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LICHENS: BIOASSAY FOR AIR POLLUTION
IN A METROPOLITAN AREA (NASHVILLE, TENNESSEE)

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ABSTRACT

The high cost of buying and maintaining air pollution monitoring equipment has led to a search for better and less expensive ways of measuring pollution levels. Bioassay by means of lichens seems to have great potential for this purpose. The appropriateness of lichens for use as indicators of atmospheric pollution goes beyond matters of economy and encompasses the more important questions of how and why *living organisms* respond to atmospheric pollutants.

In this study corticolous lichens were utilized in calculating indices of atmospheric pollution (IAP's). IAP's were calculated for twelve stations along an east-west transect through the Nashville, Tennessee area. No significant correlations between 1970 pollution levels and IAP measurements was found. Thus, it was concluded that IAP's are probably of little use in monitoring pollution levels on a daily, weekly, or monthly basis. However, the low IAP's found near the center of the city and the comparatively high IAP's found in peripheral areas appear to be very definitely related to long-range effects of pollution in these areas.

Low variability in bark pH was correlated with an efficient buffering system. The source of the buffer chemicals appears to come from various external sources of particulate matter. The

lack of correlation between bark pH and IAP suggests that pollutants selectively restrict the distribution of lichens by some method(s) other than by changing the pH of the substrate.

This and other studies show that corticolous lichens are sensitive to air pollution and quantitative measurements suggest their presence, coverage, and frequency can be used to bioassay pollution levels in metropolitan areas.

INTRODUCTION

The air pollution problems which have resulted from urbanization and industrialization are so well known as to preclude the need for documentation. Nevertheless, it seems appropriate to mention that Nashville ranks twentieth in sulfur dioxide levels (Bontrager, *personal communication*) and twenty-fifth in suspended particulate matter levels (U. S. Dept. of H. E. W., 1969) among American cities.

If certain lichen species can be identified which are reliable indicators of common aerial pollutants, then it logically follows that distributional studies of these

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species could provide the capability of mapping the polluted portions of large metropolitan areas. This has, in fact, been done in Montreal (LeBlanc and DeSloover, 1970) and in Kopmanholmen, Sweden (Moberg, 1969). Such information should prove valuable to city planners, government officials, and the public in general.

Early publications by Nylander (1866) and Johnson (1879) attest to the fact that botanists have long recognized the marked sensitivity of corticolous lichens to air borne pollutants. Observations showing the great ability of lichens to accumulate a variety of materials from dilute solutions have been cited as a possible explanation for this keen sensitivity (Smith, 1962). Rao and LeBlanc (1966) have shown that chlorophyll a is degraded to phaeophytin and Mg^{++} in lichens exposed to sulfur dioxide—a fact which is interesting, but which does not account for lichens being more sensitive than other plants containing the pigment.

During the past two decades, a copious reservoir of literature related to the effects of aerial pollutants on corticolous lichens has been amassed both in Europe (Barkmann, 1958; Rydzak, 1958; Brightman, 1959; Fenton, 1960; Skye, 1958, 1968; Skye and Hallberg, 1969; Moberg, 1969; and Gilbert, 1970a, 1970b) and in North America (Brodo, 1966; Rao and LeBlanc, 1966; LeBlanc and Rao, 1967; DeSloover, 1968; and LeBlanc and DeSloover, 1970).

With the exception of Rydzak (1958), these researchers have emphasized the important role played by SO_2 in reducing the abundance of lichens. Although most workers concede that SO_2 is not the only factor contributing to the reduction of the lichen flora in large urban areas, the majority opinion is that it is probably the major factor in most instances. Rydzak does not deny that there is a notable reduction in both the types and general abundance of lichens in and around cities. However, in explaining this reduction, he emphasizes the higher temperatures and lower humidities associated with urban microclimates. Gilbert (1970a) has presented convincing arguments against Rydzak's claims. The difficulty in resolving the controversy is obvious. Both polluted air and heated, dehumidified air are carried by prevailing winds and the decrease in vegetation is reflected in the wind patterns (Skye, 1958).

A statistic called the "index of atmospheric pollution" (IAP), (LeBlanc and DeSloover, 1970), rather than the biological scale described by Gilbert (1970b), was employed in this study due to its unlimited geographic applicability. This method is based on the assumption that the lichen and/or bryophyte flora of any given area will possess some relatively toxiphobic and toxitolerant species. The exact interpretation of an IAP value is still somewhat uncertain, however, since investigators have not correlated IAP's with measured pollution levels. Although lichen floras of most localities probably do possess a mixture of toxiphobic and toxitolerant species, this has not yet been adequately shown. Therefore, studies need to be conducted in various geographic locations in order to establish the

usefulness of IAP's as measures of atmospheric pollution.

Past lichen research in Tennessee has been restricted, for the most part, to taxonomic studies which have been limited to a few taxa or to local regions within the state (Calkins, 1890; Degelius, 1941; Mozingo, 1961; and Phillips, 1963, 1970). None of these studies have dealt with the effects of aerial pollutants on lichens of this area. Although a number of lichen species found in the Nashville area are common to areas which have been previously studied, (LeBlanc and DeSloover, 1970; and Brodo, 1966) the lichen flora of the Nashville area is sufficiently different from these floras to warrant a separate study (Phillips, 1963; Brodo, 1966; and LeBlanc and DeSloover, 1970).

The principal objective of this study was to determine if there is a demonstrable diminution in the abundance and luxuriance of lichens (as measured by IAP) as the center of the Nashville Metropolitan Area is approached; and if so, to determine if the decrease can be correlated with 1970 levels of SO_2 , suspended particulate matter, dust fall, or with some combination of these aerial pollutants.

DESCRIPTION OF THE STUDY SITE

The city of Nashville is located on the Cumberland River, in the Northwestern corner of the Central Basin of Middle Tennessee near the escarpment of the so-called Highland Rim. This rim rises to a height of 300 to 400 feet above the average elevation of the basin, forming an amphitheater about the City from the southeast to the northeast, the area to the south, southeast, and east being more or less open, but undulating (U. S. Department of Commerce, 1970).

The climate of Nashville is described as "Cfa" by the Koppen system of climate classification (Kendall and Glendinning, 1958). The mean annual temperature is 59.5°F. The coldest month (January) and the warmest (July) have mean temperatures of 39.0°F. and 79.5°F. respectively. The mean annual precipitation is approximately 46 inches and is distributed rather uniformly throughout the year. Precipitation reaches a maximum in early spring and a minimum during the fall months (U. S. Department of Commerce, 1970).

During the summer months, winds are predominantly from the south at an average speed of 7.6 miles per hour. In the winter, winds are often from the north and northwest at speeds of approximately ten miles per hour. Easterly and westerly winds are not common and are of low velocity when they do occur (U. S. Department of Commerce, 1963). Considering the fact that pollution levels are approximately three times as high during the winter months (according to soiling index data gathered by the Metropolitan Nashville Health Department) as in the summer months, the net movement of pollutants by winds is from northwest to southeast.

MATERIALS AND METHODS

Location of sampling stations. Using topographic maps published by the United States Geological Survey and road maps distributed by petroleum companies, twelve suitable sampling sites were located and studied during the spring of 1971. Care was taken to insure that all twelve sample sites were as nearly ecologically equivalent as possible. Such factors as shelter, substrate, light, and moisture have been generally recognized as having considerable effect on the distribution of lichens. It is believed that by sampling only from free-standing trees of the same species, and of approximately the same size, that the effects of the first three factors are reasonably well controlled. Although no definite statement can be made concerning local

variations in moisture, it is believed that the area is relatively free of any gradients in rainfall or relative humidity.

Figure 1 shows that the stations follow the meandering course of the Cumberland River, thus forming an east-west transect through Nashville. Also shown in Figure 1 is the location of pollution monitoring stations maintained since 1969 by the Metropolitan Health Department.

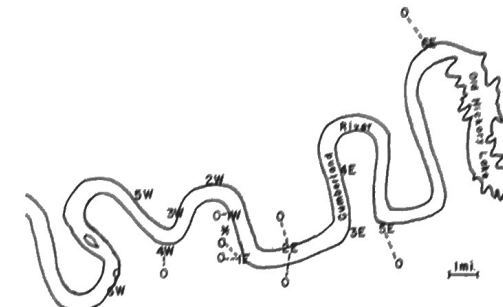


Figure 1. A map showing the location of sample stations. Lichen collecting stations are denoted by a numeral and a letter. O denotes a pollution monitoring station maintained by the Metropolitan Health Department; * denotes the State Capitol; and dashed lines denote stations paired for regression analysis.

The sampling procedure. Once a sample site was located, ten free-standing trees (*Celtis occidentalis* L.) having a diameter at breast height (dbh) of ten to twenty inches were selected. An occasional difficulty arose in locating enough trees meeting these criteria. When this occurred, a closely related tree species (*Celtis tenuifolia* Nutt.) was substituted. In two instances (stations 1W and 5E) lichen samples were collected from only eight trees.

The trunk of each tree was carefully examined from the base to a height of six feet. A hand lens was used to establish the identity of all foliose lichens present. Whenever the identity of a specimen could not be ascertained in the field, its frequency of occurrence (f) was recorded. Then the specimen was taken to the laboratory where tools such as spot testing reagents, dissecting scopes, UV light sources, etc. were available and positive identification could be made.

Assessment of the frequency of occurrence followed the arbitrary scale of LeBlanc and DeSloover (1970) with the following exception. An "f" value was estimated for each species of lichen on each of the sample trees. The frequency of occurrence for a species was then taken as the arithmetic mean of the individual "f" values for a particular station. For example, if a particular lichen was found on three of the ten trees in a sample and individual "f" values were estimated as 4, 3, and 2 respectively, the "f" value for the species would be computed by summing the individual "f"s and dividing by the number of trees in the sample. Therefore, in this case the "f" value would be 0.9.

Computation of indices of atmospheric pollution. An index of atmospheric pollution was calculated for each of the twelve sample stations, for any station, $IAP = \frac{Q \times f}{10}$, where "Q" is the ecological index of each species and "f" is the frequency of occurrence described above.

The ecological index (Q) of a species was determined by adding together the number of species of lichens occurring with it at a station and then taking the average of the sums for all the stations where the species was present (LeBlanc and DeSloover, 1970). For example, *Xanthoria candelaria* (L.) Arn. has a mean number of 9.0 "companion" species. In one instance eight species were present besides *X. candelaria* and in the other instance ten "companion" species were present. When all other variables are held constant except air pollution levels, high values of "Q" reflect a low degree of pollution tolerance. Conversely, as values of "Q" decrease, an increasing tolerance to pollutants is indicated.

Comparison of pollution levels with IAP values. Pollution monitoring stations maintained by the Metropolitan Health De-

partment (Figure 1) were located relatively near seven of the twelve stations used in this study. Therefore, it was possible to make comparisons between the IAP values computed for these stations and the various pollutant levels measured by the Health Department. These data were subjected to a regression analysis using an IBM Model 1130 computer. Only 1970 pollution data were available for analysis.

Measurement of bark pH. Dead bark flakes were collected at each station and their pH taken using a Delta-Matic Model 145 pH meter and glass electrodes manufactured by Instrument Laboratories of Boston, Massachusetts.

At each station, bark samples were removed from several different heights along the tree trunk and from several different trees in order to maximize the chance of representing local variation. Care was taken in order to exclude lichen thalli from the sample.

When measuring the pH, deionized water was boiled to remove dissolved gases and then cooled to 20°C in a carbon dioxide free atmosphere. One hundred ml of water was added to each 5.0 g sample of bark, the electrodes immersed in this mixture, and the pH measured immediately. These samples were then allowed to stand in air at room temperature for 24 hours and the pH was measured again.

Measurement of the buffer capacity of bark samples. The buffer capacity of hackberry bark was determined by measuring the amount of N/50 HCl needed to lower to pH 3.2 a sample consisting of 2 g bark flakes in 20 ml of deionized water. Several hours was allowed between additions of HCl in order to allow time for equilibrium to be established. The buffer capacity was taken as the mean of three replicates. This method is similar to one described by Gilbert (1970a).

RESULTS

Description of flora. Sixteen species of foliose lichens were identified from the 116 free-standing hackberry trees examined. The most commonly occurring individuals were members of such genera as *Physcia*, *Pyxine*, *Parmelia*, and *Candelaria*. Only two species were present at all twelve stations—*Physcia millegrana* Degel., and *Candelaria concolor* (Dicks.) B. Stein. A complete list of the lichens which were identified and their accompanying ecological indices (Q) is provided in Table I.

TABLE I. A List of the Species Identified and Their Ecological Indices

SPECIES	Q
<i>Candelaria concolor</i> (Dicks.) B. Stein	5.6
<i>Parmelia boilliana</i> Muell. Arg.	7.0
<i>Parmelia caperata</i> (L.) Ach.	8.5
<i>Parmelia galbana</i> Ach.	8.5
<i>Parmelia hypotrappa</i> Nyl.	7.7
<i>Parmelia perforata</i> (Jacq.) Ach.	7.8
<i>Parmelia rufecta</i> Ach.	7.0
<i>Physcia millegrana</i> Degel.	5.6
<i>Physcia orbicularis</i> (Neck.) Thoms.	9.0
<i>Physcia orbicularis rubropulchra</i> Degel.	8.3
<i>Physcia stellaris</i> (L.) Nyl.	6.9
<i>Physcia syncolla</i> Tuck.	7.0
<i>Physcia tribacoides</i> Nyl.	10.0
<i>Pyxine caesiopruinosa</i> (Tuck.) Imsh.	6.5
<i>Pyxine sordidata</i> (Ach.) Mont.	7.0
<i>Xanthoria candelaria</i> (L.) Arn.	9.0

Obviously sweeping conclusions about the relative pollution sensitivity of a species cannot be made when the sample is small and restricted to a small portion of

the geographic range of the species. Therefore, care must be exercised in interpreting the meaning of the values for "Q" given in Table I. This is not to say, however, that the values are not significant. It should be noted that the relatively low values of "Q" computed for *Physcia stellaris* (L.) Nyl., *P. millegrana* and *C. concolor* in this study are confirmed by LeBlanc and DeSloover in their 1970 publication concerning air pollution in Montreal, Canada.

The relationship of IAP measurements to pollution patterns. In an effort to ascertain what air qualities IAP's reflect, IAP data from the seven stations near pollution monitoring stations maintained by the Metropolitan Health Department (Figure 1) were compared via regression analysis with the 1970 mean annual values for sulfation, suspended particulates and dust fall. A negative correlation was registered between each of these factors and the IAP. Sulfation values (which bear a direct relationship to parts per million of SO₂) showed a higher correlation with the IAP's than dust fall or suspended particulates. However, none of the correlations were significant at the $\alpha = .05$ level. Table II shows the correlations (r) between IAP levels and the various pollutants and combinations of pollutants.

TABLE II. The Relationship of IAP to 1970 Pollution Levels

IAP as a function of:	(r)
Sulfation (SO ₂)	-0.562
Suspended particulates	-0.359
Dust fall	-0.043
SO ₂ plus suspended particulates	-0.592
SO ₂ plus suspended particulates plus dust fall	-0.636

F-ratio tests indicate that the correlation coefficients given for IAP as (1) a function of SO₂ levels; (2) a function of SO₂ plus suspended particulate levels; and (3) a function of SO₂ plus suspended particulates plus dust fall levels are not significantly different at the .05 level.

The low correlations observed between IAP's and the various pollutants dramatically indicate what LeBlanc and DeSloover had suspected. That is, that IAP's reflect long-term effects of pollution and that a particular IAP value may not accurately reflect the pollution level at a particular place at a particular point in time. The reason for the poor correlation between IAP's and the mean annual 1970 pollution measurements is more easily understood when consideration is given to the fact that a low IAP may not accurately indicate the pollution level in an area which has recently been cleared of pollution sources. Also, high IAP's do not necessarily indicate pure air in cases where an area has recently acquired new sources of contamination. Low correlations in such instances are probably to be expected.

It is interesting, concerning IAP measurements, that two distinct lows occur—one at station 4W and an-

other in the vicinity of stations 1W and 1E. The low at station 4W is probably related to the nature of adjacent land usage. Several colleges and numerous low income type dwellings in the vicinity have long utilized low-grade fossil fuels for heating purposes during the winter months. These sources undoubtedly contribute significantly to the high SO₂ levels recorded for the area. Recently some of the colleges and homes have installed modern heating systems which should help to alleviate the future pollution problems of this community. The area also includes numerous large industrial plants whose towering smoke stacks can be seen pouring particulates and presumably other pollutants into the atmosphere.

Stations 1W and 1E are near the downtown area and the low IAP's are probably associated with the high pollution levels which have been found here for many years. The City's highest sulfation levels were recorded in this area in 1970. Motorized vehicles are probably the chief sources of pollution in this area.

A second point of interest concerning the IAP measurement is that the stations east of the State Capitol Building show higher IAP's on the average than do those stations west of the Capitol. This is probably related to present usage in these areas and to the fact that western stations are closer to the primary centers of pollution. The IAP values for all stations are given in Figure 2.

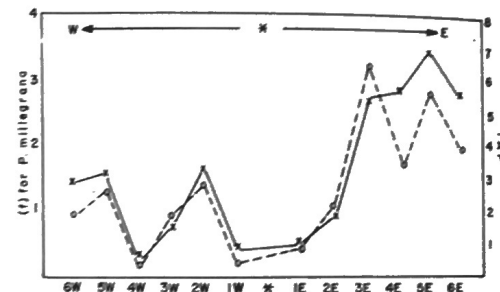


Figure 2. A comparison of IAP's and "f" values for *P. millegrana*. O denotes points for IAP's; X denotes points for "f" values; * denotes State Capitol Building.

Communities adjacent to stations 3E, 4E, 5E, and 6E are primarily middle-class residential areas of comparatively recent origin. Many of the houses have been occupied for only a few years and most of them have never utilized coal as a source of heat.

Most land adjacent to stations west of the Capitol is devoted to old, low income houses, colleges, and industrial plants. It is believed that the low IAP's generally observed for these stations reflect the long-term effects of consistently high pollution levels.

Another interesting feature concerns the IAP for station 2W. Pollution is now, and probably has long been, high in this vicinity. Yet, the IAP (2.8) is respectably high. The reason for this is not yet apparent. Inspection of topographic maps shows that the station (elevation 400 feet) is bounded on the west, north,

and east by hills which rise to elevations of about 550 feet. The station is bounded on the west by a small forest. It is possible that these factors offer some degree of protection to the trees of this sample.

The high degree of correlation between IAP values and the frequency of occurrence (f) of *P. millegrana* (Figure 2) was a noteworthy finding. This finding suggests that it may be possible to monitor pollution levels with a single lichen species. Generally speaking, an assemblage of indicators yield more information than a single indicator species. Therefore, this finding was surprising. Further study will be needed in order to determine exactly how much correlation there is between the frequency of occurrence of *P. millegrana* and air pollution. It is possible that the frequency of occurrence of *P. millegrana* may be most useful when rapid reconnaissance is of prime importance.

Bark pH. The bark pH was strikingly uniform for all twelve stations. When the pH was measured in gas-free water immediately after placing the bark in the water, the range was 6.22 to 6.56. When measured again after the mixture had stood in air for twenty-four hours, the range was 5.59 to 5.85. These results had not been anticipated since researchers (Gilbert, 1970a; Skye and Hallberg, 1969) had reported a lowering of bark pH as pollution sources (specifically, SO₂ sources) were approached. Therefore, an explanation for the observed pH values was sought.

The hypothesis that hackberry bark is intrinsically well buffered was simple and attractive. In order to test this hypothesis, a control organism known to lack an intrinsically well-buffered bark was needed. Ash (*Fraxinus americana* L.) was chosen for this purpose. Gilbert (1970a) has shown that the bark pH of a closely related species (*Fraxinus excelsior*) fluctuates with the level of atmospheric SO₂ in Northern England.

When pH measurements were made on samples of ash bark from the various stations, the bark pH proved again to be very uniform (5.23 to 5.87) from station to station. This result led to the suspicion that something was being deposited on the bark of these trees which could act as a buffering system. In order to test this hypothesis, bark samples were obtained from some hackberry trees growing in Nashville and from some growing in Rutherford County (a relatively low pollution area), about fifteen miles south of Nashville. The average amount of acid necessary to reduce the pH to 3.2 was 6.5 ml for the Nashville bark, but only 3.2 ml for the Rutherford County bark. One ml was necessary to reduce the pH of a deionized water blank to the 3.2 level. On the basis of the above evidence, it was concluded that the bark of trees in Nashville collects something which serves as a buffer against increasing acidity. The nature of the buffer was not determined in this study but is undergoing further investigation.

DISCUSSION AND CONCLUSIONS

Results from this and other studies make it apparent that urbanization and industrialization play a significant role in the destruction of native vegetation.

Although all vegetation is probably to some degree sensitive to pollutants, lichens are among the most toxiphobic of our native plants. It seems ironic that these same plants should lend themselves so well to the study of pollution problems.

According to LeBlanc and DeSloover (1970), IAP's express a response of epiphytic vegetation to the long-range effects of pollution. The results of this project generally support this conclusion. The poor correlations between IAP's and mean annual 1970 levels of pollution are interpreted as meaning that IAP's cannot be depended upon to give accurate estimates of current pollution levels in all cases. Contributing to the poor correlations observed are such factors as: (1) the recent introduction or elimination of major pollution sources, and (2) the fact that lichens are more affected by low SO₂ concentrations for prolonged periods of time than by high concentrations for short durations (LeBlanc, 1969).

Because IAP's cannot give accurate day-by-day accounts of pollution levels, their value as pollution indicator is limited. Nevertheless, IAP's may prove extremely useful in obtaining information on pollution patterns over substantial periods of time.

The authors would like to suggest one modification on computing IAP's in future research of this type. In studying the formula for computing the index of atmospheric pollution given by LeBlanc and DeSloover (1970) as $IAP = \sum(Q \times f) / 10$, it will be observed that the value for "Q" has an upper limit. This limit is the number of different epiphytic species which are capable of growing under a given set of environmental conditions. This limit will vary, of course, from one geographic location to another. It will also be observed that when the limit for "Q" approaches one, IAP values approximate f/10. Thus the variables "Q" and "f" are not proportionately represented when "Q" approaches one or when "Q" is large. Hence, it logically follows that an IAP for a region which boasts only a few epiphytes will not be comparable to one which has a large number of different epiphytes. In order to avert this difficulty in the future, it is suggested that a modified formula be used. The suggested modification is given below:

$$IAP = \sum(Q_i \times f_i) / 10$$

$$\text{Where: } Q_i = \frac{Q - \bar{Q}}{Q_i}$$

Q = the ecological index as defined by LeBlanc and DeSloover (1970).

\bar{Q} = the mean of all the values of Q.

Q_i = the standard deviation of the Q values.

This modified computational formula should make intergeographic comparisons of IAP and Q more meaningful.

The high correlation between IAP's and "f's" for *P. millegrana* was one of the more surprising findings of this study. It is not known at present if this finding is coincidental or if it represents a real relationship. If the relationship is not coincidental, there are at least two reasons for the correlation. One is that *P. millegrana*

na is very abundant (high "f") and therefore contributes significantly to the IAP measurements to begin with. A second reason is that *P. millegrana* is pollution tolerant and is capable of exhibiting a frequency (f) gradient. It should be noted that a single toxiphobic species cannot exhibit a frequency gradient because they will be restricted, in distribution, to those zones with relatively pure air. Therefore, since they are *not present* in zones of intermediate and high pollution respectively, their frequency of occurrence (f) cannot be as an indicator of pollution levels.

Although it is probable that no single species can approach an assemblage of species in total "yield of information" it is believed that a properly chosen lichen species can serve as a useful indicator of atmospheric pollution when rapid reconnaissance is required.

In explaining the low lichen frequencies near centers of pollution, most investigators have given considerable attention to the role played by sulfur dioxide. They have suggested that SO₂ dissolves in bark moisture and produces sulfurous acid. Sulfurous acid is, in turn, responsible for the lower pH levels observed in polluted areas. The lower pH values play a role in selectively favoring the growth and survival of certain lichen species and in inhibiting the growth and survival of others.

Changes in bark pH cannot, however, be considered the only factor involved in reducing lichen frequencies near pollution sources. The results of this study indicate that bark pH of hackberry and ash is independent of IAP's and mean annual 1970 levels of sulfur dioxide. The noteworthiness of the station-to-station consistency of pH level has been mentioned previously. This consistency is probably related to the well-buffered bark of the sample trees. The 6.5 ml of N/50 HCl needed to lower the bark pH to 3.2 was considered very high. Gilbert (1970a) reports that *Salix alba* bark requires 2.3 ml, *Fraxinus excelsior* bark 1.7 ml, and asbestos 6:4 ml of this acid in order to lower the pH to the same level.

Comparisons between the buffer capacity of hackberry bark obtained in Rutherford County and bark obtained from Nashville indicate that much of this buffer capacity of the Nashville bark is derived from extrinsic sources.

Possible sources of chemicals imparting this high buffer capacity include soot and suspended particulate matter. Soot deposits noted on Nashville trees, especially at stations 1E, 1W, 4W, and 6W plus Gilbert's (1970a) report that soot can play a role in preventing low pH's, may be cited as implicating soot as a source of buffer chemicals. The high positive correlation (based on 1970 data) between SO₂ levels and levels of suspended particulates may be considered suggestive evidence in favor of the idea that buffer chemicals are derived from suspended particulates. This correlation means that where SO₂ levels are high, (tending to acidify bark) accompanying high levels of particulates exist and could, presumably, supply sufficient buffer to prohibit pH changes.

In studying pollution patterns in Northeast England, Gilbert observed that both bark pH and lichen abundance decreased as town centers were approached. He noticed, however, that lichen abundance began to decrease before pH decreased. On the basis of this evidence, he concluded that substrate pH, *per se*, is not causally related to lichen abundance. This conclusion is different from that of Skye and Hallberg (1969) and Moberg (1969). The results of this study support Gilbert's conclusion.

Little is known of the events which occur at the cellular and molecular levels when lichen thalli are exposed to pollutants. Pollutants, such as SO₂, have been shown (Syke, 1968; Gilbert 1970 a) to affect thallus pH as well as the pH of the substrate. Therefore, the buffer capacity of the thallus itself is probably important to the survival of the lichen.

It is easy to imagine the rather drastic changes which might be produced by large pH shifts. For example, the action and activity of many enzymes would undoubtedly be altered, membrane permeabilities would be altered, etc. Thus in looking to areas for future research, the molecular and cellular responses of lichens to pollutants appears to represent a new and exciting frontier.

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