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**Tese de Doutorado**

# BIOMONITORAMENTO DA QUALIDADE DO AR COM USO DE LIQUENS NA CIDADE DE PORTO ALEGRE, RS

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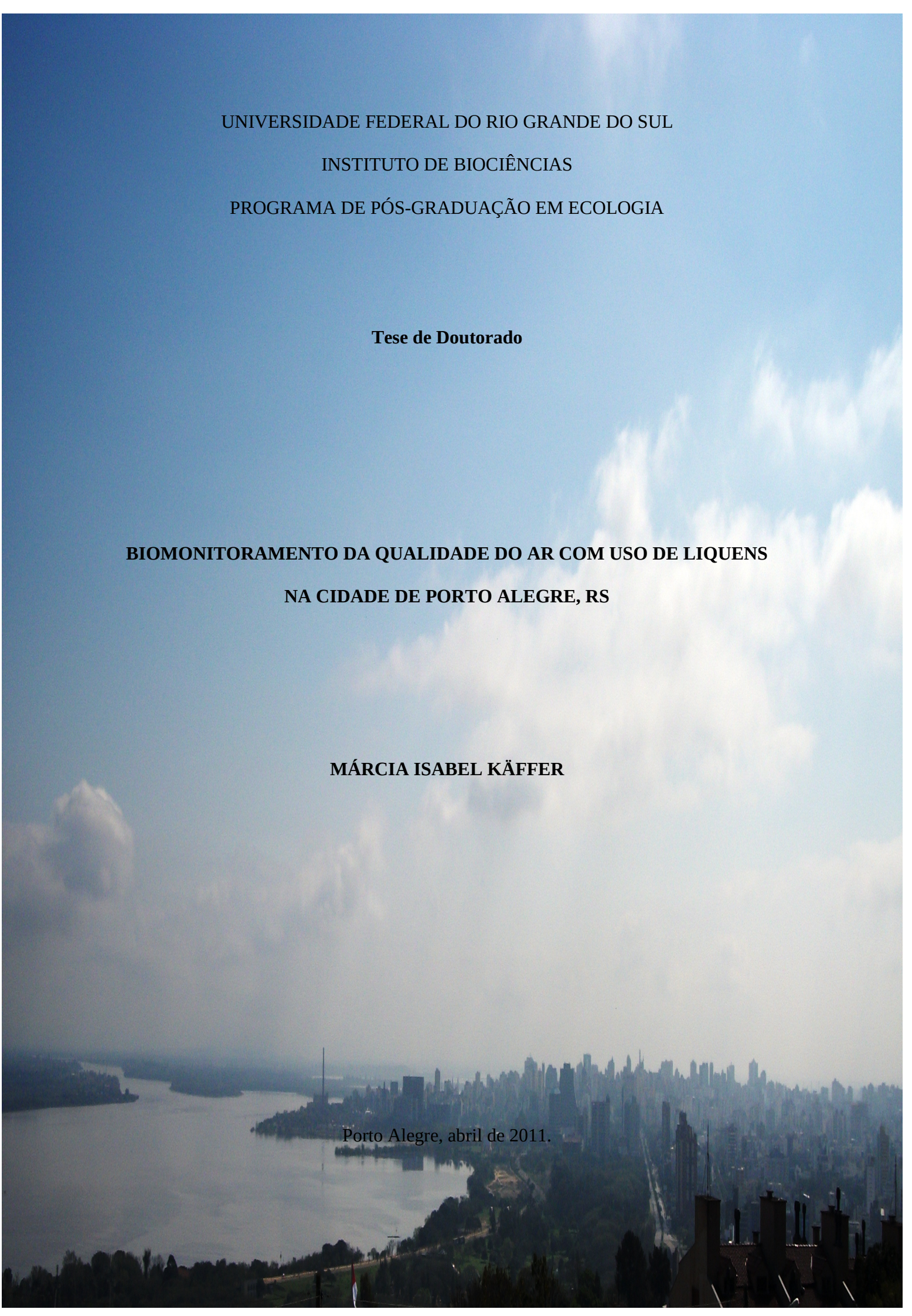

Porto Alegre, abril de 2011.

# BIOMONITORAMENTO DA QUALIDADE DO AR COM USO DE LIQUENS NA CIDADE DE PORTO ALEGRE, RS

**Márcia Isabel Käffer**

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Doutora em Ciências com ênfase em Ecologia.

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“*Sucesso se alcança convertendo cada passo em uma meta*”

Arie Dov Bem Abraham

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**RESUMO**

Liquens são organismos em simbiose, considerados indicadores biológicos e, assim como os biomarcadores de genotoxicidade, são amplamente empregados para avaliar a qualidade do ar em áreas urbanas. Desta forma, este estudo teve por objetivos analisar a comunidade liquênica na cidade de Porto Alegre, RS, através do mapeamento da micota liquenizada e comparar a influência de determinados poluentes nas estruturas morfofisiológicas de algumas espécies liquênicas e na atividade mutagênica e citotóxica do material particulado atmosférico. A comunidade liquênica foi analisada, a fim de verificar alterações na sua estrutura, e danos morfofisiológicos em duas espécies (*Parmotrema tinctorum* e *Teloschistes exilis*), pela ação de determinados poluentes. Foram analisadas 30 estações amostrais, distribuídas em 29 áreas da cidade, e uma de referência no Parque Estadual de Itapuã, RS. Foram empregados o método do elástico para mapear os liquens, o método dos transplantes para analisar a ação de alguns poluentes atmosféricos e o ensaio *Salmonella*/microssoma para avaliar a mutagenicidade e citotoxicidade dos extratos orgânicos do PM10. Foram registrados 144 táxons liquênicos. Alterações na estrutura da comunidade liquênica foram verificadas nas áreas analisadas. Concentrações de poluentes, incluindo HPAs, foram constatadas nas espécies expostas, além de danos morfofisiológicos. Atividade mutagênica também foi verificada nos primeiros meses de exposição dos liquens, bem como a presença de nitrocompostos no ar. Na avaliação geral da comunidade liquênica urbana, constatou-se que fatores, como tráfego veicular, variáveis climáticas e topografia das estações amostradas, podem ter contribuído para os resultados encontrados. A utilização conjunta entre os bioindicadores e os biomarcadoress de mutagênese proporcionou a avaliação da qualidade do ar e o diagnóstico da presença de compostos agressivos ao meio ambiente. O emprego dos liquens como indicadores de alterações em ambientes urbanos são recomendados, podendo servir como ferramenta para programas de monitoramento nas cidades.

Palavras chave: Distribuição vertical, Fator de Classificação Ambiental, Fungos liquenizados, HPAs, Poluição atmosférica, Teste de Ames

**ABSTRACT**

Lichens are symbiotic organisms considered as biological indicators and like genotoxicity biomarkers, are widely used to evaluate air quality in urban areas. Thus, the present study aims to analyze the lichen community in Porto Alegre, RS, by mapping lichenized mycota and also aims to compare the influence of certain pollutants morphophysiological structures of some lichen species and in the mutagenic and cytototoxicity activity in atmospheric particulate matter. The lichen community was analyzed in order to verify structure alterations, in addition to morphophysiological damages in two species (*Parmotrema tinctorum* and *Teloschistes exilis*) caused by the action of specific pollutants. Thirty sample stations were analyzed, distributed into twenty-nine areas in the city and a reference area in the State Park of Itapuã, RS. The band rubber method was used for mapping lichens, the transplant methodology was used in order to analyze the action of some atmospheric pollutants and the *Salmonella*/microsome assay was used to evaluate mutagenicity and cytotoxicity of PM10 organic extracts. One hundred and forty-four lichen taxa were registered. Alterations in the structure of the lichen community were verified in the analyzed areas. Pollutant concentrations, including PAHs were observed in the exposed species, in addition to morphophysiological damages. Mutagenic activity was also verified in the first months of lichen exposure, as well as the presence of nitrocompounds in the air. A general evaluation of the urban lichen community verified that traffic flow, climate variables and topography of the sampled stations may have contributed to the results obtained. The use of both bioindicators and mutagenesis biomarkers provided an evaluation of air quality and helped to determine of the presence of environmental-aggressive compounds. The use of lichens as indicators of alterations in urban environments is recommended and may serve as a tool for monitoring programs in cities.

Keywords: Air pollution, Environmental Classification Factor, lichenized fungi, PHAs, phytosociology, *Salmonella*/microssome assay, Vertical distribution

## LISTA DE ABREVIAÇÕES

**Português/Inglês**

CONAMA – Conselho Nacional do Meio Ambiente

CO – Monóxido de Carbono

DAP/DBH – Diâmetro a Altura do Peito

EPTC – Companhia de Transporte e Circulação

FEPAM – Fundação Estadual de Proteção Ambiental

FCA/ECF – Fator de Classificação Ambiental

HAS – Herbário Alarich Schultz

HC – Hidrocarbonetos

IPA/IAP – Índice de Pureza Atmosférica

I – Lugol/ Lugol's solution

KOH – Hidróxido de Potássio/ Potassium hydroxide

MP – Material Particulado/ Particulate Matter

MP10/PM10 – Partículas menores ou iguais a 10 m/ Particulate Matter up to 10 m

$NO_x$ – Óxidos de Nitrogênio

PAN – Nitrato de Peroxiacetila

PAHs/HPAs – Hidrocarbonetos Policíclicos Aromáticos

P – Parafenilenodiamina/ Paraphenylenediamine

S9 – Fração de metabolização

TCL – Thin Layer Chromatography

USEPA – US Environmental Protection Agency

UV – Ultra-violeta/ Ultraviolet light lamp

VI – Valor de Importância

CO – Organic Carbon

CMGc – Morphological lichen groups crustose

CMGfo - Morphological lichen groups foliose, micro-foliose, squamulose

CMGfr - Morphological lichen groups fruticose

NMS – Non-metric Multidimensional Scaling

IV – Indication Value

WHO – World Health Organization

IVF – Index of Photobiont Vitality

Chl_a – Chlorophyll *a*

Chl_b – Chlorophyll *b*

EOM – Extracted Organic Matter

Q – Environmental Index

LV – Light vehicle

MV – Medium vehicle

HV – Heavy vehicle

MT – Motorbikes

# SUMÁRIO

## LISTA DE FIGURAS

**Artigo 2. Corticolous lichens as environmental indicators in urban areas in southern Brazil**



**Artigo 3. Evaluation of lichen community in urban area, southern Brazil, in relation to different environmental factors**

**Artigo 4. Caracterização da comunidade liquênica corticícola de Porto Alegre e áreas adjacentes, RS, Brasil**

Figura 1. Mapa da cidade de Porto Alegre, RS, Brasil com representação das estações amostradas.

**Artigo 5. Use of lichens and genotoxicity bioindicators for the evaluation of air quality in an urban environment, southern Brazil**

Figure 1. Map of Porto Alegre, RS, Brazil and representation of sampled stations. Caption: ESC - Santa Cecília (2), EJB - Jardim Botânico (4), EA – Anchieta (13).

Figure 2. Mutagenic activity in samples of air at stations EJB and EA with TA98 strain with (+S9) and without (-S9) hepatic fraction metabolism. Values expressed in rev/$m^3$. Caption: EJB - Jardim Botânico, EA – Anchieta.

Figure 3. Mutagenicity (rev/µg) in samples of air observed at stations EJB and EA, expressed in the nitrocompound strains. Caption: EJB - Jardim Botânico, EA – Anchieta.

# LISTA DE TABELAS

Table 3. Values for the Index of Atmospheric Purity (IAP), richness, cover, diversity and percentage of morphological groups, at sampling stations in Porto Alegre and in the reference area, Brazil. Caption: Cr = crustose, Esq = squamulose, Fo = foliose, Fr = fruticose, Microf = microfoliose; E1-E30 defined in Table 2.

Table 4. Values for the Environmental Classification Factor (ECF), richness, cover, diversity and percentage of morphological groups, at sampling stations in Porto Alegre and in the reference area, Brazil. Caption: Cr = crustose, Esq = squamulose, Fo = foliose, Fr = fruticose, Microf = microfoliose; E1-E30 defined in Table 2.

**Artigo 3. Evaluation of lichen community in urban area, southern Brazil, in relation to different environmental factors**

Table 1. List of acidophytic, nitrophytic and indifferent lichen registered at the sampled stations, Porto Alegre, Brazil. Caption: cr = crustose, squa = squamulose, fo = foliose, fr= fruticose, microf = microfoliose.

Table 2. ANOVA table for the relationship between lichen richness and trunk height. Variation sources represent Height (fixation height of lichen on the trunk); Stations (30 sampled stations). Caption: df – degrees of freedom, SS = Sum-of-Squares, MS = Mean-Square, F = ration, P = Probabilities.

Table 3. Correlation analysis of richness of acidophytic, nitrophytic and indifferent lichen taxa, vertical distribution, fragmentation and average size of lichen thallus, surface bark pH and environmental variables. Caption: UR = relative humidity of air

**Artigo 4. Caracterização da comunidade liquênica corticícola de Porto Alegre e áreas adjacentes, RS, Brasil**

Tabela 1. Dados fitossociológicos dos táxons liquênicos corticícolas nas áreas amostradas na cidade de Porto Alegre e nas áreas adjacentes, RS, Brasil. Legenda: FA: freqüência absoluta, FR: freqüência relativa; CA: cobertura absoluta; CR: cobertura relativa; VI: valor de importância Legenda: Novos registros para a ciência (**), novas citações para o RS (*).

**Artigo 5. Use of lichens and genotoxicity bioindicators for the evaluation of air quality in an urban environment, southern Brazil**

Table 1. Concentration of pollutants absorbed by *Parmotrema tinctorum* and *Teloschistes exilis* thalli at the three analyzed stations in different exposure periods. Caption: ESC - Santa Cecília, EJB - Jardim Botânico, EA – Anchieta.

Table 2. Morphophysiological analyses of *Parmotrema tinctorum* and *Teloschistes exilis* thalli at the three analyzed stations in different exposure periods. Caption: CO = Organic Carbon, Chl = chlorophyll, IVF = Index of Photobiont Vitality. Caption: ESC - Santa Cecília, EJB - Jardim Botânico, EA – Anchieta, CO = Organic Carbon, Chl = chlorophyll, IVF = Index of Photobiont Vitality.

Table 3. PAHs profile in thalli of lichen samples at the three sampled stations. Caption: ESC – Santa Cecília, EJB - Jardim Botânico, EA – Anchieta.

Table 4. Concentrations of PM10 and $O_3$ at EJB and EA stations in the evaluated period. Caption: EJB - Jardim Botânico, EA – Anchieta.

## 1. INTRODUÇÃO GERAL

A poluição do ar no ambiente urbano é um problema existente nos últimos séculos ocasionada principalmente, por queima de florestas, combustíveis fósseis e descargas industriais (Jacobson, 2000). O acréscimo no número de fontes poluidoras, tanto fixas quanto móveis, especialmente nos grandes centros urbanos, aumentou consideravelmente a concentração de poluentes atmosféricos.

Os poluentes da atmosfera podem ser classificados em primários e secundários. Os primeiros são lançados no ar, diretamente da fonte, sendo responsáveis por mais de 98% da poluição nos principais centros urbanos. Destacam-se: monóxido de carbono (CO), óxidos de enxofre ($SO_x$), hidrocarbonetos (HC), material particulado (MP) e óxidos de nitrogênio ($NO_x$). Os poluentes secundários são formados na atmosfera através da reação química entre poluentes primários e componentes naturais da atmosfera, como ozônio ($O_3$), ácido fluorídrico, nitrato de peroxiacetila (PAN), entre outros (Oliveira & Kummrow, 2008). Além destes, os metais pesados e os hidrocarbonetos policíclicos aromáticos (HPAs) também estão presentes na atmosfera urbana e podem adsorver-se ao material particulado.

Devido ao incremento desses poluentes no ar, faz-se necessário cada vez mais buscar medidas de diagnóstico e controle, principalmente nas grandes cidades. O emprego de bioindicadores como método de detecção de alterações ambientais é uma ferramenta eficaz e relativamente rápida, pois apresenta menores custos, e, quando associado aos sistemas mecânicos, pode fornecer informações de grande valor (Oliveira & Kummrow, 2008).

De acordo com Arndt & Schweizer (1991), o biomonitoramento pode ser realizado através de organismos indicadores, teste e monitores. Os organismos indicadores são definidos como indivíduos ou comunidades que podem fornecer informações sobre as condições de um ecossistema. Organismos-teste são usados em ensaios toxicológicos de laboratório. Normalmente são altamente padronizados e detectam riscos imediatos aos seres vivos. Os

monitores incluem todos os organismos vivos que são empregados para os monitoramentos qualitativo e quantitativo dos níveis de poluição no ambiente e de seus efeitos.

O biomonitoramento pode também ser realizado empregando bioensaios que representam uma ferramenta importante na avaliação do risco dos poluentes para o meio ambiente. Desta maneira, a utilização de biomarcadores para avaliação do potencial efeito tóxico de compostos químicos vem se intensificando. Os biomarcadores são definidos como uma resposta biológica a um ou vários compostos químicos que fornecem informações sobre exposição e, em alguns casos, efeitos tóxicos em nível de organismo (Guaratini et al., 2008). Entre estes, há os que permitem definir a presença de substâncias que alteram o material genético com efeitos mutagênicos e/ou potencialidade carcinogênica.

Entre os bioindicadores, as plantas e microrganismos são sensíveis suficientemente para reagir às emissões que influenciem o metabolismo, os processos fisiológicos e as modificações morfológicas. Quanto ao biomonitoramento, este pode ser classificado em dois tipos: ativo e passivo. O ativo consiste em transplante ou transferência de material biológico, previamente padronizado, de área sem influência de poluentes provenientes de zonas urbanas ou industriais para área a ser monitorada. O monitoramento passivo constata os danos nos bioindicadores em campo, por exemplo, através de observação e análise da vegetação no local, relacionando estes com as condições do ambiente. No monitoramento passivo, a comunidade liquênica pode ser avaliada através de informações qualitativas obtidas por meio de listas de espécies e mapas de distribuição da comunidade, ou de métodos quantitativos, utilizando cálculos de diferentes índices para estimar a qualidade do ar. Entre estes, está o Índice de Pureza Atmosférica (IPA) desenvolvido por Le Blanc & De Sloover (1970) que é baseado na sensibilidade dos liquens. Diferentes versões têm sido propostas para este índice (Herzig et al., 1989; Asta & Rolley, 1999), sendo abordado por muitos pesquisadores (Estrabou, 1998; Calvelo & Liberatore, 2004; Gombert et al., 2004; Calvelo et al., 2009; McCarthy et al., 2009). Segundo

Hawksworth & Rose (1970) este método tem sido, entre os numéricos, o mais utilizado e seus resultados são os que revelam maior correlação entre poluição, urbanização e industrialização.

Entre os organismos, os liquens têm sido reconhecidos como bioindicadores da qualidade do ar, desde o início do século IXI (Hawksworth et al., 2005). São empregados em programas de biomonitoramento (Geebelen & Hoffmann, 2001; Van Herk et al., 2002; Nimis & Purvis, 2002; Brunialti & Giordani, 2003; Mikhailova, 2007) e, inclusive, recomendados como bioindicadores em protocolos governamentais de diversos países (McCune, 2000), seja para avaliar determinadas espécies em relação aos poluentes (Gonzáles & Pignata, 1997, 2000; Minganti et al., 2003; Saiki et al., 2003; Fuga et al., 2008; Conti et al., 2009; Daillant et al., 2009) ou para verificar alterações na estrutura da micobiota liquenizada em função de contaminantes atmosféricos (Hawksworth, 1973; Scutari & Theinhardt, 2001; Estrabou et al., 2004; Gombert et al., 2006; Giordani, 2007; Saipunkaew et al., 2007; Calvelo et al., 2009).

Os liquens são organismos simbiontes constituídos pela associação de um fungo (micobionte) e uma alga (fotobionte), resultando num talo (Hawksworth & Hill, 1984). Variam em sua complexidade, desde formas muito simples até estruturas morfológicas e anatômicas muito complexas. São separados em formas ou tipos morfológicos: crostosos, esquamulosos, foliosos, microfoliosos e fruticosos. Os crostosos se caracterizam pela forma aderida ao substrato, podendo o talo estar totalmente imerso no substrato. Nos tipos esquamulosos, o talo é composto por pequenas escamas que crescem agregadas (formando manchas) ou espalhadas nas fendas das cascas das árvores. Os foliosos e microfoliosos possuem estrutura laminar e dorsiventral (possuem lado de baixo e de cima) e normalmente aderem-se ao substrato por muitos pontos de seu lado inferior. Os fruticosos possuem talo cilíndrico ou achatado, muitas vezes ramificado, que cresce pendente de rochas, troncos ou galhos de árvores. Os liquens ocorrem em vários substratos podendo se fixar em troncos e ramificações de árvores (corticícolas), rochas (saxícolas), solos (terrícolas) folhas (folícolas) e,

praticamente, em qualquer tipo de substrato que se encontre estável por algum tempo (Hale, 1983).

A grande sensibilidade dos liquens está estreitamente relacionada com sua biologia. A alteração do balanço simbiótico entre o fotobionte e o micobionte pode ser evidenciada com rapidez, através da ruptura desta associação. Anatomicamente, os liquens não possuem estômatos nem cutícula, o que significa que gases e aerossóis podem ser absorvidos pelo talo e difundir-se rapidamente pelo tecido onde está o fotobionte. A ausência destas estruturas tampouco permite excretar as substâncias tóxicas, ou selecionar as que são absorvidas (Valencia & Ceballos, 2002). Dentre os efeitos que os poluentes podem ocasionar nos liquens, estão: inibição de crescimento e desenvolvimento do talo, alterações nos processos metabólicos e mudanças anatômicas e morfofisiológicas (Barkman, 1958; Baddeley et al., 1973; Coppins, 1973; Gries, 1996; Schlensog & Schroeter, 2001). O fotobionte é o primeiro a ser afetado pela poluição, ocorrendo desenvolvimento das anormalidades no talo, branqueamento da clorofila e desenvolvimento de áreas pardas nos cloroplastos. A clorofila degrada-se em feofitina pela ação de soluções de dióxido de enxofre, ainda que em baixas concentrações (Barkman, 1958; Bargagli & Mikhailova, 2002).

O monitoramento da qualidade do ar pode ser realizado através de bioensaios. Entre estes, o ensaio *Salmonella*/microssoma ou Teste de Ames tem sido empregado como biomarcador precoce para a presença de substâncias carcinogênicas e genotóxicas, também em ambientes urbanos (Hughes et al., 1980; Claxton, 1983; Vargas et al., 1998; Monarca et al., 2001; Ducati & Vargas, 2003; Claxton & Woodall, 2007; FEPAM, 2008; Apel et al., 2010). O princípio do teste emprega diferentes tipos de linhagens da bactéria *Salmonella typhimurium*, modificadas geneticamente e deficientes na síntese do aminoácido histidina. Estas apresentam mutações do tipo deslocamento do quadro de leitura ou substituição de pares de base do DNA. Essas linhagens são incapazes de crescer em meio de cultura mínimo sem histidina, a menos que

ocorram mutações que restaurem a sua capacidade de síntese. As linhagens comumente empregadas para avaliar a qualidade do ar são: TA98, em ausência e presença do sistema de metabolização P450 de mamíferos *in vitro* (S9 mix) (Umbuzeiro & Vargas, 2003) e as linhagens YG1021 e YG1024 que apresentam alta atividade de enzimas específicas, permitindo maior sensibilidade para nitrocompostos - como nitroarenos (YG1021) e dinitroarenos (YG1024) - e mutagênicos detectados em ensaios diretos (Watanabe, 1989; 1990). Já os ensaios em presença de S9mix são biomarcadores da ocorrência de compostos do tipo HPAs em extratos orgânicos de material particulado atmosférico (Vargas, 2003; Claxton et al., 2004; Pereira et al., 2010).

Além da poluição atmosférica, fatores como urbanização, direção dos ventos, alterações climáticas, altas temperaturas e redução da umidade dentro das cidades (Case & Krouse, 1980; Boonpragob, 2002; Insarov & Schroeter, 2002; Gombert et al, 2004; Saipunkaew et al., 2007), substrato (Hale, 1957; Brodo, 1973; Jesberger & Sheard, 1973; Hawksworth & Hill, 1984; Marcelli, 1996; Schmidt et al., 2001), composição de macro e micro nutrientes da casca das forófitas (Hawksworth, 1975), luminosidade (Honegger, 1996; Brunialti & Giordani, 2003; Martinez et al., 2006) e acidez ou alcalinidade da casca das forófitas (Brodo, 1973) podem alterar a comunidade liquênica. O pH pode ser crítico para a reprodução de vários espécimes (Hale, 1957) e diferenças nos valores de pH da casca podem inibir o estabelecimento dos organismos, propiciando o favorecimento de liquens acidófilos ou nitrófilos. Os liquens apresentam uma maior sensibilidade a amônia, sendo que mudanças na composição da micobiota liquenizada sugerem que, ao longo do tempo, exposição a baixas concentrações (3 $g/m^3$ a 8 $g/m^3$) pode afetar as espécies, possivelmente levando à extinção (Van Herk et al., 2003, Cape et al., 2009). O aumento da presença de espécies nitrófilas em áreas urbanas também tem sido atribuído ao $NO_x$ (Van Dobben & Ter Braak, 1999; Van Herk, 2001; Wolseley et al., 2005; Frati et al., 2007; Berthelsen et al., 2008; Cape et al., 2009). A presença

ou ausência destas espécies pode indicar o grau de eutrofização, também em áreas urbanas (Van Herk, 2001; Wolseley et al., 2006; Hauck, 2010).

O emprego de liquens como bioindicadores da qualidade do ar no Brasil restringe se aos estudos de determinadas espécies liquênicas para detectar a presença de contaminantes atmosféricos, especialmente $SO_2$ e metais pesados (Martins-Mazzitelli, 1990; Coccaro et al., 2000; Saiki et al., 2003, 2007a, 2007b; Liberman et al., 2005, Prochnow & Porto, 2005; Raposo Jr. et al., 2007; Fuga et al., 2008). Para os estudos com o ensaio *Salmonella*/microssoma, são relatados na literatura os efeitos tóxicos, genotóxicos e a potencialidade carcinogênica de alguns poluentes, especialmente de HPAs e seus nitroderivados (Sato et al., 1995; Vargas et al., 1998; De Martinis et al., 1999; Ducatti & Vargas, 2003; Vargas, 2003; Varella et al., 2004; Ré-Poppi & Silva, 2005; Coronas et al., 2008; Pereira et al., 2010). No entanto, até o momento, não há citações publicadas para o país de trabalhos que correlacionem a ocorrência de danos morfológicos nas espécies de liquens e a presença das respostas mutagênicas relacionadas a compostos orgânicos. Quanto aos estudos que ligam a estrutura da comunidade liquênica a fatores ambientais, estes estão restritos a regiões não urbanizadas (Marcelli, 1992; Martins, 2006; Cáceres et al., 2008; Fleig & Grüninger, 2008; Käffer et al., 2009, 2010). E, em termos de análise da micobiota liquenizada, para a região metropolitana de Porto Alegre, existe somente a contribuição de Martins et al. (2008) que relacionou alterações na estrutura da comunidade liquênica com atividades desenvolvidas num contexto urbano-industrial.

Desta forma, o presente estudo teve por objetivos avaliar a comunidade liquênica na cidade de Porto Alegre, RS, através do mapeamento da micobiota e comparar a influência de determinados poluentes nas estruturas morfofisiológicas de algumas espécies liquênicas e na atividade mutagênica e citotóxica do material particulado atmosférico. E, como objetivos específicos: (i) verificar a estrutura da comunidade liquênica quanto a composição, riqueza,

cobertura e diversidade nas diferentes estações analisadas, no município de Porto Alegre e numa área de referência; (ii) diferenciar zonas de acordo com o grau de salubridade do ar nas áreas analisadas, através de índices quantitativos; (iii) propor um fator de correção à fórmula original do IPA; (iv) verificar diferenças na estrutura da comunidade liquênica em relação a diferentes fatores ambientais; (v) caracterizar a presença de contaminantes químicos característicos da contribuição urbana nas espécies liquênicas, durante o período de exposição; (vi) caracterizar a presença de danos morfofisiológicos no talo das espécies liquênicas, durante o período de exposição; (vii) avaliar atividade mutagênica e citotoxicidade de extratos de compostos orgânicos, obtidos a partir do material particulado do ar (PM10), nos mesmos períodos de exposição dos liquens; (viii) comparar ocorrência de danos morfológicos nas espécies bioindicadoras e presença de contaminantes químicos com atividade mutagênica e citotoxicidade de extratos de compostos orgânicos, obtidos a partir do material particulado do ar.

Considerando estes objetivos a presente tese apresenta cinco capítulos correspondentes aos artigos científicos resultantes do projeto e das análises desenvolvidas.

O primeiro artigo “Novas ocorrências de liquens corticícolas crostosos para a região sul do Brasil” (publicado na Acta Botanica Brasílica, vol.24 n.4, p. 948-951, 2010) apresenta os novos registros de liquens crostosos para o Brasil e Rio Grande do Sul.

O segundo artigo “Corticolous lichens as environmental indicators in urban areas in southern Brazil” (publicado na Ecological Indicators, vol. 11, p. 1319-1332, 2011) avalia a estrutura da comunidade liquênica quanto a composição, riqueza, cobertura e diversidade nas diferentes estações analisadas, no município de Porto Alegre e numa área de referência. Este manuscrito também apresenta a classificação destas estações baseada no Índice de Pureza

Atmosférica complementado pelo Fator de Classificação Ambiental, que está sendo proposto neste trabalho.

O terceiro artigo “Evaluation of lichen community in urban area, southern Brazil, in relation to different environmental factors” (submetido para *Ecological Indicators*) analisa as modificações da comunidade liquênica em relação a fatores como a eutrofização do ambiente com indicação de espécies acidófilas e nitrófilas, a distribuição vertical das espécies, a fragmentação do talo dos liquens e a preferência dos táxons pelos forófitos.

O quarto artigo “Caracterização da comunidade liquênica corticícola de Porto Alegre e áreas adjacentes, RS, Brasil” (submetido para Acta Botanica Brasilica) mostra os resultados da análise florística da micobiota liquenizada através de dados de freqüência, cobertura e valor de importância dos táxons. Neste trabalho também foi elaborada uma chave de sistemática para identificação dos táxons registrados na área de estudo.

O quinto artigo “Use of lichens and genotoxicity bioindicators for the evaluation of air quality in an urban environment, southern Brazil” (submetido à *Environmental Pollution*) apresenta os resultados do monitoramento ativo com a caracterização da presença de contaminantes atmosféricos, incluindo HPAs, na área urbana de Porto Alegre, além de demonstração dos danos morfofisiológicos das espécies amostradas. Este trabalho também compara ocorrência de danos morfológicos nas espécies bioindicadoras e presença de contaminantes químicos com atividade mutagênica e citotoxicidade, através do ensaio *Salmonella*/microssoma.

## 2. Artigo 1

**Novas ocorrências de liquens corticícolas crostosos para a região sul do Brasil**

* Artigo publicado na Acta Botanica Brasilica vol.24 n.4 p. 948-951, 2010. Co-autores: Marcela Eugenia da Silva Cáceres[3], Vera Maria Ferrão Vargas[1,4], Suzana Maria de Azevedo Martins[2]

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**RESUMO** – (Novas ocorrências de liquens corticícolas crostosos para a região sul do Brasil). São apresentadas 26 novas espécies de liquens crostosos para o Brasil e Estado do Rio Grande do Sul, sendo três destas novas ocorrências também para o Brasil e 23 novos registros para o Estado. Os liquens foram analisados em 300 forófitos distribuídos em 33 bairros da cidade de Porto Alegre e na área do Parque Estadual de Itapuã, Viamão, RS.

**Palavras-chave:** distribuição, forófitas, fungos liquenizados, Porto Alegre, RS

**ABSTRACT** – (New records of corticolous crustose lichens from southern Brazil). Twenty six new records of corticolous crustose lichen species to the Brazil and State of Rio Grande do Sul are presented; three are new records for Brazil and 23 new records for the state. Lichens were analyzed in 300 host-trees distributed in 33 districts of the city of Porto Alegre and the Parque Estadual de Itapuã, Viamão, RS.

**Key words**: distribution, host-trees, lichenized fungi, Porto Alegre, RS.

## Introdução

Os liquens fazem parte de um grupo extremamente diverso, variando em sua complexidade, desde formas muito simples até estruturas morfológicas e anatômicas muito complexas. São separados em formas ou tipos, entre os principais estão os crostosos, esquamulosos, foliosos, filamentosos e fruticosos. Os fungos liquenizados crostosos, também denominados microliquens não possuem córtex inferior e aderem-se ao substrato por toda sua superfície inferior através das hifas da medula (Nash 1996; Marcelli 2006). Aproximadamente 75% dos liquens formam talos crostosos (Ahmadjian 1993). Entretanto, ainda são poucos os estudos que contemplem os liquens crostosos no Brasil.

Marcelli (2008) apresenta 2874 espécies liquênicas, sendo que 49,4% pertencem ao grupo dos fungos liquenizados crostosos. Cáceres (2007) em levantamento realizado em vários

tipos de ecossistemas na região nordeste do Brasil registrou 437 táxons crostosos, dos quais 18 foram mencionados como novos para a ciência, e 14 novas combinações. Dal Forno (2009) em trabalho realizado com a família Graphidaceae em vegetação de restinga, na região sul do Paraná, relacionou 57 espécies, sendo oito novas para a ciência e 44 novas ocorrências para o estado do Paraná. Para o Rio Grande do Sul já foram registrados 412 táxons crostosos (Spielmann 2006). Ainda há contribuições de Martins (2006) com citação de 43 táxons crostosos, dos quais três constituíam-se na época, em novos gêneros, 20 espécies como novos registros para o Estado e sete novas espécies para o Brasil.

O presente trabalho tem por objetivo apresentar as novas ocorrências de liquens corticícolas crostosos para o Brasil e estado do Rio Grande do Sul. As espécies liquênicas aqui apresentadas foram registradas no âmbito de um trabalho mais abrangente, de estudo da micota liquenizada como bioindicadores da qualidade do ar, em área urbana da cidade de Porto Alegre, RS, Brasil.

**Material e métodos**

Área de estudo - O trabalho foi realizado na cidade de Porto Alegre, capital do estado do Rio Grande do Sul, Brasil, que abrange uma área de 502,5 $km^2$, está localizada na região da Depressão Central, entre as coordenadas 30°01’53’’ de latitude sul e 51°13’19’’ de longitude oeste, às margens do lago Guaíba. O município está inserido nas unidades geomorfológicas do Escudo-riograndense e da Depressão Central, além de sofrer influência da Planície Costeira (Vieira, 1984). O clima é subtropical úmido apresentando temperatura média anual de 19,4°C, umidade relativa média do ar de 76% e índice pluviométrico de 1.324 mm anuais (Livi, 1998).

Amostragem e identificação - Os liquens foram coletados em 300 forófitas distribuídos em praças, parques e/ou ruas, em 32 bairros da cidade e no Parque Estadual de Itapuã, localizado no distrito de Viamão (50º 50’ e 51º 05’W e 30º 20’ e 30º 27’S) distante 57 km de Porto

Alegre, sendo este denominado estação controle. As atividades de campo ocorreram entre o período de julho de 2007 a junho de 2008.

As identificações foram realizadas com auxílio de microscópio estereoscópico e óptico, através de secções anatômicas dos talos e frutificações. Testes de coloração através de hidróxido de potássio 20% (KOH) e lugol (reação I) para determinar presença de substâncias e/ou reações no córtex, himênio, asco e ascósporos foram realizados quando necessário. A identificação foi baseada nas seguintes bibliografias: Aptroot (2008), Cáceres (2007), Dal Forno (2009), Lücking & Rivas-Plata (2008), Lücking *et al*. (2008, 2009), Rivas Plata *et al*. (2009) e Sipman (2006). O material coletado se encontra incorporado ao Herbário Prof. Dr. R.H. Alarich Schultz (HAS) do Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul.

**Resultados e Discussão**

Foram registradas 26 espécies de liquens crostosos, distribuídos em 10 famílias com 15 gêneros, sendo três novas ocorrências para o Brasil: *Graphis dolichographa* Nyl., *Phaeographis intricans* (Nyl.) Staiger e *Pyrenula mucosa* (Vain.) R. C. Harris, e 23 novos registros para o Rio Grande do Sul: *Anisomeridium tamarindii* (Fée) R. C. Harris, *Bacidia russeola* (Kremp.) Zahlbr., *Bactrospora myriadea* (Fée) Egea & Torrente, *Chapsa cinchonarum* (Fée) A. Frisch, *Coenogonium subdilutum* (Malme) Lücking, Aptroot & Sipman, *Cratiria lauricassiae* (Fée) Marbach, *Enterographa compunctula* (Nyl.) Redinger, *Fissurina instabilis* (Nyl.) Nyl., *Graphis archerii* Dal-Forno & Eliasaro, *Graphis geraensis* Redinger, *Graphis kakaduensis* A. W. Archer, *Graphis parallela* (Müll. Arg.) Cáceres & Lücking, *Graphis paraserpens* Lizano & Lücking, *Graphis rigidula* Müll. Arg., *Graphis schiffneri* Zahlbr, *Graphis submarginata* Lücking, *Haematomma personii* (Fée) A. Massal., *Lecanora caesiorubella* Ach., *Malcolmiella vinosa* (Eschw.) Kalb & Lücking, *Pertusaria carneola* (Eschw.) Müll. Arg., *Pertusaria wulfenioides* De Lesd., *Phaeographis punctiformis* (Eschw.)

Müll. Arg. e *Pyrenula pyrenuloides* (Mont.) R. C. Harris. Na tabela 1 se encontra a relação das espécies, as novas ocorrência para o país e/ou Estado, a distribuição geográfica, assim como o local onde o espécime foi registrado.

Tabela 1. Relação das novas ocorrências das espécies liquênicas registradas nas áreas de estudo. Legenda: RS = Rio Grande do Sul.

| Família/Espécies | Nova ocorrência | Localização | | Distribuição geográfica |
|---|---|---|---|---|
| | | Porto Alegre | Viamão | |
| **Coenogoniaceae** | | | | |
| *Coenogonium subdilutum* (Malme) Lücking, Aptroot & Sipman | RS | x | x | Neotropical |
| | | | | |
| **Graphidaceae** | | | | |
| *Chapsa cinchonarum* (Fée) A. Frisch | RS | x | x | Pantropical |
| *Graphis archerii* Dal-Forno & Eliasaro | RS | x | x | |
| *Graphis dolichographa* Nyl. | Brasil | x | | Neotropical e Paleotropical Oriental |
| *Graphis geraensis* Redinger | RS | x | x | Pantropical |
| *Graphis kakaduensis* A. W. Archer | RS | x | x | Pantropical |
| *Graphis parallela* (Müll. Arg.) Cáceres & Lücking | RS | x | | Pantropical |
| *Graphis paraserpens* Lizano & Lücking | RS | x | | Pantropical |
| *Graphis rigidula* Müll. Arg. | RS | x | x | Neotropical |
| *Graphis schiffneri* Zahlbr | RS | x | x | Pantropical |
| *Graphis submarginata* Lücking | RS | x | | Pantropical |
| *Fissurina instabilis* (Nyl.) Nyl. | RS | x | | Pantropical |
| *Phaeographis intricans* (Nyl.) Staiger | Brasil | x | | Pantropical e Temperada |
| *Phaeographis punctiformis* (Eschw.) Müll. Arg. | RS | | x | Pantropical |
| | | | | |
| **Lecanoraceae** | | | | |
| *Haematomma personii* (Fée) A. Massal. | RS | x | x | Pantropical |
| *Lecanora caesiorubella* Ach. | RS | x | x | Pantropical |
| | | | | |
| **Monoblastiaceae** | | | | |
| *Anisomeridium tamarindii* (Fée) R. C. Harris | RS | x | x | Pantropical |
| | | | | |
| **Pertusariaceae** | | | | |
| *Pertusaria carneola* (Eschw.) Müll. Arg. | RS | x | | Pantropical |
| *Pertusaria wulfenioides* De Lesd. | RS | | x | Endêmica para o Sudoeste da América do Norte |
| | | | | |
| **Pilocarpaceae** | | | | |
| *Malcolmiella vinosa* (Eschw.) Kalb & Lücking | RS | x | x | Pantropical |
| | | | | |
| **Physciaceae** | | | | |
| *Cratiria lauricassiae* (Fée) Marbach | RS | x | x | Subtropical |
| | | | | |
| **Pyrenulaceae** | | | | |
| *Pyrenula mucosa* (Vain.) R. C. Harris | Brasil | x | x | Pantropical |
| *Pyrenula pyrenuloides* (Mont.) R. C. Harris | RS | | x | Pantropical |
| | | | | |
| **Ramalinaceae** | | | | |
| *Bacidia russeola* (Kremp.) Zahlbr. | RS | x | | Pantropical |
| | | | | |
| **Roccellaceae** | | | | |
| *Bactrospora myriadea* (Fée) Egea & Torrente | RS | | x | Pantropical, Temperada |
| *Enterographa compunctula* (Nyl.) Redinger | RS | x | | Pantropical |

O maior número de espécies (13) pertence à família Graphidaceae, seguida dos exemplares de Lecanoraceae, Pertusariaceae e Pyrenulaceae com dois representantes cada. Os forófitos onde os liquens foram coletados pertencem às espécies: *Brachychyton populneum* (Schott & Endl.) R. Br., *Enterolobium contortiisiliquum* (Vell.) Morong, *Hovenia dulcis* Thunb., *Ligustrum japonicum* Thunb., *Melia azedarach* L., *Myrsine umbellata* Mart., *Peltotphorum dubium* (Spreng.) Taub. e *Tabebuia heptaphylla* (Vell.) Toledo.

As espécies citadas como novas ocorrências para o país possuem distribuição geográfica distinta. *Graphis dolichographa* foi registrada para Austrália e Colômbia (Lücking *et al*. 2009), *Phaeographis intricans* para Austrália, Costa Rica, El Salvador, Guiana, Singapura, Venezuela e Estados Unidos e *Pyrenula mucosa* para Costa Rica e Papua Nova Guiné (GBIF 2009). Os espécimes liquênicos registrados pela primeira vez para o estado do Rio Grande do Sul foram referenciados especialmente para a região Nordeste, litoral de São Paulo e do Paraná, com exceção de *Chapsa cinchonarum* que possui novo registro para a região sul e para o RS, tendo distribuição pantropical com amostras coletadas na Colômbia, África e Brasil (Frisch 2006). *Coenogonium subdilutum*, *Enterographa compunctula*, *Fissurina instabilis*, *Graphis parallela* foram citadas para o estado de Alagoas; *Anisomeridium tamarindii*, *Bactrospora myriadea*, *Cratiria lauricassiae*, *Graphis geraensis*, *G. paraserpens*, *G. rigidula*, *G. schiffneri*, *G. submarginata*, *Phaeographis punctiformis*, *Haematomma personii*, *Lecanora caesiorubella* e *Pertusaria carneola* para Pernambuco; *P. wulfenioides* para Sergipe; *Graphis kakaduensis* para Alagoas e Pernambuco; *Malcolmiella vinosa* em Alagoas, Pernambuco e Sergipe; *Pyrenula pyrenuloides* para Alagoas, Pernambuco, Sergipe e Rio Grande do Norte (Cáceres 2007). *Bacidia russeola* foi registrada em Pernambuco e São Paulo (Cáceres 2007, Marcelli 1992). Dal Forno (2009) cita pela primeira vez *Graphis archerii* para a região litorânea do Paraná.

A freqüência destas espécies nas áreas avaliadas foi diferenciada, sendo que *Anisomeriudium tamarindii* ocorreu em 86% dos pontos seguida de *Graphis parallela* (66,7%), *Pertusaria carneola* (63,3%); *Graphis submarginata* e *Haematomma personii* (50%); *Cratiria lauricassiae* (36,7%); *Coenogonium subdilutum* (30%); *Graphis kakaduensis* e *G. schiffneri* (23,3%); *G. archerii*, *G. geraensis* e *Pyrenula mucosa* (20%); *G. dolichographa*, *C. cinchonarum*, *Enterographa compunctula*, *Fissurina. instabilis*, *Lecanora caesiorubella* (10%); *Graphis rigidula*, *Malcolmiella vinosa* e *Phaeographis intricans* (6,7%) e as demais espécies ocorreram em 3,3% das áreas. Das áreas estudadas, a maior ocorrência (56,7%) de espécies foi identificada para a área do Parque Estadual de Itapuã considerada estação controle, porém cabe salientar que esta diferença não foi tão acentuada em relação ao conjunto das estações estudadas, que apresentou na sua totalidade um número de espécies significante, com um total de 144 táxons. Das espécies crostosas citadas neste estudo, A. *tamarindii* e *P. carneola* foram consideradas pela primeira vez como bioindicadoras da qualidade do ar, juntamente com outras seis espécies pertencentes ao grupo morfológico crostoso (Käffer et al. em preparação).

Os novos registros destas espécies crostosas para a área estudada ressaltam a importância e abrangência de estudos deste grupo de liquens. Nota-se aqui que a metodologia empregada para o estudo comparativo entre os forófitos das várias áreas analisadas na cidade de Porto Alegre e no Parque Estadual de Itapuã foi essencial para o registro desta diversidade liquênica, anteriormente desconhecida para todo o estado.

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## 3. Artigo 2

**Corticolous lichens as environmental indicators in urban areas in southern Brazil***

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ABSTRACT

Lichens are considered bioindicators and, as such, are widely used for air quality monitoring, especially in urban-industrial areas. The present paper proposes an evaluation of lichen communities in urban areas. The corticolous lichen community was assessed at 29 sampling stations in the city of Porto Alegre, in addition to a reference area located in the State Park in the city of Viamão, southern Brazil. The rubber band method was used for lichen mapping; three hundred host-trees were analyzed, at 11 different height levels. Lichens were evaluated in terms of composition, richness, cover and diversity, and sampling stations were classified based on the Index of Atmospheric Purity (IAP) complemented by the Environmental Classification Factor (ECF). The inclusion of ECF as a complement to the original IAP formula is proposed in the present study and not only richness and cover components are considered, but also data on the lichenized mycota composition (considering its different morphological forms). One hundred and thirty-one (131) taxa have been identified, out of which 13 specimens may be considered as indicators of urban areas, and the predominance of taxa belonging to the crustose and foliose morphological group was verified. The sampling stations were classified into five zones, ranging from lichen-free zones to optimal zones for lichen development. The use of lichen proved to be efficient to both evaluate air quality and identify alterations of urban microclimates. The application of an ECF-based correction factor is expected to complement the use of IAP, making it a more sensitive index, since an analysis of a multivaried information profile was deemed necessary, especially in regions where specimen diversity is higher.

Keywords: Air pollution, Biomonitoring, Environmental Classification Factor, Lichenized mycota, IAP

## 1. Introduction

Lichens are fungi which cultivate photobionts among the hyphae of their mycelium (Marcelli, 2006). They are widely used as indicators of air quality (Hawksworth et al., 2005), and are able to react to the effects of aerial emissions both at a cellular and at a population and/or community level (Purvis et al., 2007).

The first studies using lichens date from the nineteenth century and were carried out by Nylander, who used abundance data in order to measure the effects of atmospheric pollution (Seaward, 1997; Temmerman et. al., 2004). Since then, various studies have been carried out by using lichens in biomonitoring programs (Monge-Nájera et al., 2002.; Van Herk et al., 2002; Anze et al., 2007) as well as recommended as bioindicators in government protocols of numerous countries, such as the Netherlands, England, Germany, the USA, among others (McCune, 2000), either to evaluate specific species in relation to pollutants (Minganti et al., 2003; Saiki et al., 2003; Fuga et al., 2008) or to verify any alterations in the lichenized mycota due to atmospheric contaminants (Hawksworth, 1973; Estrabou et al., 2004; Saipunkaew et al., 2007; Calvelo et al., 2009).

In Brazil, only a few studies have been carried out using lichens as indicators of air quality and the existing ones are limited to the use of determined lichen species to specially evaluate the presence of sulfur dioxide in the air (Martins-Mazzitelli, 1990; Saiki et al., 2003; Fuga et al., 2008). In terms of analysis of the lichenized mycota in the Metropolitan Region of Porto Alegre, in the southern region of Brazil, the only existing contribution was made by Martins et al. (2008), who evaluated alterations in the lichen community structure in an urban-industrial context.

Abiotic factors such as pollution levels, extension of the urban area, number of inhabitants, wind direction, climate changes, high temperatures and reduction of humidity in the cities are

parameters used in order to correlate level of pollution with damages to the lichenized mycota (Case and Krouse, 1980; Gombert et al., 2004; Saipunkaew et al., 2007).

Lichen community may be evaluated either through qualitative information obtained from lists of species and distribution maps of the community or quantitative methods by calculating different indexes in order to estimate the air quality. Among such indexes, the Index of Atmospheric Purity (IAP), developed by Le Blanc and De Sloover (1970), is based on the sensitivity of lichens. Different versions of this index have been proposed (Herzig et al., 1989; Asta and Rolley, 1999), and approached by many researchers (Gombert et al., 2004; Calvelo et al., 2009).

According to Hawsworth and Rose (1970), the IAP has been the most widely used index among all numerical methods and its results reveal a higher correlation between pollution, urbanization and industrialization. However, whenever IAP is applied, scores are not considered/provided to data related to the lichen community composition (that is, to the different morphological forms of lichen species, which play an important role in the community structure due to sensitivity differences in relation to the action of atmospheric pollutants). Thus, one of the objectives of this paper is the inclusion of the Environmental Classification Factor (ECF) as a possible complement to be associated to the original IAP formula. The IAP complement makes the index more sensitive, especially in regions where species richness and composition are higher. In such places it was deemed necessary to analyze a wider data group, which includes not only richness values, but also the cover values of morphological groups not related to the lichen community composition.

The Environmental Classification Factor (ECF) considered in this study includes a scale according to the percentage of morphological-groups cover (crustose, foliose micro-foliose, squamulose and fruticose), considering their sensitivity to pollutants. According to De Sloover

and Le Blanc (1968) and Wetmore (1981) crustose lichens are more pollution-resistant, whereas fruticose lichens are more sensitive.

Lastly, the other objectives of the present paper are: (i) to verify the composition, richness, cover and diversity of the lichen community at sampling stations both in Porto Alegre and in a reference area, (ii) to identify specimens as current indicators of urban environments at the analyzed stations and (iii) to verify whether lichen community and ECF values are correlated with the following environmental variables: DBH, bark surface pH and traffic flow.

## 2. Materials and methods

### 2.1. Study Area

The city of Porto Alegre encompasses an area of 496.8 $km^2$, of which 30% represents rural area. It is located in the Central Depression region, at 30°01'S' and 51°13'W, on Lake Guaiba, in the state of Rio Grande do Sul, Brazil. The estimated population is 1.439 million inhabitants (IBGE, 2007). The region is characterized by a humid subtropical climate, with annual average temperature of 19.4°C, average relative humidity of 76% and annual average rainfall of 1.324 mm (Livi, 1998). The vegetation found in the urban area is composed of around one million trees comprising more than 200 species, including Brazilian and regional native species as well as species from other countries and continents, such as *Ligustrum japonicum* Thunb., *Jacaranda mimosaefolia* Don., *Tabebuia chrysotricha* (Mart. ex DC.) Standl., *T. avellanedae* Lor. ex Griseb, among others (Sanchotene et al., 1998).

The study also includes the State Park of Itapuã as a reference area, in the city of Viamão (50º 50' 51º 05'W 30º 20' 30º 27'S), situated in the metropolitan region, at a distance of 57 km from Porto Alegre.

The study was undertaken during the period comprised between July 2007 and June 2008, at 29 sampling stations distributed into the 33 city districts, as follows: E1 - Chácara das

Pedras (66º79’W 48º45’S), E2 – Santa Cecília (66º76’W 48º04’S), E3 – Centro 1 (66º78’W 47º92’S), E4 – Jardim Botânico (66º75’W 48º29’S), E5 – Bom Fim (66º77’W 47º92’S), E6 - Santa Tereza (66º72’W 47º89’S), E7 – Partenon (66º73’W 48º18’S), E8 – Agronomia (66º72’W 48º74’S), E9 – Hípica (66º63’W 48º11’S), E10 – Humaitá (66º83’W 48º17’S), E11 – Jardim Lindóia (66º80’W 48º56’S), E12 – Petrópolis (66º77’W 48º28’S), E13 – Anchieta (66º83’W 48º39’S), E15 – Sarandi (66º83’W 48º78’S), E16 – Jardim Leopoldina (66º78’W 48º92’S), E17 – Cascata/Glória (66º71’W 48º21’S), E18 – Menino Deus (66º74’W 47º85’S), E19 – Tristeza/Vila Assunção (66º69’W 47º53’S), E20 - Nonoai/Vila Nova (66º71’W 47º78’S), E21 – Belém Novo (66º57’W 48º19’S), E22 – Lomba do Pinheiro/Belém Velho (66º67’W 48º26’S), E23 – Higienópolis (66º78’W 48º23’S), E24 – Jardim Itu-Sabará (66º79’W 48º62’S), E25 – Ipanema (66º79’W 47º749’S), E26 – Bela Vista (66º77’W 48º20’S), E27 – Bom Jesus (66º76’O 48º57’S), E28 – Passo d’ Areia (66º80’O 48º38’S), E29 – Centro 2 (66º76’W 47º71’S), E30 – Ponta Grossa (66º61’W 47º98’S) and the reference area E14 – State Park of Itapuã located in the city of Viamão (66º43’’W 49º64’S), summing up 30 stations (Fig. 1). The sampling areas were defined according to their distribution in the city of Porto Alegre, number of inhabitants in a given area and anthropic pressure, such as intense vehicle traffic.

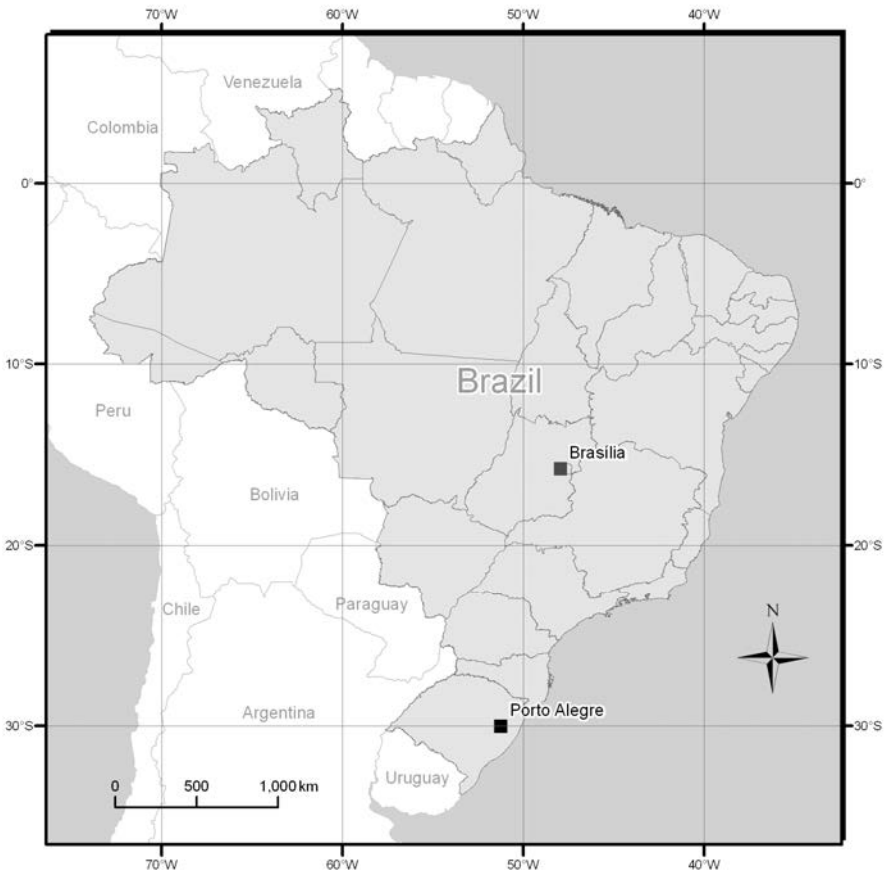


Figure 1. Map of Brazil with location of Porto Alegre.

2.2. Sampling and identification

Ten phorophytes were sampled at each analyzed station in order to study the lichen community, totalizing 300 phorophytes, under the following conditions: erect trunks and no ramifications under 150 cm, similar bark structure and diameter at breast height (DBH) over 20 cm. They were selected near local parks and/or squares considering the features previously mentioned, in addition to similarities regarding the bark structure.

Lichens were registered from 50 cm to 150 cm above ground level for each selected phorophyte at each station. In order to investigate the composition, richness, cover and diversity of lichen on trunks, the rubber band method (Fig. 2) was used (Marcelli, 1992). All the species that reached the rubber band were identified in loco or collected for posterior

confirmation. The identification of specimens was carried out with the help of both stereoscopic and optical microscopes, through anatomical cross-sections of the thalli and fructifications. External characteristics were also analyzed, such as color and thallus aspect, length and width of lobes, presence of picnids and aspect of rizines, cilia and apothecia. Color tests with potassium hydroxide 20% (KOH), sodium hypocrite (C), paraphenylenediamine (P), lugol's solution (I reaction) and ultraviolet light lamp (long wave), Thin Layer Chromatography (TLC) with solvent C and Microcrystallization tests were used to verify the presence of substances in the cortex, pith, hymenium, ascus and ascophores. In addition, specialized bibliography was consulted for each taxonomic group and material from Herbarium Prof. Dr. Alarich Schultz (HAS) at the Museum of Natural Sciences of the Zoobotanic Foundation of RS, Brazil was analyzed. The collected material was herborized and moved into the Herbarium catalogue.

Figure 2. Rubber band method.

For each analyzed phorophyte, species was identified, structure was observed and bark surface pH was measured as well as DBH determined. The pH level of bark surfaces in

phorophytes was verified through a portable digital pH meter, model PH-1700 – Instrutherm, after lichen mapping (Käffer et al., 2009).

## 2.3. Environmental variables

Information on traffic flow was obtained in relation to 21 stations (excluding stations E1, E6, E11, E14, E16, E18, E21, E24 and E26) and refers to punctual one-day sampling data. Four different categories were considered: (LV) = light-vehicles: cars and small vans; (MV) = medium-vehicles: mini and micro buses; (HV) = heavy-vehicles: trucks and buses and (MT) = motorbikes. Traffic flow data was provided by the Public Company of Transportation and Circulation (EPTC) in Porto Alegre.

## 2.4 Data analysis

Data analysis only considered species whose thalli represented at least 0.5 cm of the 10 phorophytes analyzed in each area. Species richness was considered as the total number of lichen species occurring in the 10 phorophytes analyzed at each station. The cover percentage of each species was calculated as the total sum of all units included in the rubber band of the identified species, at all height levels, in the 10 sampled phorophytes at each station (11 height levels, 100 units per level, 10 phorophytes). Diversity was based on the Shannon-Wiener Diversity Index (Krebs, 1999).

For each sampled area, IAP was used (Le Blanc and De Sloover, 1970), adapted to the sampling protocol proposed by Marcelli (1992), considering frequency data, cover and the environmental index of each species.

The formula expressed by: $IAP = \Sigma_n (Q \times f)/10$, where n= number of lichen species found in the 10 phorophytes in each area; Q= environmental index for each species and f= frequency values (1 to 5). The environmental index (Q) for each species is calculated as the average of

all accompanying species in each sampled area. The frequency scale varies according to the cover percentage of the species (Daubenmire, 1968), following an estimate: 1 – species occurs in one or two phorophytes and has a low cover value (0-25%); 2 – species occurs in up to five phorophytes or has a low cover value; 3 – species occurs in up to five phorophytes and has a medium cover level in some trees (25.1 to 75%); 4 - species occurs in between six and 10 phorophytes or has a high cover level in some tress (75.1 to 100%) and 5 - species occurs in eight to 10 phorophytes and has a high cover level in many phorophytes.

2.4.1. Environmental Classification Factor (ECF)

The Environmental Classification Factor (ECF) proposed in the present study incorporates the IAP parameters as a correction factor through the scale of cover percentage of the morphological lichen groups (crustose, foliose, micro-foliose, squamulose and fruticose), as described in table 1. The ECF is calculated as follows: ECF = (CMGc+CMGfo+CMGfr) x IAP/100, where CMGc= cover scale of crustose morphological groups; CMGfo = cover scale of foliose, micro-foliose and squamulose morphological groups; CMGfr = cover scale of fruticose morphological groups; IAP = Index of Atmospheric Purity.

The cover scale of morphological groups was elaborated considering three morphological groups (CMGc+CMGfo+CMGfr) and cover percentages (classified into five intervals), in which values were associated in order to create the scale. For crosstable CMG x Cover we have 15 cells, then 15 is the maximum value of the scale. The situation in which a higher cover of lichens belonging to the crustose morphological group exists, is equivalent to an unfavorable environmental scenario and, thus, value 1 is associated. On the other hand, a higher cover of lichens belonging to the fruticose morphological group is equivalent to the most favorable environmental situation. The classification of morphological groups cover

intervals was based on data from the present study as well as from other studies carried out by Martins and Käffer (unpublished data) in urban-industrial areas.

Table 1. Ordening system for creating the scale used for the correction expression.

| Cover scale of crustose morphological groups | | Cover scale of foliose, micro-foliose and squamulose morphological groups | | Cover scale of fruticose morphological groups | |
|---|---|---|---|---|---|
| Cover (%) | Scale | Cover (%) | Scale | Cover (%) | Scale |
| 0 - 20 | 9 | 0 - 20 | 10 | Absent | 1 |
| 20.1 - 40 | 7 | 20.1 - 40 | 8 | 0.1 - 1.5 | 5 |
| 40.1 - 60 | 5 | 40.1 - 60 | 6 | 1.6 - 3.0 | 7 |
| 60.1 - 80 | 3 | 60.1 - 80 | 4 | 3.1 - 4.5 | 10 |
| 80.1 - 100 | 1 | 80.1 - 100 | 2 | 4.6 - above | 15 |

- cover +

CMGc= cover scale of crustose morphological groups, CMGfo = cover scale of foliose, microfoliose and squamulose morphological groups, CMGfr = cover scale of fruticose morphological groups. Environmental Classification Factor (ECF).

2.4.2. Ordination analysis

In order to verify possible patterns between the sampling stations and the composition of the lichen community, a Non-metric Multidimensional Scaling method was used (NMS) as ordination analysis, using the relative Sørensen coefficient of dissimilarity as classification method (McCune et al., 2002). Statistical analyses were carried out with the statistics software PC-ORD 4.0 (McCune and Mefford, 1999).

2.4.3. Indicator species analysis

Indicator species analysis was performed to detect species that can be classified as typical of a given sampling station in an urban area. For that purpose, a Monte-Carlo test was performed on the frequency and abundance data of the lichen species (McCune et al., 2002). Statistical analyses were carried out with the statistics software PC-ORD 4.0 (McCune and Mefford, 1999).

2.4.4. Correlation analysis

ECF and original IAP data were correlated with richness, cover, diversity and morphological groups values as well as with traffic flow values. Traffic flow was also correlated with richness, cover, diversity and morphological groups, using the Pearson correlation. Statistical analyses were carried out with the statistics software PC-ORD 4.0 (McCune and Mefford, 1999).

## 3. Results

3.1. Composition of the lichen community

One hundred and thirty-one taxa were identified, divided into 50 genera and 22 families, of which 30 are new registers for the State of Rio Grande do Sul. Six of them belong to the foliose group: *Hyperphyscia cochlearis* Scutari, *Phyllopsora breviuscula* (Nyl.) Müll. Arg., *Physcia krogie* Moberg, *Physcia lacinulata* Müll. Arg., *Physcia undulata* Moberg and *Punctelia* sp., whereas twenty-three belong to the crustose group. In Brazil, three new species have been registered (Käffer et al., 2010). Five are considered new species to science: *Bulbothrix* sp., *Canoparmelia* sp. 1, *Canoparmelia* sp. 2, *Platygramme* sp. and *Phaeographis* sp. 1. (Table 2). *Canoparmelia* sp. 3 (foliose lichen) is cited as a new occurrence in the American continent (in preparation).

Table 2. Lichen taxa registered at sampling stations (E) in Porto Alegre and in the reference area in Viamão, RS.

| Taxa | Sampling stations (E) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Habit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| *Anisomeridium* sp. * | x | | x | x | x | x | x | x | x | x | x | | x | x | x | x | | x | x | x | x | x | | x | x | x | x | x | x | x | Cr |
| *Arthonia* sp. | | | | | | x | | | x | x | | | x | | | x | | | x | x | x | | | x | x | | x | | x | x | Cr |
| *Bactrospora* sp.* | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | Cr |
| *Baculifera* sp. | | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | Cr |
| *Brigantiaea leucoxantha* (Spreng.) R. Sant. & Hafellner | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | Cr |
| *Bulbothrix* sp. *** | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | Fo |
| *Bulbothrix isidiza* (Nyl.) Hale | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | Fo |
| *Caloplaca* sp. | | | | x | | x | | | x | | | | | | | x | | | | | | | | | | | | | | x | Cr |

Table 2. continuation

| Taxa | Sampling stations (E) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Habit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| *Candelaria concolor* (Dicks.) Stein | x | | | | x | x | | x | | x | x | | | | | x | x | x | x | x | | | | x | x | x | | | | | Microf |
| *Canoparmelia carneopruinata* (Zahlbr.) Elix & Hale | x | | | | x | x | | x | x | x | x | | | x | | x | x | x | x | x | x | | x | x | x | x | x | | x | x | Fo |
| *Canoparmelia caroliniana* (Nyl.) Elix & Hale | x | | | | x | x | x | | x | | x | | | x | | x | | | x | x | x | | | x | | x | x | x | | x | Fo |
| *Canoparmelia* sp. 3 **** | | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | Fo |
| *Canoparmelia texana* (Tuck.) Elix & Hale | x | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | | x | x | x | x | | x | x | x | x | x | x | | x | Fo |
| *Canoparmelia* sp.1 *** | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | Fo |
| *Canoparmelia* sp.2 *** | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Fo |
| *Carbacanthographis* sp. | x | | | x | | | | | x | x | | | | | | x | | | | x | | | | | x | | | | x | x | Cr |
| *Chapsa* sp. * | | | | | | | | | | | | | | x | | | | | | | | | | | | x | | | x | | Cr |
| *Cladonia atthi* S. Stenroos | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | Squa. |
| *Coccocarpia pellita* (Ach.) Müll.Arg. ex R.Sant. | | | | x | | | | x | | | | | | | | | | | | | | | | | | | x | | | | Microf |
| *Coenogonium* sp. * | | | | | | | | | | | x | | | x | | | | | | | | x | | | | | x | | | | Cr |
| *Cratiria americana* (Fée) Kalb & Marbach | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | Cr |
| *Cratiria* sp. * | | | | x | | x | x | | | | | | | x | | x | | x | | | | | | | | | | | | | Cr |
| *Dirinaria applanata* (Fée) D. D. Awasthi | x | x | x | x | x | x | x | x | x | | x | | x | x | | x | | x | x | x | x | | | | x | x | x | | | | Fo |
| *Dirinaria confluens* (Fr.) D. D. Awashti | x | x | x | x | x | x | x | x | x | x | x | | | x | x | x | | x | x | x | x | x | x | | x | x | x | | x | x | Fo |
| *Dirinaria picta* (Sw.) Schaer. ex Clem. | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | Fo |
| *Enterographa* sp. * | | | | | | | | | | | | | x | | | x | | | | | | | | | | | | | | | Cr |
| *Fissurina* sp. * | x | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | x | Cr |
| *Glyphis cicatricosa* Ach. | x | | x | x | x | x | x | x | x | x | x | | x | x | | x | | | x | x | x | | | x | x | x | x | x | x | x | Cr |
| *Glyphis scyphulifera* (Ach.) Staiger | | | | | x | x | x | x | | x | x | | | x | | | | x | x | | | | | | x | | x | | x | | Cr |
| *Graphis* sp.1 * | | | | | | | x | x | | | | | | | | | | | | | | | | | | | | | x | | Cr |
| *Graphis caesiocarpa* Redinger | x | | x | x | x | | | x | x | x | | x | | x | | x | | | | x | x | x | | x | x | | x | x | x | x | Cr |
| *Graphis* sp. 2** | | | | | | | x | | x | | | | | | | | | | | | | | | x | | | | | | | Cr |

Table 2. continuation

| Taxa | Sampling stations (E) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Habit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| *Graphis dupaxana* Vain. | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | Cr |
| *Graphis* sp. 3* | | | | | | | | | | | | | | x | | | | | | | | | | | x | | | | | x | Cr |
| *Graphis* sp. 4* | | | x | | | | x | | | x | | | | x | | | | | | | | | | x | x | | | | | | Cr |
| *Graphis librata* C. Knight. | | | | | | | | x | | x | | | | | | | | | | | | | | | x | | | | | | Cr |
| *Graphis* sp. 5* | x | | | x | x | | | x | x | x | | | | x | | x | | | x | x | x | x | | x | x | | x | x | x | x | Cr |
| *Graphis* sp. 6* | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | Cr |
| *Graphis pavoniana* Fée | x | | | x | x | | | x | x | | | | | | | | x | | | x | | x | | | x | | x | x | | | Cr |
| *Graphis* sp.7* | | | | | x | | | | | | | | | x | | | | | | | | | | | | | | | | | Cr |
| *Graphis* sp. 8* | | | | | x | | | | x | | | | | x | | x | | | | | | | | | x | | | x | x | | Cr |
| *Graphis* sp. 9* | x | | | x | | | | | x | x | x | x | | | | x | | | x | x | x | | | | x | | | | | | Cr |
| *Haematomma* sp.* | | | | x | | x | x | | x | | | | | x | | | | x | x | | x | | | | x | | | | | x | Cr |
| *Herpothallon rubrocinctum* (Ehrenb.) Aptroot & Lücking | | | | | | x | | | | x | | | | | | | x | | | | | x | | | | | | | | | Cr |
| *Heterodermia albicans* (Pers.) Swinscow & Krog | x | | | | x | x | | x | | x | x | x | | x | | | x | x | x | x | x | x | | x | x | x | x | x | | x | Fo |
| *Heterodermia* cf. *albicans* (Pers.) Swinscow & Krog | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | Fo |
| *Heterodermia diademata* (Taylor) Awasthi | x | | | x | x | x | x | | x | | | | | x | | | x | x | x | x | x | | x | x | x | x | x | | | x | Fo |
| *Heterodermia obscurata* (Nyl.) Trevis. | | | | x | | | | x | | | | | | | | | x | | | | | | | | | | x | | x | | Fo |
| *Heterodermia speciosa* (Wulf.) Trevis. | | | | | | | | | | | | | | x | | | | | | | | | | | x | | | | | | Fo |
| *Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt | x | | | x | | x | x | | x | | | x | | | | | | | x | x | | | | x | | x | | | | | Microf |
| *Hyperphyscia cochlearis* Scutari * | x | | | | | x | | | | | x | x | | | | x | | | | x | | | | | | x | | x | x | | Microf |
| *Hyperphyscia syncolla* (Tuck.) Kalb | | | | | | | | | | | | | | x | | | | | | | | x | | | | | | | | | Microf |

Table 2. continuation

| Taxa | Sampling stations (E) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Habit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| *Hypotrachyna livida* (Taylor) Hale | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | Fo |
| *Hypotrachyna polydactyla* (Krog & Swinscow) Nash | | x | | x | x | | x | | x | | | | | x | | | | | | | | | | | x | | x | x | | x | Fo |
| *Lecanora* aff. *achroa* Nyl. | | | x | x | | x | x | x | | | | x | x | x | | | | x | x | | x | | | | x | x | x | x | x | x | Cr |
| *Lecanora* aff. *albella* (Pers.) Ach. | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | | | Cr |
| *Lecanora* cf. *argentata* (Ach.) Malme | | | | | | x | | x | x | | | | | | | | | x | | | | | | | | | | | | | Cr |
| *Lecanora concilianda* Vain | x | | | x | x | x | x | x | x | x | | | | x | x | x | | | x | x | x | | | x | x | x | x | x | x | | Cr |
| *Lecanora* sp. 3* | | | | x | | | | | | | | | x | x | | | | | | | | | | | | | | | | | Cr |
| *Lecanora* cf. *symmicta* (Ach.) Ach. | x | | x | x | x | x | x | x | x | x | x | | x | x | x | x | | x | x | x | x | x | | x | x | x | x | x | x | x | Cr |
| *Lecanora* grupo *subfusca* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | Cr |
| *Lecanora* sp. 1 | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | Cr |
| *Lecanora* sp. 2 | | | | | | | | | | | | | | x | | | | | | | | | | | | | | x | | | Cr |
| cf. *Lepraria* sp. | | x | x | x | x | x | | x | | | | x | x | x | | x | | x | | x | | x | x | | | | x | | | | Squa. |
| *Leptogium austroamericanum* (Malme) Dodge | | | | | | | | | | | | | | | | | x | x | | | | | | | | | | | | | Fo |
| *Leptogium azureum* (Sw.) Mont. | | | | | | | | x | | | | | | | | | | | | | | x | | | | | | | | | Fo |
| *Leptogium denticulatum* Nylander | | | | | | | | | | | | | | x | | | x | | | | | x | | | | x | | | | | Fo |
| *Malcolmiella* sp. * | | | | | | | | | | | | | | | | | | | | | | x | | | | | | | | | Cr |
| *Myelochroa lindmanii* (Lynge) Elix & Hale | x | | | x | x | x | x | x | x | x | | | | x | | x | | x | | x | | x | x | x | x | x | x | x | | x | Fo |
| *Normandina pulchella* (Borrer) Nyl. | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | Squa. |
| *Ochrolechia pallescens* (L.) A.Massal. | | | | x | x | x | x | x | x | x | | | | x | | x | | x | x | | x | | | | x | | | | | | Cr |
| *Opegrapha* sp. 2 | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | Cr |
| *Opegrapha* sp. 3 | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | Cr |
| *Opegrapha* sp.1 | | | | | | | | x | | | | | | x | | | | | | x | | x | | x | x | x | | | | x | Cr |
| *Parmelinopsis minarum* (Vainio) Elix & Hale | x | | | x | | | | x | x | | | | | x | | | x | | x | | | | | | x | | x | | | | Fo |

Table 2. continuation

| Taxa | Sampling stations (E) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Habit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| *Parmotrema austrosinense* (Zahlbr.) Hale | | | | | | x | | | x | x | | | | x | | | | x | x | x | x | | | x | x | x | | | | x | Fo |
| *Parmotrema catarinae* Hale | | | | | | | | | | | | | | | | | | | | | x | | | | | | | | | | Fo |
| *Parmotrema cetratum* (Ach.) Hale | | | | | | | | x | x | | | | | x | x | | | x | x | | | | | | x | | | | | x | Fo |
| *Parmotrema consors* (Nyl.) Krog & Swinscow | | | | | | | | | x | | | | | | | | | | | | | | | | | | | x | | | Fo |
| *Parmotrema eciliatum* (Nyl.) hale | | | | | | | | | | | | | | | | | | | x | | | | | | | | | | | | Fo |
| *Parmotrema flavomedullosum* Hale | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | Fo |
| *Parmotrema haitiense* (Hale) Hale | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | Fo |
| *Parmotrema homotomum* (Nyl.) Hale | | | | | | | | x | | | | | | x | | | | | | | | | | | | | | | | | Fo |
| *Parmotrema melanothrix* (Mont.) Hale | | | | | | | | | | | | | | x | | | | | | | x | | | | | x | | | | | Fo |
| *Parmotrema mesotropum* (Müll.Arg.) Hale | | | | | | | | | | | | | | x | | | | | x | | | | | | x | | | | | x | Fo |
| *Parmotrema muelleri* (Vain.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch | | | | | x | | | x | | | | | | | | | x | | | | | | | | x | | | | | | Fo |
| *Parmotrema pilosum* (Stizenb.) Krog & Swinscow | | | | | | | | | | | | | | | | | | | x | | | | | | | | | | | | Fo |
| *Parmotrema praesorediosum* (Nyl.) Hale | x | | | | | x | | | x | x | | | | x | | | | | x | | | | | | x | | | | | x | Fo |
| *Parmotrema recipiendum* (Nyl.) Hale | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | Fo |
| *Parmotrema reticulatum* (Taylor) M. Choisy | | | | x | | | | x | x | x | x | | | x | | | | x | x | | | | | | x | | x | | | x | Fo |
| *Parmotrema subcaperatum* (Kremp.) Hale | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | Fo |
| *Parmotrema subsumptum* (Nyl.) Hale | x | | | | x | | | | | | | | | | | | | | x | | | | | | | | | | | | Fo |
| *Parmotrema tinctorum* (Nyl.) Hale | x | | | x | x | x | x | x | x | x | | | | x | | | x | x | x | | x | x | | | x | | | | x | | Fo |
| *Pertusaria* sp.* | x | | x | x | | x | x | x | x | x | x | | | x | x | x | | | | x | | | | x | x | x | | | x | x | Cr |
| *Pertusaria flavens* Nyl. | x | | x | x | x | x | x | x | x | x | x | x | x | x | | x | | x | | | | | | x | x | x | | | x | x | Cr |
| *Phaeographis lecanographa* (Nyl.) Staiger | | | | | x | | | x | x | | | | | x | | x | | | | | | | | | | | x | | | x | Cr |
| *Phaeographis lobata* (Eschw.) Müll. Arg. | x | | x | | | x | x | x | x | x | | | | x | | x | | | x | x | x | x | | | x | | | x | | x | Cr |

Table 2. continuation

| Taxa | Sampling stations (E) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Habit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| *Phaeographis subtigrina* (Vain.) Zahlbr. | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | Cr |
| *Phaeographis* sp. 1** | | | | | | | | | x | x | | | | | | | | | | | | | | | | | | | | | Cr |
| *Phaeographis* sp. 2 *** | | | x | | | | | x | | | | | | | | x | | x | | | | | | | | | | | | | Cr |
| *Phyllopsora breviuscula* (Nyl.) Müll.Arg. * | | | | | | | | x | | | | x | | | | | | | | | | x | | x | | | | | | | Squa. |
| *Physcia aipolia* (Ehrenb. ex Humb.) Fürnrohr | x | | | x | x | x | | x | x | x | | | | x | | x | x | x | x | x | x | x | x | x | x | | x | | x | | Fo |
| *Physcia alba* (Fée) Müll.Arg. | x | | | | x | | | | | | | | | x | | | | | | | | | | | x | | | | | | Fo |
| *Physcia atrostriata* Moberg | | | | | x | | | | | | | | | | | | | | | | | x | | x | | | | | | | Fo |
| *Physcia crispa* Nyl. | x | | | x | x | x | | | x | | | | | | | | | | x | x | | | | x | | | | | | | Fo |
| *Physcia krogie* Moberg* | | | | x | x | | x | | | | | | | | | | | | x | | | | | | x | x | x | | | | Fo |
| *Physcia lacinulata* Müll.Arg. * | | | | | | | | | | | | | | | | | | | | x | | | | | | | | | | | Fo |
| *Physcia poncinsii* Hue | x | | x | | x | x | | x | | x | | | | | | | x | | x | x | x | x | | x | x | x | x | | | | Fo |
| *Physcia stellaris* (L.) Nyl. | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | Fo |
| *Physcia undulata* Moberg * | | | | | | | | | | | | | | | | | | | x | | | | | | | | | | | | Fo |
| *Platygramme* aff. *arthonioides* (Vainio) Zahlbr. | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | Cr |
| *Platygramme caesiopruinosa* (Fée) Fée | | | | x | | | | x | x | | | | | | | | | | | | x | | | | x | | | x | x | x | Cr |
| *Platygramme* sp. *** | | | | x | | | | | | x | | x | | | | x | | | | | | | | | x | | | | | | Cr |
| *Porina* sp. | | | | | | | | | | | | | | | | | | | | | | x | | | | | x | | | | Cr |
| *Punctelia constantimountium* Sérus. | | | | x | x | | | x | | | x | | | x | | | | | | | | x | | x | | | x | | | | Fo |
| *Punctelia* sp. * | x | | | x | x | x | x | x | x | | x | | | x | | x | x | x | x | x | | x | | x | x | x | x | | | x | Fo |
| *Punctelia riograndensis* (Lynge) Krog | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | Fo |
| *Pyrenula* sp.** | | | | | x | x | | x | | | | | | | | | | | | | | | | x | | | | | | | Cr |
| *Pyxine berteriana* (Fée) Imsh. | x | | | | | x | | | | | | | | | | x | | | x | | | | | | | | | | | | Fo |
| *Pyxine cocoës* (Sw.) Nyl. | | | | | x | x | | | x | | x | | | x | | | | | | x | | | x | | | | | | | | Fo |
| *Pyxine subcinerea* Stirt. | x | | | x | | x | | | x | x | x | | | x | | x | | x | x | x | x | x | | | x | x | x | x | x | | 46 |

Table 2. continuation

| Taxa | Sampling stations (E) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Habit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| *Ramalina aspera* Räsänen | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | Fr |
| *Ramalina celastri* (Sprengel) Krog & Swinscow | | | | x | | x | | | | | | | | x | | | | | | | x | | | | x | | | | | x | Fr |
| *Ramalina complanata* (Sw.) Ach. | | | | | | | | | | | | | | | | | | x | | | | | | | x | | | | | | Fr |
| *Ramalina peruviana* Ach. | | | | x | | | | x | | | | | | | | | x | | | | | | | | | | | | | x | Fr |
| *Ramboldia haematites* (Fée) Kalb | | | | x | | x | x | x | | | | | | x | | | | | | | | | | | | | | | | | Cr |
| *Rinodina* sp.1 | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | x | | Cr |
| *Rinodina* sp. 2 | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | Cr |
| *Tephromella americana* (Fée) Kalb | | | | x | | x | x | x | x | | | | | x | x | | | | x | | x | | | | x | | | | | x | Cr |
| *Trypethelium nitidiusculum* (Nyl.) R. C. Harris | | | | x | | | | | x | | | | | | | x | | | | | x | | | | x | x | x | | x | x | Cr |
| *Usnea* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | Fr |

E1 - Chácara das Pedras, E2 – Santa Cecília, E3 – Centro 1, E4 – Jardim Botânico, E5 – Bom Fim, E6 - Santa Tereza, E7 – Partenon, E8 – Agronomia, E9 – Hípica, E10 – Humaitá, E11 – Jardim Lindóia, E12 – Petrópolis, E13 - Anchieta, E14 – Parque Estadual de Itapuã, E15 – Sarandi, E16 – Jardim Leopoldina, E17 – Cascata/Glória, E18 – Menino Deus, E19 – Tristeza/Vila Assunção, E20 - Nonoai/Vila Nova, E21 – Belém Novo, E22 – Lomba do Pinheiro/Belém Velho, E23 – Higienópolis, E24 – Jardim Itu-Sabará, E25 – Ipanema, E26 – Bela Vista, E27 – Bom Jesus, E28 – Passo d' Areia, E29 – Centro 2, E30 – Ponta Grossa. Taxa prepended by an asterisk (*) indicate a new citation for the state, two asterisks (**) new registers in Brazil, three asterisks (***) a new species to science and four asterisks (****) a new citation for the American continent. Cr = crustose, Squa = squamulose, Fo = foliose, Fr = fruticose, Microf = microfoliose.

Concerning the morphological group, 46.6% belong to crustose taxa, 42.7% to the foliose group, 3.8% to the microfoliose group, 3.8% to the fruticose group and, 3.1% to the squamulose group.

Families representing the highest number of taxa were Parmeliaceae (25.9%), Physciaceae (21.4%), Graphidaceae (19.8%) and Lecanoraceae (9.2%), whereas genera with the largest species representativeness were *Parmotrema* (13.7%), followed by *Graphis* (9.9%) and *Lecanora* and *Physcia* both representing 6.9%.

The most frequent species were *Dirinaria picta* (Sw.) Schaer. ex Clem. found at all stations, followed by *Canoparmelia texana* (Tuck.) Elix & Hale and *Anisomeridium* sp. found at 26 stations and *Dirinaria confluens* (Fr.) D. D. Awashti and *Lecanora* cf. *symmicta* (Ach.) Ach. found at 26 stations (Table 2).

3.2. Richness, Cover, Diversity and Morphological Groups by sampling stations

At the sampling stations, the highest richness and diversity values (62 and 1.7, respectively) were registered in the reference area (E14), followed by station E25 (55 and 1.7). Stations E2 and E15 provided the lowest richness (6 and 9) and diversity (0.8 and 0.9) rates. On the subject of cover, stations E25 and E10 revealed the highest values (495.1 and 419.4) whereas stations E2 and E15 showed the lowest values (10.7 and 17, consecutively). Species characterized by the highest cover values were *Dirinaria picta*, found at stations E9, E10, and E21, along with *Canoparmelia texana*, registered at stations E4, E11, E18 and E19, both specimens belonging to category 5 in the cover scale, that is, representing a higher than 100% cover, considering all analyzed phorophytes. The distribution of morphological groups at the stations was different. The highest percentage of crustose taxa (69.2%) was registered at E29, foliose taxa (83.3%) at E2, microfoliose and squamulose taxa (both 16.7%) at E12 and fruticose taxa (7.3%) at E30 (Table 3).

Table 3. Values for the Index of Atmospheric Purity (IAP), richness, cover, diversity and percentage of morphological groups, at sampling stations in Porto Alegre and in the reference area, Brazil.

| Sampling stations (E) | IAP | Classification | Richness | Cover | Diversity | Morphological groups % | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | cr | fo | microf | squam | fr |
| E14 | 434.2 | excellent | 62 | 317.5 | 1.7 | 50.0 | 45.2 | 1.6 | 1.6 | 1.6 |
| E25 | 388.0 | excellent | 55 | 495.1 | 1.7 | 50.9 | 43.6 | 1.8 | 0.0 | 3.6 |
| E9 | 384.2 | excellent | 49 | 364.8 | 1.6 | 53.1 | 44.9 | 2.0 | 0.0 | 0.0 |
| E4 | 360.6 | excellent | 52 | 407.9 | 1.6 | 51.9 | 38.5 | 3.8 | 1.9 | 3.8 |
| E8 | 314.5 | excellent | 50 | 386.6 | 1.6 | 52.0 | 38.0 | 4.0 | 4.0 | 2.0 |
| E30 | 312.6 | excellent | 41 | 303.5 | 1.5 | 51.2 | 41.5 | 0.0 | 0.0 | 7.3 |
| E6 | 300.4 | excellent | 45 | 216.7 | 1.6 | 42.2 | 46.7 | 6.7 | 2.2 | 2.2 |
| E19 | 269.2 | excellent | 29 | 364.2 | 1.5 | 34.1 | 63.4 | 2.4 | 0.0 | 0.0 |
| E5 | 262.4 | excellent | 42 | 212.5 | 1.5 | 35.7 | 59.5 | 2.4 | 2.4 | 0.0 |
| E10 | 255.6 | excellent | 35 | 419.4 | 1.4 | 60.0 | 37.1 | 2.9 | 0.0 | 0.0 |
| E16 | 250.2 | excellent | 35 | 203.0 | 1.5 | 60.0 | 31.4 | 5.7 | 2.9 | 0.0 |
| E1 | 243.3 | excellent | 39 | 145.8 | 1.5 | 35.9 | 56.4 | 7.7 | 0.0 | 0.0 |
| E7 | 211.9 | excellent | 32 | 199.0 | 1.4 | 59.4 | 34.4 | 3.1 | 0.0 | 3.1 |
| E20 | 211.2 | excellent | 41 | 207.2 | 1.5 | 38.2 | 50.0 | 8.8 | 2.9 | 0.0 |
| E21 | 208.2 | excellent | 31 | 350.1 | 1.4 | 48.4 | 48.4 | 0.0 | 0.0 | 3.2 |
| E27 | 206.5 | excellent | 38 | 215.0 | 1.5 | 36.8 | 52.6 | 2.6 | 7.9 | 0.0 |
| E26 | 195.2 | excellent | 30 | 187.6 | 1.4 | 33.3 | 56.7 | 10.0 | 0.0 | 0.0 |
| E18 | 190.3 | excellent | 29 | 273.3 | 1.4 | 34.5 | 55.2 | 3.4 | 3.4 | 3.4 |
| E24 | 174.2 | excellent | 31 | 131.2 | 1.4 | 45.2 | 45.2 | 6.5 | 3.2 | 0.0 |
| E11 | 135.7 | excellent | 22 | 149.2 | 1.3 | 36.4 | 54.5 | 9.1 | 0.0 | 0.0 |
| E22 | 118.5 | excellent | 27 | 102.4 | 1.4 | 40.7 | 48.1 | 3.7 | 7.4 | 0.0 |
| E29 | 117.9 | excellent | 26 | 92.2 | 1.3 | 69.2 | 26.9 | 3.8 | 0.0 | 0.0 |
| E28 | 111.0 | excellent | 22 | 83.0 | 1.3 | 54.5 | 40.9 | 4.5 | 0.0 | 0.0 |
| E3 | 78.9 | excellent | 15 | 52.7 | 1.1 | 66.7 | 26.7 | 0.0 | 6.7 | 0.0 |
| E17 | 78.1 | excellent | 18 | 59.5 | 1.2 | 11.1 | 77.8 | 5.6 | 0.0 | 5.6 |
| E23 | 63.0 | normal | 9 | 127.5 | 0.9 | 0.0 | 88.9 | 0.0 | 11.1 | 0.0 |
| E12 | 52.6 | normal | 12 | 54.2 | 1.0 | 41.7 | 25.0 | 16.7 | 16.7 | 0.0 |
| E13 | 49.5 | normal | 12 | 39.7 | 1.0 | 66.7 | 25.0 | 0.0 | 8.3 | 0.0 |
| E15 | 32 | transition | 9 | 17 | 0.9 | 55.6 | 44.4 | 0.0 | 0.0 | 0.0 |
| E2 | 19.9 | transition | 6 | 10.7 | 0.8 | 0.0 | 83.3 | 0.0 | 16.7 | 0.0 |

Cr = crustose, Esq = squamulose, Fo = foliose, Fr = fruticose, Microf = microfoliose; E1-E30 defined in Table 2.

3.3. Index of Atmospheric Purity and Environmental Classification Factor

The Index of Atmospheric Purity at sampling stations varied from 19.9 to 434.2. The lowest values were registered at stations E2 and E15 whereas the highest values were registered at stations E14, E25, E4 and E30 (Table 3).

ECF values varied from 2.4 to 93.8 and stations were classified into five different zones: three lichen-absent areas (E2, E13 and E15) and six lichen-poor areas (E3, E12, E22, E23, E28 and E29). Ten stations were classified as transition areas (E1, E5, E11, E16, E17, E19, E20, E24, E26 and E27), seven as normal areas for the development of lichenized mycota (E6, E7, E8, E9, E10, E18 and E21) and four as excellent areas for the development of lichens (E4, E14, E25 and E30). The analyzed stations were classified into five zones, based on ECF values, considering a scale variation from 1.0 to above 75.5, established by Le Blanc and De Sloover (1970) to IAP: Zone I – lichen-absent (1.0-5.5), Zone II – lichen-poor (5.6-15.5), Zone III – transition area (15.6-35.5), Zone IV – normal (35.6-75.5) and Zone V – excellent (higher than 75.6) (Table 4 and Fig. 3).

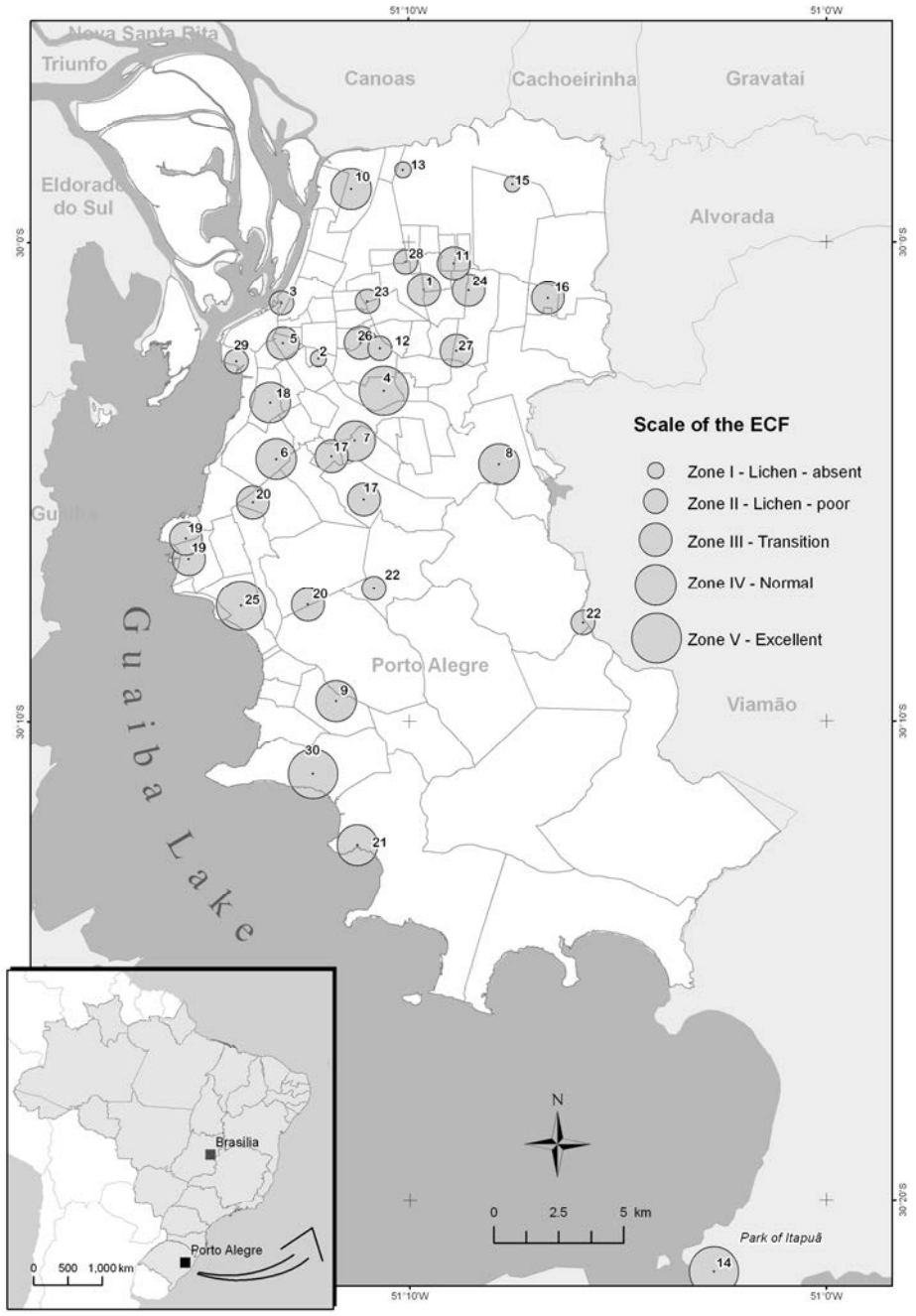


Figure 3. Map of Porto Alegre, RS, Brazil and representation of sampling stations according to the scale of the Environmental Classification Factor (ECF).

Table 4. Values for the Environmental Classification Factor (ECF), richness, cover, diversity and percentage of morphological groups, at sampling stations in Porto Alegre and in the reference area, Brazil.

| Sampling stations (E) | ECF | Classification | Richness | Cover | Diversity | Morphological groups % | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | cr | fo | microf | squam | fr |
| E4 | 93.8 | excellent | 52 | 407.9 | 1.6 | 51.9 | 38.5 | 3.8 | 1.9 | 3.8 |
| E25 | 81.5 | excellent | 55 | 495.1 | 1.7 | 50.9 | 43.6 | 1.8 | 0.0 | 3.6 |
| E30 | 81.3 | excellent | 41 | 303.5 | 1.5 | 51.2 | 41.5 | 0.0 | 0.0 | 7.3 |
| E14 | 78.2 | excellent | 62 | 317.5 | 1.7 | 50.0 | 45.2 | 1.6 | 1.6 | 1.6 |
| E8 | 56.6 | normal | 50 | 386.6 | 1.6 | 52.0 | 38.0 | 4.0 | 4.0 | 2.0 |
| E6 | 54.1 | normal | 45 | 216.7 | 1.6 | 42.2 | 46.7 | 6.7 | 2.2 | 2.2 |
| E7 | 48.7 | normal | 32 | 199.0 | 1.4 | 59.4 | 34.4 | 3.1 | 0,0 | 3.1 |
| E9 | 46.1 | normal | 49 | 364.8 | 1.6 | 53.1 | 44.9 | 2.0 | 0.0 | 0.0 |
| E21 | 43.7 | normal | 31 | 350.1 | 1.4 | 48.4 | 48.4 | 0.0 | 0.0 | 3.2 |
| E18 | 40.0 | normal | 29 | 273.3 | 1.4 | 34.5 | 55.2 | 3.4 | 3.4 | 3.4 |
| E10 | 35.8 | normal | 35 | 419.4 | 1.4 | 60.0 | 37.1 | 2.9 | 0.0 | 0.0 |
| E16 | 35.8 | normal | 35 | 203.0 | 1.5 | 60.0 | 31.4 | 5.7 | 2.9 | 0.0 |
| E19 | 32.3 | transition | 29 | 364.2 | 1.5 | 34.1 | 63.4 | 2.4 | 0.0 | 0.0 |
| E5 | 31.5 | transition | 42 | 212.5 | 1.5 | 35.7 | 59.5 | 2.4 | 2.4 | 0.0 |
| E1 | 29.2 | transition | 39 | 145.8 | 1.5 | 35.9 | 56.4 | 7.7 | 0.0 | 0.0 |
| E20 | 25.3 | transition | 41 | 207.2 | 1.5 | 38.2 | 50.0 | 8.8 | 2.9 | 0.0 |
| E27 | 24.8 | transition | 38 | 215.0 | 1.5 | 36.8 | 52.6 | 2.6 | 7.9 | 0.0 |
| E26 | 23.4 | transition | 30 | 187.6 | 1.4 | 33.3 | 56.7 | 10.0 | 0.0 | 0.0 |
| E24 | 20.9 | transition | 31 | 131.2 | 1.4 | 45.2 | 45.2 | 6.5 | 3.2 | 0.0 |
| E17 | 20.3 | transition | 18 | 59.5 | 1.2 | 11.1 | 77.8 | 5.6 | 0.0 | 5.6 |
| E11 | 16.3 | transition | 22 | 149.2 | 1.3 | 36.4 | 54.5 | 9.1 | 0.0 | 0.0 |
| E22 | 14.2 | lichen-poor | 27 | 102.4 | 1.4 | 40.7 | 48.1 | 3.7 | 7.4 | 0.0 |
| E29 | 14.1 | lichen-poor | 26 | 92.2 | 1.3 | 69.2 | 26.9 | 3.8 | 0.0 | 0.0 |
| E28 | 13.3 | lichen-poor | 22 | 83.0 | 1.3 | 54.5 | 40.9 | 4.5 | 0.0 | 0.0 |
| E3 | 8.7 | lichen-poor | 15 | 52.7 | 1.1 | 66.7 | 26.7 | 0.0 | 6.7 | 0.0 |
| E23 | 7.6 | lichen-poor | 9 | 127.5 | 0.9 | 0.0 | 88.9 | 0.0 | 11.1 | 0.0 |
| E12 | 6.3 | lichen-poor | 12 | 54.2 | 1.0 | 41.7 | 25.0 | 16.7 | 16.7 | 0.0 |
| E13 | 5.0 | lichen-absent | 12 | 39.7 | 1.0 | 66.7 | 25.0 | 0.0 | 8.3 | 0.0 |
| E15 | 3.8 | lichen-absent | 9 | 17.0 | 0.9 | 55.6 | 44.4 | 0.0 | 0.0 | 0.0 |
| E2 | 2.4 | lichen-absent | 6 | 10.7 | 0.8 | 0.0 | 83.3 | 0.0 | 16.7 | 0.0 |

Cr = crustose, Esq = squamulose, Fo = foliose, Fr = fruticose, Microf = microfoliose; E1-E30 defined in Table 2.

### 3.4. Lichen community patterns among sampling stations

The NMS (Non-metric Multidimensional Scaling) ordination analysis provided an ordination model of the sampling stations in relation to the ECF values. The analysis produced six axes and the percentage of explained variation for the first two axes was 56.3% (41.0% for

the first and 15.3% for the second). The first axis is not correlated with any of the analyzed parameters. The second axis is correlated with stations where higher values for the following variables were found: ECF (r = 0.756, p< 0.001), richness (r = 0.844, p< 0.001), cover (0.794, p< 0.001) and diversity (r = 0.906, p< 0.001) (Fig. 4). The following variables obtained low correlation values: bark surface pH (r = - 0.013, p< 0.001), DHB of phorophytes (r = - 0.053, p< 0.001) and traffic flow (r = - 0.127, p< 0.001). *Leptogium austroamericanum* (Malme) Dodge is related to axis 1, while *Anisomeridium* sp., *Dirinaria picta*, *Canoparmelia carneopruinata* (Zahlbr.) Elix & Hale, *Glyphis cicatricosa* Ach. and *Pertusaria flavens* Nyl. are related to axis 2.

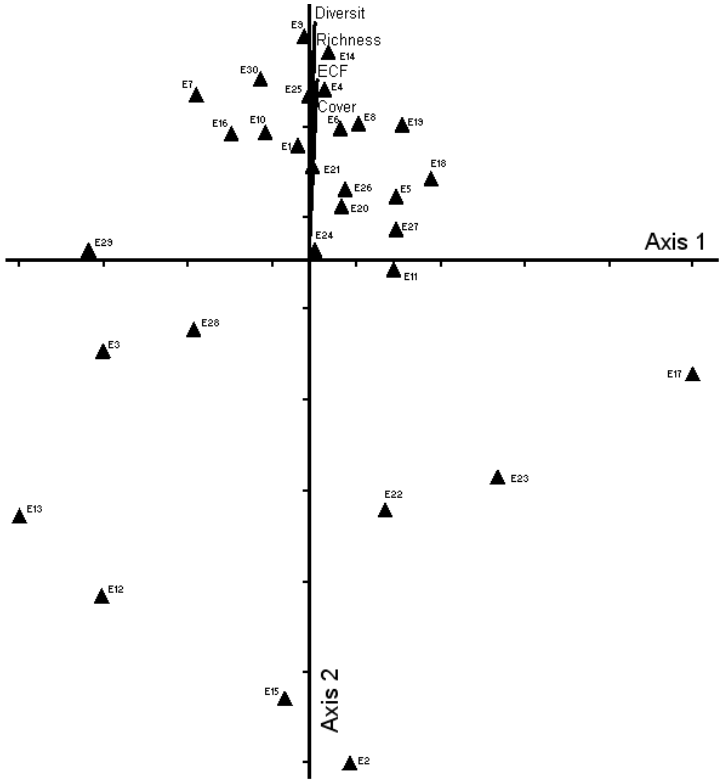


Figure 4. Representation of the variation in lichen community at sampling stations in Porto Alegre and in the reference area, Brazil, through ordination analysis with the use of the Non-metric Multidimensional Scaling method (NMS) based on the Sørensen dissimilarity coefficient. Lines indicate a correlation in axis 2 with data referring to richness, cover, diversity and ECF.

3.5. Lichen species as indicators of urban areas

Of all identified taxa, 13 were considered as indicator species in urban areas according to the richness values of each species, at the sampling stations. Taxa belonging to the crustose (46.1%) and foliose (46.1%) groups revealed the highest predominance whereas fruticose group registered only one species, representing 7.7% of all specimens. Taxa with the highest indication values (IV), considering statistically significant values only, were: *Anisomeridium* sp. (IV = 30.5, P = 0.0160), *Canoparmelia texana* (Tuck.) Elix & Hale (IV = 25.0, P = 0.2070), *Dirinaria picta* (Sw.) Schaer. ex Clem. (IV = 23.0, P = 0.1420), *Glyphis cicatricosa* Ach. (IV = 34.4, P = 0.0170), *Graphis caesiocarpa* Redinge (IV = 30.8, P = 0.124), *Graphis* sp. 5 (IV = 33.9, P = 0.0550), *Heterodermia obscurata* (Nyl.) Trevis. (IV = 35.8, P = 0.850), *Lecanora concilianda* Vain (IV = 38.4, P = 0.0130), *Parmotrema tinctorum* (Nyl.) Hale (IV = 53.1, P = 0.0020), *Parmotrema reticulatum* (Taylor) M. Choisy (IV = 38.7, P = 0.0560), *Pertusaria flavens* Nyl. (IV = 38.9, P = 0.0020), *Physcia aipolia* (Ehrenb. ex Humb.) Fürnrohr (IV = 40.3, P = 0.0060) and *Ramalina celastri* (Sprengel) Krog & Swinscow (IV = 47.5, P = 0.0250).

3.6. Environmental variables and Correlation analysis

Along the analyzed period, the highest peak observed for traffic flow was registered at station E29, counting 179,069 vehicles in a sampled day, considering high traffic levels were also registered for medium vehicles (21,656), heavy vehicles (20,565) and motorbikes (14,349) at this station. The highest register for light vehicles (124,064) was observed at station E4.

Positive and significant correlations were registered between ECF data and richness ($r = 0.856$, $p < 0.001$), cover (0.825, $p < 0.001$), diversity ($r = 0.776$, $p < 0.001$) and fruticose morphological groups ($r = 0.663$, $p < 0.001$). Positive correlations were observed for crustose

morphological groups (r = 0.253, p< 0.178). Negative correlations were registered for foliose (r = - 0.182, p< 0.335), micro-foliose (r = - 0.169, p< 0.372) and squamulose (r = - 0.448, p< 0.013) morphological groups, in addition to traffic flow (r = - 0.051, p< 0.827). Considering IAP data, positive and significant correlations were found for richness (r = 0.964, p< 0.001), cover (0.873, p< 0.001) and diversity (r = 0.915, p< 0.001) whereas negative correlations were found for squamulose morphological groups (r = - 0.514, p< 0.001). Positive results were found for crustose (r = - 0.271, p< 0.001) and fruticose (r = 0.343, p< 0.063) morphological groups whereas foliose (r = - 0.163, p< 0.388) and micro-foliose (r = - 0.082, p< 0.665) morphological groups as well as traffic flow (r = - 0.098, p< 0.673) registered negative results. Positive correlations were verified between traffic flow and species richness (r = 0.006, p< 0.980) as well as for foliose (r = - 0.123, p< 0.596) and micro-foliose (r = - 0.099, p< 0.668) morphological groups, whereas cover (r = - 0.212, p< 0.357), diversity (r = - 0.023, p< 0.922) and crustose (r = - 0.114, p< 0.622), squamulose (r = - 0.061, p< 0.792) and fruticose (r = - 0.016, p< 0.945) morphological groups registered negative values.

## 4. Discussion

The composition of corticolous lichen community resulted differentiated in terms of species composition, revealing a higher number of taxa (131) if compared to other studies carried out in urban-industrial areas (Loppi et al., 2002, Gombert et al., 2004; Canseco et al., 2006; Saipunkaew et al., 2007; Calvelo et al., 2009). Marcelli (1998) estimates 150 lichen species are expected to exist in Brazilian urban areas. Martins et al. (2008) registered 45 taxa for an urban-industrial area in the metropolitan region of Porto Alegre and, Martins-Mazzitelli et al. (1999) describes 72 taxa in the city of Porto Alegre.

In urban areas, lichen community is not as diverse as in natural environments and is usually composed of species which tolerate the excess of light and wind. The genus *Parmotrema* associated with species from Pyxinaceae family and some crustose species are typical in urbanized areas (Marcelli, 1998). Broadly speaking, lichen landscapes are mainly dominated by Parmeliaceae and Physciaceae, while *Parmotrema*, *Heterodermia*, *Hypotrachyna* and *Canoparmelia* are characteristic genera of lichen communities in all areas. Family Parmeliaceae represents the highest species dominance and richness (Marcelli, 1998), and are also characteristic of highly illuminated areas. The largest species diversity related to this family in the analyzed areas corroborates to the studies carried out in the State (Käffer and Martins-Mazzitelli, 2005; Martins, 2006; Martins et al., 2008), as well as may be considered as one of the most studied families. Physciaceae is typified as the second most abundant family in number of species in Brazil (Marcelli, 1998). Family Graphidaceae occurs specially in forests of tropical and subtropical regions around the world, on the trunks and twigs of trees and bushes (Staiger et al, 2006), usually in less-illuminated areas (Marcelli, 1998). Members of this family were frequent in the studied areas, especially on genus *Graphis*.

The highest frequency of some species such as *Dirinaria picta* and *Canoparmelia texana*, found in the current study, corroborates to other studies (Saipunkaew et al., 2005; Martins et al., 2008). *Dirinaria picta* is a widely distributed species in continental areas, occurring in natural forests, parks and avenues (Swinscow and Krog, 1988). It has been considered the pioneer species in a community study carried out in restinga areas in southern Brazil (Martins 2006). *Canoparmelia texana* is a species limited to branches and twigs in forest environments in Brazil and rarely occurs in phorophytes exposed to luminosity conditions. In urban areas, however, due to the absence of more aggressive competing species, it occupies their spaces with thallus growth and, consequently, reaches large trunk areas (Marcelli, 1998). The frequency (100% and 86.7%) of these two species in the studied area may be strongly related

to the thallus reproduction. Both reproduce directly, through soredia. De Sloover and Le Blanc (1968) report that sulfur dioxide ($SO_2$) is known to stimulate the production of soredia and isidia. Another common taxon at sampling stations was *Anisomeridium* sp., considered a cosmopolitan species in tropical and temperate regions, with a greater diversity in the tropics (Aptroot, 2002). In the current study it occurred in 86.7% of the sampling areas and showed a medium cover level. Thus, it may be considered that *Dirinaria picta*, *Canoparmelia texana* and *Anisomeridium* sp. are taxa that characterize the urban area of Porto Alegre.

The results of richness, cover and diversity of species at the sampling stations clearly demonstrate the structure differences in the lichen community. The highest percentages of richness and diversity were registered at the reference station, in the State Park, and at station E25, which is a district characterized by the predominance of residences and low traffic flow (15,140 vehicles/day), while the lowest values of such parameter, including cover, were registered at stations E2 and E15 (Fig. 5), where traffic flow is intense (67,216 and 53,662 vehicles/day, respectively) and possibly because their geographic locations are influenced by atmospheric pollutants originating from the surrounding areas, especially station E15. Such station is located near industrial towns. The morphological groups at the stations also followed patterns of distribution similar to those obtained for richness and cover data of the species. The highest percentage of foliose lichens was registered at station E2, while fruticose lichens were absent in 66.7% of the analyzed areas. The highest percentage of the latter group was found at station E30, characterized by the predominance of small rural properties and/or leisure areas, characterized by low rates of traffic flow (12,515 vehicles/day). Crustose lichens were the most frequent, registered in 93.3% of the sampling areas. The highest percentage was found at station E29. Widely speaking, foliose lichens have an average sensitivity to pollution, while fruticose lichens are more sensitive and crustose lichens are more resistant (De Sloover and Le Blanc, 1968; Wetmore, 1981). Studies carried out in urban areas in Argentina, Bolivia, Costa

Rica, Thailand and Italy report low species richness and diversity in central areas with a stronger anthropic influence, especially in those where traffic flow is more intense (Monge-Nájera et al., 2002; Calvelo and Liberatore, 2004; Saipunkaew et al., 2005, 2007; Canseco et al. 2006; Calvelo et al., 2009).

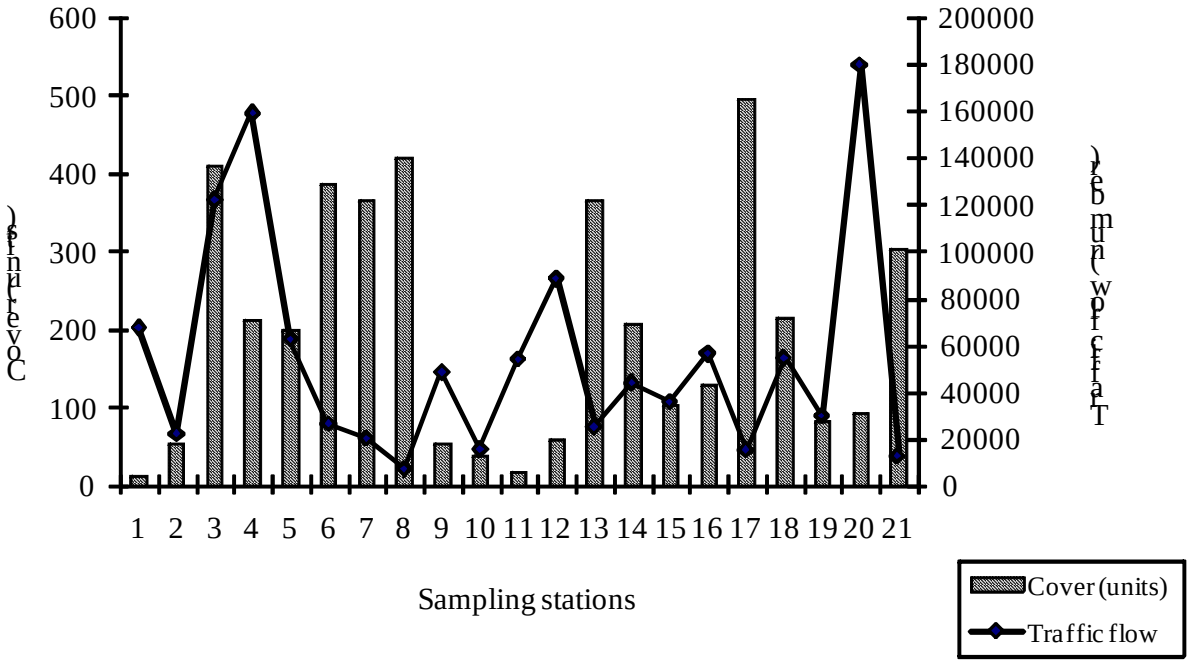


Figure 5. Relationship between cover of lichen species and traffic flow at the sampling stations. Caption: E1-E30 defined in Table 2.

When evaluating data through original IAP it is possible to observe that all sampling stations fall within the scope of transition zones considered excellent for the development of lichenized mycota. However, stations such as E2, E15, E13 and E12 show species richness and cover values featuring a higher percentage of crustose and foliose morphological groups considered resistant or mid-tolerant to pollution. Thus, these stations were classified based on IAP (complemented by ECF) as lichen-poor or lichen-absent zones, considering the presence of features such as species richness and cover and, most important, the species composition due to the dominance of taxa belonging to crustose and foliose morphological groups. A

similar behavior was registered when original IAP and an ECF complement were compared at stations classified as normal or excellent for lichen development (Tables 3 and 4). Based on the original IAP, for example, stations E3, E11, E23 and E29, located in central and northern areas of the city, were classified as excellent and normal for the development of lichen, whereas if based on the ECF they were classified as a poor or transition zones. These areas offer a higher potential for the accumulation of atmospheric pollutants along the year and are characterized as built-in and/or depression areas, of scarce vegetation and intense vehicle flow. Due to such features, the dispersion conditions of pollutants become hindered specially when associated with surface thermal inversions. According to Lima et al. (1998), the surface thermal inversion phenomenon may be characterized by significant air cooling at the surface at night, especially in depression areas, resulting in great instability of air at the surface and, consequently, making the dispersion of air pollutants difficult. The combination of such factors in dense urban areas results in the increase of local air temperatures if compared to the surrounding areas, whereas the opposite occurs in greener regions with a lower population density (Hasenack and Ferraro, 1998; Ferraro and Hasenack, 2000). As a result, the use of a complement as the IAP correction factor (ECF) enables a classification of sampling stations in accordance with factors such as richness, cover and especially taxa composition, reflecting not only the real lichenized mycota for such areas, but also the conditions for its development. Along with such aspects, lichen salubrity is at jeopardy in these areas as lichens suffering with parasites as well as necroses and/or chlorosis have been registered, especially in foliose taxa. Although different IAP versions have been used by various researchers resulting in successful relations, it is observed that for environments characterized by higher species richness and differentiation of taxon composition, such as those found in South America, an adjustment in the use of IAP is deemed necessary, such as the ECF complement developed in the current study.

In relation to indicator taxa in urban areas, the present study registered a predominance of species belonging to the crustose and foliose morphological groups, distributed along the sampling stations classified by ECF as normal, lichen-poor and lichen-absent zones.

As for environmental variables, the highest differences were registered for traffic flow. Livi et al. (1998) report that one of the first premises for the evaluation of pollutant dispersion conditions in the atmosphere of a big city is the identification of intense traffic roads, namely those with a minimum flow of 40.000 vehicles in a 12-hour period. Other important factors are natural obstacles (geographical depression areas) or built-up obstacles which may obstruct or impair the efficient and constant dispersion of such pollutants. In the current study, 30% of the analyzed areas registered an increased rate of traffic flow, especially in the central area of the city.

The alterations observed in the lichen community in Porto Alegre and in the reference area, such as the large number of crustose taxa, the decrease or even absence of the fruticose group, are indicative that some city areas are suffering with the effects of accelerated growth, especially factors such as intense traffic flow and property expansion. Nimis (1986) affirms that pollution affects microclimate and consequently the presence of lichens in urban areas. Around 30% of the analyzed stations were characterized as lichen-absent or lichen-poor areas, whereas only 13.3% were considered excellent areas, including here the reference area, where lichens enjoy a better development and are more exuberant. Loppi et al. (2002) report that urban environments are highly complex and both air pollution and lichen species are influenced by factors such as the area topography and climate. Thus, in addition to an intense traffic flow, factors such as the topography of the stations and aspects related to the landscape structure, such as the existence of parks and a larger number of tree-lined streets, for example, result in the formation of microclimates in urban areas. The results observed show that the lichen community turns out to be sensitive when changes of such urban microclimates are

evaluated. Rydzark (1968) considers climatic adversities in urban areas may influence the distribution of certain species in anthopized areas. Hawksworth et al. (2005) reports that sensitive species may be absent due to the shortage of vegetative propagules other than to the contamination levels. In this study, the following variables revealed low correlations with the lichenized mycota: traffic flow and characteristics of phorophytes. On the other hand, ECF revealed significant results in relation to the fruticose morphological group, considered as sensitive to atmospheric pollutants.

The current study demonstrates that corticolous lichens are efficient organisms to evaluate the atmospheric quality associated to urban microclimates and may serve as a tool in monitoring programs in cities. However, the use of IAP in its original formula must be used carefully, since results may be masked, especially if lichen community is not considered as a whole. In this case, the application of a correction factor such as the implementation of the ECF, developed in the present study, would complement the use of this index, making IAP more sensitive, considering the need to analyze a multivaried information profile, especially in regions where species diversity is higher. The present study also points out the importance of an urban monitoring plan with the use of equipments and bioindicator organisms for implementing air quality programs.

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## 4. Artigo 3

# Evaluation of lichen community in urban area, southern Brazil, in relation to different environmental factors [*]

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ABSTRACT

Lichens are organisms widely used to evaluate the air quality, especially in urban areas. The main objective of the current paper was to analyze the lichen community in relation to environmental factors. Lichens have been mapped on 300 phorophytes in order to evaluate the influence of surface bark pH and the presence of acidophytic and nitrophytic species as well as to verify differences in the vertical distribution of species, lichen thallus fragmentation and possible preferences of taxa for phorophytes. One hundred thirty one lichen taxa have been identified, out of which 13.7% represent nitrophytic species, 12.2% represent acidophytic species and 74.1% other species. Significant differences have been observed in terms of vertical distribution, the amount of generalist and specialist in height species as well as thallus fragmentation, average lichen size and availability for specimen fixation on phorophytes among the sampled stations. The current paper has evidenced that lichen community has been modified and that different environmental factors have contributed for the results obtained. Climate changes, air pollution, topography of the sampled stations and landscape structure may also have influenced the results. The study recommends the use of lichen as bioindicators in urban areas.

Keywords: Bark pH, Bioindicators, Lichen, Phorophytes, Vertical distribution

## 1. Introduction

Lichens are considered bioindicator organisms, being widely used by different researchers in order to monitor environmental changes in urban-industrial areas, especially air pollution (Hawksworth and Rose, 1976; Calvelo and Liberatore, 2004; Jovan and McCune, 2005; Gombert et al., 2004, 2006; Washburn and Culley, 2006; Saipunkaew et al,. 2007; Calvelo et al., 2009; McCarthy et al,. 2009).

Lichens were first acknowledged as possible air quality bioindicators in the beginning of the 19$^{th}$ century and sulphur dioxide was the main factor influencing growth, distribution and salubrity of species (Hawksworth et al., 2005). Currently, in addition, a wide range of other components (such as fluorides, metal, radioactive metal, polycyclic aromatic hydrocarbon and particulate matter) as well as eutrophication and acid rain may be detected and monitored by using lichens.

Among the effects air pollution may cause to lichen communities are inhibition of growth and development of thallus, changes in the metabolic processes, anatomical and morphophysiological changes (Barkman, 1958; Baddeley et al., 1973; Coppins, 1973; Gries, 1996; Schlensog and Schroeter, 2001; Glavich and Geiser, 2008). However, in addition to those, factors such as substrate features (Hale, 1957; Brodo, 1973; Jesberger and Sheard, 1973; Hawksworth and Hill, 1984; Marcelli, 1996; Schmidt et al., 2001), macro and micro nutrient composition of phorophyte barks (Hawksworth, 1975), luminosity, humidity (Honegger, 1996; Brunialti and Giordani, 2003; Martinez et al., 2006) and acidness or alkalinity of phorophyte barks (Brodo, 1973) also affect the distribution and establishment of the lichenized mycota. The pH levels may be critical for the reproduction of several specimens (Hale, 1957) and differences in the levels of bark pH may inhibit the establishment of organisms and propitiate nitrophytic and acidophytic lichens. Lichens present a higher sensitivity to ammonia and changes in the composition of lichenized mycota suggest that, over time, the exposition to low concentrations (3 g/m$^3$ to 8 g/m$^3$) may affect species and possibly conduce to extinction (Van

Herk et al., 2003; Cape et al., 2009). The increasing presence of nitrophytic species in urban areas has also been accredited to Nitrogen Oxides ($NO_x$) (Van Dobben and Ter Braak, 1999; Van Herk, 2001; Wolseley et al., 2005; Frati et al., 2007; Berthelsen et al., 2008; Cape et al., 2009). The presence or absence of such species may indicate the eutrophication level both in urban and forest areas (Van Herk, 2001; Wolseley et al., 2006; Sparrius, 2007; Fleig and Grüninger, 2008; Pinho et al., 2008; Hauck, 2010). Moreover, whereas some species are tolerant to increasing levels of atmospheric nitrogen, others are highly sensitive, including many high conservation value species, pointed out as ecological continuity indicators (Copins and Copins, 2002).

In Brazil, few studies relate lichen community structures to environmental factors and the existent ones are restricted to non-urban areas (Marcelli, 1992; Martins, 2006; Cáceres et al., 2008; Fleig and Grüninger, 2008; Käffer et al., 2009, 2010). Hence, the present paper aims at: (i) Analyzing the relationship between surface pH of phorophyte barks and the lichen community, indicating acidophytic and nitrophytic species at the sampled stations in the city of Porto Alegre and in a reference area in the city of Viamão, Rio Grande do Sul, Brazil; (ii) Verifying how vertical distribution of species and its occurrence in relation to phorophytes may be altered by air pollution; (iii) Investigating the presence of generalist and specialist in height species at the different sampled stations; (iv) Verifying whether differences exist as for fragmentation and average thallus height of lichen species at the sampled stations; (v) Verifying the existence of site availability for lichen fixation and preferences for specific types of phorophyte barks.

## 2. Material and Methods

### 2.1. Study Area

The city of Porto Alegre encompasses an area of 496.8 $km^2$, of which 30% represents rural area and is located in the Central Depression region, at 51º 01’ and 51º 16’W and 29º 57’ and 30º 16’S, on Lake Guaiba, in the state of Rio Grande do Sul, Brazil. The region is characterized by a humid subtropical climate, with an annual average temperature of 19.4°C, average relative humidity of 76% and annual average rainfall of 1.324 mm and predominant southeastern winds (Ferraro and Hasenack, 2000). As for the vegetation found in the urban area, there are around one million trees comprising more than 200 species, including Brazilian and regional native species as well as from other countries and continents, such as ***Ligustrum japonicum*** Thunb., ***Jacaranda mimosaefolia*** Don., ***Tabebuia chrysotricha*** (Mart. ex DC.) Standl., ***T. avellanedae*** Lor. ex Griseb, among others (Sanchotene et al., 1998). The study also includes the State Park of Itapuã, in the city of Viamão (50º 50’ and 51º 05’W and 30º 20’ and 30º 27’S), in the metropolitan region of Porto Alegre, 57 km away, as a reference area.

The study was undertaken during the period between July 2007 and June 2008 at 30 sampling stations divided into the 33 city districts, as follows: E1 (66º79’W 48º45’S), E2 (66º76’W 48º04’S), E3 (66º78’W 47º92’S), E4 (66º75’W 48º29’S), E5 (66º77’W 47º92’S), E6 (66º72’W 47º89’S), E7 (66º73’W 48º18’S), E8 (66º72’W 48º74’S), E9 (66º63’W 48º11’S), E10 (66º83’W 48º17’S), E11 (66º80’W 48º56’S), E12 (66º77’W 48º28’S), E13 (66º83’W 48º39’S), E15 (66º83’W 48º78’S), E16 (66º78’W 48º92’S), E17 (66º71’W 48º21’S), E18 (66º74’W 47º85’S), E19 (66º69’W 47º53’S), E20 (66º71’W 47º78’S), E21 (66º57’W 48º19’S), E22 (66º67’W 48º26’S), E23 (66º78’W 48º23’S), E24 – Jardim Itu-Sabará (66º79’W 48º62’S), E25 (66º79’W 47º749’S), E26 (66º77’W 48º20’S), E27 (66º76’O 48º57’S), E28 (66º80’O 48º38’S), E29 (66º76’W 47º71’S), E30 (66º61’W 47º98’S) and a

reference area E14 – State Park of Itapuã located in the city of Viamão (66º43’’W 49º64’S), summing up 30 stations (Fig. 1).

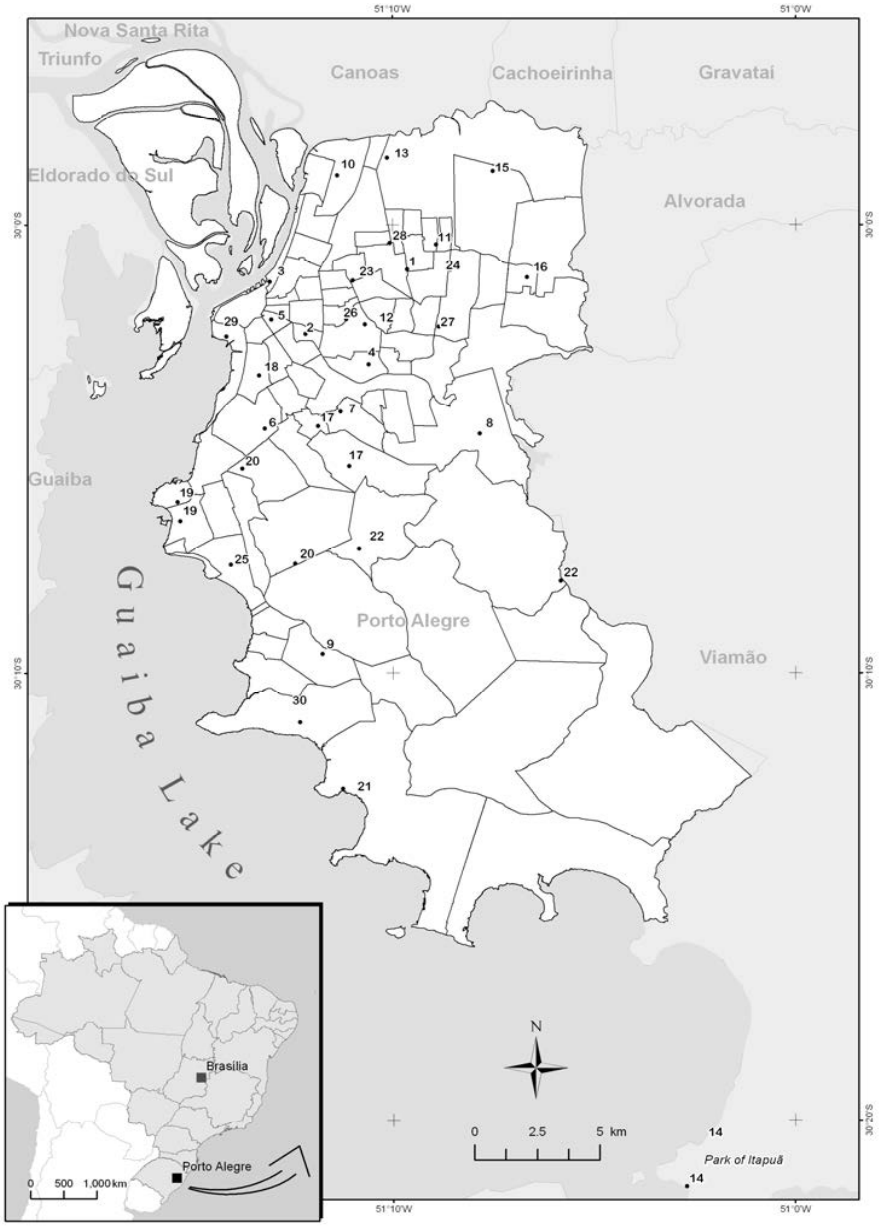


Figure 1. Map of Porto Alegre, RS, Brazil and representation of sampled stations.

2.2. Sampling and identification

Lichens were bordered from 50 cm to 150 cm high from the ground for each selected phorophyte and at each station using the elastic method (Marcelli, 1992). All the species that reached the elastic were identified in loco or collected for posterior confirmation. The identification of specimens was carried out with the help of stereoscopic and optical microscopes, through anatomical sections of the thallus and fructifications, color tests and additional techniques usually used in lichen taxonomy. Collected material is herborized and catalogued at Herbarium Prof. Dr. Alarich Schultz (HAS) at the Museum of Natural Sciences of the Zoobotanic Foundation of RS, Brazil.

2.3. Phorophytes characterization

Three hundred phorophytes with erect trunk, without ramifications, under 150 cm high, diameter at breast height (DBH) over 20 cm and classified according to type and superficial pH of barks were sampled. They were selected near parks and/or squares in the city, preferably of the same species. The bark of each phorophyte specie was classified into either furrowed or smooth using specific bibliography for each species.

The identification of phorophyte species followed the APG II system (2003). The pH of bark surfaces in phorophytes was verified through a portable digital pH meter, model PH-1700 – Instrutherm, after lichen mapping (Käffer et al., 2009). The values of surface bark pH were classified into acidic (0 a 6.9); neutral (7.0) and alkaline (7.1 a 14) (Raven et al., 2001).

2.4. Environmental variables

During the lichen sampling period daily concentrations of pollutants responsible for urban pollution, such as Particulate Matter ($PM_{10}$) and Ozone ($O_3$),were obtained at three stations: E2, E3 and E4 (which meet the environmental monitoring protocol carried out by

Environmental Protection Foundation of Rio Grande do Sul (FEPAM), but do not comprise all districts evaluated in the present study); climate data: temperature (ºC), relative humidity of air (%), and precipitation (mm), apart from the total number of vehicles circulating in the nearby areas of the sampled stations. The information about traffic flow is related to punctual data corresponding to a one-day sample data and divided into four different categories: light-vehicles (cars and small vans), medium-vehicles (mini and micro buses), heavy-vehicles (trucks and buses) and motorbikes. Data about pollutants was provided by automatic and manual nets of the FEPAM, climate data was provided by the National Institute of Meteorology – 8° District, whereas traffic flow data was provided by the Public Company of Transportation and Circulation (EPTC) in Porto Alegre.

## 2.5 Data analysis

In order to analyze the existent relationship between surface bark pH of phorophytes and the lichen community, the following data were used: phorophyte richness, surface bark pH values and richness of lichenized mycota in each phorophyte. Lichens were classified into acidophytic (surface bark pH between 0 and 6.9), nitrophytic (surface bark pH between 7.1 and 14), and indifferent (found at any pH level).

To investigate possible differences in the vertical distribution of species among the different sampled stations, two aspects were taken into consideration: 1) if lichen richness changed according to height in a different way at each of the 30 sampled stations; and 2) if differences among the sampled stations existed as for how species were more specialists or generalists at a specific height category. In this case, categories represent the difference between the maximum and minimum height of the trunk fixation. Height class specialists occur in only one fixation-amplitude category, whereas generalists occur in different categories.

To analyze if lichens richness changed at 11 height levels (from 50 cm to 150 cm high from the ground), richness values of species found at each level in all sampled phorophytes were used. Richness values were then compared to those from the other stations, considering height, diameter at breast height and the DBH x Height interaction as independent variables in a General Linear Model (GLM). Possible differences in the amount of generalist or height class specialists were verified by comparing the frequency distribution graphs of species found at one or more height levels. Differences in average thallus size of lichens on trunks at the different sampled stations were analyzed by using the total sum of size of each thallus fragment found on the trunk of each phorophyte divided by the total amount of fragments. The estimated average thallus size was compared at each sampled station through ANOVA.

In order to investigate the existence of available sites for lichen fixation, average values of DBH and surface bark pH of phorophytes were evaluated through ANOVA, whereas the differences in the characteristics of phorophyte barks were evaluated through the Contingency Table.

Spearman correlation analysis between richness of acidophytic, nitrophytic and indifferent taxa were carried out at the sampled stations, as well as between fragmentation and lichen thallus average size and the following environmental variables were considered: surface bark pH of phorophytes, climate factors (temperature, relative humidity of air and precipitation) and traffic flow as well as the correlation between the surface bark pH and traffic flow. The correlation analysis between the previous mentioned factors and the pollutant concentration variables ($MP_{10}$ e $O_3$) were carried out only at the stations where monitoring existed. All statistical analysis were carried out by using the statistics software Systat 10 (2000) package.

## 3. Results

### 3.1. Relationship between surface bark pH and lichens

One hundred thirty-one lichen taxa have been registered, of which 16 have been classified as acidophytic, 18 as nitrophytic and 97 as indifferent, considering they occurred in phorophytes presenting acid, alkaline or neutral pH (Table 1). Acidophytic species presenting the highest frequency at the sampled stations were: ***Parmotrema muelleri*** (Vain.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch, ***Enterographa*** sp. and ***Leptogium azureum*** (Sw.) Mont., whereas nitrophytic species were: ***Graphis*** sp.7., ***Graphis dupaxana*** Vain. and ***Lecanora*** sp. 1. Stations presenting the highest frequency of nitrophytic lichen species were respectively E14, E1, E4, E5 and E7, whereas the highest frequency of acidophytic species was found at stations E8 and E27

Table 1. List of acidophytic, nitrophytic and indifferent lichen registered at the sampled stations, Porto Alegre, Brazil.

| Taxa | Bark pH | | | Habit |
|---|---|---|---|---|
| | Aciddophytic | Nitrophytic | Indifferent | |
| *Canoparmelia* sp. 3 | x | | | fol |
| *Cladonia atthi* S. Stenroos | x | | | squa |
| *Enterographa* sp. | x | | | cr |
| *Hypotrachyna livida* (Taylor) Hale | x | | | fol |
| *Lecanora* aff. *albella* (Pers.) Ach. | x | | | cr |
| *Lecanora* grupo *subfusca* | x | | | cr |
| *Leptogium austroamericanum* (Malme) Dodge | x | | | fol |
| *Leptogium azureum* (Sw.) Mont. | x | | | fol |
| *Normandina pulchella* (Borrer) Nyl. | x | | | squa |
| *Parmotrema flavomedullosum* Hale | x | | | fol |

Table 1. continuation

| Taxa | Bark pH | | | Habit |
|---|---|---|---|---|
| | Aciddophytic | Nitrophytic | Indifferent | |
| *Parmotrema muelleri* (Vain.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch | x | | | fol |
| *Parmotrema pilosum* (Stizenb.) Krog & Swinscow | x | | | fol |
| *Physcia lacinulata* Müll.Arg. | x | | | fol |
| *Physcia stellaris* (L.) Nyl. | x | | | fol |
| *Physcia undulata* Moberg | x | | | fol |
| *Usnea* sp. | x | | | fr |
| *Bactrospora* sp. | | x | | cr |
| *Canoparmelia* sp. 2 | | x | | fol |
| *Cratiria americana* (Fée) Kalb & Marbach | | x | | cr |
| *Graphis dupaxana* Vain. | | x | | cr |
| *Graphis* sp. 6 | | x | | cr |
| *Graphis* sp. 7 | | x | | cr |
| *Heterodermia* cf. *albicans* (Pers.) Swinscow & Krog | | x | | fol |
| *Lecanora* sp. 1 | | x | | cr |
| *Lecanora* sp. 2 | | x | | cr |
| *Opegrapha*sp. 2 | | x | | cr |
| *Parmotrema catarinae* Hale | | x | | fol |
| *Parmotrema eciliatum* (Nyl.) hale | | x | | fol |
| *Parmotrema haitiense* (Hale) Hale | | x | | fol |
| *Parmotrema subcaperatum* (Kremp.) Hale | | x | | fol |
| *Platygramme* aff. *arthonioides* (Vainio) Zahlbr. | | x | | cr |
| *Punctelia riograndensis* (Lynge) Krog | | x | | fol |
| *Ramalina aspera* Räsänen | | x | | fr |
| *Rinodina* sp. 2 | | x | | cr |
| *Anisomeridium* sp. | | | x | cr |
| *Arthonia* sp. | | | x | cr |
| *Baculifera* sp. | | | x | cr |
| *Brigantiaea leucoxantha* (Spreng.) R. Sant. & Hafellner | | | x | cr |
| *Bulbothrix* sp. | | | x | fol |
| *Bulbothrix isidiza* (Nyl.) Hale | | | x | fol |
| *Caloplaca* sp. | | | x | cr |
| *Candelaria concolor* (Dicks.) Stein | | | x | fol |
| *Canoparmelia carneopruinata* (Zahlbr.) Elix & Hale | | | x | fol |
| *Canoparmelia caroliniana* (Nyl.) Elix & Hale | | | x | fol |
| *Canoparmelia* sp.1 | | | x | fol |
| *Canoparmelia texana* (Tuck.) Elix & Hale | | | x | fol |
| *Carbacanthographis* sp. | | | x | cr |
| cf. *Lepraria* sp. | | | x | squa |
| *Chapsa* sp. | | | x | cr |
| *Coccocarpia pellita* (Ach.) Müll.Arg. ex R.Sant. | | | x | fol |
| *Coenogonium* sp. | | | x | cr |
| *Cratiria* sp. | | | x | cr |

Table 1. continuation

| Taxa | Bark pH | | | Habit |
|---|---|---|---|---|
| | Aciddophytic | Nitrophytic | Indifferent | |
| *Dirinaria applanata* (Fée) D. D. Awasthi | | | x | fol |
| *Dirinaria confluens* (Fr.) D. D. Awashti | | | x | fol |
| *Dirinaria picta* (Sw.) Schaer. ex Clem. | | | x | fol |
| *Fissurina* sp. | | | x | cr |
| *Glyphis cicatricosa* Ach. | | | x | cr |
| *Glyphis scyphulifera* (Ach.) Staiger | | | x | cr |
| *Graphis* sp. 1 | | | x | cr |
| *Graphis caesiocarpa* Redinger | | | x | cr |
| *Graphis* sp. 2 | | | x | cr |
| *Graphis* sp. 3 | | | x | cr |
| *Graphis* sp. 4 | | | x | cr |
| *Graphis librata* C. Knight. | | | x | cr |
| *Graphis* sp. 5 | | | x | cr |
| *Graphis pavoniana* Fée | | | x | cr |
| *Graphis* sp. 8 | | | x | cr |
| *Graphis* sp. 9 | | | x | cr |
| *Haematomma* sp. | | | x | cr |
| *Herpothallon rubrocinctum* (Ehrenb.) Aptroot & Lücking | | | x | cr |
| *Heterodermia albicans* (Pers.) Swinscow & Krog | | | x | fol |
| *Heterodermia diademata* (Taylor) Awasthi | | | x | fol |
| *Heterodermia obscurata* (Nyl.) Trevis. | | | x | fol |
| *Heterodermia speciosa* (Wulf.) Trevis. | | | x | fol |
| *Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt | | | x | fol |
| *Hyperphyscia cochlearis* Scutari | | | x | fol |
| *Hyperphyscia syncolla* (Tuck.) Kalb | | | x | fol |
| *Hypotrachyna polydactyla* (Krog & Swinscow) Nash | | | x | fol |
| *Lecanora* aff. *achroa* Nyl. | | | x | cr |
| *Lecanora* sp. 3 | | | x | cr |
| *Lecanora* cf. *argentata* (Ach.) Malme | | | x | cr |
| *Lecanora* cf. *symmicta* (Ach.) Ach. | | | x | cr |
| *Lecanora concilianda* Vain | | | x | cr |
| *Leptogium denticulatum* Nylander | | | x | fol |
| *Malcolmiella* sp. | | | x | cr |
| *Myelochroa lindmanii* (Lynge) Elix & Hale | | | x | fol |
| *Ochrolechia pallescens* (L.) A.Massal. | | | x | cr |
| *Opegrapha* sp. 1 | | | x | cr |
| *Opegrapha* sp. 3 | | | x | cr |
| *Parmelinopsis minarum* (Vainio) Elix & Hale | | | x | fol |
| *Parmotrema austrosinense* (Zahlbr.) Hale | | | x | fol |
| *Parmotrema cetratum* (Ach.) Hale | | | x | fol |
| *Parmotrema consors* (Nyl.) Krog & Swinscow | | | x | fol |
| *Parmotrema homotomum* (Nyl.) Hale | | | x | fol |

Table 1. continuation

| Taxa | Bark pH | | | Habit |
|---|---|---|---|---|
| | Aciddophytic | Nitrophytic | Indifferent | |
| *Parmotrema melanothrix* (Mont.) Hale | | | x | fol |
| *Parmotrema mesotropum* (Müll.Arg.) Hale | | | x | fol |
| *Parmotrema praesorediosum* (Nyl.) Hale | | | x | fol |
| *Parmotrema recipiendum* (Nyl.) Hale | | | x | fol |
| *Parmotrema reticulatum* (Taylor) M. Choisy | | | x | fol |
| *Parmotrema subsumptum* (Nyl.) Hale | | | x | fol |
| *Parmotrema tinctorum* (Nyl.) Hale | | | x | fol |
| *Pertusaria* sp. | | | x | cr |
| *Pertusaria flavens* Nyl. | | | x | cr |
| *Phaeographis* sp. | | | x | cr |
| *Phaeographis lecanographa* (Nyl.) Staiger | | | x | cr |
| *Phaeographis lobata* (Eschw.) Müll. Arg. | | | x | cr |
| *Phaeographis* sp. | | | x | cr |
| *Phaeographis subtigrina* (Vain.) Zahlbr. | | | x | cr |
| *Phyllopsora* sp. | | | x | squa |
| *Physcia aipolia* (Ehrenb. ex Humb.) Fürnrohr | | | x | fol |
| *Physcia alba* (Fée) Müll.Arg. | | | x | fol |
| *Physcia atrostriata* Moberg | | | x | fol |
| *Physcia crispa* Nyl. | | | x | fol |
| *Physcia krogie* Moberg | | | x | fol |
| *Physcia poncinsii* Hue | | | x | fol |
| *Platygramme caesiopruinosa* (Fée) Fée | | | x | cr |
| *Platygramme* sp. | | | x | cr |
| *Porina* sp. | | | x | cr |
| *Punctelia constantimountium* Sérus. | | | x | fol |
| *Punctelia* sp. | | | x | fol |
| *Pyrenula* sp. | | | x | cr |
| *Pyxine berteriana* (Fée) Imsh. | | | x | fol |
| *Pyxine cocoës* (Sw.) Nyl. | | | x | fol |
| *Pyxine subcinerea* Stirt. | | | x | fol |
| *Ramalina celastri* (Sprengel) Krog & Swinscow | | | x | fr |
| *Ramalina complanata* (Sw.) Ach. | | | x | fr |
| *Ramalina peruviana* Ach. | | | x | fr |
| *Ramboldia haematites* (Fée) Kalb | | | x | cr |
| *Rinodina* sp. 1 | | | x | cr |
| *Tephromella americana* (Fée) Kalb | | | x | cr |
| *Trypethelium nitidiusculum* (Nyl.) R. C. Harris | | | x | cr |

cr = crustose, squa = squamulose, fo = foliose, fr= fruticose, microf = microfoliose.

3.2. Vertical distribution of lichens on phorophytes

Significant differences have been registered in terms of richness of lichen taxa, between the height levels in phorophytes and the sampled stations (Table 2). In 63.3% of the stations,

the amount of species was homogeneously distributed at the different height levels. Station E2 distinguished itself for the absence of taxa below 110, whereas at stations E13, E15, E28, E29 and E30 most species occurred from 130cm to 150cm from the ground (Fig. 2).

Table 2. ANOVA table for the relationship between lichen richness and trunk height. Variation sources represent Height (fixation height of lichen on the trunk); Stations (30 sampled stations).

| Richness | | | | | |
|---|---|---|---|---|---|
| Source | df | SS | MS | F | P |
| Height | 1 | 404.25 | 404.250 | 63.77 | 0.00 |
| Sampling stations | 28 | 2666.56 | 95.234 | 15.02 | 0.00 |
| DBH | 1 | 1.505 | 1.505 | 0.24 | 0.63 |
| Sampling stations x Height | 29 | 946.85 | 32.650 | 5.15 | 0.00 |
| Error | 270 | 1711627 | 6.339 | | |

df – degrees of freedom, SS = Sum-of-Squares, MS = Mean-Square, F = ration, P =

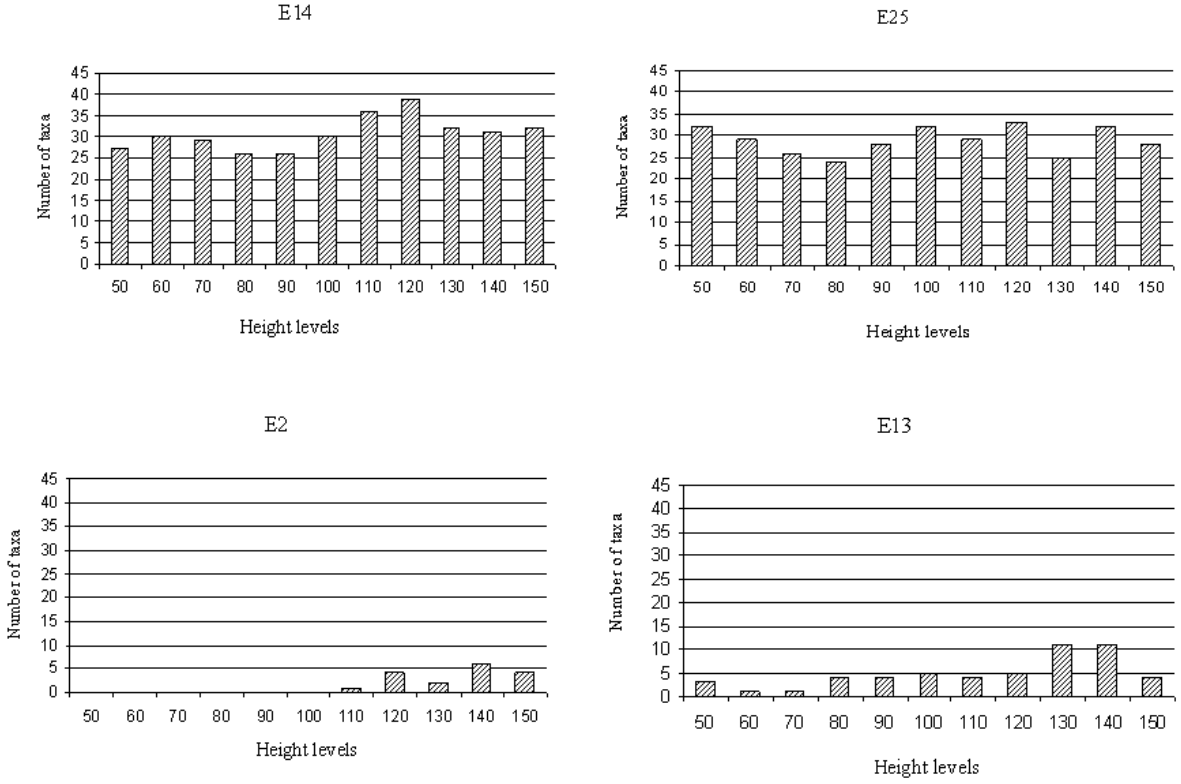


Figure 2. Richness of species registered at different height levels of phorophyte trunks at the sampled stations.

The amount of generalist and specialist in height species was also different at the different sampled stations. Around 46% of the stations presented generalist species at different height levels. The remaining stations presented a greater amount of height class specialist lichen taxa and at stations E7, E11, E12, E13, E15 and E28 more than 10 specimens occurred only at a specific height level (Fig. 3).

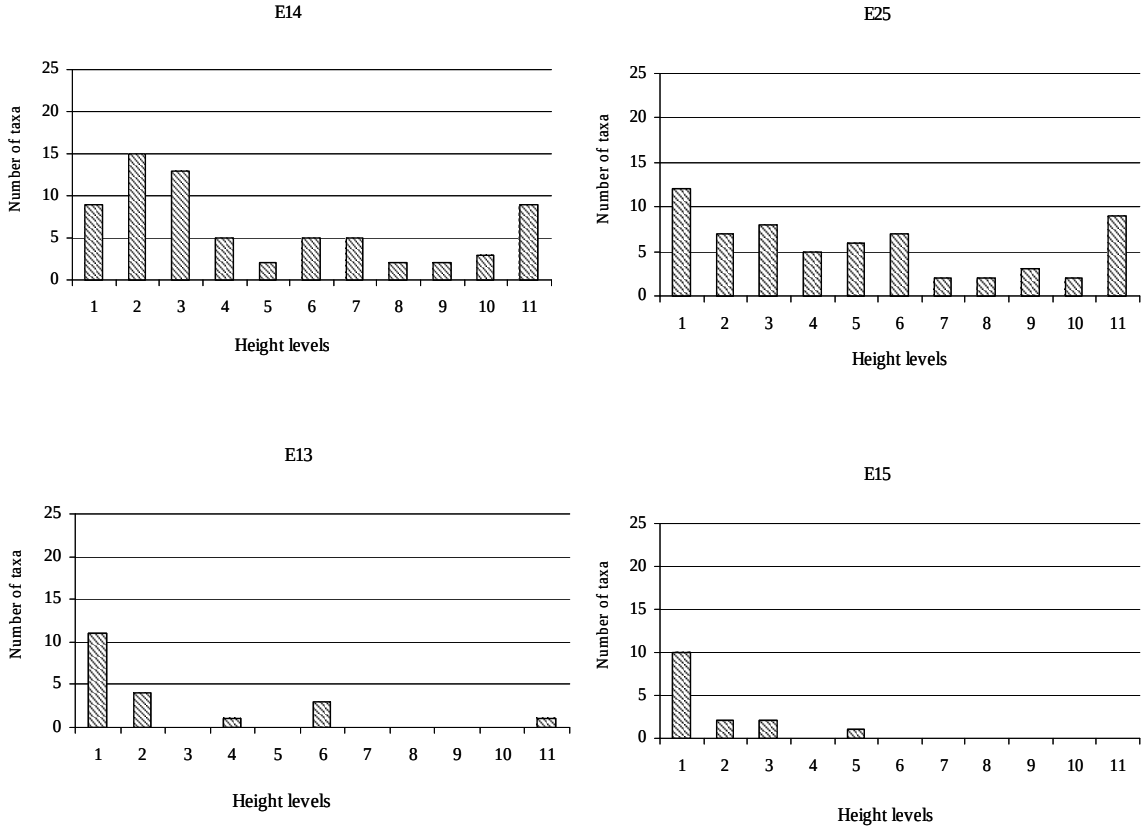


Figure 3. Richness of lichen species registered in fixation amplitude categories on phorophyte trunks at the sampled stations.

3.3. Fragmentation and size of lichens on trunks

Analyses for the evaluation of possible variations in terms of fragmentation and average size of lichen species on the trunks of phorophytes demonstrated the occurrence of significant differences at the different sampled stations ($F= 10.30$; $gl = 29,270$; $P< 0.001$) and ($F= 3.49$; gl

= 29,270; P< 0.001). Larger and more numerous fragments have been reported at stations E25, E4, E14 and E9, whereas smaller fragments have been reported at stations E2 and E15.

3.4. Phorophyte characteristics

Three hundred phorophytes, divided into eight species and seven families, have been analyzed (Fig. 4), of which ***Peltophorum dubium*** (Spreng.) Taub. presents the highest frequency (41.3%). The DHB of phorophytes varied from 33.7 to 42.4, whereas the average pH varied from 6.9 to 7.2. Out of all sampled individuals, 49.7% presented an acid surface bark pH, 45.7% presented alkaline pH and 4.6% presented neutral pH. The highest frequency of individuals with acid pH was registered at stations E20, E10 and E29, whereas stations E7, E13, E28 and E14 presented a predominance of individuals with alkaline pH.

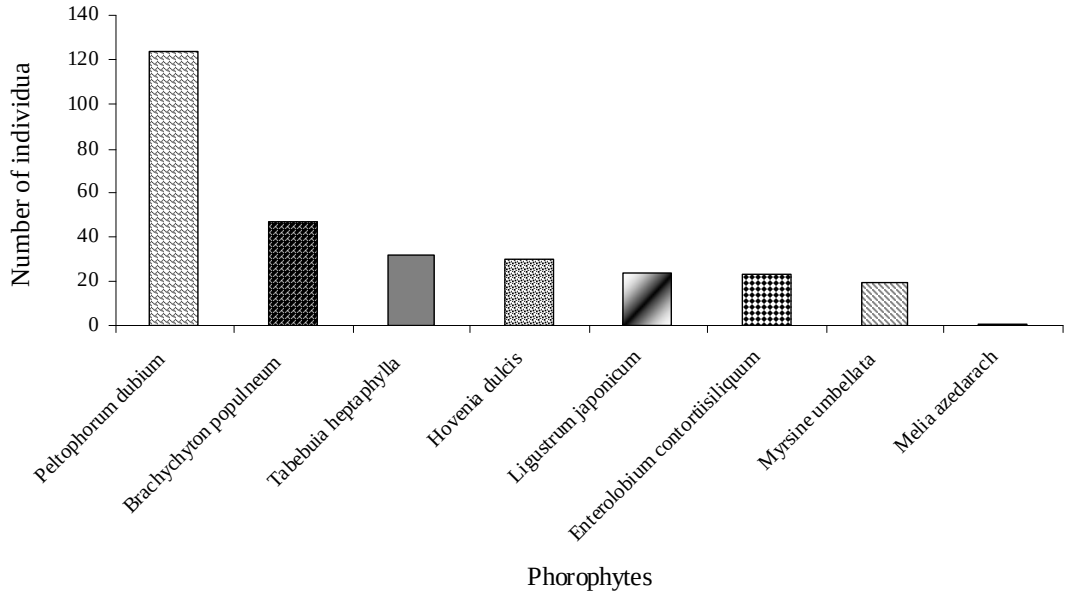


Figure 4. Frequency distribution of phorophytes at the sampled stations.

The analyses of availability for fixation sites registered significant differences between the sampled stations and the surface bark pH (F = 4.27; gl = 29, 270, P< 0.001) and between the

stations and the DHB of phorophytes (F = 3.51; gl = 29, 270, P< 0.001). Differences have also been registered as for the bark structure of phorophytes at the sampled stations. Furrowed barks occurred at a lower frequency than expected at stations E7, E15, E20, E21, E22, E24, E25, E29 and E30. As for smooth bark phorophytes, a higher frequency than expected occurred at stations E6, E12, E15, E20, E22, E24, E25, E29 and E30 (Fig. 5A, 5B).

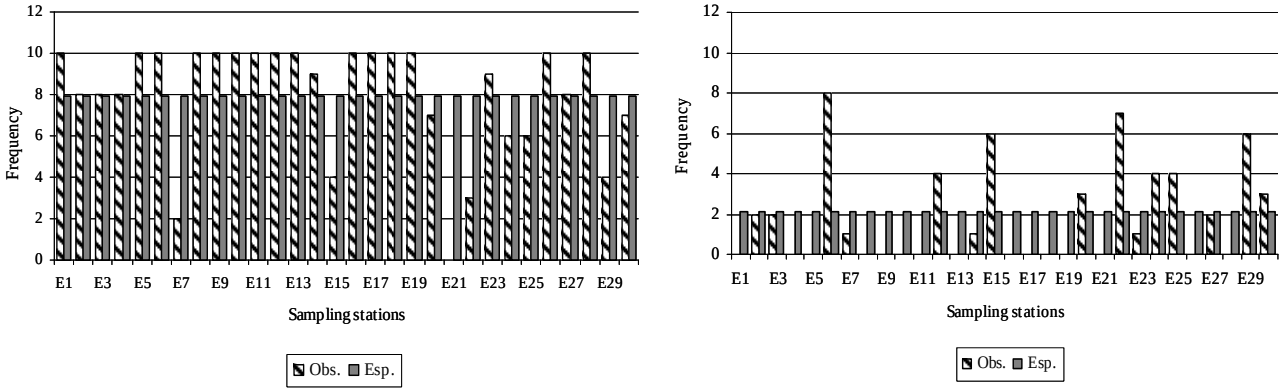


Figure 5. Occurrence frequency of furrowed (A) and smooth (B) bark structure in different phorophytes at the sampled stations

3.5. Environmental variables and Correlation analysis

The results of pollutant concentration associated with urban air pollution are in accordance with the standards demanded by Brazilian legislation (National Council for the Environment, CONAMA, 1992) as well as by the World Health Organization (OMS, 2006). Of the climate variables: temperature, relative humidity of air and precipitation, only precipitation varied, considering low volumes have been registered if compared to levels registered in the years prior to the samples. As for vehicle flow, the highest register occurred at station E29 with 179,069 vehicles in a one-day sampling. This same station also registered high flow rates of

medium-size vehicles (21,656), heavy vehicles (20,565) and motorcycles (14,349). Station E4 registered the highest flow rate of light vehicles (124,064).

The results of correlation analysis comparing richness of acidophytic, nitrophytic and indifferent taxa, fragmentation and average size of lichen thallus at the sampled stations and the environmental variables and climate factors as well as the surface bark pH were not significant and varied between positive and/or negative values (Table 3). However, when stations E2, E3 and E4 are analyzed (considering data on pollutants concentration), significant correlations are found when comparing the richness of acidophytic species and the concentrations of $O_3$ ($r = 0.866$, $P = <0.05$), $PM_{10}$ ($r = -0.866$, $P = <0.05$); the richness of nitrophytic species and the concentration of $O_3$ ($r = 0.866$, $P = <0.05$) and $PM_{10}$ ($r = -0.866$, $P = <0.05$); the richness of indifferent species, fragmentation and average thallus size and the concentration of $PM_{10}$ ($r = -1.00$, $P = <0.05$), as well as the surface bark pH and the concentrations of $O_3$ ($r = 0.866$, $P = <0.05$) and $PM_{10}$ ($r = -1.00$, $P = <0.05$).

Table 3. Correlation analysis of richness of acidophytic, nitrophytic and indifferent lichen taxa, vertical distribution, fragmentation and average size of lichen thallus, surface bark pH and environmental variables.

| Environmental variables | pH of the bark surface | Traffic flow | Temperature | UR |
|---|---|---|---|---|
| Richness of acidophytic taxa | r = -0.087 | r = -0.032 | r = 0.114 | r = -0.309 |
| Richness of nitrophytic taxa | r = 0.414 | r = -0.023 | r = -0.111 | r = -0.251 |
| Richness of indifferent taxa | r = 0.048 | r = 0.079 | r = -0.050 | r = -0.150 |
| Vertical distribution | r = 0.027 | r = -0.188 | r = -0.508 | r = 0.295 |
| Fragmentation lichen thallus | r = 0.008 | r = -0.087 | r = 0.023 | r = -0.195 |
| Average size of lichen thallus | r = -0.143 | r = -0.066 | r = 0.102 | r = -0.167 |
| Surface bark pH | | r = -0.011 | | |

$P < 0.05$
Caption: UR = relative humidity of air

**4. Discussion**

Various works carried out in European urban-industrial areas establish a relationship between the predominance of nitrophytic lichen species and decrease of acidophytic species and the amounts of ammonia and other nitrogenated components originating from the traffic flow and/or agricultural and industrial activities (Van Dobben and Ter Braak, 1998; Van Herk, 2001; Frati et al., 2006, 2007; Sparrius et al., 2007; Cristofolini et al., 2008; Pinho et al., 2008; Svoboda et al., 2010), as well as from the surface bark pH of phorophytes (Van Herk ,1999). In the current study, the occurrence of nitrophytic (13.7%) and acidophytic (12.2%) species was not relevant and the highest frequency (74%) was found for taxa known as indifferent. Species classified as nitrophytic by the bibliography, such as *Candelaria concolor* (Dicks.) Stein, *Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt e *Physcia aipolia* (Ehrenb. ex Humb.) Fürnrohr, were registered at the sampled stations only in substrates in which surface

bark pH varied from acid to alkaline, here classified as indifferent. A higher occurrence of indifferent taxa in the lichen community indicates they have no restrictions to develop in certain substrates and are well adapted to different acid or alkaline levels of phorophytes. The lichen species which colonize indifferent hosts tend to present a greater distribution due to the greater substrate offer (Valencia and Ceballos, 2002). As for the surface bark pH of phorophytes, an increase of its levels is related to an increasing concentration of atmospheric ammonia (Van Herk, 1999), or dust (Kricke and Loppi, 2002). Variations in the concentration of nitrogen ions in the phorophytes barks may influence the development of lichen species and favor the establishment of nitrophytic lichen (Kermit and Gauslaa, 2001; Wolseley et al., 2006; Fleig and Grüninger, 2008). As for the sampled stations, E14 registered the highest number of nitrophytic species, probably because of agricultural and grazing activities in the region, though this area may be influenced by the atmospheric pollution originating from other anthropogenic sources. Due to their morphophysiological structure, lichens largely depend on atmospheric nutrient deposition, especially nitrogen supply. In rural environments, the main source of nitrogen deposition is ammonia and, in urban environments, near highways, nitrogen oxides (Hargreaves et al., 1992; Pitcairn et al., 1995; Gilbert et al., 2003; Larsen Vilsholm et al,. 2009). Nitrogen oxides result from the oxidation of nitrogen in certain fuels, such as mineral coal and oil (Gilbert et al., 2003). Traffic contributes to the deposition of both ammonia and nitrates (Gadson and Power, 2009). Vehicle emissions may result in high concentrations of nitrogen and influence the composition of species (Frati et al., 2006). By this mean, the association of factors such as climate variations and anthropogenic activities, such as air pollution, may have influenced the occurrence of nitrophytic species in the analyzed areas.

Some differences have been noticed at the different sampled stations when evaluating the vertical distribution of lichen species, the occurrence of generalist species and specialist in

height species, as well as fragmentation and average size of lichen thallus, which demonstrates changes in the structure of the urban lichen community, especially at stations E2, E12, E13 and E15. Käffer et al. (unpublished data) relates that at these stations lower percentages of richness and coverage of species have also been registered, though such places were classified as lichen-desert or lichen-poor areas. These areas are located in regions with higher potential for the accumulation of atmospheric pollutants along the year, being characterized as either edified or depression regions, featuring scarce vegetation and heavy traffic flow. Due to these features, the dispersion conditions of pollutants are in disadvantage, especially when associated with thermal inversions of the surface. According to Lima et al. (1998), the phenomenon of thermal inversion of the surface is characterized by a significant nocturne cooling of the air layer near the surface, especially in depression regions, resulting in great air instability near the surface and, consequently, making the dispersion of air pollutants difficult.

The changes registered in the structure of the lichen community at the sampled stations may also be associated with temperature differences in different areas of the city, as a result of urban forestry. In urban areas, heat islands are formed and are characterized by alterations related to changes in the usual temperatures of the land surface, winds and evaporation rates as well as in the additional heat caused by human activity. The combination of the previous factors in dense urban areas results in an increase of the local air temperature when compared to the surrounding areas, whereas in regions with lower population densities and larger green areas the opposite occurs (Hasenack and Ferraro, 1998; Ferraro and Hasenack, 2000). The impacts of urban environment include changes in the structure and morphology of the lichen community (Gries, 1996). The structural and morphophysiological effects include reduction of the thallus size, reduction or total loss of the apothecium in some taxa, increase in the number of vegetative propagules, reduction in the amount of individual taxa (probably as a result of thallus decrease) and frequency, along with the total loss of sensitive species (Le Blanc and

Rao, 1973; Sigal and Nash, 1983; Zambrano and Nash, 2000; Scutari and Theinhardt, 2001; Fremstad et al., 2005; Glavich and Geiser, 2008). Variations in the vertical distribution of lichen species are frequently accredited to differences in luminosity and humidity conditions to which phorophytes are submitted (Hale, 1957; Harris, 1972; Sipman and Harris, 1989; Marcelli, 1992; Fleig and Grüninger, 2008), as well as to the inclination of their trunks in the environment (Moe and Botnen, 2000; Cristofolini et al., 2008). Some lichen groups tend to dominate specific height levels, resulting in different composition of species on the trunks (Moe and Botnen, 2000).

In relation to site availability for lichen fixation, there are only a few studies in Brazil about the structure of phorophytes and lichen community, which are limited to non-urban environments (Marcelli, 1992, Cáceres et al., 2008; Fleig and Grüninger, 2008; Käffer et al., 2009, 2010). In the current study, features such as DBH, surface bark pH and bark structure resulted in a huge contribution due to the structure of the lichenized mycota community at the sampled areas. The results found corroborate with other works carried out in urban-industrial areas (Mitchell et al., 2005; Frati et al., 2007; Washburn and Culley, 2006; Larsen et al., 2007; Cristofolini et al., 2008). Factors such as atmospheric pollution and bark structure of phorophytes have possibly contributed to variations in the surface bark pH as well as to the composition of lichen species, standing out that the greater number of lichen-absent individuals was registered at stations E2 and E15. At these stations, species ***Brachychyton populneum*** Schott and ***Enterolobium contortiisiliquum*** (Vell.) Morong registered the highest rate of absence of lichen taxa, whereas at other stations these same species registered from one to five lichen species, which demonstrates the existence of microclimatic regions within the city. Besides, atmospheric pollution may have directly influenced the found results. In polluted areas, the bark pH of specific phorophyte specie may vary widely, with differences reaching high and low pH values at the different sample units. Alongside, bark properties may change

under the effects of pollution, consequently changing the establishment of the lichenized mycota (Otnyukova and Serkretenko, 2008). However, we cannot only consider one pollutant as the cause of surface bark pH variations in phorophytes, since they may be influenced by other different factors. According to Larsen et al. (2007), it is necessary to consider the synergy effect between different pollutants, the bark pH and the interactions with climate factors. Besides, bark pH may vary according to the tree species, age and local conditions in which phorophytes are found (Wolseley et al., 2005).

Another factor that may have contributed for the differences found at each sampled stations and the lichen development is the characteristics of the phorophytes barks. Changes in the substrate texture are one of the most obvious effects for an easy colonization of lichen species (Brodo, 1973). However, such lichen specificity for the substrate may also be related to other variables, such as porosity and water retention on the phorophytes barks (Jesberger and Sheard, 1973; Kuusinen, 1996; Schmidt et al., 2001). Changes in the physical-chemical properties of the bark might influence the establishment of species (Ellis and Coppins, 2007). As trunk barks grow older, they sustain micro-niches due to variations in the exposure and inclination of the trunk as well as to the bark structure (James et al., 1977). Urban environments are highly complex and different emission sources secrete a variety of pollutants, making it difficult to determine which factor is influencing the lichen community. In this study it was found that many factors contributed to structural changes in the lichen community and that the influence of climate variations, specially at some stations, the topography of some areas, atmospheric pollution, landscape structure, bark surface pH and structure, besides the DBH of phorophytes, contributed for the obtained results. Thus, the use of lichens as indicators of alterations in urban environments are recommended, specially for monitoring programs. Yet, one must be conscious to evaluate a group of factors that may modify the structure of a lichen community, apart from specific atmospheric pollutants.

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## 5. Artigo 4

# Caracterização da comunidade liquênica corticícola de Porto Alegre e áreas adjacentes, RS, Brasil [*]

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**ABSTRACT** - (Characterization of the corticolous lichen community from Porto Alegre and adjacent areas, RS, Brazil). Lichens are symbiotic organisms found in a great variety of habitats, however, in urban areas the lichen community can be changed due to the influence of anthropogenic factors. This study aims to analyze the corticolous lichen community composition in Porto Alegre and adjacent areas, as well as to investigate the frequency, coverage and importance value of the reported taxa, providing a key for the urban species. Three hundred phorophytes distributed in 30 sampling stations divided among 33 city districts were analyzed. One hundred forty-four lichenized fungi taxa were recorded with three probable new citations for Brazil and two new records for Rio Grande do Sul State. The most important species in the community were *Canoparmelia texana* (Tuck.) Elix & Hale and *Dirinaria picta* (Sw.) Schaer. ex Clem, which showed the highest values of importance, frequency and coverage. The high number of taxa found in the studied areas represents a significant contribution to lichenological research, mainly for use in air quality monitoring programs and to evaluate forest ecosystems structure.

**Key words**: species composition, identification key, lichenized fungi, phytosociology

**Introdução**

Liquens são organismos simbiontes muito diversos, desenvolvendo-se em lugares extremos, sejam em áreas florestais, de monoculturas, industriais e/ou urbanas. Estima-se que o número de espécies de liquens conhecidas mundialmente varie de 13.500 a 20.000 (Sipman & Aptroot 2001).

Em áreas urbanas a comunidade liquênica é menos diversificada do que em ambientes naturais, sendo estimado para o Brasil em torno de 150 espécies, sendo geralmente constituída por espécies tolerantes ao excesso de iluminação e ao/de vento. Os gêneros ***Parmotrema*** A. Massal. e ***Rimelia*** Hale & Fletcher, associados a espécies da família Pyxinaceae e a algumas espécies crostosas são típicos de áreas urbanizadas. Em muitas áreas centrais do Brasil, espécies dos gêneros ***Pyxine*** Fries e ***Canoparmelia*** Elix & Hale aparecem com alta freqüência. Em locais menos urbanizados, com estradas não asfaltadas e próximas de matas naturais, são registradas espécies fruticosas de gêneros como ***Usnea*** Dill. ex Adans., ***Ramalina*** Ach. e ***Teloschistes*** Norman, além dos cianoliquens do gênero ***Leptogium*** (Ach.) Gray.

Para o Estado do Rio Grande do Sul já foram registrados 912 espécies de fungos liquenizados (Spielmann 2007), sendo que os primeiros estudos com a comunidade liquênica foram realizados a partir de 1900, com os trabalhos de Malme e Redinger que incluíram coletas nas áreas de Porto Alegre (Martins-Mazzitelli ***et al***. 1999). Ainda para a cidade de Porto Alegre e região metropolitana existem também as contribuições de Fleig (1985); Fleig & Medeiros Filho (1990); Osório ***et al.*** (1980); Osório & Fleig (1988) e Osório ***et al***. (1997). Foram registrados 72 táxons para Porto Alegre por Martins-Mazzitelli ***et al***. (1999), sendo nove espécies consideradas novos registros para o Estado.

Este trabalho teve por objetivos: ***i)*** analisar a composição da comunidade liquênica corticícola de Porto Alegre e áreas adjacentes da cidade; ***ii)*** verificar a freqüência, cobertura

e valor de importância dos táxons identificados; *iii)* e apresentar uma chave para identificação dos táxons registrados na área de estudo.

**Material e métodos**

A cidade de Porto Alegre abrange uma área de 496.8 km$^2$, sendo 30% de área rural, e está localizada na região da Depressão Central, entre as coordenadas 30°01'S e 51°13'W, às margens do lago Guaíba, no estado do Rio Grande do Sul, Brasil. O clima é subtropical úmido apresentando temperatura média anual de 19,4°C, umidade relativa média do ar de 76% e índice pluviométrico de 1.324 mm anuais (Livi, 1998). O estudo ainda incluiu como área de referência, o Parque Estadual de Itapuã localizado no município de Viamão (50º 50' e 51º 05'W e 30º 20' e 30º 27'S) área metropolitana de Porto Alegre, distante 57 km dessa capital.

O trabalho foi realizado no período de julho de 2007 a junho de 2008 em 30 estações de coletas, 29 distribuídas em 33 bairros da cidade, sendo estas: E1 - Chácara das Pedras, E2 – Santa Cecília, E3 – Centro 1, E4 – Jardim Botânico, E5 – Bom Fim, E6 - Santa Tereza, E7 – Partenon, E8 – Agronomia, E9 – Hípica, E10 – Humaitá, E11 – Jardim Lindóia, E12 – Petrópolis, E13 - Anchieta, E15 – Sarandi, E16 – Jardim Leopoldina, E17 – Cascata/Glória, E18 – Menino Deus, E19 – Tristeza/Vila Assunção, E20 - Nonoai/Vila Nova, E21 – Belém Novo, E22 – Lomba do Pinheiro/Belém Velho, E23 – Higienópolis, E24 – Jardim Itu-Sabará, E25 – Ipanema, E26 – Bela Vista, E27 – Bom Jesus, E28 – Passo d’ Areia, E29 – Centro 2, E30 – Ponta Grossa, e, uma área referência E14 localizada no município de Viamão (Fig. 1).

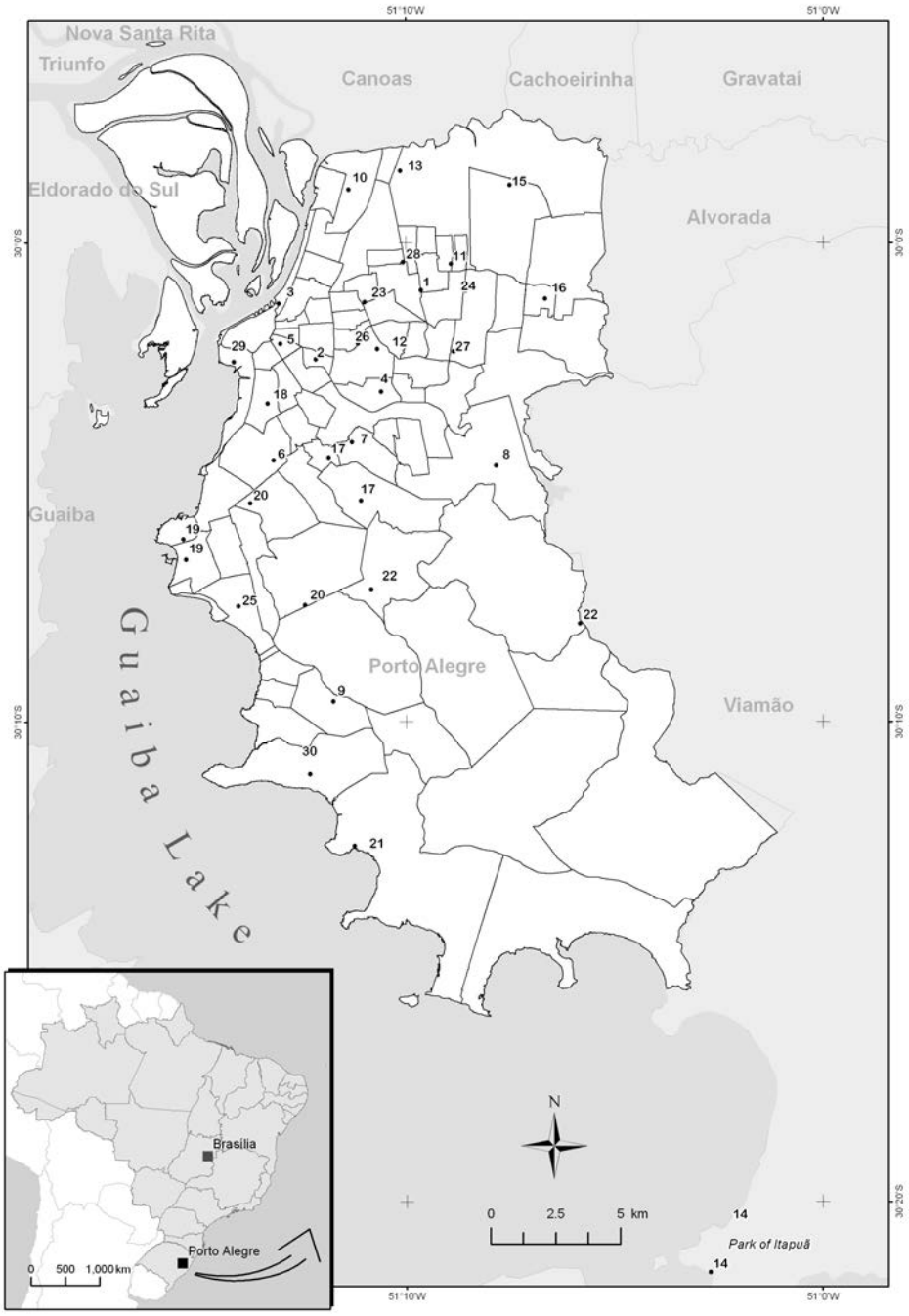


Figura 1. Mapa da cidade de Porto Alegre, RS, Brasil com representação das estações amostradas.

Para o estudo da comunidade liquênica foram amostradas 10 forófitas de troncos eretos em cada estação analisada, que não apresentassem ramificações abaixo de 150 cm e com diâmetro a altura do peito (DAP) acima de 20 cm, totalizando 300 forófitas. Estas foram selecionadas próximas a parques e/ou praças da cidade.

Para o mapeamento da micobiota liquenizada foi utilizado o método do elástico (Marcelli 1992), onde os liquens foram demarcados a partir de 50 cm acima do solo até 150 cm de altura para cada forófita selecionada. Todas as espécies que tocaram o elástico foram identificadas no campo, ou coletadas para posterior confirmação.

A identificação dos espécimes foi realizada com auxílio de microscópicos estereoscópico e óptico, com a observação de características macroscópicas e de secções anatômicas dos talos e das estruturas de reprodução. Foram analisados caracteres como cor e aspecto do talo, comprimento e largura dos lobos, presença de picnídios e aspecto das rizinas, cílios e apotécios. Testes de coloração com hidróxido de potássio (K), hipoclorito de sódio (água sanitária comercial, teste C), parafenilenodiamina (P) e lugol (I), e de fluorescência por lâmpada de luz ultravioleta de ondas longas (UV – comprimento de onda 366 nm) foram utilizados para averiguar a presença de substâncias no córtex, medula, himênio, asco e ascósporos. Foi utilizada bibliografia especializada para cada grupo taxonômico e consultados o material constante no herbário Prof. Dr. Alarich Schultz (HAS) do Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul, Brasil, sendo que o material coletado neste estudo se encontra herborizado e catalogado no próprio HAS.

A estimativa da freqüência foi baseada na presença/ausência das espécies nos troncos das forófitas nas estações de amostragens, enquanto que a cobertura de cada espécie foi calculada através da somatória de todas as unidades do elástico da espécie identificada, em todos os níveis que compreende uma faixa entre 50 a 150 cm de altura, nas 10 forófitas amostradas em cada estação (Käffer *et al*. 2011). O valor de importância (VI) de cada espécie foi calculado

em relação à comunidade como um todo somando-se os dados de freqüência e cobertura relativa.

A elaboração da chave de identificação foi baseada em diversas bibliografias (Arvidsson 1982; Swinscow & Krog 1988; Galloway 1985; Kashiwadani & Kalb 1993; Fleig *et al*. 1995; Scutari 1995; Cunha 2007; Fleig & Medeiros Filho 1990; Sipman 2002; Canêz & Marcelli 2006; Frisch 2006; Benatti & Marcelli 2007; Cáceres 2007; Fleig & Grüninger 2008; Lücking & Rivas Plata 2008; Spielmann & Marcelli 2008; Dal Forno 2009; Rivas Plata *et al.* 2010), utilizando caracteres das espécies ocorrentes na área de estudo. Em função do grande número de táxons identificados, as espécies dos gêneros dos grupos foliosos e crostosos que possuem mais de uma espécie serão apresentados em trabalhos futuros.

**Resultados e Discussão**

Foram identificados 144 táxons distribuídos em 53 gêneros de 24 famílias, e duas espécies pertencentes ao grupo dos fungos liquenizados imperfeitos. Três espécies são prováveis novos registros para a ciência: *Graphis* aff. *archerii* Dal-Forno & Eliasaro, *Hypotrachyna* aff. *punoensis* Kurok. e *Physcia* cf. *sinuosa* Moberg e, dois novos registros para o Rio Grande do Sul: *Coccocarpia stellata* Tuck., e *Parmelinopsis subfatiscens* (Kurokawa) Elix & Hale. Além destas, outras 27 espécies crostosas encontradas na área foram recentemente citadas como novas ocorrências para o Brasil e para o Rio Grande do Sul (Käffer *et al*. 2010b). Do total de táxons registrados, 45,8% são liquens crostosos, 43% foliosos (incluindo talos gelatinosos), 4,2% fruticosos, 4,2% microfoliosos e 2,8% esquamulosos. A família com maior número de táxons foi Parmeliaceae (38 espécies), seguido de Physciaceae (30), Graphidaceae (27) e Lecanoraceae (12), enquanto que os gêneros com maior representatividade do total de espécimes encontrados na área de estudo foram *Parmotrema* (13,2%), *Graphis* (9,7%),

*Physcia* (6,9%) e *Lecanora* (6,2%). Quanto ao fotobionte, 96,5% são liquenizados com clorofíceas e 3,5% com cianobactérias.

A maior riqueza (número total de espécies liquênicas ocorrentes nos forófitos analisados em cada estação) de espécies encontrada neste estudo demonstra uma adaptabilidade da micobiota liquenizada perante as condições ambientais dos grandes centros urbanos, incluindo fatores como luminosidade, umidade, tipo de forófita, assim como a poluição atmosférica. Trabalhos realizados em regiões urbano-industriais reportam baixa riqueza e diversidade de espécies para áreas similares (Scutari & Theinhardt 2001; Gobert *et al*. 2004; Saipunkaew *et al*. 2007; Calvelo *et al*. 2009).

Liquens crostosos associados a espécies foliosas das famílias Parmeliaceae e Physciaceae são característicos em áreas urbanas (Marcelli 1998). No presente estudo a maior presença de espécies crostosas corrobora com trabalhos realizados em áreas urbanas com influências industriais (Scutari & Theinhardt 2001; Monge-Najera *et al*. 2002; Saipunkaew *et al.* 2005). Em torno de 75% das espécies de liquens conhecidas atualmente formam talos crostosos (Ahmadjian 1993), entretanto este grupo ainda é pouco estudado no país, comumente passando despercebido de observadores em campo especialmente devido ao tamanho reduzido, os talos geralmente com poucos centímetros ou mesmo milímetros de diâmetro.

Quanto à composição da micobiota liquenizada em áreas urbanas do país, a mesma é caracterizada pela predominância de espécies das famílias Parmeliaceae e Physciaceae, especialmente dos gêneros *Parmotrema*, *Hypotrachyna*, *Canoparmelia* e *Heterodermia* (Marcelli 1998).

Nas áreas amostradas há predominância de liquens com clorofíceas, que têm preferência por ambientes luminosos, enquanto que a baixa ocorrência de liquens com cianobactérias está normalmente associada a ambientes úmidos e sombreada. Neste estudo, foi verificada baixa diversidade de espécies liquênicas com cianobactérias, sendo que espécimes do gênero

*Leptogium* representaram 2,1%, enquanto que *Coccocarpia* obteve 1,4% do total de táxons registrados. A ocorrência de *Leptogium* em ambientes urbanos pode ser devida a sua adaptabilidade a diferentes tipos de ambientes (Wolseley 1991), sendo que seus talos em geral crescem nas partes mais úmidas de vários tipos de ambientes, e o grau de especificidade apresentada em relação ao substrato é variável de acordo com a espécie (Sierk 1964; Swinscow & Krog 1988).

Nas análises de fitossociologia da comunidade liquênica, as espécies com maior valor de importância foram *Canoparmelia texana* (Tuck.) Elix & Hale (20,05), *Dirinaria picta* (Sw.) Schaer. ex Clem. (20,04), *Anisomeridium tamarindii* (Fée) R. C. Harris (11,52) e *Lecanora* cf. *symmicta* (Ach.) Ach. (9,94) responsáveis por 30,8% do VI total ($\sum$ = 200). Estas espécies também apresentaram maior valor de riqueza e cobertura na comunidade. Embora *Dirinaria picta* tenha ocupado o segundo lugar em termos de valor de importância, esta espécie apresentou maior riqueza (180). Na tabela 1 se encontram os táxons registrados para as áreas organizadas em ordem decrescente de Valor de Importância (VI).

Tabela 1. Dados fitossociológicos dos táxons liquênicos corticícolas nas áreas amostradas na cidade de Porto Alegre e nas áreas adjacentes, RS, Brasil.

| Táxons | Riqueza | CA | CR% | FA | FR% | VI |
|---|---|---|---|---|---|---|
| *Canoparmelia texana* (Tuck.) Elix & Hale | 154 | 939,1 | 15,01 | 0,51 | 5,04 | 20,05 |
| *Dirinaria picta* (Sw.) Schaer. ex Clem. | 180 | 885,2 | 14,15 | 0,60 | 5,89 | 20,04 |
| *Anisomeridium tamarindii* (Fée) R. C. Harris | 131 | 452,4 | 7,23 | 0,44 | 4,29 | 11,52 |
| *Lecanora* cf. *symmicta* (Ach.) Ach. | 129 | 357,6 | 5,72 | 0,43 | 4,22 | 9,94 |
| *Glyphis cicatricosa* Ach. | 103 | 261,8 | 4,19 | 0,34 | 3,37 | 7,55 |
| *Dirinaria confluens* (Fr.) D. D. Awashti | 103 | 132,7 | 2,12 | 0,34 | 3,37 | 5,49 |
| *Canoparmelia carneopruinata* (Zahlbr.) Elix & Hale | 76 | 186,5 | 2,98 | 0,25 | 2,49 | 5,47 |
| *Physcia aipolia* (Ehrenb. ex Humb.) Fürnrohr | 72 | 146,7 | 2,35 | 0,24 | 2,36 | 4,70 |
| *Phaeographis lobata* (Eschw.) Müll. Arg. | 49 | 190,0 | 3,04 | 0,16 | 1,60 | 4,64 |
| *Heterodermia albicans* (Pers.) Swinscow & Krog | 72 | 135,7 | 2,17 | 0,24 | 2,36 | 4,52 |
| *Punctelia* sp. | 59 | 154,3 | 2,47 | 0,20 | 1,93 | 4,40 |
| *Graphis parallela* (Müll. Arg.) Cáceres & Lücking | 80 | 102,3 | 1,64 | 0,27 | 2,62 | 4,25 |
| *Dirinaria applanata* (Fée) D. D. Awasthi | 64 | 124,1 | 1,98 | 0,21 | 2,09 | 4,08 |
| *Pertusaria flavens* Nyl. | 73 | 82,9 | 1,33 | 0,24 | 2,39 | 3,71 |
| *Myelochroa lindmanii* (Lynge) Elix & Hale | 55 | 115,1 | 1,84 | 0,18 | 1,80 | 3,64 |
| *Parmotrema tinctorum* (Nyl.) Hale | 55 | 101,4 | 1,62 | 0,18 | 1,80 | 3,42 |
| *Heterodermia diademata* (Taylor) Awasthi | 60 | 85,2 | 1,36 | 0,20 | 1,96 | 3,32 |
| *Lecanora concilianda* Vain. | 64 | 76,9 | 1,23 | 0,21 | 2,09 | 3,32 |
| *Ochrolechia pallescens* (L.) A.Massal. | 61 | 63,7 | 1,02 | 0,20 | 2,00 | 3,01 |
| *Lecanora* aff. *achroa* Nyl. | 50 | 84,5 | 1,35 | 0,17 | 1,64 | 2,99 |
| *Graphis caesiocarpa* Redinger | 57 | 56,5 | 0,90 | 0,19 | 1,86 | 2,77 |
| *Canoparmelia caroliniana* (Nyl.) Elix & Hale | 46 | 73,8 | 1,18 | 0,15 | 1,50 | 2,68 |
| Grupo *Lepraria* | 45 | 73,5 | 1,18 | 0,15 | 1,47 | 2,65 |
| *Pyxine subcinerea* Stirt. | 48 | 60,1 | 0,96 | 0,16 | 1,57 | 2,53 |
| *Pertusaria carneola* (Eschw.) Müll. Arg. | 55 | 40,8 | 0,65 | 0,18 | 1,80 | 2,45 |
| *Physcia poncinsii* Hue | 42 | 55,2 | 0,88 | 0,14 | 1,37 | 2,26 |
| *Parmotrema austrosinense* (Zahlbr.) Hale | 39 | 51,9 | 0,83 | 0,13 | 1,28 | 2,11 |
| *Parmotrema reticulatum* (Taylor) M. Choisy | 32 | 65,7 | 1,05 | 0,11 | 1,05 | 2,10 |
| *Arthonia* sp. | 39 | 47,6 | 0,76 | 0,13 | 1,28 | 2,04 |
| *Glyphis scyphulifera* (Ach.) Staiger | 39 | 32,7 | 0,52 | 0,13 | 1,28 | 1,80 |
| *Tephromella americana* (Fée) Kalb | 36 | 37,6 | 0,60 | 0,12 | 1,18 | 1,78 |
| *Parmelinopsis minarum* (Vain.) Elix & Hale | 29 | 51,6 | 0,82 | 0,10 | 0,95 | 1,77 |
| *Hyperphyscia cochlearis* Scutari | 35 | 36,3 | 0,58 | 0,12 | 1,14 | 1,73 |
| *Candelaria concolor* (Dicks.) Stein | 36 | 32,2 | 0,51 | 0,12 | 1,18 | 1,69 |
| *Haematomma personii* (Fée) A. Massal. | 36 | 30,7 | 0,49 | 0,12 | 1,18 | 1,67 |
| *Heterodermia obscurata* (Nyl.) Trevis. | 17 | 67 | 1,07 | 0,06 | 0,56 | 1,63 |
| *Physcia krogie* Moberg | 23 | 46,6 | 0,74 | 0,08 | 0,75 | 1,50 |
| *Punctelia constantimountium* Sérus. | 18 | 56,5 | 0,90 | 0,06 | 0,59 | 1,49 |

Tabela 1. continuação

| Táxons | Riqueza | CA | CR% | FA | FR% | VI |
|---|---|---|---|---|---|---|
| *Physcia crispa* Nyl. | 26 | 39,6 | 0,63 | 0,09 | 0,85 | 1,48 |
| *Trypethelium nitidiusculum* (Nyl.) R. C. Harris | 26 | 32,6 | 0,52 | 0,09 | 0,85 | 1,37 |
| *Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt | 28 | 24,5 | 0,39 | 0,09 | 0,92 | 1,31 |
| *Parmotrema praesorediosum* (Nyl.) Hale | 24 | 31,2 | 0,50 | 0,08 | 0,79 | 1,28 |
| *Parmotrema cetratum* (Ach.) Hale | 22 | 30,7 | 0,49 | 0,07 | 0,72 | 1,21 |
| *Hypotrachyna polydactyla* (Krog & Swinscow) Nash | 27 | 17,4 | 0,28 | 0,09 | 0,88 | 1,16 |
| *Platygramme caesiopruinosa* (Fée) Fée | 24 | 16,3 | 0,26 | 0,08 | 0,79 | 1,05 |
| *Graphis pavoniana* Fée | 19 | 25,7 | 0,41 | 0,06 | 0,62 | 1,03 |
| *Carbacanthographis* sp. | 20 | 20,1 | 0,32 | 0,07 | 0,65 | 0,98 |
| *Opegrapha* sp.1 | 17 | 25,7 | 0,41 | 0,06 | 0,56 | 0,97 |
| *Graphis submarginata* Lücking | 22 | 11,5 | 0,18 | 0,07 | 0,72 | 0,90 |
| *Cratiria lauricassiae* (Fée) Marbach | 22 | 10,3 | 0,16 | 0,07 | 0,72 | 0,88 |
| *Pyxine cocoës* (Sw.) Nyl. | 17 | 10,8 | 0,17 | 0,06 | 0,56 | 0,73 |
| *Platygramme* sp. | 16 | 12,1 | 0,19 | 0,05 | 0,52 | 0,72 |
| *Phaeographis lecanographa* (Nyl.) Staiger | 16 | 11,6 | 0,19 | 0,05 | 0,52 | 0,71 |
| *Ramboldia haematites* (Fée) Kalb | 16 | 10,2 | 0,16 | 0,05 | 0,52 | 0,69 |
| *Caloplaca* sp. | 17 | 6,4 | 0,10 | 0,06 | 0,56 | 0,66 |
| *Ramalina peruviana* Ach. | 15 | 9 | 0,14 | 0,05 | 0,49 | 0,63 |
| *Herpothallon rubrocinctum* (Ehrenb.) Aptroot & Lücking | 9 | 20,9 | 0,33 | 0,03 | 0,29 | 0,63 |
| *Lecanora* cf. *argentata* (Ach.) Malme | 11 | 12 | 0,19 | 0,04 | 0,36 | 0,55 |
| *Graphis kakaduensis* A. W. Archer | 12 | 9,5 | 0,15 | 0,04 | 0,39 | 0,54 |
| *Ramalina celastri* (Sprengel) Krog & Swinscow | 11 | 10,4 | 0,17 | 0,04 | 0,36 | 0,53 |
| *Coenogonium subdilutum* (Malme) Lücking, Aptroot & Sipman | 13 | 5,8 | 0,09 | 0,04 | 0,43 | 0,52 |
| *Physcia lacinulata* Müll.Arg. | 2 | 24,6 | 0,39 | 0,01 | 0,07 | 0,46 |
| *Graphis geraensis* Redinger | 11 | 6,1 | 0,10 | 0,04 | 0,36 | 0,46 |
| *Phyllopsora breviuscula* (Nyl.) Müll.Arg. | 9 | 8,5 | 0,14 | 0,03 | 0,29 | 0,43 |
| *Leptogium denticulatum* Nylander | 6 | 14,1 | 0,23 | 0,02 | 0,20 | 0,42 |
| *Physcia alba* (Fée) Müll.Arg. | 9 | 7,8 | 0,12 | 0,03 | 0,29 | 0,42 |
| *Parmotrema homotomum* (Nyl.) Hale | 9 | 6,7 | 0,11 | 0,03 | 0,29 | 0,40 |
| *Graphis schiffneri* Zahlbr. | 9 | 6,7 | 0,11 | 0,03 | 0,29 | 0,40 |
| *Physcia atrostriata* Moberg | 6 | 12,7 | 0,20 | 0,02 | 0,20 | 0,40 |
| *Graphis librata* C. Knight. | 9 | 6,1 | 0,10 | 0,03 | 0,29 | 0,39 |
| *Parmotrema subsumptum* (Nyl.) Hale | 6 | 6,9 | 0,11 | 0,02 | 0,20 | 0,31 |
| *Pyrenula mucosa* (Vain.) R. C. Harris | 6 | 5,9 | 0,09 | 0,02 | 0,20 | 0,29 |
| *Pyxine berteriana* (Fée) Imsh. | 7 | 3,8 | 0,06 | 0,02 | 0,23 | 0,29 |
| *Lecanora caesiorubella* Ach. | 5 | 7,6 | 0,12 | 0,02 | 0,16 | 0,29 |
| *Coccocarpia pellita* (Ach.) Müll.Arg. ex R.Sant. | 5 | 6,4 | 0,10 | 0,02 | 0,16 | 0,27 |
| *Porina* sp. | 6 | 4 | 0,06 | 0,02 | 0,20 | 0,26 |
| *Parmotrema mesotropum* (Müll.Arg.) Hale | 5 | 5,5 | 0,09 | 0,02 | 0,16 | 0,25 |
| *Graphis rigidula* Müll. Arg. | 2 | 11,3 | 0,18 | 0,01 | 0,07 | 0,25 |
| *Graphis archerii* Dal-Forno & Eliasaro | 6 | 2,6 | 0,04 | 0,02 | 0,20 | 0,24 |
| *Opegrapha* sp. 3 | 5 | 4,2 | 0,07 | 0,02 | 0,16 | 0,23 |

Tabela 1. continuação

| Táxons | Riqueza | CA | CR% | FA | FR% | VI |
|---|---|---|---|---|---|---|
| *Parmotrema muelleri* (Vain.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch | 5 | 4 | 0,06 | 0,02 | 0,16 | 0,23 |
| *Phaeographis* sp. | 5 | 3 | 0,05 | 0,02 | 0,16 | 0,21 |
| *Parmotrema melanothrix* (Mont.) Hale | 5 | 1,9 | 0,03 | 0,02 | 0,16 | 0,19 |
| *Ramalina complanata* (Sw.) Ach. | 4 | 3,8 | 0,06 | 0,01 | 0,13 | 0,19 |
| *Parmotrema consors* (Nyl.) Krog & Swinscow | 5 | 1,7 | 0,03 | 0,02 | 0,16 | 0,19 |
| *Fissurina instabilis* (Nyl.) Nyl. | 4 | 3,7 | 0,06 | 0,01 | 0,13 | 0,19 |
| *Phaeographis subtigrina* (Vain.) Zahlbr. | 5 | 1,5 | 0,02 | 0,02 | 0,16 | 0,19 |
| *Phaeographis intricans* (Nyl.) Staiger | 4 | 3,5 | 0,06 | 0,01 | 0,13 | 0,19 |
| *Parmotrema recipiendum* (Nyl.) Hale | 4 | 3,5 | 0,06 | 0,01 | 0,13 | 0,19 |
| *Enterographa compunctula* (Nyl.) Redinger | 4 | 2,9 | 0,05 | 0,01 | 0,13 | 0,18 |
| *Leptogium austroamericanum* (Malme) Dodge | 3 | 4,8 | 0,08 | 0,01 | 0,10 | 0,17 |
| *Rinodina* sp.1 | 4 | 2,6 | 0,04 | 0,01 | 0,13 | 0,17 |
| *Leptogium azureum* (Sw.) Mont. | 4 | 2,5 | 0,04 | 0,01 | 0,13 | 0,17 |
| *Normandina pulchella* (Borrer) Nyl. | 4 | 2,5 | 0,04 | 0,01 | 0,13 | 0,17 |
| *Baculifera* sp. | 4 | 2,2 | 0,04 | 0,01 | 0,13 | 0,17 |
| *Bulbothrix isidiza* (Nyl.) Hale | 4 | 1,1 | 0,02 | 0,01 | 0,13 | 0,15 |
| *Hyperphyscia syncolla* (Tuck.) Kalb | 3 | 2,8 | 0,04 | 0,01 | 0,10 | 0,14 |
| *Graphis dolichographa* Nyl. | 3 | 2,6 | 0,04 | 0,01 | 0,10 | 0,14 |
| *Graphis dupaxana* Vain. | 1 | 6,6 | 0,11 | 0,00 | 0,03 | 0,14 |
| *Heterodermia speciosa* (Wulf.) Trevis. | 3 | 2,1 | 0,03 | 0,01 | 0,10 | 0,13 |
| *Lecanora* sp. 1 | 1 | 5,8 | 0,09 | 0,00 | 0,03 | 0,13 |
| *Chapsa cinchonarum* (Fée) A. Frisch | 3 | 1,6 | 0,03 | 0,01 | 0,10 | 0,12 |
| *Platygramme* aff. *arthonioides* (Vainio) Zahlbr. | 3 | 1,2 | 0,02 | 0,01 | 0,10 | 0,12 |
| *Brigantiaea leucoxantha* (Spreng.) R. Sant. & Hafellner | 3 | 1 | 0,02 | 0,01 | 0,10 | 0,11 |
| *Lecanora* grupo *subfusca* | 2 | 2,9 | 0,05 | 0,01 | 0,07 | 0,11 |
| *Canoparmelia* sp. 3 | 2 | 1,4 | 0,02 | 0,01 | 0,07 | 0,09 |
| *Rinodina* sp. 2 | 2 | 1,3 | 0,02 | 0,01 | 0,07 | 0,09 |
| *Cladonia atthi* S. Stenroos | 1 | 3,3 | 0,05 | 0,00 | 0,03 | 0,09 |
| *Parmotrema catarinae* Hale | 2 | 1,2 | 0,02 | 0,01 | 0,07 | 0,08 |
| *Malcolmiella vinosa* (Eschw.) Kalb & Lücking | 2 | 1,1 | 0,02 | 0,01 | 0,07 | 0,08 |
| *Usnea* sp. | 2 | 0,9 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Hypotrachyna livida* (Taylor) Hale | 2 | 0,9 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Canoparmelia* sp.1 | 2 | 0,9 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Parmotrema pilosum* (Stizenb.) Krog & Swinscow | 2 | 0,9 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Cratiria americana* (Fée) Kalb & Marbach | 2 | 0,8 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Bactrospora myriadea* (Fée) Egea & Torrente | 2 | 0,8 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Teloschistes exilis* (Michaux) Vain. | 2 | 0,8 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Parmelinopsis subfatiscens* (Kurok.) Elix & Hale* | 2 | 0,7 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Hypotrachyna* aff. *punoensis* Kurok.** | 2 | 0,6 | 0,01 | 0,01 | 0,07 | 0,08 |
| *Parmotrema flavomedullosum* Hale | 1 | 2 | 0,03 | 0,00 | 0,03 | 0,06 |
| *Heterodermia* cf. *albicans* (Pers.) Swinscow & Krog | 1 | 1,6 | 0,03 | 0,00 | 0,03 | 0,06 |
| *Punctelia riograndensis* (Lynge) Krog | 1 | 1,5 | 0,02 | 0,00 | 0,03 | 0,06 |

Tabela 1. continuação

| Táxons | Riqueza | CA | CR% | FA | FR% | VI |
|---|---|---|---|---|---|---|
| *Parmotrema haitiense* (Hale) Hale | 1 | 1,5 | 0,02 | 0,00 | 0,03 | 0,06 |
| *Opegrapha* sp. 2 | 1 | 1,1 | 0,02 | 0,00 | 0,03 | 0,05 |
| *Bulbothrix* sp. | 1 | 0,9 | 0,01 | 0,00 | 0,03 | 0,05 |
| *Ramalina aspera* Räsänen | 1 | 0,9 | 0,01 | 0,00 | 0,03 | 0,05 |
| *Parmotrema subcaperatum* (Kremp.) Hale | 1 | 0,7 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Lecanora* aff. *albella* (Pers.) Ach. | 1 | 0,7 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Graphis paraserpens* Lizano & Lücking | 1 | 0,6 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Canoparmelia* sp. 2 | 1 | 0,6 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Physcia undulata* Moberg | 1 | 0,6 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Lecanora* sp. 2 | 1 | 0,5 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Physcia stellaris* (L.) Nyl. | 1 | 0,5 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Parmotrema eciliatum* (Nyl.) Hale | 1 | 0,5 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Bulbothrix goebelli* (Zenker) Hale | 1 | 0,4 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Phaeographis punctiformis* (Eschw.) Müll. Arg. | 1 | 0,4 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Lobaria discolor* (Bory ex Delise) Hue | 1 | 0,4 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Parmotrema dilatum* (Vain.) Hale | 1 | 0,4 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Graphis* aff. *archerii* Dal-Forno & Eliasaro* | 1 | 0,4 | 0,01 | 0,00 | 0,03 | 0,04 |
| *Pyrenula pyrenuloides* (Mont.) R. C. Harris | 1 | 0,3 | 0,00 | 0,00 | 0,03 | 0,04 |
| *Bacidia russeola* (Kemp.) Zahlbr. | 1 | 0,3 | 0,00 | 0,00 | 0,03 | 0,04 |
| *Cratiria obscurior* (Stirton) Marbach & Kalb | 1 | 0,3 | 0,00 | 0,00 | 0,03 | 0,04 |
| *Physcia* cf. *sinuosa* Moberg** | 1 | 0,2 | 0,00 | 0,00 | 0,03 | 0,04 |
| *Coccocarpia stellata* Tuck * | 1 | 0,2 | 0,00 | 0,00 | 0,03 | 0,04 |
| Total | | 6255,1 | 100,00 | 10,19 | 100,00 | 200,00 |

FA: freqüência absoluta, FR: freqüência relativa; CA: cobertura absoluta; CR: cobertura relativa; VI: valor de importância. Novos registros para a ciência (**), novas citações para o RS (*).

No Brasil ainda são poucos os estudos que abordam aspectos ecológicos das comunidades liquênicas, e são restritos, em geral, a áreas não urbanizadas (Marcelli 1992; Martins-Mazzitelli et al., 2006; Cáceres *et al*. 2007, 2008; Käffer *et al.* 2009; 2010a).

Os maiores valores de importância registrados para as duas primeiras espécies (*Canoparmelia texana* e *Dirinaria picta*) estão diretamente relacionados com seus aspectos ecológicos e reprodutivos. Ambas são espécies pertencentes ao grupo dos liquens foliosos, caracterizados pela estrutura laminar e dorsiventral (Marcelli 2006), sendo que estas duas espécies se reproduzem de forma direta, através da formação de sorédios. De acordo com

Marcelli (1992), a freqüência indica a capacidade de dispersão e o estabelecimento de uma espécie no ambiente, enquanto que a cobertura está relacionada com a capacidade dos espécimes desenvolverem-se e cobrirem partes do substrato.

Em ambientes urbanos estas duas espécies (*Canoparmelia texana* e *Dirinaria picta*) ocupam o espaço preenchido anteriormente pelos talos de outros táxons menos resistentes à poluição ambiental. *Anisomeridium tamarindii* e *L*ecanora cf. *symmicta* são espécies crostosas, caracterizadas pela ausência de córtex inferior e por se aderirem ao substrato pela porção inferior da medula (Marcelli 2006). Neste estudo foi verificada a presença de talos bem desenvolvidos, ocupando grandes áreas do tronco das forófitas, sendo consideradas espécies características de ambientes urbanos.

No Brasil ainda são poucos os trabalhos sobre comunidades liquênicas em áreas urbanas, especialmente com enfoque ecológico. O grande número de táxons registrados neste estudo e sua caracterização vêm contribuir com as pesquisas na área da liquenologia, especialmente pelo fato de muitas espécies liquênicas serem amplamente empregados em programas de biomonitoramento, assim como na avaliação da estrutura de ecossistemas florestais.

Para identificação dos grupos morfológicos, assim como dos táxons registrados na área de estudo, segue a chave de identificação abaixo:

Chaves para os grupos morfológicos de liquens ocorrentes em Porto Alegre e adjacências

A. Talo, constituído por pequenas escamas, às vezes com pequenos lobos marginais; ou reduzidos a uma massa de grânulos ou isídios1.....Chave 1 - Talo esquamuloso
B. Talo dimórfico: talo primário esquamuloso; talo secundário ascendente; formado por podécio..... 2. Chave 2 – Talo dimórfico
C. Talo fixado ao substrato por um ou mais pontos (apressórios basais), formado por ramos pendentes ou ascendentes, normalmente estreitos e longos, achatados ou cilíndricos..... 3. Chave 3 - Talo fruticoso

D. Talo dorsiventral, destacando-se facilmente do substrato; com lobos largos a muito estreitos, bem definidos; crescem em forma de roseta; geralmente presos por rizinas ou tomento; córtex inferior presente ou ausente ..... 4. Chave 4 - Talo folioso ou microfolioso

E. Talo aderido ao substrato diretamente pela medula, semelhantes a manchas; geralmente sem córtex inferior, rizinas ou tomento..... 5. Chave 5 - Talo crostoso

A. Chave 1 - Talo esquamuloso

1. Talo adnato; formado por esquâmulas sub-orbiculares; com até 02 mm de diâmetro; com ou sem córtex superior; sorais irregulares geralmente nas margens..... *Normandina pulchella*

2. Talo com esquâmulas arredondadas; formando um tapete com um emaranhado de hifas distais que precedem o talo; córtex superior presente; sorais ausentes ..... *Phyllopsora breviuscula*

B. Chave 2 – Talo dimórfico

1. Talo com esquâmulas primárias; talo secundário formado por podécios ocos; ascendentes; geralmente com propágulos; às vezes de hábito pulviniforme; apotécios vermelhos; podécios não cifosos; escamas com sorais marginais; medula branca ..... *Cladonia ahtii*

C. Chave 3 - Talo fruticoso

1. Talo alaranjado; ramos cilíndricos, ascendentes; formando um tufo; disco do apotécio laranja..... *Teloschistes exilis*

1. Talo esverdeado; ramos cilíndricos ou achatados; não formando tufos; disco do apotécio não laranja ou apotécio ausente .....2

2. Talo com ramos cilíndricos; com eixo central condróide; apotécio ausente ..... *Usnea* sp.

2. Talo com ramos achatados; sem eixo central condróide; apotécio presente .....3

3. Talo com ramos dorsiventral para canaliculado; menores que 4 mm de largura; sorédios laminais para marginais; apotécios ausentes ..... *Ramalina peruviana*

3. Talo com ramos achatados; maiores de 4 mm de largura; sem sorédios apotécios presentes .....4

4. Ramos largos; acima de 10 mm de largura; superfície reticulada; pseudocifelas elevadas; arredondadas; ascósporos elipsóide-fusiforme; bicelulares; até 12 m comprimento .. *Ramalina aspera*

4. Ramos estreitos; menores que 10 mm de largura .....5

5. Ramos canaliculados; 3–5mm de largura; superfície não reticulada; pseudocifelas punctiformes ascósporos curto-fusiformes; bicelular; 13–14 m comprimento .. *Ramalina complanata*

5. Ramos lanceolados a planos; 3–5mm de largura; superfície não reticulada; pseudocifelas planas a achatadas; elipsóides ou lineares; ascósporos curto-fusiforme; bicelular; menores que 15 m comprimento ..... *Ramalina celastri*

D. Chave 4 - Talo folioso ou microfolioso (incluindo gelatinosos)

1. Talo de coloração cinza-azulado a cinza-chumbo ou negro; com cianobactéria .....2

1. Talos de coloração cinza-claro a verde ou amarelo-citrino; com clorofícea .....3

2. Talo cinza-chumbo; não gelatinoso quando úmido; superfície inferior com tomento; lobos flabeliformes; com estrias concêntricas ..... *Coccocarpia*

2. Talo cinza-azulado ou negro; gelatinoso quando úmido; superfície inferior não tomentosa; lobos não flabeliformes; sem estrias concêntricas..... *Leptogium*

3. Talo amarelo-citrino; lobos alongados ou muito divididos; até 1,0 mm de largura; sorédios presentes..... *Candelaria concolor*

3. Talo não amarelo – citrino; cinza-claro a verde; lobos estreitos; sorédios presentes ou ausentes .....4

4. Talo cinza-esverdeado; verde quando úmido; lobos largos (1–2 cm de largura); bordo liso ou crenado; superfície superior de lisa a fraco-escrobiculada; sem sorédios ou isídios; lado inferior claro; rizinas branco-enegrecidas .....*Lobaria discolor*

4. Talo cinza-oliváceo a esverdeado; lobos estreitos (menores que 2 cm de largura); sorédios e isídios presentes ou ausentes; lado inferior claro ou escura; rizinas ausentes ou presentes.....5

5. Lobos com cerca de 0,5 mm de largura; córtex superior paraplectenquimatoso; rizinas ausentes; medula K-; apotécio ausente ou presentes; ascósporos com um septo; 14–21 m comprimento ..... *Hyperphyscia*

5. Lobos maiores que 0,5 mm de largura; rizinas presentes; medula K+ ou K-; apotécio ausente ou presentes; ascósporos simples ou bicelulares .....6

6. Superfície inferior com uma margem nua ou com poucas rizinas; com ou sem cílios marginais; se cílios presentes; não bulbados; lobos largos; máculas presentes ou ausentes ..... *Parmotrema*

6. Superfície inferior com rizinas estendendo-se até a margem; simples ou dicotômicas; cílios marginais presentes; máculas presentes ou ausentes.....7

7. Lobos entre 0,7 a 1,5 mm de largura; cílios marginais bulbados; máculas ausentes ou presentes; medula branca; rizinas simples ..... *Bulbothrix*

7. Lobos entre 0,7 a 3,0 mm de largura; cílios marginais não bulbados ou sem cílios; rizinas simples ou ramificadas presentes ou não ..... 8

8. Lobos com até 5 mm largura; córtex superior não maculado; rizinas ramificadas dicotomicamente; medula branca ..... *Hypotrachyna*

8. Lobos maiores que 5 mm largura; córtex superior com máculas ou máculas ausentes; rizinas simples; medula amarela ou branca ..... 9

9. Córtex superior sem máculas; cílios presentes; isídios presentes; medula amarela; superfície inferior negra; pseudocifelas ausentes ..... *Myelochroa lindmanii*

9. Córtex superior com máculas ou máculas se presentes fracas; cílios presentes ou ausentes; isídios presentes ou ausentes; medula branca; superfície inferior branca, castanha ou negra; pseudocifelas presentes ou ausentes ..... 10

10. Córtex superior com máculas ausentes ou raramente fracas; cílios ausentes; isídos presentes ou ausentes; superfície inferior branca, castanho ou negra; pseudocifelas presentes ..... *Punctelia*

10. Córtex superior com máculas ausentes ou fracas; cílios presentes; isídios presentes ou ausentes; pseudocifelas ausentes; córtex inferior ausente ou presente; lobos redondos ou alongados ..... 11

11. Lobos de 0,3 a 2,0 mm de largura; ápice dos lobos redondo a truncados; margem rizinada; cílios simples; negros; sorédios ausentes; isídios presentes; córtex inferior presente; superfície inferior negra ..... *Parmelinopsis*

11. Lobos acima de 2,0 mm de largura; ápice dos lobos arredondado; margem não rizinada; cílios presentes ou ausentes; sorédios ausentes ou presentes; isídos ausentes ou presentes; córtex inferior presente ou ausente; superfície inferior branca, castanho ou negra ..... 12

12. Lobos de 1,0 a 8,0 mm de largura; estreita margem nua (< 5 mm); cílios ausentes; sorédios presentes ou ausentes; isídios presentes ou ausentes; superfície inferior negra a raramente castanha; ascósporos simples, hialinos ..... *Canoparmelia*

12. Lobos com até 2,0 mm de largura; sem estreita margem nua; cílios ausentes; sorédios presentes ou ausentes; isídios ausentes; superfície inferior branca, castanha ou negra; ascósporos marrons, bicelulares ..... 13

13. Lobos dicotômicos, lineares; córtex superior prosoplectenquimatoso; sorédios ausentes ou presentes; máculas presentes; medula branca; córtex inferior presente ou ausente; pigmento amarelo ou vermelho, às vezes presentes; rizinas presentes ..... *Heterodermia*

13. Lobos não dicotômicos, plicados ou não; adpressos ou soltos no ápice; córtex superior paraplectenquimatoso; máculas presentes ou ausentes; medula branca ou amarela; sorédios ausentes ou presentes; cílios ausentes; rizinas ausentes ou presentes.....14

14. Talo de coloração cinza-metálico; lobos confluentes, adpressos, estreitos (até 2 mm de largura); máculas ausentes; sorédios presentes ou ausentes; medula branca ou pigmentada; superfície inferior negra; rizinas ausentes..... *Dirinaria*

14. Talo de coloração cinza-claro, esverdeado, azulado, rosetado; córtex superior K+ amarelo ou K-; sem rizinas densas na zona distal; presença de máculas ou não; sorédios presentes ou ausentes; isídios ausentes; medula branca a amarela; superfície inferior clara, marrom a negra .....15

15. Talo cinza-claro, cinza-esverdeado a azulado; córtex superior sempre K+ amarelo; UV-, superfície superior com pruínas ou não; medula branca; superfície inferior clara, marrom a negra.....*Physcia*

15. Talo cinza-claro ou esverdeado; córtex superior K+ ou K-; UV+ amarelo ou UV-; superfície superior com pruínas ou não; medula branca ou pigmentada; superfície inferior negra.....*Pyxine*

D. Chave 4 – Talo crostoso

1. Talo pulverulento, granuloso a leproso, amarelo-esverdeado; com grânulos que se espalham sobre o córtex pulverulento..... grupo *Lepraria*

1. Talo não pulverulento, nem granuloso ou leproso; orbicular ou crostoso; ascomas presente ou ausente; isídios presentes ou ausentes.....2

2. Talo orbicular, cinza-esverdeado, ou cinza-alaranjado e parte central avermelhada; ascomas e ascósporos ausentes; isídios presentes..... *Herpothallon rubrocinctum*

2. Talo crostoso; acinzentado; ascomata ascósporos presentes; isídios ausentes .....3

3. Peritécios presentes.....32

3. Apotécios ou lirelas presentes.....4

4. Ascoma lireliforme; lirelas alongadas e, ou ramificadas .....25

4. Ascoma apotécio obriculares a angulares, porém não alongados.....5

5. Apotécios agrupados em estromas ou pseudoestroma.....6

5. Apotécios isolados .....7

6. Apotécios peritecióides; himênio e ponta do asco I+ azul; ascos com 2 a 8 ascósporos; ascósporos grandes (maiores que 30  m comprimento), hialinos, simples..... *Pertusaria*

6. Apotécio com disco exposto, disco marrom, irregularmente ramificado; himênio I-; ascos com 8 ascósporos; ascósporos I+ marrons a violeta escuro, elipsóide-fusiforme (menores que 30 m comprimento), hialinos, septos transversais ou muriformes ..... *Glyphis*

7. Apotécio com margem da mesma cor do talo, com camada de alga (lecanorino); apotécio séssil; himênio e ponta do asco I+ azul.....8

7. Apotécio com margem própria, não da mesma cor do talo, às vezes da mesma cor do disco, ausente de algas, ou margem ausente;, imerso erumpente, raramente séssil; himênio às vezes I-, raramente I+ laranja-avermelhado, KI+ azul, ou I+ azul (ápice do asco geralmente I-).....14

8. Ascósporos simples.....9

8. Ascósporos transversalmente septados.....12

9. Ascósporos maiores que 20 m comprimento, 1 a 8 por asco.....10

9. Ascósporos menores que 20 m comprimento, 8 por asco .....11

10. Ascósporos com paredes grossas, maiores que 50 m comprimento..... *Pertusaria*

10. Ascósporos com paredes finas, menores que 50 m comprimento.....*Ochrolechia*

11. Talo não espesso, K+ amarelo; apotécio imerso, adnatos ou sésseis no talo; disco negro, amarelo, creme ou branco; ápice I+ azul ou I-; himênio hialino; K-; hipotécio hialino..... ..... *Lecanora*

11. Talo espesso, K-; apotécio séssil, raramente imerso no talo; disco negro; ápice I+ azul; himênio violeta; K-; hipotécio violeta ..... *Tephromela americana*

12. Apotécio com disco vermelho, K+vermelho-púrpura; ascósporos hialinos .....13

12. Apotécio com disco marrom a negro, K-; ascósporos marrons .....*Rinodina*

13. Ascósporo fusiforme, 5–7 septos transversais, hialinos, 30–40 × 3,5 µm comprimento ..... .....*Haematomma personii*

13. Ascósporo elipsóide, polarilocular, hialino, 14–16 × 3,5 µm comprimento..... *Caloplaca* sp.

14. Apotécio com margem proeminente sobre o disco, imerso a erumpente, raramente séssil; himênio às vezes I-, raramente I+ laranja-avermelhado, KI+ azul ou I+ azul (ápice do asco geralmente I+).....15

14. Apotécio com margem própria, ausente de camadas de alga, ou margem com cor diferente da cor do talo, ou da mesma cor do disco, ou margem ausente; himênio I+ azul ou I+ laranja-avermelhado .....17

15. Apotécio arredondado para angular; margem geralmente sem pruína; himênio I-, fortemente insperso (com gotas de óleo); ascósporo com um ou mais septos transversais ou muriformes e lúmen em forma de lente ..... 16

15. Apotécio arredondado; margem sem pruína; disco exposto e pruinoso; himênio I + azul ou I-, não insperso; ascósporo marrom, muriforme ..... *Glyphis scyphulifera*

16. Córtex talino sem cristais, apotécio discóide para urceolado; margem inteira e disco exposto; excípulo hialino, ou marrom para carbonizado; ascósporo marrons, transversalmente septados, 30–42 × 8–9 m; himênio I- ..... *Phaeographis lobata*

16. Córtex talino com cristais; apotécio arredondado para angular; margem inteira e disco exposto, com leve pruína; excípulo hialino; ascósporo hialino, (3)5–7 septos transversais, 18.3–27.4 × 6.1 m; himênio I+ alaranjado ..... *Chapsa cinchonarum*

17. Apotécio com margem própria, disco e margem negros a (lecideíno); com pigmento negro ou carbonizado, mas às vezes coberto por pruína ..... 18

17. Apotécio com margem pálida ou incolor, mas não negra, ou margem ausente (biatorino) ..... 20

18. Ascósporo marrom; 1 a 3 septos transversais; himênio I- ..... 19

18. Ascósporo hialino, 3 septos ou mais (13–22 septos); himênio I+ laranja-avermelhado ..... ..... *Bactrospora myriadea*

19. Talo com protalo negro; conídios oblongo-elipsóide, menores que 6 m comprimento; epitécio K-; excípulo com 3 camadas, uma dela pálida ..... *Cratiria*

19. Talo com protalo negro, conídios baciliformes, maiores que 6 m comprimento; epitécio K+ verde excípulo com uma camada ..... *Baculifera* sp.

20. Apotécio arredondado, adnato, ou levemente aumentado sobre o talo, raramente com uma zona marginal pálida ou sem margem; ascósporos hialinos, clavados, 3-6 septos transversais ..... *Arthonia*

20. Apotécio distintamente proeminente para séssil, geralmente com margem distinta ou fina, muito raramente emarginada ..... 21

21. Ascósporos simples ..... 22

21. Ascósporos transversalmente septados ou muriforme ..... 23

22. Disco do apotécio e margem vermelho brilhante, K+ púrpura; ácido norstíctico presente; ascósporo hialino, 8 por asco, 12.0–16.4 × 6.0–9.0 m comprimento; paráfises simples; himênio I-

..... *Ramboldia haematites*

22. Disco do apotécio marrom-pálido para marrom-enegrecido, K-; substâncias químicas ausentes; ascósporo hialino, 8 por asco, 12.2–21.3 × 6.1–9.1 m comprimento; paráfises simples; himênio I+ azul ..... *Malmidea vinosa*

23. Ascósporos transversalmente septados; apotécio não pruinoso ..... 24

23. Ascósporos muriforme; apotécio pruinoso ..... ..... ..... *Brigantieae leucoxantha*

24. Apotécio com margem não proeminente; disco amarelado; ascósporo elipsóide, hialino, 1 septo transversal, 6.0–12.2 × 3.0–6.1 m comprimento, I-; himênio I- ..... ..*Coenogonium subdilutum*

24. Apotécio com margem proeminente; disco laranja-amarronzado ou escurecido variavelmente incolor; ascósporo fusiforme, hialino, 6–7(8) septos transversais, I-; himênio I+ azul-avermelhado ..... *Bacidia russeola*

25. Lirelas agrupadas em (pseudo) estroma ..... 26

25. Lirelas solitárias, às vezes agrupadas, mas não em (pseudo) estroma ..... 28

26. Himênio I+ azul; ascósporo fusiforme ..... *Enterographa compunctula*

26. Himênio I-; ascósporos elípticos a raramente globosos ..... 27

27. Excípulo e hipotécio carbonizados; ascósporos hialinos, 3–6 septos transversais, 35–42 × 7–9 m comprimento, asco I+ violeta-azulado ou vermelho-vinho; margem das lirelas não fissurinadas ..... .....*Glyphis cicatricosa*

27. Excípulo não carbonizado; ascósporos hialinos, muriformes, 31.4 × 13.2 m comprimento; ascos I+ azul-violeta; margem das lirelas fissurinadas ..... *Fissurina instabilis*

28. Ascósporos marrons ..... 29

28. Ascósporos hialinos ..... 30

29. Apotécios orbiculares, irregularmente circulares, ovais, ou lireliformes; disco exposto, cinza ou marrom; himênio insperso, I+ alaranjado, ou I-; excípulo não carbonizado; ascósporos muriformes ou transversalmente septados, I+ vermelho-vinho ..... *Phaeographis*

29. Apotécios lireliformes; disco exposto, negro; himênio insperso, I-; excípulo apicalmente carbonizado; ascósporo muriforme, 4–6 por asco, I+ amarelo-avermelhado *Platygramme*

30. Ascósporos I- ..... 31

30. Ascósporos I+ azul-violeta ..... *Graphis*

31. Lirelas não pruinosas, disco exposto a fechado; excípulo não carbonizado a lateralmente carbonizado I-, himênio hialino ou I+ azul, ascósporos I-, clavados, hialinos, 1(2)–3 septos transversais ..... *Opegrapha*

31. Lirelas com pruína branca eminente; disco não exposto; excípulo carbonizado I-; himênio hialino I+ alaranjado; ascósporos I-, oblongos, hialino, submuriforme*Carbacanthographis*

32. Ascósporo marrons (pigmentados) ..... *Pyrenula*

32. Ascósporos hialinos .....33

33. Paráfises não ramificadas..... *Trypethelium nitidiusculum*

33. Paráfises ramificadas .....34

34. Talo cinza-verdoso; peritécios isolados ou confluentes; poro apical; ascósporos com células cilíndricas, alongado-fusiformes, 1–7 septos transversais, às vezes com gelatina no epispório, (halonado) .....*Porina* sp.

34. Talo esbranquiçado a acinzentado, peritécio erumpente, hemisférico para cônico, poro apical, ascósporos claviformes, geralmente com constrição mediana, macro-cefálico (uma célula menor que a outra), 01 septo transversal; epispório sem gelatina, sem halo .....*Anisomeridium tamarindii*

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## 6. Artigo 5

Use of lichens and genotoxicity bioindicators for the evaluation of air quality in an urban environment, southern Brazil

* Artigo submetido para Environmental Pollution. Co-autores: Andréa T. Lemos [a,c], Miriam Anders Apel [d], Jocelita Vaz Rocha [c], Suzana Maria de Azevedo Martins [b], Vera Maria Ferrão Vargas [a,c*]

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ABSTRACT

Biological indicators such as lichens and genotoxicity bioindicators are widely used to monitor the air quality in urban environments. *Parmotrema tinctorum* and *Teloschistes exilis* (lichens) have been used to verify the presence of pollutants, including PAHs and analyze morphophysiological alterations in the thallus of species caused by their action. Species were exposed during seven months, at three sampling stations in an urban area, in southern Brazil. Mutagenicity and cytotoxicity of PM10 organic extracts were assessed in the *Salmonella*/microssome assay at two stations. Higher concentrations of S, Pb, Cr and Zn were registered in the last period of exposure and more significant morphophysiological damages were verified in the lichens. Naphthalene and Phenanthrene were the dominating PAHs at the analyzed stations and periods. A more distinct mutagenic activity was verified during the first months of exposure of lichens. In addition, the presence of nitro composites was detected through YG1021 and 1024 strains. Factors such as traffic flow and topography contributed to the results obtained. Lichens and biomarkers allowed the evaluation of air quality and the presence of environmentally-aggressive compounds.

Keywords: Airborne particulate matter, Biomonitoring, Chlorophyll, PHAs, *Salmonella*/microssome assay

## 1. Introduction

Biological indicators have been widely used to monitor air quality, particularly in urban areas. Among them, epiphytic lichens have been known since the beginning of the 19th century as sensitive to phytotoxic gases such as $SO_2$, $NO_x$, heavy metals, fluoride as well as to chlorinated hydrocarbons (Hawksworth and Rose, 1970; Mitchell et al., 2000; Minganti et al., 2003; Giordano et al., 2005; Conti et al., 2009). In addition, their sensitivity to Polycyclic Aromatic Hydrocarbons (PAHs) and $O_3$ has been verified over the last decades (Blasco et al., 2006; Gombert et al., 2006; Augusto et al., 2010). Yet, transplant methodology is the most used.

Lichens are symbiotic organisms consisting in the association of a fungus (mycobiont) and an alga (photobiont), resulting in the formation of a thallus (Hawksworth and Hill, 1984). Their high sensitivity is closely related to their biology. Alterations in the symbiotic balance between the photobiont and the mycobiont may be promptly evidenced through the rupture of this association. Anatomically, lichens have no stomata or cuticle, which means gases and aerosols may be absorbed by the thallus and quickly spread over the tissue where the photobiont is. The absence of such structures restrains absorbed toxic substances to be excreted or selected (Valencia and Ceballos, 2002).

Among the effects pollutants may cause to lichens are growth and development inhibition of the thallus, alterations in the metabolic processes as well as morphological alterations (Barkman, 1958; Baddeley et al., 1973; Gries, 1996; Schlensog and Schroeter, 2001). The photobiont is the first to be affected and the development of abnormalities in the thallus as well as chlorophyll bleaching and the development of yellowish brown areas in the chloroplasts are observed. Chlorophyll converts into pheophytin by the action of sulfur dioxide solutions even in low concentrations (Barkman, 1958; Bargagli and Mikhailova, 2002).

Air quality may be analyzed through biomarkers and mutagenic effects may be detected by the Salmonella microsome assay or Ames Test. Such assay is the most used worldwide to detect mutagenic substances with carcinogenic potentialities (Claxton et al., 2004), in large (Ducati and Vargas, 2003; Vargas, 2003; Umbuzeiro and Vargas, 2003), industrial urban areas (Vargas, 2003; FEPAM, 2008; Coronas et al., 2009) or in small towns (Pereira et al., 2010). The test uses different strain types of genetically modifies *Salmonella typhimurium*, deficient in histidine amino acid synthesis. Such strains are unable to develop in a minimal growth medium without histidine, unless mutations occur and reestablish their synthesis capacities. Frameshift or base pair substitution mutations are observed in vitro assays with or without rat metabolization fractions (Maron and Ames, 1983). Studies with the use of *Salmonella*/microsome assay to analyze the presence of specific classes of substances which may be adsorbed onto particulate matter, specially PAHs and their nitroderivates, are cited by the literature (Claxton, 1983; Vargas et al., 1998; Monarca et al., 2001; Ducati and Vargas, 2003; Vargas, 2003; Claxton et al., 2004; Claxton et al., 2007; Apel et al, 2010; Pereira et al, 2010).

Of all methodologies used to evaluate and monitor air quality with the use of bioindicators, the transplant methodology has been applied in several countries, even with lichens. It consists of transplanting or transferring biological material, previously standardized, from an area not influenced by pollutants from urban or industrial areas to an area to be monitored. (Martins – Mazzitelli et al., 2006). In Brazil, there are not many studies with lichens with use of this methodology to evaluate the presence of atmospheric contaminants, and the existent ones were carried out to detect $SO_2$ and heavy metals (Coccaro et al., 2000; Prochnow and Porto, 2005; Saiki et al., 2007a, 2007b; Fuga et al., 2008). However, in studies with the use of *Salmonella*/microsome assay, toxic and genotoxic effects as well as the carcinogenic potentialities of some pollutants, especially PAHs, are cited in the literature (Vargas et al.,

1998; De Martinis et al., 1999; Ducatti and Vargas, 2003; Vargas, 2003; Varella et al., 2004; Coronas et al., 2008; Appel et al., 2010; Pereira et al., 2010). Despite that, there are no published citations for the country correlating the presence of compounds with mutagenic properties related to organic compunds and the occurrence of morphological damages in lichen species, as well as the absorption of PAHs in the lichen thalli. Thus, the objectives of the present manuscript are: (i) characterize the presence of sulfur, cadmium, lead, chrome and mercury, usually representative of the urban contribution in lichen species exposed for the long study period, in Porto Alegre; (ii) verify the presence and types of PAHs in the thalli of exposed species; (iii) characterize the presence of morphological damages in the thalli of lichen species during the exposure period; (iv) evaluate the mutagenic activity and cytotoxicity of organic compound extracts obtained from particulate matter in the air (PM10), during the same lichen exposure periods; (v) compare the occurrence of morphological damages in bioindicator species and the presence of chemical contaminants with mutagenic and citotoxic activity in atmospheric particulate matter.

## 2. Materials and methods

### 2.1. Study Area

The city of Porto Alegre encompasses an area of 496.8 $km^2$, of which 30% represents rural area. It is located in the Central Depression region at 51º 01’ and 51º 16’W and 29º 57’ and 30º 16’S, on Lake Guaiba, in the state of Rio Grande do Sul, Brazil. The estimated population is 1,409 million inhabitants (IBGE, 2010). The region is characterized by a humid subtropical climate, with annual average temperature of 19.4°C, average relative humidity of 76% and annual average rainfall of 1.324 mm (Livi, 1998). The predominance of winds is from south-east to north-east (Embrapa, 2010).

The study was undertaken during the period comprised between July 2007 and June 2008, at three stations: ESC, EJB and EA located in the following neighborhoods: Santa Cecília, Jardim Botânico and Anchieta (Fig. 1). The sampling areas were defined according to their distribution in the city of Porto Alegre, total number of inhabitants per area, anthropic pressure, traffic flow and the presence of semi-automatic equipment used by the Foundation of Environmental Protection of Rio Grande do Sul (FEPAM) to monitor the air quality.


Figure 1. Map of Porto Alegre, RS, Brazil and representation of sampled stations. Caption: ESC - Santa Cecília (2), EJB - Jardim Botânico (4), EA – Anchieta (13).

## 2.2. Lichen Samples

### 2.2.1 Material Sampling and Preparation

For analyses of pollutants and morphophysiological damages, two standardized lichen species were selected: ***Parmotrema tinctorum*** (Nyl.) Hale, a foliose lichen, and ***Teloschistes exilis*** (Michx.) Vain., a fruticose lichen. The specimen selection occurred in accordance with distribution and abundance in the State. Specimens were collected in the cities of Caraá (29°43'S' e 50°20'W) and Tapes (30º38'S' e 51°22'W) approximately 97 km and 117 km away from Porto Alegre, respectively. The samples remained in the laboratory for 20 days for acclimatation, objecting their physiological adaptation and homogenization before being exposed at the sampling stations.

### 2.2.3 Sampler Set Up and Exposure Period

***Parmotrema tinctorum*** samples were exposed in 55 x 35 cm plastic screens while ***T. exilis*** samples were placed in perforated bags, both fastened to the table nettings sheltered by "sombrite" covers 120 cm above the ground. At each station a table with samplers was arranged near FEPAM's semi-automatic equipments. Before sample exposure, analyses of pollutant concentrations as well as morphophysiological evaluations, known as control analyses, were carried out. Lichens were exposed for seven months and after two, five and seven months samples were collected for evaluation of pollutants absorbed by the thallus as well as of morphophysiological damages.

### 2.2.4 Concentration of pollutants in lichen tissue

After each exposure period, the concentrations of pollutants were evaluated in the lichen samples exposed at the sampled areas: sulfur (S) and heavy metals: lead (Pb), cadmium (Cd), zinc (Zn), chrome (Cr) and mercury (Hg). After the removal of samples, they were cleaned

and substrate residues were removed. The analyses of pollutants were carried out using atomic emission spectrophotometry (AES) through the wet nitric-perchloric digestion (ICP-OES). The samples were submitted to a high temperature using argon flame (plasma), whose analytes were dissociated into atoms and excited by higher energy states. The method detection limit was 0.01% for S, 1.0 mg/kg for Pb, 0.1 mg/kg for Cd, 1.0 mg/kg for Zn and 0.2 mg/kg for Cr (Boss and Fredeen, 2004, Standard Methods, 1995). The evaluation of mercury was carried out through atomic absorption spectrophotometry/cold steam generation after oxidation by wet digestion (EPA 7471, 2007), with detection limit of 0.01 mg/kg (Standard Methods, 1995). Such analyses were carried out by the Soils Laboratory of the Federal University of Rio Grande do Sul, Brazil.

### 2.2.5 Morphophysiological Analysis

#### 2.2.5.1 Concentration of Organic Carbon (CO) in lichen tissue

The analysis of CO in the thallus of lichen species was carried out after each exposure period at all assessed stations, following the same sample preparation procedure used for the evaluation of pollutant concentrations. As for organic carbon, the analysis was carried out through wet combustion using the Walkley-Black method with external heat and detection limit of 0.01% (Standard Methods, 1995). Such analyses were carried out by the Soils Laboratory of the Federal University of Rio Grande do Sul, Brazil.

#### 2.2.5.2 Live, dead and plasmolyzed cells counting and IVF

Cell counting was carried out using the neutral-red test 5% (Le Blanc, 1971; Mendez and Fournier, 1980) through which it is possible to determine the percentage of live, dead and plasmolyzed cells of lichen species. For the evaluation of the vitality state of lichen thalli after the exposure period at the sampled stations, the Index of Photobiont Vitality (IVF) proposed

by Calvelo et al. (2010) was used. The formula is expressed by: IVF = [V+(Pl/2)/M+(Pl/2)] where, V = amount of live cells, Pl = amount of plasmolyzed cells and M = amount of dead cells.

2.2.5.3 Chlorophyll Analysis

For the analysis of chlorophyll (Chl_a, Chl_b), pieces of each lichen sample were cut, placed in a chemistry tube with 10 ml of 96% ethanol, according to the technique proposed by Knudson et al. (1977). The reading was carried out through absorbance, in the wavelength range comprised between 649 and 665 using spectrophotometer Digimed DME-21.

2.2.5.4 Analysis of external morphological damages

The observation of external morphological alterations in lichen samples was carried out through photographic comparison (Le Blanc et al., 1976) in the different exposure periods at the sampled stations, by comparing changes in the color of the thalli.

2.2.6. Analysis of PAHs Profile in lichen samples

Analyses to verify the absorption of PAHs were carried out in two lichen species (***P. tinctorum*** and ***T. exilis***), at the three sampled stations (ESC, EJB and EA) and in the different exposure periods (2m, 5m and 7m). Eighteen PAHs were selected, out of which $15^{(*)}$ were considered as priority by the US Environmental Protection Agency (USEPA, 1985), due to health side effects, considering they are more toxic and represent a higher possibility of exposure to human population. The PAHs analyzed were: *Acenaphthene, *Acenaphthylene, Aminoanthracene, *Anthracene, *Benzo(a)anthracene, *Benzo(a)pyrene, *Benzo(b)fluoranthene, *Benzo(ghi)perylene, *Benzo[k]fluoranthene, *Chrysene, *Dibenzo(a,h)anthracene, Dibenzofurane, *Phenanthrene, *Fluoranthene, *Fluorene, *Naphthalene, Nitropyrene and *Pyrene.

2.2.6.1 Reagents

The mix of polycyclic aromatic hydrocarbons naphthalene, acenaphtylene, acenaphthene, fluorene, anthracene, phenanthrene, dibenzofurane, chrysene, pyrene, benzo(a)-pyrene, fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)anthracene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene; aminoanthracene and 1-nitropyrene were purchased from Sigma- Aldrich (USA).

2.2.6.2 Lichen Extraction

To 0.2 g of spiked lichen samples, approximately 0.1 g of Florisil and 0.1 g of anhydrous sodium sulphate were added to each sample and then submitted to four sequential extractions with 15 mL dichloromethane for 15 min at 20 °C in ultrasonic bath each one. The combined extract was concentrated to 1mL in rotary evaporator at 40 °C before purification. The clean-up step was performed in a glass column filled with alumina (3%) activated at 550 ºC for 5 h. A 2 g of anhydrous sodium sulphate were added to the top of the column. The analytes were eluted with 20 mL hexane – dichloromethane (3:1) and concentrated to 500 µL under nitrogen stream. All the extracts were gravimetrically controlled. The final extract was analyzed by GC–MS, operating in selected ion monitoring (SIM) mode, as described below (Blasco et al., 2006; Domeño et al., 2006).

2.2.6.3 Gas chromatography–mass spectrometry (GC–MS)

The analytes were identified using a Shimadzu QP-5000-quadrupole MS system. Separations were carried out on a DB-5 fused silica capillary column (25 m, 0.25 mm; film thickness 0.25 µm). The analysis was performed using helium as carrier gas at 1 mL/min. The GC system was equipped with a split/splitless injector operating in the splitless mode with the purge valve open at 1.0 min. The oven temperature was held 50 ºC for 3 min, increased to 160 ºC by a temperature ramp of 20 ºC/min and to 205 ºC by 4 ºC/min, held 205 ºC for 1 min,

ramped at 5 ºC/min to 261 ºC, held 1 min, increased at 10 ºC/min to 280 ºC, maintained 5 min, ramped at 10 ºC/min to 300 ºC and held for 5 min. The MS operating conditions were: EM 70 eV, injector temperature 270 ºC, operating SIM (selected ion monitoring) mode, using the following characteristics masses: 1-nitropyrene, 247; aminoanthracene, 193; naphthalene, 128; acenaphthylene and acenaphthene, 153; dibenzofurane, 168; fluorene, 166; phenantrene and antracene, 178; fluoranthene and pyrene, 202; benz[*a*]anthracene and chrysene, 228; benz[*b*]fluoranthene, benz[*k*]fluoranthene and benz[*a*]pyrene, 252; dibenz[*a,h*]anthracene, 278; and benz[*g,h,i*]perylene, 276 (Blasco et al., 2006; Domeño et al., 2006).

## 2.3. Mutagenic Study of Atmospheric Particulate Matter PM10

### 2.3.1 Particulate Material Sample

The samples of atmospheric particulate material were collected simultaneously to the exposure of lichen samples at two of the studied stations (EJB e EA), through high volume air samplers (AVG MP10, 1200/CCV). The samples were collected weekly, every 24 hours, using Teflon filters (TX40HI20WW, 254X203mm) for particles with diameter up to 10 μm (PM10). Filters were weighted and stabilized before and after samples (45% humidity) for the determination of particulate concentration, expressed in μg/m³ units of sampled air (ABNT, 1988) and grouped in monthly pools. The volume of air passing through each filter varied from 1684.5 to 2361m³, with average values of 2004.12 ± 233.81m³.

### 2.3.2 Extraction of Organic Compounds

One-fourth of each filter was used in the extraction of organic compounds through the ultrasound technique with dichloromethane solvent (DCM, CASRN. 75-09-2), (Vargas et al., 1998). The amount of extracted organic matter (EOM) in μg/m³ was calculated as the result of the total amount of EOM per filter divided by the sampled volume of air. The percentage of extraction output was also calculated. Previously to the bioessays, extracts were dried with

gaseous nitrogen and resuspended in dimethyl sulfoxide. The extracts were analyzed as representative pools of the lichen exposure periods (2, 5 and 7 months of exposure). For the comparative evaluation by the fifth month of exposure, 40% of the extract concentration in the second period was analyzed in relation to the pools from the first two months whereas the other 60% related to the extract pool from the three months comprised by the second period. For the evaluation by the seventh month, 28.6% of the concentration related to the extract pool from the first two months was analyzed whereas 42.8% related to the extract pool from the three subsequent months comprised by the second period and the reprresentative 28.6% of the pool from the last two months of exposure were analyzed.

2.3.3 *Salmonella*/microsome Assay

The mutagenicity of organic extracts was determined using the *Salmonella*/microsome assay (Maron and Ames, 1983), by microsuspension method as described by Kado et. al (1983). *Salmonella typhimurium* TA98 strain with and without metabolization (S9 mix fraction) in order to measure the presence of frameshift mutations. The presence of nitroderivate mutagenic compounds was investigated in the absence of metabolization through YG1021 and YG1024 strains, which present a high activity of specific enzymes allowing a higher sensitivity for nitrocompounds such as nitroarenes (YG1021) and dinitroarenes (YG1024) (Watanabe, 1989; 1990).

Six concentrations of each sample (1.25; 2.50; 10.00; 20.00 and 40.00 µg/plate) were tested in duplicate, in addition to the negative (5µl dimethyl sulfoxide solvent – DMSO; 100µl liquid nutrient medium) and positive (4-nitroquinoline oxide- 4NQO, 0.5µg/plate, CASRN. 56-57-5; 2 – nitrofluorene - 2NF, 0.15 µg/plate, CASRN. 607-57-8; and 2- aminofluorene - 2AF, 1µg/plate, CASRN. 153-78-6 from Sigma Chemical Company, St. Louis, MO) controls for the different strains.

The cytotoxicity of the organic extracts was tested in TA98 strain through the bacterial growth culture with nutrient agar after dilution in phosphate buffer (pH = 7.4) resulting in a 100-200-cell concentration.

2.4 Environmental Variables

During the period lichens the exposition, concentrations of pollutants were obtained: the samples of particulate matter (PM10) were collected weekly, every 24 hours and Ozone ($O_3$) the daily concentrations for stations EJB and EA, in addition to climate data: temperature (ºC), relative humidity of air (%) and precipitation (mm) available for the city.

Information on traffic flow refers to punctual one-day sampling data. Four different categories were considered: (LV) = light-vehicles: cars and small vans; (MV) = medium-vehicles: mini and micro buses; (HV) = heavy-vehicles: trucks and buses and (MT) = motorbikes. Pollutant data (PM10 and $O_3$) was obtained from manual and automatic nets installed by FEPAM at the sampled stations while climate data was obtained from the National Institute of Meteorology – 8th District. Traffic flow data was provided by the Public Company of Transportation and Circulation (EPTC) in Porto Alegre.

2.5 Statistical Analysis

Anova was used for the comparison of pollutant concentrations absorbed by lichen samples, percentages of CO, percentage of live, dead and plasmolyzed cells, IVF and chlorophyll concentrations (Chl_a, Chl_b) among the sampled stations. Significance level was $P<0.001$. Spearman correlation analyses were also carried out between the following variables: PM10, $O_3$ and climate data (temperature, relative humidity and precipitation) with the pollutant concentration absorbed by lichen thalli, the percentage of CO, live, dead and plasmolyzed cells, IVF and chlorophyll concentration, as well as between the concentrations of pollutants absorbed by lichens with morphophysiological variables (CO, live, dead and

plasmolyzed cells, IVF and chlorophyll) with significance level of $P<0.01$ and $P<0.05$. All statistical analyses were carried out using SPSS Statistics Software (Statistical Package Social Science Inc., Chicago, IL.).

The dose-response curves for the evaluation of mutagenic activity were run with SALANAL Software (*Salmonella* Assay Analysis, version 1.0 of Research Triangle Institute, RTP, Carolina do Norte, USA) applying the linear regression choosing the linear or Bernstein model as described in Vargas et al. (1998). Mutagenic activity analyses of samples were considered positive whenever they presented significant Anova ($p\leq0.05$) and a significant dose-response curve ($p\leq0.05$). Results were expressed as the number of revertents per µg of extract (rev/µg) and revertents per cubic meter of sampled air ($rev/m^3$ = rev/µg X MOE).

Cytotoxicity analysis was carried out by comparing the percentage obtained of surviving colonies per plate in relation to the negative control. Values below 60% characterize samples with toxic cellular effect (Vargas et al., 1993).

Spearman correlation analyses have been carried out between the morphophysiological damages caused by pollutants in lichens and mutagenic and cytotoxicity responses of organic extracts using SPSS Software.

## 3. Results

### 3.1 Pollutant Concentration in lichen tissue

The results of pollutant concentrations absorbed by lichens can be seen in Table 1. During the exposure period values above those registered for control samples were detected, for all analyzed pollutants, in both species. Higher S values were observed in *P. tinctorum*, at station EA, by the fifth month of exposure (0.18%), followed by ESC (0.15%) and EJB by the second and fifth months, both with 0.14%, whereas for *T. exilis*, the highest values were at EA (0.21%), followed by ESC and EJB (0.18%) by the fifth month of exposure. The

concentrations of Pb were higher at EA by the seventh month of exposure both in ***P. tinctorum*** (13mg/kg) and ***T. exilis*** (7 mg/kg), followed by EJB and ESC, both with 6 mg/kg. Regarding Cr, the highest concentrations were found by the fifth month in ***P. tinctorum*** and ***T. exilis,*** at EA (19 mg/kg and 6 mg/kg, respectively) and, by the seventh month, at EJB and ESC (both with 9 mg/kg) in ***P. tinctorum*** and at ESC in ***T.exilis*** (5 mg/kg). The highest zinc concentrations were registered by the seventh month in ***P. tinctorum***, at ESC (659 mg/kg), followed by EA (153 mg/kg) and EJB (97 mg/kg), whereas in ***T. exilis*** the highest values were found at ESC (275 mg/kg), followed by EA (75 mg/kg) and EJB (42 mg/kg). As for Hg, the highest concentrations were registered by the seventh month for ***T. exilis*** at ESC (0.16 mg/kg) and for ***P. tinctorum***, by the fifth month, at EJB (0.17 mg/kg). For both species no significant differences were found between the concentrations of pollutants absorbed by lichens and the sampled stations. Significant correlations were verified in ***T. exilis*** between the concentration of sulfur and relative humidity ($rp = 0.865$, $p \leq 0.01$) and between zinc and PM10 ($rp = 0.886$, $p \leq 0.05$) for both species.

Table 1. Concentration of pollutants absorbed by *Parmotrema tinctorum* and *Teloschistes exilis thalli* at the three analyzed stations in different exposure periods.

| Station sampling | Exposure time (months) | *Parmotrema tinctorum* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Elements | | | | | |
| | | S (%) | Pb (mg/kg) | Cr (mg/kg) | Cd (mg/kg) | Zn (mg/kg) | Hg (mg/kg) |
| Control | | 0.09 | 2.0 | 3.0 | 0.1 | 44.0 | 0.10 |
| ESC | 2 | 0.10 | 4.0 | 6.0 | < 0.2 | 177.0 | 0.08 |
| | 5 | 0.15 | 5.0 | 6.0 | < 0.2 | 659.0 | 0.03 |
| | 7 | 0.14 | 6.0 | 9.0 | 0.3 | 362.0 | 0.13 |
| EJB | 2 | 0.10 | 5.0 | 6.0 | 0.2 | 72.0 | 0.09 |
| | 5 | 0.14 | 4.0 | 6.0 | < 0.2 | 82.0 | 0.17 |
| | 7 | 0.14 | 6.0 | 9.0 | 0.2 | 97.0 | 0.09 |
| EA | 2 | 0.10 | 4.0 | 7.0 | 0.2 | 50.0 | 0.12 |
| | 5 | 0.18 | 11.0 | 19.0 | 0.2 | 114.0 | 0.11 |
| | 7 | 0.14 | 13.0 | 12.0 | 0.3 | 153.0 | 0.10 |

| Station sampling | Exposure time (months) | *Teloschistes exilis* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Elements | | | | | |
| | | S (%) | Pb (mg/kg) | Cr (mg/kg) | Cd (mg/kg) | Zn (mg/kg) | Hg (mg/kg) |
| Control | | 0.12 | 1.0 | 2.0 | 0.06 | 17.0 | 0.11 |
| ESC | 2 | 0.15 | 2.0 | 3.0 | < 0.2 | 185.0 | 0.08 |
| | 5 | 0.18 | 2.0 | 5.0 | < 0.2 | 242.0 | 0.11 |
| | 7 | 0.17 | 4.0 | 3.0 | < 0.2 | 275.0 | 0.16 |
| EJB | 2 | 0.14 | 3.0 | 3.0 | < 0.2 | 31.0 | 0.10 |
| | 5 | 0.18 | 2.0 | 2.0 | < 0.2 | 37.0 | 0.09 |
| | 7 | 0.14 | 2.0 | 2.0 | < 0.2 | 42.0 | 0.09 |
| EA | 2 | 0.14 | 2.0 | 3.0 | < 0.2 | 23.0 | 0.14 |
| | 5 | 0.21 | 5.0 | 6.0 | < 0.2 | 57.0 | 0.13 |
| | 7 | 0.17 | 7.0 | 5.0 | < 0.2 | 75.0 | 0.13 |

ESC - Santa Cecília, EJB - Jardim Botânico, EA – Anchieta. S= Sulphur, Pb = Lead; Cr = Chrome, Cd = Cadmium, Zn = Zinc, Hg = Mercury. Detection limit: 0.01% = S; 1.0mg/kg = 1.0 mg/kg = Pb, 0.1 mg/kg = Cd, 1.0 mg/kg = Zn, 0.2 mg/kg = Cr, 0.01 mg/kg = Hg.

3.2 Concentration of CO in lichen tissue

During the lichen exposure period a reduction of CO was observed in relation to the control sample for both species, at the evaluated stations and periods (Table 2). The lowest concentrations were registered in *P. tinctorum* by the second month of exposure, at ESC (28%) and at EJB (39%) and, by the fifth month at EA (36%), while for *T. exilis*, at EJB (36%), followed by ESC (36%) by the second month and, at EA by the fifth and seventh

months of exposure (38%). No significant differences were observed for CO concentrations in the lichen samples in relation to the stations.

Table 2. Morphophysiological analyses of *Parmotrema tinctorum* and *Teloschistes exilis* thalli at the three analyzed stations in different exposure periods.

| Station sampling | Exposure time (months) | *Parmotrema tinctorum* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Morphologycals dates | | | | | | |
| | | CO (%) | Chl_a | Chl_b | Live cels | Dead cels | Plasmolyzed cells | IVF |
| Control | | 43.0 | 2.07 | 1.36 | 65.7 | 17.8 | 16.4 | 2.80 |
| ESC | 2 | 28.0 | 0.24 | 0.55 | 66.7 | 31.2 | 2.1 | 2.10 |
| | 5 | 39.0 | 2.41 | 3.18 | 41.9 | 54.6 | 3.5 | 0.77 |
| | 7 | 37.0 | 1.36 | 1.10 | 62.9 | 2.4 | 34.7 | 4.06 |
| EJB | 2 | 39.0 | 0.09 | 0.59 | 52.3 | 43.9 | 3.8 | 1.18 |
| | 5 | 42.0 | 0.15 | 5.80 | 42.4 | 53.4 | 4.2 | 0.80 |
| | 7 | 40.0 | 0.85 | 2.02 | 59.3 | 11.0 | 29.7 | 2.87 |
| EA | 2 | 40.0 | 0.58 | 0.15 | 67.5 | 29.0 | 3.5 | 2.25 |
| | 5 | 36.0 | 2.79 | 16.54 | 47.4 | 47.4 | 5.1 | 1.00 |
| | 7 | 37.0 | 1.00 | 0.26 | 58.8 | 8.8 | 32.4 | 3.00 |

| Station sampling | Exposure time (months) | *Teloschistes exilis* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Morphologycals dates | | | | | | |
| | | CO (%) | Chl_a | Chl_b | Live cels | Dead cels | Plasmolyzed cells | IVF |
| Control | | 40.0 | 1.45 | 2.63 | 64.4 | 29.2 | 6.4 | 2.09 |
| ESC | 2 | 37.0 | 0.41 | 0.95 | 53.0 | 42.4 | 4.5 | 1.24 |
| | 5 | 38.0 | 3.43 | 3.97 | 35.7 | 58.7 | 5.6 | 0.63 |
| | 7 | 39.0 | 1.03 | -0.30 | 47.6 | 5.5 | 46.9 | 2.45 |
| EJB | 2 | 36.0 | 0.32 | 1.90 | 48.6 | 41.7 | 9.7 | 1.15 |
| | 5 | 37.0 | 2.61 | 4.43 | 36.6 | 53.3 | 10.1 | 0.71 |
| | 7 | 39.0 | 1.82 | 0.12 | 51.0 | 6.0 | 43.0 | 2.63 |
| EA | 2 | 39.0 | 0.50 | 0.90 | 61.2 | 24.7 | 14.1 | 2.15 |
| | 5 | 38.0 | 3.33 | 7.64 | 27.0 | 53.5 | 19.5 | 0.58 |
| | 7 | 38.0 | 3.33 | 8.10 | 30.5 | 9.4 | 60.1 | 1.53 |

ESC - Santa Cecília, EJB - Jardim Botânico, EA – Anchieta, CO = Organic Carbon, Chl = chlorophyll, IVF = Index of Photobiont Vitality. Detection limit: 0.01% = CO.

3.3 Live, dead and plasmolyzed cell counting and IVF

Differences in the percentage of live, dead and plasmolyzed cells and in IVF were verified in relation to the control samples, as well as between the stations and the periods analyzed (Table 2). The lowest percentages of live cells were registerd by the fifth month of exposure, in both species at all stations while the highest percentages of dead and plasmolyzed cells were

registered in the same exposure period. The same pattern was observed for IVF. No significant differences were observed between the analyzed periods for both species. Significant correlations were verified in *P. tinctorum*, between sulfur concentrations and the percentage of live cells ($rp = -0.674$, $p \leq 0.05$), Cd and the percentage of dead cells ($rp = -0.725$, $p \leq 0.05$) and between the concentration of Pb ($rp = 0.777$, $p \leq 0.05$), Cr ($rp = 0.716$, $p \leq 0.05$) and Cd ($rp = 0.728$, $p \leq 0.05$) and plasmolyzed cells. For *T. exilis* significant correlations were observed between the concentration of S and IVF ($rp = -0.710$, $p \leq 0.05$) and between the percentage of dead cells and the concentration of $O_3$ in the air ($rp = -0.900$, $p \leq 0.05$).

3.4 Chlorophyll Analysis

Variations in Chl_a and Chl_b were registerd at all stations and exposure periods for both species, especially in relation to the control samples (Table 2). The lowest chlorophyll values were identified at station EJB in relation to the other sampled stations, especially in *P. tinctorum*. The lowest values were registered in *P.tinctorum* for Chl_a (0.09 mg $g^{-1}$) and Chl_b (0.59 mg $g^{-1}$) by the second month and for Chl_a (0.15 mg $g^{-1}$) and Chl_b (5.80 mg $g^{-1}$) by the fifth month, whereas in *T. exilis* values were Chl_a (0.32 mg $g^{-1}$) and Chl_b (1.90 mg $g^{-1}$) by the second month and Chl_a (2.61 mg $g^{-1}$) and Chl_b (4.43 mg $g^{-1}$) by the fifth month. However, by the seventh month of exposure a reduction was registered: Chl_a (1.82 mg $g^{-1}$) and Chl_b (0.12 mg $g^{-1}$). Station ESC registered values inferior to those registered at EA for both species and periods, and the lowest values were registered by the fifth and seventh months of exposure (Table 2). No significant differences were registered between the periods and both species exposed. Significant correlations were verified in *T. exilis* between Chl_a values and the concentration of $O_3$ in the air ($rp = -0.900$, $p \leq 0.05$).

3.5 Analysis of External Morphological Damages

Damages on the external appearance of lichen thalli were verified in the first months of exposure, in both species. At stations EA and EJB chorotic thalli were registered and at station ESC brownish stains were verified in both species. By the fifth month of exposure chlorosis became more intense, with medulla exposure in both species at all sampled stations. By the seventh month, both chlorosis and necrosis were present, in addition to the medulla exposure, especially at ESC. Another aspect registered in the thalli of *P. tinctorum* was an increase of vegetative propagules.

3.6 *Salmonella*/microsome Assay

Mutagenic activity was verified in the analysis of organic extracts obtained from PM10 collected at two sampled stations (EJB and EA), with significant values according to Anova and regression analysis run in the Salanal Software. Responses were observed for frameshift damages without metabolism, varying in rev/$m^3$ of sampled air from 15.4±1.0 to 7.0±0.70 (EJB) and from 13.0±1.00 to 9.2±0.46 (EA). Higher values were observed in the first (two months) and second (five months) exposure periods to EJB and EA, respectively. The highest values were found at EA in the second and third exposure periods. After metabolic activation, reduction and constancy in responses were observed at each station, being higher at EA (7.4±0.59 to 7.1±0,67) than at EJB (5.4±0.42 to 4.2±0.49) (Fig. 2). When analyzing the presence of nitrocompounds through YG strains, higher and more significant values for YG1021 strains were observed when compared to TA98 parental strain during the different exposure periods (Fig. 3).

When cytotoxicity was analyzed, survival rates of bacteria below 60% were found during the two months of lichen exposure. The lowest percentages were observed for TA98+S9, at stations EA (40%) and EJB (57.2%). By the fifth month of exposure, cytotoxicity tests

indicated a survival rate of 55.7% for TA98, at EJB and 53.8% for TA98+S9 at EA. By the seventh month toxicity was found only for TA98 strains, with a survival rate of 58.6% at EA.

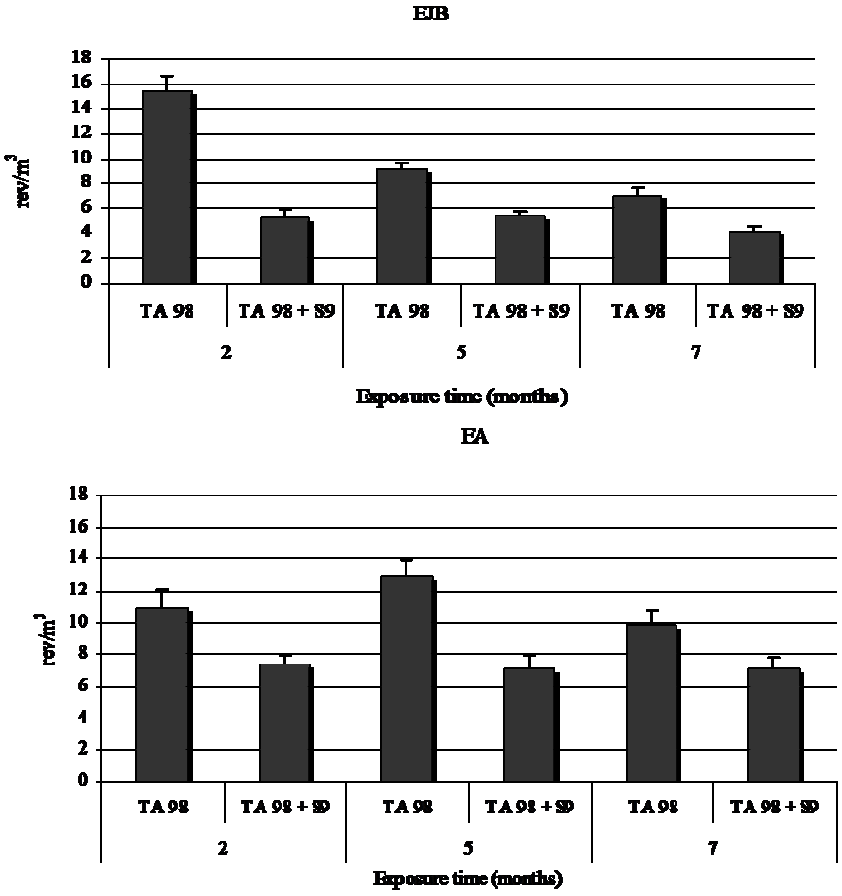


Figure 2. Mutagenic activity in samples of air at stations EJB and EA with TA98 strain with (+S9) and without (-S9) hepatic fraction metabolism. Values expressed in rev/$m^3$. Caption: EJB - Jardim Botânico, EA – Anchieta.

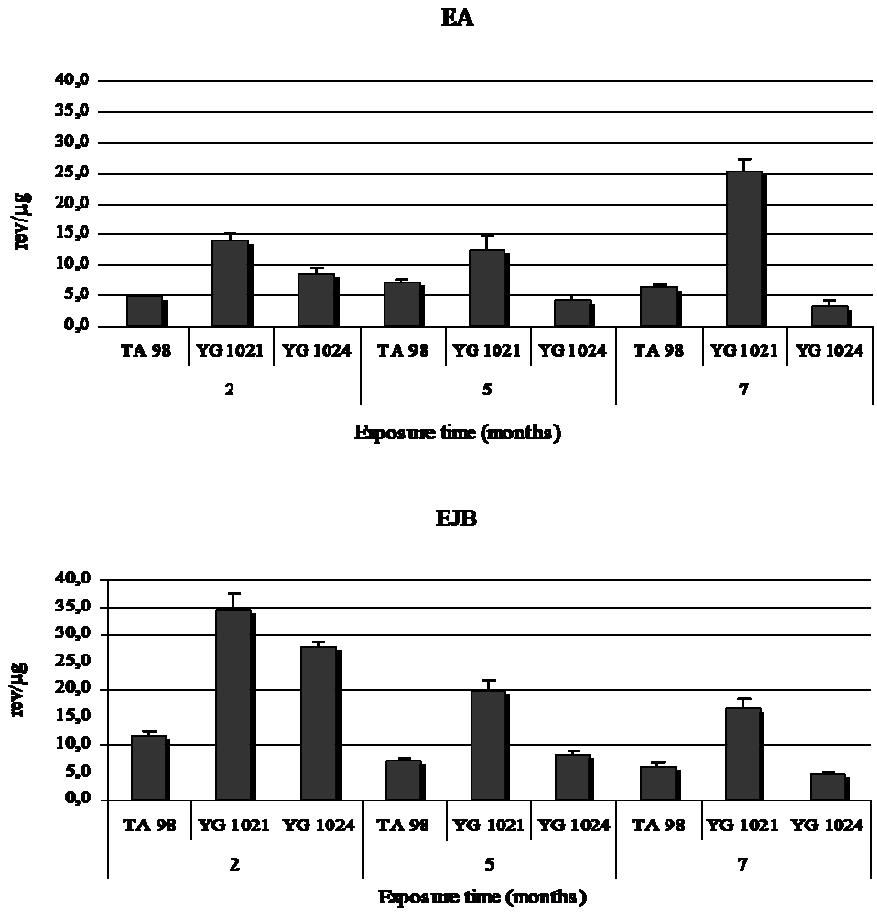


Figure 3. Mutagenicity (rev/µg) in samples of air observed at stations EJB and EA, expressed in the nitrocompound strains. Caption: EJB - Jardim Botânico, EA – Anchieta.

3.7 Analyses of PHAs profile in the lichen samples

In lichen species the presence of some PAHs was detected at all sampled stations, both in the control samples and in the different exposure periods (Table 3). In the *P. tinctorum* samples some traces of Acenaphthene were registered at station ESC by the second month of exposure. The presence of such compound in control samples and traces of Anthracene at station EJB were detected by the fifth month of exposure. The presence of Naphthalene was registered at stations ESC and EJB, by the fifth month of exposure and traces were found at EA during the same period. The presence of Phenanthrene was detected in the control sample and at stations ESC and EA, by the second month of exposure while during the same period

traces of PAH were detected at station EJB. By the fifth month of exposure traces of PAH at EA and the presence at EJB were detected. By the seventh month, traces of PAH were found at EJB whereas the presence of PAH was found at EA.

In *T. exilis* samples the presence of Acenaphthene was detected at station ESC, by the second month of exposure. By the fifth month, traces of this PAH were registered at station EJB while at ESC it was present. By the seventh month the presence of this substance was verified at all stations. Traces of Anthracene were registered in the control sample and, at station EJB, by the fifth month. The presence of Acenaphthylene was registered at all stations analyzed only by the seventh month of exposure. Traces of Naphthalene were detected in the control sample and by the second month at stations EJB and EA. By the fifth month, the presence of this compound as well as traces of ESC were registered at stations EJB and EA while by the seventh month only traces of such compunds were found. The presence of Phenanthrene was verified in the control sample and at station ESC. Traces of such substance were found at station EJB by the second month. By the fifth month the presence of Phenanthrene was verified at sation ESC as well as traces were observed at sation EA. By the seventh month the presence of this PAH was found at all stations.

Table 3. PAHs profile in thalli of lichen samples at the three sampled stations.

*Parmotrema tinctorum*

| PAHs | Station sampling/Exposure time | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | ESC | | | EJB | | | EA | | |
| | | 2 | 5 | 7 | 2 | 5 | 7 | 2 | 5 | 7 |
| Acenaphthene | + | traces | - | - | - | - | - | - | - | - |
| Acenaphtylene | - | - | - | - | - | - | - | - | - | - |
| Aminoanthracene | - | - | - | - | - | - | - | - | - | - |
| Anthracene | - | - | - | - | traces | - | traces | - | - | - |
| Benzo(a)anthracene | - | - | - | - | - | - | - | - | - | - |
| Benzo(a)pyrene | - | - | - | - | - | - | - | - | - | - |
| Benzo(b)fluoranthene | - | - | - | - | - | - | - | - | - | - |
| Benzo(g,h,i)peryilene | - | - | - | - | - | - | - | - | - | - |
| Benzo(k)fluoranthene | - | - | - | - | - | - | - | - | - | - |
| Chrysene | - | - | - | - | - | - | - | - | - | - |
| Dibenzo(a,h)anthracene | - | - | - | - | - | - | - | - | - | - |
| Dibenzofurane | - | - | - | - | - | - | - | - | - | - |
| Phenanthrene | + | + | - | - | traces | + | traces | + | traces | + |
| Fluoranthene | - | - | - | - | - | - | - | - | - | - |
| Fluorene | - | - | - | - | - | - | - | - | - | - |
| Naphthalene | - | - | + | - | - | + | - | - | traces | - |
| Nitropyrene | - | - | - | - | - | - | - | - | - | - |
| Pyrene | - | - | - | - | - | - | - | - | - | - |

*Teloschistes exilis*

| PAHs | Station sampling/Exposure time | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | ESC | | | EJB | | | EA | | |
| | | 2 | 5 | 7 | 2 | 5 | 7 | 2 | 5 | 7 |
| Acenaphthene | - | + | + | + | - | traces | + | - | - | + |
| Acenaphtylene | - | - | - | + | - | - | + | - | - | + |
| Aminoanthracene | - | - | - | - | - | - | - | - | - | - |
| Anthracene | traces | - | - | - | - | traces | - | - | - | - |
| Benzo(a)anthracene | - | - | - | - | - | - | - | - | - | - |
| Benzo(a)pyrene | - | - | - | - | - | - | - | - | - | - |
| Benzo(b)fluoranthene | - | - | - | - | - | - | - | - | - | - |
| Benzo(g,h,i)peryilene | - | - | - | - | - | - | - | - | - | - |
| Benzo(k)fluoranthene | - | - | - | - | - | - | - | - | - | - |
| Chrysene | - | - | - | - | - | - | - | - | - | - |
| Dibenzo(a,h)anthracene | - | - | - | - | - | - | - | - | - | - |
| Dibenzofurane | - | - | - | - | - | - | - | - | - | - |
| Phenanthrene | + | + | + | + | traces | - | + | - | traces | + |
| Fluoranthene | - | - | - | - | - | - | - | - | - | - |
| Fluorene | - | - | - | - | - | - | - | - | - | - |
| Naphthalene | traces | traces | traces | traces | traces | + | traces | - | + | - |
| Nitropyrene | - | - | - | - | - | - | - | - | - | - |
| Pyrene | - | - | - | - | - | - | - | - | - | - |

ESC – Santa Cecília, EJB - Jardim Botânico, EA – Anchieta. Presence (+), Absence (-).

3.8 Environmental Variables

The pools of Particulate Matter (PM10) analyzed during the three periods of exposure varied from 181 g/m$^3$ in the first period (relative to six weeks of sampling, average per filter of 30 g/m$^3$) at station EJB to 569.4 g/m$^3$ at station EA with higher values by the seventh month of exposure, relative to nine weeks of sampling (weekly average per filter of 63.25 g/m$^3$). Five elevated weekly peaks of PM10 were verified, from 61 to 150 g/m$^3$, especially at station EA, relative to seven months of lichen exposure (Table 4). The values of $O_3$ varied from 15.01 to 42.75 gm$^3$. For the period comprised between August 2007 and June 2008, the air temperature varied from 13ºC to 24.5ºC, relative humidity varied from 68% to 84.8% and precipitation varied from 50.3 to 174.9 mm. No correlation was verified between the concentrations of pollutants in lichens, morphophysiological damages and climate variables.

As for traffic flow, the highest value was registered at station EJB with 121.650 vehicles in a sampling day. High levels of light vehicles (96.519), medium-sized vehicles (8.505), heavy vehicles (5.170) and motorcycles (11.457) were registered at this station. Station ESC obtained the second highest total volume of vehicles (67.216), while station EA obtained 15.288.

Table 4. Concentrations of PM10 and $O_3$ at EJB and EA stations in the evaluated period.

| Station sampling | Exposure time (months) | PM10 ∑C µg/m$^3$ | $O_3$ |
|---|---|---|---|
| EJB | 2 | 181 | 125.71 |
| | 5 | 300 | 82.99 |
| | 7 | 228.1 | 121.37 |
| EA | 2 | 222 | ------- |
| | 5 | 524 | ------- |
| | 7 | 569.4 | ------- |

| Station sampling | Exposure time (months) | PM10 ∑C µg/m$^3$ | Média/filtro |
|---|---|---|---|
| EJB | 2 | 181 | 30.17 |
| | 5 | 300 | 27.27 |
| | 7 | 228.1 | 25.34 |
| EA | 2 | 222 | 31.71 |
| | 5 | 524 | 34.93 |
| | 7 | 569.4 | 63.27 |

EJB - Jardim Botânico, EA – Anchieta.

3.9 Comparison between morphological damages in lichens and mutagenic tests of organic extracts

When comparing the analyses carried out in lichen species and mutagenesis and cytotoxicity tests of organic extracts, the same pattern of results was observed, that is, an increasing concentration of pollutants in lichens and consequent morphophysiological alterations with the presence of mutagenic and citotoxic activities in the samples of organic extracts in the periods and stations analyzed. Significant correlations were verified in ***P. tinctorum*** between Chl_a values and plasmolyzed cells ($rp = -0.886$, $p \leq 0.05$) with YG1024 strain and the percentage of live cells ($rp = -0.829$, $p \leq 0.05$) and TA98-S9 strain. For ***T. exilis*** the correlations were found between TA98-S9 strains and the percentage of plasmolyzed cells ($rp = -0.886$, $p \leq 0.05$) and CO ($rp = -0.971$, $p \leq 0.01$). Such results show the increase in the

mutagenicc activity in the organic extracts of PM10 is related to the decrease in the percentage of live cells, CO and chlorophyll values, as well as to the increase of plsmolyzed cells.

**4. Discussion**

The comparison between the responses of biomarkers and bioindicators as parameters of air quality aims at defining the sensitivity of organisms in relation to different contaminant groups. The strategy used in the present study allowed to evaluate the concentrations of some atmospheric pollutants, including PAHs absorbed by lichens, morphophysiological damages in the thalli of specimens in order to define the response of two lichen species exposed during two, five and seven months to urban pollution, through the transplant methodology; evaluate and relate the presence of organic mutagenic compounds adsorbed onto PM10 during the same period of exposure, using different strains from the *Salmonella*/microsome assay as biomarkers. The literature already brings information of such assay as sensitive to specific compound groups with known mutagenic and carcinogenic action, such as PAHs and their nitroderivates (Claxton et al., 2004; Vargas, 2003; Ducatti and Vargas, 20003).

An increase in the concentration of pollutans in the thalli was registered in two lichen species, especially S, Pb, Cr, Hg and Zn at all stations in relation to the concentrations found in the control samples. Among the analyzed pollutants, Zn showed higher volumes at station ESC. Studies carried out by several researchers in urban areas and/or with industrial influences report the accumulation of these pollutants in the thalli of lichen species (Coccaro et al., 2000; Minganti et al., 2003; Prochnow and Porto, 2005; Gombert et al., 2006; Saiki et al., 2007a, 2007b; Fuga et al., 2008; Bermudez et al., 2009; Calvelo et al., 2009). Pollutants such as S, Pb, Cr and Zn are related to traffic flow and industrial emissions. Heavy vehicles (buses and trucks) are responsible for the high fractions of sulfur and nitrogen oxides emissions, while light gasoline and alcohol-powered vehicles are the main responsible for the emissions of

carbon monoxide and hydrocarbonets (Teixeira et al., 2008). In relation to Cr, the main sources are the metallurgical and siderurgical industries, among others (Liberman et al., 2005). Zinc is related to many anthropogenic sources. However, traffic flow and industrial emissions are the main potential sources of this metal (Minganti et al., 2003). In *P. tinctorum* a higher accumulation of pollutants was verified in comparison to *T. exilis*. High rates of pollutant accumulation depend on the lichen species and the element, which shows the sensitivity of the species in the accumulation mechanism (Garty et al., 2001; Minganti et al., 2003). Both species used in the present study feature distinct growth forms. *Parmotrema tinctorum* belongs to the foliose group, which usually have a dorsiventral organization and thalli with well defined surfaces attached to the substract. *Teloschistes exilis* belongs to the fruticose group, is highly branched and features a single attachment to the surface. The growth form determines the orientation on the surface of the specimen for the accumulation of airborne pollutant and some studies demonstrate folisoe lichens accumulate a larger quantity of elements if compared to the fruticose group (Glenn et al., 1995; St. Clair et al., 2002).

When analyzing morphophysiological damages to lichen species, the following findings were verified: alterations in the percentage of organic carbon, of dead and plamolyzed cells, of IVF and chlorophyll values, especially Chl_a. All these alterations cause damages to the external appearance of lichens resulting in chlorosis, necrosis and medulla exposition. The alterations were more pronounced by the fifth and seventh months of exposure and are related to an increase in the concentration of pollutants, especially S, Cr, Pb and Zn in both species and at all sampled stations. Similar results were registerd in other studies, in which morphophysiological damages in thalli of lichen species were observed, especially in chlorophyll and in the percentage of dead and plasmolyzed cells (Zambrano and Nash, 2000; Calvelo and Liberatore, 2004; Garty et al., 2007; Basile et al., 2008; Baruffo et al., 2008;

Bajpai et al., 2010; Carreras et al., 2009). The type of response varies according to the pollutant, its concentration and exposure time.

Transplanted samples usually show a degradation of chlorophyll and are positively correlated to the pollution level (Garty et al., 1993; González and Pignata, 2000; Boonpragob, 2002), mainly by traffic flow and other urban emissions (Carreias et al., 1998). The chlorophyll_a is more sensitive to pollutants than Chl_b (Rao and Le Blanc, 1966; Gries, 1996). The presence of chloroses and necroses as well as the increased number of dead cells demonstrate the damages caused to lichens, possibly because of urban pollution. According to Calvelo and Liberatore (2004), the percentage of dead cells may reveal an important variable for the evaluation of damages to lichen thalli and, thus, for the estimation of atmospheric contamination. Thallus darkening occurs as a result of chlorophyll degradation (Puckett et al., 1973). Such disturbances not necessarily cause the lichen death, but may cause changes in the morphology of species (Hale, 1983).

Another clear alteration signal was the increased number of vegetative propagules in *P. tinctorum*, especially by the seventh month of exposure. De Sloover and Le Blanc (1968) relate that sulfur dioxide ($SO_2$) is known to estimulate the production of soredia and isidia. The response of each organism may also be strongly influenced by climate conditions as well as physiology and nourishment conditions (Nash, 1996). Even though the highest levels of pollutant concentrations were verified by the seventh month of exposure, more pronounced damages were found by the second and fifth month of exposure, especially for the levels of chlorophyll, dead cells and IVF. For plasmolyzed cells, the highest percentage was found by the seventh month of exposure and is related to the vitality state of photobiont cells, once they can recover or die. Such results may be related to the morphology and physiology of species and their capacity to recover, considering the environmental alterations. During the period of lichen exposure, different morphological responses were observed. The first damages were

identified by the second month as a decrease in the percentage of CO in species, followed by a decrease in cholophyll values, an increase in the percentage of dead cells, a decrease of IVF, followed by thallus necrosis after five months of exposure. An increased percentage of plasmolyzed cells and enlargement of the necrotic area with medulla exposure occurred by the seventh month. Such results may indicate the loss of CO interferes with the capacity of photobionts to carry out photosyntesys with consequent death of cells. When analyzing organic extracts based on PM10, station EA showed steadier levels of high values during most periods, even though higher levels were found at station EJB during the first exposure period. Cytotoxicity was also present at station EA. Such responses are related to the different contributions from both stations, predominantly urban at EJB while EA was influenced by an urban/industrial area. Although a decrease occurred in assays in the presence of S9, the significant response observed reveals the presence of PAHs-like compounds, with discrete sensitivity to this biomarker (Claxton et al., 2004; Pereira et al., 2010) as previously observed in different studies (Vargas et al., 1998; Ducatti and Vargas, 2003). It is important to report the increase of genotoxins at EJB if compared to previous studies (Vargas et al., 1998; Ducatti and Vargas, 2003) resulting in a decrease in the air quality over time, absence of mutagenic activity (Vargas et al., 1998) until the present results.

The comparison betwen rev/µg responses observed between TA98 strain and YG1021/1024 strains, evidence the presence of mononitroarene-type PAHs derivates identified by YG1021 strains, during the three exposure periods at both stations. In previous studies at the same stations, a higher presence of mononitroarenes was observed in the urban area in relation to dinitroarenes (Vargas et al., 1998; Ducatti and Vargas, 2003; Coronas et al., 2008; Pereira et al., 2010). Although mutagenicity of samples cannot be attributed to a specific group of compounds, much of it is due to nitro-PAH lactones and to simpler nitro-PAH (Lewtas, 2007). This supports the importance of co-pollutants in the mutagenic activity of

airborne particles. Reactive gases ($NO_X$, $O_3$) under photoactive conditions can produce mutagenic compounds from non-mutagenic organic compounds commonly present in ambient air (Claxton et al., 2004).

The comparison between mutagenesis and cytotoxicity assays and the results obtained from the lichen samples revealed morphological alterations in the lichen thalli, for the same periods, and significant correlations ($p \leq 0.05$) indicating a probable influence of the presence of organic compounds adsorbed onto PM10, detected by the biomarker, and the effects in the exposed species. It was possible to observe an increase in the direct mutagenesis in organic extracts of PM10 related to a decrease in the percentage of live cells, CO and chlorophyll values, as well as an increase of plasmolyzed cells in *P. tinctorum*.

Atmospheric particles comprise a complex mixture of different elements and compounds, constituted by, for example, sulfates, nitrates, organic compounds (PAHs, nPAHs) and heavy metals (Pb, Zn, Cr, Cd) (Dallarosa et al., 2008). They are originating from different sources which include vehicle emissions (especially gas oil powered engines), combustion emissions and those resulting from industrial processes, contruction among others (Oliveira and Kummrow, 2008). In addition, the absortion of S, Pb, Cr, Zn and PAHs in tissues indicate that lichen species suffer damages due to the presence of such pollutants.

The presence and/or traces of some PAHs were verified at different stations and in different periods both in samples of exposed lichens and control samples, especially by the fifth month. Naphthalene and Phenanthrene were the predominanting compounds in lichen samples, at all stations and in the same periods. Naphthalene is a semivolatile PAH present in the atmosphere in the gas phase (Reisen et al., 2003) and considered one of the most abundant (Albinet et al., 2007) contributing to mutagenicity observed in the vapor stream phase in air samples (Gupta et al., 1996). Phenanthrene originates especially from vehicle emissions, mainly from gas oil powered vehicles (Blasco et al., 2006). Studies carried out in urban areas

with the use of lichens to detect PAHs cite the presence of such compounds in exposed samples, associating the occurrence with traffic flow (Owczarek et al., 2001; Guidotti et al., 2003; Domeño et al., 2006; Blasco et al., 2006, 2007; Shukla and Upreti, 2009). In Brazil no published studies on this topic exist.

Aromatic Polycyclic Hydrocarbons with three and/or four rings in the structure are present in great part of vehicle emissions and partially or totally present in the vapor stream phase in the atmosphere (Guidotti et al., 2003). Considering Phenanthrene and Anthracene are in this phase, it may have been easier to be bioaccumulated by lichens. The presence and/or traces of some PAHs in the control samples indicate that even areas away from the urban region suffer with the influence of atmospheric pollution. Studies carried out in nearby cities, in the metropolitan region of Porto Alegre, report the presence of PAHs, especially Dibenzo(a,h)anthracene, Fluoranthene, Benzo(a)anthracene, Benzo(a)pyrene, Chrysene, Benzo(ghi)perylene and Indeno [1,2,3-cd]pyrene associated in the fraction of PM10 (Dallarosa et al., 2008). Benzo(ghi)perylene and Indeno [1,2,3-cd]pyrene were found in high concentrations and are related to mutagenic and citotoxic responses from organic extracts analyzed in this region (Pereira et al., 2010).

The occurrence of such PAHs in the present manuscript corroborates with other studies carried out in urban areas and are especially related to traffic flow and /or other characterisic emissions (Vasconcellos et al., 2003; Dallarosa et al., 2005, 2008; Ré-Poppi and Silva, 2005; Rehwagen et al., 2005; Pereira et al., 2010).

As for the concentration of pollutants, the results found are in accordance with the patterns demanded by the Brazilian legislation - Resolution CONAMA 03/90, which establishes PM10 patterns as daily concentrations of 150 $g/m^3$ and annual averages of 50 $g/m^3$. However, weekly peaks above the established by WHO (2006) were observed, which establishes daily averages of 50 $g/m^3$, more frequently at station EA. As for $O_3$, the concentration verified is in

accordance with the legislation which establishes 160 $g/m^3$ for a one-hour concentration (CONAMA, 1992). In Porto Alegre, the main source of pollutant emission is the transportation sector, which corresponds to mobile sources, based on the gasoline, gas oil and alcohol combustion. Fixed sources which use combustion from gas oil and coal burning are also reported (Lima et al., 1998; Teixeira et al., 2008).

Climate variables are also in accordance with the patterns for the region. The pollutant concentrations also depend on metereological conditions (Simpson, 1994), once factors such as wind direction and speed, thermal inversion and precipitation may contribute to transportation, dispersion and deposition of atmospheric pollutants (Dallarosa et al., 2005).

More pronounced morphological alterations, highest concentrations of pollutant in the lichen thalli as well as a more pronounced occurrence of PAHs were registered at station ESC. At the other stations, the highest concentrations of pollutants, especially S, Pb and Zn, the highest percentage of dead and plasmolyzed cells and chlorophyll were registered at EA when compared to EJB, especially by the fifth and seventh exposure period. In mutagenesis analysis the results were more frequently high at EA by the different periods and by the first period at EJB. At stations ESC and EA alterations in the structure of the urban lichen community were also verified, especially in the composition, richness and coverage of species. In addition, such areas were also classified as lichen-absent and lichen-poor areas (Käffer et al., 2011). Such areas are located in regions of higher potential for the accumulation of atmospheric pollutants over the year, characterized as edification and/or depression regions, with scarse vegetation and high traffic flow. As a result, the dispersion conditions of pollutants become hindered. In addition, station EJB is located in an area of intense traffic flow and receives contributions of particulate matter originating from the burning of residues from a hospital area (Braga et al., 2005; Dallarosa et al., 2008). Station EA is located in the city outskirts, near roads of intense traffic flow, the airport and a small industrial district which includes a siderurgy company

(Vargas et al, 1998; Dallarosa et al., 2005, 2008; Teixeira et al., 2008). Thus, the features of these areas contributed to the results obtained.

Out of the lichen species used in the present study *P. tinctorum* turned out to be more appropriate for the evaluation of atmospheric quality in urban environments and for the detection of some PAHs, once *T. exilis* demonstred higher sensitivity to the action of atmospheric pollutants.

Traffic flow, climate variables and topography of the sampled stations may have contributed to the results observed. On the other hand, urbanized regions are highly complex, where different emission sources release a variety of pollutants, what makes it difficult to determine which factor influences lichen species. It must be also considered that the biological effect is the result of a mixture of compounds and not only the action of a specific component.

The present manuscript reveals that a set of factors contributed to the morphophysiological alterations of lichens. The use of lichens as bioindicators and mutagenesis biomarkers, through *Salmonella*/microsome assay, contributed to the evaluation of air quality and to the diagnosis of the presence of environmental-aggressive compounds. They showed a differentiated sensitiveness in relation to the group of compounds present in the mixture, making it possible to map areas with potential risk to the environment. The inclusion of the *Salmonella*/microsome assay showed the advantage of defining the presence of mutagenic compounds with carcinogenic potentiality with risks to human health.

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## 7. CONSIDERAÇÕES FINAIS

O presente estudo proporcionou avaliar o desenvolvimento da micobiota liquenizada corticícola, em diferentes estações amostradas, na cidade de Porto Alegre, RS, tendo em vista que as alterações ambientais ocasionadas pela influência da poluição atmosférica em área urbana possam vir a interferir na estrutura desta comunidade liquênica, assim como no seu estabelecimento no meio ambiente.

Foram constatadas diferenças na estrutura da comunidade liquênica quanto a composição, riqueza, diversidade e cobertura dos táxons. A grande diversidade de espécies registradas (144 táxons) indica sua adaptabilidade às condições ambientais urbanas, como ação de poluentes atmosféricos, luminosidade, umidade e tipo de forófita. Do total de táxons registrados, oito são novos para a ciência, uma citação é nova para o Continente americano, três registros são novos para o Brasil e 32, para o Rio Grande do Sul. De acordo com Marcelli (1998), são esperadas em torno de 150 espécies para áreas urbanas do Brasil.

A composição das espécies liquênicas diferenciou-se nas áreas analisadas, sendo que *Canoparmelia texana* (Tuck.) Elix & Hale, *Dirinaria picta* (Sw.) Schaer. ex Clem., *Anisomeridium tamarindii* (Fée) R. C. Harris, *Glyphis cicatricosa* Ach., *Parmotrema tinctorum* (Nyl.) Hale, *Parmotrema reticulatum* (Taylor) M. Choisy, *Graphis caesiocarpa* Redinger, *Graphis parallela* (Müll. Arg.) Cáceres & Lücking, *Lecanora concilianda* Vain. e *Ramalina celastri* (Sprengel) Krog & Swinscow foram consideradas espécies indicadoras de áreas urbanas. As duas primeiras espécies também apresentaram o maior Valor de Importância (VI), estando este relacionado aos valores de riqueza e cobertura das mesmas, mostrando, desta forma, que os aspectos ecológicos e reprodutivos destes táxons são determinantes na estrutura da comunidade. Por serem do grupo morfológico folioso e apresentarem talos maiores, ambas preenchem o espaço anteriormente ocupado por táxons menos resistentes às adversidades ambientais ocasionadas pela poluição atmosférica, o que lhes confere sua

capacidade de competidoras por espaço. Em áreas urbanas do país, a composição da micobiota liquenizada é caracterizada pela dominância de espécimes da família Parmeliaceae e Physciaceae, especialmente dos gêneros *Parmotrema*, *Hypotrachyna*, *Heterodermia* e *Canoparmelia*, assim como do grupo morfológico crostoso (Marcelli, 1998).

Nos últimos anos, tem se intensificado e aprimorado o emprego de índices para estimar a qualidade do ar através da sensibilidade dos liquens. No entanto, estes foram elaborados para serem aplicados em regiões da Europa e Estados Unidos, onde a comunidade liquênica é menos diversificada. Desta forma, a utilização de um fator de correção à fórmula original do Índice de Pureza Atmosférica (IPA), o Fator de Classificação Ambiental (FCA), proposto neste estudo, foi importante para tornar o índice mais sensível. Consideramos a necessidade de analisar um perfil multivariado de informações, especialmente em regiões onde a diversidade de espécies é maior. Assim, com o emprego do FCA, foi possível mapear e diferenciar as estações amostradas de acordo com a salubridade do ar. As estações localizadas nas áreas norte (E28 – Passo d' Areia, E23 – Higienópolis, E13 - Anchieta e E15 – Sarandi), leste (E22 – Lomba do Pinheiro/Belém Velho) e central (E3 – Centro 1, E29 – Centro 2, E2 – Santa Cecília, E12 – Petrópolis) da cidade foram classificadas como zonas pobres e/ou ausentes em liquens mostrando a influência da poluição atmosférica (especialmente ocasionada pelo tráfego veicular), das condições climáticas e características geográficas das áreas avaliadas. Estas áreas (especialmente E2, E3, E13 e E15) estão localizadas em regiões de maior potencial de acumulação de poluentes atmosféricos, ao longo do ano, especialmente de metais pesados como chumbo e zinco, além de HPAs e são caracterizadas como regiões edificadas e/ou de depressão de relevo, assim como de vegetação escassa, grande circulação de veículos e indústrias. Em função destas características, as condições de dispersão de poluentes tornam-se prejudicadas (Braga et al., 2005; Dallarosa et al., 2005, 2008; Teixeira et al., 2008).

Diferenças na estrutura da comunidade liquênica foram verificadas nas estações amostradas quanto à presença de espécies acidófilas, nitrófilas e indiferentes; distribuição vertical; espécies especialistas e generalistas em altura; fragmentação do talo, assim como preferência por forófitas. A maior ocorrência (97) de espécies indiferentes, ou seja, que ocorreram tanto em forófitas com pH ácido, como, básico ou neutro, indica que estas não possuem restrições para se estabelecerem em determinados substratos, estando bem adaptadas às diferentes condições de acidez ou alcalinidade das forófitas. As espécies liquênicas que colonizam hospedeiros indiferenciados tendem a ter uma ampla distribuição pela maior oferta de substratos (Valencia & Ceballos, 2002). Nas mesmas estações que foram classificadas como zonas pobres e/ou ausentes de liquens, registraram se as maiores alterações quanto a distribuição vertical, ocorrência de espécies generalistas e especialistas em altura e fragmentação do talo das espécies. As modificações registradas na estrutura da comunidade liquênica entre as estações analisadas podem também estar associadas às diferenças de temperatura entre as áreas da cidade, fato este relacionado com a arborização urbana.

A utilização de duas espécies liquênicas: ***Parmotrema tinctorum*** (Nyl.) Hale e ***Teloschistes exilis*** (Michx.) Vain., através da exposição destas, em três estações da cidade (E2, E4 e E13), mostrou a presença de contaminantes característicos da contribuição urbana, sendo verificado acúmulo de poluentes, especialmente de S, Pb, Cr, Hg e Zn em relação às amostras testemunho. Com estas espécies, também foi possível verificar a ocorrência de alguns HPAs, especialmente de Naftaleno e Fenantreno, que predominaram nas amostras liquênicas, constantes na lista de poluentes prioritários pela Agência de Proteção Ambiental dos Estados Unidos (USEPA, 1985). No entanto, não foi detectada a presença daqueles considerados como de maior risco carcinogênico pela International Agency For Research On Cancer - IARC (2010). Entre os mais tóxicos, que apresentam maior possibilidade de risco à população humana estão: Dibenzo(a,h)antraceno, Benzo(a)antraceno, Benzo(b)fluoranteno,

Benzo(k)fluoranteno, Benzo(a)pireno, e Indeno [1,2,3-cd]pireno. Em função da exposição aos poluentes, foram constatados danos morfofisiológicos nas espécies como: aumento do percentual de células mortas, diminuições do Índice de Vitalidade do Fotobionte (IVF), dos valores de clorofila e Carbono Orgânico, cloroses, necroses e exposição de medula. As maiores alterações, ao longo do período, foram verificadas na estação E2, seguida da E13 e E4.

Os impactos do ambiente urbano incluem mudanças na estrutura da comunidade e na morfologia dos liquens (Gries, 1996). Os efeitos morfofisiológicos e estruturais incluem redução no tamanho do talo, diminuição ou perda total de apotécios em alguns táxons, aumento no número de propágulos vegetativos, redução na abundância de táxons individuais (provavelmente resultado da diminuição do talo) e na sua freqüência, simultaneamente com completa perda de espécies sensíveis (Le Blanc & Rao, 1973; Sigal & Nash, 1983; Zambrano & Nash, 2000; Scutari & Theinhardt, 2001; Fremstad et al., 2005; Glavich & Geiser, 2008).

Adicionalmente à exposição dos bioindicadores, os testes de mutagênese e citotoxicidade dos extratos orgânicos do material particulado atmosférico realizados (através do teste de Ames) mostraram aumento da atividade mutagênica, especialmente nas linhagens TA98 (em ausência de metabolização), YG1021 e 1024. Estes resultados indicaram a presença de derivativos de HPAs do tipo mononitroarenos identificados pela linhagem YG1021. A atividade mutagênica, verificada com a linhagem TA98 (-S9), pode estar associada à presença de nitroarenos (DeMarini et al., 1996), identificadas pelas as linhagens YG1021 e 1024 que permitem diferenciar a contribuição de atividade mutagênica de diferentes classes de nitrocompostos, indicando, através de padrões de resposta, as diferenças em áreas urbanas e industriais (Coronas, 2008). A presença de atividade mutagênica em misturas complexas está relacionada a poucas classes de compostos (DeMarini et al., 1996). No entanto, a contribuição significativa dos HPAs para a atividade mutagênica não os tornam uma classe dominante de

mutágenos em amostras de ar. A presença de outros poluentes, como por exemplo, os gases reativos ($NO_x$ e $O_3$) sob condições fotoativas poderiam produzir compostos mutagênicos a partir de compostos orgânicos não mutagênicos frequentemente presentes no ar (Claxton et al., 2004).

Os resultados obtidos neste estudo demonstraram que os liquens vêm sofrendo alterações tanto na estrutura da comunidade como através dos danos morfofisiológicos nas duas espécies amostradas, evidenciando diferenciações nas regiões norte, leste e central da cidade, sendo estas mais afetadas pela urbanização e antropização. A aplicação do Fator de Classificação Ambiental como complementação à fórmula original do IAP proporcionou definir as áreas mais impactadas pelo desenvolvimento acelerado dos centros urbanos.

A utilização conjunta entre os bioindicadores e os biomarcadoress de mutagênese, através do ensaio *Salmonella*/microssoma, proporcionou a avaliação da qualidade do ar e o diagnóstico da presença de compostos agressivos ao meio ambiente urbano. Ambos mostraram sensibilidade diferenciada frente aos grupos de compostos presentes na mistura, possibilitando ainda o mapeamento de áreas com potencial risco ao ambiente. Além disso, associá-los ao ensaio *Salmonella*/microssoma teve a vantagem de definir a presença de compostos mutagênicos com potencialidade carcinogênica destacando os riscos à saúde humana.

Na avaliação geral da comunidade liquênica urbana, constatou-se que fatores como tráfego veicular, variáveis climáticas, assim como topografia das estações amostradas pode ter contribuído para os resultados encontrados. Por outro lado, as regiões urbanizadas são altamente complexas, uma vez que diferentes fontes de emissões liberam uma variedade de poluentes, tornando-se difícil determinar qual fator poderia estar influenciando as espécies liquênicas. Deve-se considerar também que o efeito biológico avalia uma mistura de compostos e não apenas a ação de um componente específico.

Desta forma, as avaliações biológicas empregadas permitiram associar informações à análise da poluição atmosférica na cidade de Porto Alegre, podendo servir como ferramentas para programas de monitoramento nas áreas urbanizadas. Os estudos através dos liquens revelaram as modificações no ambiente urbano, proporcionando o mapeamento das principais regiões da cidade que apresentam alterações ocasionadas pela contaminação do ar, enquanto que, o ensaio *Salmonella*/microssoma permitiu definir classes de compostos, seus efeitos mutagênicos e potencialidade carcinogênica através de biomarcadores.

Este estudo também proporcionou um diagnóstico mais amplo da salubridade do ar, servindo de alerta para os órgãos governamentais como base de ações que promovam a saúde ambiental da cidade.

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# APÊNDICES – FIGURAS COMPLEMENTARES

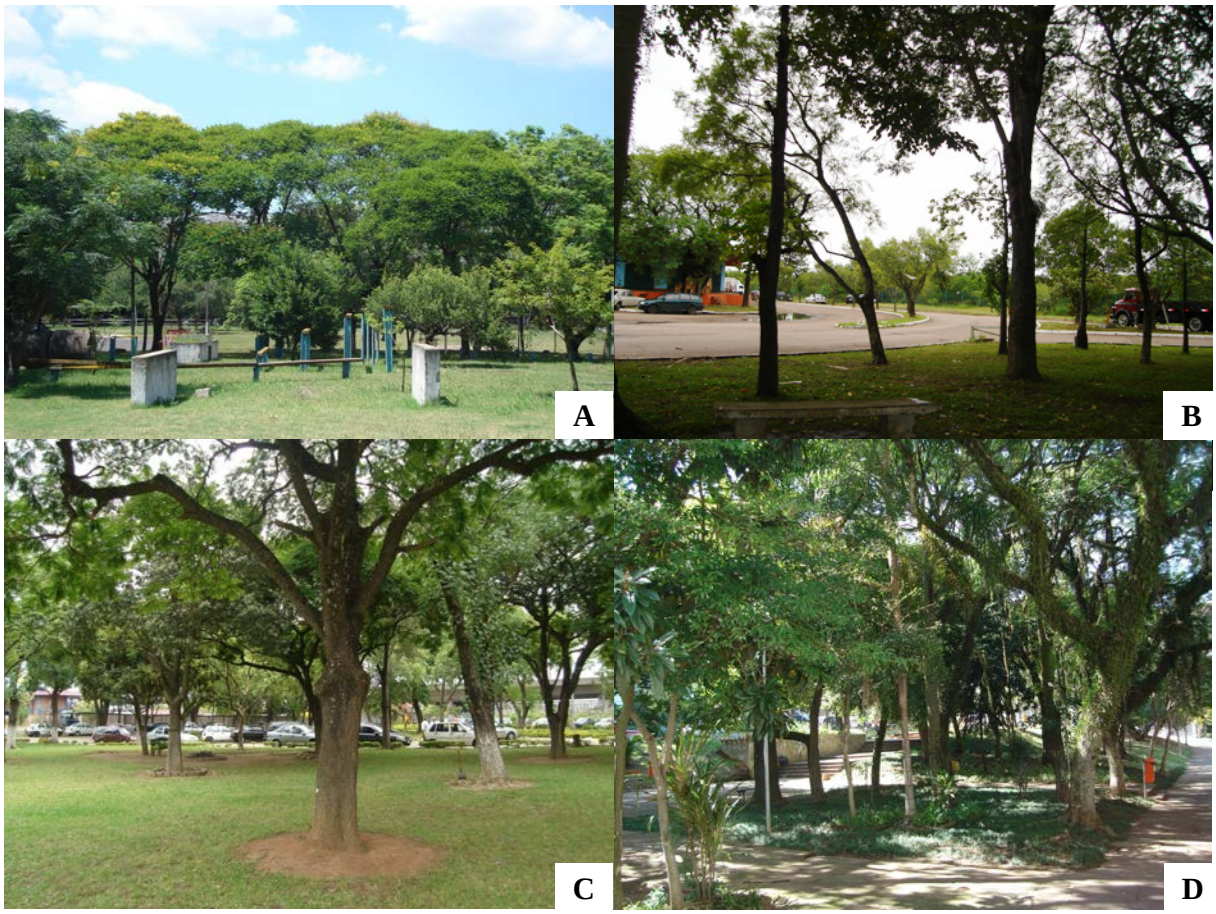


**Figura A1.** Vista geral das estações amostradas: E2 – Santa Cecília (A); Ceasa - E13 (B); E3 - Centro 2 e E12 – Petrópolis (D).

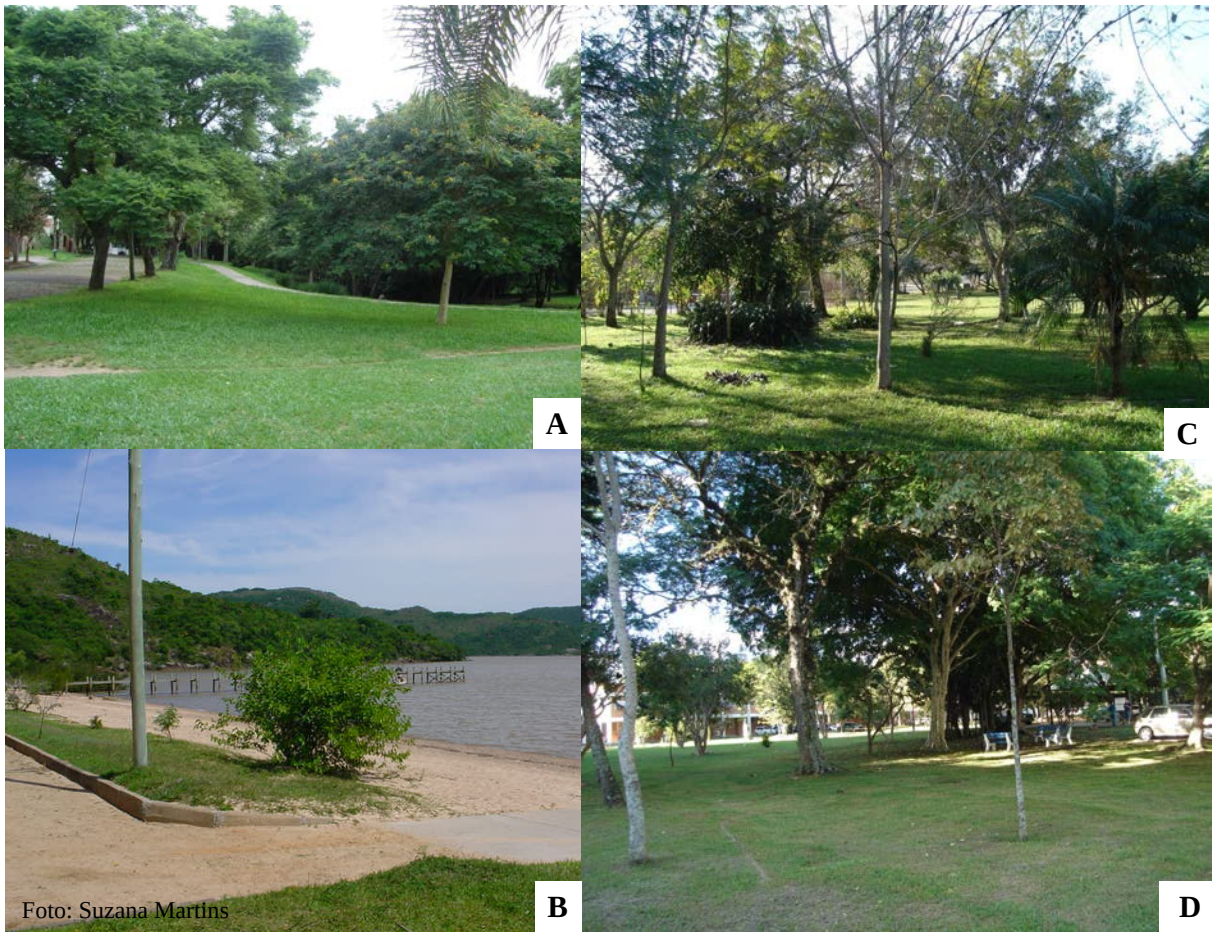


**Figura A2.** Vista geral das estações amostradas: E24 – Jardim Itu-Sabará (A); E14 – Parque Estadual de Itapuã (B); E25 – Ipanema (C) e E18 – Menino Deus (D).

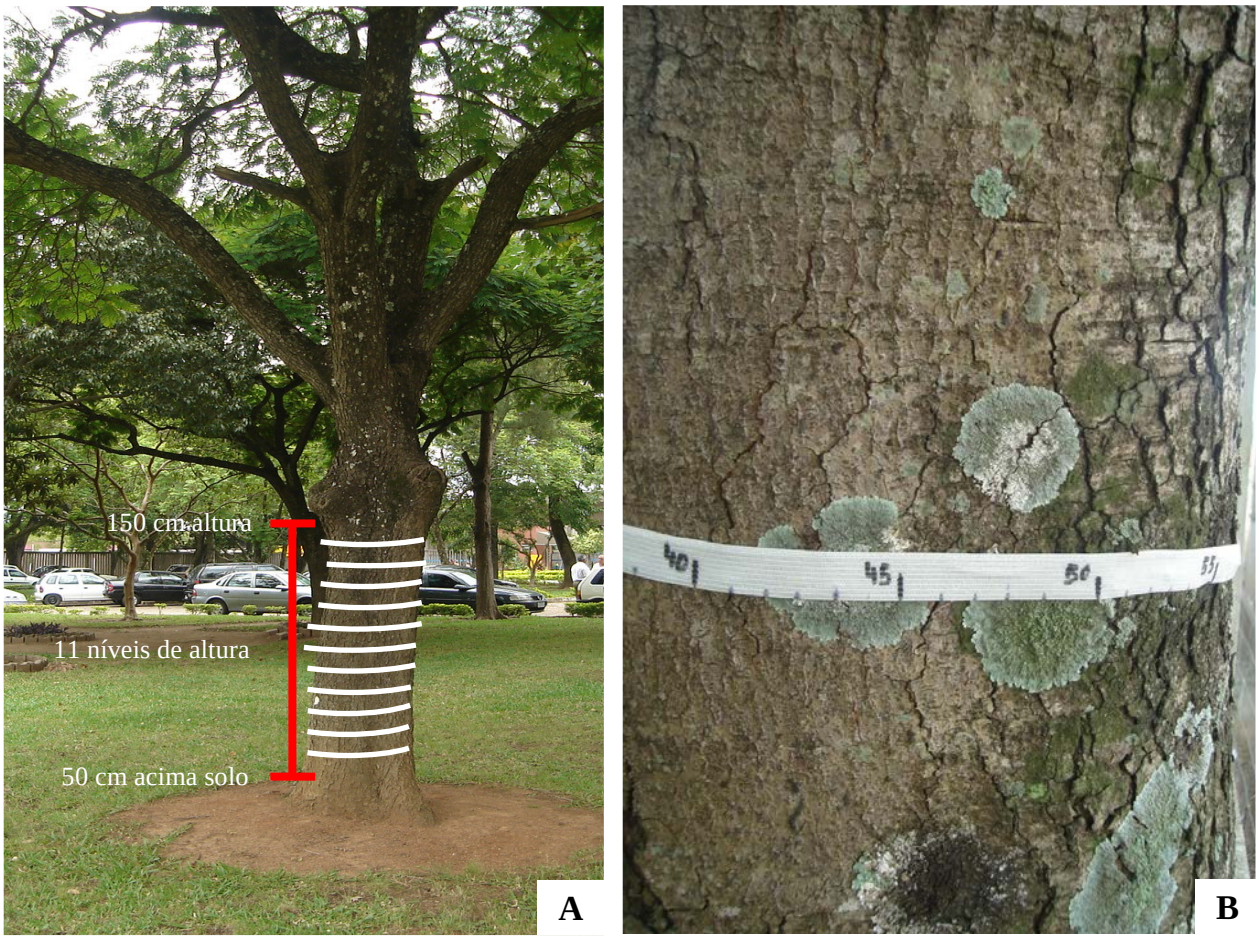


**Figura A3.** Faixa de altura do mapeamento dos liquens no forófito (A) e Método do elástico (B).

**Figura A4.** Análise do pH nos forófitos amostrados realizada em campo.


**Figura A5.** Espécies de forófitos utilizados para amostragem dos liquens: *Brachychyton populneum* Schott (A) e *Ligustrum japonicum* Thunb. (B).


**Figura A6.** Espécies de forófitos utilizados para amostragem dos liquens: *Peltophorum dubium* (Spreng.) Taub. e *Myrsine umbellata* Mart. (B).


**Figura A7.** Espécies de forófitos utilizados para amostragem dos liquens: *Enterolobium contortiisiliquum* (Vell.) Morong. (A) e *Hovenia dulcis* Thunb. (B).

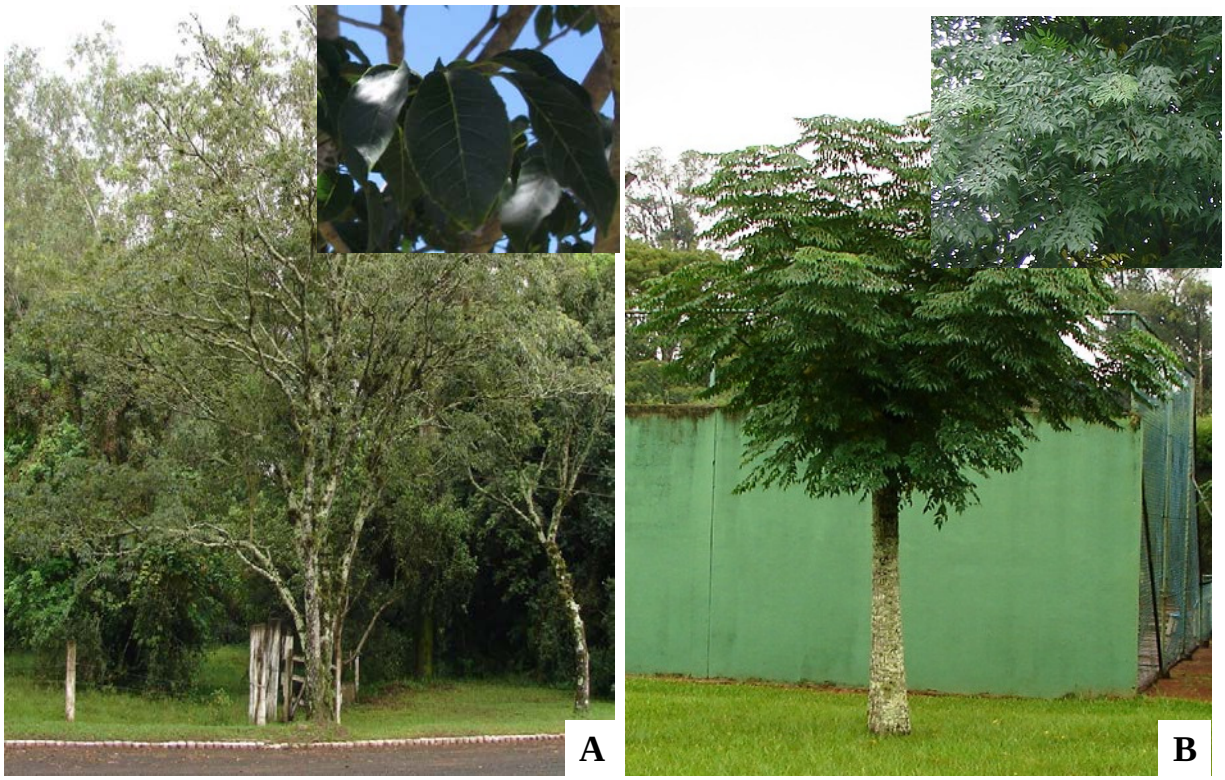


**Figura A8.** Espécies de forófitos utilizados para amostragem dos liquens: *Tabebuia avellananedae* Lorentz ex Griseb (A) e *Melia azedarach* Linn. (B).

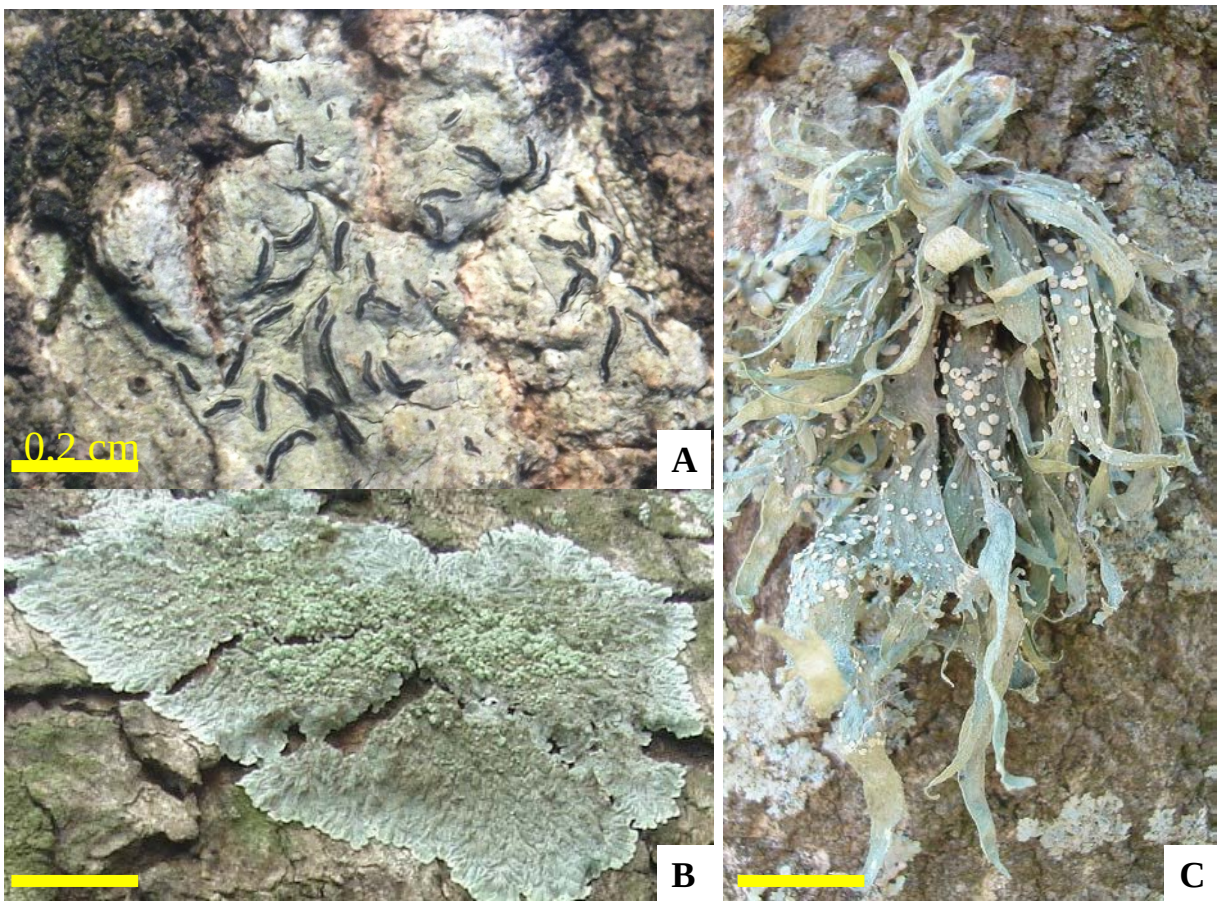


**Figura A9:** Espécies liquênicas registradas nas estações amostradas: *Graphis caesiocarpa* Redinger (A), *Dirinaria picta* (Sw.) Schaer. ex Clem. (B) e *Ramalina celastri* (Sprengel) Krog & Swinscow (C). Barra = 1cm, exceto quando registrado na figura.

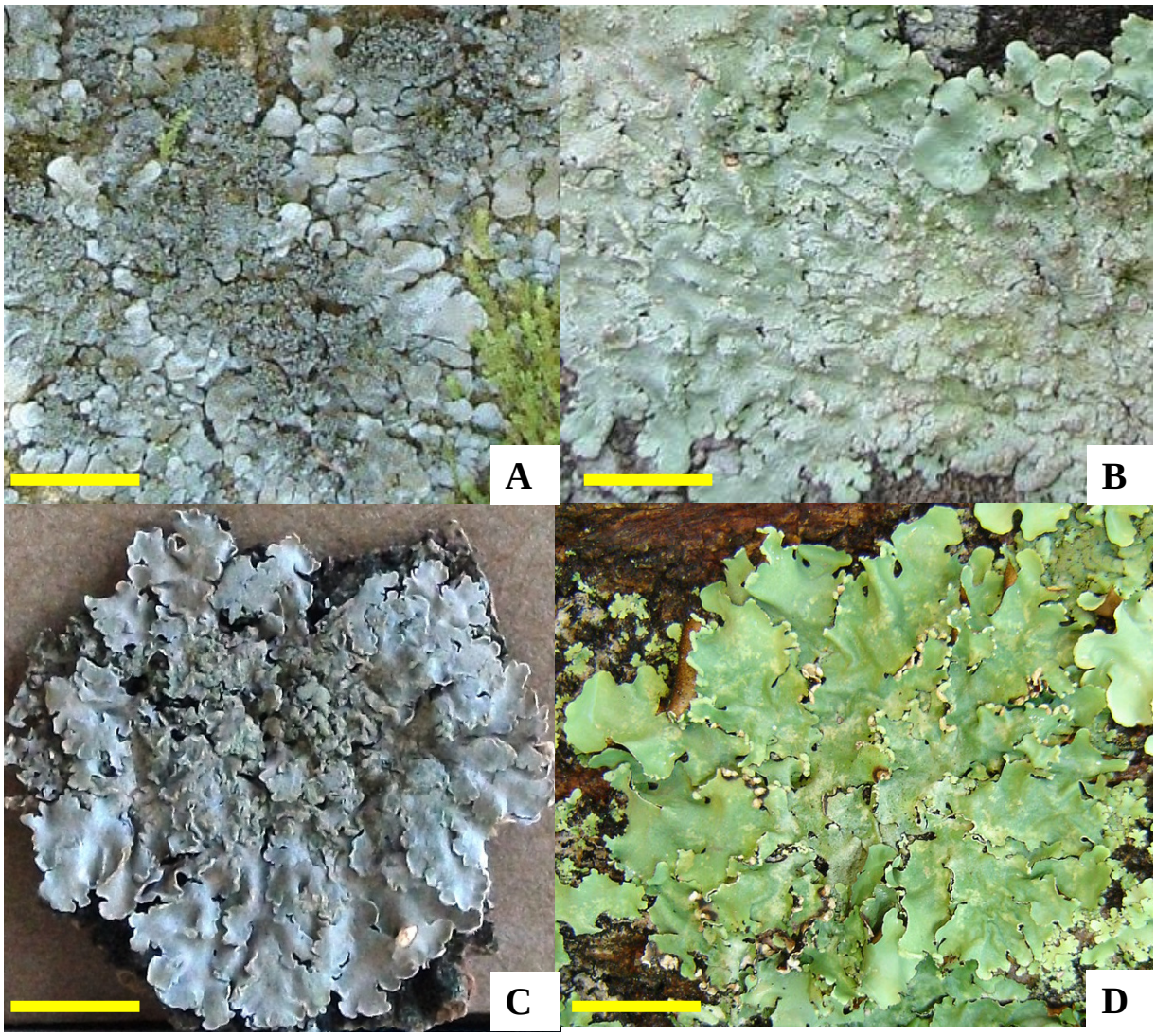


**Figura A10:** Espécies liquênicas registradas nas estações amostradas: *Coccocarpia pellita* (Ach.) Müll.Arg. ex R.Sant. (A), *Canoparmelia texana* (Tuck.) Elix & Hale (B), *Physcia undulata* Moberg (C) e *Parmotrema reticulatum* (Taylor) M. Choisy (D). Barra = 1cm

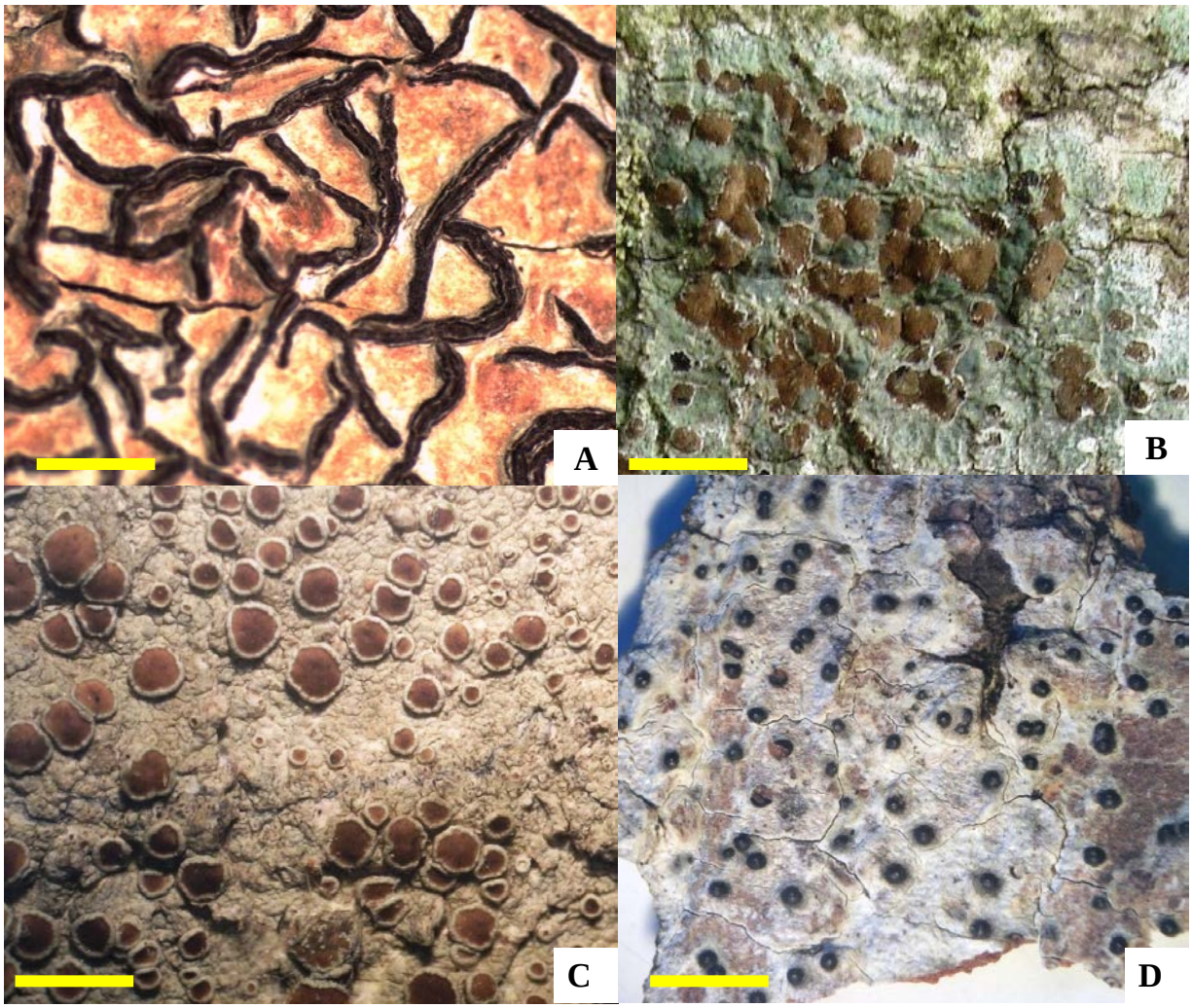


**Figura A11:** Espécies liquênicas registradas nas estações amostradas: *Graphis rigidula* Müll. Arg. (A), *Glyphis cicatricosa* Ach. (B), *Lecanora concilianda* Vain (C) e *Anisomeridium tamarindii* (Fée) R. C. Harris (D). Barra = 0,2 cm.


**Figura A12.** Espécies bioindicadoras empregadas no método dos transplantes: *Parmotrema tinctorum* (Nyl.) Hale (A) e *Teloschistes exilis* (Michaux) Vain. (B).


**Figura A13:** Mesa com as espécies liquênicas expostas na estação E2 – Santa Cecília: *Parmotrema tinctorum* (Nyl.) Hale (A) e *Teloschistes exilis* (Michaux) Vain. (B).


**Figura A14:** *Parmotrema tinctorum* (A) e *Teloschistes exilis* (B) aos cinco meses de exposição, na estação E2 – Santa Cecília apresentando manchas marrons e esbranquiçadas no talo.

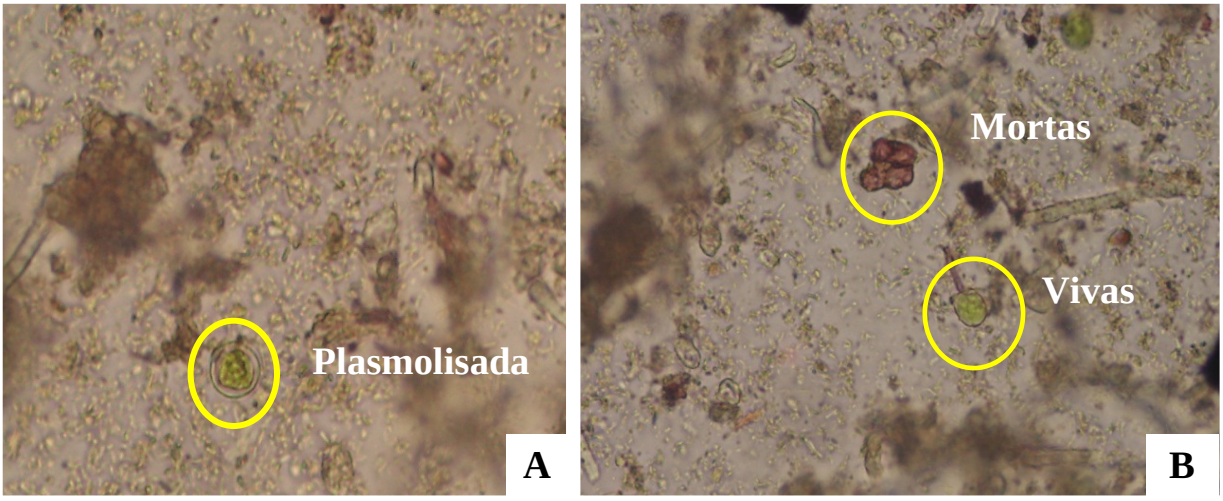


**Figura A15:** Contagem de células em amostras liquênicas demonstrando as células plasmolisadas (A) e mortas e vivas (B), no material testemunho.

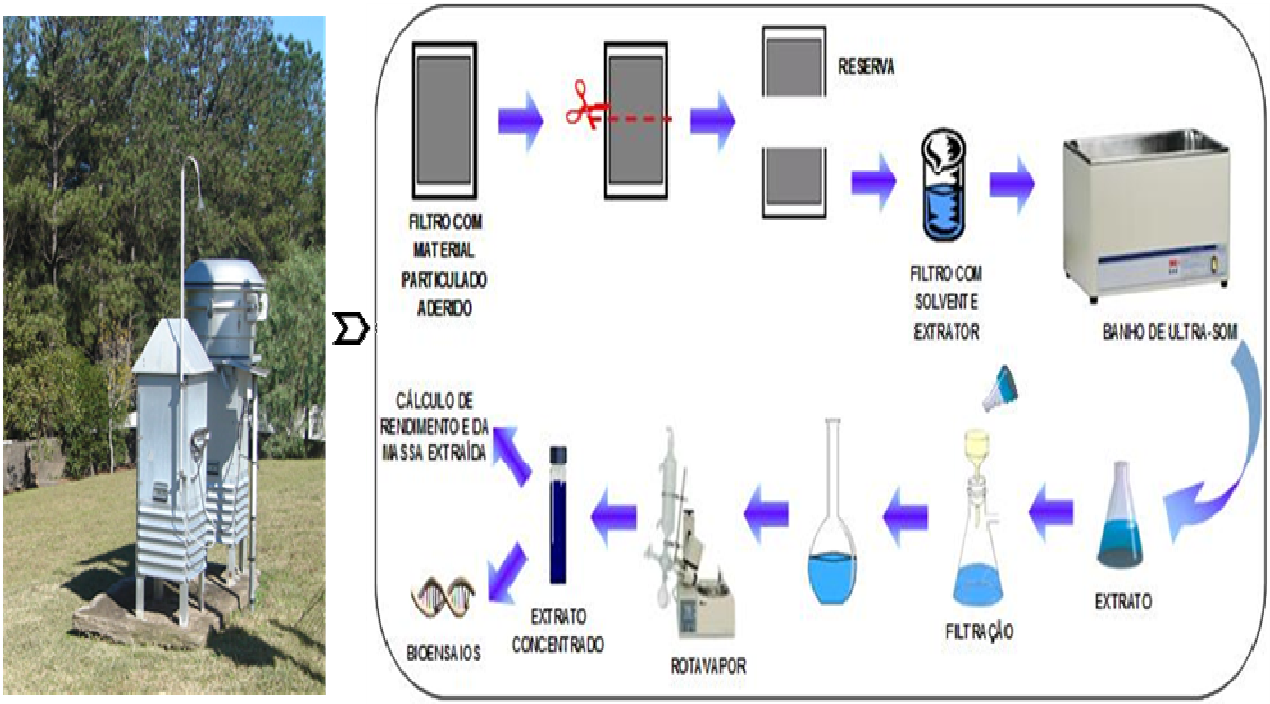


**Figura A16.** Amostradores para coleta de Material Particulado, seguido do esquema de extração dos compostos orgânicos do ar.

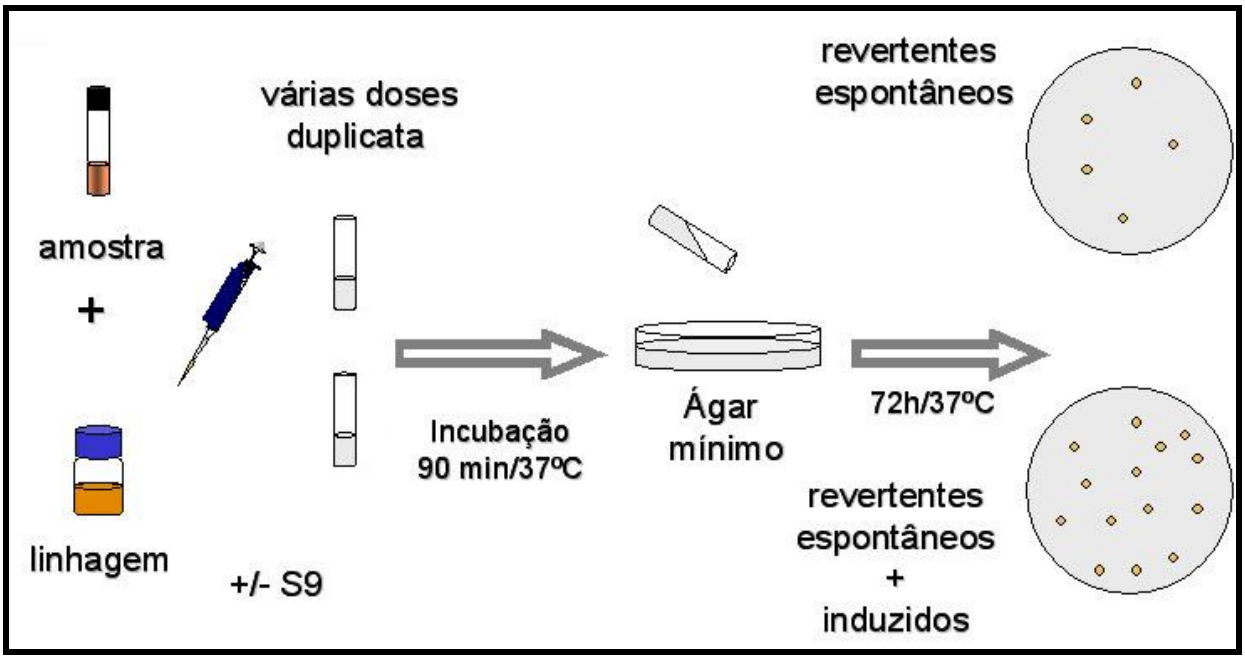


**Figura A17.** Esquema do teste de Ames. Fonte: Umbuzeiro & Vargas, 2003.

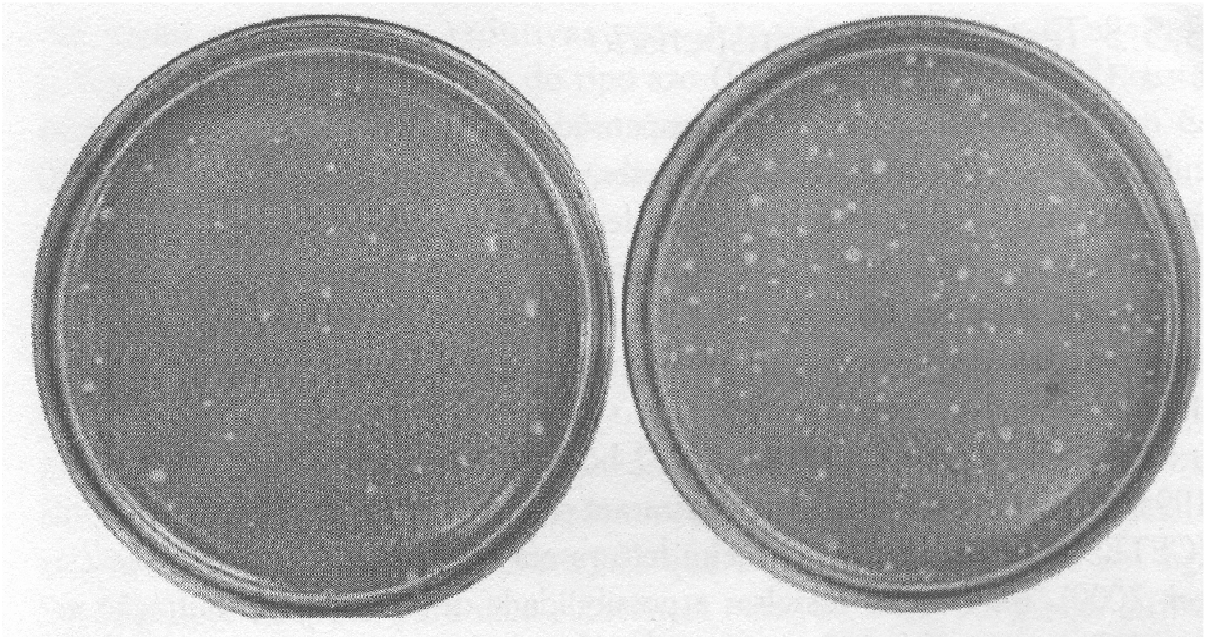

**Figura A18.** Ilustração da resposta observada no ensaio para controle negativo e mutagênese induzida. Fonte: Umbuzeiro & Vargas, 2003.