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Tese de Doutorado

Efeitos da poluição atmosférica como fator de estresse ambiental na estrutura e na funcionalidade das comunidades de liquens

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Porto Alegre, abril de 2016.

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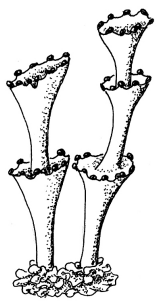
"QUANDO O MAR JÁ ERA MAR, A TERRA NÃO PASSAVA DE UMA ROCHA NUA. OS LIQUENS, VINDOS DO MAR, FIZERAM AS CAMPINAS. ELES INVADIRAM, CONQUISTARAM E VERDEJARAM O REINO DA PEDRA. ISSO OCORREU NO ONTEM DOS ONTENS, E CONTINUA OCORRENDO. ONDE NADA VIVE, OS LIQUENS VIVEM: NAS ESTEPES GELADAS, NOS DESERTOS ARDENTES, NO ALTO MAIS ALTO DAS MAIS ALTAS MONTANHAS. OS LIQUENS VIVEM ENQUANTO DURA O MATRIMÔNIO ENTRE AS ALGAS E SEUS FILHOS, OS FUNGOS. SE O MATRIMÔNIO SE DESFAZ, SE DESFAZEM OS LIQUENS."

(BOCAS DO TEMPO - EDUARDO GALEANO)



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RESUMO

O conjunto de alterações causadas pela poluição atmosférica é considerado uma fonte importante de estresse ambiental. A poluição é hoje uma das maiores preocupações ambientais do mundo, pois afeta todas as fontes de recursos naturais que as populações humanas utilizam para a sobrevivência. Agravando esse quadro, somam-se as modificações na paisagem, que intensificam ainda mais os efeitos da poluição. As comunidades de liquens, entre as mais sensíveis em nível de ecossistema, são capazes de demonstrar sinais precoces como resposta às mudanças ambientais e ser úteis como biondicadoras e biomonitoras dessas mudanças. Avaliações em nível de indivíduos e de comunidades de liquens podem ser ferramentas de monitoramento ambiental, assim como uma abordagem com base na estrutura funcional dessas comunidades. Ainda, o uso de atributos funcionais pode permitir compreender como os liquens são capazes de se adaptar funcionalmente à forte pressão ambiental da poluição, além de servir como nova ferramenta para o biomonitoramento da qualidade do ar. Assim sendo, o objetivo geral desta tese foi avaliar os efeitos da poluição atmosférica e da paisagem (como descritora de poluição) na estrutura e na resposta funcional das comunidades de liquens. A partir dos resultados obtidos demonstramos que os liquens de áreas urbanas têm clara influência da poluição atmosférica e das mudanças na paisagem como estresse ambiental, tanto em nível estrutural quanto funcional. No primeiro artigo, avaliamos a qualidade do ar em cada um dos municípios amostrados, contribuindo para a gestão destas áreas com informações relevantes em nível de saúde pública e ambiental. No segundo, demonstramos que tanto a riqueza, cobertura e composição de espécies, além da vitalidade dos liquens, são afetadas pela poluição atmosférica e pelas mudanças da paisagem, sendo a vitalidade e a composição de espécies os melhores indicadores para avaliar os efeitos de múltiplos distúrbios ambientais neste tipo de áreas urbanas. No terceiro artigo, verificamos que alguns atributos funcionais de liquens podem ser bons indicadores da qualidade do ar, relacionados ao tipo de alga, tipo de crescimento, estratégia reprodutiva e à presença de proteção no talo. Fechamos a tese com a descrição de uma nova espécie encontrada, até o momento, somente em áreas pouco urbanizadas. Portanto, novas formas de se utilizar os liquens como bioindicadores e biomonitores de qualidade do ar foram propostas, ampliando ainda mais a aplicação prática desses organismos.

Palavras-chave: atributos funcionais, ecologia de comunidades, fungos liquenizados, indicadores ecológicos, qualidade do ar, monitoramento ambiental.

ABSTRACT

The set of changes caused by air pollution is an important source of environmental stress. Pollution is one of the greatest environmental concerns worldwide in the present days, since it affects all natural resources that people need to survive. Worsening this panorama, there are serious changes in land cover intensifying the effects of pollution. Lichens, which are among the most sensitive organisms in the ecosystem, are able to show early signs as responses to environmental changes. Besides, they can also be useful as bioindicators and biomonitors of such changes. Evaluations using single lichen species and/or lichen communities can be tools of environmental monitoring, as well as an approach based on the functional structure of these communities. The use of functional traits may allow the understanding of how lichens are able to functionally adapt to the strong pressure of pollution. Furthermore, this is also a promising method to be used as a new tool for air quality biomonitoring. Therefore, the main objective of this thesis was to evaluate the effects of air pollution and land cover changes (as surrogates of this pollution) on lichen communities structure and functional response. From the results found, we could demonstrate that lichens from urban areas have clear influence of atmospheric pollution and land cover changes as environmental stresses. The effects are both in structure and function of these communities. In the first paper, we evaluated the air quality in each of the studied cities, contributing to the environmental management of these areas with relevant information for public and environmental health. In the second, we demonstrated that lichen species richness, cover and composition, besides thallus vitality, are affected by air pollution and land cover changes. Among these parameters, we showed that vitality and species composition are the best indicators to evaluate multiple disturbances in this type of urban environment. In the third paper, we verified that some functional traits can be good indicators of air quality, namely the ones related to the type of lichen algae, growing form, reproduction strategy and the presence of any protection (physical or chemical) in the thallus. We ended the thesis with the description of a new species which is so far only found in low urbanized areas. Thus, new means of using lichens as bioindicators and biomonitors of air quality were proposed, helping to expand the application of these organisms in environmental studies and actions.

Keywords: functional traits, community ecology, lichenized fungi, ecological indicators, air quality, environmental monitoring.

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INTRODUÇÃO GERAL

O termo estresse está relacionado à teoria da elasticidade e foi proposto pelo matemático Augustin-Louis Cauchy, em 1821, sendo definido como uma unidade de força para uma dada unidade de área (Kranner *et al.* 2010). Em biologia, um fator de estresse pode ser definido como qualquer influência externa com efeito prejudicial sobre determinado organismo ou conjunto de organismos (Beckett *et al.* 2008). Mudanças físicas, químicas e bióticas nos ecossistemas, causadas ou não por atividades humanas podem ser consideradas produtores de estresse ambiental (Grime 1977, Fränzle 2003). Dentre essas mudanças, o conjunto de alterações causadas pela poluição atmosférica é considerado uma fonte importante de estresse ambiental (Beckett *et al.* 2008). Entender os efeitos do estresse de fonte antrópica nos ecossistemas é essencial e pode servir para mitigar esse impacto no futuro, especialmente considerando o constante aumento da pressão nos sistemas naturais com o crescimento das populações humanas (Allan *et al.* 2013).

A poluição é hoje uma das maiores preocupações ambientais do mundo, pois afeta todas as fontes de recursos naturais que as populações utilizam para a sobrevivência (Cansaran-Duman *et al.* 2010). A qualidade do ar, especialmente afetada em ambientes urbanos, vem sendo fortemente comprometida pela queima de combustíveis fósseis e descargas industriais, aumentando de forma considerável os níveis de dióxido de enxofre (SO_2), monóxido de carbono (CO), óxidos de nitrogênio (NO_X), material particulado (PM_{10} e $PM_{2.5}$), partículas ultrafinas (menores de 100 nanômetros), hidrocarbonetos policíclicos aromáticos (HPAs) e metais pesados, além de oxidantes fotoquímicos como o ozônio (O_3) (Martins *et al.* 2008). Como resultado desses aumentos observam-se os efeitos na saúde humana, a exemplo do incremento da incidência de uma série de doenças cardiopulmonares, como câncer de pulmão, ataques cardíacos e asma (Pope III *et al.* 2002). Estima-se que,

somente em 2012, esse tipo de poluição causou cerca de 3,7 milhões de mortes no mundo (WHO 2013).

Além dos efeitos à saúde humana, as emissões atmosféricas das últimas décadas estão também alterando o clima mundial. De acordo com os relatórios do Painel Intergovernamental sobre Mudanças Climáticas (IPCC - Intergovernmental Panel on Climate Change), as emissões recentes dos gases de efeito estufa de fonte antropogênica são as maiores da história. Esses gases, junto com outros poluentes como metais pesados e compostos orgânicos, são as principais causas do aquecimento global (Pachauri *et al.* 2014). Como cada parte do planeta está interligada com todas as outras partes através de transporte atmosférico, emissões de um continente podem atingir o outro, tendo a poluição do ar não apenas impacto no clima local, mas atingindo também uma escala global (Ramanathan & Feng 2009).

Agravando o quadro da poluição atmosférica, somam-se as modificações na paisagem, como a transformação de florestas e áreas verdes naturais em áreas urbanas. Essas transformações intensificam ainda mais os efeitos da poluição na saúde humana e ambiental, além de alterar o clima das nossas cidades (Foley *et al.* 2005). Um fenômeno bastante comum nessas áreas é o efeito de "ilha de calor urbana" (UHI – Urban Heat Island), causado principalmente pelo calor lançado dos veículos, ares-condicionados, ou outras fontes de calor das cidades, além do calor armazenado e depois novamente irradiado por grandes estruturas urbanas ou poluentes atmosféricos (Rizwan *et al.* 2008). Esse fenômeno de alteração climática local se caracteriza por temperaturas mais altas do que o registrado em áreas naturais ou rurais adjacentes, diminuição da umidade do ar, alterações nos padrões locais de ventos e mudanças nos padrões de precipitações (Munzi *et al.* 2014).

Dentro deste contexto de estresse ambiental, as comunidades de liquens, que estão entre as mais sensíveis em nível de ecossistema (Pinho *et al.* 2011), são capazes de

demonstrar sinais precoces como resposta às mudanças ambientais (Weissman *et al.* 2006). Estes organismos podem viver e crescer em uma ampla variedade de substratos, como rochas, madeira, solo, folhas e no córtex das árvores (Umaña & Sipman 2002), ou em qualquer superfície que se mantenha estável por certo tempo (telhas de barro, vidros, monumentos). A sensibilidade dos liquens pode ser explicada pela sua biologia, pois não possuem uma camada de proteção e usam quase toda sua superfície como um mecanismo eficiente para absorver água e nutrientes da atmosfera (Szczepaniak & Biziuk 2003; Hawksworth *et al.* 2005). Ao fazer isso, como consequência negativa, eles absorvem e concentram os poluentes presentes no ar (Nash III 2008).

Portanto, devido às suas necessidades fisiológicas e ecológicas, liquens são organismos muito sensíveis e amplamente utilizados como indicadores ecológicos para o monitoramento dos efeitos das mudanças ambientais (Geiser & Neitlich 2007, Giordani & Incerti 2008) e das mudanças em decorrência da poluição atmosférica (Conti & Cecchetti 2001, Käffer *et al.* 2011). Algumas espécies de liquens apresentam boa resistência ao estresse ambiental e uma capacidade efetiva de acumulação de poluentes atmosféricos, enquanto outras são sensíveis a determinados poluentes, que causam danos fisiológicos às vezes irreversíveis a essas espécies (Cansaran-Duman *et al.* 2010).

Estes danos fisiológicos, que são respostas precoces ao estresse ambiental, podem gerar a morte do indivíduo ou diminuição da eficiência reprodutiva (Hoffmann & Hercus 2000) e, consequentemente, mudanças em nível de comunidade, como alterações na composição ou na riqueza de espécies. Sendo assim, não só avaliações em nível de indivíduos de liquens podem ser uma ferramenta de monitoramento ambiental, como também em nível de comunidade. Esta última abordagem permite uma visão mais ampla e de mais longo prazo acerca de determinado local, integrando os efeitos de uma série de estressores e características ambientais (Pinho *et al.* 2004). Liquens são, portanto, organismos

bioindicadores, pois demonstram sintomas particulares em resposta à mudanças ambientais (como mudanças fisiológicas específicas e o desaparecimento de espécies sensíveis), e também biomonitores, podendo ser usados para monitorar mudanças ambientais ao longo de determinado tempo (Hawksworth *et al.* 2005).

Alguns países Europeus (em especial França, Alemanha, Itália, Suíça e Holanda), além dos Estados Unidos, tem políticas nacionais de utilização de liquens para monitorar os efeitos da poluição atmosférica (Nimis & Purvis 2002). No Brasil, no entanto, existem apenas alguns esforços pontuais a esse respeito, como a exigência da inclusão de monitoramento com liquens em alguns estudos de grande impacto ambiental no estado do Rio Grande do Sul (Martins *et al.* 2008). Além disso, poucos estudos no Brasil relacionam mudanças nas comunidades de liquens com a poluição atmosférica. Os primeiros artigos no país com esse tema datam de 2008 e depois disso apenas alguns trabalhos seguiram (Martins *et al.* 2008, Käffer *et al.* 2011, 2012, Käffer & Martins 2014). Importante ressaltar também que, até o momento, nenhum estudo avaliou os efeitos de mudanças na paisagem com alterações nos padrões de organização dos liquens.

Além de acessar os efeitos da poluição atmosférica a partir dos organismos e da estrutura das comunidades de liquens, pode-se também extrair tais informações a partir de uma abordagem funcional, ferramenta potencialmente mais universal do que a abordagem estrutural (Garnier *et al.* 2007). O uso de atributos funcionais pode permitir compreender como os liquens são capazes de se adaptar funcionalmente à forte pressão ambiental da poluição, além de servir como nova ferramenta para o biomonitoramento da poluição atmosférica. No entanto, poucos estudos tem sido realizados com o uso dessa abordagem para avaliar o estresse ambiental (Pinho *et al.* 2011, Llopp *et al.* 2012, Matos *et al.* 2015). De maneira geral, a influência das condições ambientais nos atributos funcionais de liquens é pouco documentada (Ellis & Coppins 2006, Giordani *et al.* 2012, Marini *et al.* 2011) e pouco

se sabe sobre o comportamento destes atributos em regiões de clima tropical ou subtropical (Cáceres *et al.* 2007, 2008, Koch *et al.* 2013), reforçando ainda mais a importância de novos estudos para melhor elucidar a aplicação dos atributos de liquens como bioindicadores (Giordani *et al.* 2012).

Objetivo e estrutura da tese

O objetivo geral desta tese foi avaliar os efeitos da poluição atmosférica e da paisagem (como descritora de poluição) na estrutura e na resposta funcional das comunidades de liquens. A tese está estruturada em quatro capítulos, cada um correspondendo a um artigo científico, descritos abaixo:

Capítulo 1 – Air quality assessment in urban areas from southern Brazil using lichen transplants. Neste capítulo, a qualidade do ar em diferentes áreas urbanas foi avaliada através de quatro formas: 1) analisando a concentração de alguns poluentes absorvidos pela espécie de líquen biomonitora; 2) quantificando danos fisiológicos observados nesta mesma espécie após exposição; 3) acessando alguns poluentes medidos por amostradores automáticos e semi-automáticos de ar; e 4) a partir de dados de poluentes modelados para essas áreas. Além de avaliar a qualidade do ar, neste capítulo também testamos se havia diferenças entre estes municípios, com o objetivo de apontar quais as principais características em relação à poluição atmosférica dessas áreas para uso em futuros estudos e como ferramenta de gestão e proteção da saúde pública e ambiental nestes municípios.

Capítulo 2 – The application of lichens as ecological surrogates of air pollution in the subtropics: a case study in south Brazil. Os objetivos deste capítulo foram: a) investigar quais as principais variáveis responsáveis pelas alterações em comunidades de liquens em um gradiente urbano de poluição nos subtrópicos e b) testar quais métricas apresentam uma melhor performance em tal gradiente. Para responder a essas questões, a diversidade de

liquens foi avaliada em diferentes áreas, usando a composição, riqueza de espécies, cobertura de liquens nos troncos das árvores e a vitalidade de exemplares transplantados. O conteúdo de poluentes nos liquens, poluentes modelados e características de paisagem foram utilizados como preditores de poluição atmosférica. Nossa hipótese foi de que os principais causadores de mudanças nas comunidades de liquens estariam associados à urbanização, o que pode sustentar ainda mais o uso de liquens como indicadores de poluição atmosférica nos subtrópicos.

Capítulo 3 – Lichen functional structure as indicator of urban stress in a subtropical region. Neste capítulo, buscamos entender como a composição funcional de comunidades de liquens respondia à poluição atmosférica como estresse ambiental, identificando os atributos funcionais mais relevantes. Além disso, avaliamos as diferenças na diversidade funcional dessas comunidades através de índices de riqueza, equitabilidade e diversidade (Rao) funcional. Nossas principais hipóteses para este capítulo foram que: 1) atributos que permitem dispersão mais rápida e ampla (como sorédios) e maior proteção (física e química) ao talo dos liquens poderiam ter maiores frequências em ambientes mais poluídos; e 2) os índices de diversidade funcional teriam maiores valores em áreas menos poluídas, pois a poluição funcionaria como um filtro ambiental para esses parâmetros.

Capítulo 4 – New species of *Graphis* (Graphidaceae: Lichenized Ascomycota) from the Atlantic Forest, Brazil. Este capítulo encerra a tese com a descrição de uma nova espécie de líquen, encontrada, até o momento, somente em áreas secundárias de Mata Atlântica (nordeste do Rio Grande do Sul) sem impacto considerável de urbanização. A descrição dessa nova espécie é também uma homenagem a Dra. Suzana de Azevedo Martins, co-orientadora desta tese.

ARTIGO 1

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Air quality assessment in urban areas from southern Brazil using lichen transplants

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Abstract

Based on the constant need to monitor air pollution and on the high importance of monitoring it with biological organisms, the present work has as main objective to assess air quality in different urban areas by measuring: 1) atmospheric compunds absorbed by a biomonitor lichen species; 2) physiological damages observed in this lichen; 3) pollutants measured by automatic/semi-automatic air samplers and 4) by modeled pollution data for these areas. Besides assessing air quality, this work also tested whether there were differences among the studied cities, aiming to point out which are the main pollution characteristics of these areas so that this information can be used in future studies. The monitoring was carried out in four mainly industrial cities and three mainly rural cities in southern Brazil. The foliose epiphytic lichen *Parmotrema tinctorum* was used as biomonitor and transplanted to the sites in 2013 and 2014. Physiological tests and the content of S, Cu, Zn, Fe, Mn, Cr, Ni, Pb, V and Al were measured in the lichen. We also had other pollution variables available such as PM_{10}, SO_2, O_3, NO_2 and CO for some cities, as well as modeled concentration of $PM_{2.5}$ and NO_X for all sites. This study contributes to the knowledge of the air quality on the northeastern region of Rio Grande do Sul, southern Brazil, and can be a tool for environmental health protection and a guideline for future studies.

Keywords: air pollution, biomonitoring, greenhouse gases, heavy metals, lichenized fungi, particulate matter.

1. Introduction

Air pollution from biomass burning, fossil fuel combustion or from industrial emissions can spread far beyond the limits of the country that generated it (Akimoto, 2003). Besides being globally spread, these emissions are also changing the world climate. According to the latest report of the IPCC (Intergovernmental Panel on Climate Change) (Pachauri et al., 2014) recent anthropogenic emissions of greenhouse gases are the highest in history and together with other anthropogenic drivers, such as heavy metals or organic compounds, are extremely likely to have been the dominant cause of the observed warming. In addition to climate changing, environmental pollution is also one of the greatest concerns for public health (Cansaran-Duman et al., 2010). Loss of air quality has been related to several cardiopulmonary diseases, such as lung cancer, heart attacks and asthma (Pope III et al*.,* 2002) and it is estimated that air pollution caused around 3.7 million deaths worldwide only in 2012 (WHO, 2013).

Therefore, it is very important that local actions to control and monitor air quality are adopted, not only considering regional population health, but also thinking on a greater and global impact of air pollution. For this purpose, the use of biological monitors, such as trees (Domingos et al., 2000; Alves et al., 2001), grasses (Crittenden and Read, 1979), mosses (Bignal et al., 2008; Gerdol et al., 2014) or lichens (Carreras and Pignata, 2002; Loppi and Frati, 2006; Riddell et al., 2012; Zverina et al., 2014) are good options. We will here focus on lichens, which are long considered as good tools to monitor air pollution and air quality, as well as the early impact of pollutants (Hawksworth et al., 2005).

The use of lichens for monitoring atmospheric pollution has been known since independent observations made in England, Munich and Paris in the 1800s documented that lichens were disappearing from urban areas (Nash III, 2008). Some years later and until nowadays, many studies have shown and proved that lichens are indeed good biomonitors

and bioindicators of air pollution of different kinds and with different origins (Conti and Cecchetti, 2001; Käffer et al., 2012; Pinho et al., 2011). Besides disappearing when the level of pollution reaches too high concentrations, lichens can also show early signs of atmospheric contamination, such as physiological damages, external changes and accumulation of air particles (Carreras and Pignata, 2002; Backor and Loppi, 2009; Riddell et al., 2012).

The reason why lichens are so sensitive to pollution can be explained by their biology. Lichens do not have a cuticle to protect their thalli and use almost all their surfaces as an efficient mechanism for water and nutrients uptake from the atmosphere (Szczepaniak and Biziuk, 2003; Hawksworth et al., 2005). However, by absorbing these nutrients directly, they also absorb and concentrate the pollutants that are present in the air (Nash III, 2008), which can damage important physiological processes or even cause cells death (Raposo-Junior et al., 2007).

One of the possible methods to monitor air pollution with lichens is the active monitoring method. It consists on the exposure of a well-defined biomonitor species under relatively controlled conditions (Szczepaniak and Biziuk, 2003). The transplantation of the suitable organisms occurs from unpolluted or less polluted areas to the polluted sites under consideration, during a defined exposure time (Ceburnis and Valiulis, 1999). This strategy allows a rapid and accessible evaluation, especially where there is no other way to measure the atmospheric pollution (Aras et al., 2011).

Based on the constant need to monitor air pollution and on the importance of monitoring it with the help of biological organisms, the present work had as main objective to assess air quality in different urban areas using the following approaches: 1) analysis of the amount of pollutants absorbed by the biomonitor lichen species; 2) observation of physiological damages in the lichen thalli of this species after exposure; 3) measurement of pollutants by automatic/semi-automatic air samplers and 4) modeled pollution data for these

areas. Besides assessing air quality, this work also tested whether there were differences among the studied cities, aiming to point out which are the main pollution characteristics of these areas. This assessment can then be used in future studies of air quality in south Brazil and also as a tool of environmental and public health protection.

2. Methods

2.1. Study area

The atmospheric monitoring was carried out in seven cities: Esteio (ES), Triunfo (TR), Charqueadas (CH), Montenegro (MO), Santo Antônio da Patrulha (SA), Caraá (CA) and Maquiné (MA), which were not more than 150 km far from each other and located in the northeastern region of the state of Rio Grande do Sul, southern Brazil (Fig. 1).

These cities have great importance for the state economy holding many industries (Esteio, Charqueadas and Montenegro), a Petrochemical Complex (Triunfo), and an important agricultural area of family farming (Santo Antônio da Patrulha, Maquiné and Caraá). A description of the main industrial activities, the demographic density and the rural practices of the monitored cities are detailed in Koch et al. (2016), as well as further information about their climate, elevation and vegetation, with little variation among these cities. On some of them (only Charqueadas, Esteio and Santo Antônio da Patrulha) there are air-monitoring systems with automatic and semi-automatic air samplers. The elevation of all sampling areas ranges from 10 to 75 m above sea level and the climate is classified as subtropical humid, Cfa type according to the updated Köppen–Geiger classification (Peel et al., 2007).

2.2. Sample preparation and lichen exposure

The foliose epiphytic lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale was chosen as the biomonitor species. This species has known tolerance to air pollution, besides being already used in other monitoring studies in southern Brazil (Käffer et al., 2012) and having great abundance and occurrence in Brazil (Spielmann and Marcelli, 2009). Samples of *P. tinctorum* were collected on trunks of planted *Eucalyptus* spp. and *Pinus* spp in the city of Tapes (Fig. 1) in the beginning of 2013 and 2014 to be later exposed in potentially polluted sites. Lichens were collected around 100 km far from the closest exposure area. There are no industries near the area of the lichen collections, but there are rice crops in the region. Before being exposed, the lichen samples were left in the laboratory for acclimation during ten days (Käffer et al., 2012).

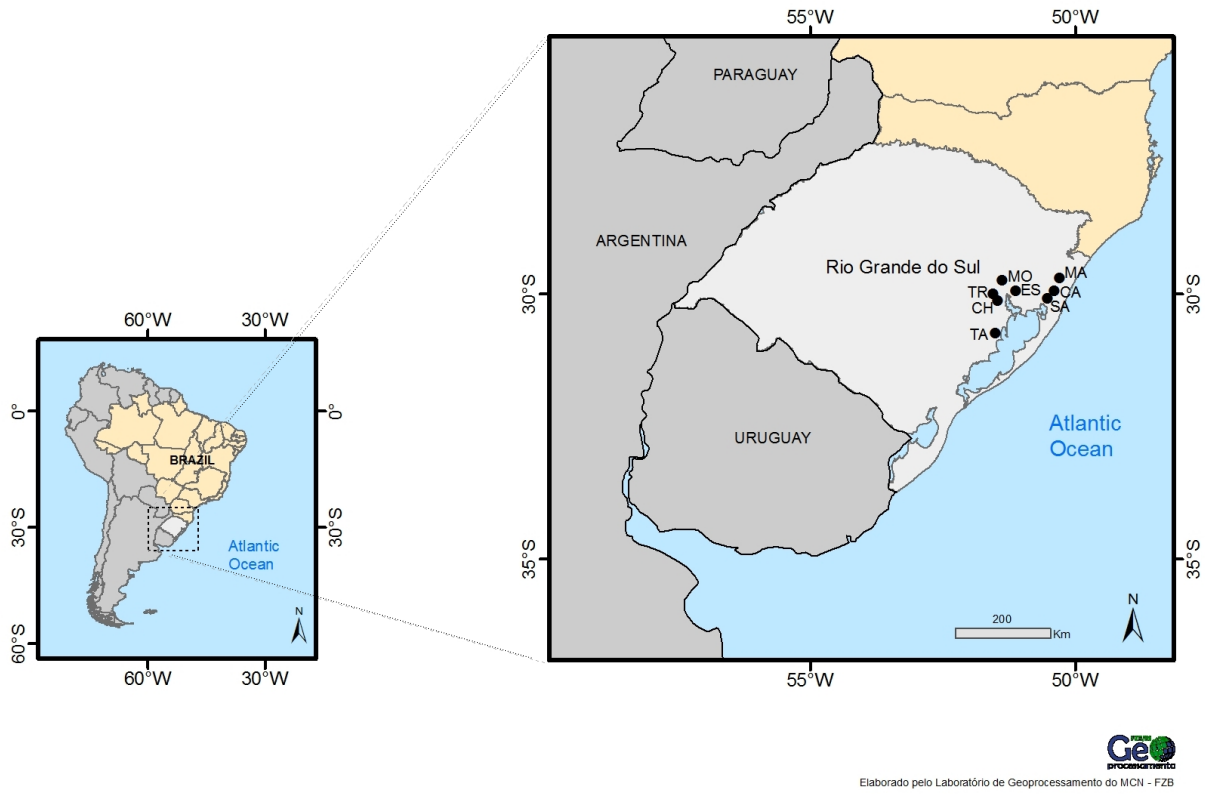

Fig. 1. Location of the seven monitored cities (CA, MA, SA, CH, TR, ES and MO) and the city where lichen samples of *Parmotrema tinctorum* were collected (TA) to be later exposed to monitor air pollution. Abbreviations: CA = Caraá; MA = Maquiné; SA = Santo Antônio da Patrulha; CH = Charqueadas; TR = Triunfo; ES = Esteio; MO = Montenegro; TA = Tapes.

Lichen thalli were then exposed in 55 x 35 cm synthetic nettings in proper and standardized structures being around 120 cm from the ground and covered with shading covers, to reduce the exposure to the sunlight. They were placed vertically on this structure, to simulate almost the same position they were found on the trees (Fig. 2). When available, lichen transplants were placed close to existing automatic/semi-automatic air samplers. Before exposing the samples of *P. tinctorum* in the seven sites, we made chemical control analyses, to evaluate metal and sulphur contents and prior existing physiological damages.

The lichen samples were exposed from March to October 2013 and from April to November 2014. Sub-samples of these materials were taken after two, five and seven months of exposure, in all the seven cities monitored, in both years. At the end, we monitored 14 months in each city and had a total of six sub-samples for each of them (with the only exception of the city of Maquiné, which we only monitored in 2014).

Fig. 2. Thalli of *Parmotrema tinctorum* exposed for biomonitoring atmospheric pollutants.

2.3. Physiological analyses

For each of the six sub-samples from each site, we made physiological tests in order to evaluate lichen responses to atmospheric pollution. We first removed all the remaining pieces of bark and cleaned the lichen thalli with the help of a brush. We then macerated part of the thalli and counted the percentage of live algal cells, under the microscope using the neutral-red test at 5% (Le Blanc, 1971; Calvelo and Liberatore, 2004). With the same macerated samples, the Chlorophyll a and b analyses were undertaken, according to the method of Boonpragob (2002), by using test tubes containing 10 mL of 96% ethanol and 5 cm^2 of lichens. Reading was expressed through absorbance in the wavelengths of 649 and 665 nm, using the spectrophotometer Digimed DME-21. Chlorophyll contents and the percentage of live algal cells are methods for analyzing physiological damages on the lichen photobiont. The content of organic carbon, which is a measure of physiological damage on the lichen mycobiont, was analyzed through the wet combustion method using the Walkley-Black method with external heat (APHA, 2005), performed by the Laboratory of Soil Analyses at the Universidade Federal do Rio Grande do Sul.

2.4. Chemical analyses

For the same sub-samples we also evaluated the total content of the following pollutants in the lichen thalli: sulphur (S), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), chromium (Cr), nickel (Ni), lead (Pb), vanadium (V) and aluminum (Al). The analyses were undertaken using inductively coupled plasma optical emission spectrometry (ICP-OES) (Boss and Fredeen, 2004) with previous nitric-perchloric wet digestion of samples. Samples were dried at 65^{o}C before being macerated. The detection limits were as follows: S = 0.01%; Cu = 0.3 mg/kg; Zn = 1.0 mg/kg; Fe = 2.0 mg/kg; Mn = 2.0 mg/kg; Cr = 0.4 mg/kg; Ni = 0.4 mg/kg; Pb = 2.0 mg/kg; V = 0.3 mg/kg; Al = 0.10%. These chemical analyses were made by

the Laboratory of Soil Analyses at the Universidade Federal do Rio Grande do Sul and were validated with the use of spikes and the certified reference material SRM 1515, for Apple Leaves and Domestic Sludge SRM 2781.

2.5. Other pollution variables

For some of the monitored cities we had other pollution variables available, such as particulate matter (PM_{10}), sulphur dioxide (SO_2), ozone (O_3), carbon monoxide (CO) and nitrogen dioxide (NO_2). These data were obtained through automatic and semi-automatic air samplers of the Environmental Department of the State of Rio Grande do Sul (FEPAM - Fundação Estadual de Proteção Ambiental Henrique Luiz Roessler) and were only available for the cities of Charqueadas, Esteio and Santo Antônio da Patrulha, but in some cases not for all the monitoring period and not including all parameters. The data sampled by these air samplers were then compared with the standards of air quality in Brazil (CONAMA, 1990) and also with the guidelines of the World Health Organization (WHO, 1999, 2006).

We also assessed modeled atmospheric concentrations of some air pollution elements, such as NO_X and $PM_{2.5}$, from the Chemistry Coupled Aerosol-Tracer Transport Modeling System (CCATT-BRAMS; Freitas et al., 2009; Longo et al., 2013), for each of the studied areas. Data were provided by the Group of Modeling of the Atmosphere and its Interfaces from the Universidade Federal de Pelotas, state of Rio Grande do Sul (http://ccatt.ufpel.edu.br). This model has been recently implemented to the state and data were only available for 2014 on.

2.6. Statistical analyses

Aiming to test whether the monitored sites had differences on air quality, one-way ANOVAs (Analyses of Variance) were made with each set of variables analyzed: pollutants

absorbed by the biomonitor lichen species, physiological damages on the lichen thalli, and the contents of particulate matter and other gases (measured and modeled).

Finally, a PCA (Principal Component Analysis) was performed to see how the sampled areas were distributed based on the pollution variables measured (a matrix of the sites described by averages of metals, sulphur and modeled pollutants). The result scores were plotted in a biplot and the variables scores were scaled by the square root of eigenvalues. This analysis was made with the "rda" function of CRAN software R using vegan package (R Core Team, 2015).

3. Results

3.1. Lichen physiological responses

Regarding the physiological damages on the thalli of *Parmotrema tinctorum* (Tab. 1), it was only possible to find significant differences for the percentage of live algal cells ($F = 2.84$; $df = 7$; $P = 0.02$) (Fig. 3). Esteio was the city with the lowest values of this parameter while Montenegro and Maquiné had the highest values (Tab. 1). Percentages of live algal cells also showed a decrease in almost all sites after exposure, with the exception of Maquiné and Montenegro in 2014 (Tab.1).

3.2. Air pollutants monitoring

Most of air pollutants analyzed through the lichen biomonitor, the air samplers and the modeled data, significantly varied among cities (Tab. 2, Fig. 4 and 5).

Regarding the metals absorbed by the lichen *P. tinctorum*, differences were found in the mean concentration of copper, zinc, iron, chromium and nickel, with higher values in Charqueadas and Montenegro in general. For the other heavy metals and also for sulphur

contents, no differences were found among sites. However, when comparing these results with the observed before exposure, in the control sample, it is possible to note that almost all pollutants had higher concentrations after the exposure time (Fig. 4).

Table 1 Averages of lichen physiological parameters measured to evaluate the damages on the photobiont (Chl a, Chl b and percentage of live cells) and on the mycobiont (percentage of organic carbon) after exposure. Thalli of *Parmotrema tinctorum* were exposed during seven months in 2013 and 2014.

Site	Year	Organic Carbon (%)	Chl a (mg/g)	Chl b (mg/g)	Live Cells (%)
Tapes (before exposure)	2013	39.0	0.6	0.9	59.1
	2014	40.0	1.8	1.1	46.7
Maquiné	2014	40.0±3.0	1.9±1.0	1.1±1.0	48.6±3.4
Caraá	2013	40.7±2.5	0.7±0.3	4.1±4.6	41.8±8.0
	2014	43.0±1.0	1.8±0.7	0.9±1.0	43.0±1.0
Triunfo	2013	41.3±0.9	1.4±0.8	3.6±2.9	41.3±8.8
	2014	40.0±3.0	5.5±2.7	1.4±1.8	28.4±12.7
Santo Antônio da Patrulha	2013	41.7±2.6	1.6±0.9	1.1±0.2	43.9±9.7
	2014	40.0±0.0	1.0±0.7	1.1±0.8	44.2±7.3
Montenegro	2013	43.0±2.8	1.7±0.6	0.2±0.1	47.4±.1.0
	2014	39.0±3.0	2.4±1.0	1.2±0.6	48.6±5.7
Charqueadas	2013	40.7±1.9	1.3±0.7	1.2±0.4	40.0±6.2
	2014	40.5±0.5	2.7±1.8	1.2±0.6	37.8±10.8
Esteio	2013	40.7±3.3	2.0±1.1	0.6±0.4	35.9±4.0
	2014	41.0±1.0	3.2±1.5	1.3±1.4	29.6±3.8
ANOVA		**n/s**	**n/s**	**n/s**	**P < 0.05**

Results of the ANOVAs comparing sites are shown. Abbreviations: ANOVA = Analysis of Variance; n/s = not significant. Values shown are the mean of the three exposure periods (two, five and seven months after exposure) and the standard deviations of the same data.

High contents of pollutants in the lichen thalli were found even in rural sites, such as sulphur, zinc, chromium, nickel, lead, vanadium and aluminum. Regarding chromium, it is important to note that it had a considerable peak in CA and SA in 2013 (more than 160 mg/kg), which did not repeat in 2014. It is also important to highlight the peak of sulphur in

the beginning of 2014, reaching all sampled cities. In the industrial sites (MO, TR, CH and ES) all pollutants increased after exposure when compared to the control (Fig. 4).

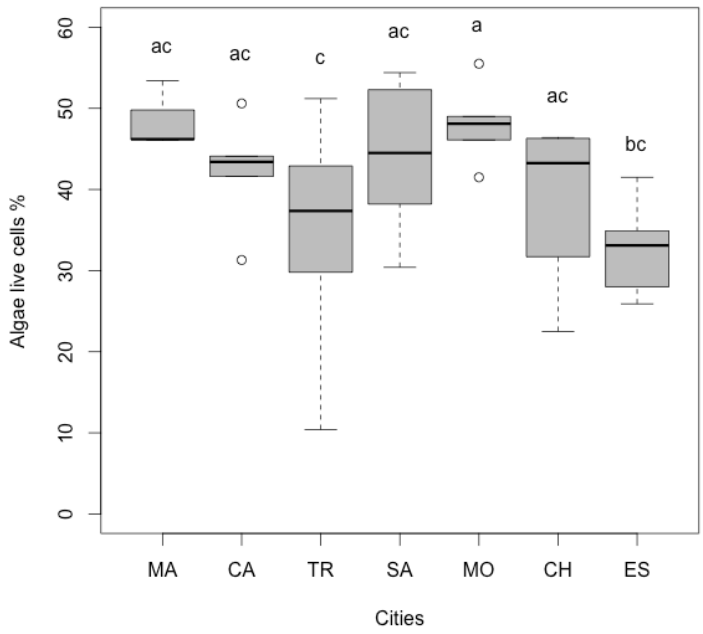

Fig. 3. Boxplot of the percentage of live algae cells in the different monitored cities. The lines in the boxes represent means and the bars confidence intervals. Different letters represent statiscally significant differences tested by Analysis of Variances (ANOVAs), considering $P<0.05$. Abbreviations: MA = Maquiné; CA = Caraá; TR = Triunfo; SA = Santo Antônio da Patrulha; MO = Montenegro; CH = Charqueadas; ES = Esteio.

Data from the air samplers were not available for all monitored cities during the period when the lichens were exposed (2013 and 2014). However, based on the data evaluated, significant differences among some cities can be noted (Tab. 2). The modeled contents of NO_x and $PM_{2.5}$ (Fig. 5) also significantly varied among cities in 2014, both being higher in Esteio, Montenegro, Triunfo and Charqueadas, all mainly industrial cities.

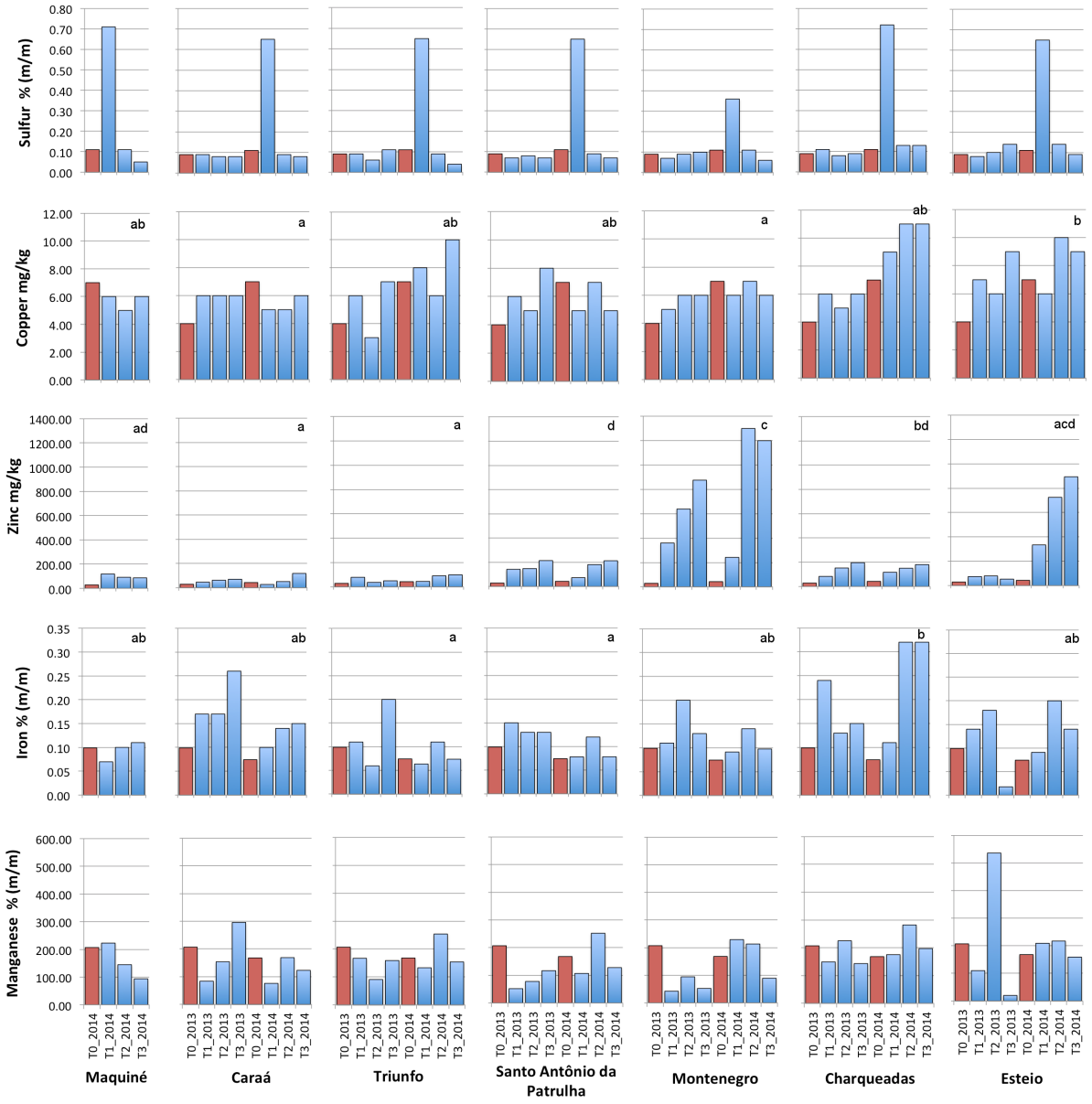

Fig. 4. Contents of sulphur and metals absorbed by the lichen *Parmotrema tinctorum* during seven months of exposure in 2013 and 2014. The red column represents the basal values, prior to exposure in the cities. Different letters represent statiscally significant differences tested by Analysis of Variances (ANOVAs), considering $P<0.05$. T0 = contents prior to exposure; T1 = after two months; T2 = after five months; T3 = after seven months (continues).

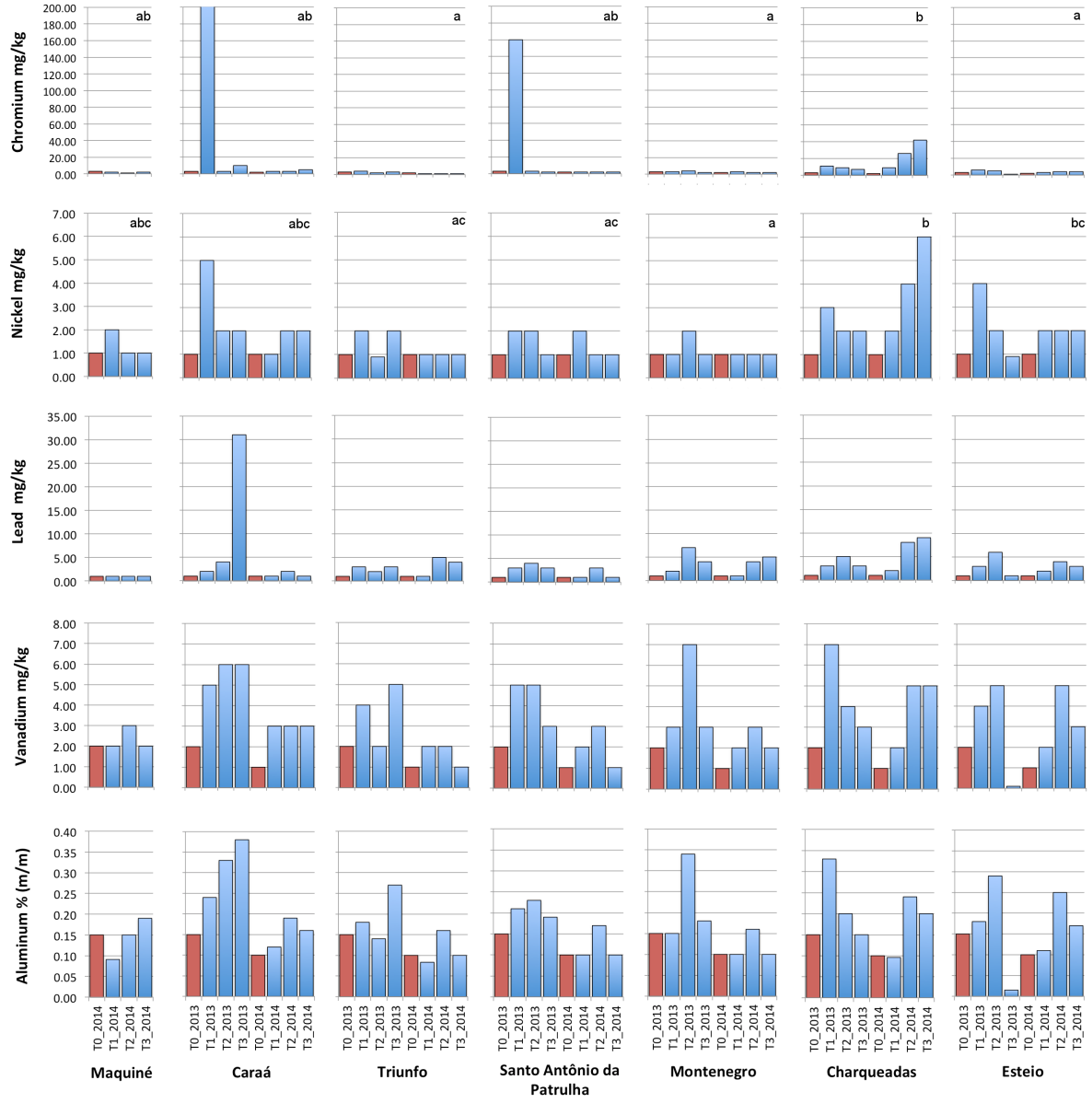

Fig. 4. (continuation). Contents of sulphur and metals absorbed by the lichen *Parmotrema tinctorum* during seven months of exposure in 2013 and 2014. The red column represents the basal values, prior to exposure in the cities. Different letters represent statiscally significant differences tested by Analysis of Variances (ANOVAs), considering $P<0.05$. T0 = contents prior to exposure; T1 = after two months; T2 = after five months; T3 = after seven months.

Considering PM_{10}, Charqueadas (CH) had statistically higher values than Santo Antônio da Patrulha (SA) (F = 7.2; df = 1; P = 0.02), which also happened for the modeled $PM_{2.5}$, and even higher values were found for Triunfo (TR), Montenegro (MO) and Esteio (ES) (the highest) (Fig. 5). Particulate matter in CH also exceeded the recommended values for health security (WHO 2006) (Tab. 2). Measured contents of SO_2, showed statistical differences among CH, ES and SA, with CH having higher concentrations than ES and SA (F = 37.5; df = 2; P < 0.0001). Regarding NO_2, ES had higher values of measured concentration than CH (F = 29.6; df = 1; P = 0.0001), which also happened for modeled NO_x (Fig. 5). This pollutant showed also higher values in 2014 in all industrial cities (CH, TR, MO and ES) (Fig. 5). Charqueadas also showed greater values of measured ozone in the two years when compared to Esteio (F = 102.2; df = 1; P < 0.0001). Contents of carbon monoxide were not significant different when comparing CH and ES.

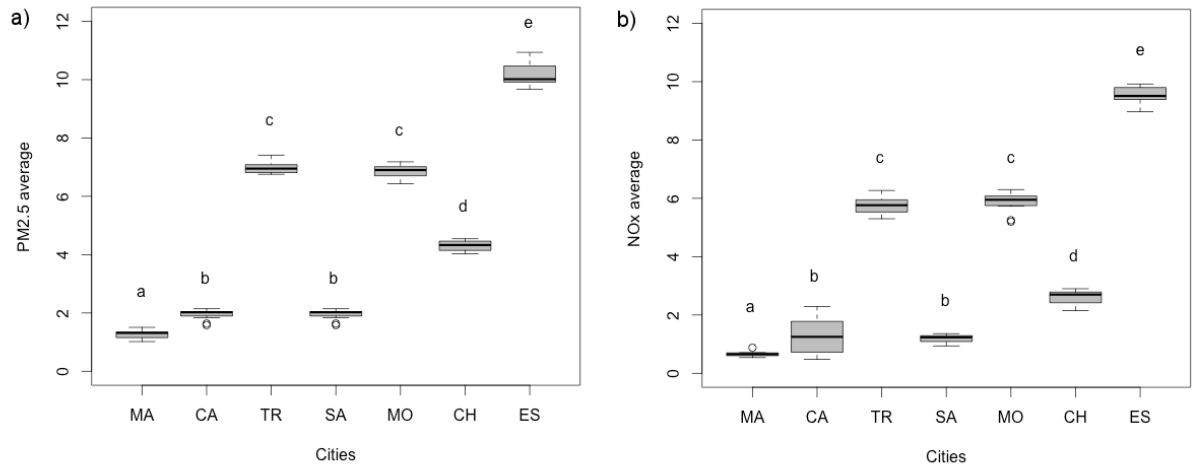

Fig. 5. Boxplots of the monthly averages of modeled $PM_{2.5}$ in $\mu g/m^3$ (a) and NO_x in ppb (b). The lines in the boxes are means and the bars confidence intervals. Different letters represent statiscally significant differences tested by Analysis of Variances (ANOVAs), considering P<0.05. Abbreviations: CA = Caraá; MA = Maquiné; SA = Santo Antônio da Patrulha; MO = Montenegro; TR = Triunfo; ES = Esteio; CH = Charqueadas.

Table 2 Means and standard deviations for PM10 and the greenhouse gases that were sampled by the automatic and the semi-automatic air samplers in 2013 and 2014.

City	Measuring period	PM_{10} (μg/m^3)	SO_2 (μg/m^3)	NO_2 (μg/m^3)	O_3 (μg/m^3)	CO (ppm)
Charqueadas	2013	33.7±0.6	13.4±0.9	15.5±0.9	32.4±2.1	0.3±0.0
	2014	29.8±0.7	15.5±0.5	13.6±0.5	38.3±4.4	0.9±0.0
Esteio	2013	m/d	3.6±0.2	25.2±1.6	20.3±1.2	0.4±0.0
	2014	m/d	5.4±0.5	19.9±0.5	19.3±3.0	0.4±0.0
Santo Antônio	2013	19.6±11.8	6.9*	m/d	m/d	m/d
	2014	m/d	m/d	m/d	m/d	m/d
ANOVA		P < 0.05	P < 0.0001	P < 0.001	P < 0.001	n/s
CONAMA (1990)		50.0	40.0	100.0	160.0 (one-hour mean)	35.0 (one-hour mean)
WHO (1999/2006)		20.0	20.0 (daily mean)	40.0	100.0 (eight-hour mean)	9.0 (eight-hour mean)

Results of the ANOVAs comparing sites are shown, as well as the air quality standards (annual means, except when mentioned differently) according to the Brazilian legislation (CONAMA, 1990) and the World Health Organization guidelines (WHO, 1999, 2006). *Only one month of data available. Abbreviations: ANOVA = Analysis of Variance; m/d = missing data; ns = not significant. Values shown are the mean of the three exposure periods (two, five and seven months after exposure) and the standard deviations of the same data.

3.3. Main pollution composition in each city

Cities were grouped based on their pollution characteristics through a principal components analysis and the ordination diagram of these patterns is shown in Figure 6. Axis 1 explained 43.4% of the observed variance and axis 2, 28.8%. The city of Caraá (CA), seem to be more related to higher contents of chromium, while Charqueadas (CH), is closely related to many different metals and sulfur. A greater relation of Mn, Cu and modeled NO_X and $PM_{2.5}$ is found to Esteio (ES). In the meanwhile, Zn is more important in Triunfo (TR) and Montenegro (MO), but also related to ES.

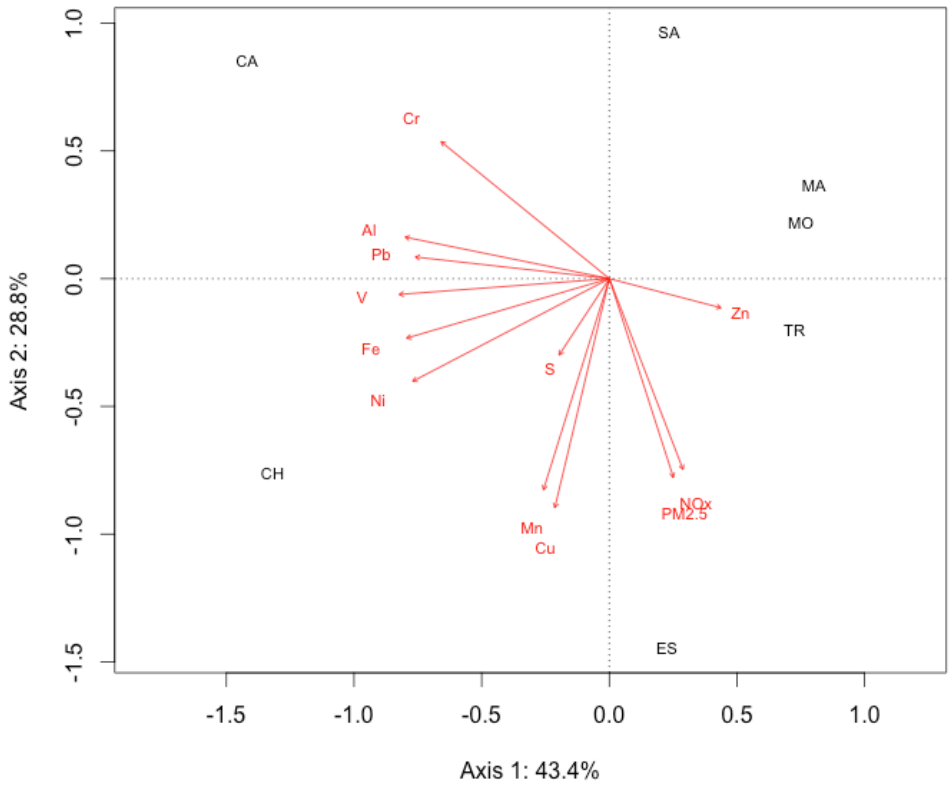

Fig. 6. Diagram of ordination of a PCA (Principal Component Analysis) based on a matrix of sites (cities) described by the pollution variables. Sites are the black letters and the variables, the red ones. Abbreviations: CA = Caraá, CH = Charqueadas, TR = Triunfo, MO = Montenegro, MA = Maquiné, SA = Santo Antônio da Patrulha, Cr = Cromium, Al = Aluminum, Pb = Lead, V = Vanadium, Fe = Iron, Ni = Nickel, S = Sulphur, Mn = Manganese, Cu = Copper, PM2.5 = $PM_{2.5}$, NOx = NO_x.

4. Discussion

The results of the present study provide a good idea of how actually is the atmosphere quality in the monitored sites. There were some differences among mainly industrial cities from mainly rural ones, but there were also urban influences in all of them. Besides, the present work is a pioneer study on air pollution biomonitoring with lichens in most of the sampled cities, showing relevant concentrations of some air pollutants with negative impact on public health and environmental quality.

4.1. Physiological responses

The physiological responses of the transplanted lichen *Parmotrema tinctorum* showed that most of the exposed thalli suffered stress after exposure. The overall pattern was an increase in these parameters, with the exception of the percentages of live algal cells, which had a decrease after the exposure months. It is possible to highlight the cities of Montenegro and Maquiné, with the greatest percentages of live algae cells, while Triunfo and Esteio had the lowest values.

Many authors have demonstrated that lichens are very much sensitive to contents of particulate matter, PAHs (Polycyclic Aromatic Carbons), NO_2 and SO_2, showing physiological damages when exposed to these pollutants (van Herk et al., 2003; Riddell et al., 2012; Käffer et al., 2012). Organic compounds were not evaluated by this work, but are probably also reflecting in loss of lichen vitality in the industrial cities, namely PAHs, dioxins, furans and others, which tend to accumulate in biota, as a result of atmospheric deposition (Augusto et al., 2013). Some authors have noted that the percentage of dead algae cells is related to air pollution (Calvelo and Liberatore, 2004; Käffer et al., 2012) and here we show that live algae cells can also be a good parameter.

4.2. Air pollution monitoring

Almost all the pollutants absorbed by *P. tinctorum* transplanted thalli increased after exposure when compared to the control. This pattern was already expected and also found in other studies (Käffer et al., 2012). Lichens are known to accumulate and retain many heavy metals in quantities that exceed their physiological requirements because they are able to keep metals extracellularly as oxalate crystals or turn them into lichen acids (Backor and Loppi, 2009).

Zinc contents were higher in cities with industrial activities as Triunfo, Esteio and Montenegro. Esteio has also a great demographic density (2,918 inhabitants/km^2, which is one of the highest in the state of Rio Grande do Sul) and thus an intense daily traffic. According to Minganti et al. (2003) the main potential sources of zinc are indeed industrial emissions and traffic flow. Sulphur peaks were registered in the beginning of 2014 in all sampled sites. These peaks do not seem to be related to local pollution events, but instead to some global emissions as maybe a volcanic eruption (Thomas and Prata, 2011) or other important font.

Regarding the observed peak of chromium in the monitored sites of Caraá and Santo Antônio da Patrulha recorded in 2013, it could be either related to leather wastes burning in any of the small shoe factories in these cities or could have been released (accidentally or not) by some metalworking industry, since it only occurred in one month and did not repeat during the monitored period. In the urban environment of Porto Alegre, the largest and most populated city of Rio Grande do Sul, Käffer et al. (2012) found 19.0 mg/kg as the mean highest concentration of chromium in *P. tinctorum*, while in our study it was more than three times higher (Caraá = 73.0 mg/kg). Charqueadas also had higher contents of chromium (averaging 17.4 mg/kg), but constantly along the monitoring period. In Europe, a review of chromium averages in mossess between 1990 and 2000 demonstrated that no country had higher concentrations than 8.5 mg/kg during this period and the trend expected for the next years was a reduction in chromium emissions (Harmens et al., 2007).

Despite only having data of PM_{10}, SO_2, NO_2, O_3 and CO for some sites, this information was valuable to understand the differences among these sites. It is important to note that all these pollutants were under the levels required by the National Patterns of Air Quality (CONAMA, 1990). However, if we consider the guidelines of the World Health Organization for particulate matter (PM_{10}), Charqueadas exceeded the suggested limit for

health security, which is an annual mean of 20 μg/m^3 (WHO 2006) and Santo Antônio da Patrulha is also very close to reach the maximum, considering the few data available. Charqueadas has a greater demographic density and more intense industrial activity, while Santo Antônio has not many industries, but had lately a great increment in the percentage of vehicles (82% increase in seven years) (Silva et al., 2015).

Particulate matter may include a wide range of chemical species, as elemental and organic carbon compounds; oxides of silicon, aluminum and iron; trace-metals; sulphates; nitrates and ammonia (Bergamaschi et al., 2007). The great danger of higher values of PM_{10} is because it is associated with increased rates of bronchitis, reduced lung functions (WHO, 2000), and also an increase of cancer chances, since many compounds of the particulate matter are also mutagenic (Coronas et al., 2008, 2009). In a recent study, Coronas et al. (2008) analyzed the mutagenic activity of airborne particulate material (PM_{10}) in Triunfo through the *Salmonella*/microsome assay, and found positive genotoxic activity, which they related to the presence of some PAHs (Polycyclic Aromatic Hydrocarbons). The concentration of PM_{10} found by this study in 2008 was even higher than the registered here (up to 100 μg/m^3). In Esteio, Coronas et al. (2009) found mutagenic activity in all samples of particulate matter (PM_{10}), using the same method. Similar results were found in the study of Pereira et al. (2013) for the city of Montenegro and also for Santo Antônio da Patrulha. In this last city, Silva et al. (2015) recorded peaks of 64 μg/m^3 of $PM_{2.5}$, much higher than the limit defined by WHO (25 μg/m^3 for this parameter), besides high values of PAHs.

Almost all the other greenhouse gases differed among sites, with the exception of Carbon Monoxide. This gas is highly related to vehicular emissions (Mayer, 1999) and has been controlled by the Brazilian government directly in the car industries, through several actions. Since 2003, when this program started, two thirds of the emissions have been reduced in the country (IBAMA, 2014). So, considering what we observed in this study, it is

indeed true that emissions of CO are controlled, since the emissions did not change between sites and are much smaller than the WHO recommendations (WHO, 1999).

Regarding the other gases, contents of SO_2 and O_3 had higher concentrations in Charqueadas, while NO_2 had higher concentrations in Esteio, which is comparable to the results found through modeled data of NO_x. The higher concentration of SO_2 in Charqueadas can be due to the coal-fired power plant, since they are well-known sources of SO_2, heavy metals and other elements (Garty, 2000). While the higher concentration of ozone could be a result of pollutants coming from other sites, since this greenhouse gas arises from the oxidation of nitrogen oxides, carbon monoxides and/or volatile organic compounds (Jenkin and Clemitshaw, 2000). Nitrogen dioxide (NO_2), which had the higher values in Esteio, has as its main sources the burnt of fossil fuels, and so it is closely related to motor vehicle traffic (Mayer, 1999). The city of Esteio is indeed the one with the highest demographic density from our study (2,918 inhabitants/km^2) and consequently holds the greatest vehicular fleet, summing around 43,100 vehicles (IBGE 2014).

Based on the Principal Component Analysis, an air pollution profile could be assessed for the seven monitored cities. The most polluted cities considering data of metals, sulphur, modeled $PM_{2.5}$ and NO_x seem to be Charqueadas and Esteio, with high contents of many pollutants as already discussed. In the first, there is a thermoelectric power plant, in the second, important petrochemical industries and in both these cities there are large steel manufacturing industries. These, together with other kinds of industries and also traffic emissions, are probably the main air pollution sources in these cities, which had also high concentrations of measured gases (SO_2, NO_2 and O_3) and PM_{10}.

The cities of Triunfo and Montenegro, mainly industrial cities, had also impacts of air pollution. Triunfo holds a large Petrochemical Complex, while Montenegro is in the main direction of the winds from this complex. However, organic compounds that were not

evaluated would probably better separate these cities from the mainly rural ones (SA, MA and CA). Caraá (CA) seemed to be highly influenced by the huge peak of chromium in 2013, while Santo Antônio da Patrulha (SA) and Maquiné (MA) were characterized by lower concentrations of the analyzed pollutants.

5. Conclusion

The present study shows novel information about the air quality of Rio Grande do Sul, southern Brazil. It is also highlights that air pollution is spread everywhere and not only limited to dense populated and industrial cities. We demonstrated here that even apparently not polluted sites, as small rural cities with family farming plantations as the main economic activity, could be affected by external (or internal and discreet) sources of some pollutants.

As pointed by Carreras and Pignata (2002), although it is still hard to determine the exact sources of elements that reach the lichen thalli, the knowledge about their distribution patterns is indeed of great value for society. This information can be of great importance for decision makers on, for example, where to reinforce health attention or where the air quality is already too saturate for new pollution sources installation.

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ARTIGO 2

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The application of lichens as ecological surrogates of air pollution in the subtropics: a case study in south Brazil

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Abstract – The use of lichens as ecological surrogates has been an important tool to evaluate the impact of air pollution in both ecosystem and human health, but remains underused in the subtropics due to lack of knowledge. Aiming to support the application of lichen as ecological surrogates of the effects of air pollution in the subtropics we hypothesized that urbanization was an important driver of changes on lichen diversity, composition and vitality. For that, we quantified several lichen diversity metrics (richness, cover and community composition) and photobiont vitality in relation to atmospheric pollution or its surrogates (modeled pollutant gases, pollutants in lichen thallus and land cover). We confirmed that air pollution was a key driver for lichen diversity. Changes in lichen community composition and vitality were very significantly related to air pollution, and integrated the effect of multiple stressors (particulate matter, NO_x and Cu), being thus powerful ecological indicators of air pollution in the subtropics.

Keywords – atmospheric pollution, biomonitoring, bioindicators, environmental stress, industrial pollution, urban pollution.

1. Introduction

In urban environments, many types of pollutants often act together making it difficult to unveil their effects (Pinho et al. 2008a). Bioindicators are good options to understand the effects of multiple pollutants on ecosystems since they integrate the results of air pollution over time, accounting even for unknown pollutants (Conti and Cecchetti 2001). Lichens - symbiotic organisms composed by a fungus and a photosynthetic partner (either an algae or a cyanobacteria) - have been widely used as indicators of environmental changes (Nimis et al. 2002) and as good surrogates of air pollution (Conti and Cecchetti 2001, Käffer et al. 2012, Pinho et al. 2011, Augusto et al. 2013, Branquinho et al. 2015).

Despite being very diverse in the subtropics, lichen communities are still poorly understood in these regions (Li et al. 2013). Especially, not much is known regarding lichens and air quality in the southern subtropics. In Brazil, only a few studies relate lichens and air pollution (Fuga et al. 2008, Martins et al. 2008, Käffer et al. 2011, 2012, Käffer and Martins 2014), none of them covering a larger urbanization gradient. These studies mainly showed that air pollution seems to affect lichen photobiont vitality and lichen community composition. In Käffer et al. (2011), some lichen species from an urban environment showed to be more sensitive to air pollution and urbanization, while others were more tolerant to this type of stress.

Metrics of lichen diversity such as species richness, lichen cover and lichen community composition have been successfully used to quantify the effects of atmospheric pollutants on ecosystems (Hawksworth et al. 2005, Käffer et al. 2012). On the other hand, lichen capacity to accumulate pollutants has also been used over these decades as a cost-effective method to assess pollutants deposition (Carreras and Pignata 2002, Branquinho et al. 2008). Pollutants accumulation may, in turn, result in physiological damage (either in the algae or in the fungal component), translated into a loss of vitality (Branquinho et al. 2011)

that can be used as early signs of air quality impacts (Carreras and Pignata 2002, Backor and Loppi 2009). These three approaches (lichen community patterns, pollutants accumulation and thallus damage) are within the most widely used to track the effects of global change (Branquinho et al. 2015), and also used by some authors in southern subtropics (Fuga et al. 2008, Martins et al. 2008, Käffer et al. 2011, 2012, Käffer and Martins 2014).

Most studies using lichens as ecological indicators are focused on the northern hemisphere, namely in Europe and North America, while the southern subtropics have received much less attention. In fact, there is still a lack of lichenological knowledge in this region: not even lichen diversity is well known, what can be noted by the continuous number of new species being described every year (Canêz and Marcelli 2009, Gumboski and Eliasaro 2011, Aptroot et al. 2014, Feuerstein et al. 2014, Feuerstein and Eliasaro 2015). The relation of lichen communities with environmental changes is even less documented in Brazil (Käffer et al. 2011, Koch et al. 2013). Besides, in this region nothing is known about the influence on lichen communities of land cover changes as a result of urbanization.

Thus, since different lichen biomonitoring metrics respond to distinct drivers in different environments (e.g. for SO_2, lichen species richness is a good metrics but for N pollution lichen community composition is a better predictor) (Branquinho et al. 2015) it is important to understand which are the best metrics to be used in urban environments from the subtropics. Our study aims to answer two major questions: a) What are the main drivers of change for lichens in an urban/industrial pollution intensity gradient in the subtropics? and b) Which lichen metrics perform better under such conditions in the subtropics? To answer these questions, lichen diversity in an urban context in south Brazil was assessed using species richness, cover and composition, and lichen vitality was evaluated with transplanted thalli. Lichen thallus contents, modeled atmospheric pollutants and land cover characteristics were used as surrogates of air pollution. We hypothesized that the main environmental

drivers of change on lichen diversity, composition and vitality are associated with increasing urbanization, described by higher concentrations of pollutants, higher demographic density and changes on land cover, supporting the use of lichens as bioindicators of air pollution in the southern subtropics.

2. Methods

2.1 Study area

The study was carried out in seven cities: Esteio (ES), Triunfo (TR), Charqueadas (CH), Montenegro (MO), Santo Antônio da Patrulha (SA), Caraá (CA) and Maquiné (MA). These cities are separated by no more than 150 km and are located in the northeastern region of the state of Rio Grande do Sul, southern Brazil (Fig. 1). They vary from small (27.7 km^2) to large (1049.8 km^2) in size and hold different main economic activities and different sources of atmospheric pollution (Table 1).

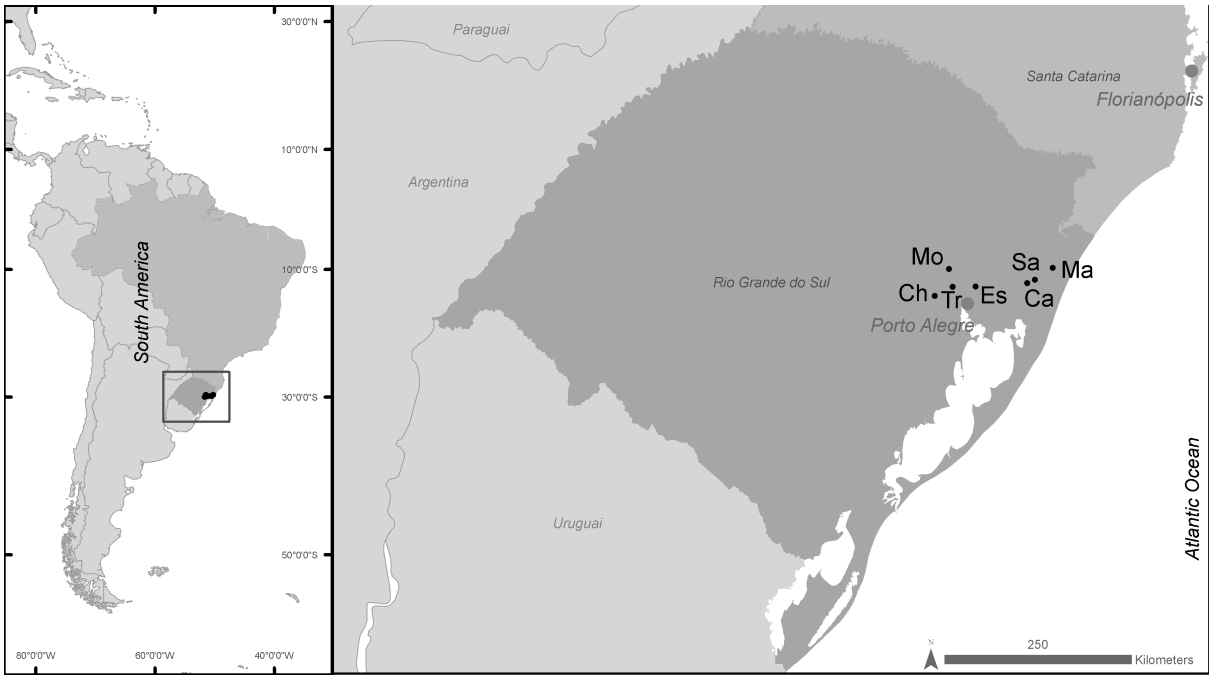

Figure 1. Location of the seven cities (MA, CA, TR, SA, MO, CH and ES) where lichen diversity was assessed. Abbreviations: MA = Maquiné; CA = Caraá; SA = Santo Antônio da Patrulha; ES = Esteio; MO = Montenegro; TR = Triunfo; CH = Charqueadas.

Table 1 Population density, area and detailed economic characteristics of each of the seven studied cities.

City	Population Density	Area	Main industrial activities	Main rural practices
Maquiné (MA)	11	621.7	No industries	Vegetables
Caraá (CA)	25	294.3	Small shoe factories	Vegetables and tobacco
Triunfo (TR)	32	818.8	Large petrochemical industries	Fruits and rice crops
Santo Antônio da Patrulha (SA)	38	1049.8	Shoe factories, agricultural machinery industries	Rice crops
Montenegro (MO)	140	424.0	Food and metalworking industries, tanneries, direct influence of a large petrochemical plant	Fruits
Charqueadas (CH)	163	216.5	Steel manufacturing, thermoelectric power plant	Rice crops
Esteio (ES)	2918	27.7	Large petrochemical industries, steel manufacturing	No rural area

*Population density is given by inhabitant/km^2, and area in km^2. Source: IBGE, 2014 (http://cidades.ibge.gov.br/).

2.2 Sampling design

Cities were selected based on similar climate, altitude and vegetation, on different population density and industrial activities. The altitude ranged from 10 to 75 m above sea level and the climate is classified as subtropical humid, Cfa type according to the updated Köppen–Geiger classification (Peel et al. 2007).

In each city, four sampling units were chosen based on the presence of a vegetation stand with at least four suitable trees to sample lichen diversity. The exception was Maquiné, where six sampling units were selected, due to the highest habitat heterogeneity of this site. Trees had to comply with the following criteria: straight unbranched trunk below 1.50 m, with at least 18 cm circumference at breast height (CBH, 1.30 m above the ground) and without smooth or peeling cortex. The study comprised a total of 30 sampling units (SUs), 14

situated in cities with a few industries and lower population density (CA, MA and SA) and 16 SUs located in cities with a great number of industries and higher demographic density (CH, ES, MO and TR) (Table 1).

2.3 Lichen diversity

In each SU, four selected trees were sampled for lichen diversity. The use of a single tree species (phorophyte) was not possible due to different urban green areas among cities (as in Llop et al. 2012). Not only Brazil accounts for one of the highest tree species diversity in the world (more than 40,000 species of Angiospermae) (Lewinsohn and Prado 2005) but urban areas hold also many planted exotic trees. According to Cáceres et al. (2007), in Brazilian tropical rainforests, understory lichens are not directly affected by the phorophyte species, but by microclimate and phorophyte bark characteristics. So, in order to ensure as little variation as possible driven by bark characteristics, lichens were only sampled in trees with smooth to medium fissured barks, comprising a total of 23 tree species, from 15 families (Appendix A).

Epiphyte lichens growing on the trunks from 50 to 150 cm from the ground were sampled, following the Rubberband Method (Marcelli 1992), and sampling duration of each tree varied from one to two hours. A rubber band gradually marked in percentage cover classes from 0 to 100% was placed at regular intervals of 10 cm, totalizing 11 levels of height. The rubber bands length varied according to the stem circumference and so the percentage coverage was always relative to the tree size (Koch et al. 2013). Afterwards, lichen cover per phorophyte was calculated as the sum of the percentage cover (standardized according to the stem circumference) of all the 11 levels of height. Also, the total cover per lichen species in each SU was the sum of cover in all the four trees of each SU. Lichen specimens were identified in the field or collected for posterior identification in the

laboratory. Data was used to assess the following lichen diversity metrics: total species richness, total lichen cover and species composition. Values for cover and species relative cover were averaged per sampling site.

2.4 Local site variables

Bark superficial pH, circumference at breast height and canopy openness were measured for each tree and the surrounding tree vegetation structure was characterized, considering a radius of approximately 5 m. Bark pH was measured in the field using a portable digital pH meter. Canopy openness (COP) was quantified with hemispherical photographs (averaging four pictures taken close to each tree). Photographs were taken 1 m above the ground and the canopy openness was estimated using the software Gap Light Analyzer (Frazer et al. 1999). The characterization of the surrounding vegetation was determined summing all basal areas of trees (TBA) growing inside a 5 m radius (around each tree sampled), and counting the number of tree individuals (NTI). For these, only trees with CBH higher than 18 cm were accounted.

2.5 Lichen transplants: photobiont vitality and atmospheric pollutants

Lichen transplants were used to assess atmospheric pollution in the studied sites. This method was chosen due to the low lichen natural biomass availability in some areas. The foliose epiphytic lichen species selected for the transplants, *Parmotrema tinctorum* (Despr. ex Nyl.) Hale, has a known tolerance to air pollution and was successfully used in other monitoring studies in the region (Käffer et al. 2012). Samples of *P. tinctorum* were collected in a less polluted city (30°38'17.7" S and 51°22'35.6" W) to be later exposed in the study sites. Prior to exposition, lichens were acclimated in the laboratory for ten days, for physiological adaptation and homogenization (Käffer et al. 2012). Chemical and

physiological analyses were made to evaluate initial condition of these lichens.

Lichen thalli were exposed inside of 55 x 35 cm synthetic mesh bags placed vertically in proper standardized structures, at around 120 cm from the ground and covered with shading. Each lichen transplant was composed by approximately 12 lichen thalli and exposed from March to October 2013 and from April to November 2014, next to the trees where lichen diversity was evaluated and always in the central region of each city (supposed to be more polluted). Sub-samples of these materials were taken after two, five and seven months of exposure, from all the cities monitored, on both years. At the end, 14 months were monitored in each city, with a total of six sub-samples for each of them (with the exception of the city of Maquiné, where only 2014 was monitored). The average concentration during the exposure period was then used in the statistical analysis.

Photobiont vitality was assessed by counting the percentage of live algal cells (Käffer et al. 2012) on each of the sub-samples from each site. Prior to the analysis, lichen thalli were cleaned with a brush and part of the sub-samples were macerated. The percentage of live algal cells was counted under the microscope using the neutral-red test at 5% (Le Blanc 1971, Calvelo and Liberatore 2004).

The other part of the same composed sub-samples was used to measure the contents of metals and sulfur. Total contents of sulfur and metals were determined with inductively coupled plasma optical emission spectrometry (ICP-OES) (Boss and Fredeen 2004), after acid digestion of samples. Samples were validated with spikes and with the certified reference material SRM 1515, for Apple Leaves and Domestic Sludge SRM 2781. The following metals were determined: copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), chromium (Cr), nickel (Ni), lead (Pb), vanadium (V) and aluminum (Al).

2.6 Modeled pollutants

Modeled atmospheric concentrations of some important air pollution elements, such as NO_X and $PM_{2.5}$, were retrieved from the Chemistry Coupled Aerosol-Tracer Transport Modeling System (CCATT-BRAMS; Freitas et al. 2009; Longo et al. 2013), for each of the studied city. This model incorporates data measured on physic-chemical stations, when available. Data were provided by the Group of Modeling of the Atmosphere and its Interfaces from the Universidade Federal de Pelotas, Rio Grande do Sul (http://ccatt.ufpel.edu.br).

2.7 Land cover

Land cover was extracted from the vegetation cover map of Rio Grande do Sul (Hasenack et al. 2015). The original cover map had 15 land cover categories, which were aggregated in six types of land use: (1) water and wetlands (WA), comprising rivers and natural wetlands; (2) urban and/or industrial areas (UR); (3) open fields (FI), comprising humid and dry fields (grasslands), including degraded fields; (4) native forests (FO) with more than 70% of forest cover; (5) tree plantations (TP), including existing and cut tree plantations; (6) agriculture (AG), including dry agriculture, rice crops and small-scale agriculture. The area of each land cover category was calculated for several different circular buffers, starting 50 m away from the center of the sampling unit and going up to 12800 m. Buffer size to be used in subsequent analysis was decided using non-parametric correlations (Spearman) with lichen community parameters (lichen species richness and cover), as in Munzi et al. (2014). A buffer of 3200 m was chosen for subsequent analysis as it exhibited the greatest number of significant correlations ($P<0.05$) with lichen species richness and cover. Distance of each sampling unit to the coast (DC) and the number of pollution sources (PS) (such as industries, coal power plants and sand extraction sites) were determined for

each sampling unit. All land cover and landscape analyses were done with the software ArcMap 10.2.2 (ESRI 2014).

2.8 Statistical analyses

To explore differences on lichen species richness and cover between sites a Mann-Whitney pairwise test was performed. These analyses were done with CRAN software R using function 'wilcox.test' from STATS package (R Core Team 2015). Sampling units were considered independent samples.

Analyses of correlation were performed to determine which lichen metrics (photobiont vitality, species richness and lichen cover) better describes this subtropical urban and industrial stress gradient (translated by pollution, land cover and local variables). Spearman rank R correlations (ρ) were calculated to account for non-linearity of the relationships and considered significant for $P<0.05$.

A Non-metric Multidimensional scaling (NMDS) ordination was performed to detect the most prominent environmental gradients driving lichen community composition. The analysis was based on a matrix of sampling units described by lichen species cover, using PC-ORD v. 6 (McCune and Mefford 2011). Prior to the analysis, the lichen cover matrix was relativized to minimize the effects of local site characteristics unrelated to the environmental gradient of interest (Matos et al. 2015). Bray-Curtis distance was used, since it is one of the most effective measures of species dissimilarities for community data (McCune et al. 2002). Data underwent 500 iterations per run, and the best one (lowest stress) was chosen. A Monte Carlo test was made to test the significance of the ordination, when compared with randomized data. The coefficient of determination between original plot distances and distances in the final ordination was used to assess the variability of lichen community represented by each axis. Pollution, land cover and local sites variables were overlaid in the

ordination and significant correlations between these variables and the ordination axes were assessed with Spearman rank correlations (ρ).

3. Results

3.1 Lichen vitality, species richness and cover

A total of 255 lichen species, belonging to 29 families, were identified (Appendix B). The highest species richness per sampling unit was found in the city of Montenegro (42), and the lowest in Triunfo (8) (Fig. 2a). Regarding lichen cover percentage, the highest values were found in Maquiné (60%) and the lowest in Esteio (7%) (Fig. 2b). Almost none local site variables were significantly correlated to lichen species richness and cover. The exception was the number of tree individuals (NTI), which was slightly correlated to a decrease on lichen cover (Tab. 2).

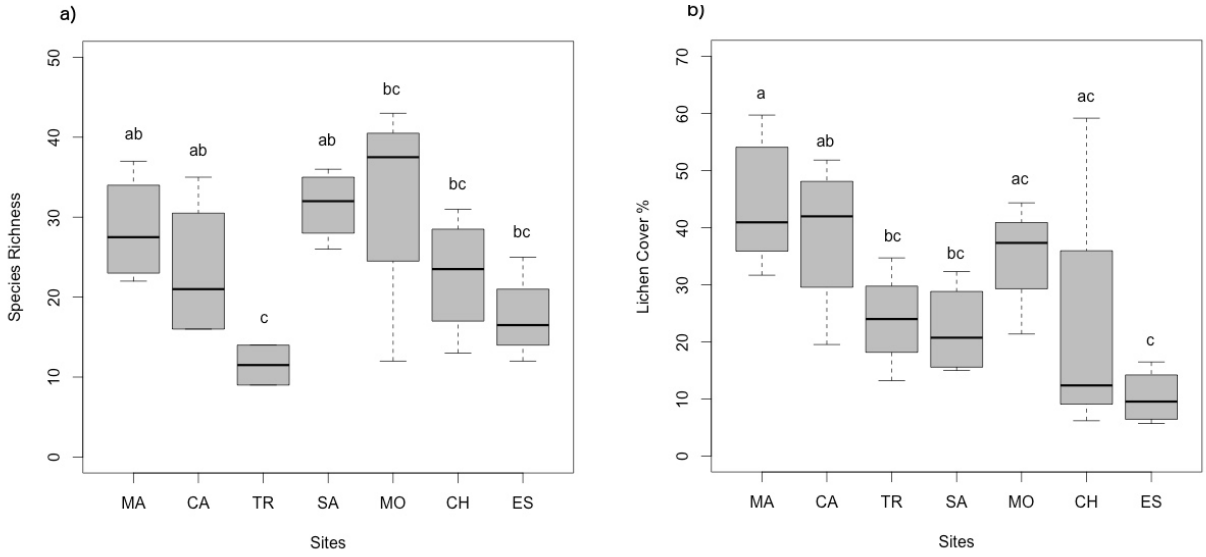

Figure 2. Box plot of lichen species richness (a) and percentage of lichen cover (b) in each city. Lines inside boxes represent mean and whiskers the standard error. Different letters represent significant differences ($P<0.05$). Cities are sorted from low (left) to high (right) following population density, and in the same order as shown in Table 1. Abbreviations: MA = Maquiné; CA = Caraá; TR = Triunfo; SA = Santo Antônio da Patrulha; MO = Montenegro; CH = Charqueadas; ES = Esteio.

Table 2. Spearman correlations (ρ) between environmental variables and lichen metrics.

Type of variable	Environmental variable	Species Richness	Lichen Cover	Photobiont vitality
Local site	Canopy Openness (COP)	ns	ns	na
	Total Trunk Basal Area (TBA)	ns	ns	na
	Number of Tree Individuals (NTI)	ns	-0.38 (0.04)	na
	Phorophyte Circumference (PC)	ns	ns	na
	Phorophyte pH (pH)	ns	ns	na
Modeled pollution	NO_X Average (NOx)	ns	ns	-0.70 (≤ 0.0001)
	$PM_{2.5}$ Average (PM2.5)	-0.44 (0.02)	-0.48 (0.007)	-0.82 (≤ 0.0001)
Pollutants on lichens	Sulfur % (S)	ns	ns	ns
	Copper mg/kg (Cu)	ns	-0.43 (0.02)	-0.67 (≤ 0.0001)
	Zinc mg/kg (Zn)	ns	ns	ns
	Manganese mg/kg (Mn)	ns	-0.45 (0.01)	-0.44 (0.01)
	Iron % (Fe)	ns	ns	ns
	Chromium mg/kg (Cr)	ns	ns	ns
	Nickel mg/kg (Ni)	ns	ns	-0.41 (0.02)
	Lead mg/kg (Pb)	ns	ns	-0.44 (0.01)
	Vanadium mg/kg (V)	ns	ns	-0.45 (0.01)
	Aluminum % (Al)	ns	ns	-0.40 (0.03)
Land cover	Water and wetlands (WA)	ns	ns	ns
	Urban and/or industrial (UR)	ns	ns	-0.53 (0.0003)
	Open fields (FI)	-0.48 (0.007)	-0.58 (0.0008)	-0.80 (≤ 0.0001)
	Native forests (FO)	ns	ns	0.62 (0.0003)
	Tree plantations (TP)	-0.42 (0.02)	-0.38 (0.04)	ns
	Agriculture (AG)	ns	0.38 (0.04)	ns
Others	Number of pollution sources (PS)	-0.40 (0.03)	-0.50 (0.005)	-0.88 (≤ 0.0001)
	Distance from the coast (DC)	ns	ns	-0.50 (0.005)
	Population density (PD)	ns	ns	-0.65 (≤ 0.0001)

* P-values are shown between parentheses and considered significant for $P<0.05$. Abbreviations: ns = not significant; na = not applicable.

Concerning the modeled pollutants, $PM_{2.5}$ was negatively correlated with all lichen metrics, showing a stronger pattern with lichen vitality, and NO_x levels were only significantly and negatively correlated with lichen vitality (Tab. 2). The monthly averages of these pollutants in each city are shown in Table 3.

From the contents of elements measured in lichens (Appendix C), Mn and Cu were the only negatively related to more than one lichen metric (lichen cover and photobiont vitality) (Tab. 2). Species richness was unrelated to element concentrations and lichen cover was correlated with only two of them (Cu and Mn). Lichen vitality was negatively correlated with almost all elements measured in lichens (Tab. 2).

Considering the effects of land cover, species richness and lichen cover were negatively correlated with open fields and tree plantations areas (Tab. 2). Lichen cover also showed a positive correlation with agricultural areas, while photobiont vitality was negatively related to open fields. This physiological feature was also negatively related to urban and industrial areas, distance to coast and population density, and positively correlated with native forest and agriculture. All metrics (lichen species richness, cover and vitality) were negatively correlated with the number of pollution sources.

3.2 Lichen communities' composition

The NMDS ordination of lichen species composition suggested three axes, with a final stress of 19.1%, lower than would be expected by chance (P = 0.004). The first axis (Fig. 3) explained 30.8% of the lichen community structure, while the second and the third axes accounted only for 14.3% and 13.9% respectively. Individual correlations of axes site scores with pollution, local site and land cover variables were calculated to assess which axis represented the urbanization gradient of interest. Axis one showed the strongest relationships with pollution and land cover variables. Although the second and third axes seem also to be related to pollution, the relationships were weaker, hereafter we will assume the first axis as the main representative of species composition and the remaining will not be further discussed. Environmental variables with significant correlations equal to or higher than ±0.4

with the first axis were overlaid in the solution (Fig. 3). Variables related to tree diameter or bark characteristics were not strongly correlated to these axes (ρ <0.4).

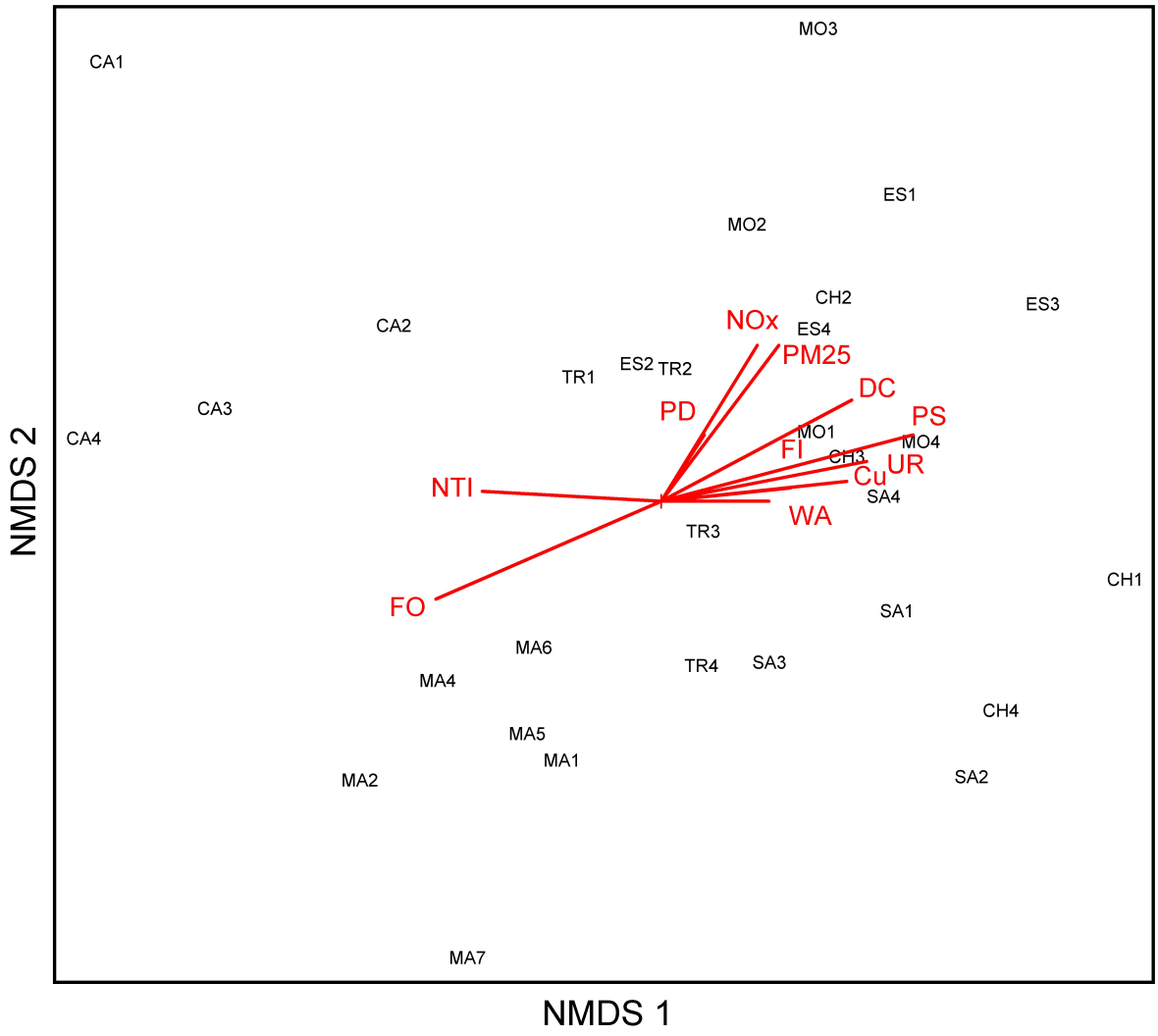

Figure 3. Non-metric multidimensional scaling (NMDS) ordination of sites according to lichen species composition. Vectors represent significant correlations (P<0.05 and with ρ higher than ±0.4) between environmental variables and ordination site scores. Abbreviations: MA = Maquiné; CA = Caraá; SA = Santo Antônio da Patrulha; ES = Esteio; MO = Montenegro; TR = Triunfo; CH = Charqueadas; FO = forest cover; NTI = number of tree individuals; NOx = modeled NOx average; PM25 = modeled $PM_{2.5}$ average; DC = distance from the coast; PS = number of pollution sources; FI = open fields cover; UR = urban cover; WA = water and wetlands Cu = Copper; PD = population density. (Final stress = 19.1; First axis explains 30.8% of the variability and the second 14.3%)

The pollution gradient represented by the first axis of the NMDS was related to many urbanization-related variables and showed on the left side the less polluted sites (CA and MA) and on the right side the most polluted ones. The environmental factor more significantly and positively related with the less polluted sites were the percentage of surrounding forest (FO) and the number of available dispersal sources (number of trees - NTI) (Fig. 3), while the polluted ones were mainly related with high concentrations of NO_x, particulate matter ($PM_{2.5}$) and copper (Cu). The polluted sites were also those further distant from the coast, with higher number of pollution sources (PS) and higher land cover percentages of urban (UR), open fields (FI) and wet areas (WA).

To visualize the results in a map, sites were color-coded into 9 classes based on their ordination scores in the first axis (Fig. 4). A pie chart around each sampling site was added including the relative proportion of land cover categories. Cities located in the eastern side of the map (MA and CA) are those with the lowest axis value (left side of the NMDS plot). Urbanization and forest cover were the main land cover categories changing along this gradient.

4. Discussion

It seems that the first response to air pollution in urban environments in subtropical areas is associated with photobiont vitality, followed by changes in lichen community composition and finally changes in species cover and richness in the most polluted sites. Indeed Loppi (2013) stated that monitoring lichens at an ecophysiological level could allow the detection of early stress symptoms, although lichen community diversity indexes could also respond to air pollution. As hypothesized, lichen diversity metrics and photobiont vitality changed mainly in response to environmental drivers associated with urbanization (particulate matter, NO_x and Cu). Community composition and photobiont vitality were the

best metrics integrating multiple stressors and were associated with several underlying environmental drivers of air pollution. A decrease in total richness and cover with increasing urbanization and proximity to industrial areas was also found, a pattern similar to that observed in other studies (Cristofolini et al. 2008, Nimis et al. 2002, Pinho et al. 2004, Pinho et al. 2008b).

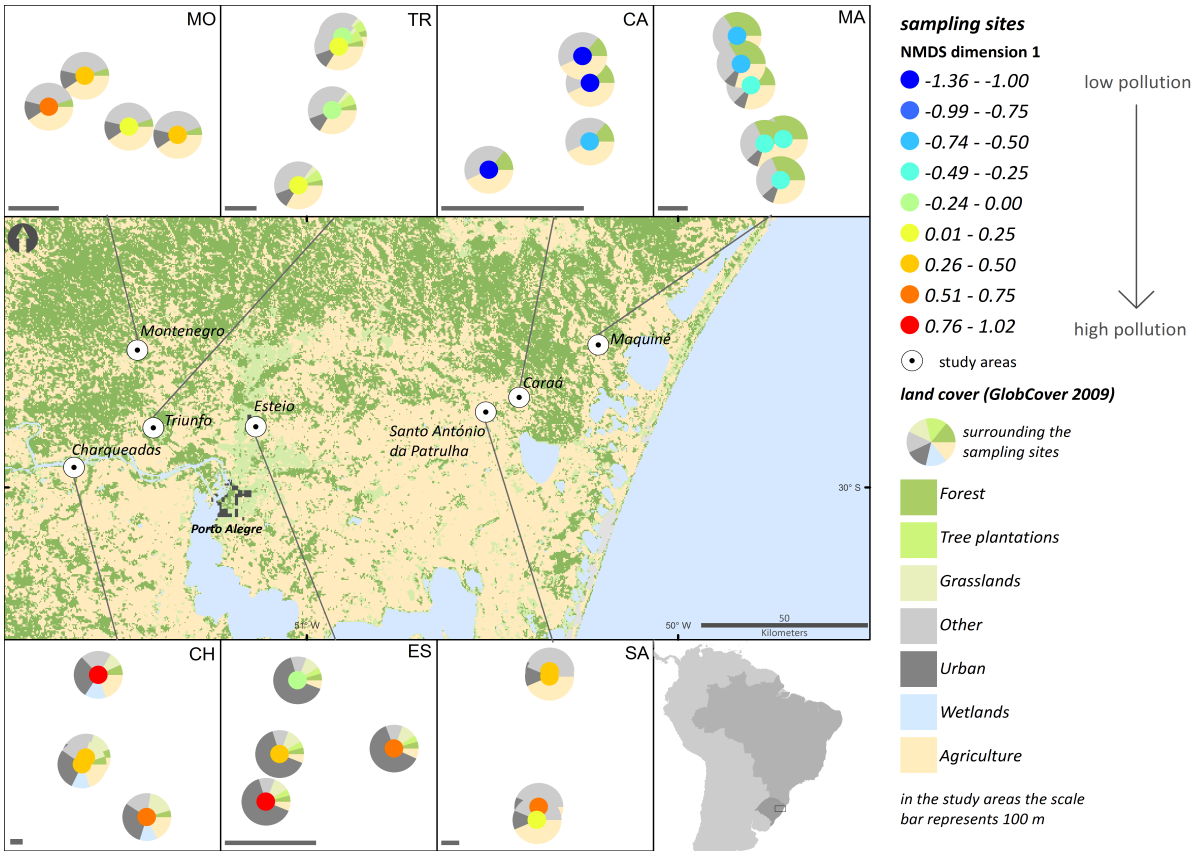

Figure 4. Characterization of each sampling site according to its position in the pollution gradient. Different colors represent different range of score values of the first axis of the non-metric multidimensional scaling (NMDS) ordination. Blue and green tonalities correspond to less polluted areas (negative axis values), while yellow, light orange, dark orange, and red represent areas with greater pollution (positive axis values). Color pies surrounding the sites correspond to the percentage of land cover categories based on a buffer of 3200m. Abbreviations: CH = Charqueadas; MO = Montenegro; TR = Triunfo; ES = Esteio; SA = Santo Antônio da Patrulha; CA = Caraá; MA = Maquiné.

Subtropical areas are expected to have less dry deposition than other climates such as Mediterranean and semiarid, thus we could expect a lower influence of fine particles in this

climate. In fact that does not happen, fine particulate matter was the pollutant with the more negative effect in lichen communities, it affected all lichen metrics tested. This negative effect on lichens was previously observed in other works with other climates in Europe, Asia and in the United States (Saipunkaew et al. 2007, Marmor et al. 2010, Paoli et al. 2014), but never with such a strong effect in the subtropics or tropics. In a previous work in the subtropics (Käffer et al. 2012) only the effect of this pollutant on photobiont vitality was showed. Fine particles can interfere with lichen physiology in many different ways: by displacing macronutrients from the cell wall, by carrying toxic pollutants inside the cells and by changing the pH (Bergamaschi et al. 2007, Branquinho 2001).

Most sources of atmospheric pollution based on sulphur have been decreasing all over the world due to the implementation of clean technologies in industrial areas thus we expect that urban and industrial areas have considerable dominance of nitrogen oxides; in fact this atmospheric pollutant was the second most important pollutant affecting negatively lichen vitality and species composition but did not influence lichen species richness and cover. Previous works in Europe found N compounds in the atmosphere to be related to a loss of lichen diversity and to changes in species composition (Pinho et al. 2008b). According to Munzi et al. (2012), nitrogen oxides can be transformed with precipitation in nitrate, which can interfere with lichen physiology and cover, and at very high concentrations can lead to species disappearance.

Manganese was negatively related to both lichen cover and photobiont vitality, as previously found by Carreras and Pignata (2002) in Southern hemisphere. Hauck and Paul (2005) showed that indeed high manganese concentrations could damage lichen photobiont and affect lichen reproduction, by inhibiting growth of soredia, for example. This element may be originated in soil, or from iron and steel industries (Parekh 1990), which are present in some of the studied areas (SA, MO, CH and ES). Copper had also important relation with

lichen species composition and negative correlation with lichen cover and vitality, probably due to its ability to enter lichen cells causing serious physiological damages that reduce lichen vitality and affects lichen diversity, particularly in what concerns the membrane damage (Branquinho et al. 2011).

Land cover was used as a surrogate of air pollution, to reflect changes from natural to urban/industrial landscapes. As seen in Europe (Pinho et al. 2008a, 2008b), land-cover categories related to air pollution (as urban cover, open areas and the number of pollution sources) influenced lichen communities, integrating a more complete overview of the anthropogenic disturbance that affects these organisms.

Open fields comprise large open areas likely to emit dust and they were negatively related to all the lichen metrics as also shown in other works in Europe (Pinho et al. 2008a; Llop et al. 2012). On the other hand, the amount of native forest areas was associated with changing species composition and improving lichen vitality. Besides helping filtering pollution (Bolund and Hunhammar 1999) forests can influence the dispersion of some lichen propagules that are not able to disperse through far distances (Sillett et al. 2000). Conversely, the increase of surrounding urban areas was negatively related to lichen vitality. Urbanization seemed to be also important for species composition and can be reflecting the Urban Heat Island Effect (UHI) (Munzi et al. 2014), air pollution (Llop et al. 2012) and less quantity of available lichen propagules due to habitat fragmentation (Styers et al. 2010).

The observed relation of distance to coast with lichen vitality and species composition is probably reflecting the impact of saline elements on lichen physiological processes (Matos et al. 2011). A greater number of pollution sources and higher population density were also important for a decrease in lichen diversity, photobiont vitality and were both related to species composition, highlighting the negative effects of pollution on lichens.

Local/micro environmental characteristics did not influence lichen diversity metrics considerably, excepting the number of tree individuals, which was related to lichen composition and cover. However, it is not possible to discard that could also be some effect of tree bark texture, not evaluated here. Regarding NTI, since a relation with TBA and COP was not observed, it can be hypothesized that the presence of more neighbor trees could be increasing propagule availability (Sillett et al. 2000) and so increasing lichen cover and affecting composition.

Overall, both lichen diversity and vitality decreased in urban and industrial areas in the studied subtropical region (southern Brazil) under the effect of multiple environmental drivers related to pollution and land cover. The changes observed were in accordance with what was already seen in the northern hemisphere (Conti and Cecchetti 2001, Pinho et al. 2008b) and independent from local conditions. Based on these results, the evaluation of lichen vitality associated with lichen community composition seem to be the most indicated method for a sensitive and integrated analysis.

Photobiont vitality, here related to the percentage of photobiont cells, was more sensitive than species richness or cover to the disturbances in the studied environmental gradient. A similar pattern was reported by other authors studying lichen metrics related to air pollution (Garty 2000). Furthermore, the analysis of live algal cells, a very cost-time effective technique, has seldom been used as a vitality measure (Käffer et al. 2012) but could have a more regular use in this kind of studies in the future.

Lichen species richness and cover were related to less air pollution variables than photobiont vitality. In fact, in the absence of high concentrations of pollutants with great deleterious effect on lichens, these metrics may show highly variable responses to air pollution (Cristofolini et al. 2008). On the other hand, changes on lichen species composition were able to reflect the disturbance gradient along the studied cities. This effect can be related

to the difference of sensitivity that each species or each group of species have to air pollution (van Haluwyn and van Herk 2002). Changes in species composition have been pointed out as fundamental measures in the context of global change (Matos et al. 2015; Dornelas et al. 2014), and our work corroborates their potential also in the subtropics.

5. Conclusions

Based on the evaluated data it is possible to state that lichens from subtropical disturbed areas are more related to pollution and land cover changes, as a result of urbanization, than to local site characteristics. This relation is seen on species richness, cover and composition and also on lichen vitality. Fine particulate matter ($PM_{2.5}$), open fields (sources of dust) and the number of pollution sources impacted all these parameters. NO_X, Cu, Mn and urban cover also had a negative effect on lichen communities, which was opposed by the existence of forest cover. Based on these results, we indicate lichen vitality and lichen composition as the best surrogates to evaluate and integrate the effects of multiple environmental disturbances in this type of subtropical urban areas.

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Appendices

Appendix A Sampled phorophyte diversity at each studied city

Species	Family	MA	CA	TR	SA	MO	CH	ES
Banara parviflora (A. Gray) Benth.	Salicaceae		X					
Bauhinia forficata Link	Fabaceae					X		
Brachychiton populneus (Schott et Endl.) R. Br.	Sterculiaceae					X		
Casearia sylvestris Sw.	Salicaceae				X			
Cedrela fissilis Vell.	Meliaceae					X		
Cupania vernalis Cambess.	Sapindaceae							X
Guapira opposita (Vell.) Reitz	Nyctaginaceae	X						
*Handroanthus pulcherrimus (*Sandwith) S.O.Grose	Bignoniaceae	X	X		X	X	X	X
Hovenia dulcis Thunb.	Rhamnaceae						X	
Jacaranda mimosifolia D. Don	Bignoniaceae						X	X
Jacaranda puberula Cham.	Bignoniaceae	X						
Leucaena leucocephala (Lam.) De Wit	Fabaceae						X	
Ligustrum lucidum Aiton	Oleaceae				X			
Luehea divaricata Mart. & Zucc.	Malvaceae							X
Mangifera indica L.	Anacardiaceae							X
Melia azedarach L.	Meliaceae						X	
Myrsine coriacea (Sw.) R.Br.	Myrsinaceae	X	X	X				
Myrsine umbellata Mart.	Myrsinaceae				X	X		
NI1								X
NI2			X					
Peltophorum dubium (Spreng.) Taub.	Fabaceae					X	X	
Persea gratissima Gaertn.	Lauraceae	X						
Sapium glandulosum (L.) Morong.	Euphorbiaceae			X				
Schinus terebinthifolius Raddi	Anacardiaceae				X		X	
Verbenoxylum reitzii (Mold.) Tronc.	Verbenaceae		X					

Abbreviations: MA = Maquiné; CA = Caraá; TR = Triunfo; SA = Santo Antônio da Patrulha; MO = Montenegro; CH = Charqueadas; ES = Esteio.

Appendix B Table of the 255 species sampled in all studied sites.

Family	Species
Arthoniaceae	*Arthonia* aff. *cinnabarina* (DC.) Wallr.
	Arthonia cinnabarina (DC.) Wallr.
	Arthonia sp.1
	Arthonia sp.2
	Arthonia sp.3
	Arthonia sp.4
	Cryptothecia sp.1
	Cryptothecia sp.2
	Cryptothecia sp.3
	Herpothallon echinatum Aptroot, Lücking & Will-Wolf
	Herpothallon roseocinctum (Fr.) Aptroot, Lücking & G. Thor
	Herpothallon rubrocinctum (Ehrenb.) Aptroot, Lücking & G. Thor
	Herpothallon sp.1
Brigantiaeaceae	*Brigantiaea leucoxantha* (Sprengel) R. Sant. & Hafellner
Candelariaceae	*Candelaria concolor* (Dicks.) Arnold
Cladoniaceae	*Cladonia* cf. *ahtii* S. Stenroos
	Cladonia subradiata (Vain.) Sandst.
	Cladonia subsquamosa Kremp.
Coccocarpiaceae	*Coccocarpia palmicola* (Spreng.) Arv. & D.J. Galloway
Coenogoniaceae	*Coenogonium* cf. *bacilliferum* (Malme) Lücking, Aptroot & Sipman
	Coenogonium geralense (Henn.) Lücking
	Coenogonium linkii Ehrenb.
	Coenogonium nepalense (G. Thor & Vězda) Lücking
Collemataceae	*Leptogium* aff. *denticulatum* Tuck.
	Leptogium cf. *azureum* (Sw.) Mont.
	Leptogium cf. *cyanescens* (Pers.) Körb.
	Leptogium chloromelum (Ach.) Nyl.
	Leptogium cyanescens (Pers.) Körb.
	Leptogium denticulatum Tuck.
	Leptogium isidiosellum (Riddle) Sierk
	Leptogium marginellum (Sw.) Gray
	Leptogium milligranum Sierk
Crocyniaceae	*Crocynia pyxinoides* Nyl.
Fissurinaceae	*Fissurina instabilis* Nyl.
	Fissurina sp.1
	Fissurina sp.2
Graphidaceae	*Chapsa* aff. *albida* (Nyl.) Lücking & Sipman
	Chapsa aff. *tibellii* Mangold
	Chapsa leprieurii (Mont.) Frisch
	Chapsa sp.1
	Diorygma sp.1
	Glyphis cicatricosa Ach.
	Glyphis scyphulifera (Ach.) Staiger
	Graphidaceae (esterile)
	Graphidaceae sp.1
	Graphis aff. *albotecta* (Redinger) Staiger

	Graphis aff. *dracaenae* Vain.
	Graphis aff. *duplicatoinspersa* Lücking
	Graphis aff. *elegans* (Borrer ex Sm.) Ach.
	Graphis aff. *inversa* R.C. Harris
	Graphis aff. *leptocarpa* Fée
	Graphis aff. *submarginata* Lücking
	Graphis calcea (Fée) A. Massal.
	Graphis cf. *anfractuosa* (Eschw.) Eschw.
	Graphis cf. *angustata* Eschw.
	Graphis cf. *aurit*a Eschw.
	Graphis cf. *erythrocardia* Müll. Arg.
	Graphis cf. *subcontorta* (Müll. Arg.) Lücking & Chaves
	Graphis cincta (Pers.) Aptroot
	Graphis duplicata Ach.
	Graphis elegans (Borrer ex Sm.) Ach.
	Graphis elongata Zenker
	Graphis furcata Fée
	Graphis granulata Fée
	Graphis handelii Zahlbr.
	Graphis immersella Müll. Arg.
	Graphis intricata Eschw.
	Graphis leptocarpa Fée
	Graphis lineola Ach.
	Graphis longispora D.D. Awasthi
	Graphis longula Kremp.
	Graphis pinicola Zahlbr.
	Graphis plurispora (Redinger) Lücking & Chaves
	Graphis rhizocola (Fée) Lücking & Chaves
	Graphis rimulosa (Mont.) Trevis.
	Graphis schiffneri Zahlbr.
	Graphis sp. 1
	Graphis sp. 2
	Graphis sp. 3
	Graphis sp. 4
	Graphis sp. 5
	Graphis streimannii A.W. Archer
	Graphis striatula (Ach.) Spreng.
	Graphis tenella Ach.
	Hemithecium chlorocarpum (Fée) Trevis.
	Hemithecium rufopallidum (Vain.) Staiger
	Phaeographis cf. *scalpturata* (Ach.) Staiger
	Phaeographis dendritica (Ach.) Müll. Arg.
	Phaeographis lecanographa (Nyl.) Staiger
	Phaeographis lobata (Eschw.) Müll. Arg.
	Phaeographis schizoloma (Müll. Arg.) Müll. Arg.
	Platygramme caesiopruinosa (Fée) Fée
	Platygramme reticulata Fée
Gyalectaceae	*Ramonia intermedia* Kalb
Haematommataceae	*Haematomma persoonii* (Fée) A. Massal.

Hygrophoraceae	*Dictyonema* sp.1
Lecanoraceae	*Lecanora achroa* Nyl.
	Lecanora albella (Pers.) Ach.
	Lecanora concilianda Vain.
	Lecanora helva Stizenb.
	Lecanora sp.1
	Lecanora sp.2
	Lecanora sp.3
	Ramboldia haematites (Fée) Kalb
Letrouitiaceae	*Letrouitia domingensis* (Pers.) Hafellner & Bellem.
Lobariaceae	*Crocodia aurata* (Ach.) Link
	Ricasolia aff. *discolor* (Bory) Hue
	Ricasolia aff. *tenuis* Vain.
	Ricasolia sp.1
Malmideaceae	*Malmidea flavopustulosa* (M. Cáceres & Lücking) M. Cáceres & Kalb
	Malmidea vinosa (Eschw.) Kalb, Rivas Plata & Lumbsch
Monoblastiaceae	*Anisomeridium* aff. *leptospermum* (Zahlbr.) R.C. Harris
	Anisomeridium leptospermum (Zahlbr.) R.C. Harris
	Anisomeridium sp.1
	Anisomeridium subprostans (Nyl.) R. C. Harris
	Anisomeridium tamarindii (Fee) R. C. Harris
NI	N.I.3
Ochrolechiaceae	*Ochrolechia africana* Vain.
Pannariaceae	*Pannaria rubiginosa* (Thunb. ex Ach.) Delise
Parmeliaceae	*Bulbothrix isidiza* (Nyl.) Hale
	Bulbothrix regnelliana Jungbluth, Marcelli & Elix
	Canoparmelia caroliniana (Nyl.) Elix & Hale
	Canoparmelia texana (Tuck.) Elix & Hale
	Crespoa carneopruinata (Zahlbr.) Lendemer & B.P. Hodk.
	Hypotrachyna aff. *endochlora* (Leight.) Hale
	Hypotrachyna costaricensis (Nyl.) Hale
	Hypotrachyna polydactyla (Krog & Swinscow) T.H. Nash
	Hypotrachyna sp.1
	Hypotrachyna sp.2
	Myelochroa lindmanii (Lynge) Elix & Hale
	Parmelinopsis minarum (Vain.) Elix & Hale
	Parmotrema aff. *herrei* (Zahlbr.) A.A. Spielm. & Marcelli
	Parmotrema aff. *muelleri* (Vain.) O. Blanco, A. Crespo, Divakar, Elix &
	Parmotrema austrosinense (Zahlbr.) Hale
	Parmotrema clavuliferum (Räsänen) Streimann
	Parmotrema conferendum Hale
	Parmotrema consors (Nyl.) Krog & Swinscow
	Parmotrema homotomum (Nyl.) Hale
	Parmotrema indicum Hale
	Parmotrema internexum (Nyl.) Hale ex DePriest & B.W. Hale
	Parmotrema muelleri (Vain.) O. Blanco, A. Crespo, Divakar, Elix &
	Parmotrema pilosum (Stizenb.) Krog & Swinscow
	Parmotrema praesorediosum (Nyl.) Hale
	Parmotrema pseudoreticulatum (Tav.) Hale

	Parmotrema sancti-angeli (Lynge) Hale
	Parmotrema sp.1
	Parmotrema subcaperatum (Kremp.) Hale
	Parmotrema tinctorum (Despr. ex Nyl.) Hale
	Protoparmelia capitata Lendemer
	Punctelia canaliculata (Lynge) Krog
	Punctelia constantimontium Sérus.
	Punctelia crispa Marcelli, Jungbluth & Elix
	Punctelia hypoleucites (Nyl.) Krog
	Punctelia nebulata Elix & J. Johnst.
	Punctelia neutralis (Hale) Krog
	Punctelia osorioi Canêz & Marcelli
	Punctelia sp.1
	Punctelia sp.2
	Punctelia subrudecta (Nyl.) Krog
	Usnea angulata Ach.
	Usnea ceratina Ach.
	Usnea cf. *grandisora* Truong & P. Clerc
	Usnea perhispidella J. Steiner
	Usnea sp.1
	Usnea sp.2
	Usnea subelegans (Vain.) Motyka
Pertusariaceae	*Pertusaria* aff. *carneola* (Eschw.) Müll. Arg.
	Pertusaria carneola (Eschw.) Müll. Arg.
	Pertusaria flavens Nyl.
	Pertusaria sp.1
	Pertusaria sp.2
	Pertusaria sp.3
	Pertusaria sp.4
	Pertusaria sp.5
	Pertusaria sp.6
	Pertusaria sp.7
	Pertusaria sp.8
Physciaceae	*Buellia* sp.1
	Cratiria lauricassiae (Fée) Marbach
	Dirinaria applanata (Fée) D.D. Awasthi
	Dirinaria confluens (Fr.) D.D. Awasthi
	Dirinaria picta (Sw.) Clem. & Shear
	Heterodermia aff. *diademata* (Taylor) D.D. Awasthi
	Heterodermia aff. *speciosa* (Wulfen) Trevis.
	Heterodermia albicans (Pers.) Swinscow & Krog
	Heterodermia casarettiana (A. Massal.) Trevis.
	Heterodermia diademata (Taylor) D.D. Awasthi
	Heterodermia galactophylla (Tuck.) W.L. Culb.
	Heterodermia lutescens (Kurok.) Follmann
	Heterodermia microphylla f. *granulosa* (Kurok.) J.C. Wei
	Heterodermia obscurata (Nyl.) Trevis.
	Heterodermia sp.1
	Heterodermia sp.2

	Heterodermia sp.3
	Heterodermia sp.4
	Heterodermia speciosa (Wulfen) Trevis.
	Hyperphyscia adglutinata (Flörke) H. Mayrhofer & Poelt
	Hyperphyscia cochlearis Scutari
	Hyperphyscia tuckermanii Lynge
	Physcia aff. *stellaris* (L.) Nyl.
	Physcia aipolia (Ehrh. ex Humb.) Fürnr.
	Physcia alba (Fée) Müll. Arg.
	Physcia atrostriata Moberg
	Physcia crispa Nyl.
	Physcia erumpens Moberg
	Physcia krogiae Moberg
	Physcia poncinsii Hue
	Physcia sinuosa Moberg
	Physcia sorediosa (Vain.) Lynge
	Physcia sp.1
	Physcia tenuis Moberg
	Physcia tribacia (Ach.) Nyl.
	Physcia tribacioides Nyl.
	Physcia undulata Moberg
	Pyxine berteriana (Fée) Imshaug
	Pyxine cocoës (Sw.) Nyl.
	Pyxine coralligera Malme
	Pyxine petricola Nyl.
	Pyxine sp.1
	Pyxine subcinerea Stirt.
Pilocarpaceae	*Bapalmuia confusa* Kalb & Lücking
	Byssoloma chlorinum (Vain.) Zahlbr.
	Byssoloma leucoblepharum (Nyl.) Vain.
	Calopadia puiggarii (Müll. Arg.) Vězda
	Calopadia sp.1
	Calopadia sp.2
	Calopadia subcoerulescens (Zahlbr.) Vězda
Porinaceae	*Porina africana* Müll. Arg.
	Porina atlantica (Erichsen) P. M. Jørg.
	Porina conspersa Malme
	Porina mastoidea Fée
	Porina sp.1
	Porina sp.2
	Porina tetracerae (Ach.) Müll. Arg.
Pyrenulaceae	*Distopyrenis americana* Aptroot
	Pyrenula aff. *pyrenuloides* (Mont.) R.C. Harris
	Pyrenula microcarpa Müll. Arg.
	Pyrenula mucosa (Vain.) R.C. Harris
	Pyrenula neosandwicensis Aptroot
Ramalinaceae	*Bacidia* aff. *medialis* (Tuck.) Zahlbr.
	Bacidia aff. *russeola* (Kremp.) Zahlbr.
	Bacidia fluminensis (Malme) M. Cáceres & Lücking

	Bacidia russeola (Kremp.) Zahlbr.
	Phyllopsora breviuscula (Nyl.) Müll. Arg.
	Phyllopsora buettneri (Müll. Arg.) Zahlbr.
	Phyllopsora confusa Swinscow & Krog
	Phyllopsora corallina (Eschw.) Müll. Arg.
	Phyllopsora furfuracea Zahlbr.
	Ramalina aff. *puiggarii* Müll. Arg.
	Ramalina aspera Räsänen
	Ramalina celastri (Spreng.) Krog & Swinscow
	Ramalina peruviana Ach.
Teloschistaceae	*Caloplaca erythrantha* (Tuck.) Zahlbr.
	Teloschistes exilis (Michx.) Vain.
Trypetheliaceae	*Trypethelium nitidiusculum* (Nyl.) R.C. Harris
	Trypethelium ochroleucum (Eschw.) Nyl.

Appendix C Mean values and standard deviations of each pollutant absorbed by the lichen *Parmotrema tinctorum* used as biomonitor during seven months of exposure in 2013 and 2014.

Site	Year	Sulfur (% m/m)	Copper (mg/kg)	Zinc (mg/kg)	Iron (% m/m)	Manganese (mg/kg)	Chromium (mg/kg)	Nickel (mg/kg)	Lead (mg/kg)	Vanadium (mg/kg)	Aluminum (% m/m)
Control	2013	0.09	4.00	29.00	0.10	207.00	3.00	1.00	1.00	2.00	0.15
	2014	0.11	7.00	43.00	0.07	168.00	2.00	1.00	1.00	1.00	0.10
Maquiné	2014	0.29±0.30	5.67±0.47	99.33±14.06	0.09±0.02	154.00±53.05	1.67±0.47	1.33±0.47	1.00±0.00	2.33±0.47	0.14±0.04
Caraá	2013	0.08±0.00	6.00±0.00	59.30±9.98	0.20±0.04	178.00±88.20	73.00±94.09	3.00±1.41	12.33±13.22	5.67±0.47	0.32±0.06
	2014	0.27±0.27	5.33±0.47	65.33±38.51	0.13±0.02	122.67±37.97	3.67±0.94	1.67±0.47	1.50±0.50	3.00±0.00	0.16±0.03
Triunfo	2013	0.09±0.02	5.33±1.70	55.33±17.56	0.12±0.06	139.00±34.10	3.00±0.82	1.63±0.52	2.67±0.47	3.67±1.25	0.20±0.05
	2014	0.26±0.28	8.00±1.63	78.33±23.80	0.08±0.02	180.33±52.79	1.00±0.00	1.00±0.00	3.00±2.00	1.67±0.47	0.11±0.03
Santo Antônio da Patrulha	2013	0.07±0.00	6.33±1.25	168.00±32.62	0.14±0.01	82.33±26.71	55.00±74.25	1.67±0.47	3.33±0.47	4.33±0.94	0.21±0.02
	2014	0.27±0.3	5.67±0.94	155.67±59.90	0.09±0.02	162.33±63.98	2.00±0.00	1.33±0.47	2.00±1.00	2.00±0.82	0.12±0.03
Montenegro	2013	0.09±0.01	5.67±0.47	625.00±210.89	0.15±0.04	62.67±22.53	3.00±0.82	1.33±0.47	4.33±2.05	4.33±1.89	0.22±0.08
	2014	0.18±0.13	6.33±0.47	914.00±476.93	0.11±0.02	177.00±62.57	2.33±0.47	1.00±0.00	2.50±1.50	2.33±0.47	0.12±0.03
Charqueadas	2013	0.09±0.01	5.67±0.47	144.67±46.51	0.17±0.05	172.33±37.32	9.00±1.63	2.33±0.47	3.67±0.94	4.67±1.70	0.23±0.08
	2014	0.33±0.28	10.33±0.94	150.00±25.73	0.25±0.10	218.00±46.14	25.67±13.47	4.00±1.63	6.33±3.09	4.00±1.41	0.18±0.06
Esteio	2013	0.11±0.02	7.33±1.25	68.67±11.26	0.11±0.07	222.00±225.05	4.00±2.16	2.30±1.28	3.30±2.10	3.00±2.10	0.16±0.12
	2014	0.29±0.25	8.33±1.70	651.00±234.53	0.14±0.04	195.00±25.66	3.67±0.47	2.00±0.00	3.00±0.82	3.33±1.25	0.18±0.06

ARTIGO 3

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Lichen functional structure as indicator of urban stress in a subtropical region

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Abstract – Urban environments have multiple stressors that are able to affect all forms of life, being air pollution one of the most concerning of these stressors. Lichens, which are widely known as good biomonitors and indicators of air quality, can be a valuable tool to air pollution monitoring in urban environments, in a cost effective and efficient way. The objective of this work was to identify lichen functional traits that respond to urban and industrial air pollution in a subtropical region. We focused on: 1) how lichen functional composition responded to air pollution as an environmental stress, identifying the most relevant response traits and 2) to understand if there were differences on lichen functional diversity under different stress conditions. For that, we assessed lichen functional composition and diversity in seven cities with different air pollution characteristics, comprinsing a total of 28 sampling units. Air pollution showed to negatively affect lichen functional traits, decreasing the frequency of lichens with cyanophycea as the main algae, of loose attached crustose thallus and lichens with isidia as main reproduction structure. On the other hand, this stress factor increased foliose lichens with narrow lobes, lichens with pruina (both disc and thallus) and containing terpenes. Functional richness of lichen communities also showed to be influenced by air pollution, land cover changes and bark structure, which could be related to niche reduction. Lichen functional traits showed to be good indicators of air pollution and the use of a functional approach with this purpose proved to be effective in the subtropics for the first time.

Keywords – air pollution, ecological indicators, land cover, lichen functional traits, functional richness, subtropics.

1. Introduction

Urban environments hold a complexity of multiple stressors, which can affect all biological forms of life living in these areas (Mayer, 1999; Rizwan et al., 2008). The most concerning stressors are the ones related to air pollution, but there are also loss of biodiversity and climatic alterations, all being able to directly affect ecosystem and human health in these environments (Munzi et al., 2014). Urban air pollution and changes on air quality are important issues worldwide mainly considering their proved negative and serious effect on human health even in low concentrations (Brunekreef and Holgate, 2002). Thus, finding tools to identify these stressors through ecological indicators are of great concern.

Lichens are widely known as good biomonitors and indicators of air quality. As ideal ecological indicators, lichen communities can be used to assess the current condition of the environment (Niemi and McDonald, 2004). This is possible due to their sensibility and close relation with the atmosphere (Hawksworth et al., 2005). Lichens do not have cuticle or stomata as plants have, and because of this reason, they happen to absorb many pollutants along with the nutrients they need to survive (Bergamaschi et al., 2007).

Environmental stressors such as air pollution not only affect lichen communities composition (van Haluwyn and van Herk, 2002), as also influences their functional organization (Llop et al., 2012; Pinho et al., 2008; 2012), namely their functional traits composition or functional diversity patterns. Functional traits can be directly linked to some function in one organism and can be divided in response (effect on the organisms) or effect trait (effect on ecosystems) (de Bello et al., 2010). In the present work the focus was given to response traits. Supposedly, these traits naturally evolved to allow species to have a great fitness and to adapt to changing environments. However, man-made ecosystems can have a lot more stressors, and also completely different from natural ecosystems, and thus it is important to understand lichen functional responses to these stressors.

Not much is known about the effects of air pollution on functional traits patterns in lichen communities (Nelson et al., 2015), besides some works undertaken in Europe (Giordani et al., 2012; Llop et al., 2012). Mainly functional studies on lichens and air pollution concern to lichen functional groups, based on previous grouping of species due to their sensitivity or tolerance to air pollutants (Llopp et al., 2012; Pinho et al., 2008; 2011; 2012). Unfortunately, there is not enough information in the subtropics on species-specific tolerance to air pollution and so this type of study has not been accomplished so far. However, a few attempts to integrate lichen traits in the subtropics can be cited (Calvelo et al., 2009; Käffer et al., 2011), more commonly comprising only lichen growth forms.

Since air pollution is an important issue and stressor in urban environments, the use of lichens as indicators can be a valuable tool to air quality monitoring, in a cost effective and efficient way. Based on this, the present work had as main objectives to: 1) understand how lichen functional composition responded to air pollution as an environmental stress, identifying the most relevant response traits and 2) what were the differences on lichen functional diversity (namely diversity, evenness and richness) under different stress conditions. Our main hypotheses were that: 1) traits that allow faster and wider dispersion (as soredia) and more protection to lichen thallus (physical and chemical protection) could have higher frequency on more polluted environments; and 2) lichen functional diversity indexes would show higher values on less polluted areas, since pollution would function as an environmental filter to these parameters.

2. Methods

2.1. Study area

The present study was carried out in seven cities (Esteio, Triunfo, Charqueadas, Montenegro, Santo Antônio da Patrulha, Caraá and Maquiné), located no more than 150 km

apart from each other, and situated in the northeastern region of the state of Rio Grande do Sul, southern Brazil. These cities have different atmospheric pollution characteristics, mainly due to urban and industrial pollution sources (more detailed information in Koch et al. (2016)).

2.2. Sampling design

All selected cities have similar climate, altitude and vegetation. The altitude ranged from 10 to 75 m above sea level and the climate is classified as subtropical humid, Cfa type according to the updated Köppen–Geiger classification (Peel et al., 2007).

Four sampling units were chosen in a vegetation stand with at least four suitable trees to sample lichen diversity. Trees had to comply with the following criteria: straight unbranched trunk below 1.50 m, with at least 18 cm circumference at breast height (CBH, 1.30 m above the ground) and without smooth or peeling cortex. The study comprised a total of 28 sampling units.

2.3. Environment and pollution variables

We assessed local site variables for each tree, such as bark superficial pH, bark structure (percentage of smooth to low fissured bark trees), canopy openness and surrounding tree vegetation structure (total basal area – TBA, and the number of trees inside a 5 m radius). Details on these measures were reported in Koch et al. (2016).

Lichen transplants were used to assess atmospheric pollution in the studied sites as described in Koch et al. (*in press*). Samples of *P. tinctorum* were collected in a less polluted city to be later exposed in the study sites. Each lichen transplant was exposed during seven months in 2013 and seven months in 2014, next to the trees where lichen diversity was evaluated. Sub-samples of these materials were taken after two, five and seven months of

exposure to measure total contents of sulfur and metals. Contents of copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), chromium (Cr), nickel (Ni), lead (Pb), vanadium (V), aluminum (Al) and sulphur (S) were determined with inductively coupled plasma optical emission spectrometry (ICP-OES) (Boss and Fredeen, 2004), after acid digestion of samples.

Modeled atmospheric concentrations of some important air pollution elements, such as NO_X and $PM_{2.5}$, were retrieved from the Chemistry Coupled Aerosol-Tracer Transport Modeling System (CCATT-BRAMS; Freitas et al., 2009; Longo et al., 2013), for each of the studied city. This model incorporates data measured on physic-chemical stations, when available. Data were provided by the Group of Modeling of the Atmosphere and its Interfaces from the Universidade Federal de Pelotas, Rio Grande do Sul (http://ccatt.ufpel.edu.br).

2.4. Land cover variables

Land cover was extracted from the vegetation cover map of Rio Grande do Sul (Hasenack et al., 2015) as detailed described in Koch et al. (*in press*). Four types of land use related to possible sources of air pollution (negative or positive) was considered: (1) urban and/or industrial areas (UR); (2) open fields (FI); (3) native forests (FO) with more than 70% of forest cover; and (4) agriculture (AG). The area of each land cover category was calculated for several different circular buffers, starting 50 m away from the center of the sampling unit and going up to 12800 m. A buffer of 3200 m was chosen for posterior analysis as it exhibited the greatest number of significant correlations ($P<0.05$) with lichen richness and cover. All land cover and landscape analyses were carried out with the software ArcMap 10.2.2 (ESRI, 2014).

2.5. Functional traits

Prior to traits assessment, all species with only one single occurrence were excluded, to reduce noise in the analyses. A complete database of 255 species sampled in 112 trees was used (Koch et al. 2016), after the exclusions resulting in 123 lichen species (Appendix A).

For these species, four groups of lichen functional traits were evaluated: 1. type of photobiont – chlorococcoid, trentepohlia, cyanophycea; 2. type of growth form – crustose attached, crustose loose attached, foliose narrow lobes, foliose wide lobes, fruticose, squamulose; 3. main type of reproductive strategy – apothecia, thallus fragmentation, isidia, lirellae, perithecia, picnidia, soredia; 4. physical/chemical protection – UV protection, microbiological protection, pruina on thallus and/or on the ascoma disc, presence of terpenes (Table 1).

All these traits were obtained from literature and can be easily assessed, which, as highlighted by Matos et al., (2015), is of great importance when seeking for universal ecological indicators. All traits were binary variables, summing 20 variables and chosen based on previous use in other studies (Cáceres et al., 2008; Giordani et al., 2012; Matos et al., 2015). Growth form and type of algae, for example, have known physiological relations with changes in light and humidity (Lakatos et al., 2006; Marini et al., 2011). In addition to that, type of reproduction and type of reproductive structure are related here to lichen dispersion ability and establishment.

2.6. Statistical analyses

Functional analyses were based on the community-level weighted means of trait values (CWM) approach (Lavorel, 2008) and on diversity indexes, as Functional Richness (FRic), Functional Evenness (FEve) and Functional Diversity (Rao). These parameters were all calculated through the FD Package (Laliberté and Legendre, 2010) using CRAN software

R (R Core Team, 2016). Differences on lichen functional traits and on functional diversity indexes among cities were assessed with Mann-Whitney pairwise tests.

To test the first hypothesis that lichen functional traits would respond to pollution, first a Non-metric Multidimensional scaling (NMDS) ordination was performed on a matrix of sampling units described by lichen species cover, using PC-ORD v.6 (McCune and Mefford, 2011). Then, environmental variables and CWMs with significant correlations with the ordination axes (assessed through Pearson correlations) were overlaid on the NMDS ordination. Lichen cover matrix was relativized to minimize the effects of local site characteristics unrelated to the environmental gradient of interest (Matos et al., 2015). Bray-Curtis distance measure was used, since it has showed to be one of the most effective measures of species dissimilarities for community data (McCune et al., 2002). Data underwent 500 iterations per run, and the best one (lowest stress) was chosen. A Monte Carlo test was made to test the significance of the ordination, when compared with randomized data. The coefficient of determination between original plot distances and distances in the final ordination was used to assess the variability of lichen community represented by each axis. In order to better observe differences of air pollution among sites in the final ordination plot, cities were divided in two groups based on a principal component analysis ordination (PCA) (Appendix B). This ordination was made considering all pollution variables evaluated (excluding variables related to host trees or light availability).

Model selection based on the Akaike Information Criterion with small sample correction (AICc) was made for all traits and the averaging importance of model terms was calculated. Assessing modeled-averaged importance of terms allows robust estimates of model parameters (Johnson and Omland, 2004) and was used to demonstrate the influence of pollution variables on lichen functional traits, separating the effect of local variables (host tree and light availability). All possible additive models were tested.

Table 1. Traits description based on literature and abbreviations.

Classification	Trait	Abbreviation	Description
Type of photobiont	Chlorococcoid	chlo	With green chlorococcoid algae
	Trentepohlia	tren	With green filamentous (*Trentepohlia*) algae
	Cyanolichen	cyan	With cyanobacteria
Type of growth form	Crustose Attached	catt	Firmly and entirely attached to the substrate by the lower surface
	Crustose Loose Attached	cloo	Entirely, but loosely attached to the substrate, with a marginal and underlying prothallus
	Foliose Narrow Lobes	fnlo	Partly attached to the substrate, with leaf-like form and narrow lobes (< 3mm)
	Foliose Wide Lobes	fwlo	Partly attached to the substrate, with leaf-like form and wide lobes (> 3mm)
	Fruticose	frut	Attached to the substrate at only one point, mainly with shrubby or beard-like form
	Squamulose	squa	Composed of small scales
Main Reproduction Strategy	Apothecia	apot	Cup or disc-shaped bodies containing spores (sexual reproduction)
	Thallus fragmentation	frag	Small parts of thallus which can generate a new individual, as filidia, lobules and others
	Isidia	isid	Asexual reproductive structures, usually cylindrical, which also increase thallus surface area
	Lirellae	lire	Linearly elongated apothecia, sometimes closed by thickened walls
	Perithecia	peri	Closed apothecia, sometimes immersed in the thallus
	Soredia	sore	Powder or grains containing both algae and fungus (asexual reproduction)
Physical/Chemical Protection	Pruina Disc	pdis	Fine wooly or granular covering on the upper portion of the apothecia
	Pruina Thallus	ptha	Fine wooly or granular covering on the thallus, usually at lobe tips (younger portion)
	UV Protection	uvpr	Lichen metabolites able to protect against UV rays (e.g. anthraquinones; divaricatic, secalonic or usnic acid)
	Microbiological Protection	mipr	Lichen metabolites able to protect against bacteria or other fungi (e.g. atranorin; salazinic, stictic or usnic acid)
	Terpenes	terp	Organic compound able to protect against herbivory

For the second hypothesis, in order to assess the importance of the selected environmental variables (those with greater correlation with the community) on the functional diversity indexes (FRic, FEve and Rao), a model selection was made. The matrix of environmental variables was prior standardized to avoid different weights due to sampling

unit differences. All possible additive models were tested through the Akaike Information Criterion with small sample correction (AICc). Besides selecting the best models, the averaging importance of model-terms was also assessed. All analyses on model selection were run out in CRAN software R (R Core Team 2016) with packages 'glmulti' (Calcagno and Mazancourt, 2010) and 'bblme' (Bolker, 2016).

3. Results

Lichen functional traits responded to air pollution in urban stressed environments. The type of algae, growth form, reproduction strategy and thallus protection were the traits which responded to the stress gradient. Among the indexes of functional diversity, functional richness had the most important response to air pollution.

3.1. Functional traits composition

The NMDS ordination of lichen species composition suggested three axes, with a final stress of 14.9%, lower than would be expected by chance (P =0.001). The first axis (Fig. 1) explained 32.2% of the lichen community structure, while the second and the third axes accounted for 18.1% and 13.5% respectively. Based on the highest explanation, first and second axes were plotted in the solution (Fig 1). Environmental variables and functional traits with significant correlations (Pearson r) equal to or higher than ±0.5 with the first and/or the second axes were overlaid in the solution (Fig. 1). The variables TBA (total basal area) (r = 0.3) and PD (population density) (r = 0.3) had lower correlation but were also plotted in the ordination diagram. The first was included to have a metric of light availability (with higher correlation than canopy openness), and the second, included as another metric of urbanization.

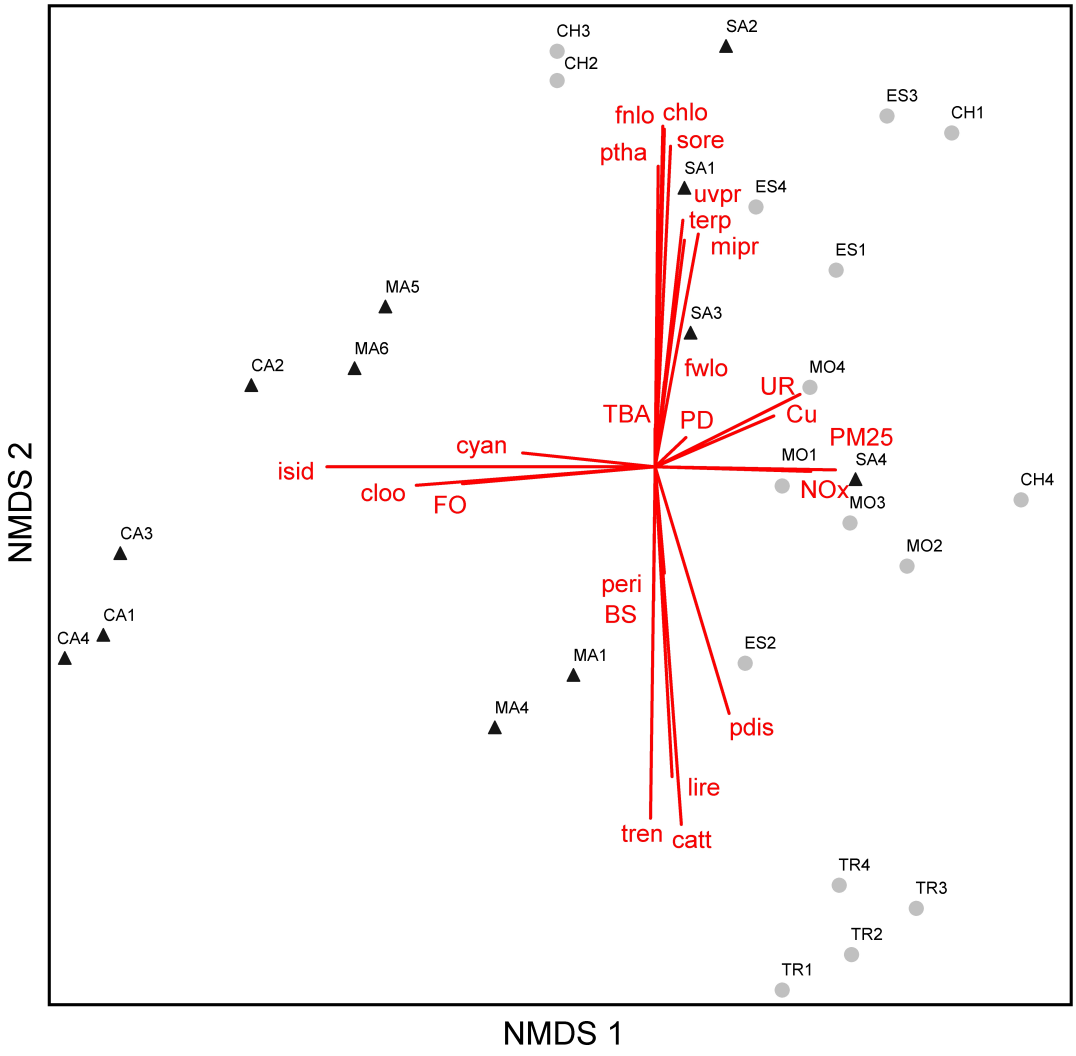

Figure 1. Non-metric multidimensional scaling (NMDS) ordination of sites according to lichen species composition and biplot of traits and environmental variables. Abbreviations: MA = Maquiné; CA = Caraá; SA = Santo Antônio da Patrulha; ES = Esteio; MO = Montenegro; TR = Triunfo; CH = Charqueadas; FO = forest cover; UR = urban cover; Cu = copper, BS = bark structure; NOx = NOx modeled average; PM25 = $PM_{2.5}$ modeled average; PD = population density; TBA = total basal area; fnlo = foliose narrow lobes; chlo = chlorococcoid green algae; sore = soredia; ptha = pruina on thallus; uvpr = UV protection; terp = terpenes; mipr = microbiological protection; fwlo = foliose wide lobes; pdis = pruina on disc; lire = lirelae; catt = crustose attached; tren = *Trentepohlia* green algae; peri = perithecis; cloo = crustose loose attached; isid = isidia; cyan = cyanolichen. (Final stress = 14.9%; First axis explains 32.2% of the variability and the second 18.1%).

According to the NMDS biplot (Fig. 1) and the CWM (Appendix C) highest values, cyanophycea (cyan), isidia (isid) and crustose loose attached (cloo) were most related to cities with greatest forest surrounding land cover (CA and MA). Conversely, lichen traits

such as, foliose narrow lobes (fnlo), soredia (sore), pruina on thallus (ptha), UV protection (uvpr), microbiological protection (mipr) and terpenes (terp) were most related to urban stressed cities, related to a higher concentration of Cu, $PM_{2.5}$ and NO_x, with greater population density and higher urban surrounding cover.

Table 2. Modeled-averaged importance of terms used as stress variables affecting lichen functional traits. Only traits with greatest correlation with the NMDS ordination axes are shown.

Trait	Pollution-related variables						Local variables	
	NO_X	$PM_{2.5}$	Cu	UR	FO	PD	BS	TBA
Chlorococcoid (chlo)	*1.0*	*1.0*	*1.0*	*1.0*	0.1	*1.0*	*1.0*	0.1
Cianophycea (cyan)	***0.6***	***0.7***	**0.3**	**0.3**	**0.2**	**0.4**	**0.2**	**0.2**
Trentepohlia (tren)	*1.0*	*1.0*	*1.0*	*1.0*	0.2	*0.9*	*1.0*	0.1
Crustose Attached (catt)	*1.0*	*1.0*	*1.0*	*0.9*	0.4	*1.0*	*1.0*	0.1
Crustose Loose Attached (cloo)	***0.7***	***0.6***	**0.5**	***1.0***	**0.5**	***1.0***	**0.3**	**0.3**
Foliose Narrow Lobes (fnlo)	***0.7***	**0.5**	**0.3**	***0.8***	***0.9***	**0.3**	**0.2**	**0.2**
Isidia (isid)	***0.8***	***0.7***	***0.6***	***1.0***	**0.4**	***1.0***	**0.2**	**0.3**
Lirellae (lire)	*1.0*	*1.0*	*1.0*	*0.9*	*0.9*	1.0	*1.0*	0.1
Perithecia (peri)	0.4	0.4	0.3	0.2	0.2	0.2	*1.0*	0.2
Soredia (sore)	*0.7*	*0.7*	*0.8*	*0.8*	0.2	0.5	*1.0*	0.2
Pruina Disc (pdis)	***0.7***	***0.7***	***0.7***	**0.3**	**0.3**	***0.7***	**0.5**	**0.3**
Pruina Thallus (ptha)	***0.7***	**0.5**	**0.3**	***1.0***	**0.3**	**0.5**	**0.1**	**0.2**
UV Protection (uvpr)	*1.0*	*0.9*	*1.0*	*1.0*	0.1	*0.6*	*1.0*	*0.6*
Microbiological Protection (mipr)	*0.9*	*0.8*	*1.0*	*1.0*	0.2	0.4	*1.0*	0.4
Terpenes (terp)	***0.8***	**0.5**	**0.3**	***0.9***	***0.8***	**0.4**	**0.2**	**0.5**

Abbreviations: Cu = copper, UR = urban cover, FO = forest cover, PD = population density, BS = bark structure, TBA = total basal area. In bold are highlighted the traits with highest importance based on pollution and land cover changes and in italic the variables with highest importance (greater than 0.5).

The results of modeled-averaged importance of terms estimated for each lichen functional trait reinforced the patterns observed in the NMDS diagram (Tab. 2, Appendix D). Traits cianophyceae, crustose loose attached, foliose narrow lobes, isidia, pruina on disc,

pruina on thallus and terpenes showed higher importance of pollution variables, instead of local variables (bark structure or light availability). The other traits had influence of all types of variables (pollution and local), with great importance of bark structure (Tab. 2, Appendix D).

3.2. Functional diversity indexes

Functional Diversity (Rao) did not differ among cities. Functional Evenness and Richness did show some differences, being more expressive in the last (Fig. 2). According to the results of a previous study (Koch et al. *in press*), to the NMDS ordination (Fig. 1) and the PCA of the environmental variables (Appendix B), we considered cities of CA, MA and SA as low stress sites and MO, TR, CH and ES as stressed sites. Based on this, only Functional Richness responded to stress, with greatest values in less stressed sites (Fig. 2).

Considering model selection results, both pollution and bark structure had important influence determining lichen functional diversity indexes, while light availability showed only little importance (Tab. 3, Fig. 4). All best models on Functional Diversity (Rao) included bark structure (BS) and also included NO_X or $PM_{2.5}$. These were the three parameters with the highest modeled-averaged importance for Rao Diversity in the studied communities (Fig. 4). For Functional Evenness, urbanization and population density were present in all models (Tab. 3) and had the greatest importance (Fig. 4). Finally, models on Functional Richness showed that this index is influenced by almost all variables at the same time (Tab. 3), with lower importance of light availability (TBA) and population density (Fig. 4).

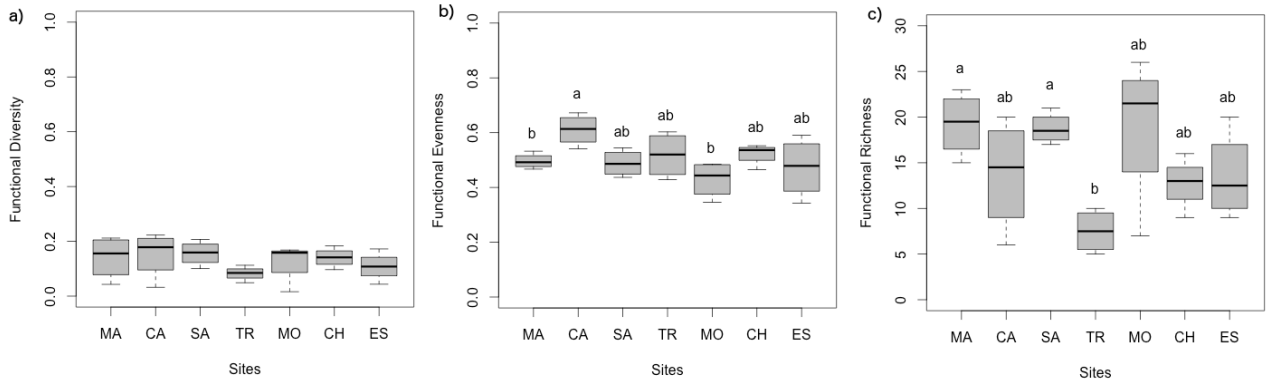

Figure 2. Functional Diversity (Rao), Functional Evenness and Functional Richness in all the sampled sites. Different letters represent significant differences ($P<0.05$).

Table 3. Best models for each of the lichen functional diversity matrix tested. Models were based on Akaike's Information Criterion corrected for small sample size (AICc). Models within 2 units of ΔAICc are shown and also the weights of each model.

Response variable	Model	AICc	Weight
Functional diversity (Rao)	Rao ~ $PM_{2.5}$ + UR + BS	74.69	0.07
	Rao ~ NO_X + UR + BS	74.83	0.06
	Rao ~ NO_X + PD + BS	75.02	0.06
	Rao ~ NO_X + UR + FO + BS	75.74	0.04
	Rao ~ NO_X + $PM_{2.5}$ + Cu + PD + BS	75.89	0.04
	Rao ~ $PM_{2.5}$ + PD + BS	76.03	0.04
	Rao ~ $PM_{2.5}$ + UR + FO + BS	76.23	0.03
	Rao ~ BS	76.27	0.03
	Rao ~ $PM_{2.5}$ + BS	76.58	0.03
	Rao ~ NO_X + BS	76.71	0.03
Functional Evenness	FEve ~ UR + PD	79.20	0.12
	FEve ~ Cu + UR + PD	79.99	0.08
	FEve ~ UR + PD + BS	80.93	0.05
	FEve ~ Cu + UR + PD + BS	81.46	0.04
Functional Richness	FRic ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + PD + BS	70.30	0.39
	FRic ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + BS	70.37	0.38
	FRic ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + BS + TBA	74.14	0.06

Abbreviations: Cu = Copper, UR = urban cover, FO = forest cover, PD = population density, BS = bark structure, TBA = Total Basal Area.

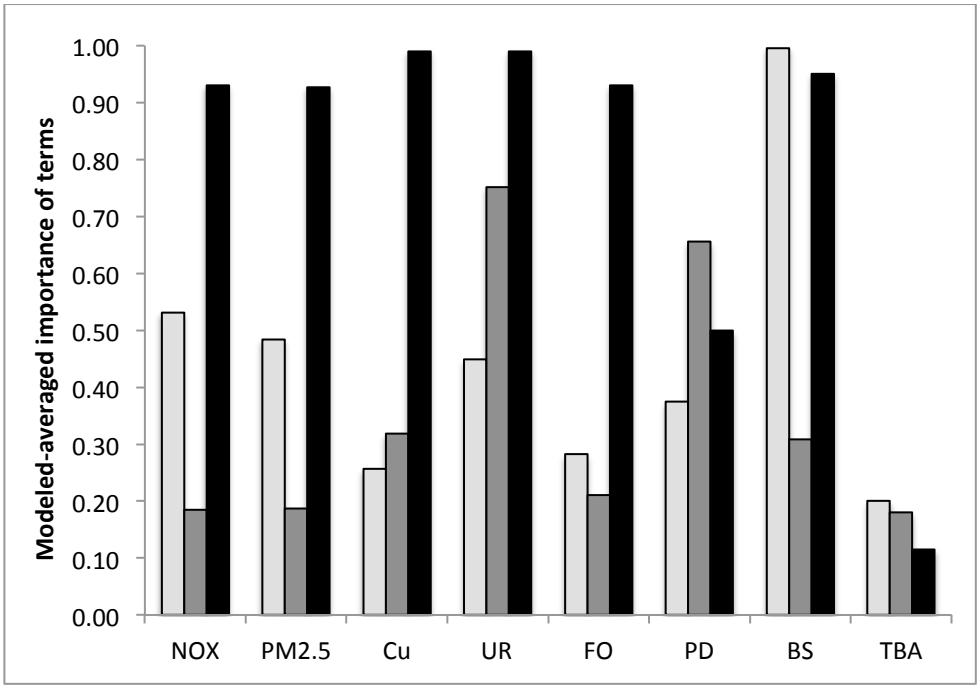

Figure 3. Modeled-averaged importance of terms used as stress variables affecting functional diversity indexes. Light grey bars correspond to Functional Diversity (Rao), grey bars to Functional Evenness and black bars to Functional Richness. Abbreviations: NOX = NO_X, PM25 = $PM_{2.5}$, Cu = Copper, UR = urban cover, FO = forest cover, PD = population density, BS = bark structure, TBA = Total Basal Area.

4. Discussion

A functional approach based on lichen functional traits was for the first time applied in an urban stressed environment in the subtropics. Lichens are good indicators of air pollution and some of their functional traits are indeed affected by air quality, as also functional richness.

4.1. Lichen functional traits responding to air pollution

Air pollution showed to negatively affect lichen functional traits, decreasing the frequency of cyanolichens, of loose attached crustose thallus and lichens with isidia as main reproduction structure. Oppositely, this stress factor increased foliose lichens with narrow lobes, lichens with pruina (both disc and thallus) and containing terpenes.

The negative effect of pollution and environmental changes on cyanolichens may be due mainly to two reasons: first, lichens with cyanobacteria need liquid water in the thallus for photosynthesis to be accomplished (Gauslaa, 2014); second, their predominant gelatinous thallus that help them to reach higher levels of water saturation, also facilitate the deposition of pollutants (Bargagli and Mikhailova, 2002). Indeed, many cyanolichens are reported as sensitive or moderate sensitive to air pollution (Will-Wolf et al., 2002), sometimes disappearing in urban areas (Calvelo et al., 2009). Moreover, cyanolichens seem to have lower respiration rates in lighter conditions than green algae lichens (Palmqvist, 2000). Thus, they are usually more adapted to forest areas than urban and stressed environments, with probably competition limitations in this type of environments.

The evaluation of growth form was used in some pollution and land use-intensity studies with lichens (Llop et al., 2012; Käffer et al., 2011; Stofer et al., 2006). Generally, higher frequency of crustose lichens is related to higher air pollution, while foliose and fruticose lichens are considered as more sensitive to this stress (van Haluwin and van Herk, 2002; Käffer et al., 2011). Opposing this idea, crustose loose attached lichens were here related to more natural and less polluted environments. It may be particularly applied to tropical areas, with a great diversity of loose attached crustose lichens, as from genus *Cryptothecia* and *Herpothallon* for example (Aptroot et al., 2009). This type of lichen is formed by loosely interwoven mycobiont hyphae, allowing them to deal well with higher levels of water availability (as in humid forests) (Lakatos et al., 2006). However, this thallus organization is probably not favorable in polluted environments, since their loose characteristic may allow the entrance of more pollutants. This paper is the first to suggest a negative relation of this type of lichen with urbanization in a subtropical area, which should be also tested in future studies.

Most foliose lichens are known to tolerate moderate to high levels of pollution (Llop et al., 2012) and higher levels of aridity (Matos et al., 2015). They also respond to changes in land cover, increasing in more altered landscapes (Stofer et al. 2006). Foliose narrow-lobed species (as from family Physciaceae) have been reported in some works to tolerate high levels of eutrophication (Pinho et al., 2011; Nimis and Martellos, 2008). This trait was tested here for the first time in the subtropics as a functional trait, and showed to be influenced by both land cover changes and air pollution, increasing with urbanization.

Another important lichen response to environmental change is the reproduction strategy. Smaller propagules, related to longer dispersions, are supposed to increase in frequency along a stress gradient (Stofer et al., 2006). Isidia, which can be considered as big propagules and more difficult to be detached from the lichen thallus, have then lower dispersion efficiency when compared to soredia. Besides, isidia also increase lichen surface to allow more photosynthesis rates (Büdel and Scheidegger, 2008), which may not be an advantage in polluted environments since it probably also increase pollutants absorption. This corroborates the pattern found here, where the trait isidia was positively related to areas surrounded by greater forest cover and negatively related to urban sites. Meanwhile, soredia, with their small size, enable lichens to quickly colonize available substrates at shorter and longer distances (Nelson et al., 2015) and was here reported to increase in more urbanized areas.

As well as plants, lichens have developed a number of protection strategies against stressors. Among these strategies is the production of chemical compounds, usually called secondary metabolites (Elix and Stocker-Wörgötter, 2008). Terpenes, also produced by plants, are known to work against herbivory and pathogenic fungi (Rundel, 1978), but here we showed that there may be also an advantage for species to have this organic compound in human-stressed environments. The physiological reason for this pattern has to be tested yet

so that we can understand whether there is a real relation. In the present paper, together with the chemical compounds increasing UV and microbiological protection, a higher frequency of terpenes showed to be related with increasing urban stress.

It is important to highlight the novelty of including the presence of pruina as a lichen functional response trait to air pollution. This fine powder, mainly consisting of calcium oxalate, is produced by some lichens and usually located in new portions of the thallus and on the apothecia (Giordani et al., 2003). Pruina has been demonstrated to indeed have a protective function against herbivory, excessive light and mechanical damages (Büdel and Scheidegger, 2008; Giordani et al., 2003). The present work suggests that besides having these functions, the presence of pruina may be helping to increase the frequency of species containing it in urban polluted sites. Since pruina production is linked to oxidative stress in lichens (Caviglia and Modenesi, 1999), the stress produced by more polluted environments may be favoring these species.

4.2. Functional indexes and air pollution

Functional richness was the functional diversity index that showed a closer relation to environmental stress in the present paper. This index describes the volume of trait space within a community and may be also related to the breadth of environmental conditions that are suitable for species within a community (Butterfield and Suding, 2013). Lower values of functional richness were found in the higher stressed cities, which may indicate that some of the resources (niches) potentially available to the community are unused (Mason et al., 2005).

Despite being related to more urbanized and polluted cities, when exploring the models and the modeled-averaged importance of terms, functional richness did not show only influence of pollution, but also of bark structure. Pollution may be reducing niches for communities and working as an environmental filter, as also bark structure (here computed as

higher percentage of smooth to low fissured barks). Many lichen species have evolved specific requirements for substrates which are products of late sucession (as old trees or dry decaying wood, usually fissured), making them more sensitive to anthropogenic disturbances (Scheidegger and Goward, 2002). This is the first study to find a relation with this index in lichen communities and environmental stress. We suggest that it should be tested in other studies so that a clearer relation could be found.

5. Conclusions

Lichen functional traits are good indicators of air pollution and the use of a functional approach with this purpose proved to be effective in the subtropics for the first time. The type of algae in the lichen, the growth form, the reproduction strategy and the presence of thallus protection showed to be good options when studying the stress effect on the epiphytic communities from urban environments. Besides, functional richness of lichen communities also showed to be influenced by air pollution, land cover changes and bark structure, which seemed to be related to niche reduction.

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Appendices

Appendix A. List of sampled species and their functional traits.

Species	Family	Type of Photobiont	Type of Growth Form	Main Reproduction Strategy	Physical/Chemical Protection			
					UV protection	Antimicrobial compound	Other compounds	Pruina
Anisomeridium aff. *leptospermum* (Zahlbr.) R.C. Harris	Monoblastiaceae	tren	catt	peri	na	na	abs	abs
Anisomeridium leptospermum (Zahlbr.) R.C. Harris	Monoblastiaceae	tren	catt	peri	na	na	abs	abs
Anisomeridium sp.1	Monoblastiaceae	tren	catt	othe	na	na	abs	na
Anisomeridium subprostans (Nyl.) R. C. Harris	Monoblastiaceae	tren	catt	peri	abs	abs	abs	abs
Anisomeridium tamarindi (Fee) R. C. Harris	Monoblastiaceae	tren	catt	peri	abs	abs	abs	abs
Arthonia aff. *cinnabarina* (DC.) Wallr.	Arthoniaceae	tren	cloo	apot	pre	abs	abs	disc
Arthonia cinnabarina (DC.) Wallr.	Arthoniaceae	tren	cloo	apot	pre	abs	abs	disc
Arthonia sp.1	Arthoniaceae	tren	cloo	apot	na	na	abs	na
Arthonia sp.2	Arthoniaceae	tren	cloo	apot	na	na	abs	na
Arthonia sp.3	Arthoniaceae	tren	cloo	apot	na	na	abs	na
Arthonia sp.4	Arthoniaceae	tren	cloo	apot	na	na	abs	na
Bacidia aff. *medialis* (Tuck.) Zahlbr.	Ramalinaceae	chlo	cloo	apot	abs	abs	abs	abs
Bacidia aff. *russeola* (Kremp.) Zahlbr.	Ramalinaceae	chlo	cloo	apot	na	na	abs	na
Bacidia fluminensis (Malme) M. Cáceres & Lücking	Ramalinaceae	chlo	cloo	apot	na	na	abs	na
Bacidia russeola (Kremp.) Zahlbr.	Ramalinaceae	chlo	cloo	apot	na	na	abs	na
Bapalmuia confusa Kalb & Lücking	Pilocarpaceae	chlo	cloo	apot	na	na	abs	na
Brigantiaea leucoxantha (Sprengel) R. Sant. & Hafellner	Brigantiaeaceae	chlo	cloo	apot	pre	pre	abs	all
Buellia lauri-cassiae (Fée) Müll. Arg.	Physciaceae	chlo	catt	apot	pre	pre	nore	abs
Buellia sp.1	Physciaceae	chlo	catt	apot	na	na	abs	na
Bulbothrix isidiza (Nyl.) Hale	Parmeliaceae	chlo	fowl	isid	pre	pre	abs	abs
Bulbothrix regnelliana Jungbluth, Marcelli & Elix	Parmeliaceae	chlo	fonl	apot	pre	pre	nore	abs
Byssoloma chlorinum (Vain.) Zahlbr.	Pilocarpaceae	chlo	cloo	apot	abs	abs	abs	abs

Byssoloma leucoblepharum (Nyl.) Vain.	Pilocarpaceae	chlo	catt	apot	abs	abs	abs	abs
Calopadia puiggarii (Müll. Arg.) Vězda	Pilocarpaceae	chlo	cloo	apot	abs	abs	abs	abs
Calopadia sp.1	Pilocarpaceae	chlo	cloo	othe	abs	abs	abs	abs
Calopadia sp.2	Pilocarpaceae	chlo	cloo	othe	abs	abs	abs	abs
Calopadia subcoerulescens (Zahlbr.) Vězda	Pilocarpaceae	chlo	cloo	apot	abs	abs	abs	abs
Caloplaca erythrantha (Tuck.) Zahlbr.	Teloschistaceae	chlo	cloo	apot	pre	abs	abs	na
Candelaria concolor (Dicks.) Arnold	Candelariaceae	chlo	fonl	sore	abs	abs	othe	abs
Canoparmelia caroliniana (Nyl.) Elix & Hale	Parmeliaceae	chlo	fowl	isid	pre	pre	abs	tha
Canoparmelia texana (Tuck.) Elix & Hale	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Chapsa aff. *albida* (Nyl.) Lücking & Sipman	Graphidaceae	tren	catt	apot	na	na	abs	na
Chapsa aff. *tibellii* Mangold	Graphidaceae	tren	catt	apot	abs	abs	abs	disc
Chapsa leprieurii (Mont.) Frisch	Graphidaceae	tren	catt	apot	abs	abs	abs	disc
Chapsa sp.1	Graphidaceae	tren	catt	apot	na	na	abs	na
Cladonia cf. *ahtii* S. Stenroos	Cladoniaceae	chlo	squa	sore	abs	pre	abs	abs
Cladonia subradiata (Vain.) Sandst.	Cladoniaceae	chlo	squa	sore	pre	pre	nore	abs
Cladonia subsquamosa Kremp.	Cladoniaceae	chlo	squa	sore	abs	pre	nore	abs
Coccocarpia palmicola (Spreng.) Arv. & D.J. Galloway	Coccocarpiaceae	cyan	fowl	isid	abs	abs	abs	abs
Coenogonium cf. *bacilliferum* (Malme) Lücking, Aptroot & Sipman	Coenogoniaceae	tren	catt	apot	na	na	abs	na
Coenogonium geralense (Henn.) Lücking	Coenogoniaceae	tren	catt	apot	abs	abs	abs	abs
Coenogonium linkii Ehrenb.	Coenogoniaceae	tren	othe	apot	abs	abs	abs	abs
Coenogonium nepalense (G. Thor & Vězda) Lücking	Coenogoniaceae	tren	catt	apot	abs	abs	abs	disc
Crespoa carneopruinata (Zahlbr.) Lendemer & B.P. Hodk.	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	tha
Crocodia aurata (Ach.) Link	Lobariaceae	chlo	fowl	sore	abs	abs	othe	abs
Crocynia pyxinoides Nyl.	Crocyniaceae	chlo	squa	sore	pre	pre	terp	abs
Cryptothecia sp.1	Arthoniaceae	tren	cloo	sore	na	na	abs	na
Cryptothecia sp.2	Arthoniaceae	tren	cloo	sore	na	na	abs	na
Cryptothecia sp.3	Arthoniaceae	tren	cloo	sore	na	na	abs	na
Dictyonema sp.1	Hygrophoraceae	cyan	othe	frag	na	na	abs	abs
Diorygma sp.1	Graphidaceae	tren	cloo	lire	na	na	abs	na

Dirinaria applanata (Fée) D.D. Awasthi	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Dirinaria confluens (Fr.) D.D. Awasthi	Physciaceae	chlo	fonl	apot	pre	pre	terp	tha
Dirinaria picta (Sw.) Clem. & Shear	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Distopyrenis americana Aptroot	Pyrenulaceae	tren	catt	peri	abs	abs	abs	abs
Fissurina instabilis Nyl.	Fissurinaceae	tren	catt	lire	abs	abs	abs	abs
Fissurina sp. 1	Fissurinaceae	tren	catt	lire	na	na	abs	na
Fissurina sp. 2	Fissurinaceae	tren	catt	lire	na	na	abs	na
Glyphis cicatricosa Ach.	Graphidaceae	tren	catt	lire	abs	abs	abs	disc
Glyphis scyphulifera (Ach.) Staiger	Graphidaceae	tren	catt	apot	abs	abs	abs	abs
Graphidaceae sp. 1	Graphidaceae	tren	catt	sore	na	na	abs	na
Graphis aff. *albotecta* (Redinger) Staiger	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis aff. *dracenae* Vain.	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis aff. *duplicatoinspersa* Lücking	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis aff. *elegans* (Borrer ex Sm.) Ach.	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis aff. *inversa* R.C. Harris	Graphidaceae	tren	catt	lire	abs	abs	othe	na
Graphis aff. *leptocarpa* Fée	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis aff. *submarginata* Lücking	Graphidaceae	tren	catt	lire	na	na	abs	disc
Graphis calcea (Fée) A. Massal.	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis cf. *anfractuosa* (Eschw.) Eschw.	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis cf. *angustata* Eschw.	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis cf. *aurita* Eschw.	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis cf. *erythrocardia* Müll. Arg.	Graphidaceae	tren	catt	lire	abs	abs	nore	na
Graphis cf. *subcontorta* (Müll. Arg.) Lücking & Chaves	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis cincta (Pers.) Aptroot	Graphidaceae	tren	catt	lire	abs	abs	nore	abs
Graphis duplicata Ach.	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis elegans (Borrer ex Sm.) Ach.	Graphidaceae	tren	catt	lire	abs	abs	nore	abs
Graphis elongata Zenker	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis furcata Fée	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis granulata Fée	Graphidaceae	tren	catt	lire	abs	abs	abs	na
Graphis handelii Zahlbr.	Graphidaceae	tren	catt	lire	abs	abs	nore	abs

Graphis immersella Müll. Arg.	Graphidaceae	tren	catt	lire	abs	pre	abs	abs
Graphis intricata Eschw.	Graphidaceae	tren	catt	lire	abs	abs	nore	abs
Graphis leptocarpa Fée	Graphidaceae	tren	catt	lire	abs	pre	abs	abs
Graphis lineola Ach.	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis longispora D.D. Awasthi	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis longula Kremp.	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis pinicola Zahlbr.	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis plurispora (Redinger) Lücking & Chaves	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis rhizocola (Fée) Lücking & Chaves	Graphidaceae	tren	catt	lire	abs	abs	abs	na
Graphis rimulosa (Mont.) Trevis.	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis schiffneri Zahlbr.	Graphidaceae	tren	catt	lire	abs	abs	nore	na
Graphis sp. 1	Graphidaceae	tren	catt	lire	abs	pre	abs	abs
Graphis sp. 2	Graphidaceae	tren	catt	lire	abs	pre	abs	abs
Graphis sp. 3	Graphidaceae	tren	catt	lire	abs	pre	nore	abs
Graphis sp. 4	Graphidaceae	tren	catt	lire	na	na	abs	abs
Graphis sp. 5	Graphidaceae	tren	catt	lire	na	na	abs	abs
Graphis streimannii A.W. Archer	Graphidaceae	tren	catt	lire	na	na	abs	na
Graphis striatula (Ach.) Spreng.	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Graphis tenella Ach.	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Haematomma persoonii (Fée) A. Massal.	Haematommataceae	chlo	catt	apot	pre	pre	abs	abs
Hemithecium chlorocarpum (Fée) Trevis.	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Hemithecium rufopallidum (Vain.) Staiger	Graphidaceae	tren	catt	lire	abs	abs	abs	abs
Herpothallon echinatum Aptroot, Lücking & Will-Wolf	Arthoniaceae	tren	cloo	isid	abs	abs	othe	abs
Herpothallon roseocinctum (Fr.) Aptroot, Lücking & G. Thor	Arthoniaceae	tren	cloo	isid	pre	pre	abs	abs
Herpothallon rubrocinctum (Ehrenb.) Aptroot, Lücking & G. Thor	Arthoniaceae	tren	cloo	isid	abs	abs	othe	abs
Herpothallon sp.1	Arthoniaceae	tren	cloo	isid	na	na	abs	abs
Heterodermia aff. *diademata* (Taylor) D.D. Awasthi	Physciaceae	chlo	fonl	na	pre	pre	terp	abs
Heterodermia aff. *speciosa* (Wulfen) Trevis.	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Heterodermia albicans (Pers.) Swinscow & Krog	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Heterodermia casarettiana (A. Massal.) Trevis.	Physciaceae	chlo	fonl	sore	pre	pre	nore	abs

Heterodermia diademata (Taylor) D.D. Awasthi	Physciaceae	chlo	fonl	apot	pre	pre	abs	abs
Heterodermia galactophylla (Tuck.) W.L. Culb.	Physciaceae	chlo	fonl	sore	pre	pre	abs	abs
Heterodermia lutescens (Kurok.) Follmann	Physciaceae	chlo	fonl	sore	pre	pre	abs	abs
Heterodermia microphylla f. granulosa (Kurok.) J.C. Wei	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Heterodermia obscurata (Nyl.) Trevis.	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Heterodermia sp. 1	Physciaceae	chlo	fonl	sore	na	na	abs	na
Heterodermia sp. 2	Physciaceae	chlo	fonl	sore	pre	pre	nore	abs
Heterodermia sp. 3	Physciaceae	chlo	fonl	sore	pre	pre	abs	abs
Heterodermia sp. 4	Physciaceae	chlo	fonl	frag	pre	pre	abs	tha
Heterodermia speciosa (Wulfen) Trevis.	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Hyperphyscia adglutinata (Flörke) H. Mayrhofer & Poelt	Physciaceae	chlo	fonl	sore	abs	abs	abs	tha
Hyperphyscia cochlearis Scutari	Physciaceae	chlo	fonl	sore	abs	abs	abs	tha
Hyperphyscia tuckermanii Lynge	Physciaceae	chlo	fonl	frag	abs	abs	abs	na
Hypotrachyna aff. *endochlora* (Leight.) Hale	Parmeliaceae	chlo	fowl	sore	pre	pre	seca	tha
Hypotrachyna costaricensis (Nyl.) Hale	Parmeliaceae	chlo	fowl	isid	pre	pre	abs	tha
Hypotrachyna polydactyla (Krog & Swinscow) T.H. Nash	Parmeliaceae	chlo	fonl	frag	pre	pre	abs	abs
Hypotrachyna sp. 1	Parmeliaceae	chlo	fowl	sore	na	na	abs	na
Hypotrachyna sp. 2	Parmeliaceae	chlo	fowl	isid	pre	pre	abs	abs
Lecanora achroa Nyl.	Lecanoraceae	chlo	catt	apot	pre	pre	terp	disc
Lecanora albella (Pers.) Ach.	Lecanoraceae	chlo	catt	apot	pre	pre	abs	disc
Lecanora concilianda Vain.	Lecanoraceae	chlo	catt	apot	pre	pre	abs	abs
Lecanora helva Stizenb.	Lecanoraceae	chlo	catt	apot	pre	pre	abs	disc
Lecanora sp. 1	Lecanoraceae	chlo	catt	sore	na	na	abs	na
Lecanora sp. 2	Lecanoraceae	chlo	catt	apot	na	na	abs	na
Lecanora sp. 3	Lecanoraceae	chlo	catt	apot	na	na	abs	na
Leptogium aff. *denticulatum* Tuck.	Collemataceae	cyan	fowl	isid	abs	abs	abs	abs
Leptogium cf. *azureum* (Sw.) Mont.	Collemataceae	cyan	fowl	apot	abs	abs	abs	abs
Leptogium cf. *cyanescens* (Pers.) Körb.	Collemataceae	cyan	fowl	isid	abs	abs	abs	abs
Leptogium chloromelum (Ach.) Nyl.	Collemataceae	cyan	fowl	apot	abs	abs	abs	abs
Leptogium cyanescens (Pers.) Körb.	Collemataceae	cyan	fowl	isid	abs	abs	abs	abs

Leptogium denticulatum Tuck.	Collemataceae	cyan	fowl	apot	abs	abs	abs	abs
Leptogium isidiosellum (Riddle) Sierk	Collemataceae	cyan	fowl	isid	abs	abs	abs	abs
Leptogium marginellum (Sw.) Gray	Collemataceae	cyan	fowl	apot	abs	abs	abs	abs
Leptogium milligranum Sierk	Collemataceae	cyan	fowl	isid	abs	abs	abs	abs
Letrouitia domingensis (Pers.) Hafellner & Bellem.	Letrouitiaceae	chlo	catt	apot	abs	abs	othe	abs
Malmidea flavopustulosa (M. Cáceres & Lücking) M. Cáceres & Kalb	Malmideaceae	chlo	catt	apot	pre	abs	abs	abs
Malmidea vinosa (Eschw.) Kalb, Rivas Plata & Lumbsch	Malmideaceae	chlo	catt	apot	abs	abs	abs	abs
Myelochroa lindmanii (Lynge) Elix & Hale	Parmeliaceae	chlo	fowl	isid	pre	pre	seca	tha
N.I. 1		tren	catt	peri	na	na	abs	na
Ochrolechia africana Vain.	Ochrolechiaceae	chlo	catt	apot	pre	pre	abs	disc
Pannaria rubiginosa (Thunb. ex Ach.) Delise	Pannariaceae	cyan	fonl	apot	abs	abs	othe	tha
Parmelinopsis minarum (Vain.) Elix & Hale	Parmeliaceae	chlo	fonl	isid	pre	pre	abs	abs
Parmotrema aff. *herrei* (Zahlbr.) A.A. Spielm. & Marcelli	Parmeliaceae	chlo	fowl	othe	pre	pre	abs	abs
Parmotrema aff. *muelleri* (Vain.) O. Blanco	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema austrosinense (Zahlbr.) Hale	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema clavuliferum (Räsänen) Streimann	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema conferendum Hale	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema consors (Nyl.) Krog & Swinscow	Parmeliaceae	chlo	fowl	apot	pre	pre	abs	abs
Parmotrema homotomum (Nyl.) Hale	Parmeliaceae	chlo	fowl	apot	pre	pre	abs	abs
Parmotrema indicum Hale	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema internexum (Nyl.) Hale ex DePriest & B.W. Hale	Parmeliaceae	chlo	fowl	isid	pre	pre	abs	abs
Parmotrema muelleri (Vain.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema pilosum (Stizenb.) Krog & Swinscow	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema praesorediosum (Nyl.) Hale	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema pseudoreticulatum (Tav.) Hale	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema sancti-angeli (Lynge) Hale	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Parmotrema sp. 1	Parmeliaceae	chlo	fowl	apot	pre	pre	abs	abs
Parmotrema subcaperatum (Kremp.) Hale	Parmeliaceae	chlo	fowl	apot	pre	pre	abs	abs
Parmotrema tinctorum (Despr. ex Nyl.) Hale	Parmeliaceae	chlo	fowl	isid	pre	pre	abs	abs

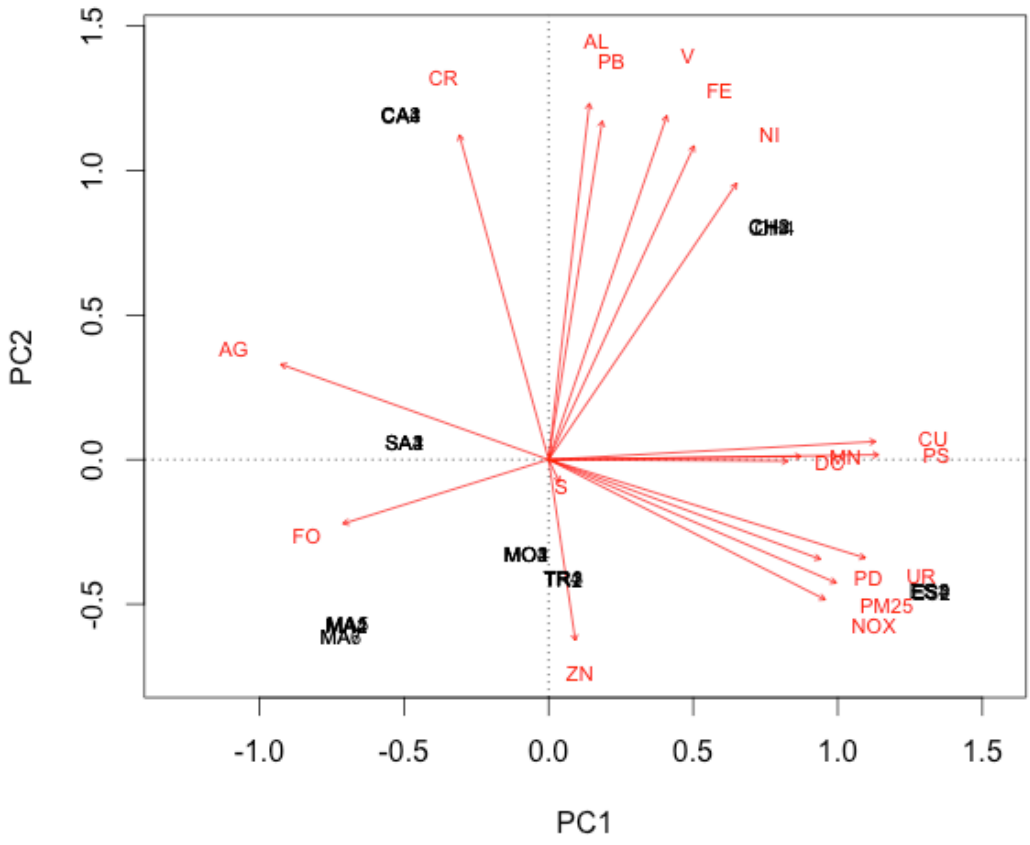
Pertusaria aff. *carneola* (Eschw.) Müll. Arg.	Pertusariaceae	chlo	catt	peri	na	na	abs	na
Pertusaria carneola (Eschw.) Müll. Arg.	Pertusariaceae	chlo	catt	peri	abs	abs	abs	na
Pertusaria flavens Nyl.	Pertusariaceae	chlo	catt	peri	abs	abs	othe	na
Pertusaria sp.1	Pertusariaceae	chlo	catt	peri	na	na	abs	na
Pertusaria sp.2	Pertusariaceae	chlo	catt	sore	na	na	abs	na
Pertusaria sp.3	Pertusariaceae	chlo	catt	peri	na	na	abs	na
Pertusaria sp.4	Pertusariaceae	chlo	catt	peri	na	na	abs	na
Pertusaria sp.5	Pertusariaceae	chlo	catt	sore	na	na	abs	na
Pertusaria sp.6	Pertusariaceae	chlo	catt	peri	na	na	abs	na
Pertusaria sp.7	Pertusariaceae	chlo	catt	peri	na	na	abs	na
Pertusaria sp.8	Pertusariaceae	chlo	catt	othe	na	na	abs	na
Phaeographis cf. *scalpturata* (Ach.) Staiger	Graphidaceae	tren	catt	lire	abs	abs	abs	disc
Phaeographis dendritica (Ach.) Müll. Arg.	Graphidaceae	tren	catt	lire	abs	abs	othe	disc
Phaeographis lecanographa (Nyl.) Staiger	Graphidaceae	tren	catt	lire	abs	abs	othe	disc
Phaeographis lobata (Eschw.) Müll. Arg.	Graphidaceae	tren	catt	lire	abs	abs	abs	disc
Phaeographis schizoloma (Müll. Arg.) Müll. Arg.	Graphidaceae	tren	catt	lire	abs	pre	abs	disc
Phyllopsora breviuscula (Nyl.) Müll. Arg.	Ramalinaceae	chlo	squa	apot	abs	abs	abs	abs
Phyllopsora buettneri (Müll. Arg.) Zahlbr.	Ramalinaceae	chlo	squa	frag	abs	pre	abs	tha
Phyllopsora confusa Swinscow & Krog	Ramalinaceae	chlo	squa	frag	abs	abs	abs	abs
Phyllopsora corallina (Eschw.) Müll. Arg.	Ramalinaceae	chlo	squa	isid	abs	abs	abs	abs
Phyllopsora furfuracea Zahlbr.	Ramalinaceae	chlo	squa	isid	abs	abs	othe	abs
Physcia aff. *stellaris* (L.) Nyl.	Physciaceae	chlo	fonl	apot	na	na	abs	all
Physcia aipolia (Ehrh. ex Humb.) Fürnr.	Physciaceae	chlo	fonl	apot	pre	pre	abs	all
Physcia alba (Fée) Müll. Arg.	Physciaceae	chlo	fonl	apot	abs	abs	abs	disc
Physcia atrostriata Moberg	Physciaceae	chlo	fonl	sore	pre	pre	abs	tha
Physcia crispa Nyl.	Physciaceae	chlo	fonl	sore	pre	pre	abs	tha
Physcia erumpens Moberg	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Physcia krogiae Moberg	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Physcia poncinsii Hue	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Physcia sinuosa Moberg	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha

Physcia sorediosa (Vain.) Lynge	Physciaceae	chlo	fonl	sore	na	na	terp	tha
Physcia sp. 1	Physciaceae	chlo	fonl	sore	na	na	abs	na
Physcia tenuis Moberg	Physciaceae	chlo	fonl	sore	na	na	terp	abs
Physcia tribacia (Ach.) Nyl.	Physciaceae	chlo	fonl	sore	na	na	terp	tha
Physcia tribacoides Nyl.	Physciaceae	chlo	fonl	sore	na	na	terp	abs
Physcia undulata Moberg	Physciaceae	chlo	fonl	sore	na	na	terp	tha
Platygramme caesiopruinosa (Fée) Fée	Graphidaceae	tren	catt	lire	abs	abs	abs	disc
Platygramme reticulata Fée	Graphidaceae	tren	catt	lire	abs	abs	abs	disc
Porina africana Müll. Arg.	Porinaceae	tren	catt	peri	abs	abs	abs	abs
Porina atlantica (Erichsen) P. M. Jørg.	Porinaceae	tren	catt	peri	abs	abs	abs	abs
Porina conspersa Malme	Porinaceae	tren	catt	isid	abs	abs	abs	abs
Porina mastoidea Fée	Porinaceae	tren	catt	peri	na	na	abs	abs
Porina sp. 1	Porinaceae	tren	catt	peri	na	na	abs	na
Porina sp. 2	Porinaceae	tren	catt	peri	na	na	abs	na
Porina tetracerae (Ach.) Müll. Arg.	Porinaceae	tren	catt	peri	abs	abs	abs	abs
Protoparmelia capitata Lendemer	Parmeliaceae	chlo	catt	sore	abs	abs	othe	abs
Punctelia canaliculata (Lynge) Krog	Parmeliaceae	chlo	fowl	apot	pre	pre	abs	abs
Punctelia constantimontium Sérus.	Parmeliaceae	chlo	fowl	apot	pre	pre	abs	abs
Punctelia crispa Marcelli, Jungbluth & Elix	Parmeliaceae	chlo	fowl	frag	pre	pre	abs	abs
Punctelia hypoleucites (Nyl.) Krog	Parmeliaceae	chlo	fowl	othe	pre	pre	abs	abs
Punctelia nebulata Elix & J. Johnst.	Parmeliaceae	chlo	fowl	apot	pre	pre	abs	tha
Punctelia neutralis (Hale) Krog	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Punctelia osorioi Canêz & Marcelli 2010	Parmeliaceae	chlo	fowl	frag	pre	pre	abs	abs
Punctelia sp .2	Parmeliaceae	chlo	fowl	frag	pre	pre	abs	abs
Punctelia sp. 1	Parmeliaceae	chlo	fowl	sore	na	na	abs	abs
Punctelia subrudecta (Nyl.) Krog	Parmeliaceae	chlo	fowl	sore	pre	pre	abs	abs
Pyrenula aff. *pyrenuloides* (Mont.) R.C. Harris	Pyrenulaceae	tren	catt	peri	abs	abs	abs	abs
Pyrenula microcarpa Müll. Arg.	Pyrenulaceae	tren	catt	peri	abs	abs	abs	abs
Pyrenula mucosa (Vain.) R.C. Harris	Pyrenulaceae	tren	catt	peri	abs	abs	abs	abs
Pyrenula neosandwicensis Aptroot	Pyrenulaceae	tren	catt	peri	abs	abs	abs	abs

Pyxine berteriana (Fée) Imshaug	Physciaceae	chlo	fonl	apot	pre	pre	terp	tha
Pyxine cocoës (Sw.) Nyl.	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Pyxine coralligera Malme	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Pyxine petricola Nyl.	Physciaceae	chlo	fonl	apot	pre	pre	terp	tha
Pyxine sp.1	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Pyxine subcinerea Stirt.	Physciaceae	chlo	fonl	sore	pre	pre	terp	tha
Ramalina aff. *puiggarii* Müll. Arg.	Ramalinaceae	chlo	frut	apot	pre	pre	abs	abs
Ramalina aspera Räsänen	Ramalinaceae	chlo	frut	apot	pre	pre	diva	abs
Ramalina celastri (Spreng.) Krog & Swinscow	Ramalinaceae	chlo	frut	apot	pre	pre	abs	abs
Ramalina peruviana Ach.	Ramalinaceae	chlo	frut	sore	pre	pre	abs	abs
Ramboldia haematites (Fée) Kalb	Lecanoraceae	chlo	catt	apot	pre	pre	nore	abs
Ramonia intermedia Kalb	Gyalectaceae	chlo	catt	apot	abs	abs	abs	abs
Ricasolia aff. *discolor* (Bory) Hue	Lobariaceae	chlo	fowl	apot	na	na	abs	abs
Ricasolia aff. *tenuis* Vain.	Lobariaceae	chlo	fowl	frag	na	na	abs	abs
Ricasolia sp.1	Lobariaceae	chlo	fowl	na	na	na	abs	abs
Teloschistes exilis (Michx.) Vain.	Teloschistaceae	chlo	frut	apot	pre	abs	abs	abs
Trypethelium nitidiusculum (Nyl.) R.C. Harris	Trypetheliaceae	tren	catt	peri	abs	abs	abs	abs
Trypethelium ochroleucum (Eschw.) Nyl.	Trypetheliaceae	tren	catt	peri	pre	abs	abs	abs
Usnea angulata Ach.	Parmeliaceae	chlo	frut	frag	pre	pre	nore	abs
Usnea ceratina Ach.	Parmeliaceae	chlo	frut	isid	pre	pre	seca	abs
Usnea perihispidella J. Steiner	Parmeliaceae	chlo	frut	isid	pre	pre	abs	na
Usnea sp. 2	Parmeliaceae	chlo	frut	frag	pre	pre	abs	na
Usnea sp. 3	Parmeliaceae	chlo	frut	sore	pre	pre	abs	na
Usnea sp.1	Parmeliaceae	chlo	frut	frag	pre	pre	abs	na
Usnea subelegans (Vain.) Motyka	Parmeliaceae	chlo	frut	frag	pre	pre	abs	na

Abbreviations: tren = *Trentepohlia*, chlo = chlorococcoid, cyan = cyanolichen, catt = crustose attached, cloo = crustose loose attached, fowl = foliose wide lobes, fonl = foliose narrow lobes, squa = squamulose, frut = fruticose, peri = perithecia, apot = apothecia, isid = isidia, sore = soredia, frag = fragmentation, lire = lirellae, pre = presence, abs = absence, pre = presence, terp = terpenes, seca = secalonic acid, nore = norestictic acid, diva = divaricatic acid, tha = thallus, othe = other, na = not available.

Appendix B. Diagram of ordination of a PCA (Principal Component Analysis) based on a matrix of sites (cities) described by 18 pollution variables (including pollutants and land cover variables). Sites are the black letters and the red ones are the variables.



Abbreviations: CA = Caraá, CH = Charqueadas, TR = Triunfo, MO = Montenegro, MA = Maquiné, SA = Santo Antônio da Patrulha, CR = Chromium, AL = Aluminum, PB = Lead, V = Vanadium, FE = Iron, NI = Nickel, S = Sulphur, MN = Manganese, CU = Copper, ZN = Zinc, NOX = modeled NOx average; PM25 = modeled PM2.5 average; DC = Distance from the coast; PS = number of pollution sources; UR = Urban cover, FO = Forest cover, AG = Agriculture cover, PD = population density.

Appendix C. Community Weighted Mean values (CWMs) of each trait in each of the city sampled.

TRAITS	MA		CA		TR		SA		MO		CH		ES	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
chlo	**0.67**^{a}	0.24	**0.47**^{a}	0.26	0.08^{b}	0.06	**0.87**^{a}	0.10	**0.84**^{a}	0.10	**0.90**^{a}	0.06	**0.80**^{a}	0.31
cyan	**0.02**^{a}	0.02	**0.04**^{a}	0.03	0.00^{b}	0.00	**0.03**^{a}	0.03	0.00^{b}	0.00	0.00^{b}	0.00	0.00^{b}	0.00
tren	0.31^{b}	0.25	0.49^{b}	0.24	**0.92**^{a}	0.06	0.10^{b}	0.10	0.16^{b}	0.10	0.10^{b}	0.06	0.20^{b}	0.31
catt	0.32^{b}	0.29	0.22^{b}	0.15	**0.95**^{a}	0.07	0.16^{b}	0.15	0.18^{b}	0.13	0.21^{b}	0.19	0.21^{b}	0.30
cloo	0.04^{b}	0.02	**0.33**^{a}	0.14	0.05^{b}	0.07	0.00^{c}	0.00	0.00^{c}	0.00	0.00^{c}	0.00	0.01^{c}	0.01
fnlo	0.07^{b}	0.05	0.16^{ab}	0.13	0.00^{b}	0.00	**0.49**^{a}	0.16	0.23^{ab}	0.22	**0.59**^{a}	0.19	0.32^{ab}	0.19
fwlo	**0.51**^{a}	0.23	0.11^{b}	0.07	0.00^{b}	0.00	**0.34**^{a}	0.09	**0.56**^{a}	0.25	0.20^{ab}	0.10	0.46^{ab}	0.23
frut	**0.04**^{a}	0.03	0.00^{b}	0.00	0.00^{b}	0.00	0.01^{ab}	0.01	0.01^{ab}	0.01	0.00^{b}	0.00	0.00^{b}	0.01
squa	0.01	0.02	0.17	0.30	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
apot	0.06^{ab}	0.06	0.18^{ab}	0.17	0.09^{ab}	0.06	**0.20**^{a}	0.07	0.05^{b}	0.03	0.21^{ab}	0.21	0.04^{b}	0.03
frag	0.00^{b}	0.00	0.01^{b}	0.01	0.00^{b}	0.00	0.03^{b}	0.03	0.00^{b}	0.00	0.00^{b}	0.00	**0.16**^{a}	0.07
isid	0.22^{b}	0.06	**0.47**^{a}	0.11	0.00^{c}	0.00	0.14^{b}	0.05	0.08^{bc}	0.07	0.04^{c}	0.03	0.07^{c}	0.01
lire	0.27^{ab}	0.26	0.07^{b}	0.07	**0.65**^{a}	0.08	0.02^{b}	0.02	0.08^{b}	0.09	0.03^{b}	0.03	0.02^{b}	0.04
peri	0.01^{b}	0.01	0.07^{ab}	0.11	**0.22**^{a}	0.14	0.07^{ab}	0.08	0.07^{a}	0.03	0.07^{ab}	0.08	0.14^{ab}	0.25
sore	0.44^{abc}	0.34	0.21^{c}	0.17	0.03^{bc}	0.05	0.53^{ac}	0.13	**0.71**^{a}	0.09	0.65^{ac}	0.19	0.57^{abc}	0.28
pdis	0.14^{ab}	0.13	0.02^{b}	0.03	**0.39**^{a}	0.10	0.05^{b}	0.05	0.07^{b}	0.09	0.12^{ab}	0.20	0.04^{b}	0.03
ptha	**0.22**^{a}	0.07	**0.12**^{a}	0.08	0.00^{b}	0.00	**0.46**^{a}	0.19	**0.24**^{a}	0.22	**0.53**^{a}	0.23	**0.29**^{a}	0.12
uvpr	0.65^{ab}	0.25	0.27^{bc}	0.18	0.07^{c}	0.05	**0.68**^{a}	0.11	**0.78**^{a}	0.12	**0.62**^{a}	0.12	0.69^{ab}	0.28
mipr	0.65^{ab}	0.25	0.24^{bc}	0.17	0.13^{c}	0.07	**0.68**^{a}	0.11	**0.78**^{a}	0.11	**0.67**^{a}	0.11	0.69^{ab}	0.28
terp	0.06^{c}	0.03	0.09^{c}	0.07	0.04^{c}	0.06	0.37^{ab}	0.15	0.21^{abc}	0.21	**0.48**^{a}	0.09	0.20^{bc}	0.08

Abbreviations: SE = Standard Error; chlo = chlorococcoid; cyan = cyanolichen; tren = Trentepohlia; catt = crustose attached; cloo = crustose loose attached; fnlo = foliose narrow lobes; fwlo = foliose wide lobes; frut = fruticose; squa = squamulose; apot = apothecia; frag = thallus fragmentation; isid = isidia; lire = lirellae; peri = perithecia; sore = soredia; pdis = pruina on disc; ptha = pruina on thallus; uvpr = UV protection; mipr = microbiological protection; terp = terpenes. Different letters represent significant differences ($P<0.05$). In bold are highlighted the highest values of each trait.

Appendix D. Best models for each of the lichen functional trait evaluated. Models were based on Akaike's Information Criterion corrected for small sample size (AICc). Models within 2 units of ΔAICc are shown and the weights of each model.

Response variable	Model	AICc	Weight
Chlorococcoid (chlo)	chlo ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + BS	42.81	0.75
	chlo ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + BS + TBA	46.76	0.10
Cyanolichen (cyan)	cyan ~ NO_X + $PM_{2.5}$	73.86	0.10
	cyan ~ $PM_{2.5}$	75.31	0.05
	cyan ~ $PM_{2.5}$ + UR + PD	76.02	0.03
Trentepohlia (tren)	tren ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + BS	44.89	0.69
	tren ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + PD + BS	48.40	0.12
Crustose Attached (catt)	catt ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + BS	60.39	0.50
	catt ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + PD + BS	61.76	0.25
	catt ~ NO_X + $PM_{2.5}$ + Cu + FO + PD + BS	64.64	0.06
Crustose Loose Attached (cloo)	cloo ~ NO_X + $PM_{2.5}$ + UR + FO + PD	63.24	0.17
	cloo ~ Cu + UR + PD + TBA	65.31	0.06
Foliose Narrow Lobes (fnlo)	fnlo ~ NO_X + UR + FO	69.95	0.18
	fnlo ~ $PM_{2.5}$ + UR + FO	72.01	0.07
Foliose Wide Lobes (fwlo)	fwlo ~ Cu + UR + BS + TBA	49.70	0.19
	fwlo ~ Cu + UR + PD + BS + TBA	50.63	0.12
	fwlo ~ NO_X + Cu + UR + BS + TBA	51.28	0.09
	fwlo ~ Cu + UR + FO + PD + BS	51.39	0.08
	fwlo ~ Cu + UR + PD + BS	51.45	0.08
	fwlo ~ $PM_{2.5}$ + Cu + UR + BS + TBA	51.67	0.07
	fwlo ~ NO_X + $PM_{2.5}$ + Cu + UR + BS + TBA	52.28	0.05
Fruticose (frut)	frut ~ FO + BS	70.68	0.10
	frut ~ FO + BS + TBA	71.61	0.06
	frut ~ FO	72.07	0.05
	frut ~ $PM_{2.5}$ + FO + BS	72.76	0.04
Squamulose (squa)	squa ~ UR + TBA	80.26	0.11
	squa ~ UR + BS + TBA	80.71	0.09
Apothecia (apot)	apo ~ NO_X + FO	81.18	0.06
	apo ~ $PM_{2.5}$ + FO	81.64	0.05
	apo ~ NO_X	82.09	0.04
	apo ~ $PM_{2.5}$	82.63	0.03
	apo ~ NO_X + Cu	82.66	0.03
	apo ~ $PM_{2.5}$ + Cu	82.94	0.03
	apo ~ $PM_{2.5}$ + Cu + FO	82.95	0.03
	apo ~ NO_X + Cu + FO	82.97	0.03
	apo ~ NO_X + FO + TBA	83.04	0.02
	apo ~ NO_X + TBA	83.36	0.02
Thallus fragmentation (frag)	frag ~ PD	48.54	0.09
	frag ~ $PM_{2.5}$ + PD	48.93	0.08

	frag ~ NO_X + PD	49.42	0.06
	frag ~ Cu + PD	49.56	0.05
	frag ~ UR + PD	50.54	0.03
	frag ~ PD + TBA	50.60	0.03
Isidia (isid)	isid ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + PD	47.49	0.18
	isid ~ NO_X + $PM_{2.5}$ + Cu + UR + PD	48.22	0.12
	isid ~ NO_X + UR + FO + PD	49.30	0.07
	isid ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + TBA	49.33	0.07
	isid ~ NO_X + UR + PD + TBA	49.70	0.06
Lirellae (lire)	lire ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + PD + BS	49.56	0.76
	lire ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + PD + BS + TBA	54.07	0.08
Perithecia (peri)	peri ~ $PM_{2.5}$ + BS	76.54	0.10
	peri ~ NO_X + BS	76.98	0.08
	peri ~ Cu + BS	77.96	0.05
	peri ~ $PM_{2.5}$ + Cu + BS	78.53	0.04
	peri ~ NO_X + Cu + BS	78.54	0.04
	peri ~ UR + BS	78.57	0.04
Soredia (sore)	sore ~ NO_X + $PM_{2.5}$ + Cu + UR + BS	65.16	0.25
	sore ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + BS	66.15	0.15
	sore ~ Cu + UR + PD + BS	67.29	0.09
Pruina Disc (pdis)	prui_disc ~ NO_X + $PM_{2.5}$ + Cu + UR + PD	76.45	0.13
	prui_disc ~ NO_X + $PM_{2.5}$ + Cu + PD	77.45	0.08
	prui_disc ~ NO_X + $PM_{2.5}$ + Cu + FO + PD + BS	77.57	0.08
	prui_disc ~ NO_X + $PM_{2.5}$ + Cu + FO + PD	78.07	0.06
	prui_disc ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + BS	78.70	0.04
Pruina Thallus (ptha)	ptha ~ $PM_{2.5}$ + UR + PD	73.52	0.09
	ptha ~ NO_X + UR + PD	73.58	0.09
	ptha ~ NO_X + $PM_{2.5}$ + Cu + UR	74.10	0.07
	ptha ~ NO_X + UR + FO	74.39	0.06
	ptha ~ NO_X + UR	74.44	0.06
	ptha ~ NO_X + $PM_{2.5}$ + UR	75.35	0.04
	ptha ~ NO_X + UR + FO + PD	76.20	0.02
UV Protection (uvpr)	uvpr ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + BS + TBA	57.08	0.39
	uvpr ~ NO_X + $PM_{2.5}$ + Cu + UR + BS	58.57	0.18
	uvpr ~ NO_X + $PM_{2.5}$ + Cu + UR + BS + TBA	58.98	0.15
	uvpr ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + BS	60.73	0.06
Microbiological Protection (mipr)	mipr ~ NO_X + $PM_{2.5}$ + Cu + UR + BS	57.70	0.26
	mipr ~ NO_X + $PM_{2.5}$ + Cu + UR + BS + TBA	57.96	0.23
	mipr ~ NO_X + $PM_{2.5}$ + Cu + UR + PD + BS + TBA	59.36	0.11
	mipr ~ NO_X + $PM_{2.5}$ + Cu + UR + FO + BS	59.71	0.09
Terpenes (terp)	terp ~ NO_X + UR + FO + TBA	67.45	0.15
	terp ~ $PM_{2.5}$ + UR + FO + TBA	69.74	0.05

Abbreviations: Cu = Copper, UR = urban cover, FO = forest cover, PD = population density, BS = bark structure, TBA = total basal area.

ARTIGO 4

Este artigo foi submetido à revista "Phytotaxa" (F.I. 1.318 – Qualis Capes: B1)

New species of *Graphis* (Graphidaceae: Lichenized Ascomycota) from the Atlantic Forest, in southern Brazil

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ABSTRACT: (New species of *Graphis* [Graphidaceae: Lichenized Ascomycota] from the Atlantic Forest, in southern Brazil). In this paper we describe a new species of the lichen genus *Graphis*, named *Graphis suzanae* Koch & Feuerstein. This species is characterized mainly by lirellae with entire labia, disc partially exposed with orange pigment, thick lateral thalline margin, excipulum laterally carbonized, hymenium clear and ascospores transversely septate. *G. suzanae* is here described in detail and a picture is also presented. The chemistry of the species was assessed through TLC analysis. The results of this paper contribute to the knowledge of lichens as well as to the whole biodiversity of Atlantic Forests.

Key words: microlichens, Rio Grande do Sul, tropical rainforests.

Introduction

The genus *Graphis* Adans. is the largest in the lichen family *Graphidaceae*. It comprises more than 400 species (around 18% of the family richness), mostly with tropical to subtropical distribution and found mainly on tree trunks (Lücking 2009; Barcenas-Peña *et al.* 2014). According to Staiger (2002) and Lücking (2009), *Graphis* is characterized by crustose lichens with sessile to immersed lirellae, with well-developed labia, carbonized excipulum, mostly clear hymenium (not inspersed), hyaline, I+ violet-blue, and distoseptate ascospores with lens-shaped lumina. Lücking *et al.* (2009) published an important worldwide key to this genus, which helped new descriptions to be made, especially of tropical species, as we present here. However, even with new descriptions appearing every year, it is estimated that globally around 1,800 species of Graphidaceae might be undiscorved yet (Lücking *et al.* 2014).

In Brazil, recent studies have discovered many new species of *Graphis*, showing that in fact there is much to be studied in this country. In the north and northeastern regions of Brazil, publications of Cáceres (2007), Cáceres *et al.* (2012, 2014) have added eight new species to Brazilian diversity. In Lumbsch *et al.* (2011) more two species were described for the central region of the country. From southern Brazil, there are also few works about *Graphis*. For the state of Paraná, Dal-Forno (2009) reported 30 *Graphis* species, among these four were new (described in Dal-Forno & Eliasaro 2010), and some years later, Feuerstein & Eliasaro (2015) published five new *Graphis* species from the same state. In the state of Rio Grande do Sul, where the whole lichen diversity comprises more than 900 species (Spielmann 2006), only 38 species of *Graphis* are known (Käffer *et al.* 2014). Recently, Käffer et al. (2014) described a new species of *Graphis* for Rio Grande do Sul, but there are no complete studies on this genus for this state.

Tropical rain forests support a high number from all kind of species, besides a great quantity of endemism, and are one of the most endangered ecosystems in the world (Myers *et al.* 2000). The Atlantic forest used to occupy around 150 million of hectares but today it is reduced to less than 16% of the original cover (Ribeiro *et al.* 2009). However, this ecosystem is still considered the one with the highest biodiversity in Brazil, and so in constant need of conservation actions (Myers *et al.* 2000).

The objective of this paper is to describe a new species of *Graphis* (Graphidaceae: Lichenized Ascomycota) found in Atlantic forests from southern Brazil. The paper will contribute to the knowledge of this lichen genus as well as to the whole biodiversity of this important ecosystem.

Material and Methods

The samples of *Graphis suzanae* were found in two areas in the northeastern region of Rio Grande do Sul state (Figure 1), south of Brazil, where the climate is classified as subtropical humid (Cfa type) according to Köppen–Geiger classification (Peel *et al.* 2007). The holotype was collected in the city of Maquiné (25º32'02"S 48º18'51"W). This region is characterized by valley slopes ranging from 30 to 800 m of elevation (Becker *et al.* 2004) and the original vegetation is primarily composed of Atlantic rain forest remnants, with some small areas of Semideciduous Forests and Mixed Araucaria Forests (Sevegnani & Baptista 1996). It corresponds to the southern distribution limit of the Atlantic Rainforest, one of the most important and rich tropical forests in the world (Myers *et al.* 2000). The paratype was collected in Caraá, a city situated around 26 km far from where the holotype was collected. This city is in an ecotone of the same forests types than Maquiné, but with less prevalence of original Atlantic Forests. There are also many valley slopes and some old forests in the region.

All morphological characters were studied on dry specimens using a dissecting microscope. Free hand sections were made to analyze anatomical characteristics of the thalli and the ascoma and put in water to be observed under light microscopy. Color tests were also carried out both on the thalli and the ascoma with KOH (10%) and after subjected to TLC using solvent C (Culberson & Ammann 1979).

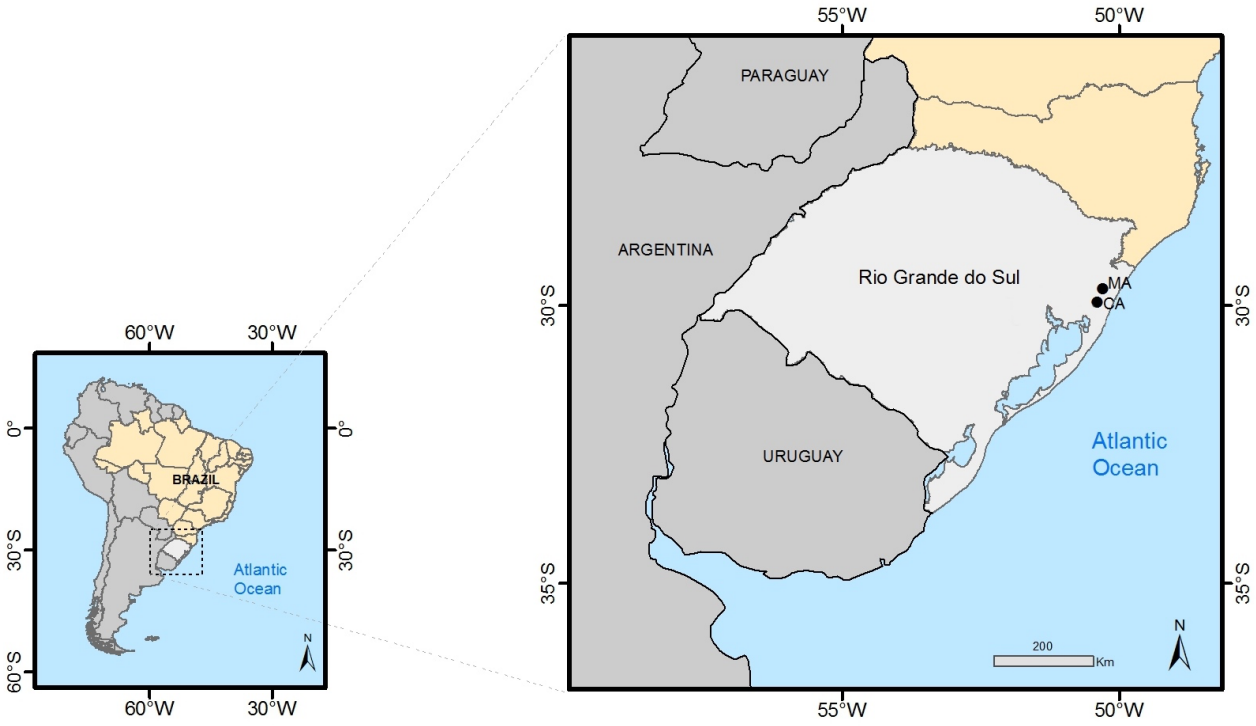

FIGURE 1 Location of the two sampled areas where the new species *Graphis suzanae* was found. Abbreviations: CA = Caraá; MA = Maquiné.

Taxonomic treatment

Graphis suzanae Koch & Feuerstein sp. nov. (Fig. 2)

MycoBank No.: X

Lirellae with entire labia, disc partially exposed with orange pigment, lateral thalline margin thick, excipulum laterally carbonized, hymenium clear, ascospores transversely septate, 40–44 µm long.

Type: BRAZIL. RIO GRANDE DO SUL: Maquiné, Forqueta locality; 308 m elev., 29º32'43.72"S, 50º11'54.77"W; on tree bark in secondary forest patch of Atlantic rainforest in initial successional stage; 26 July 2011, *NMKOCH 550* (HAS 90994).

Thallus crustose, continuous, soredia and isidia absent; surface grayish white, opaque, smooth to irregular; cortex present. Lirellae elongated, isolated, a little sinuous, simple to rarely branched, ends acute to rounded, erumpent to prominent, 0.4–1.9 mm long and 0.1–0.2 mm wide, black; disc partially exposed with orange pigment; thalline margin laterally thick; labia entire, convergent; excipulum laterally carbonized. Hymenium clear, 90–110 µm high, 100–110 µm wide, I–; epithecium orange, 15–23 µm; hypothecium hyaline, 20–25 µm; paraphyses simple, filiform, 1.0–1.5 µm thickness, hyaline with apex orange; periphysoids absent; ascospores hyaline, transversely septate, 7–9–locular, ellipsoid, I+ violet-blue, 40–44 × 8–10 µm, 8/ascus.

Chemistry. K+ purple; TLC: tetra-hydroxy-anthraquinone-1, 3, 6, 8.

Etymology. We dedicate this species to our professor and friend Dr. Suzana de Azevedo Martins, for her contributions to the knowledge of Brazilian lichens and for promoting several lichen studies in southern Brazil.

Discussion. Among *Graphis* species with orange pigmentation K+ purple restricted to the epithecium and/or the hymenium, only four are known: *Graphis chromothecia* R. C. Harris, *G. inversa* R. C. Harris, *G. hodgesiana* Lendemer and *G. tamiamiensis* Lendemer. However, *G. chromothecia* differs by the completely carbonized excipulum, inspersed hymenium and the production of norstictic acid (Lücking 2009; Lendemer 2010).

The other species, although similar regarding the laterally carbonized excipulum and the hymenium, which are also not inspersed, differ by the production of norstictic acid and the ascospores characteristics. *Graphis hodgesina* has muriform ascospores which are also bigger than those from *G. suzanae* (50–100 x 20–30µm), *G. tamiamiensis* has submuriform ascospores and smaller, not exceeding 35µm, and *G. inversa*, has even smaller ascospores (23–30 µm) and absent thalline margin (Lücking *et al.* 2009; Lendemer 2010).

So far, *G. suzanae* has been found only in two localities (Maquiné and Caraá), where it grows on bark in Atlantic Rainforest fragments in early stages of succession.

Specimens examined. BRAZIL. RIO GRANDE DO SUL: Caraá; 77 m elev., 29˚47'03.83"S, 50˚25'38.20"W; secondary forest patch of Atlantic rainforest and Semideciduous forest, 15 November 2014, *NMKOCH 551* (HAS 90995).

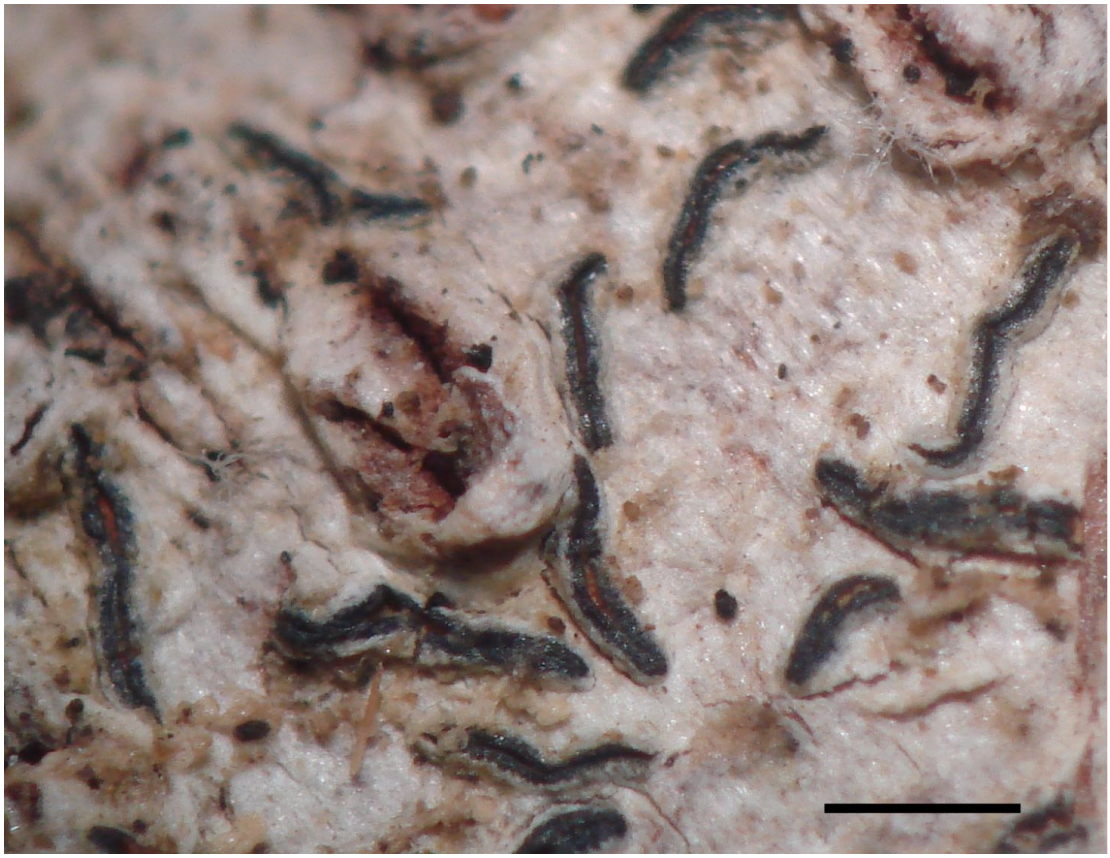

FIGURE 2. *Graphis suzanae*, holotype: overall aspect. Scale = 0.5 mm.

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CONSIDERAÇÕES FINAIS

A presente tese demonstra que as comunidades de liquens de áreas urbanas têm clara influência da poluição atmosférica e das mudanças na paisagem como estresse ambiental, tanto em nível estrutural quanto funcional. Demonstramos novas formas de se utilizar os liquens como bioindicadores e biomonitores de qualidade do ar, ampliando ainda mais a aplicação prática desses organismos, e finalizamos a tese com a descrição de uma nova espécie para a qual, até o momento, só se tem o registro em áreas pouco poluídas e pouco urbanizadas.

Optamos por iniciar a tese com uma abordagem mais local (Capítulo 1) discutindo a qualidade do ar em cada um dos sete municípios amostrados, contribuindo para a gestão destas áreas com informações relevantes em nível de saúde pública e ambiental. Ressaltamos também que a poluição atmosférica está amplamente distribuída e não se limita somente aos municípios densamente populosos e industrializados. Como demonstrado nos resultados desse capítulo, até mesmo locais aparentemente não poluídos, áreas predominantemente rurais e com pequenas propriedades, podem ser afetadas por fontes externas, ou internas pouco pronunciadas, de alguns poluentes. Os dados obtidos com esse estudo podem ser, portanto, de grande importância para tomadores de decisão sobre, por exemplo, onde reforçar atenção à saúde ou onde a qualidade do ar já está muito saturada para a instalação de novas fontes de poluição.

Seguindo a linha e o objetivo geral da tese, no segundo capítulo testamos o uso de diferentes métricas, com base na estrutura das comunidades de liquens e na vitalidade de uma espécie biomonitora, como indicadores de poluição atmosférica. A partir dos dados avaliados nesse capítulo é possível afirmar que os liquens de áreas subtropicais impactadas estão mais relacionados à poluição e às mudanças da paisagem, como resultado de urbanização, do que a

outras características locais. Esta relação pode ser percebida na riqueza, cobertura e composição de espécies, e também na vitalidade dos liquens. Material particulado fino ($PM_{2.5}$), campos abertos e o número de fontes poluidoras impactaram todos esses parâmetros. Óxidos de nitrogênio (NO_X), cobre (Cu), manganês (Mn), percentual de cobertura florestal e urbana foram também importantes variáveis influenciando mudanças nas comunidade de liquens. Nesse capítulo, indicamos que a vitalidade dos liquens e a composição de espécies seriam os melhores indicadores para avaliar e integrar os efeitos de múltiplos distúrbios ambientais neste tipo de áreas urbanas com influência industrial nos subtrópicos.

No terceiro capítulo, no qual testamos os efeitos da poluição como fator de estresse ambiental afetando os padrões funcionais das comunidades de liquens, demonstramos que alguns atributos funcionais testados podem ser bons indicadores da qualidade do ar. O uso de uma abordagem funcional provou ser, portanto, efetiva como indicadora de poluição atmosférica nos subtrópicos pela primeira vez, além de trazer resultados inéditos em nível mundial quanto à resposta dos atributos funcionais de liquens.

O tipo de alga do líquen, o tipo de crescimento, a estratégia reprodutiva e a presença de proteção física e química no talo mostraram ser boas opções para se entender os efeitos do estresse nas comunidades epifíticas em ambientes urbanos. Em áreas com maior estresse ambiental, observamos a diminuição de liquens com cianofícea como alga principal, com talos crostosos pouco aderidos e liquens com isídios como a principal forma de reprodução. Ao mesmo tempo, a urbanização e a poluição aumentaram a frequência de liquens foliosos com lobos estreitos, de liquens com pruína e contendo terpenos. Além disso, a riqueza funcional das comunidades de liquens demonstrou ser também influenciada pela poluição atmosférica e pelas mudanças na paisagem.

Para encerrar a tese, no último e quarto capítulo fechamos com a descrição de uma nova espécie do gênero *Graphis* (*Graphis suzanae* Koch & Feuerstein), cujo nome foi dado

em homenagem à Dra. Suzana de Azevedo Martins, co-orientadora desta tese e renomada liquenóloga brasileira. O resultado deste capítulo contribui não só para o conhecimento da diversidade de liquens, mas também para o conhecimento da biodiversidade da Mata Atlântica, uma vez que essa espécie parece estar relacionada com esse tipo de vegetação. Além disso, a descoberta de uma nova espécie reforça a importância da manutenção de áreas poucos urbanizadas, pois essas áreas podem conter uma diversidade nem mesmo conhecida.

Como mensagem final, salientamos que os liquens podem e devem ser mais amplamente usados como biomonitores e biondicadores de mudanças ambientais e de poluição atmosférica, pois consistem em uma ferramenta sensível e acessível de monitoramento. E sugerimos ainda que mais trabalhos testem a relação funcional das comunidades de liquens com o estresse ambiental, para que o uso dessa abordagem se torne ainda mais eficiente.

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