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The Influence of Vehicular Air Pollution on Lichen Abundance in Two Central Ontario Forests

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Abstract
Lichens have been used extensively as a bioindicator to test for damage by vehicular air pollution in urban and rural areas, however, the degree of influence vehicular air pollution has on lichen in forest ecosystems is not as well understood. The influence of vehicular emissions on two forests in southern Ontario was assessed by studying changes in lichen species composition and density on trees. The sites included in this study were Indian Point Provincial Park and Mark S. Burnham Provincial Park, located next to low-use and high-use roads respectively to compare effects of point source vehicular emissions. Horizontal transects, parallel with the road, were established at predetermined distances from the edge of the forest. All sugar maple trees with a diameter at breast height (DBH) of 10cm or greater, and which were within 2.5m of each transect were sampled for percent cover of microlichen and macrolichen. The study found a significantly greater percent coverage of macrolichens at Indian Point than at Burnham ($p<0.001$, $t=-5.481$), but significantly less microlichens ($p < 0.001$, $t = 3.470$). There was a decrease in macrolichen coverage as distance from the road increased at Indian Point, unlike Burnham which exhibited no change. A positive relationship between microlichen coverage and distance from the road was found in both sites. These findings suggest that, up to and possibly over 100 m into the forest, the Burnham Park site is in a constant state of disturbance due to anthropogenic air pollution activities in the surrounding area. However, further studies investigating the role of a natural forest edge on lichen tree coverage should be conducted before concrete conclusions can be made on the influence of air pollution on lichen populations in forests.

Keywords
Lichens — Bioindicators — Air pollution — Epiphytes — Ontario

1. Introduction

Atmospheric pollutants such as ozone (O_3), carbon dioxide (CO_2), sulphur dioxide (SO_2), nitrogen oxide (NO_x) and ammonia (NH_3) can all have a negative effect on ecosystems and human health (Percy & Ferretti, 2004), Atmospheric monitoring programs have been established across Canada in the form of monitoring stations which house equipment such as precipitation collectors and air samplers (Grant et al., 2009). These stations can be expensive to install and require regular maintenance, therefore can be few and sparsely distributed. Biomonitoring, which is the use of living material (bioindicators) to measure pollutants in an ecosystem, offers an alternative monitoring method, as it is an easy and cost-effective method to measure atmospheric pollution (Goffinet & Shaw, 2000). Biomonitoring can provide insight into ecosystem health by a measure of species composition and diversity.

Lichens have been shown to be an effective biomonitoring species for a range of pollutants from NO_x to heavy metals (Freedman, 2010). Noss (1990) outlines several characteristics that make a species an effective bioindicator: the ability to sufficiently indicate small changes in an ecosystem, demonstrating a broad geographical distribution, and ease of use and cost effectiveness during sampling or collection (Noss, 1990). Epiphytic lichens, which are lichens that live on the surface of plants, have been used by forest research scientists as bioindicators since 1866 and, most recently, as a tool to monitor air quality (Conti & Cecchetti, 2001). Lichens are highly susceptible to subtle atmospheric changes due to their lack of cuticle, and consequently, can be used as a tool not only to indirectly quantify emissions but also to display its effects (Conti & Cecchetti, 2001). The symbiosis of fungi and alga to form lichens allow lichens to adapt easily to a variety of environments and to flourish globally, enabling comparisons of atmospheric pollution impacts over a variety of scales (Nash, 1996). Given enough time, lichens attach themselves to virtually any surface in nature, therefore, by examining changes in the species composition of lichen communities it is possible to make inferences about levels of air pollution in each area.

Epiphytic lichens are found in many ecosystems worldwide, and they are abundant in Ontario's forests, where they have been used to monitor air pollution in regions across Canada (Freedman, 2010). Forests are sensitive to air pollution; forest ecosystems can act as buffers or sinks for low levels of pollution, but many forests, especially smaller ones, are unable to respond to higher quantities and concentrations

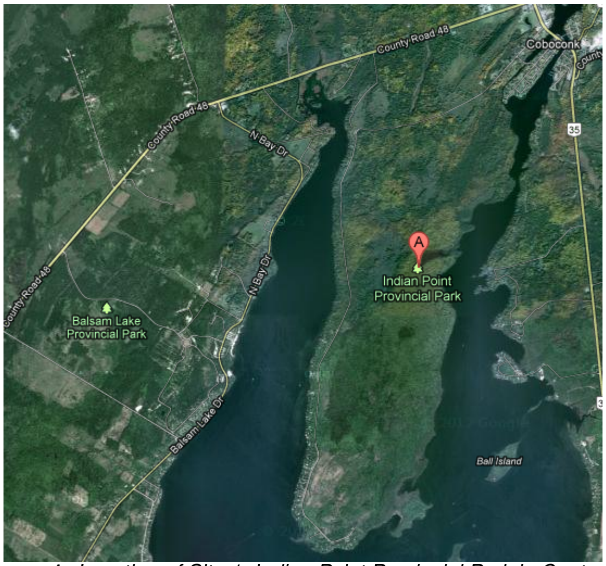

Figure 1. *Location of Site 1, Indian Point Provincial Park in Central Ontario, Canada. The red line represents Indian Point Road along which the sampling site was set up adjacent to. The red circle is the location of sampling site 1.*

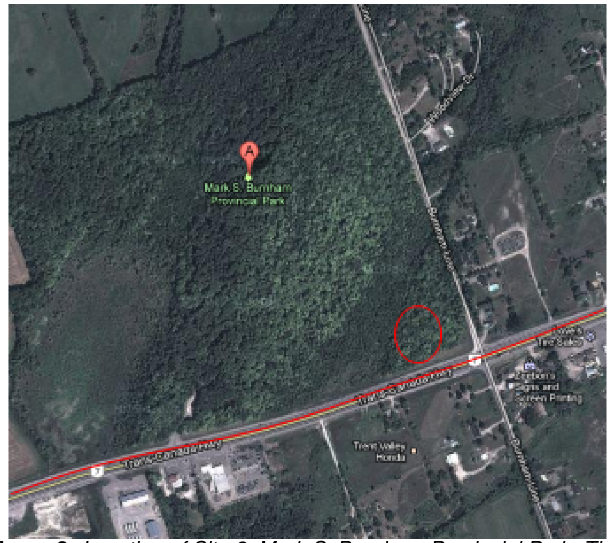

Figure 2. *Location of Site 2, Mark S. Burnham Provincial Park. This site is located along the Trans Canada Highway, near Peterborough, Ontario, Canada. The red line denotes the highway and the red circle is the location of the sampling area within site 2.*

of pollutants (Smith, 1974). When exposed to these higher levels of air pollution, individual forest species can exhibit reductions in growth or reproduction while ecosystems can experience significant changes in biogeochemical (Agnan, et al., 2013) and hydrological cycling (Smith, 1974). The monitoring of the relationship between air quality and forest health is crucial due to the ecological, recreational and economic significance of forests. One of the major sources of air pollution affecting forest ecosystems is generated through transportation, particularly by automobiles. Emissions from vehicles typically generate much of the air pollution in urban environments (Paoli et al., 2013). In Canada 70,000 tonnes of SO_2 and 1,240,000 tonnes of NO_x are emitted annually (Freedman, 2010). Southern Ontario is home to a large and widely mobile population with a high dependence on vehicles for everyday activities; this has led to the formation of an extensive and highly used road network spanning the southern part of the province. It is logical to assume that this high usage will, in turn, lead to a larger than normal presence of gases associated with air pollution in these areas. As most of southern Ontario's remaining forests are small and located close to roadsides, it is important to consider that the health of these forest ecosystems may be threatened by the presence of vehicular sourced air pollution. This study assessed the influence of vehicular emissions on two forests in southern Ontario by evaluating changes in lichen species composition and density on sugar maple trees (Acer saccharum). The sites used in this study were Indian Point Provincial Park, located at the northern end of Balsam Lake (Figure 1), and Mark S. Burnham Provincial Park east of Peterborough (Figure 2). The sites chosen for this study represented both a region of low and high traffic density to allow for comparison between locations and at increasing distance from the source of emission.

2. Materials and Methods

2.1 Study Sites

Indian Point Provincial Park is categorized as an undeveloped provincial park and is located on the north end of Balsam Lake, in Central Ontario. The park encompasses an area of 947 hectares (Ministry of Natural Resources, 2004). It is a mixed coniferous and deciduous forest consisting mainly of Red and sugar maple; *Acer rubrum*, *Acer Saccharum*, Ironwood; *Ostrya virginiana*, White Pine; *Pinus strobus*, Eastern White-Cedar; *Thuja occidentalis*, White Ash- *Fraxinus americana*, and Basswoods; *Tilia Americana* (Resources, Indian Point, 2004). The sampling site was set up beside Indian Point Road, a small road with an annual average daily traffic volume of 120 cars per day (Ontario. 2012) which runs parallel with the east shore of Balsam Lake (Figure 1).

Mark S. Burnham Park is a smaller park with an area of 43 hectares, and like Indian Point Provincial Park, is mostly deciduous forest consisting of Red and sugar maple; Acer rubrum, Acer saccharum, Beech; Fagus americanus, Elm; Ulmus americana, Eastern Hemlock; Tsuga canadensis and Eastern White-Cedar; Thuja occidentalis. The sampling site was set up parallel to the section of the Trans Canada Highway that is adjacent to the forest (Figure 2). This highway has an annual average daily traffic volume of 18,800 cars per day (Ontario, 2012).

2.2 Lichen Survey

For this study, 50 m transects parallel with the road were established 10 m, 20 m, 50 m and 100 m from the edge of the forest. To minimize variation in the age and species of epiphytic lichen, we sampled only sugar maple trees with a diameter at breast height (DBH) of 10 cm or greater. Each tree was sampled for percent cover and species of lichen.

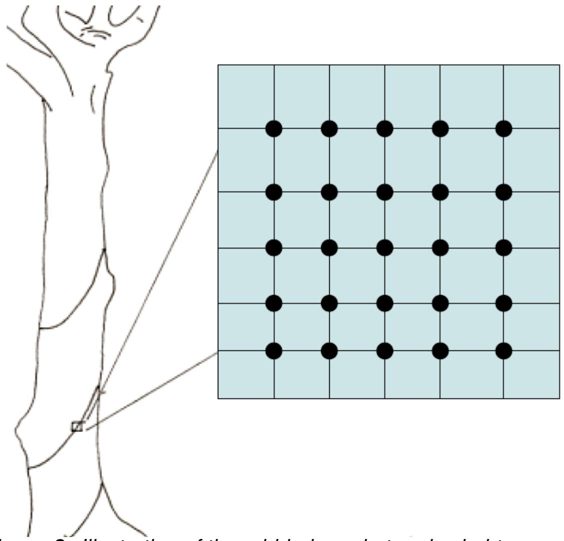

Figure 3. *Illustration of the gridded quadrat and spiral-transect method. Nodes on the tree trunk are set up at 5cm and 100 cm on one side and on the opposite side at 50cm and 150cm. The gridded quadrat is then used at 20cm intervals along the spiral. This method provides data that gives an accurate representation of lichen cover upon the whole tree (Wolseley, 2006).*

The spiral-transect method was used (Figure 3). A starting node was marked, 5 cm above the base of the trunk of the tree. Subsequent nodes were marked on the same side of the trunk at 100 cm from the base, and on the opposite side at 50 cm and 150 cm. A linear transect with sampling points at 20 cm intervals was then aligned in a spiral around the tree. A gridded quadrat was placed at each sampling point and the presence or absence of lichen was determined at each intersection on the grid. This data was then used to provide an accurate representation of percent cover of lichen on the tree (Wolseley, 2006)

2.3 Data Analysis

This study required the testing of a pair of null hypotheses, the first being that there was no significant difference in macrolichen and microlichen coverage on sugar maple trees between the Burnham Park site and the Indian Point Road site; the second being that there was no significant change in macrolichen and microlichen coverage as the distance away from the road increased. To test the first hypothesis, a t-test at a 95% significance level, was conducted assuming each sample was independent. To test the second hypothesis, four simple logarithmic regression tests were conducted: two for macrolichen coverage at each site and two for microlichen coverage at each site. These tests were conducted with a 5% significance level and the samples were also assumed to be independent. The sample size for Burnham was 40, and for Indian Point Road, 38

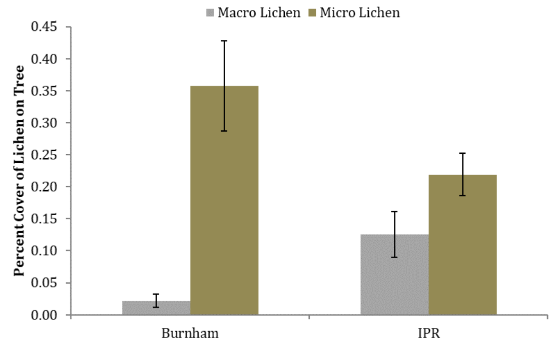

Figure 4. *Mean percent cover of lichen on each sample site. The mean percent cover of both macro (grey) and micro (brown) lichen on sugar maple trees at both the Burnham and Indian Point sites. Error bars describe a 95% confidence range of the mean values.*

3. Results

3.1 Lichen Species

Lichens occur in four basic growth forms; crustose which grows tight against the substrate, squamulose which is a tight clustered unit, foliose which is leaf-like and fruticose which have free standing branching tubes. For this study, lichens were split into two categories: microlichens which consist of only crustose, and macrolichens consisting of squamulose, foliose and fruticose. The most commonly occurring macrolichens were Wreath lichen *Phaeophyscia rubropulchra*, Tube lichen *Hypogymnia physodes*, and Candle Flame lichen *Candelario concolor*. The microlichens most commonly seen were Orange lichen *Caloplaca holocarpa*, and Common Script lichen *Graphis scripta*.

The results in Figure 4 indicate that there were significant differences in both microlichen and macrolichen between the sites. There was significantly more macrolichen at Indian Point than at Burnham ($p<0.001$, $t=-5.481$), but significantly less microlichen ($p=0.001$, $t=3.470$). This demonstrates a very strong relationship between the percent cover of microlichen and macrolichen between each site; however, it also points to the possibility that there could be a relationship between the percent cover of macrolichen and microlichen. A statistical analysis of the relationship between macrolichen and microlichen suggested that there was a relationship but it was only verging on the 5% level of significance ($p=0.052$, $t=-1.971$). Another possible confounding variable for the site to site analysis of macrolichen and microlichen was the possible influence of tree age on macrolichen and microlichen coverage. On visual inspection, it appeared that Burnham was an older forest and had reached more of a climax community than the site at Indian Point. Using tree diameter at breast height (DBH) as a surrogate, a scatter plot of tree DBH and macrolichen coverage did not quite indicate a significant relationship between the two variables at either site to a 5% significance level ($p=0.079$, $t=-1.778$).

Figure 5 illustrates the relationship between the coverage of macrolichen growth, as the distance from the road increases.

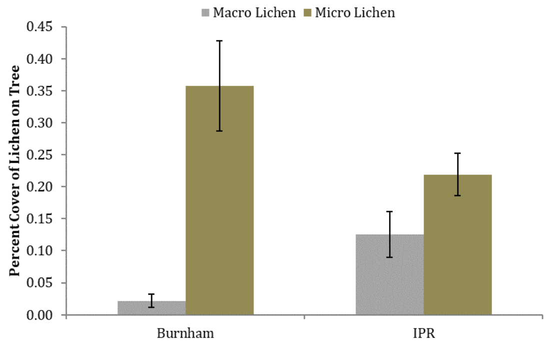

Figure 5. *Percent cover of macrolichen over distance from road. The logarithmic regression lines of percent macrolichen cover on sugar maple trees at both the Burnham and Indian Point sites as the distance from the road increases. Points illustrate distribution of each site's data*

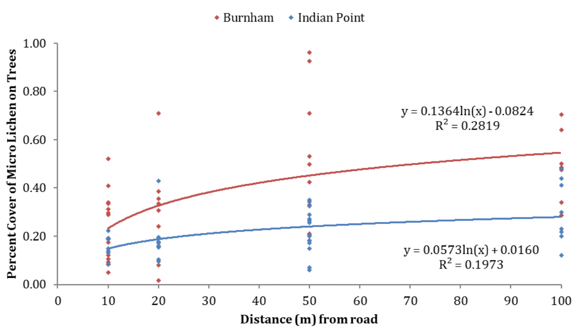

Figure 6. *Percent cover of microlichen over distance from road. The logarithmic regression lines of percent microlichen cover on sugar maple trees at both the Burnham and Indian Point sites as the distance from the road increases. Points illustrate distribution of each site's data*

The relationship was significant at the Indian Point site; here there was found to be a strong negative correlation whereas the distance from the edge of the forest increased, the percent coverage of macrolichen decreased ($p<0.001$, $t=-4.278$). This correlation fits most strongly with a logarithmic line which indicates that lichen coverage was most strongly impacted by the edge and that at distances equal than or greater than 100m away from the road, macrolichen coverage plateaus. The Burnham site, however, had no correlation, with distance having no apparent impact upon the macrolichen coverage within the forest ($p= 0.442$, $t=-0.777$). There was also a significant difference between both the slopes of the Burnham and Indian Point regression lines ($p<0.001$, $t=-6.816$).

Figure 6 demonstrates the effect of distance from a road at both Indian Point and Burnham sites on the percent coverage of microlichen. Statistical analysis of this data set indicated that there was a significant relationship between distance and microlichen coverage at both sites (Burnham, $p<0.001$, $t=3.862$; IPR, $p=0.005$, $t=2.975$). However, there was not a significant difference between either the y-intercepts or the slopes of the two regression lines suggesting that there was very little or no impact on this relationship due to differences between the study sites ($p=0.148$, $t=1.461$).

4. Discussion

The results of this study support its general hypothesis that significant differences in lichen coverage would be observed by site characteristics (road usage) and distance from the road. Figure 4 indicates a larger difference between the two types of lichen at the Burnham Park site than at Indian Point. It was initially suspected that this difference could be due to variations in size and age of trees, rather than due to external site influences as it has been noted that both species richness and composition of lichen communities increase with tree age (Nascimbene, 2009). By using tree DBH as a surrogate for tree age, it was possible to examine the relationship tree age might have with lichen coverage. Our results indicated a possible relationship; however, similarities in average DBH between the stands led us to believe that both forest stands were of similar ages and would therefore not be affected by this possibility.

Examination of the differences in macrolichen and microlichen abundance between the Burnham Park and Indian Point sites suggested that microlichen is the more dominant of the lichen species at these sites (Figure 4). This apparent dominance is most likely due to succession tendencies. When examining the natural succession of lichen, the initial stages consist of a micro lichen crustose, followed by macrolichens such as foliose and fruticose (Bergamini et al., 2007). Assuming that both sites are of a similar age, both sites should exhibit similar natural succession patterns, however, the Burnham Park site was not. The vehicular traffic and associated emissions occurring at a higher density surrounding the Burnham Park site could be disturbing the natural succession of both microlichens and macrolichens. Disturbance is defined as any event, either natural or anthropogenic, that changes the existing condition of an ecosystem (Freedman, 2010), and it has been shown that in some habitats, a re-occurring disturbance can continually return succession to an earlier stage and therefore disrupt its natural pathway (Krohne, 2001). Burnham Park is situated along a busy highway, within 10 km of Peterborough, and is also in the direct path of migrating air pollution originating from the City of Toronto and along the Highway 401 corridor; all these factors are considered anthropogenic disturbances. Based on this knowledge, it is possible that the Burnham Park site is in a constant state of disturbance due to anthropogenic activities in the surrounding area. This would explain the high amounts of microlichen and low amounts of macrolichens observed at Burnham Park.

The differences in trends between both sites were also illustrated in Figure 5. The regression line of macrolichen cover at Indian Point clearly demonstrates the impact that the forest edge has on macrolichen. Indian Point has extremely low levels of traffic (local sources of air pollution) and as equally impacted as Burnham by long distance sources of air pollution; this would suggest that the difference between lichen coverage at the road and 100 m from the road in Indian

Point may only be due to a natural edge effect. A natural edge effect imposes different living conditions on the outer edge of the ecosystem than in the understory such as increased light and wind levels as well as varying water availability (Brunialti et al, 2013). These conditions can potentially improve the growth or reproductive conditions for macrolichens. However, this relationship is not replicated at Burnham, which instead showed no significant change in macrolichen coverage as the distance from the road increases. It is possible that the positive lichen growing conditions, which existed at the forest edge along Indian Point Road, did not exist at Burnham due to the influence of the high traffic vehicle emissions. The consequences of elevated vehicular air pollution on macrolichens has been well documented (Conti & Cecchetti, 2001), and this study's findings of decreased macrolichen density within 300 m of a vehicular pollution point source is also consistent with previous findings (Fahrig & Coffey, 2012).

Changes over distance were also seen in microlichen coverage in both sites. Unlike in Figure 5, Figure 6 suggests that microlichens in each site share equal distance distributions. The Burnham site had a slightly greater percent cover and stronger correlation than Indian Point, however, this difference was negligible. Interestingly, the microlichen distribution was opposite to the microlichen distribution at Indian Point. In other words, at Burnham, percent coverage of microlichens increased as the distance from the road grew. This result is likely related to microlichen's role as a primary colonizer in degraded, exploited or polluted environments (Bergamini et al., 2007). It is possible that the large coverage of macrolichens close to the road at Indian Point has succeeded pioneer microlichen species leading to a lower coverage of microlichens at the roadside. This same reasoning cannot be applied to Burnham Park. Instead, it can only be assumed that air pollution changes the conditions at the forest edge, substantially reducing the ability of lichens to exist. It might be possible to attribute the lack of microlichens close to the road in Burnham to the low amount of microlichens in that area due to the succession theory of lichen previously outlined.

Possible differences in forest edge characteristics (other than air pollution levels) between the two sites could account for some of this study's findings. Previous lichen studies have found that moisture levels are one of the regulating factors governing lichen community growth and diversity (Tretiach et al., 2011; Fahrig & Coffey, 2012). Further, differences in wind could play a large role in lichen spore dispersal, thereby affecting the ability of lichens to become established. Additional studies investigating the role of varying natural forest edges on lichen tree coverage should be conducted before concrete conclusions can be made on the influence of air pollution on lichen populations in forests.

5. Acknowledgements

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