

Effect of air Pollution on Chlorophyll Content and Lichen Morphology in Northeastern Louisiana

Author(s): Jessica M. Wakefield and Joydeep Bhattacharjee

Source: *Evansia*, 28(4):104-114. 2011.

Published By: The American Bryological and Lichenological Society, Inc.

DOI: <http://dx.doi.org/10.1639/079.029.0404>

URL: <http://www.bioone.org/doi/full/10.1639/079.029.0404>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Effect of air pollution on chlorophyll content and lichen morphology in Northeastern Louisiana

Jessica M. Wakefield
United States Forest Service, Ozark-St. Francis National Forest,
1001 East Main Street, Mountain View, AR 72560
E-mail: jmwakefield@fs.fed.us

Joydeep Bhattacharjee
Plant Ecology Laboratory, Department of Biology,
University of Louisiana, Monroe, LA 71209
E-mail: joydeep@ulm.edu

Abstract. Unlike deciduous plants, lichen thalli over time accumulate elements present in the air, such as lead, zinc, sulfur, nitrogen, and radionuclides in their tissues. Therefore, lichens may constitute ideal bioindicators to monitor air quality in both urban and rural environments. We evaluated the impact of air pollution on lichens in and around the city of Monroe, Louisiana. Lichen discs of *Ramalina stenospora*, *Physcia sorediosa*, and *Parmotrema perforatum* were transplanted from the core of a wildlife management area (lower levels of air pollution) to areas of potentially higher levels of air pollution and monitored over time. We measured percent bleaching, chlorophyll content and morphological characteristics of the thalli over a 12-month period. Percent bleaching, chlorophyll content and morphological characteristics of the thalli differed significantly over time among sites with contrasting pollution levels. Therefore, variables such as percent bleaching and chlorophyll content of lichen thalli, which are relatively easily determined, can be successfully used to estimate air quality in an area.

Keywords. transplant, bioindicator, bleaching, *Ramalina*, *Physcia*, *Parmotrema*

INTRODUCTION

As environmental contamination becomes more prevalent today than in the past, scientists are often called upon to come up with methods of detecting and quantifying atmospheric pollution. The concentration of funding and research is often upon the immediate effect of a catastrophe or in areas that have a human interface, while in most cases the concern for damage done to the structure and function of an ecosystem often goes under-researched (Landis & Yu 2004). Elaborate equipment is available for continuous monitoring of atmospheric pollution levels however, such equipment is expensive and often not easily deployable at remote locations. Of the many sources of air pollution, the most significant is burning of fossil fuels with the production of sulfur and nitrogen oxides, volatile organic compounds, and carbon dioxide, which in turn contribute to acid rains and global warming (Duffus & Worth 2006).

In order to develop an early warning system and to possibly avoid detrimental effects of these pollutants, the use of lichens as bioindicators to monitor atmospheric quality in both urban and rural environments has been emphasized. For over 140 years, lichens have been known to be extremely

sensitive to air pollution due to the impact of pollutants on primary metabolic functions in both the alga and the fungus (Brodo et al. 2001). The most sensitive species may become locally extinct in urban areas or near industrial facilities, while a few, pollution tolerant species will survive and some may even flourish in sites with poor air quality (Riddell et al. 2007). Unlike many vascular plants, lichens have no deciduous parts and hence cannot avoid continuous exposure to pollutants. The lack of stomata and cuticle in lichens means that aerosols and gases may be absorbed over the entire thallus surface and readily diffuse to the photobiont layer.

As with many organisms, the accumulation and processing of macro and micronutrients in lichens is essential for physiological functioning and critical to the growth and development (Nash 1996). Since atmospheric contaminants get accumulated in lichen thallus during the process, estimating the quantities of such contaminants assists researchers in determining the levels of pollutants present in the environment. The use of biological material, combined with analytical techniques, allows improvement of the sensitivity and accuracy of traditional chemical methods (Gadzala-Kopciuch et al. 2004). Today, lichens are used as bioindicators because they accumulate lead, zinc, sulfur, nitrogen, and radionuclides in their tissues. Lichens are also very sensitive to disturbances, natural or anthropomorphic; this creates a need for locating and conserving undisturbed habitats and unique ecosystems.

Lichens are long-lived and can be monitored in almost any season with relatively easy in-field identification that usually does not call for removing large samples from the field and carrying out elaborate laboratory analyses (except for in-depth chemical analyses). During the period of 1973-1988, approximately 1500 papers were published on the effects of air pollution on lichens (Richardson 1988; Ahmadjian 1993). Many different methods can be applied to use lichens to monitor air quality. For example, species composition of lichen communities was used to demonstrate the improvements in air quality in the Ohio Valley (Showman 1990, 1997) and to show oxidant air pollutant gradients in southern California (Sigal & Nash III 1983). Some of the areas of lichen research that have received attention, especially with regard to air quality monitoring, include evaluation of bleaching in lichen thalli due to degradation of chlorophyll (Kardish 1987; Riddell et al. 2007; Von Arb et al. 1990), and morphological changes in the lichen tissues (Eversman & Sigal 1987; Holopainen & Karenlampi 1984). Due to high costs of complex chemical analyses, and complicated and time-consuming procedures of sample preparation, analysts search for quicker and more specific methods, including bioindicators such as lichens, which enable detection of changes taking place in ecosystems.

Different volatile organic compounds present in the atmosphere in high concentrations have been shown to have damaging effects on the surrounding environment and human health. The presence of sensitive lichen species is a good indication that average annual SO₂ levels are < 15 ppb. If only tolerant species are present, then average annual SO₂ levels are probably > 30 ppb. A lichen desert can result from very high ambient SO₂ concentrations (Glavich & Geiser 2008).

In this study we evaluate the effectiveness of using lichens as “bio-monitors” in identifying areas and ecosystems that are at a risk of damage from high levels of air pollution. We used lichen disc transplants from tree barks to compare pollution levels at five different sites. Lichens were transplanted from areas of known lower pollution concentrations to areas of potentially higher degrees of air pollution and monitored over time. We used the transplant technique developed by Brodo (1961), where bark discs bearing epiphytic lichens and mosses are placed onto trees at selected sites in a polluted area for monitoring. This also has been satisfactorily used by ecologists to assess the effects of pollution (Brodo 1966; LeBlanc, Comeau & Rao 1971; LeBlanc & Rao 1973; Peck et al. 2000; Garty et al. 2001).

A detailed literature review revealed that there are no field studies carried out on lichens as bioindicators of air quality in the southeastern United States which is geographically home to some of the most unique bottomland hardwood forest systems in the nation. With rapid urban development, setting up of industries and increased vehicular traffic, Monroe, (northeast Louisiana) provides an ideal setting for use of lichens as bioindicators of air quality. The objectives of this study were to evaluate the following: a) If levels of air pollution differed between forest interiors and

exteriors as indicated by possible changes in lichen morphology post deployment of the lichen discs. A difference between the two is expected if the trees in the exterior part of the forest 'shielded' the interior part from pollutants that are airborne, in essence supporting the positive role of edge-effects on forests. b) If the predicted difference in air quality (based on their proximity to major pollution sources, among the research sites) is 'indicated' by quantifiable changes in lichen morphology over time. Using the outcome of the above, we evaluated the effectiveness of using lichens as bioindicators of air quality.

MATERIALS AND METHODS

Study sites: Five sites surrounding Monroe, Louisiana were selected within Union and Ouachita Parishes (**Fig. 1**); one city park (Restoration Park in West Monroe), one wildlife management area (Russell Sage), and three national wildlife refuges (D'Arbonne, Upper Ouachita and Black Bayou Lake). Sites were selected based on their proximity to and around Monroe, as well as, their relative similarities of vegetation (mostly bottomland hardwood forest with species of oak, bald-cypress and hickory and an understory composed of blackberry, greenbrier, pepper-vine, etc.). All sites included mature bottomland hardwoods that partially flooded throughout the year and harbored the same lichen species. Restoration Park was chosen because of its location near the city and industrial sites. Black Bayou National Wildlife Refuge was chosen because it is north of the city and it is considered to be a more urban refuge with higher vehicular traffic. The other three sites were selected based on their distance from the city and most pollution sources. In this study, we refer to major roads, industries, and urban settlements as sources of pollution. We established two monitoring stations within each site and mapped them using ArcGIS. One station represented an outer edge of the site, close to a pollution source (Station 1) with assumed higher pollution levels while the other station (Station 2) was set up in the interior, in a more secluded and pristine area within the site. Each interior monitoring station (Station 2) was located at least 600 m from any road or pollution source. This set up allowed us not only to compare relative levels of pollution among the different sites but also compare stations (exterior vs. interior) within each site.

Lichen transplants: All lichens were collected from the core of Russell Sage Wildlife Management Area (reference site) due to its remote location (no roads in a 2 km radius) away from any pollution source. Lichen discs were removed from the bark of randomly selected hardwood trees using a 5 cm diameter corer. The corer was made from a hole-saw drill bit and had to be hammered into the bark, then the disc was removed carefully from the bit. We used two commonly occurring lichens, *Ramalina stenospora* Müll. Arg. and *Physcia solediosa* (Vain.) Lynge for the disc transplants (**Fig. 2**). The genus *Ramalina* has been used in multiple studies of air pollution (Garty et al. 1993, 1997, 2001; Kardish et al. 1987; Riddell et al. 2007). Three discs of each species (*R. stenospora* and *P. solediosa*) were placed on a 31 cm² untreated plywood board using exterior wood glue (**Fig. 3**). Since each disc had lichens adhered on the outer surface of the bark, we used glue to attach in the opposite side to make sure the glue did not come into contact with the lichen at any point during transplantation. Three lichen boards containing a total of eighteen discs were placed at each station. *Parmotrema perforatum* (Jacq.) A. Massal. covered twigs were also collected at the reference site to be trans located to other study sites. The twigs were approximately 25 cm long and attached vertically to a 31 cm wooden dowel rod using plastic zip ties. One wooden rod containing three lichen twigs was placed at each station. All boards and wooden rods were nailed to trees at each station, about 1.8 m from the ground to avoid animal disturbance. Each board and the suspended twigs faced the same cardinal direction. Lichen discs were also placed at the collection site to be used as control.

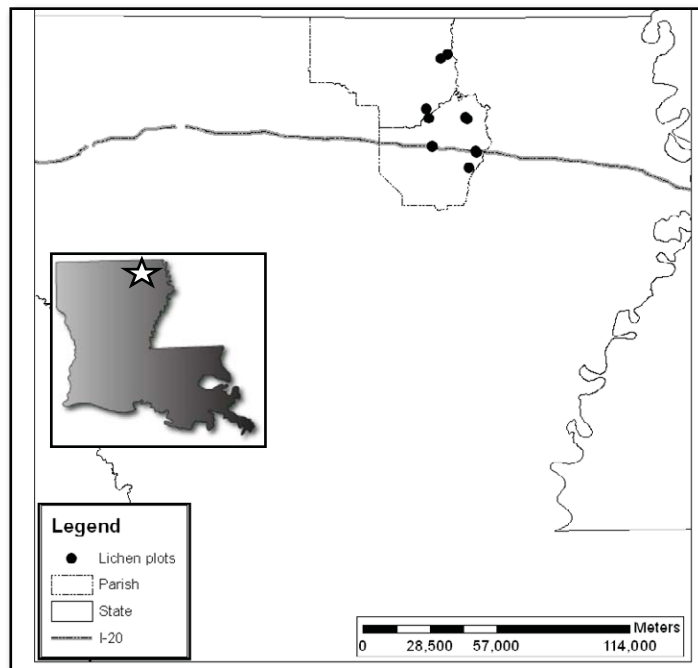


Figure 1. Location of study sites, in Union and Ouachita Parishes, with reference to the state of Louisiana, USA. Inset, map of site location (star) within state.

Thallus bleaching and chlorophyll assessment: To estimate the proportion of thallus bleached over the 12 months of deployment in the different sites, we used a standardized photographic technique. In this method, we photographed the lichen discs containing *Physia. solediosa* (**Fig. 3** top row), before deployment, in the lab under controlled lighting conditions, using a fixed focal length lens and a black background mat. Then at the end of the deployment, brought the discs back to the lab and photographed them again under identical conditions. Bi-monthly site visits were made to ensure that the lichen disc boards are undisturbed or branches from trees made no contact with the discs. At the time of photographing the lichen discs, thalli were sprayed with distilled water and let to stand for approximately 30 seconds, which allowed bleached spots to stand out more readily. Bleaching of the lichen thallus on each disc was calculated from photographs by overlaying a gridded transparency (with each grid measuring 2.54 cm²) on the lichen disc photo in a flat computer screen. Bleaching before deployment (if any) in these thalli was calculated by counting the number of squares covered by bleached portions divided by the total area covered by the thallus on the disc. This provided the baseline data of proportions of bleaching. At the end of deployment, we carried out the same calculations and then determined the difference in bleaching proportions between initial deployment and 12 months into it.

Chlorophyll content in the lichen thallus was estimated through destructive sampling as portions of thalli were removed from the discs for each analysis. Chlorophyll content was measured three times over the 12-month period. To determine chlorophyll content, discs with *R. stenospora* (**Fig.3** bottom row) thalli were removed carefully from the bark using a scalpel, air dried overnight, and divided into subsamples of ~ 0.02 g. The subsamples were each placed in 10 ml of dimethyl sulfoxide (DMSO) overnight and in the dark (Ronen & Galun 1984). Optical densities were then measured using a spectrophotometer (Spectronic™ GENESYS™ 20) at 645 nm and 665 nm. Chlorophyll content was calculated using the equation developed by Arnon (1949)

$$\text{mg chlorophyll g dw}^{-1} = \frac{(20.20D_{645} + 8.020D_{665}) \times 10 \text{ ml}}{1000 \text{ ml} \times \text{gdw}}$$

Morphological measurements: Overall health of the lichen was assessed using measurements of external and internal components, such as, thallus thickness, cortex thickness, and algal layer thickness. Apothecia obtained from *Parmotrema perforatum* from the twigs were carefully sliced off the thallus and placed in carrot pith. The pith was then sliced in a rotary microtome set at 10 microns. Thin slices were selected by first placing all the sliced material in a petri dish of water and separating the best sections and wet-mounting them on glass slides. Multiple slices were examined under a compound microscope for each apothecium taken. Photographs of each slice were taken using a built-in digital camera (Moticam[®] 2000) on the microscope at three different magnifications (Fig. 4). Measurements of thickness (in μm) were taken for each cellular component of the lichen in three repetitions using the program Motic Images Plus[®]. The camera was re-calibrated before each use.



Figure 2. Lichen disc of *Physcia solediosa* glued on plywood board.



Figure 3. Lichen transplant assembly containing 3 discs of *Physcia solediosa* (top row) and 3 discs of *Ramalina stenospora* (bottom row).

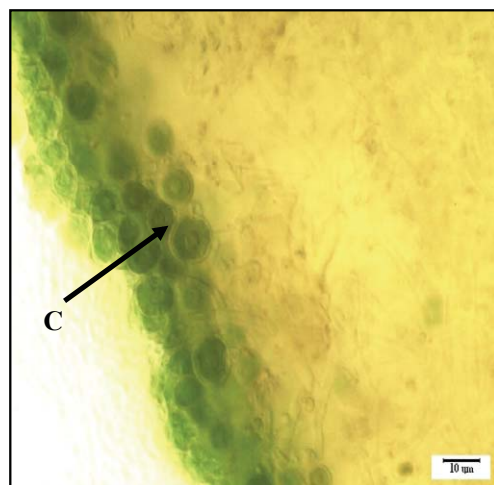
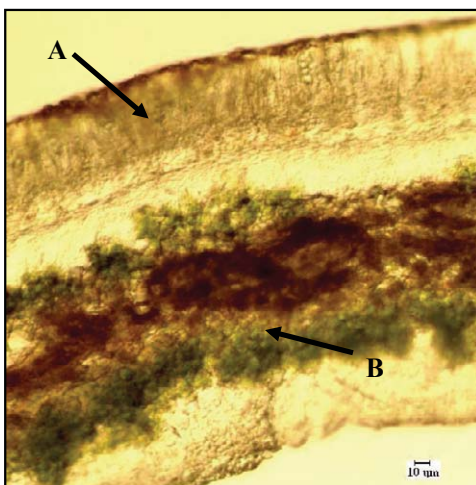


Figure 4. Photograph of *Parmotrema perforatum* thallus section. Section of entire thallus showing upper cortex (A) and algal layer (B), left photo. Single algal cell (C) within the thallus, right photo.

Air quality data: The study sites did not have any permanent air quality monitoring stations. We collected ambient whole air samples for VOCs (volatile organic compounds) using grab sampling by Summa-type six-liter air canisters provided by the Louisiana Department of Environmental Quality (LADEQ). The canisters are made of high purity, passivated, stainless steel that is designed to maintain the stability and integrity of a sample while being transported for analysis. The samples were analyzed at LDEQ's lab (in Baton Rouge, LA) using gas chromatograph separation with flame ionization detector. These air samples were analyzed for VOCs, in particular benzene and ethane since both of these have been treated by the US EPA as "toxic air pollutants" or "hazardous air pollutants", that are known or suspected to cause cancer or other serious health effects, such as reproductive effects or birth defects, or adverse environmental effects. Both of these gases are associated with vehicular exhaust (Power et al. 1996). Air samples were taken at each station during 3 different intervals during the study, once during the fall (October), then during winter (December) and then the final sample during summer, June (**Fig. 5**).

Data analyses: To evaluate potential difference in thallus bleaching among sites and stations we used a two-factor analyses of variance (ANOVAs), with bleaching as the dependent variable and site and stations as the two factors. We used another two-factor ANOVA for estimating differences in chlorophyll content among the sites and stations. Differences in morphological measurements of thalli among stations were analyzed using a multiple factor ANOVA. All data were tested for standard assumptions for the test carried out and transformations (for proportion data) were carried out as needed and means reported in the results have been back transformed. We present the values of the controls (from Russell Sage Wildlife Management Area) for each of the variables measured in respective tables and figures. We did not include them in the analyses as the control site was not differentiated into stations (exterior and interior).

RESULTS

Air quality – Mean concentration of benzene in the air samples was highest in Black Bayou NWR (0.27 ppbv, S.E. = 0.03) closely followed by Russell Sage WMA (0.24 ppbv; S.E. = 0.02). Upper Ouachita WMA had the lowest concentration (0.10 ppbv; S.E. = 0.01) and D'Arbonne NWR and Restoration Park had intermediate levels (0.17 and 0.15; S.E. = 0.05 and 0.05 respectively) of benzene. Upper Ouachita WMA had the highest concentration of ethane (7 ppbv, S.E. = 0.40) followed by D'Arbonne, Black Bayou NWR, Restoration Park and Russell Sage WMA (5.41, 4.66, 2.95 and 2.74; S.E. = 0.07, 0.19, 0.07 and 0.05 respectively). No statistical tests were conducted on these values since there was no replicate air samples collected at each of these sites.

Bleaching and chlorophyll content - Effect of site on thallus bleaching: The main effect test suggested significant differences ($F_{9,80} = 2.41$, $P = 0.02$) in thallus bleaching among the 5 sites. Test of simple main showed that the amount of thallus bleaching in and among the sites Upper Ouachita NWR, Restoration Park and D'Arbonne NWR, were not significantly different from one another (13%, 15% and 11% respectively). Further, within each site, the amount of bleaching between stations did not differ ($P = 0.15$). The interaction between the site and station was not significant ($P = 0.06$). Black Bayou NWR had the highest amount of bleaching (16%) and Russell Sage had the lowest amount of bleaching (10%).

Effect of site on chlorophyll content: The amount of chlorophyll in thalli during each sampling period was first tested for any potential difference based on the position of the stations within each site. Results indicated that the amount of chlorophyll in the lichen thalli was independent of the position of the station within each site for all the three sampling periods ($T1 - F_{1,8} = 0.13$, $P = 0.73$; $T2 - F_{1,8} = 1.52$, $P = 0.25$; $T3 - F_{1,8} = 0.03$, $P = 0.87$). Because there was no station effect, we carried out post-hoc analyses to evaluate the effect of site. We calculated the average chlorophyll content per station from the three subsamples per station and then carried out analysis to evaluate

differences in the chlorophyll content by site. Results revealed significant differences in the amount of chlorophyll among the sites during the first sampling ($T1 - F_{4,5} = 10.63, P = 0.01$) only. There was no difference in the chlorophyll content in the lichen thalli during subsequent samplings, time 2 and 3. Lichens in the Restoration Park had the highest amount of chlorophyll content (14.23 mg/gm dry weight of thallus) and D'Arbonne NWR had the lowest (7.4 mg/gm dry weight of thallus; **Fig. 6**)

Morphology- *Effect of site on thallus morphology:* Results of analyses using morphological data from the thallus of *Parmotrema perforatum* (thickness of thallus, cortex, algal layer and hyphal layer) indicated significant site by station interaction (**Table 1**). Whether thickness of these parts differed between stations (interior and exterior), was dependent on the specific sites under consideration. Mean values of thickness for thallus, cortex, algal layer and hyphal layer for lichens at the study sites have been presented in Table 2.

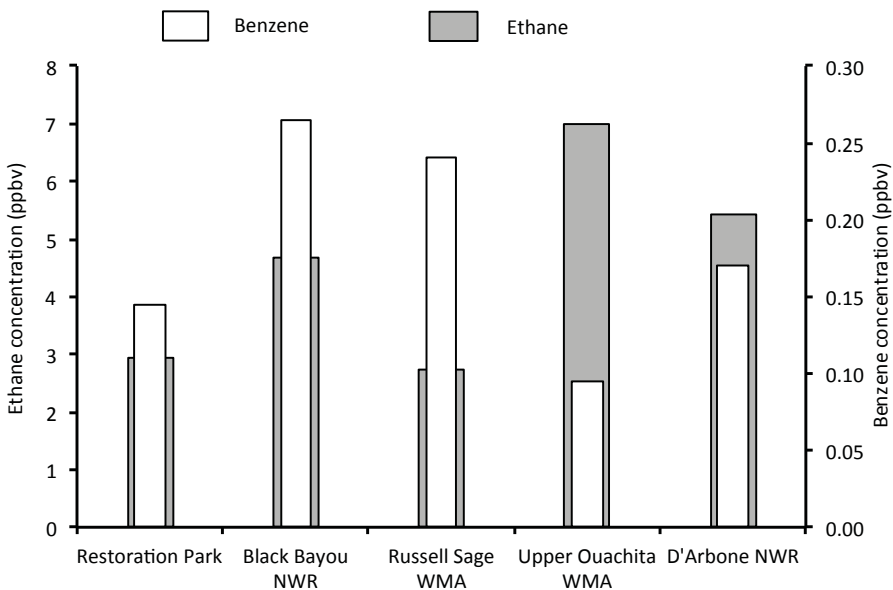


Figure 5. Mean concentration of benzene and ethane (parts per billion/volume) measured at the study locations using Summa-type air canisters. Air quality samples were taken 3 different times at each site during the study. Values of benzene and ethane in the control site were 0.12 and 1.8 ppbv respectively.

DISCUSSION

Relatively bad air quality in an area can negatively impact lichen morphology. Of all the species used in our study, *Ramalina stenospora*, *Physcia solediosa*, and *Parmotrema perforatum*, best indications of higher levels of benzene and ethane were obtained using discs of *Physcia solediosa*. This finding is also supported in a study by Shrestha and St. Clair, 2011, where *Physcia* - sp. was found to be sensitive to pollution in the intermountain areas of Utah, Colorado and New Mexico, in addition to other genera. The other genera we used to test for chlorophyll bleaching, *Ramalina stenospora* did not show consistent changes in chlorophyll content by sites. Morphological differences in the thallus of *Parmotrema perforatum* also did not reveal any clear trends to the varying levels of pollution in our sites.

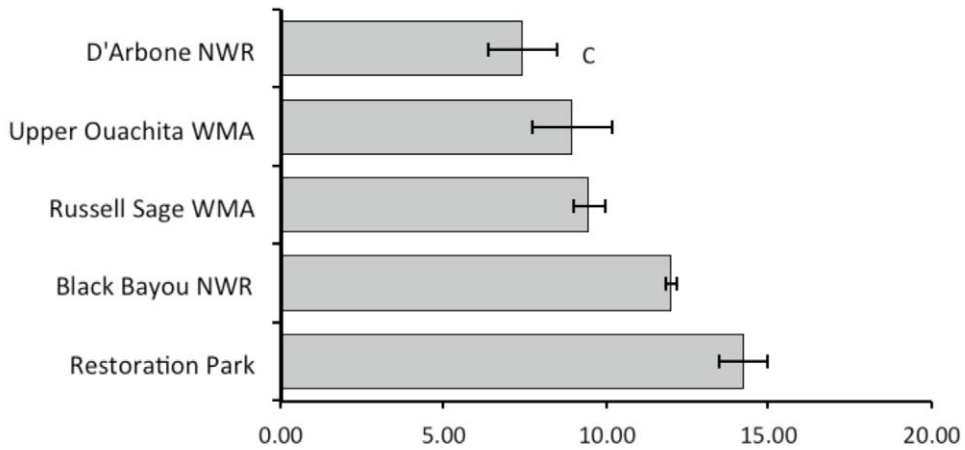


Figure 6. Difference in mean lichen thallus chlorophyll content (mg/g dry weight of thallus) during first sampling period in the five sites. Note: there was no statistical difference in the chlorophyll content among the site in the second and third samplings. Error bars indicate standard errors. Different letters on mean chlorophyll content values indicate statistical significance ($P < 0.05$). Values of mean lichen thallus chlorophyll content in the control site was 7.51 mg/g dry weight of thallus.

Table 1. Summary statistics from analysis of variance (ANOVA) for each variable in thallus of *Parmotrema perforatum*. The variable Site included Russell Sage WMA, Upper Ouachita NWR, Restoration Park, Black Bayou Lake NWR, and D'Arbonne NWR. Variable station included 'interior' and 'exterior'.

Variable (thickness in μm)	F-Value	P-Value
Thallus	500	<0.001
Site	530.7	<0.001
Station	871.7	<0.001
Site* Station	252.8	<0.001
Cortex	117.59	<0.001
Site	140.69	<0.001
Station	94.15	<0.001
Site* Station	83.9	<0.001
Hyphal layer	632.49	<0.001
Site	816.68	<0.001
Station	662.53	<0.001
Site* Station	249.09	<0.001
Algal layer	16.6	<0.001
Site	21.93	<0.001
Station	17.66	0.001
Site* Station	5.39	0.01

Table 2. Mean values of thickness (in μm) for *Parmotrema perforatum* thallus, cortex, algal layer and hyphal layer at the study sites (Russell Sage WMA, Upper Ouachita NWR, Restoration Park, Black Bayou Lake NWR, and D'Arbonne NWR). Numbers in parentheses represent the standard deviation of the mean.

Site	Station	Thallus	Cortex	Algal layer	Hyphal layer
Russell Sage WMA	Interior	156.77 (6.51)	19.20 (4.51)	0.29 (0.05)	0.606 (0.08)
	Exterior	307.10 (2.31)	67.45 (3.31)	0.36 (0.03)	1.16 (0.08)
Upper Ouachita NWR	Interior	308.38 (4.94)	75.77 (4.96)	0.41 (0.04)	0.66 (0.09)
	Exterior	424.11 (8.94)	86.31 (2.54)	0.43 (0.01)	0.54 (0.03)
Restoration Park	Interior	*	*	*	*
	Exterior	223.65 (5.45)	47.56 (0.52)	0.26 (0.02)	1.01 (0.03)
Black Bayou Lake NWR	Interior	*	*	*	*
	Exterior	265.58 (6.78)	79.26 (3.53)	0.32 (0.05)	0.98 (0.04)
D'Arbonne NWR	Interior	226.15 (3.82)	88.52 (3.94)	0.43 (0.01)	0.49 (0.08)
	Exterior	221.61 (8.45)	81.67 (4.89)	0.63 (0.10)	0.82 (0.03)
Control Site [†]		361.90	88.57	0.44	0.74

Note: * indicates missing data

[†] The control site did not have replicates or interior/exterior stations, hence no standard deviation values are available.

Careful observations of thallus bleaching can be correlated to higher levels of ethane and benzene in the area. In our study, Black Bayou Lake NWR, located in more urban settings had the highest amount of thallus bleaching among the five sites and also the highest amount of benzene concentration in air and one of the higher concentrations of ethane. This is perhaps indicative of the increased vehicular traffic in the areas. This site is adjacent to HWY 165, which is a 4-lane highway connecting Louisiana and Arkansas. In addition, there is also a boat ramp on site which is heavily used (average of 100 launched a week). Black Bayou Lake NWR is also surrounded by heavily populated localities, Sterlington, Swartz and North Monroe. On the other hand Russell Sage WMA had the lowest values of thallus bleaching among all sites. Russell Sage WMA is secluded with no road in a 2 km radius and no continuous human activities. While the concentration of benzene in Russell Sage WMA was relatively high, the concentration of ethane was among the lowest.

The amount of chlorophyll in the lichen thalli is often related to the levels of environmental stress; with greater amounts of chlorophyll under stressed conditions than under non-stressful conditions. The amount of chlorophyll in lichens at Restoration Park, which is next to the Interstate-20 and experiences high traffic volumes, was very high. It was about twice the amount in D'Arbonne NWR. Once again, this is indicative of the possibly better air quality at D'Arbonne NWR due to its greater distance to any possible sources of pollution (roads, human settlements or industries).

Morphological variables did not present a clear pattern in response to assumed pollution level differences among sites. The variations in measurements observed need to be further studied to

interpret them in relation to actual measures of air pollution at each site. Some sites had a thicker cortical layer while others had thicker algal layer. The presence of a significant interaction between sites and stations made it difficult to outline a clear pattern. Another study, by Estrabou et al. 2004 in Argentina, also reported not finding any clear pattern of thallus morphological differences in relation to varying levels of air pollution. Perhaps, a study that is carried out in a controlled environment (atmospheric chambers) will be able to reveal any direct correlation of changes in morphological measurements in lichen thalli with varying levels of common atmospheric pollutants.

Overall, station position (exterior or interior) within each site did not have an effect on the bleaching of lichen thalli. A study by Hasselrot and Grennfelt (1987) found that air pollution levels, as indicated by through fall and bulk deposition, was higher on the outer forest boundary than in the inner reaches. However, in our study sites, trees on the outer edges of the forest perhaps did not serve as a protective pollution buffer to the interior of the forest, hence organisms occurring deeper within the forests may risk the same pollution damage as the forest boundary.

While we collected air samples from all the sites three times during the study and analyzed them, we found 'spot' measurements of air samples to be temporally variable. We used these measurements more to categorize our sites as 'more' or 'less' pollution prone. We do understand that such measurements are less indicative of the 'actual' air quality in the site. To obtain accurate air pollution profile of an area, it is important to set up on-site monitoring stations, which again can be extremely expensive. Sometimes, environmental quality monitors are set up by the EPA or a state affiliate, for long-term data collection and monitoring. It would therefore be more appropriate to conduct studies such as ours in those locations to reconfirm the findings of our study.

ACKNOWLEDGEMENTS

The authors would like to thank Leonard Killmer, Kirk Cormier, Larry Baldwin, and Melvin Mitchell of the Louisiana Department of Environmental Quality for their help in providing us with the summa air canisters and analyzing the air samples. We also thank the Louisiana Department of Wildlife and Fisheries and United States Fish and Wildlife Service for permits access to our research sites. JW was supported by a graduate assistantship from the Department of Biology at the University of Louisiana, Monroe, Louisiana. We thank Alex Fotis and Matthew Reid for their help in the field and suggestions on earlier versions of the manuscript.

LITERATURE CITED

- Ahmadjian, V. (1993) *The Lichen Symbiosis*. New York: John Wiley and Sons.
- Brodo, I. M., Sharnoff, S. D. & Sharnoff, S. (2001) *Lichens of North America*. New Haven: Yale University Press.
- _____. (1961) Transplant experiments with corticolous lichens using a new technique. *Ecology* 42: 838-841.
- Daily, G. C. (1997) *Nature's Services: Societal Dependence on Natural Ecosystems*. Washington D.C.: Island Press.
- Duffus, J. & Worth, H. (2006) Toxicology and the environment: An IUPAC teaching program for chemists. *Pure and Applied Chemistry* 78: 2043-2050.
- Eversman, S. & Sigal, L. L. (1987) Ultrastructural effects of gaseous pollutants and acid precipitation on lichens. *Canadian Journal of Botany* 65: 1806-1818.
- Gadzała-Kopciuch, R., Berecka, B., Bartoszewicz, J. & Buszewski, B. (2004) Some considerations about bioindicators in environmental monitoring. *Polish Journal of Environmental Studies* 13: 453-462.
- Garty, J., Kloog, N., Cohen, Y., Wolfson, R. & Karnieli, A. (1997) The effect of air pollution on the integrity of chlorophyll, spectral reflectance response, and on concentrations on nickel, vanadium, and sulphur in the lichen *Ramalina duriaei* (De Not.) Bagl. *Environmental Research* 74: 174-187.
- _____, Karary, Y. & Harel J. (1993) The impact of air pollution on the integrity of cell membranes and chlorophyll degradation in the lichen *Ramalina duriaei* (De Not.) Bagl. transplanted to industrial sites. *Environmental Contamination and Toxicology* 24: 455-460.

- _____, Tamir, O., Hassid, I., Eshel, A., Cohen, Y., Karnieli, A. & Orlovsky, L. (2001) Photosynthesis, chlorophyll integrity, and spectral reflectance in lichens exposed to air pollution. *Journal of Environmental Quality* 30: 884-893.
- Glavich, D. A. & Geiser, L. H. (2008) Potential approaches to developing lichen-based critical loads and levels for nitrogen, sulfur and metal-containing atmospheric pollutants in North America. *The Bryologist* 111: 638-649.
- Hasselrot, B. & Grennfelt, P. (1987) Deposition of air pollutants in a wind-exposed forest edge. *Water, Air, & Soil Pollution* 34: 135-143.
- Hawksworth, D.L., Rose, F. (1976) *Lichens as Pollution Monitors*. London, UK: Edward Arnold Ltd.
- Holopainen, T. & Karenlampi, L. (1984) Injuries to lichen ultrastructure caused by sulphur dioxide fumigations. *New Phytologist* 98: 285-294 .
- Kardish, N., Ronen, R., Bubrick, P. & Garty, J. (1987) The influence of air pollution on the concentration of ATP and on chlorophyll degradation in the lichen *Ramalina duriaei* (De Not.) Bagl. *New Phytologist* 106: 697-706.
- Landis, W. G. (2004) *Introduction to Environmental Toxicology: impacts of chemicals upon ecological systems* (Third Edition). Boca Raton: CRC Press.
- LeBlanc, F. & (1973) Effects of sulphur dioxide on lichen and moss transplants. *Ecology* 54: 612-617.
- _____, F. C. (1971) Fluoride injury symptoms in epiphytic lichens and mosses. *Canadian Journal of Botany* 49: 1691-1698.
- McCune B., & Geiser, L. (1997) *Macrolichens of the Pacific Northwest*. Eugene: Oregon State University Press.
- Moss, B. (1967) A spectrophotometric method for the estimation of percentage degradation of chlorophylls to pheo-pigments in extracts of algae. *Limnology and Oceanography* 12: 335-340.
- Nash III, T. H. (1996) *Lichen Biology*. New York: Cambridge University Press.
- Peck, J. E., Ford, J., McCune, B. & Daily, B. (2000) Tethered transplants for estimating biomass growth rates of the Arctic lichen *Masonhalea richardsonii*. *The Bryologist* 103: 449-454.
- Power, H., Moussiopoulos N., Brebbia C. A. (ed.) (1996) *Urban pollution*. Volume I. Computational Mechanics Publications, Southampton United Kingdom.
- Puckett, K. J., Nieboer, E., Flora, W. P. & Richardson, D. H. S. (1973) Sulphur dioxide: its effect on photosynthetic C fixation in lichens and suggested mechanisms of phototoxicity. *New Phytologist* 72: 141-154.
- Richardson, D. H. S. (1988) Understanding the pollution sensitivity of lichens. *Botanical journal of the Linnean Society* 96: 31-43.
- Riddell, J., Nash III, T. H. & Padgett, P. (2008) The effect of HNO₃ gas on the lichen *Ramalina menziesii*. *Flora* 203: 47-54.
- Ronen, R. & Galun, M. (1984) Pigment extraction from lichens with dimethyl-sulfoxide (DMSO) and estimation of chlorophyll degradation. *Environmental and Experimental Botany* 24: 239-245.
- Sarret, G., Manceau, A., Cuny, D., Van Haluwyn, C., Déruelle, S., Hazemann, J., Soldo, Y., Eybert-Bérard, L. & Menthonnex, J. (1998) *Mechanisms of Lichen Resistance to Metallic Pollution*. *Environmental Sciences and Technology* 32: 3325-3330.
- Showman, R. (1997) Continuing lichen recolonization in the upper Ohio River Valley. *The Bryologist* 100: 478-481.
- _____. (1990) Lichen recolonization in the upper Ohio River Valley. *The Bryologist* 93: 427-428.
- Shrestha, G & L. St. Clair (2011) Comparisons of lichen floras of four locations in the Intermountain Western United States” *North American Fungi* Vol 6, Number 8 pp.1-20.
- Sigal, L. L. & Nash III, T. H. (1983) Lichen communities on conifers in southern California mountains: An ecological survey relative to oxidant air pollution. *Ecology* 64: 1343-1354 .
- Von Arb, C., Mueller, C., Ammann, K. & Brunold, C. (1990) Lichen physiology and air pollution. *New Phytologist* 115: 431-437.