANI SIPHUMA DATE
EXECUTIVE DIRECTOR: ENVIRONMENTAL MANAGEMENT AND PARKS

Kgoro ya Taolo ya Tikologo • Departement Omgewingsbestuur • Lefapha la Tsamaiso ya Tikologo
Ndzawulo ya Mafambiselo ya swa Mbango • UMnyango Wezokuphathwa Kwemvelo
Environmental Management Department

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Appendix 1B: City of Tshwane

CITY OF TSHWANE

AGRICULTURE AND ENVIRONMENTAL MANAGEMENT DEPARTMENT

Environmental Management Division

Toulouse building Fountains Resort Christina de Wit Drive Pretoria 0001
PO Box 1454 Pretoria 0001
Tel:012 3411452 Fax:012 3414595
Email: thinusp@tshwane.gov.za | www.tshwane.gov.za

My ref: 19/11/14 Date : 19 Januarie 2016
Your ref:
Contact person: Thinus Prinsloo
Division/Section/Unit: Nature Conservation

RESEARCH PROJECT AT : City of Tshwane Nature Reserves.

This serves as notice to confirm that _*Khumbudzo Walter Maphangwa*__is allowed to do his/her research project on above mentioned nature areas of the City of Tshwane. This registered student may gain free access to the reserve during the week and on Saturdays but not on Sundays and public holidays.
The study will start on __*January 2016*________ and end on _*December 2016*_______.

A Study Proposal must be submitted to this office before starting the project.

I UNDERTAKE TO-

1. accept that the Council has the right to withdraw its consent to use the reserve if I do not comply with the provisions below;
2. see to it that I at all times comply with the provisions of the By-laws relating to Public Order, Public Places, Recreation Grounds and the Nature Reserves' rules and regulations, published under Administrators Notice 55 of 18 January 1984 (as amended);
3. comply with the provisions of the Nature Conservation Ordinance 1983 (Ordinance 12 of 1983) promulgated under Administrator's Notice 519 of 14 December 1983 (as amended);
4. enter the reserve during the normal gate times and be out one hour before the gate closes;
5. work in uniform with the appropriate epaulette (if available);
6. drive my vehicle responsibly and not drive off any of the roads into the veld (leave vehicle next to road and walk in if necessary);
7. mark my vehicle with a notice that indicates my name, the study title and student reference number;
8. hand 2 copies of the completed report in at the Nature Conservation offices in the Toulouse Building at the Fountains Valley Resort.
9. answer all questions and enquiries from the public and
10. complete and indemnity form and use the nature reserve and facilities at own risk.

Yours truly

Thinus Prinsloo
Deputy Director
Nature Conservation
City of Tshwane

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Appendix 2: Pionier Museum

Maphangwa, Khumbudzo

From:	Sammy Marks Museum <marks@ditsong.org.za>
Sent:	16 September 2016 10:10
To:	Maphangwa, Khumbudzo
Cc:	caroline@ditsong.org.za
Subject:	FW: Permission Request of UNISA student to do research at Pioneer Museum

Dear Khumbudzo Maphangwa

I have taken note of your request with regard to study on the Ditsong Pioneer Museum site in Silverton. Your period of research on the Museum will be brought under the attention of the reception and security staff.
Please report to the reception on the days you will be on the site.

Kind regards
Nerina Walters
Deputy Director: Ditsong Pioneer Museum

From: Caroline Griessel [mailto:caroline@ditsong.org.za]
Sent: 26 August 2016 08:04 AM
To: 'Sammy Marks Museum'
Subject: FW: Permission Request of UNISA student to do research at Pioneer Museum

More Nerina

Sien asb. versoek wat ek ontvang het. Ek het sommer ook deel van die betekenis op google gaan opsoek. Ek benodig asb. jou toestemming.

Groete

Caroline

An **epiphyte** is a plant that grows harmlessly upon another plant (such as a tree) and derives its moisture and nutrients from the air, rain, and sometimes from debris accumulating around it. Epiphytes differ from parasites in that epiphytes grow on other plants for physical support and do not necessarily negatively affect the host. An epiphytic organism that is not a plant is called an epibiont.[1] Epiphytes are usually found in the temperate zone (e.g., many mosses, liverworts, lichens, and algae) or in the tropics (e.g., many ferns, cacti, orchids, and bromeliads).[2] Many houseplants are epiphyte species due to their minimal water and soil requirements. Epiphytes provide a rich and diverse habitat for other organisms including animals, fungi, bacteria, and myxomycetes.[3]

Epiphyte is one of the subdivisions of the Raunkiær system.

From: Maphangwa, Khumbudzo [mailto:maphakw@unisa.ac.za]
Sent: Thursday, August 25, 2016 11:20 AM
To: caroline@ditsong.org.za
Subject: Permission Request

Good day

1

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Appendix 3: Voortrekker Monument Nature Reserve

Maphangwa, Khumbudzo

From: Lizette Jansen <snrtoerisme@vtm.org.za>
Sent: 26 June 2018 08:35
To: Maphangwa, Khumbudzo
Subject: RE: Permission request at Voortrekker Nature Reserve/Monument

Good morning Khumbudzo,

You are welcome to do your study on the Voortrekker Monument site. Please arrange your visits beforehand so we can arrange free entrance and ease of access at the main gate in Eeufees Road. Please take note that we have experienced some criminal activity on site recently, especially in the nature reserve area. We have limited control over individuals who enter the premises illegally, usually by cutting a hole in the fence. The area surrounding Fort Schanskop experiences the highest crime rate at the moment. I therefore request that you be vigilant on the reserve, and that you report at the main gate on arrival.

We would also like to request that you provide us with the findings of your research study. We would very much like to make this information accessible to researchers who visit our library and archives on site.

Kind regards

Lizette Jansen

Senior Bemarkingsbestuurder/ Senior Marketing Manager

Voortrekkermonument en Natuurreservaat / Voortrekker Monument and Nature Reserve

Tel: 012 326 6770

Faks / Fax: 086 619 8719

www.vtm.org.za

From: Maphangwa, Khumbudzo [mailto:maphakw@unisa.ac.za]
Sent: 25 June 2018 08:44
To: Lizette Jansen
Subject: Permission request at Voortrekker Nature Reserve/Monument
Importance: High

Good day

As discussed last week Friday, I write to request for permission to carry out a research study titled: The biodiversity of epiphytic lichens in Pretoria and its value for ecological indication with University of South Africa (UNISA), student number: 55774830, under the supervision of Prof Memory Tekere. The research will commence from 2016 to 2019. See attached letter.

Regards,

1

Appendix 4: Ethical clearance

UNISA university of south africa

CAES RESEARCH ETHICS REVIEW COMMITTEE

Date: 10/11/2014

Ref #: **2014/CAES/157**
Name of applicant: **Mr KW Maphangwa**
Student #: **55774830**

Dear Mr Maphangwa,

Decision: Ethics Approval

Proposal: The biodiversity of epiphytic lichens in Pretoria (South Africa) and its value for ecological indication

Supervisor: Dr L Zedda

Qualification: Postgraduate degree

Thank you for the application for research ethics clearance by the CAES Research Ethics Review Committee for the above mentioned research. Final approval is granted for the duration of the project.

The application was reviewed in compliance with the Unisa Policy on Research Ethics by the CAES Research Ethics Review Committee on 06 November 2014.

The proposed research may now commence with the proviso that:

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*
2) *Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study, as well as changes in the methodology, should be communicated in writing to the CAES Research Ethics Review Committee. An amended application could be requested if there are substantial changes from the existing proposal, especially if those changes affect any of the study-related risks for the research participants.*
3) *The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study.*

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

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Note:
The reference number [top right corner of this communiqué] should be clearly indicated on all forms of communication [e.g. Webmail, E-mail messages, letters] with the intended research participants, as well as with the CAES RERC.

Kind regards,

Signature
CAES RERC Chair: Prof EL Kempen

Signature
CAES Executive Dean: Prof MJ Linington

PLEASE NOTE PROVISIONS

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

Appendix 5: Tree with termite mounds

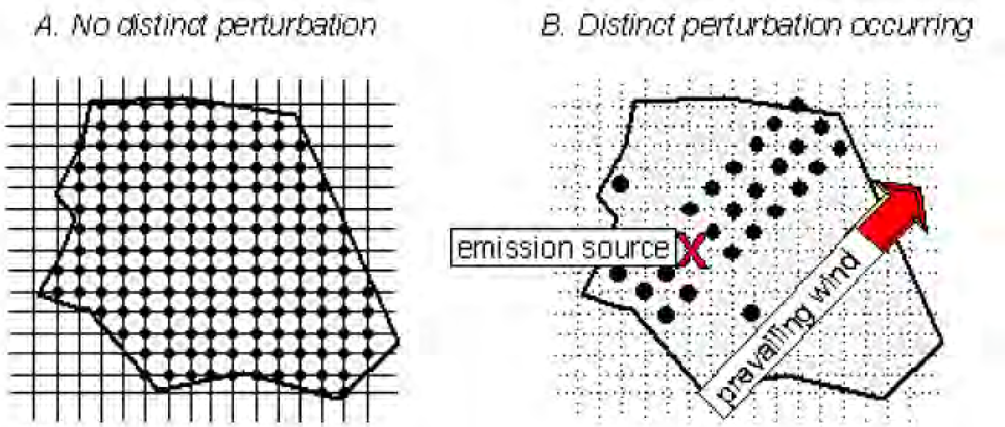
Appendix 6: *Jacaranda* tree with advertisement notice

Appendix 7: Protocol for lichen monitoring

Protocol for lichen monitoring from the "European guideline for mapping lichen diversity as an indicator of environmental stress" (Asta *et al*. 2002) that this study tested in Pretoria. Also the European standard protocol EN 16413 (2014) was consulted for applying the methodology in South Africa.

The European procedure for monitoring environmental alteration using lichens is universally applicable, but interpretation of the results has to be adapted to the regional characteristics of the lichen flora and to the prevalent types of environmental stress.

The **sampling design** defines rules to objectively select monitoring sites (sampling units). If the source of perturbation is not distinct in the monitored area, the sampling units are located at the intersections of the gridlines within a grid covering the areas (case A). In case of distinct perturbation, i.e. an emission source (case B), they are located around the emission source. Sampling density (= number of sampling units) can be variable for different geographical scales and type of study. This is calculated with a specific formula according to grid size.



The **size of each sampling unit** of a grid can vary from 0.25 km x 0.25 km to 12 km x 12 km, considering the presence of perturbation, the type of study and the geographical scale.

Sampling tactic also defines the quantity of trees to be sampled and their selection within the sampling units. The quantity of trees per sampling unit relies on its size, on the within-unit data variability and on the accessibility of appropriate trees in small areas. The recommended minimum number of trees to be investigated for sampling units of 0.25 km x 0.25 km is 3-4, in larger units of 1 km x 1 km, 6-12 trees should be investigated (see also EN 16413, 2014). If the minimal number of trees is not available, the sampling unit has to be shifted according to given rules. When many suitable trees occur in a sampling unit, trees for monitoring are selected according to a statistically valid method.

The **sampling procedure** indicates the investigation of free-standing trees, whose trunks must have an inclination lower than 10° from the vertical position, must receive direct solar radiation for at least part of the day and have a trunk circumference not less than 40 cm and not larger than 150 cm. Trees of the same size must be used within a survey for monitoring. Injured trees are not appropriate for survey purposes and they should not be considered for monitoring work. Trees evidently affected by actions such as liming, removal of the bark or of the lichens by humans or by grazing animals are additionally not appropriate and excluded from survey. If it is not possible to place at least three ladders of the grid onto one tree, the tree is not surveyed. Parts of the tree with greater than 25% cover of bryophytes are not used. A sampling grid composed of four ladders each with five quadrats sized 10 cm × 10 cm is appended vertically to the trunk so that the lower edge of the ladder is 1 m above the highest point of the ground. The four ladders of the sampling grid are placed to correspond to the four aspects (NSEW) of the tree trunk.
This sampling procedure in Pretoria is also explained in Chapter 3, section 3.8.

Data analysis consists in the **calculating of the Lichen Diversity Values** (LDV). The LDV of a sample unit is a measurable estimator of the environmental conditions in that unit. Since considerable variances in lichen development might be normal on various sides of the trunks, the frequencies must be summed independently for every aspect. The initial phase in calculating the LDV of a sampling unit (j) is to sum the frequencies of all lichen species found on selected tree (i) at different orientations (N, S, W and E). For each tree, there are four Sums of Frequencies (tree i: SF_{iN}, SF_{iE}, SF_{iS}, SF_{iW}). After that, the arithmetic mean of the Sums of Frequencies (MSF) for sampling unit j are calculated using the following formula for each aspect:

$$MSF_{Nj} = (SF_{1Nj}+SF_{2Nj}+SF_{3Nj}+SF_{4Nj}+\ldots..+SF_{nNj})/n$$

Wherein:

- MSF: Mean of the sums of frequencies of all the sampled trees of unit j
- SF: Sum of frequencies of all lichen species found at one aspect of tree i
- N, E, S, W: north, east, south, west
- n: number of trees sampled in unit j

The Lichen Diversity Value of a sampling unit j (LDV_j) is the sum of the MSFs of each aspect

$$LDV_j = (MSF_{Nj}+MSF_{Ej}+MSF_{Sj}+MSF_{Wj})$$

Example of calculating LDV

Tree 1, Unit j:

	N	E	S	W
Lichen species 1	0	5	4	2
Lichen species 2	1	3	3	2
Lichen species 3	1	2	5	2
Lichen species 4	0	0	0	0
Lichen species 5	0	5	1	5
Lichen species 6	0	1	2	5
Lichen species 7	0	4	1	4
Lichen species 8	0	4	2	1
Lichen species 9	0	1	0	5
Lichen species 10	1	1	1	0
Sums of Frequencies (SF)	**3**	**26**	**19**	**26**

Tree 2, Unit j:

	N	E	S*)	W
Lichen species 1	0	0	-	2
Lichen species 2	1	2	-	5
Lichen species 3	0	0	-	0
Lichen species 4	1	3	-	5
Lichen species 5	1	4	-	4
Lichen species 6	0	0	-	0
Lichen species 7	1	5	-	4
Lichen species 8	1	4	-	3
Lichen species 9	1	1	-	4
Lichen species 10	0	2	-	1
Sums of Frequencies (SF)	**6**	**21**	-	**28**

....

....

Tree n-1, Unit j:

	N*)	E	S	W
Lichen species 1	-	5	5	4
Lichen species 2	-	4	2	1
Lichen species 3	-	2	3	3
Lichen species 4	-	0	3	4
Lichen species 5	-	0	0	0
Lichen species 6	-	5	4	3
Lichen species 7	-	2	2	3
Lichen species 8	-	4	0	3
Lichen species 9	-	2	3	3
Lichen species 10	-	3	1	2
Sums of Frequencies (SF)	-	**27**	**23**	**26**

Tree n, Unit j:

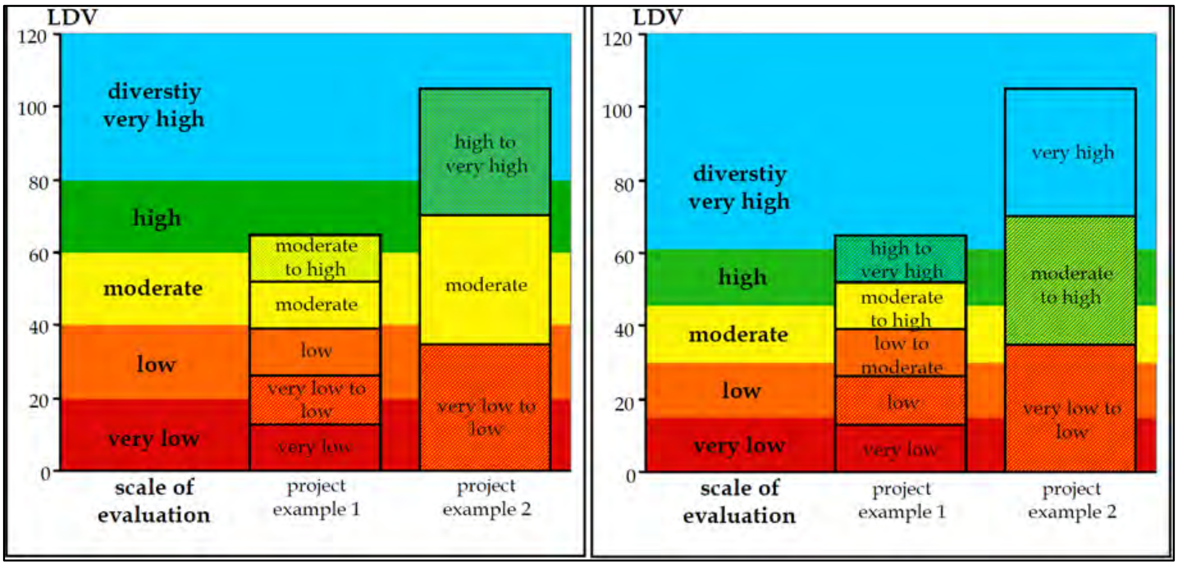
	N	E	S	W
Lichen species 1	0	5	5	5
Lichen species 2	0	3	1	4
Lichen species 3	1	4	5	2
Lichen species 4	0	3	5	3
Lichen species 5	1	4	4	4
Lichen species 6	0	3	3	4
Lichen species 7	0	0	0	0
Lichen species 8	0	3	2	0
Lichen species 9	0	0	0	0
Lichen species 10	0	0	1	3
Sums of Frequencies (SF)	**2**	**25**	**26**	**25**

	N	E	S	W
Sums of Frequencies Tree 1	3	26	19	26
Sums of Frequencies Tree 2	6	21	-	28
....	....	....	....	....
....	....	....	....	....
Sums of Frequencies Tree n-1	-	27	23	26
Sums of Frequencies Tree n	2	25	26	25
Means of Sums of Frequencies (MSF)	**3,7**	**24,8**	**22,7**	**26,3**
LDV of unit j		**77,3**		

Lichen Diversity Classes (LDC) are then defined by grouping LDV into classes. Standard errors of LDV can help to define the size of classes. If these are large, the classes will be broad. If standard errors are small, a finer distinction of lichen diversity values will be possible (smaller classes).

For data Interpretation LDV classes are assigned to an **interpretation scale** to detect geographic patterns of LDV and relate these to environmental alteration (deviation from background conditions). In the European guideline, lichen diversity is defined as “very high - high - moderate - low - very low“. Examples for the scale of different geographical areas are reported as follow.

The LDV classes are assigned to different colours in the interpretation scale.



If an interpretation scale is not available, interpretations can be based on the differences between maximum and minimum LDV values within the survey area.

Further examples from other publications are reported below.

Naturality/alteration scale obtained from Loppi *et al*. (2002) for Tyrrhenian Italy

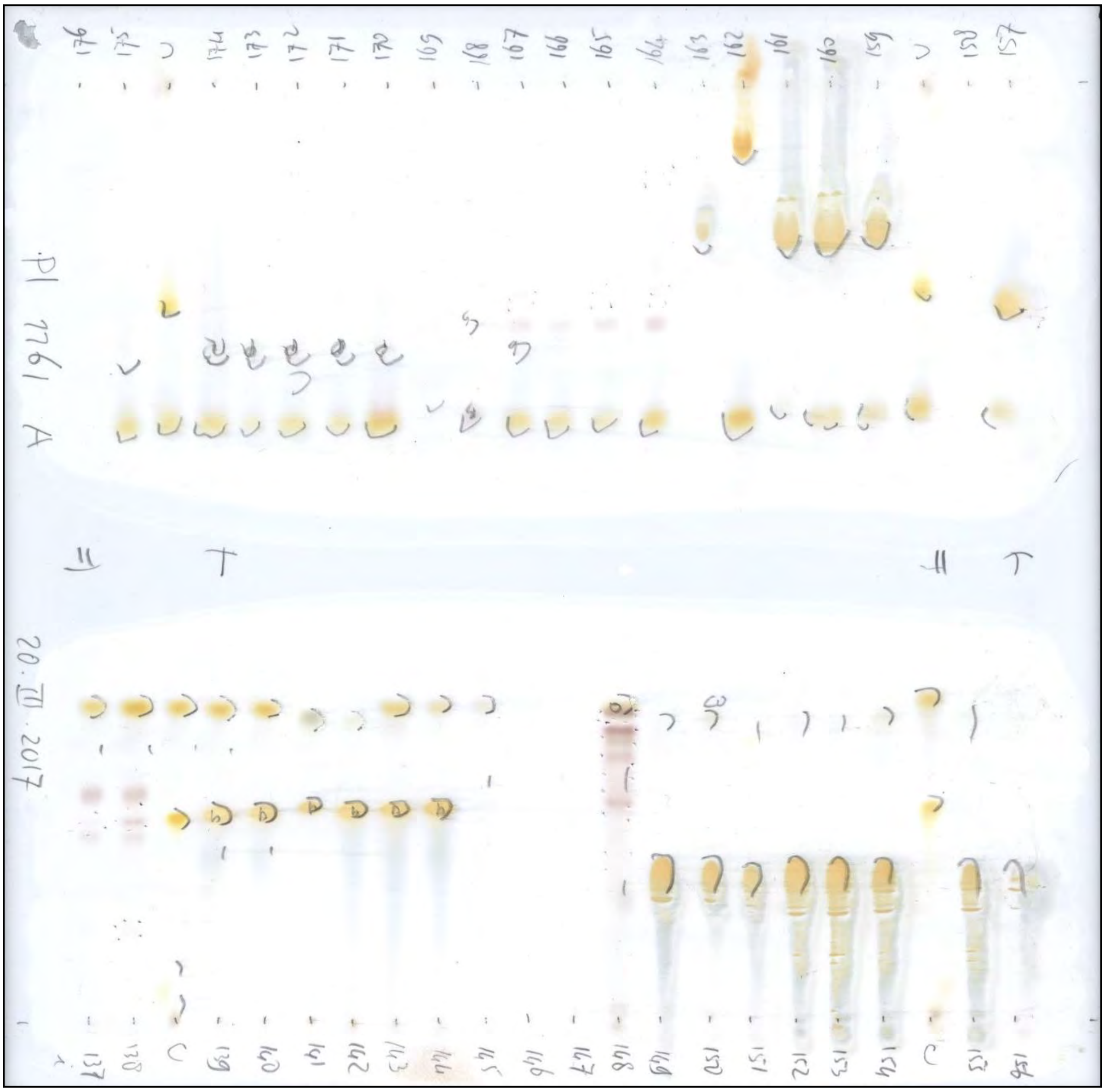
LB values	% deviation from normal conditions	Interpretation
0	100	Lichen desert
1-25	75-99	Alteration
25-50	50-75	Semi-alteration
50-75	25-50	Semi-naturality
> 75	0-25	Naturality

Naturality/alteration scale obtained by Frati and Brunialti (2006) for the town Ancona in Italy.

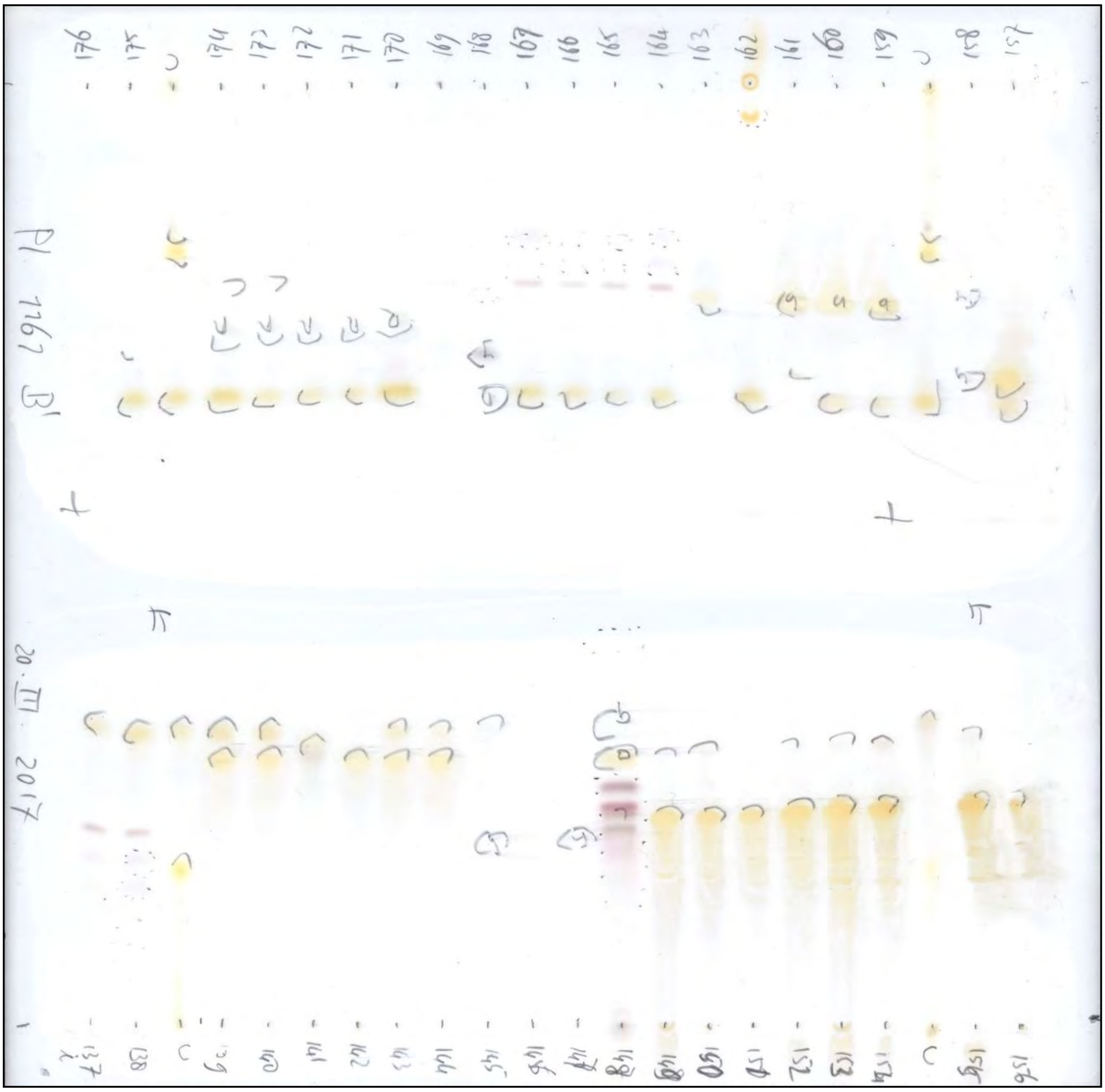
% Deviation from normal conditions	Classes	LB	LDV
0-25	1. Naturality	>70	>115
26-50	2. Semi-naturality	41-70	76-115
51-75	3. Semi-alteration	21-40	41-75
76-99	4. Alteration	1-20	1-40
100	5. Lichen desert	0	0

Appendix 8: *Jacaranda* trees

Appendix number 9A: TLC plate run with Solvent A (toluene-dioxan-acetic acid)



Appendix number 9B: TLC plate run with Solvent B (hexane-diethyl ether-formic acid)



Appendix number 9C: TLC plate run with Solvent C (toluene-acetic acid)

Appendix 10: Survey form

Date: ....

Surveyor: ....

Locality: ....

Site number ……………… Plot number:..................... Tree number: ....

Lichen species	N	E	S	W

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General Data

Altitude above sea level (in m)

GPS coordinates

Main aspect of the community i.e. principally foliose, fruticose, crustose

Tree species (South Africa)

01 Acacia karoo
02 Acacia caffra
03 Jacaranda ….

circumference of tree trunk at 150 cm above ground level (in cm)

Age of lichens

1 young and mature thalli together
2 predominantly young thalli
3 predominantly mature thalli

Lichen vitality

1 predominantly healthy thalli
2 healthy and degraded thalli
3 predominantly degraded thalli

Tree description

1 exposed, 2 sheltered

1 unshaded, 2 shaded

Bark cracks: 1 superficial, 2 moderately deep, 3 deep

☐☐ Type of terrain

00 no statement	04 slope
01 flat	05 hill top
02 valley	06 gap
03 depression	07 mountain

☐ (if not flat) Aspect

0 no statement	3 N
1 S	4 E
2 W	

☐☐ Site Landuse

00 no statement	08 backyard
01 urban area	09 private garden
02 rural area	10 fallow land
03 village	11 grassland
04 industrial area	12 pasture
05 commercial area	13 farmland
06 park/grassy plot	14 forest / nature reserve
07 memorial park	15 riparian vegetation

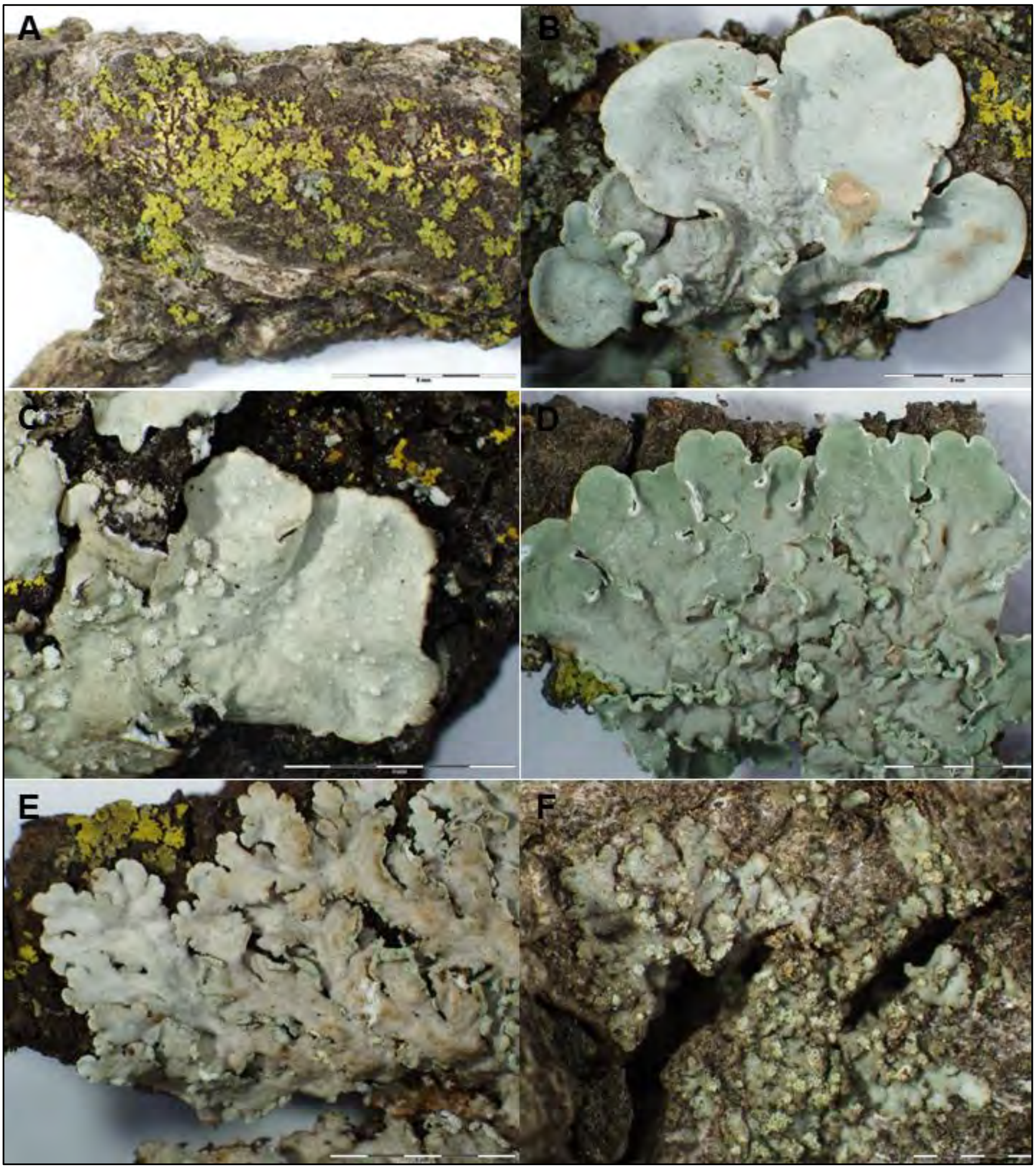
☐ Influence by traffic	☐ Distance to the road
1 dirty road	1 < 2 m
2 asphalt road, low traffic	2 < 5 m
3 main road/highway, much traffic	3 < 10 m
	4 > 10 m

☐☐ Other emission sources

01 domestic heating	06 incinerator
02 chemical industry	07 landfill
03 power plant (fossil fuelled)	08 sewerage purification plant
04 metallurgical plant	09 intensive agriculture
05 coking plant	10 lime emitting plant
	11 Other emission sources

☐☐ distance (km) and location of emission source(s) from examined tree

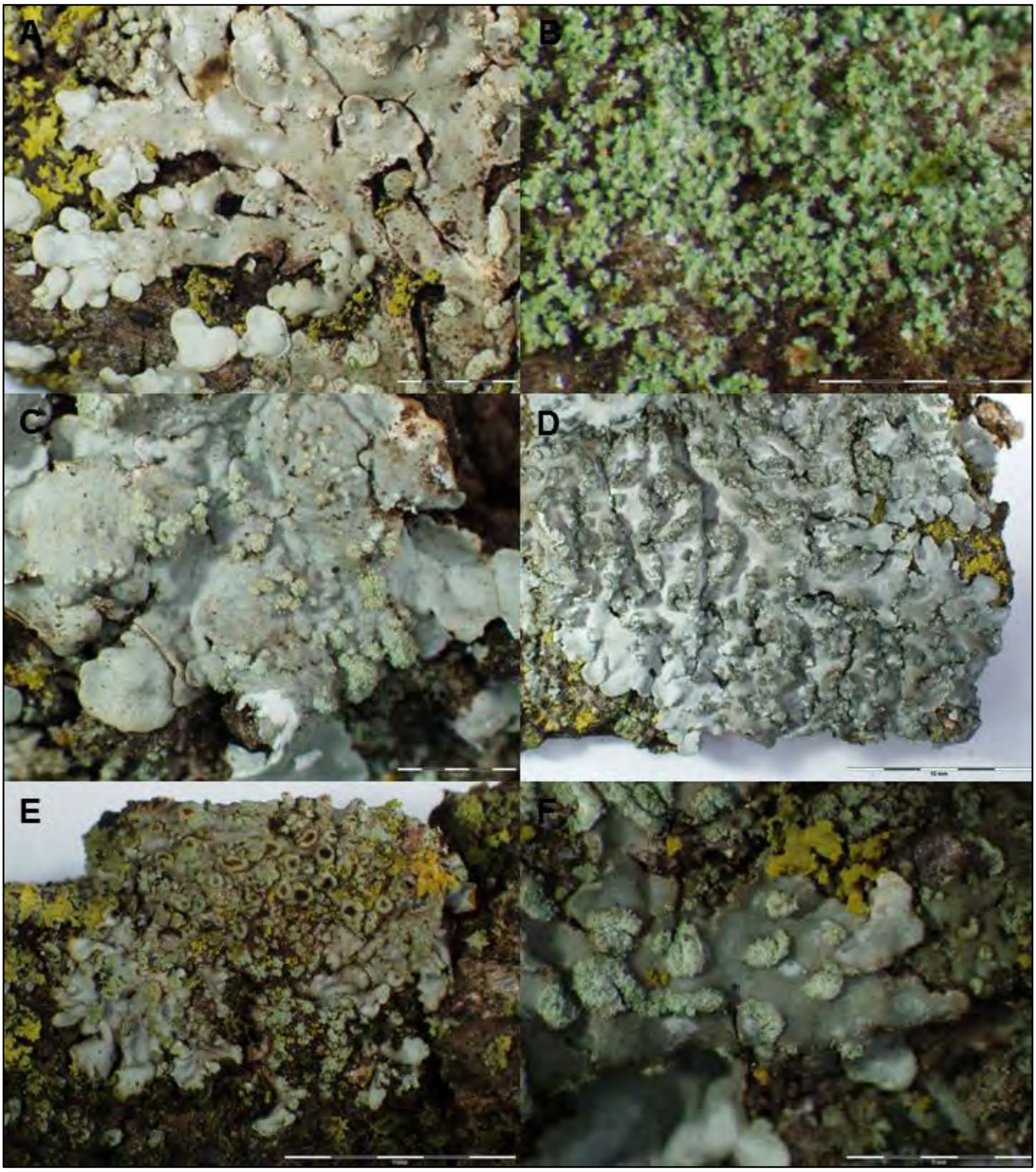
Appendix 11A: Pictures of lichens



A. *Candelaria concolor*, B. *Parmotrema austrosinense*, C. *Flavopunctelia flaventior*,

D. *F. soredica*, E. *Heterodermia speciosa* and F. *Hyperphyscia adglutinata*

Appendix 11B: Pictures of lichens

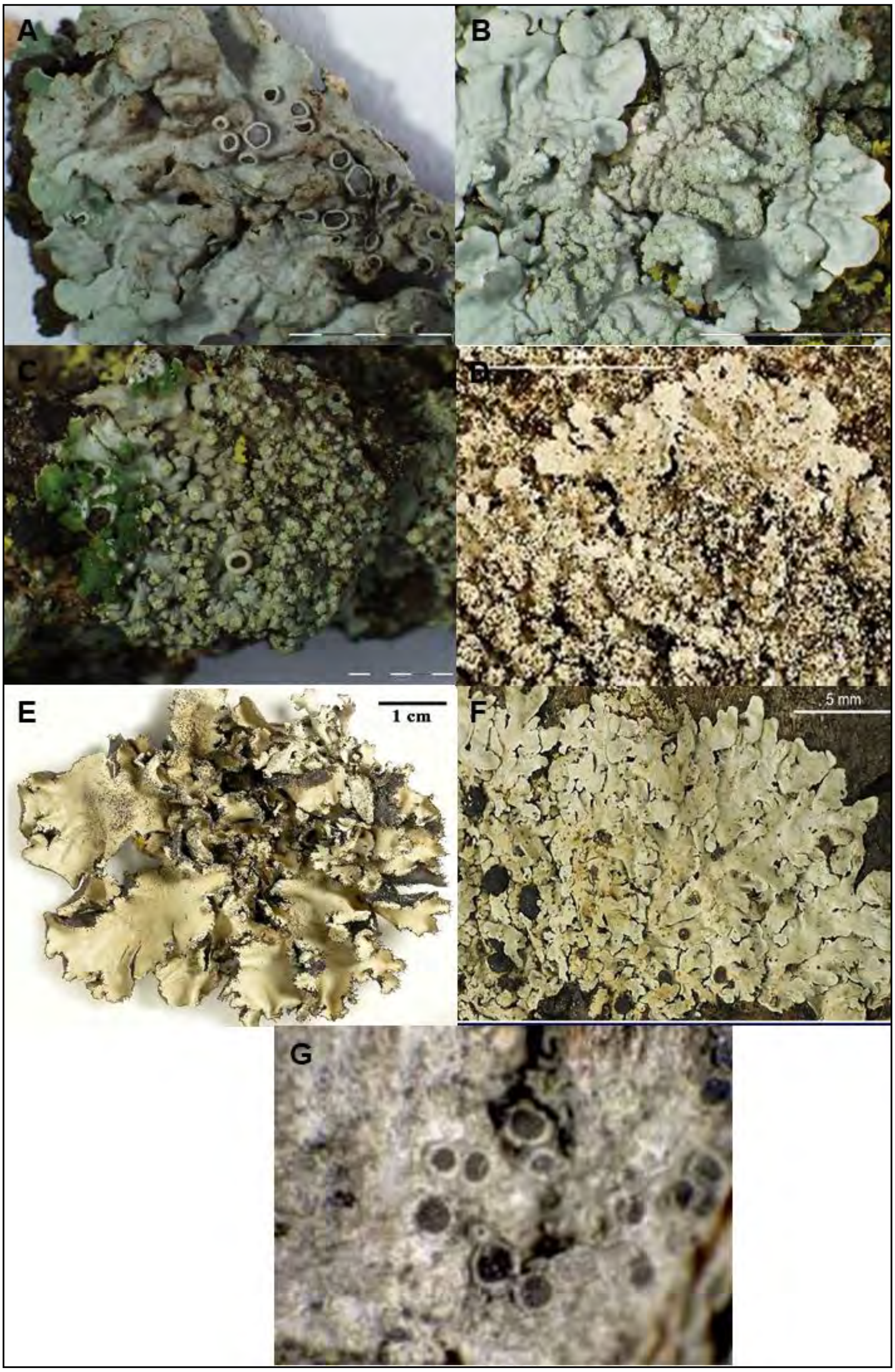


A. *Pyxine cocoes*, B. *Lepraria* spp., C. *Canoparmelia texana*, D. *Culbersonia nubila*, E. *Hyperphyscia pandani* and F. *Physcia tribacia*

Appendix 11C: Pictures of lichens

A. *Amandinea natalensis*, B. *Chrysothrix xanthina*, C. *Dirinaria applanata*, D. *Hyperphyscia granulata*, E. *Hyperphyscia pruinosa* and F. *Hyperphyscia isidiata* (*H. isidiata* picture obtained from Nimis and Martellos, 2017: http://dryades.units.it/italic, CC BY-SA4.0)

Appendix 11D: Pictures of lichens



A. *Physcia biziana*, B. *Physcia erumpens*, C. *Physcia poncinsii*, D. *Physcia undulata*, E. *Parmotrema reticulatum*, F. *Pyxine petricola* and G. *Rinodina ficta*. Picture D, E, F and G are from Nimis and Martellos, 2017: http://dryades.units.it/italic, CC BY-SA4.0

Appendix 12: TLC Retention factors (Rf) and identities of detected secondary metabolites

	TLC - Rf (retention factor)			Comments
Secondary Compound	Solvent A	Solvent A	Solvent A	
atranorin	75	73	79	
divaricatic acid	39	68	51	
lecanoric acid	28	44	22	
lichexanthone	72	66	75	
perlatolic acid	44	75	54	
salazinic acid	10	7	4	
terpenoids				"A pool of different compounds with different Rf not identifiable by TLC".
usnic acid	70	66	71	
zeorin	52	42	43	
unidentified traces				Obtained from *Lepraria* spp.

Appendix 13: Published paper

The Lichenologist 51(2): 187–191 (2019) © British Lichen Society, 2019
doi:10.1017/S0024282919000033

Short Communication

The phylogenetic position of *Culbersonia* is in the *Caliciaceae* (lichenized ascomycetes)

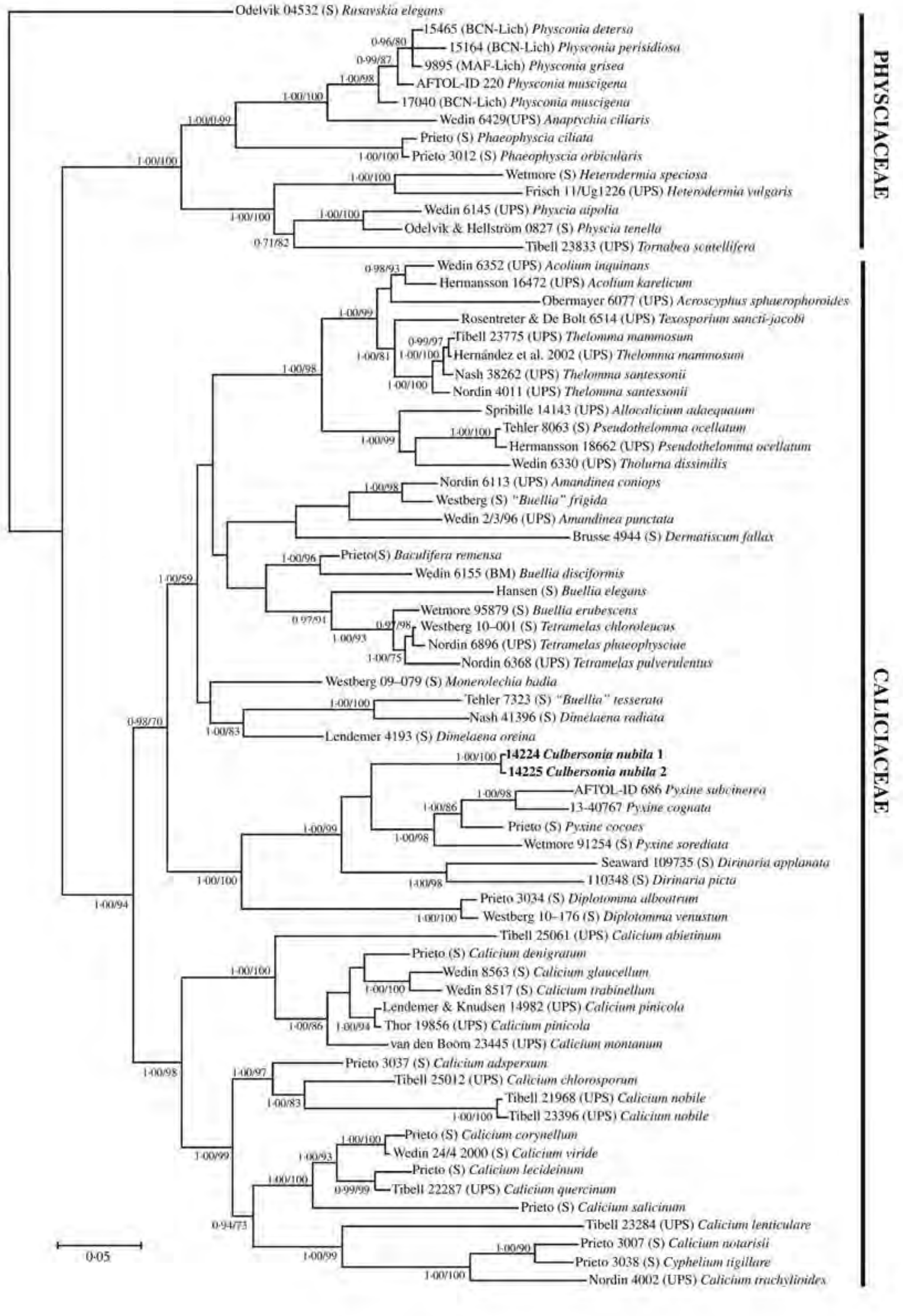
Culbersonia Essl. is a monotypic genus originally based on *C. americana* Essl., a taxon described from Arizona, United States of America (Esslinger 2000). Soon after publication, it was realized that this species had already been described in 1980, as *Pyxine nubila* Moberg, from Africa (Moberg 1980). Consequently, the combination *Culbersonia nubila* (Moberg) Essl. was made (Nash *et al.* 2002). The species combines characters of *Pyxine* Fr. with those of *Physconia* Poelt. It is widely distributed in dry subtropical regions around the world but is most common in Africa and Central America, where it grows on trees and rocks (Swinscow & Krog 1988; Moberg 2004; Obermayer *et al.* 2009).

Culbersonia is currently thought to belong in the *Physciaceae* Zahlbr. because it morphologically resembles the genus *Physconia* Poelt (Esslinger 2000), but this position has not yet been confirmed by genetic analysis (Lücking *et al.* 2016). The type species was originally described in the genus *Pyxine* Fr., which is currently classified in a different family, *Caliciaceae* Chevall (Prieto & Wedin 2017). These two families constitute the order *Caliciales* Bessey in the current sense. Almost all species in both families are lichenized. Both families contain crustose, foliose and a small number of fruticose growth forms, and together contain *c.* 600 known species. Non-mazaedioid genera, previously classified in the *Physciaceae* or *Buelliaceae* Zahlbr., have been added to the family *Caliciaceae* (which previously contained only mazaedioid taxa) after molecular work (Wedin *et al.* 2002). Many genera classified for a long time in the *Caliciales* (see e.g. Tibell 1984) are now relocated in other families, orders, and classes following molecular work. However, it is worth noting that all six genera that Tibell (1984) classified in the *Caliciaceae* still remain in that family in the present sense.

Culbersonia nubila was identified by us among lichens collected in South Africa (Maphangwa *et al.* 2018). As the phylogenetic position of this monotypic genus is uncertain, we produced genetic data from our specimens and generated a phylogenetic tree to establish the most suitable taxonomic status of *Culbersonia*.

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993) for the rDNA internal transcribed spacer 1, 5.8S and internal transcribed spacer 2 (collectively referred to as ITS), and LR0R and LR5 (Vilgalys & Hester 1990; Cubeta *et al.* 1991) were used to amplify the 28S rDNA of the nuclear ribosomal repeat. PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s respectively) with a final 72 °C step for 10 min. PCR products were checked in 1% agarose gels and positive reactions were sequenced with one of the PCR primers.

BLAST (Altschul *et al.* 1997) of 5.8S-ITS2 and 28S rDNA sequences was used to select the most closely related taxa; 5.8S-ITS2 and 28S rDNA were the only regions amplified from the samples. ITS only or 28S rDNA only phylogenies produced significant support for some clades, such as that including *Culbersonia*, *Dirinaria* and *Pyxine*, but failed to recover significant support values at the family level. Therefore, a 5-gene phylogeny was produced to add some resolution at the supraspecific level. Thus, 5.8S-ITS2, 28S rDNA, mtSSU rDNA, *Mcm7* and β-tubulin sequences of representative members of the *Caliciaceae* were downloaded from GenBank, mainly originating from Schmull *et al.* (2011) and Prieto & Wedin (2017), including most sequences available for the genus *Pyxine* when all five loci were available for the species. *Rusavskia elegans* was used as outgroup, following Prieto & Wedin (2017). Sequences were first aligned



Odelvik 04532 (S) Rusavskia elegans
15465 (BCN-Lich) Physconia detersa
15164 (BCN-Lich) Physconia perisidiosa
9895 (MAF-Lich) Physconia grisea
AFTOL-ID 220 Physconia muscigena
17040 (BCN-Lich) Physconia muscigena
Wedin 6429(UPS) Anaptychia ciliaris
Prieto (S) Phaeophyscia ciliata
Prieto 3012 (S) Phaeophyscia orbicularis
Wetmore (S) Heterodermia speciosa
Frisch 11/Ug1226 (UPS) Heterodermia vulgaris
Wedin 6145 (UPS) Physcia aipolia
Odelvik & Hellström 0827 (S) Physcia tenella
Tibell 23833 (UPS) Tornabea scutellifera
Wedin 6352 (UPS) Acolium inquinans
Hermansson 16472 (UPS) Acolium karelicum
Obermayer 6077 (UPS) Acroscyphus sphaerophoroides
Rosentreter & De Bolt 6514 (UPS) Texosporium sancti-jacobi
Tibell 23775 (UPS) Thelomma mammosum
Hernández et al. 2002 (UPS) Thelomma mammosum
Nash 38262 (UPS) Thelomma santessonii
Nordin 4011 (UPS) Thelomma santessonii
Spribille 14143 (UPS) Allocalicium adaequatum
Tehler 8063 (S) Pseudothelomma ocellatum
Hermansson 18662 (UPS) Pseudothelomma ocellatum
Wedin 6330 (UPS) Tholurna dissimilis
Nordin 6113 (UPS) Amandinea coniops
Westberg (S) "Buellia" frigida
Wedin 2/3/96 (UPS) Amandinea punctata
Brusse 4944 (S) Dermatiscum fallax
Prieto(S) Baculifera remensa
Wedin 6155 (BM) Buellia disciformis
Hansen (S) Buellia elegans
Wetmore 95879 (S) Buellia erubescens
Westberg 10–001 (S) Tetramelas chloroleucus
Nordin 6896 (UPS) Tetramelas phaeophysciae
Nordin 6368 (UPS) Tetramelas pulverulentus
Westberg 09–079 (S) Monerolechia badia
Tehler 7323 (S) "Buellia" tesserata
Nash 41396 (S) Dimelaena radiata
Lendemer 4193 (S) Dimelaena oreina
14224 Culbersonia nubila 1
14225 Culbersonia nubila 2
AFTOL-ID 686 Pyxine subcinerea
13-40767 Pyxine cognata
Prieto (S) Pyxine cocoes
Wetmore 91254 (S) Pyxine sorediata
Seaward 109735 (S) Dirinaria applanata
110348 (S) Dirinaria picta
Prieto 3034 (S) Diplotomma alboatrum
Westberg 10–176 (S) Diplotomma venustum
Tibell 25061 (UPS) Calicium abietinum
Prieto (S) Calicium denigratum
Wedin 8563 (S) Calicium glaucellum
Wedin 8517 (S) Calicium trabinellum
Lendemer & Knudsen 14982 (UPS) Calicium pinicola
Thor 19856 (UPS) Calicium pinicola
van den Boom 23445 (UPS) Calicium montanum
Prieto 3037 (S) Calicium adspersum
Tibell 25012 (UPS) Calicium chlorosporum
Tibell 21968 (UPS) Calicium nobile
Tibell 23396 (UPS) Calicium nobile
Prieto (S) Calicium corynellum
Wedin 24/4 2000 (S) Calicium viride
Prieto (S) Calicium lecideinum
Tibell 22287 (UPS) Calicium quercinum
Prieto (S) Calicium salicinum
Tibell 23284 (UPS) Calicium lenticulare
Prieto 3007 (S) Calicium notarisii
Prieto 3038 (S) Cyphelium tigillare
Nordin 4002 (UPS) Calicium trachylioides
PHYSCIACEAE
CALICIACEAE
0·05

in MEGA 5.0 (Tamura *et al.* 2011) with the ClustalW application and then corrected manually. Ambiguous regions were not removed from the alignment. ITS1 was excluded because of the insertions/deletions making alignment difficult. Gblocks (Castresana 2000) was employed to remove ambiguous positions from 5.8S-ITS2, 28S rDNA and mtSSU, while introns were manually removed from *Mcm7* and β-tubulin datasets. The final alignment included 116/254 variable sites in the 5.8S and ITS2 regions, 219/642 in the 28S rDNA, 212/513 in the mtSSU rDNA, 221/444 in the *Mcm7* gene, and 228/624 in the β-tubulin gene. The aligned loci were loaded as independent partitions (*Mcm7* and β-tubulin each split into three partitions, one for each codon position) in PAUP* 4.0b10 (Swofford 2001) and subjected to MrModeltest 2.3 (Nylander 2004). The model GTR+Γ+I was implemented for all partitions (except the 2nd position of β-tubulin, for which HKY+Γ+I was employed) in MrBayes 3.1 (Ronquist & Huelsenbeck 2003), where a Bayesian analysis was performed (two simultaneous runs, six chains, temperature set to 0·2, sampling every 100th generation) until convergence parameters were met after *c.* 890 000 generations (on which the Bayesian analysis is based), standard deviation having fallen below 0·01. A total of 8900 trees were sampled but the first 25% (2225) was discarded as burn-in; the others were used to produce a consensus tree. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis 2006) using the standard search algorithm (data partitioned, GTRMIX model, 2000 bootstrap replications). The significance threshold was set above 0·95 for posterior probability (PP) and 70% bootstrap proportions (BP).

*Specimens sequenced (*Culbersonia nubila*).* **South Africa:** *Gauteng Province*: Pretoria, Arcadia, Stanza Bopape Street, *c.* 25°44′41″S, 28°12′30″E, 1332 m, 20 vii 2016, *Maphangwa & Zedda* [KWM_0205] (PRE), no. 205 = ALV 14225, GenBank MH121318 (ITS) & MH121320 (LSU); Pretoria, Pionier Museum, Keuning Dr, *c.* 25°44′07″S, 28°18′36″E, 1317 m, 14 xi 2016, *Maphangwa* [KWM_0103] (PRE), no. 103 = ALV 14224, GenBank MH121317 (ITS) & MH121319 (LSU).

The two analyzed specimens of *Culbersonia nubila* clustered together with only a small number of different base pairs (Fig. 1). They clustered with significant statistical support with the monophyletic genera *Pyxine* and *Dirinaria* (Tuck.) Clem., nested deep within the family *Caliciaceae*, and they are unrelated to the *Physciaceae*. Therefore, we propose to classify the genus *Culbersonia* in the *Caliciaceae* in the current sense.

Morphologically, *Culbersonia* is quite distinct from other foliose lichens in the *Caliciales*. The upper surface is thickly pruinose, grey with a bluish tint, K+ rose-violet, and green when wet. The lower surface is pale tan, with the same bluish tint as on the upper surface in a broad marginal zone. *Culbersonia* shares with *Pyxine* the presence of an internal stipe in the apothecia. However, apothecia are extremely rare in *Culbersonia* and currently only one apothecium has been reported (Moberg 1980). Conidia are cylindrical to fusiform, but also rare and again only reported from one specimen (Nash *et al.* 2002). The overall aspect of the thallus, however, mostly resembles that of the genus *Physconia*, with which it also shares the pale lower surface which is almost invariably black in all species of *Pyxine* and *Dirinaria*.

The monophyletic group formed by *Culbersonia*, *Pyxine* and *Dirinaria* is morphologically distinguished from the other *Caliciaceae* by the appressed foliose growth form and the absence of a mazaedium, and ecologically by its predominance in the (sub-) tropics.

Almost all other genera in the two families *Physciaceae* and *Caliciaceae* that are represented in Fig. 1 are shown to be monophyletic. These results agree with those produced by Wedin *et al.* (2002), Helms *et al.* (2003), Gaya *et al.* (2012), Miadlikowska *et al.* (2014) and Prieto & Wedin (2017), where the lineages comprising the families of *Physciaceae* and *Caliciaceae* were shown to be significantly different. Three main clades were found within *Caliciaceae*, those of *Calicioideae*, *Buellioideae* s. str. and the clade containing *Dirinaria*, *Pyxine* and *Diplotomma* Flot., also in agreement with previous authors. The

FIG. 1. Consensus phylogram produced in MrBayes after the analysis of a combined ITS + nuLSU rDNA + mtSSU rDNA + *Mcm7* + β-tubulin-alignment of species in the families *Caliciaceae* and *Physciaceae* demonstrating the phylogenetic position of *Culbersonia nubila* nested within the *Caliciaceae.* Values next to nodes represent Bayesian PP and maximum likelihood bootstrap proportions (BP); only those nodes supported by >0·95 PP or >70% BP are annotated. Taxon names are preceded by the collectors of the voucher specimen and herbarium location for specimens not mentioned in the text. Further details of the species included in the tree can be found in Schmull et al. (2011) and Prieto & Wedin (2017).

exception is the genus *Buellia* De Not., which contains many species that should be reclassified into other genera. Such species are marked as "*Buellia*" in Fig. 1.

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Appendix 14: Generalised linear regression models (GLM) with interpolated environmental data

		Intercept	Altitude	Landuse Industrial areas and busy roads	Treespecies Acacia caffra	Tree species Acacia karroo	Tree circumference	NO_x	SO_2	Summary statistics of each model
LDV	Estimate	37,391	0,056	**-44,261**	-4,116	**17,609**	0,021	**-0,477**	-4,913	Residual SE: 22.02 (156 df) Multiple R-squared: 0.5654 Adjusted R-squared: 0.5459 F-statistic: 29 on 7 and 156 df **p-value: < 2.2e-16**
	Std Error	62,516	0,037	7,582	8,849	4,925	0,119	0,187	5,956	
	t value	0,60	1,52	-5,84	-0,47	3,58	0,18	-2,55	-0,82	
	P value	0,551	0,130	**0,000*** **	0,642	**0,000*** **	0,860	**0,012***	0,411	
Candelaria concolor	Estimate	12,877	0,010	-1,391	2,515	0,824	0,024	**-0,143**	-1,677	Residual SE: 5.146 (156 df) Multiple R-squared: 0.1475 Adjusted R-squared: 0.1092 F-statistic: 3.854 on 7 and 156 df **p-value: 0.0006775**
	Std Error	14,611	0,009	1,772	2,068	1,151	0,028	0,044	1,392	
	t value	0,88	1,17	-0,79	1,22	0,72	0,86	-3,27	-1,20	
	P value	0,379	0,243	0,434	0,226	0,475	0,392	**0,001****	0,230	
Canoparmelia texana	Estimate	-1,855	0,002	**-2,446**	-0,674	0,430	-0,001	0,010	0,333	Residual SE: 2.06 (156 df) Multiple R-squared: 0.2636 Adjusted R-squared: 0.2305 F-statistic: 7 976 on 7 and 156 df **p-value: 2.886e-08**
	Std Error	5,850	0,003	0,709	0,828	0,461	0,011	0,017	0,557	
	t value	-0.32	0,61	-3,45	-0,81	0,93	-0,12	0,59	0,60	
	P value	0,752	0,540	**0,001*** **	0,417	0,352	0,901	0,553	0,551	
Culbersonia nubila	Estimate	2,161	-0,004	**-4,222**	**-3,301**	**1,047**	0,003	0,013	**1,651**	Residual SE: 2.275 (156 df) Multiple R-squared: 0.4683 Adjusted R-squared: 0.4444 F-statistic: 19.63 on 7 and 156 df **p-value: < 2.2e-16**
	Std Error	6,459	0,004	0,783	0,914	0,509	0,012	0,019	0,615	
	t value	0,33	-0,95	-5,39	-3,61	2,06	0,21	0,69	2,68	
	P value	0,738	0,343	**0,000*** **	**0,000*** **	**0,041***	0,834	0,492	**0,008****	
Flavopunctelia flaventior	Estimate	**-16,624**	**0,015**	**-2,042**	-0,845	**1,732**	0,008	-0,007	-0,345	Residual SE: 2.925 (156 df) Multiple R-squared: 0.3179 Adjusted R-squared: 0.2872 F-statistic: 10.38 on 7 and 156 df **p-value: 1.149e-10**
	Std Error	8,305	0,005	1,007	1,176	0,654	0,016	0,025	0,791	
	t value	-2,00	3,05	-2,03	-0,72	2,65	0,50	-0,30	-0,44	
	P value	**0,047***	**0,003****	**0,044***	0,473	**0,009****	0,616	0,766	0,664	
Flavopunctelia soredica	Estimate	**-25,325**	**0,030**	**-6,870**	**-3,806**	**2,378**	-0,011	-0,050	-1,004	Residual SE: 3.84 (156 df) Multiple R-squared: 0.6084 Adjusted R-squared: 0.5908 F-statistic: 34.63 on 7 and 156 df **p-value: < 2.2e-16**
	Std Error	10,903	0,006	1,322	1,543	0,859	0,021	0,033	1,039	
	t value	-2,32	4,63	-5,20	-2,47	2,77	-0,52	-1,53	-0,97	
	P value	**0,021***	**0,000*** **	**0,000*** **	**0,015***	**0,006****	0,605	0,127	0,336	
Heterodermia speciosa	Estimate	9,695	-0,001	**-7,120**	-2,402	0,496	-0,011	-0,006	0,164	Residual SE: 3,353 (156 df) Multiple R-squared: 0.4391 Adjusted R-squared: 0.4139 F-statistic: 17.45 on 7 and 156 df **p-value: < 2.2e-16**
	Std Error	9,520	0,006	1,155	1,347	0,750	0,018	0,028	0,907	
	t value	1,02	-0,19	-6,17	-1,78	0,66	-0,60	-0,23	0,18	
	P value	0,310	0,853	**0,000*** **	0,077	0,509	0,547	0,821	0,857	
Hyperphyscia adglutinata	Estimate	1,178	-0,002	0,648	**5,785**	2,041	-0,003	0,022	1,656	Residual SE: 6.228 (156 df) Multiple R-squared: 0.0731 Adjusted R-squared: 0.03151 F-statistic: 1.758 on 7 and 156 df p-value: 0.09958
	Std Error	17,686	0,010	2,145	2,503	1,393	0,034	0,053	1,685	
	t value	0,07	-0,18	0,30	2,31	1,47	-0,10	0,41	0,98	
	P value	0,947	0,857	0,763	**0,022***	0,145	0,920	0,684	0,327	
Hyperphyscia	Estimate	1,517	-0,004	0,106	0,140	0,283	0,017	0,005	0,421	Residual SE: 1.865 (156 df) Multiple R-squared: 0.03948 Adjusted R-squared: -0.003622 F-statistic: 0,916 on 7 and 156 df p-value: 0.4958
	Std Error	5,295	0,003	0,642	0,749	0,417	0,010	0,016	0,504	
	t value	0,29	-1,14	0,16	0,19	0,68	1,65	0,30	0,83	

	P value	0,775	0,255	0,869	0,852	0,499	0,101	0,766	0,405	
Hyperphyscia pandani	Estimate	-0,085	0,000	-0,025	0,025	0,004	0,000	0,000	0,021	Residual SE: 0.1099 (156 df) Multiple R-squared: 0.04637 Adjusted R-squared: 0.003575 F-statistic: 1.084 on 7 and 156 df p-value: 0.3764
	Std Error	0,312	0,000	0,038	0,044	0,025	0,001	0,001	0,030	
	t value	-0,27	0,19	-0,66	0,56	0,17	-0,53	0,05	0,71	
	P value	0,786	0,847	0,512	0,579	0,863	0,594	0,960	0,478	
Lepraria spp.	Estimate	**33,728**	0,006	**-13,142**	**-4,942**	**2,933**	-0,019	**-0,243**	**-3,977**	Residual SE: 4.599 (156 df) Multiple R-squared: 0.6502 Adjusted R-squared: 0.6345 F-statistic: 41.42 on 7 and 156 df **p-value: < 2.2e-16**
	Std Error	13,059	0,008	1,584	1,848	1,029	0,025	0,039	1,244	
	t value	2,58	0,75	-8,30	-2,67	2,85	-0,76	-6,23	-3,20	
	P value	**0,011***	0,456	**0,000*****	**0,008****	**0,005****	0,451	**0,000*****	**0,002****	
Parmotrema austrosinense	Estimate	-5,830	0,010	-2,644	0,101	**3,959**	0,020	-0,034	-0,472	Residual SE: 5.707 (156 df) Multiple R-squared: 0.1899 Adjusted R-squared: 0.1536 F-statistic: 5.225 on 7 and 156 df **p-value: 2.256e-05**
	Std Error	16,205	0,010	1,965	2,294	1,277	0,031	0,048	1,544	
	t value	-0,36	1,09	-1,35	0,04	3,10	0,64	-0,70	-0,31	
	P value	0,720	0,276	0,180	0,965	**0,002****	0,522	0,483	0,760	
Physcia tribacia	Estimate	5,847	0,000	-1,702	**3,486**	0,175	-0,001	0,012	-0,780	Residual SE: 3.125 (156 df) Multiple R-squared: 0.2823 Adjusted R-squared: 0.2501 F-statistic: 8.766 on 7 and 156 df **p-value: 4.556e-09**
	Std Error	8,873	0,005	1,076	1,256	0,699	0,017	0,027	0,845	
	t value	0,66	0,08	-1,58	2,78	0,25	-0,07	0,45	-0,92	
	P value	0,511	0,939	0,116	**0,006****	0,803	0,942	0,654	0,357	
Physcia undulata	Estimate	-1,18	0,000	0,08	0,323	0,04	0,004	0,005	0,12	Residual SE: 0.7183 (156 df) Multiple R-squared: 0.02364 Adjusted R-squared: -0.02017 F-statistic: 0.539 on 7 and 156 df p-value: 0.8035
	Std Error	2,04	0,001	0,247	0,289	0,161	0,004	0,006	0,194	
	t value	-0,58	0,10	0,32	1,12	0,25	0,96	0,84	0,62	
	P value	0,564	0,923	0,747	0,265	0,801	0,339	0,405	0,539	
Pyxine cocoes	Estimate	**15,557**	-0,004	**-3,080**	-0,159	**1,300**	0,001	**-0,047**	-1,131	Residual SE: 2.77 (156 df) Multiple R-squared: 0.2213 Adjusted R-squared: 0.1863 F-statistic: 6.333 on 7 and 156 df **p-value: 1.485e-06**
	Std Error	7,867	0,005	0,954	1,113	0,620	0,015	0,024	0,750	
	t value	1,98	-0,87	-3,23	-0,14	2,10	0,09	-1,98	-1,51	
	P value	**0,050***	0,388	**0,002****	0,887	**0,038***	0,925	**0,049***	0,133	
Pyxine petricola	Estimate	-0,142	0,000	-0,074	-0,120	-0,042	0,000	0,002	0,069	Residual SE: 0.4752 (156 df) Multiple R-squared: 0.01117 Adjusted R-squared: -0.0332 F-statistic: 0.252 on 7 and 156 df p-value: 0.971
	Std Error	1,349	0,001	0,164	0,191	0,106	0,003	0,004	0,129	
	t value	-0,11	-0,04	-0,45	-0,63	-0,39	0,11	0,44	0,53	
	P value	0,916	0,965	0,650	0,531	0,695	0,911	0,662	0,594	

Appendix 15: Published paper

Herzogia **31** (2), 2018: 949–964

Epiphytic lichen diversity on *Jacaranda* and *Acacia* trees in Pretoria (Tshwane, Republic of South Africa)

Khumbudzo Walter Maphangwa*, Harrie J.M. Sipman, Memory Tekere & Luciana Zedda

Abstract: Maphangwa, K.W., Sipman, H.J.M., Tekere, M, & Zedda, L. 2018. Epiphytic lichen diversity on *Jacaranda* and *Acacia* trees in Pretoria (Tshwane, Republic of South Africa). – Herzogia **31**: 949–964.

The epiphytic lichen diversity in and around the city of Pretoria (Tshwane, South Africa) has been investigated in 11 sites representing different land use types. Lichens were collected from three tree species: *Jacaranda mimosifolia*, *Acacia karoo* and *A. caffra*. Twenty-four taxa were recorded and are listed with notes on their ecology and distribution. Small foliose lichens of the family Physciaceae appear to be predominant, while crustose lichens are rare and fruticose lichens absent. The highest lichen diversity is found in protected areas, and comprises predominantly subtropical to tropical species. By contrast, at strongly disturbed and contaminated sites only few species are found, mostly *Heterodermia speciosa* and the cosmopolitan *Candelaria concolor* and *Hyperphyscia adglutinata*. No substantial differences in lichen richness could be detected among the three phorophytes. However, *Jacaranda*, being more common in disturbed areas, hosted more frequently species typical for disturbed conditions.

Zusammenfassung: Maphangwa, K.W., Sipman, H.J.M., Tekere, M, & Zedda, L. 2018. Baumbewohnende Flechten auf dem Palisanderholzbaum und Akazien in Pretoria (Tshwane, Republik Südafrika). – Herzogia **31**: 949–964.

Die baumbewohnenden Flechten der Stadt Pretoria und Umgebung (Tshwane, Südafrika) wurden an elf Lokalitäten mit unterschiedlichen Landnutzungstypen untersucht. Flechtenproben wurden an drei Baumarten gesammelt: *Jacaranda mimosifolia*, *Acacia karoo* und *A. caffra*. Insgesamt wurden 24 Taxa nachgewiesen, deren Verbreitung und Ökologie skizziert werden. Kleinblättrige Flechten der Familie Physciaceae kommen am häufigsten auf den untersuchten Bäumen vor; Krustenflechten sind selten und Strauchflechten fehlen. Es überwiegen subtropische bis tropische Arten. Bei intensiven anthropogenen Störungen kommen nur wenige Arten vor, vor allem *Heterodermia speciosa* und die Kosmopoliten *Candelaria concolor* und *Hyperphyscia adglutinata*. Es konnten keine wesentlichen Unterschiede in den Artenzahlen der Flechten auf den drei Phorophyten erkannt werden. Auf dem Palisanderholzbaum kommen allerdings häufiger Arten vor, die typisch für gestörte Standorte sind.

Key words: Biodiversity, lichenized fungi, distribution, savannah biome, man-made disturbance.

Introduction

South Africa is a well-known biodiversity hotspot for phanerogams (Myers et al. 2000, Mittermeier et al. 2004). However, the lichen diversity still remains much underexplored (Crous et al. 2006, Maphangwa et al. 2011, Mayrhofer et al. 2014). Lichens are less familiar to most local researchers than vascular plants and are frequently overlooked (Mukherjee et al. 2010). Nevertheless numerous publications exist which deal with South African lichens,

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including taxonomical revisions, floristic and ecological studies on lichens from different parts of South Africa: Crombie (1876a, 1876b), van der Byl (1931), Almborn (1966, 1987, 1988), Hale (1971, 1984), Wessels & van Vuuren (1986), Brusse (1984, 1985, 1986, 1988, 1994), Kärnefelt (1986, 1987, 1988), Thomas & Bhat (1994, 1995, 1996), B*üdel* (1995), J*ürgens* & Niebel-Lohmann (1995), Elix (1999, 2002, 2003), Feuerer & Zedda (2001), Wirth (2010), Zedda & Rambold (2004, 2009, 2010), Schultz et al. (2009), Zedda et al. (2010, 2011), Fryday (2015), Ahti et al. (2016). These studies focused mainly on saxicolous and terricolous lichens while epiphytic lichens have been less explored so far. Herbarium vouchers of published South African lichens are very scattered throughout numerous herbaria in South Africa, Europe and North America and therefore difficult to check (Fryday 2015). This hinders the floristic investigation of South Africa's lichen diversity.

A checklist of South African lichens was compiled by Doidge (1950) and supplemented by Almborn (1988), Feuerer & Zedda (2001), Fryday (2015) and Ahti et al. (2016). Online versions of the checklists of Fryday (2015) and Feuerer (2016) are available and updated periodically. However, the checklist is still provisional and far from complete. Reports may be based on misidentifications because earlier collections were sometimes uncritically identified as European taxa and need to be revised (Fryday 2015). Many taxonomic groups such as the genera *Buellia*, *Lecanora* and *Lecidea*, are still poorly investigated and no reliable identification keys are available, so that the pertinent names in the checklist cannot be verified. The current checklist of lichens and lichenicolous fungi from South Africa includes ca. 1850 species. This is a low number when compared with lichen diversity of other countries such as the much better explored Great Britain (1838 taxa) (Fryday 2015) and Italy (2700 taxa) (Nimis 2016). When fully explored, the lichen diversity of South Africa could amount to 2500-3000 taxa (Fryday 2015).

The Savannah biomes are much less investigated than the Desert and Karoo biomes and only studies on lichens in savannahs in Namibia are available so far (Zedda et al. 2009). In Pretoria only some patchy epiphytic lichen collections were formerly carried out (vouchers in PRE).

The present study is a first contribution to the knowledge of the epiphytic lichen diversity in Pretoria, focusing on *Jacaranda mimosifolia* D.Don, *Acacia karoo* Hayne and *A. caffra* (Thunb.) Willd. trees. It presents a first assessment of the biodiversity of epiphytic lichens in an urban environment in South Africa.

Material and methods

Study area

The study was carried out in the city of Pretoria and its surroundings. Pretoria is located in the northern part of the Gauteng province (25°44'46"S/28°11'10"E), within the City of Tshwane Metropolitan Municipality (Republic of South Africa). The province is placed in the north-east of South Africa, in a transitional belt between the plateau of the Highveld to the south and the lower-lying Bushveld to the north. The city is composed of a central business district and three outer sections: west, east and north. It is popularly known as "The Jacaranda City" due to the thousands of *Jacaranda* trees planted in its streets, parks and gardens (Henderson 1990, Joseph 2006, Coetzee et al. 2015).

The town has a population of about 2.4 million people (Liebenberg-Enslin & Petzer 2005). It enjoys a dry, sunny climate, with occasional late afternoon downpours in the summer months of October to April. Temperatures are usually fairly mild due to the city's high altitude (1271 m a.s.l.) with an average maximum daytime temperature of 21.5 °C in January, dropping to an average maximum of around 11 °C in July (Olowoyo et al. 2010). Snow is very rare and mean annual rainfall is 784 mm (Olowoyo et al. 2013). The study area falls within the Savannah biome with the exception of one collecting site, which falls within the Grassland biome, according to the definitions of Mucina & Rutherford (2006) and Marais (2004).

Sampling sites

11 sampling sites were investigated in the period from July 2016 to February 2017. They represent nature reserves, parks and other green areas as well as residential, industrial (i. e. metal pressings) and high traffic areas.

1 *Waterkloof*. This is a residential area situated in the south-east of Pretoria around 10 km from the city centre. It falls within the Savannah biome. Sampling was done at the Rigel Avenue (25°47'45"S/28°14'37"E, elevation 1546 m a.s.l.). The area is characterized by the presence of many planted *Jacaranda mimosifolia* trees along the streets. Traffic is heavy only along the main avenues and roads, and lower compared to the city centre. Visited on 18 July 2016.

2 *Pretoria central business district*. This district is in the city centre and falls within the Savannah biome. The collecting site was chosen at the Nana Sita Street (around 25°45'02"S/28°11'33"E, elevation 1327 m a.s.l.). This street is lined up with *J. mimosifolia* trees and has heavy traffic. Visited on 19 July 2016.

3 *Groenkloof Nature Reserve*. This reserve is located adjacent to the Fountains Valley (Christina De Wit Ave), just 5 km S of the city centre. It is about 600 ha in size and falls within the Savannah biome. The vegetation approaches a semi-open thicket but is dominated by a variety of woody species, including *Acacia karoo*, *A. caffra*, *Commelina erecta* L., and *Drimia multisetosa* (Baker) Jessop (Marais 2004). Collections were made in several spots (around 25°47'22"S/28°11'54"E, elevation 1390 m a.s.l.). Visited on 19 July 2016.

4 *Magalies*. This is a mountain area 15 km NW of the city centre. It falls within the Savannah biome. The sampling site was situated along the Hornsnek Road (25°40'52"S/28°04'07"E, elevation 1453 m a.s.l.) and was characterized by the presence of *A. caffra* and other *Acacia* species and grasses. Pollution is expected to be less in this site in comparison to downtown, because there is less movement of cars, there is no industry around it and it is not a residential area. Visited on 19 July 2016.

5 *Arcadia*. This area is in the central part of the town and concerns a park around the Union Buildings. It falls within the Savannah biome. The collecting site was located at Stanza Bopape Street (around 25°44'41"S/28°12'30"E, elevation 1332 m a.s.l.) next to the Union Buildings. Traffic is very heavy. The street is lined up with many old *J. mimosifolia* trees, on the bark of which citizens often pin notices and advertisements. Visited on 20 July 2016.

6 *Pretoria West*. This area is situated 7–8 km W of the city centre, and falls within the Savannah biome. Two spots were investigated. The first was along Quagga Road (around 25°45'24"S/28°08'32"E, elevation 1340 m a.s.l.), near a coal-fired power plant belonging to the City of Tshwane. The road is busy with cars and trucks and is lined up with old *J. mimosifolia* trees. Visited on 13 September 2017. The second site was in the *Pretoria West* - industrial area at the Staal Road (25°45'50" S/28°07'52" E, elevation 1360 m a.s.l.) opposite to the metal pressings. There are also other small industries present in the area. *J. mimosifolia* trees are found along the street. There is heavy traffic all around the area. Visited on 04 February 2017.

7 *Pionier Museum*. This area is located 10 km E of the city centre, and falls within the Savannah biome. The collecting site was situated at the Keuning Dr (around 25°44'07"S/28°18'36"E, elevation 1317 m a.s.l.). The vegetation found here includes *A. karoo*, *A. caffra* and other trees. Visited on 14 November 2016.

8 *Hercules*. This residential area is located NW of the city centre. It falls within the Savannah biome. Collections were made at the Van Der Hoff Road (25°43'03"S/28°08'25"E, elevation 1293 m a.s.l.), at a site dominated by *A. karoo* and *J. mimosifolia* trees. Car traffic is very heavy. There is also coal burning by households. Visited on 18 November 2016.

9 *Sunnyside*. This area is situated less than 3 km from the city centre and falls within the Savannah biome. The collecting site was located in the Jorissen Street (around 25°45'27"S/28°12'56"E, elevation 1345 m a.s.l.). This is a busy street with heavy traffic, lined up with *J. mimosifolia* trees. Visited on 03 February 2017.

10 *Lotus Gardens*. This residential area is situated 10 km from the city centre, and falls within the Savannah biome. The collecting site was located at the WF Nkomo Street (around 25°45'28"S/28°05'34"E, elevation 1370 m a.s.l.). The area has heavy traffic. Residents also burn coal for heating their homes during winter months. Vegetation found in this site includes planted *J. mimosifolia* and other woody trees, as well as different grasses. Visited on 04 February 2017.

11 *Rietvlei Nature Reserve.* This reserve is located along the R21 highway, 18 km SSE of the centre of Pretoria and 38 km N of the OR Tambo International Airport, and covers approximately 3800 ha in size. The area falls within the Grassland biome. The tree vegetation includes *Acacia caffra*, *A. decurrens* Willd., *A. karroo*, *Euclea crispa* (Thunb.) Gürke. Collections were made at one site close to the Rietvlei Dam (around 25°52'56"S/28°15'49"E, elevation 1506 m a.s.l.). Visited on 17 February 2017.

Phorophytes

All available epiphytic lichen species were collected from both native and exotic trees. Sampling was focused on the following three tree species:

- *Jacaranda mimosifolia*, commonly known as jacaranda, is an exotic species originating from North-Western Argentina (Coetzee et al. 2015). It is a fast-growing tree, 15 to 22 m tall, with a rounded and spreading crown The young bark is smooth, but with age it becomes rougher (Henderson 1990) (van Wyk and van Wyk 1997, Olowoyo et al. 2010).
- *Acacia karoo* is a native shrub to medium-sized tree, variable in shape but typically with a somewhat rounded, spreading crown (van Wyk & van Wyk 1997, Boon 2010). Usually, it is single stemmed and branches low down. The bark is coarse and fissured. *A. karoo* is a heterogeneous species (van Wyk & van Wyk 1997, Boon 2010).
- *Acacia caffra* is a native shrub to medium-sized deciduous tree up to 12 m in height, often with a twisted trunk and rather thin, spreading branches with a somewhat rounded crown. The bark is rough, sometimes fissured and horizontally cracked forming squares (Palgrave 1977, Boon 2010).

These tree species were selected for the investigation as they are widespread across the town. Jacaranda trees are planted and widely distributed in Pretoria along most roads and avenues. *Acacia karoo* and *A. caffra* are part of the natural savannah vegetation and are able to survive in urbanized areas. Other advantages of these trees are that they are easily identified, and easily accessible for sampling (Walker 1986).

Sampling and identification methods

The lichens were mainly collected from tree trunks. The specimens (altogether 114) were investigated by macro- and microscopic observation, chemical spot tests and partly by Thin Layer Chromatography following the methodology described by Orange et al. (2001). Pictures were taken with an Olympus DP72 digital color camera for microscopes. Images are available on request.

The lichen species were identified with the aid of the interactive LIASLight online identification keys (http://www.lias.net) and numerous publications. General keys used mainly for recognition of the genera included Swinscow & Krog (1988), Brodo et al. (2001), Nash et al. (2002). More specific keys were used for *Physciaceae* (Moberg 2004), *Chrysothrix* (Elix & Kantvilas 2007, Elix 2009) and other taxa (Matzer & Mayrhofer 1996, Sipman 2003). Taxonomical nomenclature follows the databases "LIASnames" (www.lias.net), "Index Fungorum" (www.indexfungorum.org/) and "Species Fungorum" (www.speciesfungorum.org/Names/Names.asp). Voucher specimens will be stored at the South African National Biodiversity Institute (SANBI) Herbarium in Pretoria (PRE).

Results

List of species

Amandinea natalensis (Vain.) Marbach

Magalies, Hornsnek Road, on *A. caffra*, Maphangwa & Zedda KWM_0032 (PRE).

This crustose subtropical species is known only from South Africa. A detailed description is provided by Marbach (2000) and it was known before from only one locality (Howick in Natal). It is included in the identification key of Sipman (2003) and the checklist of Fryday (2015). In Pretoria, it was found once, in a rural site with moderate traffic.

Candelaria concolor (Dicks.) Arnold

Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0001 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karoo* and *A. caffra*, Maphangwa & Zedda KWM_0009, KWM_0012 (PRE). Magalies, Hornsnek Road, on *A. caffra*, Maphangwa & Zedda KWM_031, (PRE). Hercules, Van Der Hoff Road, on *A. karoo* and *J. mimosifolia*, Maphangwa KWM_0039; KWM_0045 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0079 (PRE). Pretoria West, Staal Road, on *J. mimosifolia*, Maphangwa KWM_0115 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0118 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0139 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0172 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo* and *A. caffra*, Maphangwa KWM_0177; KWM_0195 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia*, Maphangwa & Zedda KWM_0208 (PRE). Central, Nana Sita Street, on *J. mimosifolia*, Maphangwa & Zedda KWM_0210 (PRE).

This is a cosmopolitan species (Almborn 1966), common on nutrient-rich substrates, often forming luxuriant colonies along rain tracks on tree trunks or on twigs of trees near farms or towns (Brodo et al. 2001). It is widespread in tropical and temperate regions and prefers in East Africa an altitude of 1000 to 2000 m a.s.l. (Swinscow & Krog 1988). Almborn (1966) and Zedda et al. (2009) reported it in Namibia from the bark of different tree species. *C. concolor* has been reported from South Africa by Almborn (1966, 1988; according to Fryday 2015). In Pretoria, it was found in all sampling sites, irrespective of degradation of the natural vegetation or air contamination sources.

Canoparmelia texana (Tuck.) Elix & Hale

Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0004 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra* and *A. karoo*, Maphangwa & Zedda KWM_0014, KWM_0023 (PRE). Hercules, Van Der Hoff Road, on *A. karoo*, Maphangwa KWM_0041 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0094 (PRE). Lotus Gardens, WF Nkomo, on *J. mimosifolia*, Maphangwa KWM_0135 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0173 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo* and *A. caffra*, Maphangwa KWM_0203 (PRE).

This species has a pantropical distribution and it is also known from East Africa (Swinscow & Krog 1988), where it occurs in dry, sun-exposed habitats on lowlands and coastal hills at up to 1000 m a.s.l., and from Madagascar (Aptroot 2016). Fryday (2015) reported it from South Africa. In Pretoria, it was found in areas with both disturbed and undisturbed areas, epiphytic in avenues, but avoiding the most disturbed city centre. Atranorin and divaricatic acid were found by TLC in KWM_0041, KWM_0094, and KWM_0203.

Chrysothrix xanthina (Vain.) Kalb

Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo*, Maphangwa KWM_0232 (PRE).

The species has been reported from North and South America, Macaronesia, Africa, Madagascar, Asia, New Zealand and Norfolk Island (Laundon 1981, Kalb 2001, Elix & Kantvilas 2007, Fryday 2015). In Pretoria, it was found only once.

Culbersonia nubila (Moberg) Essl.

Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karoo* and *A. caffra*, Maphangwa & Zedda KWM_0011; KWM_0225 (PRE). Pionier Museum, Keuning Dr, on *A. caffra*, Maphangwa KWM_0103 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0138 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0150 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia*, Maphangwa & Zedda KWM_0205 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0218 (PRE).

C. nubila (syn. *Pyxine nubila* Moberg) has a scattered distribution in dry, subtropical areas of America, Africa, Eurasia and Australia, where it grows on trees and rocks (Swinscow & Krog 1988, Moberg 2004, Obermayer et al. 2009). It has been reported from Lesotho and South Africa, in particular from Eastern Cape, Free State, Mpumalangs and the Natal provinces (Moberg 2004). Obermayer et al. (2009) reported it from Gauteng Province. A specimen from Pretoria collected on *Jacaranda* and checked by Moberg in 2004 is present in the herbarium database of UPS (http://130.238.83.220/botanik/browserecord.php?-action=browse&-recid=226595). In Pretoria it is rather common and was found in less disturbed areas and on trees in avenues. No recognizable substances were found by TLC in KWM_0103, KWM_0138 and KWM_0204.

Dirinaria applanata (Fée) D.D.Awasthi

Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0078 (PRE).

This is a common corticolous and saxicolous lichen widespread in all tropical regions of both hemispheres (Nash et al. 2004). It is also reported from Ethiopia, Kenya, Tanzania, Uganda and South Africa where it grows in natural woodlands, parks, avenues, and plantations, from sea level up to 2300 m a.s.l. (Swinscow & Krog 1988, Fryday 2015). Aptroot (2016) reported it from Madagascar. In Pretoria, it was found only once, in a protected area.

Flavopunctelia flaventior (Stirt.) Hale

Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra* and *A. karoo*, Maphangwa & Zedda KWM_0006; KWM_0016 (PRE). Hercules, Van Der Hoff Road, on *A. karoo* and on *J. mimosifolia*, Maphangwa KWM_0044; KWM_0047 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0099 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0122 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0155 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo* and *A. caffra*, Maphangwa KWM_0186; KWM_0201 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0219 (PRE).

This species is widespread in temperate regions as well as at moderate elevations in the tropics in North and South America, Africa, Europe and India (Swinscow & Krog 1988, Nash et al. 2004, Fryday 2015). In East Africa is common (Swinscow & Krog 1988, Killmann & Fischer 2005). Schultz et al. (2009) and Zedda et al. (2009) reported it as epiphytic from Namibia. In Pretoria it was previously collected by Degelius in the Fountains Valley (UPS:BOT:L-053801). In Pretoria, it was found in the majority of the visited sites, irrespective of vegetation degradation or air contamination. Usnic and lecanoric acids were found by TLC in KWM_0099, KWM_0122, and KWM_0186.

Flavopunctelia soredica (Nyl.) Hale

Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0002 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra* and *A. karoo*, Maphangwa & Zedda KWM_0007; KWM_0224 (PRE). Hercules, Van Der Hoff Road, on *A. karoo* and on *J. mimosifolia*, Maphangwa KWM_0048 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0095 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0134 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0146 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo*, Maphangwa KWM_0189 (PRE).

This species is widespread in temperate areas of Asia, North and South America and South Africa (Nash et al. 2004, Fryday 2015). It grows on bark and wood of different tree species, rarely on rock (Nash et al. 2004). It has been reported from *Jacaranda* trees in Windhoek (Brusse 1988 as *Parmelia soredica* Nyl). Zedda et al. (2009) reported it as epiphyte from Namibia. In Pretoria, it has the same distribution as *F. flaventior*. Usnic and lecanoric acids were found by TLC in KWM_0134, KWM_0189 and KWM_0224.

Heterodermia speciosa (Wulfen) Trevis.

Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karoo* and *A. caffra*, Maphangwa & Zedda KWM_0008; KWM_0013 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0079 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0125 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0140 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0162 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo* and *A. caffra*, Maphangwa KWM_0174; KWM_0204 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia*, Maphangwa & Zedda KWM_0206 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0218 (PRE).

H. speciosa is widely distributed in subtropical to temperate areas of the world (Nash et al. 2002). The species is common on sheltered tree trunks in natural and artificial habitats, and occasionally on rock at 1100 to 3600 m altitude in East Africa (Swinscow & Krog 1988), while in North America it grows on sunny, but moist rocks or on tree trunks in humid conditions (NASH et al. 2002). It has been reported from Madagascar by APTROOT (2016) and from East Africa by SWINSCOW & KROG (1988) and KILLMANN & FISCHER (2005). FRYDAY (2015) reported it in South Africa. In Pretoria, it is rather common and was found on most sites, irrespective of vegetation degradation or air contamination. Atranorin and zeorin were found by TLC in KWM_0079, KWM_0162, and KWM_0204.

Hyperphyscia adglutinata (Flörke) H.Mayrhofer & Poelt

Hercules, Van Der Hoff Road, on *J. mimosifolia*, Maphangwa KWM_0051 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0100 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0132 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0140 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0147 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo*, Maphangwa KWM_0198 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia*, Maphangwa & Zedda KWM_0205 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0215 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra*, Maphangwa & Zedda KWM_0221 (PRE).

This species is widespread and common worldwide from tropical to temperate regions and is moderately toxitolerant (SWINSCOW & KROG 1988, PURVIS et al. 1992). Also in South Africa it seems to have a wide distribution and grows mainly on nutrient-rich or nutrient-enriched tree trunks, branches, twigs and rocks in open or partly shaded habitats (SWINSCOW & KROG 1988, MOBERG 2004, FRYDAY 2015). ZEDDA et al. (2009) recorded it in Namibia. In Pretoria, it is rather common and was found irrespective of vegetation degradation or air contamination, preferably on trees in avenues in sunny conditions.

Hyperphyscia granulata (Poelt) Moberg

Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_119 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0214 (PRE).

The species is widespread in tropical-subtropical regions of East Africa, Southern Africa (Namibia, and South Africa), Madagascar, South America and Asia (e.g. Nepal) (SWINSCOW & KROG 1988, MOBERG 2004, ZEDDA et al. 2009, SCHULTZ et al. 2009, FRYDAY 2015, APTROOT 2016). It is known from Gauteng, Natal and the Northern provinces in South Africa (MOBERG 2004). In East Africa and southern Africa it occurs on trunks, branches, and twigs usually in open sites but sometimes in partially shaded conditions, often mixed with other *Hyperphyscia* species at 850 to 2470 m a.s.l. (SWINSCOW & KROG 1988, MOBERG 2004). In Pretoria, it was found in green residential areas, on street trees.

Hyperphyscia isidiata Moberg

Hercules, Van Der Hoff Road, on *A. karoo* and *J. mimosifolia*, Maphangwa KWM_0042; KWM_0051 (PRE).

The species is uncommon and so far known from Costa Rica, Australia, Angola, South Africa and few localities in Kenya (SWINSCOW & KROG 1988, MOBERG 2004, FRYDAY 2015). It has been reported from one locality in Pretoria so far (MOBERG 2004, collected by Kotler on *Jacaranda*, UPS:BOT: L-057759). It grows on tree trunks in open situations associated with other species of *Hyperphyscia*, at 800 to 1600 m a.s.l. (SWINSCOW & KROG 1988, MOBERG, 2004). During this study, it was found in a residential area with dense traffic, on roadside trees.

Hyperphyscia pandani (H.Magn.) Moberg

Hercules, Van Der Hoff Road, on *J. mimosifolia*, Maphangwa KWM_0052 (PRE). Magalies, Hornsnek Road, on *A. caffra*, Maphangwa & Zedda KWM_0062 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0119 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0144 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0168 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. caffra*, Maphangwa KWM_00195 (PRE).

The species is known from tropical to subtropical areas of Australia, America, the Hawaiian Islands, South Africa and East Africa (SWINSCOW & KROG 1988, MOBERG 2004, FRYDAY 2015). It is corticolous on trunks, branches, and twigs of different tree species, often in association with other species of *Hyperphyscia* (SWINSCOW & KROG 1988, MOBERG 2004). It appears to be widespread in South Africa as it is known from Eastern Cape, Gauteng, Natal and Northern Provinces (MOBERG 2004). In Pretoria, it is rather common and was found in both urbanized and in protected areas, also on street trees.

Hyperphyscia pruinosa Moberg

Magalies, Hornsnek Road, on *A. caffra*, Maphangwa & Zedda KWM_0033 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_00087 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0160 (PRE).

This species is known from Australia (Moberg 1987) and in Africa from Lesotho, South Africa (Eastern and Northern Cape) (Moberg 2004, Fryday 2015), Namibia (Zedda et al. 2009) and East Africa (Moberg 2004, Swinscow & Krog 1988). In East Africa it is uncommon on old decorticated wood and on bark of trees and shrubs at 1500 to 2900 m a.s.l. (Swinscow & Krog 1988). Also in South Africa old wood and bark in open areas seem to be the preferred substrate (Moberg 2004). In Pretoria, it was found in rural areas and once in a downtown avenue with heavy traffic.

Lepraria spp.

Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra*, Maphangwa & Zedda KWM_0018 (PRE). Magalies, Hornsnek Road, on *A. caffra*, Maphangwa & Zedda KWM_0061 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0104 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0171 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo*, Maphangwa KWM_0194 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0213 (PRE).

Lepraria species are widespread worldwide, but the highest number of species is found in temperate areas (Saag et al. 2009). Species are distinguished on the basis of subtle differences in colour, thallus thickness, substrate and especially chemistry (Brodo et al. 2001). Few taxa have been reported from Africa so far: *Lepraria nigrocincta* Diedrich, Sérus & Aptroot, *L. pallida* Sipman, *L. rigidula* (de Lesd.) Tønsberg, *L. leuckertiana* (Zedda) L.Saag, *L. sipmaniana* (Kümmerl. & Leuckert) Kukwa, *L. umbricola* Tønsberg, *L. usnica* Sipman, *L. glaucella* Ach., *L. incana* (L.) Ach. and *L. yunnaniana* (Hue) Zahlbr. (Saag et al. 2006). Most of them have been found in Northern or East Africa. *L. sipmaniana*, *L. usnica*, *L. glaucella* and *L. incana* have been recorded from Southern Africa (Fryday 2015) as well. The collected specimens were very small and could therefore not be identified to species level. Either important thallus traits or characteristic secondary metabolites could be observed or detected. The genus as a whole is widespread in Pretoria, both in natural and rural vegetation and on roadside trees along heavy traffic streets, but the thalli are always poorly developed. Atranorin and unidentified traces were found by TLC in KWM_0018, KWM_0061 and KWM_0104.

Parmotrema austrosinense (Zahlbr.) Hale

Hercules, Van Der Hoff Road, on *A. karoo* and *J. mimosifolia*, Maphangwa KWM_0040; KWM_0046 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0065 (PRE). Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0117 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0144 (PRE). Sunnyside, Jorissen Street, on *J. mimosifolia*, Maphangwa KWM_0154 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo*, Maphangwa KWM_0188 (PRE). Arcadia, Stanza Bopape Street, on *J. mimosifolia*, Maphangwa & Zedda KWM_0207 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0216 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karoo* and *A. caffra*, Maphangwa & Zedda KWM_0005; KWM_0227 (PRE).

This species is widespread in tropical and temperate regions (Swinscow & Krog 1988, Brodo et al. 2001). It is known from North and South America, Africa, Australia and Oceania (Nash et al. 2002, Kukwa et al. 2012). In Africa, it has been recorded from East Africa, Madagascar, South Africa and Namibia (Almborn 1988, Swinscow & Krog 1988, Thomas & Bhat 1994, Killmann & Fischer 2005, Forbes et al. 2009, Schultz et al. 2009, Zedda et al. 2009, Trüe et al. 2012, Fryday 2015, Aptroot 2016). In East Africa it is common and widespread at 1000 to 3000 m a.s.l. (Swinscow & Krog 1988). In Pretoria it is very common, in both urbanized areas and nature reserves. Atranorin and lecanoric acid were found by TLC in KWM_0117, KWM_0188 and KWM_0216.

Parmotrema reticulatum (Taylor) M.Choisy

Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0089 (PRE).

P. reticulatum is widespread throughout the tropical and temperate regions of North and South America, Africa, Europe, southern Asia, Australasia and Oceania (Swinscow & Krog 1988, Purvis et al. 1992, Nash et al. 2002), India (Aptroot & Feijen 2002) and Oceania (Swinscow & Krog 1988, Purvis et al. 1992, NASH et al. 2002). In Africa, it has been reported from East Africa (SWINSCOW & KROG 1988), Madagascar (APTROOT 2016) and Southern Africa (DOIDGE 1950, ALMBORN 1988, FRYDAY 2015). The species is corticolous, saxicolous, and terricolous in a wide variety of natural and artificial, and more or less open habitats. In East Africa it is common and widespread at 1000 to 3000 m a.s.l. (SWINSCOW & KROG 1988). In Pretoria, it is uncommon and was found once, in a protected area. Atranorin and salazinic acid were found by TLC in KWM_0089.

Physcia biziana (A.Massal.) Zahlbr.

Lotus Gardens, WF Nkomo Street, on *J. mimosifolia*, Maphangwa KWM_0133 (PRE). Pretoria West, Quagga Road, on *J. mimosifolia*, Maphangwa KWM_0142 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. caffra*, Maphangwa KWM_0199 (PRE).

This species is Mediterranean to mild-temperate (NIMIS & MARTELLOS 2017), known from Africa, North and South America, Australia and Europe (SWINSCOW & KROG 1988, MOBERG 2004). In Africa, it has been reported from Tunisia (GUTTOVÁ et al. 2015), East Africa (SWINSCOW & KROG 1988), South Africa (ALMBORN 1988, MOBERG 2004, FRYDAY 2015) and Namibia (ZEDDA et al. 2009). *P. biziana* was known only from Mpumalanga province in South Africa so far, where it grows on tree trunks and branches in open situations (MOBERG 2004) and was collected at the Botanical Garden of Pretoria by Almborn (specimen in B and UPS:BOT: L-012954). In East Africa it is found at 1500 to 2100 m a.s.l. and is uncommon (SWINSCOW & KROG 1988). In Pretoria, it was found on roadside trees in streets with heavy traffic and once in a nature reserve.

Physcia erumpens Moberg

Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0003 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra*, Maphangwa & Zedda KWM_0021 (PRE).

The species is subtropical (NIMIS & MARTELLOS 2017) and known from South Africa, Madagascar, Asia, East Africa and North America (MOBERG 2004, APTROOT 2016). In South Africa it has been reported from scattered localities in the Eastern and Western Cape, Mpumalanga and Natal provinces (MOBERG 2004, FRYDAY 2015). It grows on trees and rocks in more or less open situations (MOBERG 2004). In Pretoria it was found only at two rather favourable sites, on roadside trees in a green residential area and in a nature reserve.

Physcia poncinsii Hue

Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0104 (PRE).

The species is known from tropical to subtropical regions of Australia, Southern Africa, Madagascar, East Africa and America (NASH et al. 2002, MOBERG 2004, FRYDAY 2015, APTROOT 2016). It is known from the Eastern and Western Cape, Mpumalanga and Natal provinces in South Africa (MOBERG 2004) and ZEDDA et al. (2009) reported it from Namibia. It grows on tree trunk, wood and rocks in open situations (MOBERG 2004). In Pretoria, it is uncommon and was found only once, in a relic of the rural landscape.

Physcia tribacia (Ach.) Nyl.

Groenkloof Nature Reserve, Christina De Wit Ave, on *Acacia caffra*, Maphangwa & Zedda KWM_0016 (PRE). Hercules, Van Der Hoff Road, on *A. karoo* and *J. mimosifolia*, Maphangwa KWM_0043; KWM_0053 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0113 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo* and *A. caffra*, Maphangwa KWM_0190; KWM_0196 (PRE).

This species is widely distributed but not common in northern and southern temperate regions (SWINSCOW & KROG 1988, NASH et al. 2002, MOBERG 2004). According to NIMIS & MARTELLOS (2017) it is Mediterranean to subtropical. It usually grows on rather exposed rocks and sometimes on bird's perches, rarely on bark (SWINSCOW & KROG 1988, MOBERG 2004). In Africa *P. tribacia* is found in South Africa, Lesotho and East Africa (SWINSCOW & KROG 1988, MOBERG 2004, FRYDAY 2015). In South Africa, it is common and known from Eastern and Northern Cape, Free State, Natal and Mpumalanga provinces (MOBERG 2004). In Pretoria it was found mainly in nature reserves and rural area, and once in an avenue with heavy traffic.

Physcia undulata Moberg

Groenkloof Nature Reserve, Christina De Wit Ave, on *A. caffra*, Maphangwa & Zedda KWM_0027 (PRE).

This species is known from Africa, South and Central America and Australia (Swinscow & Krog 1988, Moberg 2004), and has probably a tropical-subtropical distribution. In Africa it has been reported from Kenya (Moberg 1986), Namibia (Zedda et al. 2009), Lesotho and South Africa, in particular from Eastern and Northern Cape and Natal provinces (Moberg 2004, Fryday 2015). This species grows on trunks and branches of solitary trees in open sites (Nash et al. 2002) at 500 to 3000 m a.s.l. (Swinscow & Krog 1988). In Pretoria, it was found once.

Pyxine cocoes (Sw.) Nyl.

Magalies, Hornsnek Road, on *A. caffra*, Maphangwa & Zedda KWM_0030 (PRE). Groenkloof Nature Reserve, Christina De Wit Ave, on *A. karoo* and *A. caffra*, Maphangwa & Zedda KWM_0010; KWM_0223 (PRE). Hercules, Van Der Hoff Road, on *J. mimosifolia*, Maphangwa KWM_0058 (PRE). Pionier Museum, Keuning Dr, on *A. karoo*, Maphangwa KWM_0108 (PRE). Lotus Gardens, Church Street, on *J. mimosifolia*, Maphangwa KWM_0121 (PRE). Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo* and *A. caffra*, Maphangwa KWM_0181; KWM_0196 (PRE). Waterkloof, Rigel Avenue, close to house number 226, on *J. mimosifolia*, Maphangwa & Zedda KWM_0220 (PRE).

This is a pantropical species with scattered collections from the subtropics and Laurimacaronesia (Nash et al. 2002). Swinscow & Krog (1988) report it as widespread in the tropics and subtropics. It is common and widespread in East Africa, where it occurs on bark and wood of trees and shrubs, sometimes on rocks in sun or partial shade, from sea level up to about 2500 m a.s.l. and thrives in artificial habitats (Swinscow & Krog 1988). Zedda et al. (2009) and Schultz et al. (2009) reported it from Namibia, Aptroot (2016) from Madagascar. It seems to be rather common in South Africa, as Moberg (2004) and Fryday (2015) report it from Eastern and Northern Cape and Mpumalanga provinces as corticolous and saxicolous. In Pretoria, it was collected in nature reserves, rural areas and on trees along the streets in residential areas. Lichexanthone and terpenoids were found by TLC in KWM_0223.

Rinodina sp.

Rietvlei Nature Reserve, OR Tambo (R21), on *A. karoo*, Maphangwa KWM_0176 (PRE).

A recent revision of corticolous species of the genus *Rinodina* is available for Southern Africa. Four species are recorded: *Rinodina albocincta* Zahlbr., *R. australiensis* Müll.Arg., *R. capensis* Hampe and *R. ficta* (Stizenb.) Zahlbr. (Mayrhofer et al. 2014). *R. ficta* has been previously reported from Pretoria (Zwartdam) on bark of *Acacia*, Transvaal and Namibia while the other species have been found mainly in the Cape regions and along the coast so far (Mayrhofer et al. 2014). Further *Rinodina* spp. are listed by Fryday (2015). The collected specimen could not be identified to species level due to lack of well-developed ascospores, but could be most likely *R. ficta*. In Pretoria, it was found in a nature reserve.

Additional species reported from Pretoria in the literature

The following additional species of epiphytic lichens have been reported by other authors from Pretoria.

Parmelia sulcata Taylor is a pantemperate to southern boreal (CNALH 2017) and cosmopolitan species (Swinscow & Krog 1988). In East Africa it is saxicolous in the lower alpine zone at 3500 to 4200 m a.s.l. (Swinscow & Krog 1988). Olowoyo et al. (2011) reported it from Pretoria around Garankuwa, whereas Monna et al. (2006) reported it from Johannesburg.

Phaeophyscia adiastola (Essl.) Essl., known in South Africa from Western Cape, Eastern Cape and Gauteng provinces of South Africa (Moberg 2004, Fryday 2015). It is also known from Ethiopia, Kenya, Lesotho, Tanzania, Uganda, North America and Eastern Russia (Swinscow & Krog 1988, Moberg 2004). It is corticolous, but occasionally also saxicolous, in well-lit sites at 900 to 3600 m a.s.l. (Swinscow & Krog 1988). Moberg (2004) reported it from Fountains valley in Pretoria on tree bark.

Phaeophyscia orbicularis (Neck.) Moberg, known from Lesotho, Eastern Cape, Gauteng, and North West provinces in South Africa (MOBERG 2004, FRYDAY 2015). It is also known from temperate regions of the northern hemisphere growing on tree trunks in open situations (MOBERG 2004). MOBERG (2004) reported it from the zoological gardens in Pretoria.

Rinodina ficta (Stizenb.) Zahlbr., known from Italy, New Zealand and USA, and in Africa from South Africa and Namibia (ZEDDA et al. 2009, as *Rinodina* aff. *boleana*; MAYRHOFER et al. 2014). It occurs in parkland, wayside situations and in open maquis or woodlands and grows on either rough or smooth bark (GIRALT & MAYRHOFER 1991, MAYRHOFER et al. 2014). FRYDAY (2015) reported it from South Africa. Specimens from Pretoria (Zwartdam, on bark of an *Acacia* tree), Transvaal and Namibia are reported by MAYRHOFER et al. (2014).

Frequency and phytogeography of the recorded lichens

Twenty-four taxa of epiphytic lichens were observed in Pretoria during this study. The most frequent species at the investigated sites are *Candelaria concolor* (11.3% percentage occurrence), *Hyperphyscia adglutinata* (8.5%), *Parmotrema austrosinense* (8.5%) and *Heterodermia speciosa* (7.5%). *Canoparmelia texana*, *Flavopunctelia flaventior*, *F. soredica* and *Pyxine cocoes* are also rather common (each with a percentage occurrence of 6.6%). More than half of the species belong to the family Physciaceae

As shown in Fig. 1, the majority of the species recorded during this survey (51%) are subtropical to tropical (30%), tropical (12%) and subtropical (9%). The tropical-temperate (9%) and the subtropical-temperate (18%) taxa amount in the sum to 27%. The Mediterranean-subtropical (4%) and Mediterranean-mild temperate (9%) species make together 13%. The cosmopolitans are 9% of the lichen biota.

The lichen diversity on the three phorophytes, *Jacaranda mimosifolia*, *Acacia caffra* and *A. karro*, is similar (17, 17 and 18 taxa respectively). However, cosmopolitan and more disturbance-tolerant species such as *Candelaria concolor* and *Hyperphyscia adglutinata* are more common on *Jacaranda*.

As shown in Fig. 2most of the recorded species are foliose-narrow lobed (63%), followed by foliose-broad-lobed species (21%), while leprose and crustose lichens are much more rare (both 8%). No fruticose lichens were found.

Discussion

A greater diversity of lichens was found on the sites Pionier Museum, Rietvlei Nature Reserve and Groenkloof Nature Reserve as compared to Pretoria centre and Pretoria West (opposite Metal Pressings). The richer sites show lower man-made disturbance as they are found in protected areas and such characterized by low traffic. This supports the observations by FORBES et al. (2009) and OLOWOYO et al. (2010), and suggests that the lichen diversity is impoverished in Pretoria centre and Pretoria west.

The two most common lichens (*Candelaria concolor* and *Hyperphyscia adglutinata*) are cosmopolitan and very common pioneer taxa, which prefer nutrient-rich or nutrient-enriched and sun-exposed bark (KILLMANN & FISCHER 2005, ZEDDA et al. 2009) as observed in Europe too. At the most urbanized and traffic-rich sites of Pretoria, these are the only species found on bark, but they are also adapted to grow under better ecological conditions together with other species. Most frequently, they are found on *Jacaranda*, probably because it is the dominant tree in the city centre.

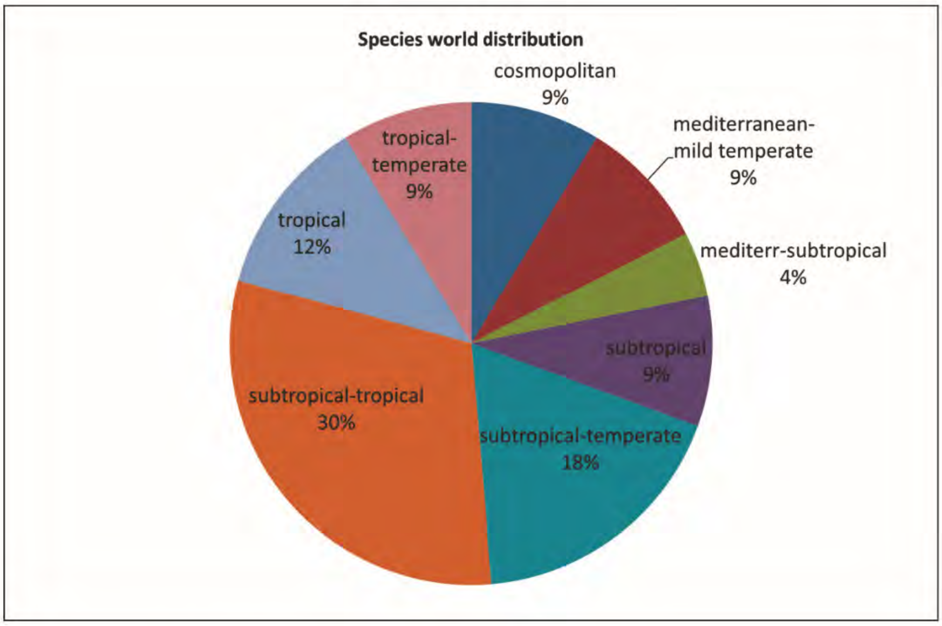

Fig. 1. World distribution (phytogeography) of the collected lichen species (n=21).

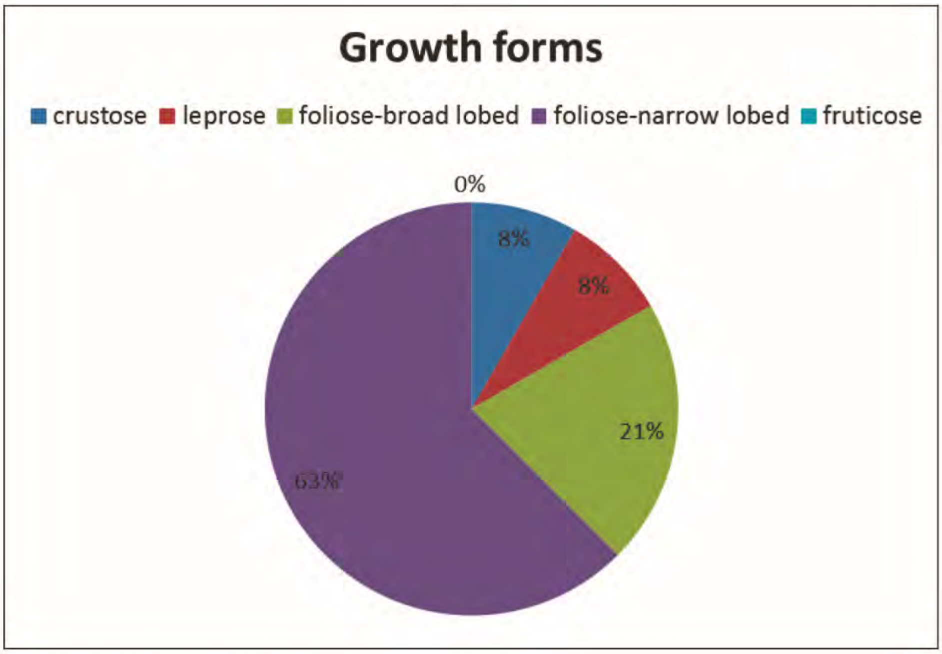

Fig. 2. Percentage occurrence of growth forms of the recorded species.

On sites with less man-made disturbance, trees are not only richer in lichen species, but these are then predominately subtropical to tropical species, such as *Pyxine cocoes*, *Hyperphyscia pandani*, *H. pruinosa*, *H. granulata* and *H. isidiata*. Also some Mediterranean to temperate elements were found here.

The finding that the majority of species belong to the Physciaceae is in agreement with the results of ZEDDA et al. (2009) with epiphytic lichens in the Namibian Savannah, who also found that most lichens belonged to this family. This may reflect the dry climate rather than any man-made influences. Remarkable is the large number of species from the genus *Hyperphyscia*.

Notable is also that so very few crustose lichens were recorded in this study, especially in the more disturbed sites. Unfortunately, little is known about the epiphytic crustose lichen diversity in South Africa, it remains unclear if the scarcity depends on man-made influences, in particular on air pollution by nitrogen compounds, or just on the dry climate. Several species of the Physciaceae are known from other regions to be nitrogen pollution tolerant, while many crustose lichens are more sensitive. For sure, the checklist of South Africa (FRYDAY 2015) contains numerous epiphytic crustose lichens, which might potentially be present in Pretoria in absence of man-made disturbance.

In conclusion, man-made disturbance and land-use in Pretoria appear to have a negative impact on lichen diversity, reducing the species number and changing the composition of morphological and geographical groups.

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Appendix 16: Papers to be published

1. Most common epiphytic lichens in Pretoria: description and identification key. Bothalia Journal
2. Feasibility study for the application of European guidelines for mapping lichen diversity as environmental indicator in Pretoria (Republic of South Africa). Journal of Environmental Monitoring and Assessment
3. Modelling lichen diversity in relation to environmental predictors. The case study of the city of Pretoria (South Africa). Ecological Indicators Journal

Appendix 17: Conference attendance

1. XXXII Congress of the Italian Lichen Society (Bologna, Italy from 18th - 20th September 2019).

Società
Lichenologica
Italiana

Torino, 18th July 2019

Mr Khumbudzo Walter Maphangwa
College of Agriculture and Environmental Sciences, Department of Environmental Sciences, University of South Africa

Object: Letter for your attendance of the 32th Congress of the Italian Lichen Society

Dear Mr. Khumbudzo Walter Maphangwa,

We have the pleasure of informing you that your below mentioned abstract has been accepted as an oral presentation in the 32th Congress of the Italian Lichen Society, to be held on 18-20 September 2019 in Bologna, Italy:

Feasibility study for the application of European guidelines for mapping lichen diversity as environmental indicator in Pretoria (Republic of South Africa)
Khumbudzo Walter Maphangwa*, Giorgio Brunialti, Luisa Frati, Marco Calderisi, Francesca Giorgolo, Memory Tekere, Luciana Zedda

To attend the congress you are expected to be member of the Italian Lichen Society (membership fee for 2019 is 30€) and to confirm your participation paying the registration fee (50€ after 1st July). With this aim, the invoice for the payment is attached to the present letter.

If you have any questions regarding your registration please feel free to contact me at segreteria@lichenologia.eu.

Best regards,

The Secretariat of the Italian Lichen Society*

*Sergio Favero Longo, PhD
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SOCIETÀ LICHENOLOGICA ITALIANA (S.L.I.)
Sede legale: c/o Museo Regionale di Scienze Naturali, via Giolitti 36, I - 10123 Torino
Sito: www.lichenologia.eu

Abstract A

Not.Soc.Lich.Ital. 32: 24 (2019)

Feasibility study for the application of European guidelines for mapping lichen diversity as environmental indicator in Pretoria (Republic of South Africa)

Khumbudzo Walter Maphangwa[1]1, Giorgio Brunialti[2], Luisa Frati[2], Marco Calderisi[2], Francesca Giorgolo[2], Memory Tekere[1], Luciana Zedda[1,3]
[1] Department of Environmental Sciences, University of South Africa; [2]TerraData srl environmetrics, Spin-off of the University of Siena; [3]BIO-Diverse, Bonn

The applicability of the European guidelines for monitoring lichen diversity as indicator of environmental stress were tested in the City of Tshwane (Pretoria) in South Africa. Three different tree species (native *Acacia karoo* and *A. caffra*, and exotic *Jacaranda mimosifolia*) were investigated under two main land use types ("Industrial areas and busy roads" and "Parks and nature reserves"). The variability of Lichen Diversity Values (LDVs) and that of the frequency of lichen species was studied by means of descriptive statistics, univariate analysis and Principal Component Analysis (PCA). A naturality/alteration interpretative scale based on the percentile deviation from natural conditions was developed and an interpolated LDV map of the study area was elaborated.
LDVs and lichen species frequencies on the selected trees are significantly different. LDVs of alien *Jacaranda* are lower than that of native *Acacia*. Also, trees in "Parks and nature reserves" have significantly higher LDVs than those of "Industrial areas and busy roads".
The PCA shows an increasing gradient of lichen diversity for positive values of Axis 1 in relation to the trees sampled in "Parks and natural reserves", with the highest distance from emission sources, where native *Acacia* trees are mainly distributed. On the contrary, most of the trees sampled in "industrial areas and busy roads" and on alien *Jacaranda* trees are characterized by negative values of Axis 1.
Although it is difficult to find suitable trees and plots by following the sampling procedures included in the European guidelines, this study shows that lichen diversity responds well to environmental disturbance, especially due to air pollution, also in Pretoria. Therefore, the European standardized monitoring method can be applied in South Africa, by adopting a stratified random sampling and a more flexible strategy for tree selection.

Abstract B

Not.Soc.Lich.Ital. 32: 25 (2019)

Modeling lichen diversity in relation to environmental predictors. The case study of the city of Pretoria (South Africa)

Giorgio Brunialti[1], Khumbudzo Walter Maphangwa[2], Luisa Frati[1], Marco Calderisi[1], Francesca Giorgolo[1], Memory Tekere[2], Luciana Zedda[2, 3]

[1]TerraData srl environmetrics, Spin-off of the University of Siena; [2]Department of Environmental Sciences, University of South Africa; [3]BIO-Diverse, Bonn

The results of a biomonitoring study carried out in the City of Tshwane Metropolitan Municipality (Pretoria) in South Africa (29 sites, 164 trees; tree species surveyed: *Acacia caffra*, *A. karoo*, *Jacaranda mimosifolia*) was used to model the relationship among epiphytic lichen diversity (LDV and lichen species) and the following environmental predictors: site and tree level variables (altitude, land use, distance from emission sources, tree circumference, tree species); climatic variables (air temperature, pressure and humidity, wind speed and direction) and pollutants (CO, NO, NO_2, NO_x, SO_2, PM10, O_3).
The dataset of air pollutants and climate variables of 5 air quality monitoring stations was obtained after a screening for data completeness and an imputation of missing data. This dataset was interpolated (Inverse Distance Weighting) to assign values to each of the 29 lichen monitoring stations.
PCA was used as explorative multivariate analysis to study the relationships among lichen diversity and the predictors. Well-developed lichen communities show an opposite trend with respect to the main atmospheric pollutants.
Generalized Linear Models were applied to fit the relationship between a set of environmental predictors and the response variables LDV and lichen species. The models were significant for LDV and for 10 lichen species. Industrial areas and the proximity of busy roads negatively affect LDV ($p<0.001$) and the frequency of many lichen species. *A. karoo* trees are positively related to lichen diversity ($p<0.001$) and the abundance of some species: *Culbersonia nubila*, *Pyxine cocoes* ($p<0.05$), *Flavoparmelia flaventior*, *F. soredica*, *Lepraria* spp., *Parmotrema austrosinense* ($p<0.01$). Atmospheric concentrations of NO_x negatively affect LDV ($p<0.05$) and the frequency of *Candelaria concolor* ($p<0.01$), *Lepraria* spp. ($p<0.001$) and *Pyxine cocoes* ($p<0.05$). *Culbersonia nubila* and *Lepraria* spp. are positively and negatively correlated with atmospheric SO_2 ($p<0.01$) respectively.

2. IX International Lichenological Symposium (IAL9), in Bonito, Mato Grosso do Sul, Brazil from 2 - 7 August 2020.