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

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

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

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. sorediosa* (**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 cm^2) on the lichen disc photo in a flat computer

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 (

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

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 parforatum* (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.

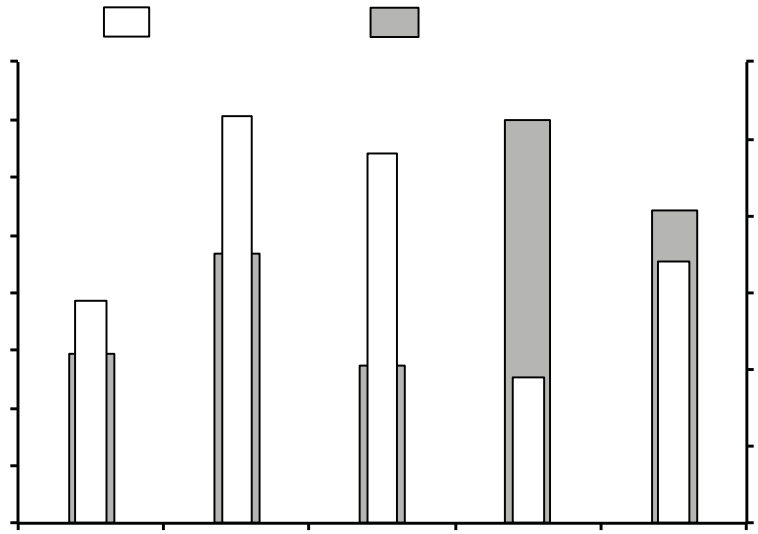

Figure 5. Mean concentration of benzene and ethane (parts per billion/volume) measured at the study locations using Summa-type air canisters. Air quality samples were taken 3 different times at each site during the study. Values of benzene and ethane in the control site were 0.12 and 1.8 ppbv respectively.

DISCUSSION

Relatively bad air quality in an area can negatively impact lichen morphology. Of all the species used in our study, *Ramalina stenospora, Physcia sorediosa,* and *Parmotrema perforatum* , best indications of higher levels of benzene and ethane were obtained using discs of *Physcia sorediosa*. 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

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

[†]

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 st