Geographic Distribution of Airborne Fluorides Near a Point Source in Southeast Ohio

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Appreciation is also extended to the 156 farmers and other landowners of Ohio and West Virginia who permitted sampling of soil, vegetation, and other materials.

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I. INTRODUCTION

Atmospheric fluorides may affect agriculture in several ways. Plants can accumulate fluoride directly from the atmosphere, which may in turn lead to visible leaf injury, damage to fruits, yield changes, and possibly other effects. Excessive dietary fluoride also can have a serious impact on cattle. Discoloration, weakening, and disintegration of the teeth, lameness, and swelling and stiffness of the joints are symptoms often ascribed to fluoride toxicosis. Normally, cattle receive relatively small amounts of fluoride in the total diet, mostly in forage and mineral supplements (44). However, the presence of an airborne source can increase fluoride in forage by as much as ten times the normal level and pose a threat to cattle health. An effective means of determining the potential for fluoride toxicosis in cattle near an airborne source is by monitoring the fluoride content of forage (21, 37, 45).

Several investigators have used natural vegetation as a bioindicator of geographic fluoride distribution (5, 7, 9, 19, 21, 24). Use of vegetation as an indicator of airborne fluoride seems justified, since only small amounts of fluoride are normally accumulated from soil by above-ground portions of most plants, including common forage species (15, 21, 27, 38, 39, 42). Generally about 10 ppm or less fluoride in leaves is considered normal for plants growing in uncontaminated air (37), although levels up to 36 ppm have been reported for alfalfa in some areas of the U.S. (44). There is less consensus regarding tolerable levels of fluoride in cattle diets. A total dietary intake of 40 ppm fluoride is often regarded as marginal in causing symptoms (37). Some research has shown that tooth markings can occur among cattle receiving one-half to one-third this amount in the daily ration (29). Other studies suggest that total nutrition may play an important role in symptom development often ascribed to chronic fluorosis (10, 37).

The overall purpose of this study was to evaluate the effectiveness of a new pollutant abatement system being installed at the fluoride source. The source is situated in the Ohio River Valley near Hannibal (Monroe County), Ohio. This report summarizes results of the pre-abatement phase and deals with the geographic distribution of fluorides in the environment prior to installation of the upgraded pollutant abatement system. These background data can then be compared with similar data collected following installation of the new abatement system. Information reported here relates specifically to fluoride distributions in forage (hay and pasture), tree foliage, and soil from 1972 through 1975.

II. METHODS FOR THE FIELD STUDY

Sampling Locations

The study area was a region about the fluoride source encompassed by a circle of 10-mile radius. It included much of Monroe County, Ohio, and portions of Marshall, Wetzel, and Tyler counties, West Virginia. Potential plot locations were first mapped on U. S. Geological Survey topographic quadrangles (scale = 1:24,000) along 20 radii spaced at 18° intervals around the source. Along each radius, plot locations were designated at one-half mile intervals out to 6 miles, and at 1-mile intervals between 6 and 10 miles.

On-site visits were made during October and November 1972 and the owners of properties suitable for sampling were asked to serve as cooperators. Sample plots (i.e., active farms or other areas) were chosen on the basis of: a) proximity to the designated location, b) presence of farming activity, and c) owner's agreement to cooperate.

A total of 156 landowners were cooperators, representing 197 sample plots. Hay was available on 115 plots and pasture forage was collected on 170 plots.

Pasture Sampling

Two fenced quadrats, 3 feet in diameter (0.16 milacre each), were established in one pasture at each plot location during April and early May 1973 (Fig. 1). Unfenced, square plots of the same area were used in unpastured locations. Corners were marked by wooden stakes and stake-wire flags.

Forage was clipped monthly from all pasture plots beginning in late May and continuing through August 1973 and October 1974. Each complete pasture sampling required 10 or 11 days. Forage was clipped 3 inches above and at ground level in adjacent halves of each quadrat in 1973 to evaluate effects that different grazing intensities might have on dietary fluoride intake of cattle, and to determine
whether significant fluoride contamination of lower portions of forage plants might occur from soil splash (Fig. 2). All clippings were done at ground level in 1974.

Clipping was done with grass shears, one pair modified with guides for the 3-inch clipping height. Weed species not ordinarily consumed by cattle (e.g., ironweed, milkweed, etc.) and dead material were removed from the sample. Harvested forage from the two quadrats was bulked according to clipping height and stored in paper bags. Bags of material collected each day were placed in large polyethylene bags and temporarily stored in a cooler (40° F), then moved to a freezer (0° F) at the OARDC laboratory after each week.

**Hay Sampling**

Hay was sampled in barns (or in several cases from stacks outdoors) during early autumn of 1972 through 1975. Ordinarily, one or two hay cuttings were made by cooperators. The first crop was generally harvested in June and the second in August, but wet conditions often resulted in only one harvest in mid- or late summer by many cooperators. Cores from 10 to 12 bales of each cutting were extracted with a “Penn State Hay Sampler” and placed in paper bags (Fig. 3). Qualitative estimates of species composition were recorded for the 1972 hay samples. Where loose hay was encountered, grab samples were collected, care being taken to avoid sampling the outer, dust-contaminated portion of the mow. These sample bags were also placed in large polyethylene bags and stored in a freezer (0° F) until preparations for fluoride analyses were made.

In addition to routine hay sampling, paired sets of hay bales (three bales per set) were established in barns at five locations to test the hypothesis that bales exposed at the top of the mow might accumulate fluoride during storage. If sampled, these could bias the estimate of average fluoride concentration for the crop. One set of bales was kept covered (top and sides) by a polyethylene sheet while the adjacent set remained uncovered (Fig. 4). The two sets were sampled initially and then periodically from July 1973 through June 1974.
Tree Foliage Sampling

The purpose of surveying fluoride levels in tree foliage was to investigate the possible use of a common tree species for making quantitative estimates of fluoride in forage. An acceptable tree species for use in surveying airborne fluoride dispersion must be capable of accumulating atmospheric fluorides efficiently, tolerate relatively high foliar concentrations of fluoride, be common and well-distributed throughout the survey area, and accumulate minimal amounts of fluoride from soil. Preliminary analyses of leaves from several tree species near the fluoride source and the OARDC Secrest Arboretum, along with observations of species typically found within the survey area, indicated several candidates. This information and data from a greenhouse study on uptake of soil fluorides (Section IV) led to the choice of black locust (*Robinia pseudoacacia* L.) as a species best meeting the above criteria.

Leaves (leaflet plus rachis) from one black locust at each plot were collected from the outer, exposed portions of the crown from September 1973 through 1975. Sample trees were selected in or on the borders of pastures in which forage quadrats were located. These were labeled to enable resampling of the same individuals in subsequent years.

Soil Sampling

Soils were sampled from pastures at 14 sites within a radius of 10 miles around the fluoride source (32). Sample locations were chosen from areas of both high and low airborne fluoride impact as identified from data on fluorides in forage (pasture grass and hay). High and low fluoride sites averaged 46 and 16 ppm pasture forage fluoride, respectively, during the 2 years in which soil data were collected.

Soils typically sampled in this study were of the Gilpin-Upshur complex. The Gilpin and Upshur series are deep, well-drained silt loams common on upper slopes and ridgetops (3, 18). By virtue of their close proximity and frequent intermixing on slopes, these two series are typically undifferentiated in mapping.

Six to eight 12-inch deep soil cores were extracted with a standard soil sampler in spring and early
fall of 1973 and 1974 within the same areas (approximately 50 x 150 feet) at each of the 14 locations. Cores were separated into surface (0-2 inch), middle (2-6 inch), and lower (6-12 inch) portions and stored in pint cardboard boxes. After air drying, the samples were gently ground and the material passing a 2-mm sieve was kept for fluoride analysis.

**Fluoride Analysis**

Plant material was oven-dried for 48 hours at 70°C (158°F) and ground to pass a 20-mesh screen in a Wiley mill. Aliquots of approximately 10 g of each sample were saved in snap-cap styrene vials for analysis.

Replicated 1 g aliquots of each sample were weighed to an accuracy of $10^{-4}$ g for fluoride determination. Since it was desired to express fluoride concentration on a standard dry weight basis (105°C, 221°F), the actual aliquot weights were increased by the average weight differential between material dried at 70°C (158°F) and 105°C (221°F). The average difference was of negligible significance for materials containing less than about 100 ppm fluoride.

The Gyoerkoes and Baretincic procedure (14) for plant fluoride analysis was compared with alkali-fusion (6, 12), direct double distillation (30, 46), and sulfuric acid extraction (22) techniques. The latter two methods yielded consistent but relatively lower results. Alkali-fusion results were quite comparable to the Gyoerkoes and Baretincic method. In each case, an ion specific electrode was used for fluoride detection. In general, Gyoerkoes and Baretincic’s procedure gave comparatively greater yields which were highly reproducible. For this reason, and because this procedure is efficient for routine analysis of large numbers of samples, it was adopted for use.

Extraction and determination procedures were basically those given by Gyoerkoes and Baretincic (14), with one exception; comparative tests revealed that removal of the ground plant material by filtration after perchloric acid extraction was unnecessary.

Fluoride concentrations were then determined from a standard curve based on a series of standards in the range of 1-1,000 ppm fluoride as NaF.

**Data Reduction**

A computer program was designed to accept the millivolt data, plot number, and sample material code for each plot. Fluoride concentrations were then calculated by the program and two sets of output were generated. A list was produced of computed individual and average fluoride concentrations (identified by plot number), material (pasture grass, hay, etc.), map coordinates of the plot, and property owner's name. A copy of the results generated for each plot was mailed to the cooperator.

The data were then used to produce computer-generated isopleth maps of fluoride concentration in

<table>
<thead>
<tr>
<th>TABLE 1.—Comparison of Fluoride Concentrations of Filtered and Unfiltered Extraction Solutions.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Material</strong></td>
</tr>
<tr>
<td>Hay†</td>
</tr>
<tr>
<td>Pasture Forage‡</td>
</tr>
<tr>
<td>Tree Leaves††</td>
</tr>
</tbody>
</table>

*Significant at $P = 0.05$.
†Mostly orchardgrass.
‡Mixed species, including orchardgrass, Kentucky bluegrass, and various broadleaved herbs.
††Black locust, shagbark hickory, and white oak.
vegetation for the study area. From these maps, estimates were made of total areas in which vegetation accumulated different levels of fluorides.

III. FLUORIDE SURVEY RESULTS

Fluoride Distribution in Hay

A qualitative estimate of the major forage species found in hay was made in 1972 (Fig. 5). Orchardgrass (*Dactylis glomerata* L.) was the most common component of both first and second hay crops. Red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) became slightly more prevalent in second crops.

Geographic fluoride distributions for first and second hay crops averaged over 3 years (1972 through 1974) are shown in Figures 6 and 7, respectively. Higher fluoride levels were found northeast and, to a lesser extent, southwest of the fluoride source. The northeastward distribution patterns are undoubtedly due to fluoride transport by prevailing winds. The authors believe the extension of relatively high fluoride levels south and southwest of the source results from down-valley transport, primarily during nocturnal periods when large-scale winds are calm. Under

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**Fig. 5.** Frequency of 1972 hay samples containing orchardgrass (OG), Kentucky bluegrass (BG), timothy (T), brome (BR), red clover (RC), and alfalfa (ALF).

**Fig. 6.** Isopleths of average fluoride concentration (ppm) in first cutting hay for 1972 through 1974.

**Fig. 7.** Isopleths of average fluoride concentration (ppm) in second cutting hay for 1972 through 1974.
FIG. 8.—Isopleths of fluoride concentration (ppm) in 1975 first cutting hay.

such conditions, which are most commonly associated with atmospheric inversions, down-slope and down-valley air flows tend to develop at night in major valleys (13). Reverse flows occur in daytime. Thus, airborne fluorides transported south of the source at night would be convected up valley slopes and dispersed onto adjacent ridges during the morning hours, especially on east-facing slopes where convective air flows develop first. Data on air movements in the Ohio River Valley are discussed in the section on pasture forage fluoride distribution (page 10).

A considerably wider fluoride distribution is apparent for second crop hay than for first crops (Figs. 6 and 7). This is also clearly reflected by the annual acreages in which different ranges of fluoride concentration prevailed (Table 2).

Randomized complete block analyses of variance were used to examine the fluoride distribution data for differences among years and between cuttings. The first analysis involved affected acreage as a variable, with the three blocks being areas within the 20-30, 30-40, and > 40 ppm fluoride isopleths. The second test was based on sample plots as blocks (N = 30). Only those plots which consistently had both first and second cutting hay were included, and fluoride concentration was the variable. The first analysis provides a general test of differences in the geographic distribution of forage fluoride over time and should reveal relatively large-scale changes, particularly those resulting from major shifts in fluoride emissions from the source. The second analysis would be more sensitive to small-scale variations not necessarily resulting in major shifts in areas within the different fluoride isopleth intervals. The authors would expect this analysis to reveal differences associated with factors having more subtle influences on forage fluoride accumulations (weather patterns, etc.), in addition to changes in fluoride emissions.

The first analysis indicated that differences in acreages between cuttings were consistent and significant ($F_{1, 14} = 24.19, P < 0.005$) over the first 3 years of observation (1972-1974). However, there was no such difference in fluoride distribution between cuttings in 1975 ($F_{1, 14} = 0.28, P > 0.10$). This similarity in hay fluoride distributions is illustrated in Figs. 8 and 9. Further, there was no difference in fluoride distribution between the 1975 first cutting hay and first cuttings in previous years ($F_{1, 14} = 1.01, P > 0.10$).

Results of the second analysis, in which plots were treated as blocks, indicated there were no differences among years in average hay fluoride levels ($F_{5, 205} = 1.00, P > 0.10$). A significant interaction revealed that fluoride levels in second crop hay in 1973 were greater than in other years. Reasons for
this difference are not clear, but could be related to meteorological factors or a temporary increase in fluoride emissions. This difference was not detected by the previous analysis of variance based on fluoride distribution (areas). Data in Table 2 indicate that a considerably larger area, and hence a greater number of plots, exceeded 40 ppm fluoride in second crop hay in 1973 than in other years. Differences in areas were not as great for the other ranges of fluoride concentration, which suggests that expansion of the area having more than 40 ppm fluoride in second crop hay was largely responsible for the significant increase in 1973.

Fluoride concentrations were significantly greater (P < 0.05) in second hay crops than in first crops each year except in 1975 when there was no difference between cuttings. This is the same result obtained by the previous analysis based on geographic fluoride distribution.

Several factors probably contribute to greater fluoride levels in second vs. first hay cuttings. The rate of standing crop biomass accumulation is greatest during the spring flush of growth, which would likely create a dilution effect on fluoride accumulating in leaf tissue. Further, the predominant forage species (i.e., orchardgrass, Kentucky bluegrass, timothy) flower during this period, resulting in a high proportion of stem tissue which accumulates little fluoride. In contrast, summer forage increases in leafiness and, consequently, in the proportion of fluoride-accumulating tissues. In addition, the typical trend of diminishing rainfall over the growing season may result in decreasing net loss of fluoride through foliar leaching, although rain splash of soil on plants has also been cited as a potential contributor to the fluoride burden of forage (28). Some aspects of soil contamination are considered further in the discussion of pasture fluoride.

Changes in fluoride emissions from the source may account for some of the significant differences in forage fluoride levels among years. Work was begun in 1973 to increase the effectiveness of the pollution control system at the source. Temporary disruption of the system during initial renovation procedures could account for the increase in 1973 second cutting hay, but this possibility could not be verified. Renovation of the emissions control system continued throughout the period of investigation but was less than half completed by 1975. There were no apparent trends in either average annual hay fluoride concentrations or in acreages affected by different hay fluoride levels (Table 2) to indicate that emissions were reduced in either 1973 or 1974. Actual reduction in fluoride emissions during this period, if any, is not known. Small-scale reductions in fluoride emissions are not likely to be reflected by the data because of random yearly variations in weather patterns, stage or date of forage harvest, and other uncontrolled variables influencing net fluoride accumulation in hay.

The significant reduction in fluoride levels in 1975 second cutting hay can be attributed to a 50% cutback in aluminum production at the source, beginning in January 1975. No fluctuations in emissions of a similar magnitude occurred during the preceding 3 years of observations. In 1975, the greatest

### TABLE 2.—Total Areas Within Which Hay Was Estimated to Contain Given Levels of Fluorides.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fluoride Concentration Range ppm</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>&gt;40</th>
<th>Total Thousand Acres*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>First Cutting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1972</td>
<td></td>
<td>41.4†</td>
<td>12.7</td>
<td>5.2</td>
<td>10.1</td>
<td>69.4†</td>
</tr>
<tr>
<td>1973</td>
<td></td>
<td>37.6†</td>
<td>8.6</td>
<td>4.0</td>
<td>11.8</td>
<td>62.0†</td>
</tr>
<tr>
<td>1974</td>
<td></td>
<td>39.4†</td>
<td>12.1</td>
<td>6.8</td>
<td>4.7</td>
<td>63.0†</td>
</tr>
<tr>
<td>1975</td>
<td></td>
<td>44.7†</td>
<td>6.8</td>
<td>5.2</td>
<td>6.0</td>
<td>62.7†</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td>40.8†</td>
<td>10.1</td>
<td>5.3</td>
<td>8.2</td>
<td>64.4†</td>
</tr>
<tr>
<td><strong>Second Cutting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1972</td>
<td></td>
<td>75.3†</td>
<td>21.4</td>
<td>10.2</td>
<td>11.7</td>
<td>118.6†</td>
</tr>
<tr>
<td>1973</td>
<td></td>
<td>54.1†</td>
<td>23.9</td>
<td>11.5</td>
<td>25.5</td>
<td>115.0†</td>
</tr>
<tr>
<td>1974</td>
<td></td>
<td>65.2†</td>
<td>18.3</td>
<td>9.7</td>
<td>19.8</td>
<td>113.0†</td>
</tr>
<tr>
<td>1975</td>
<td></td>
<td>50.6†</td>
<td>15.6</td>
<td>3.6</td>
<td>3.5</td>
<td>73.3†</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td>61.3†</td>
<td>19.8</td>
<td>8.8</td>
<td>15.1</td>
<td>105.0†</td>
</tr>
<tr>
<td><strong>Percent Reduction‡</strong></td>
<td></td>
<td>22.0†</td>
<td>26.4</td>
<td>65.7</td>
<td>81.6</td>
<td>36.5†</td>
</tr>
</tbody>
</table>

*Estimates are based on areas within different hay fluoride concentration zones on isopleth maps.
†Approximate due to lack of 10 ppm isopleth closure.
‡Percentage reduction for 1975 compared with the 1972-74 mean.
relative decrease in geographic distribution of second-cutting hay fluoride occurred within the 30-40 and > 40 ppm ranges (Table 2). Areas in which hay was affected to this extent were only 34% and 18% as large as areas of the preceding 3 years. These differences were much smaller for areas affected in the 10-20 and 20-30 ppm fluoride ranges. Thus, the 50% reduction in fluoride emissions effectively reduced the area in which second cutting hay contained 30 ppm or more fluoride. Contraction of the distribution pattern was greatest northeast of the source, but many chronically affected areas to the west and southwest remained at high (> 30 ppm) fluoride levels (Figs. 7 and 9).

A similar decrease in the distribution of fluoride in first hay crops was not evident in 1975, possibly because areas within the different fluoride ranges were small. Additional fluoride emissions reduction may be required to cause an appreciable decline in distribution of high-fluoride first cutting hay.

Fluoride Accumulation by Stored Hay

Effects of storage on the fluoride concentration of baled hay are shown in Table 3. Data for Plot 2 are incomplete and were excluded from the analyses. The average change in fluoride concentration over the 11-month measurement period indicated a net increase of 1.5 ppm in covered bales and an increase of 4.2 ppm in exposed bales. This is a mean net increase of 2.7 ppm in exposed compared to covered bales. A paired-t test indicated this difference to be non-significant (P = 0.05).

Although the number of observations was limited, it appears that exposed hay bales did not accumulate significant amounts of fluoride, even at relatively high fluoride locations (Plots 1, 4, and 5). Notably, covered hay also showed a net fluoride gain ranging from 0.8 to 2.0 ppm over the 11-month observation period. This suggests absorbance of gaseous fluoride forms, but should be examined more critically.

The apparent maximum accumulation rate of 0.5 ppm fluoride per month for exposed hay is not of practical importance to cattle diet, but should be considered when sampling hay in the mow. It is good sampling procedure to avoid undue sampling of top bales in the mow or from only the outer portions of loose hay.

Fluoride Distribution in Pasture Forage

Sampling Considerations: Unlike stored hay, which is more or less quantitatively consumed by cattle, pasture forages are selectively grazed in regard to both species and proportion of the forage utilized. Various sampling schemes have been used to select pasture samples representative of forage consumed (35). Although the main objective in this study was to monitor fluorides in pastures, it was also desirable that these levels be related to those in the diets of grazing cattle. In view of the variation in species and proportions of forages among pastures, all plants ordinarily considered palatable to cattle were harvested. However, since grazing height varies with pasture condition, cattle nutrition, and other factors that in turn affect fluoride ingestion by cattle (35), the effect of clipping height (ground level and 3 inches above ground) on forage fluoride concentration was tested during 1973.

Results of determinations made on paired samples of forage clipped at these heights indicated significantly higher fluoride concentrations in vegetation clipped at 3 inches (Table 4). The average difference for the season, however, amounted to only 2.6 ppm, which is of little biological significance; that is,

### TABLE 3.—Periodic Fluoride Concentration of Covered and Uncovered Hay Bales Stored in Barns at Different Locations Near the Fluoride Source.

<table>
<thead>
<tr>
<th>Plot No.</th>
<th>Location*</th>
<th>Treatment†</th>
<th>7/10/73 ppm</th>
<th>8/16/73 ppm</th>
<th>10/30/73 ppm</th>
<th>6/11/74 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 mi. N</td>
<td>C</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U</td>
<td>2.1</td>
<td>2.5</td>
<td>5.9</td>
<td>7.6</td>
</tr>
<tr>
<td>2</td>
<td>6.4 mi. NNE</td>
<td>C</td>
<td>21.4</td>
<td>21.6</td>
<td>17.5‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>U</td>
<td>19.7</td>
<td>19.5</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.4 mi. S</td>
<td>C</td>
<td>8.0</td>
<td>8.4</td>
<td>7.5</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U</td>
<td>9.9</td>
<td>10.5</td>
<td>10.0</td>
<td>11.8</td>
</tr>
<tr>
<td>4</td>
<td>2.5 mi. W</td>
<td>C</td>
<td>12.0</td>
<td>11.6</td>
<td>11.0</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U</td>
<td>10.4</td>
<td>10.7</td>
<td>10.0</td>
<td>15.9</td>
</tr>
<tr>
<td>5</td>
<td>3.0 mi. SW</td>
<td>C</td>
<td>6.8</td>
<td>6.6</td>
<td>8.1</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U</td>
<td>6.6</td>
<td>7.8</td>
<td>9.1</td>
<td>10.5</td>
</tr>
</tbody>
</table>

*Distance and direction from the fluoride source.
†Covered (C) or uncovered (U) bales.
‡Covered bales developed mold.
the average total dietary intake by grazing animals would not be greatly affected by this difference. However, the mean difference between clipping levels tended to increase later in the growing season (Table 4), as did pasture fluoride concentrations. This suggests that fluoride differences associated with clipping height may be greater and, hence, more important where forage fluorides are high. A second series of paired-t analyses was therefore computed, using only sample pairs in which at least one of the values was \( \geq 30 \) ppm fluoride. Results of these analyses indicate that different grazing intensities could alter actual dietary fluoride concentrations by 10 ppm or more in high fluoride areas (Table 4). Thus, cattle in such areas would ingest considerably more fluoride during periods of light grazing than by complete utilization of available forage. In conflict with these results, MacIntire et al. (28) found an inverse relationship between fluoride level and clipping height.

Fluoride levels for the two harvesting heights were linearly correlated \( (r = 0.88 \text{ to } 0.97 \text{ for the four sampling periods}) \), indicating that determinations based on one clipping height are a reasonable index of the fluoride concentration of the other. The effects, if any, of soil contamination on results of fluoride determinations on forage clipped at ground level were apparently obscured by greater fluoride accumulations in the upper, leafler portions of the standing crop. The potential effect of soil contamination on forage fluoride determinations was investigated. Dried and ground forage containing 7.3 ppm fluoride was amended with 0-200 mg soil/g plant material, and fluoride determinations were made by the usual (Gyoerkoes and Barettincic) method. This soil was collected from the surface 2 inches of a pasture within 1 mile of the fluoride source and contained 312 ppm total fluoride. A linear regression of ppm fluoride vs. percentage weight of soil contaminating the sample \( (r = 0.966) \) revealed that 10% contamination by soil increased detectable fluoride by only 7 ppm. Less than 5% soil in a sample would contribute no more than 3.5 ppm fluoride.

Other tests showed that the Gyoerkoes and Barettincic method measures about one-third of the total soil fluoride as determined by the single distillation procedure. However, this proportion increased logarithmically as soluble forms of fluoride were added. It appears that an inordinate amount of soil contamination in the study area would be required to significantly affect forage fluoride determined by either the Gyoerkoes and Barettincic method or by distillation. In some studies, soil contamination was believed to be an important source of fluoride in pasture forage (28, 35).

**Fluoride Distribution Patterns:** Isopleths of monthly and seasonal average fluoride distributions for 1973 and 1974 are shown in Figs. 10-14 and 15-20, respectively. Each pasture sampling period in 1974 was 1 to 2 weeks later than in 1973 (Table 5).²

There was a general tendency for pasture fluoride levels to increase over the growing season on many of the survey plots, thus expanding the distribution pattern (see Affected Acreage, page 16). This trend coincides with that for successive hay cuttings, and the discussion of factors influencing hay fluoride distribution also applies to pastures.

As was the case for hay, the influence of prevailing winds is clearly reflected by the northeastward extension of the pasture fluoride distribution from the source in virtually every monthly survey. The southerly extension of the fluoride distribution evident in many survey periods also was similar (e.g., Figs. 13 and 15). A hypothesis was set forth in the discussion of hay fluoride distribution that down-valley air flow in the absence of large-scale winds is responsible for this pattern. Nocturnal, down-valley air movement averaging 1 mph, a conservative rate compared with data reported by Geiger (13) for other large valleys,

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²For convenience in discussion, sampling periods will be referred to by month (May through August each year, plus October 1974).
FIG. 10 (Upper left).—Isopleths of fluoride concentration (ppm) in pasture forage collected in May 1973.

FIG. 11 (Above).—Isopleths of fluoride concentration (ppm) in pasture forage collected in June 1973.

FIG. 12 (Left).—Isopleths of fluoride concentration (ppm) in pasture forage collected in July 1973.
FIG. 13 (Above).—Isopleths of fluoride concentration (ppm) in pasture forage collected in August 1973.

FIG. 14 (Upper right).—Isopleths of 1973 average pasture forage fluoride concentration (ppm).

FIG. 15 (Right).—Isopleths of fluoride concentration (ppm) in pasture forage collected in June 1974.
FIG. 16.—Isopleths of fluoride concentration (ppm) in pasture forage collected in July 1974.

FIG. 17.—Isopleths of fluoride concentration (ppm) in pasture forage collected in August 1974.

FIG. 18.—Isopleths of fluoride concentration (ppm) in pasture forage collected in September 1974.

FIG. 19.—Isopleths of fluoride concentration (ppm) in pasture forage collected in October 1974.
TABLE 5.—Acreages Within Which Pasture Forage Was Estimated to Contain Given Levels of Fluoride.

<table>
<thead>
<tr>
<th>Fluoride Concentration Range ppm</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>≥40</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thousand Acres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1973</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 15-25</td>
<td>51.4</td>
<td>5.1</td>
<td>2.4</td>
<td>5.7</td>
<td>54.6</td>
</tr>
<tr>
<td>June 18-27</td>
<td>65.4</td>
<td>10.6</td>
<td>10.5</td>
<td>8.3</td>
<td>94.8</td>
</tr>
<tr>
<td>July 23-August 1</td>
<td>59.5</td>
<td>19.6</td>
<td>4.5</td>
<td>9.3</td>
<td>92.9</td>
</tr>
<tr>
<td>August 21-30</td>
<td>88.2</td>
<td>22.9</td>
<td>12.0</td>
<td>15.0</td>
<td>138.1</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>61.1</td>
<td>14.0</td>
<td>4.0</td>
<td>8.1</td>
<td>87.2</td>
</tr>
<tr>
<td><strong>1974</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 3-11</td>
<td>85.0</td>
<td>10.1</td>
<td>10.3</td>
<td>8.8</td>
<td>114.2</td>
</tr>
<tr>
<td>July 1-10</td>
<td>50.2</td>
<td>17.1</td>
<td>8.0</td>
<td>8.6</td>
<td>83.9</td>
</tr>
<tr>
<td>July 29-August 1</td>
<td>55.0</td>
<td>21.3</td>
<td>7.3</td>
<td>16.4</td>
<td>80.9</td>
</tr>
<tr>
<td>September 3-13</td>
<td>99.1</td>
<td>12.0</td>
<td>3.5</td>
<td>3.7</td>
<td>58.3</td>
</tr>
<tr>
<td>October 16-23</td>
<td>89.1</td>
<td>28.3</td>
<td>15.3</td>
<td>20.8</td>
<td>153.5</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>53.3</td>
<td>19.6</td>
<td>6.4</td>
<td>11.0</td>
<td>90.3</td>
</tr>
</tbody>
</table>

*Based on average isopleth maps, Figs. 14 and 20.

could transport gaseous pollutants more than 6 miles south of the source. This distance coincides with the southward extension of the 30 ppm fluoride isopleth in Figs. 7, 13, 15, and 17, indicating that such a transport mechanism is plausible.

The pasture surveys also showed that the southerly fluoride distribution is small or absent in some months (e.g., Figs. 10 and 18), presumably in response to fewer periods of nocturnal calm when down-valley air flows could develop.

A unique variation in fluoride distribution for pasture forage is the frequent occurrence of elevated fluoride levels centered on Case and Boltz Ridges in Ohio, about 6 miles north of the source and immediately north of Clarington (see especially Figs. 11, 13, 15, and 19). These ridges create a northeastward bend in the river valley at the end of a 4-mile fetch leading directly from the source. Several secondary stream valleys in the ridges are so situated as to funnel up-valley winds onto the ridgetops. It is hypothesized that air could be channeled up-valley (north) from the source in response to prevailing winds, thus transporting airborne fluoride onto these ridges. Wind data collected at a pasture forage sample plot on Case Ridge (Fig. 21) shows that winds > 1 mph were mostly southerly (up-valley), whereas winds during periods of near-calm (< 1 mph) were usually easterly (up-slope). Similar wind data for 1973 showed that airflows paralleled the river valley about 65% of the time. Thus, the possibility exists for atmospheric transport of fluorides up the Ohio River Valley and subsequently onto the Case-Boltz Ridge upland area. Such anomalous patterns of air pollution can be expected in regions of complex, irregular topography and the present example illustrates the hazard in extrapolating pollutant distribu-

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*Data provided by Ormet Corporation.*
FIG. 21.—Relative frequency of wind direction during periods less than (—) and greater than (-----) 1 mph in a pasture on Case Ridge, Ohio. Data were recorded between July 24 and Sept. 19, 1974. Radial distance represents 0-30%.

FIG. 22.—Interaction of monthly pasture forage fluoride distribution between years.

Affected Acreage: Acreages within which pasture forage contained various fluoride concentration ranges were determined from the isopleth maps and are presented in Table 5. Two variables were used in separate analyses of variance to examine the pasture forage data for yearly and monthly differences in fluoride distribution. These analyses followed the procedures outlined in the discussion of hay fluoride distribution. The first analysis involved acreage as a variable and areas within the 20-30, 30-40, and > 40 ppm fluoride isopleths as blocks (N = 3). The second analysis was based on sampling locations as blocks (N = 153) and ppm forage fluoride as the variable.

Analysis of variance on acreages indicated there was no difference in fluoride isopleth areas between years (F1, 14 = 0.01, P > 0.10), but there were significant differences among months (F5, 14 = 3.55, P = 0.05). An LSD. 05 comparison of the average May fluoride area with that of other months substantiated the earlier observation that pasture fluoride was generally more widely distributed in early spring than in summer (there was no difference between the May and June distributions). However, a significant interaction (P = 0.05) revealed that August fluoride distributions differed between years in relation to the trends for other months (Fig. 22). The opposing trend for August cannot be explained by information at hand; however, it seems reasonable to expect occasional fluctuations of this magnitude as a result of unusual weather conditions or brief changes in fluoride emissions.

The second analysis of variance, with forage fluoride concentration on plots as the variable, indicated there were no differences in mean pasture fluoride levels between years (F1, 1064 = 0.91, P > 0.10) or months (F3, 1064 = 2.04, P = 0.11). However, orthogonal means comparisons showed that spring (May plus June) fluoride levels were lower than summer (July plus August) levels (P = 0.005), which agrees with the first analysis. The two analyses were also in agreement regarding a reversal in the yearly trend for the August sample. Unlike the first analysis, fluoride levels for May and June were higher (P = 0.05) in 1974 than in 1973. Possibly some of the difference in monthly fluoride levels between years was due to a later sampling schedule in 1974.

The two analyses indicate there were no differences between years in mean pasture fluoride concentrations. This was true because differences appearing among sampling periods both within and between years proved to be compensating.

Summary: Of the two variables used in analyses, isopleth area appears most useful in relation to monitoring geographic fluoride distribution. It is less sensitive to ordinary seasonal fluctuations associated with meteorological events or small, short-term changes in fluoride emissions and an area measure
relates more directly to the practical concern of affected acreage. Therefore, it is recommended that isopleth areas be used in the analysis of variance for studying the significance of varying fluoride emissions on geographical forage fluoride distribution.

There was no detectable change in the annual geographic distribution of fluoride in either hay or pasture forages during the first 3 years of the survey (1972-74). A 50% decrease in aluminum production in 1975 apparently caused a significant reduction in second cutting hay fluoride, but no change in the distribution for first cutting hay. The reduction for second hay crops was greatest for areas affected by high fluoride levels. The area of > 40 ppm fluoride in second cutting hay declined about 82% compared with a 66% decrease in the area associated with the 30-40 ppm level.

Fluoride concentrations generally increased in successive hay cuttings and pasture samples within a given growing season, but average seasonal fluoride levels did not generally differ among years prior to the 1975 reduction in emissions. Occasional exceptions to this trend for both hay and pasture forage could not be explained on the basis of available information, and point out the need for identification and understanding of factors other than fluoride emissions that are responsible for inherent variations in the system.

Information collected between 1972-74 appears reasonably representative of the seasonal variation to be expected in the vicinity of the fluoride source under pre-abatement conditions, and should provide a useful data base for comparison with the post-abatement situation. This is partially substantiated by the observed reduction in second cutting hay fluoride distribution in 1975 after a 50% decrease in fluoride emissions.

**Distribution of Soil Fluorides**

Data in Table 6 indicate the amount of variation encountered among locations and sampling periods. Average fluoride concentrations did not vary significantly with soil depth, but were significantly higher in the spring of 1973 than subsequent samples (P = 0.01). This can be seen in Fig. 23, which also illustrates the variation among sampling periods. Aside from higher fluoride levels in the spring of 1973, no clear trends could be identified for any portions of the soil profile. Similar annual fluctuations were also evident in Israel’s data (21).

Analysis of variance also revealed a significant block effect (P = 0.005), indicating that total soil fluoride differed among locations. Examining fluoride levels averaged over the three sampling depths, seasonal trends appeared to differ considerably between the high and low atmospheric fluoride sites (Fig. 24), but the spring of 1973 results were again an exception. It should be noted that none of the

**TABLE 6.—Average Total Soil Fluoride Levels for Locations Subject to Relatively High and Low Airborne Fluorides.**

<table>
<thead>
<tr>
<th>Soil Depth</th>
<th>1973 High* ppm Total Fluoride</th>
<th>1973 Low† ppm Total Fluoride</th>
<th>1974 High* ppm Total Fluoride</th>
<th>1974 Low† ppm Total Fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>inches</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>359 ± 92</td>
<td>342 ± 58</td>
<td>364 ± 94</td>
<td>316 ± 61</td>
</tr>
<tr>
<td>Fall</td>
<td>371 ± 88</td>
<td>311 ± 56</td>
<td>353 ± 96</td>
<td>314 ± 55</td>
</tr>
<tr>
<td>2-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>343 ± 88</td>
<td>357 ± 80</td>
<td>249 ± 97</td>
<td>321 ± 63</td>
</tr>
<tr>
<td>Fall</td>
<td>354 ± 96</td>
<td>304 ± 48</td>
<td>344 ± 97</td>
<td>320 ± 64</td>
</tr>
<tr>
<td>6-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>344 ± 97</td>
<td>379 ± 92</td>
<td>347 ± 104</td>
<td>315 ± 61</td>
</tr>
<tr>
<td>Fall</td>
<td>358 ± 105</td>
<td>315 ± 53</td>
<td>341 ± 110</td>
<td>329 ± 87</td>
</tr>
</tbody>
</table>

*Eight observations per mean.  †Six observations per mean.
seasonal differences shown in Figure 24 were statistically significant.

The high airborne fluoride sites also exhibited comparatively greater fluoride concentrations at various depths within the soil profile (Fig. 25). However, the difference was significant only at the 0-2 inch depth (P = 0.05). It is also worth noting that the profile for soils from low atmospheric fluoride areas exhibited an increase in soil fluoride with depth. This agrees with the typical situation reported by Robinson and Edgington (40), but a comparatively inverted fluoride profile is evident in soils from high airborne fluoride sites. This strongly implies an atmospheric source impact on fluoride distribution within the soil profile in areas a few miles downwind from the alumina reduction plant.

Variation in soil parent materials can also impart large inherent differences in fluoride content to soils within a relatively small geographical area, as can amount and type of fertilizer applications. For example, Israel's (21) prepollution results might be an example of either or both of these factors. Robinson and Edgington (40) found identical soils treated with different fertilizers varied considerably in fluoride content.

The authors have no information on the fertilizer history of the study sites; however, comparisons of soil types are available from published soil survey data for Monroe County, Ohio, (18) and Marshall County, West Virginia (3). Soils found on 10 of the 14 sample sites were of the Gilpin-Upshur complex, and one each were Gilpin-Westmoreland and Gurnsey-Upshur. Each of two additional sites were on Vandalia-Sees and Lindside silt loams. The four sites not located on Gilpin-Upshur soils were divided between the high and low airborne fluoride areas. Thus, there is no reason to suspect that inherent soil differences are an important source of variation in fluoride concentration between these two areas.

There is a definite relationship between distance from the source and soil fluoride concentration. The amount of fluoride in soil from all three depths sampled showed a decreasing trend with distance from the source. This trend, as determined from isopleth maps of soil fluoride distribution, was very similar among the different profile depths. The northeast-southwest fluoride profile for the 0 to 2-inch soil layer is typical (Fig. 26). High fluoride levels extended farthest northeast of the source, and a secondary extension of the pattern occurred toward the southwest. This correlates well with the patterns of fluoride distribution shown for forage.

However, it must be remembered that most of the soil data points are confined to these two areas. The multiple peaks seen northeast of the source (Fig. 26) probably resulted from sparseness of data in certain areas and should not be considered significant. Generalizing from the profile, it appears that soil fluoride levels decreased rapidly southwest of the
source, but remained higher at a greater distance to the northeast. Equal levels (280 ppm) were observed about 10 miles from the source in both directions. Similarly, fluoride levels at the 2 to 6 and 6 to 12-inch depths decreased bi-directionally from the source, declining to about 280-300 ppm at about 10 miles.

Some data were obtained from locations essentially free from airborne fluorides, and these provide an indication of inherent (normal) total soil fluoride concentrations for the study area. Two such locations contained 229 and 284 ppm fluoride in the surface 2 inches of soil. Previously, 322 ppm total fluoride were found in the surface 6 inches of soil from a Monroe County vineyard relatively free from airborne fluorides. Assuming these values approximate background fluoride levels for soils in the study area, the influence of the source on soil fluoride content does not appear to extend more than 10 miles in any direction. Lack of additional data for sites more remote from the source, particularly to the northeast, casts some uncertainty on this.

Field investigations have generally failed to show a significant correlation between fluoride levels in soil and vegetation. Researchers generally agree that uptake of soil fluorides by most plants, including forage crops, is minimal and would therefore contribute little to total fluoride accumulated by plants in a contaminated atmosphere (21, 27). In this study, essentially no correlation (r = 0.14) was found between average soil surface and pasture fluoride levels.

In summary, the fluoride source has apparently affected the distribution of soil fluorides by: 1) increasing the general fluoride content of nearby soils, and 2) causing the fluoride distribution within the profile of affected soils to decrease rather than increase with depth. The data indicate a possible fluoride gain of as much as 180 ppm in surface soils 5 miles northeast of the source (the nearest agricultural land in this direction). In contrast, heavy fertilization over a 20 to 40-year period has been shown to increase fluoride levels of surface soils by nearly half this amount (40). Other investigations indicated that relatively large soil fluoride amendments are ordinarily necessary to significantly increase plant uptake or affect plant growth (15, 27, 39, 42). For example, in a study similar to this one, Israel (21) estimated that each 120 ppm increment in soil fluoride resulted in a 1 ppm gain in forage fluoride. It should be pointed out that the linear regression on which Israel's estimate was based was derived from relatively low forage fluoride levels compared to the present study, so his estimate does not necessarily apply here. In general, it appears that the observed increase in soil fluoride will not have a significant im-

FIG. 26.—Fluoride profile for the 0-2 inch soil depth.
maple to absorb fluorides from soil and to study the effects of different soil fluoride levels on their dry matter production.

Previous Work

Indigenous vegetation has been found useful as an indicator of the geographic extent and relative degree of fluoride air pollution (1, 34, 36). Accumulation of fluorides in above-ground portions of most plants is generally reported to be less than 10 ppm where atmospheric sources are negligible (30). Tea, spinach, elderberry, camellia, and perhaps lettuce apparently are capable of accumulating significant amounts of fluoride from soil (25, 31), but there is little information available on the ability of plants, especially natural vegetation, to absorb fluoride from soil. Neither has there been a thorough evaluation of indigenous species as bioestimators of atmospheric fluorides.

Significant increases in uptake have been induced in certain grasses and legumes by adding various amounts of fluorides in soluble forms to soils inherently low in calcium and phosphorus (23). However, lime or superphosphate amendments were shown partly effective in counteracting the increase in uptake due to fluoride addition (23, 25, 31). This presumably occurs through the immobilization of fluoride in insoluble forms less available to plants (31).

Although U. S. soils range in total fluoride concentration from almost none to more than 7,000 ppm (30), plant uptake may be more closely related to the water soluble fraction (4). This portion is usually relatively small due to a variety of factors, particularly the formation of alumino or phosphatic complexes and pH (25). Many questions remain concerning fluoride uptake by plants in relation to soil physical and chemical properties.

Methods

Commercially grown seedlings of shagbark hickory, black locust, and red maple (averaging 4.5, 12.8, and 13.4 inches in stem height, respectively) were bare-root transplanted in 2-gallon plastic pots (two seedlings per pot). Each pot contained 8 lb (dry weight) of equal parts of Wooster silt loam and muck soil which had been amended with 0, 10, 100, or 1,000 ppm fluoride (dry weight basis) as NaF in distilled water. After fluoride addition, these soils had total fluoride levels of amendment, but shagbark hickory leaf concentrations surpassed those of the roots (Fig. 27). Significant interactions (P = 0.05) showed that roots of red maple contained higher concentrations than those of black locust when grown in soil amended with 974 ppm fluoride. In general, root concentrations were quite high for all three species at this high level.

A study of soil fluoride uptake in tomato indicated that accumulation by roots was typically greater than that by leaves (31). An investigation by Benedict et al. (2) showed that relative fluoride accumulation by various plant organs often differed among species. The authors' findings substantiate these results.

Shagbark hickory accumulated significantly higher fluoride concentrations in both leaves and stems than the other species tested. A species-soil fluoride interaction revealed that, in shagbark hickory stems, significantly more fluoride was accumulated at all levels of amendment. There were no significant differences in either leaf or stem fluoride concentrations between black locust and red maple.

Romney et al. (41) reported relatively high stem fluoride concentrations in bean (34 ppm), tomato (19 ppm), and alfalfa (8 ppm) in a study of uptake from nutrient media. Stem concentrations of these plants were consistently lower than those of leaves.

The significantly greater stem fluoride concentration of shagbark hickory, compared to black locust
and red maple, may have resulted from accumulation in its relatively large buds. To investigate this, the authors collected current-year twigs of shagbark hickory in September 1973 at four locations near the fluoride source and determined fluoride concentrations of buds, twigs, and leaves (with rachis). Linear correlation analysis revealed that fluoride levels in buds, twigs, and leaves were all strongly related ($r \geq 0.975$, $n = 4$). Fluoride levels ranged from 20 to 44 ppm in buds, 4 to 12 ppm in twigs, and 171 to 698 ppm in leaves. Linear regression showed that fluoride levels in buds increased 3 ppm for each 1 ppm increase in twigs. Thus, it appears that bud fluoride content could have contributed significantly to the total stem concentration of shagbark hickory in the greenhouse study. Buds of red maple and black locust may also accumulate more fluoride than stems; however, the bud/stem biomass ratio is much smaller for these species, so bud fluoride would probably contribute little to total fluoride concentration. Romney et al. (41) also found comparatively high fluoride accumulations in stem apices of bean and tomato.

Fluoride accumulation by leaves is of particular interest in regard to the potential of these trees as indicators of atmospheric fluorides. At all levels of soil fluoride addition, red maple accumulated less foliar fluoride than the other two species tested (Fig. 27). Black locust foliage performed similarly up to the 106 ppm amendment and remained below 10 ppm in leaves up to the 974 ppm amendment (Fig. 27). It is concluded from these data that black locust and red maple leaves are not significant accumulators of soil fluoride. Also, foliar fluorides from soil uptake appear less in red maple than in black locust.

Shagbark hickory leaves appeared to be a definite sink for fluoride absorbed from soil (Fig. 27). This could interject a confounding effect in interpreting results of a field survey to monitor airborne fluorides. Shagbark hickory is not recommended as a satisfactory bioestimator. The apparent ability of shagbark hickory leaves to accumulate fluoride at low soil amendments (13 ppm) suggests its potential value in assessing soil fluoride in the absence of atmospheric sources.

### TABLE 7.—Mean Biomass of Tree Seedlings Grown in Fluoride Amended Soil.

<table>
<thead>
<tr>
<th>Material</th>
<th>Soil Fluoride Added, ppm</th>
<th>0</th>
<th>13</th>
<th>106</th>
<th>974</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grams Dry Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shagbark Hickory</td>
<td></td>
<td>1.5</td>
<td>1.5</td>
<td>1.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Black Locust</td>
<td></td>
<td>21.1</td>
<td>19.5</td>
<td>19.2</td>
<td>18.2</td>
</tr>
<tr>
<td>Red Maple</td>
<td></td>
<td>17.2</td>
<td>20.0</td>
<td>18.1</td>
<td>15.2</td>
</tr>
<tr>
<td>Stems</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shagbark Hickory</td>
<td></td>
<td>2.3</td>
<td>1.7</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Black Locust</td>
<td></td>
<td>15.7</td>
<td>14.6</td>
<td>14.5</td>
<td>15.5</td>
</tr>
<tr>
<td>Red Maple</td>
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<td>18.8</td>
<td>19.4</td>
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<td>13.7</td>
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<tr>
<td>Roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shagbark Hickory</td>
<td></td>
<td>7.9</td>
<td>9.1</td>
<td>8.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Black Locust</td>
<td></td>
<td>9.8</td>
<td>8.0</td>
<td>8.3</td>
<td>7.7</td>
</tr>
<tr>
<td>Red Maple</td>
<td></td>
<td>12.4</td>
<td>12.9</td>
<td>10.3</td>
<td>9.7</td>
</tr>
</tbody>
</table>

**FIG. 27.**—Fluoride accumulated by organs of shagbark hickory (—), black locust (----), and red maple (.....) in relation to soil fluoride amendment.
Dry weight determinations of plant organs for each species suggested that, in general, soil fluoride addition may have caused a slight reduction in biomass (Table 7); however, none of the differences was statistically significant. The 974 ppm amendment appeared to have a detrimental effect on biomass production of shagbark hickory roots, stems, and leaves. A similar tendency can be seen for black locust and red maple; however, a more sensitive test is needed to determine if these small differences represent a real decrease in biomass.

V. USE OF A BIOESTIMATOR FOR PREDICTING FORAGE FLUORIDE DISTRIBUTIONS

Plants have been frequently cited as useful bioindicators of various air pollutants, including fluorides (1, 36). Experienced observers use indicator plants to make qualitative estimates of the severity of pollutant exposure and geographic area affected. Few quantitative relationships have been developed to estimate pollutant concentration or distribution based on responses of indicator organisms; however, semi-quantitative methods have been designed for using lichens as indicators of air pollutant concentrations (17).

As part of the program for monitoring environmental fluorides, the authors tested the efficacy of using indigenous plant foliage as a bioestimator of the geographic distribution of forage fluoride. The term bioestimator is used to distinguish the authors' quantitative approach from the qualitative results usually derived from bioindicators. A major advantage of using a bioestimator in fluoride monitoring is the reduction in time and expense as a result of fewer collections of samples and laboratory analyses. The following discussion describes the geographical distribution of fluoride in leaves of black locust and use of this species as a bioestimator of forage fluoride.

Distribution of Fluoride in Black Locust Leaves

Black locust leaves consistently contained much more fluoride than hay or pasture samples at the same location (Fig. 28). This was to be expected, since most of the tree leaves were present and accumulating fluorides over a greater portion of the growing season, compared to forages.

The geographic distribution of black locust leaf fluoride levels for 1973 (Fig. 28) typifies patterns encountered in subsequent years. Although the general pattern follows that for forages, concentrations below 10 ppm in black locust foliage were not found within the survey area. Leaves collected on the OARDC campus at Wooster in September 1973 contained 12 ppm fluoride and seedlings grown in charcoal-filtered air in a greenhouse accumulated only 2 ppm after 3 months. It appears from these limited data that normal leaf fluoride levels in black locust in the field may be above 10 ppm by the end of the growing season.

Black locust leaves apparently tolerate high levels of fluoride. Definite leaf injury symptoms did not consistently appear at concentrations of 200 or 300 ppm fluoride; levels of 874 and 769 ppm were the respective maxima for the 1973 and 1974 surveys. Despite leaf fluoride burdens of this magnitude, sample trees displayed no signs of poor vigor beyond symptoms of foliar fluoride injury. Leaf symptoms appeared as marginal and tip necroses.

Results of the authors' surveys show that fluoride levels in black locust foliage can be used to determine the relative extent of the airborne fluoride distribution. However, these fluoride concentrations are not equivalent to those in forages.

Predictive Model

The authors' specific objective was to estimate the geographic distribution of fluorides in first and second hay crops and the mean seasonal fluoride distribution in pasture forage using fluoride determinations on a single collection of black locust leaves. Net fluoride accumulation in black locust leaves is undoubtedly influenced by many of the same environmental factors affecting hay and pasture fluoride.
One approach would be to identify and measure these factors and include them in the model, but the resulting model and the data required would be too complex for the authors' purposes. The authors therefore attempted to directly relate fluoride levels in forages to levels of fluoride in black locust leaves.

Based on this objective, a sequence of criteria for accepting or rejecting potential models was developed. The criteria were: 1) the model must explain at least 60% of the total variation in forage fluoride concentration \( r^2 \geq 0.60 \), 2) the model must not exhibit significant lack of fit \( P = 0.05 \), 3) parameters for the model must exhibit stability between years within the study area, and 4) the model must satisfactorily estimate areas subject to different ranges of forage fluoride concentration. The fourth criterion is necessarily subjective and ultimately depends upon the standards of the investigator and specific application of the model.

The authors' approach was to begin with the simple linear regression model:

\[
Y = a + bX \tag{1}
\]

in which \( Y = \text{ppm fluoride in either pasture forage or hay at a given location and } X = \text{ppm fluoride in leaves of black locust at the same location. Examination of scatter diagrams of the data indicated possible exponential relationships in some cases, so the following logarithmic transformation was also routinely tested:}

\[
\text{LOG}_{10} Y = a + bX \tag{2}
\]

Specific regressions were developed on the basis of 1973 survey data relating fluoride concentrations in first \( Y_1 \) and second \( Y_2 \) hay cuttings and mean annual pasture forage \( Y_a \) to fluoride in black locust leaves. Results of these analyses are summarized in Table 8.

Based on the first criterion for model acceptance \( r^2 \geq 0.60 \), neither model was satisfactory for first cutting hay, but models 1 and 2 explained an adequate proportion of the variation in second cutting hay and pasture forage fluoride, respectively. Tests of significance of the difference between \( r \) values for models 1 and 2 also indicated that better correlations were obtained with the linear model for second cutting hay \( (P = 0.03) \) and the exponential model for pasture forage \( (P = 0.10) \). No additional models were tested for first cutting hay, since examination of the scatter diagram indicated that a satisfactory relationship probably did not exist.

Analysis of variance tests for lack of fit \( (P) \) indicated that only the linear model \( (1) \) adequately described the relationship for second cutting hay and only the exponential model \( (2) \) was adequate in the case of pasture forage. Since these respective models explained at least 60% of the total variation in forage fluoride, they were examined further.

The 1974 data were used to test the 1973 models, and the tests of hypotheses of no difference between slope regression coefficients were not rejected at \( P = 0.10 \) \( (43) \). The 1974 \( r^2 \) values for second cutting hay and mean pasture forage regressions were 0.614 and 0.694, respectively. Compared with \( r^2 \) values for 1973 \( \text{(Table 8)} \), the fit of the models in 1974 was not significantly different \( (P = 0.05) \). In neither case was there a significant \( (P = 0.05) \) lack of fit with the 1974 data, nor were there any significant \( (P = 0.05) \) between-year differences in the estimates of slope \( (b) \). From this, the authors concluded that the proposed general models are applicable to results obtained in 1973 and 1974, and that the parameters exhibited stability \( \text{(criterion 3)} \).

Next, a comparison was made of the 1974 geographic distribution of fluorides in forage crops as estimated by the 1973 regressions and as determined from forage sampling. Figures 29 and 30 compare results for second cutting hay and mean pasture, respectively.

The regression estimate of the mean fluoride distribution pattern for pasture forage agreed reasonably well with that based on monthly samples \( \text{(Fig. 30)} \). However, the pattern predicted by regression for second cutting hay is displaced somewhat southwestward of that derived from hay sampling \( \text{(Fig. 29)} \). A similar shift in monthly pasture fluoride patterns was also seen in the late summer isopleths \( \text{(July and}

---

TABLE 8.—Trial Regression Models Fitted to 1973 Data.

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>( r^2 )</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Cutting Hay</td>
<td>46</td>
<td>0.3548†</td>
<td>( Y_1 = 3.183 + 0.82X )</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>0.3990†</td>
<td>( \text{LOG}_{10} Y_1 = 0.71907 + 0.00175X )</td>
</tr>
<tr>
<td>Second Cutting Hay</td>
<td>29</td>
<td>0.8097†</td>
<td>( Y_2 = -2.810 + 0.25X )</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>0.5374†</td>
<td>( \text{LOG}_{10} Y_2 = 0.99411 + 0.00214X )</td>
</tr>
<tr>
<td>Mean Pasture Forage</td>
<td>149</td>
<td>0.5321†</td>
<td>( Y_a = 0.511 + 0.117X )</td>
</tr>
<tr>
<td></td>
<td>149</td>
<td>0.6328†</td>
<td>( \text{LOG}_{10} Y_a = 0.69303 + 0.00218X )</td>
</tr>
</tbody>
</table>

*Dependent variable [ppm fluoride in forage].
†Value of \( r \) significant at \( P = 0.01 \).
Possibly fluoride accumulation by leaves of black locust was strongly affected by this change in pattern, but most second cutting hay had been harvested before or during the early part of this period and was therefore less influenced.

Determination of areas in which forages were subject to different ranges in fluoride levels was made from the isopleth maps. The results (Table 9) provide another indication of the reliability of the regression model for predicting the geographic extent of forage fluoride contamination. Areas obtained from the model-generated isopleths deviated on the order of 20% to 50% from those based on forage sampling in 1974. However, estimates of the total areas in which forage crops were influenced by

![FIG. 29.—Isopleths of 1974 second cutting hay fluoride concentration (ppm) based on hay sampling (-----) and estimated by regression (-----).](image)

![FIG. 30.—Isopleths of average 1974 pasture forage fluoride concentration (ppm) based on four monthly pasture samples (-----) and estimated by regression (-----).](image)

<table>
<thead>
<tr>
<th>TABLE 9.—Geographic Areas Within Which Forages Contained Different Fluoride Levels. Estimated Areas Were Derived from Isopleth Maps Based on Regression Values.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Forage</th>
<th>ppm Fluoride</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>&gt;40</th>
<th>&gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thousand Acres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second Cutting Hay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1974</td>
<td>65.2†</td>
<td>18.3</td>
<td>9.7</td>
<td>19.8</td>
<td>113.0†</td>
<td></td>
</tr>
<tr>
<td>1974 (estimated)</td>
<td>46.8†</td>
<td>14.1</td>
<td>12.1</td>
<td>24.5</td>
<td>97.5†</td>
<td></td>
</tr>
<tr>
<td>1975</td>
<td>40.0</td>
<td>22.7</td>
<td>7.2</td>
<td>3.4</td>
<td>73.3</td>
<td></td>
</tr>
<tr>
<td>1975 (estimated)</td>
<td>30.6</td>
<td>14.8</td>
<td>8.9</td>
<td>17.0</td>
<td>71.3</td>
<td></td>
</tr>
<tr>
<td>Average Pasture Forage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1974*</td>
<td>27.2</td>
<td>9.4</td>
<td>2.5</td>
<td>5.9</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>1974 (estimated)</td>
<td>32.5</td>
<td>10.0</td>
<td>5.2</td>
<td>3.1</td>
<td>50.8</td>
<td></td>
</tr>
<tr>
<td>1975 (estimated)</td>
<td>18.5</td>
<td>5.4</td>
<td>1.2</td>
<td>2.2</td>
<td>27.3</td>
<td></td>
</tr>
</tbody>
</table>

*Seasonal average based on four monthly samples.
†Approximate due to lack of 10 ppm isopleth closure.
atmospheric fluoride (fluoride levels > 10 ppm) were slightly better.

Forage fluoride levels in excess of 40 ppm are of special concern because of the potential for causing fluorosis in cattle (37). Regression estimates of these areas for hay and pasture forage in 1974 were quite good, especially considering the small total areas involved. There is also evidence that perhaps 20 to 30 ppm total dietary fluoride may induce symptoms of dental fluorosis in cattle (29). Areas affected by 30 ppm or more hay and pasture fluoride were predicted for 1974 by the model to within +24% and −17%, respectively. For 20 ppm or more fluoride, these respective percentages were +6 and −37.

Results for 1975

The fluoride survey was to have been terminated in 1975; however, fluoride distributions in that year were of great interest because of the 50% curtailment in aluminum production at the source. Therefore, the survey was continued through 1975, but was necessarily limited to a single sampling of stored hay and leaves of black locust in September. Regression models based on the combined data of 1973 and 1974 were then used to estimate fluoride levels in second cutting hay and pasture forage at each sample location for 1975 and isopleth maps of fluoride distribution were generated from the predicted values (Figs. 31 and 32).

Comparisons were made of the fluoride distributions for second cutting hay based on hay sampling (Fig. 9) and predicted values (Fig. 31) as a further test of the reliability of the regression model. The model predicted a considerably greater distribution of high (> 30 ppm) fluoride than was estimated from hay samples, whereas the pattern and total area influenced by airborne fluoride (> 10 ppm) was predicted quite accurately (Table 9, Figs. 9 and 31). The highly irregular pattern southwest of the source (Fig. 9) is partly the result of a paucity of data from this area. It is suspected that reduced hay sampling in this and other locales near the source in 1975 contributed to the discrepancy in the 30-40 ppm isopleths between actual and predicted distributions.

On the other hand, comparison of the slope of regression (b) between the model derived from 1973-74 data and that based on 1975 data revealed a lack of parameter stability (P = 0.05). Further, the
slope of the 1975 regression ($b = 0.095$) was not different from that for 1973 first cutting hay ($b = 0.082$). The $r$ values were also similar and low (0.60 and 0.53 for the 1973 first cutting and 1975 second cutting hay, respectively). This similarity of regressions further substantiates the results of analysis of variance (Section III) which indicated no difference in fluoride distribution between 1975 second cutting hay and first hay crops in all sample years.

Thus, as fluoride distributions decrease (or perhaps increase), the parameters vary and the regression model becomes statistically unsatisfactory. However, the model continued to adequately predict the area of impact of the fluoride source and may have predicted high fluoride areas better than the scarce hay fluoride data indicated.

The decrease in the slope of the regression with decreasing fluoride distribution suggests that net fluoride accumulation varies independently in leaves of black locust and the forage species in hay as atmospheric fluoride regimes change. Specifically, under low regimes other factors having differential influences on the dose responses of vegetation may come into play. These factors may be either environmental or physiological in nature, or both. They are likely the same factors which cause variation in the coefficient of fluoride accumulation ($K$) in the dose-rate relation $\Delta F = KCT$ (where $\Delta F = \text{change in fluoride level}$, $C = \text{atmospheric fluoride level}$, and $T = \text{exposure period}$) (37).

Although lack of parameter stability for the hay regression suggests that the same may be true for the pasture forage model, the adequate prediction of hay fluoride distribution in 1975 justifies estimation of 1975 pasture forage fluoride as well. The estimated isopleth map is shown in Figure 32 and affected acreages are presented in Table 9.

The overall reduction in geographic spread of fluorides in 1975 pasture forage is apparent from a comparison with isopleths for 1973 (Fig. 14) and 1974 (Fig. 20). In terms of affected area, the total acreage influenced by the source was apparently reduced by about one-third in 1975 (Table 9). This is the same relative reduction observed for second cutting hay.

It appears from these estimates that the 50% decrease in fluoride emissions effectively restricted high (> 30 ppm) average pasture fluoride concentrations to about 4,000 acres adjacent to the source. This is slightly more than one-third of the affected area in 1974. Much of this acreage that is in grazing land is owned by the fluoride emitter, so the fluoride impact on pastures of private farms should have markedly declined.

### Discussion of the Models

Finding workable relationships between estimator (black locust leaf) fluoride concentration and that in forage crops is probably influenced more by atmospheric fluoride levels near the time of estimator sampling than those earlier in the growing season. It has been shown for several species that leaves tend to decrease in fluoride content with time after fumigation (20, 23, 26). In this study, relatively higher linear correlations were generally found between fluoride levels in the estimator and pasture forage late in the growing season. This “loss of memory” by the estimator would account for the poor correlation with first cutting hay despite a good correlation with second cutting hay. It also indicates that the model can be expected to yield poorer predictions when the highest fluoride levels are attained in forage relatively early in the growing season. This was not a problem in this study; however, greatly different rates of fluoride emissions, unusual atmospheric fluoride transport or dispersion patterns, or similar events early in the growing season perhaps would not be adequately represented by the modeling approach. As the authors’ results indicated for second cutting hay, lack of parameter stability is another source of error when a large shift occurs in fluoride emissions. This problem underscores the need for frequent checks on the regression parameters through supplementary forage sampling. Some change in parameters is tolerable when, as in the authors’ survey, information from estimator-derived isopleth maps is the objective rather than estimates of fluoride levels at specific locations.

Although the models have a number of real and potential sources of error, they had practical value in this survey work. The bioestimator approach should also prove effective in similar fluoride monitoring programs, although other bioestimators may need to be developed in other locales. Another application of this technique might include monitoring of the area in which cattle should be examined for symptoms of fluorosis. In addition, sampling of a bioestimator in areas not in forage production would enable extension of the survey and thereby provide a basis for estimating the potential future impact of fluoride on livestock in such areas. The authors especially recommend this approach for investigations of long-term trends encompassing large areas. In such cases, year-to-year variations relating to meteorological anomalies would probably average out. Local peculiarities in the geographic fluoride distribution pattern, such as those created by the major river valley in this study, would be confirmed during the initial intensive forage sampling phase. Data from 2 years should be considered a minimum for develop-
ing the predictive model, and additional checks on parameter stability and reliability of the regressions are strongly recommended. As in this survey, hay sampling every other year (a relatively inexpensive procedure) provides a direct test of the hay regression and, by inference, would indicate reliability of the pasture regression.

**LITERATURE CITED**


APPENDIX

Fluoride Determination by Selective Ion Electrode [Basic procedure of Gyoerkoes and Baretincic (14)]

Reagents

Buffer: Dissolve 4.0 g Sodium Carbonate, 40 g Sodium Chloride, 40 g Sodium Citrate in 750 ml of glass-distilled water in a 1000 ml volumetric flask. Add 1 drop of BRIJ-35 wetting agent and bring to volume with glass-distilled water.

Perchloric Acid: Prepare 0.1M HClO₄ by adding 10 ml of 70% HClO₄ to 500 ml of glass-distilled water in a 1000 ml volumetric flask. Add 1 drop BRIJ-35 wetting agent and bring to volume with glass-distilled water.

Procedure

1. Dry vegetation at 70° C for 48 hours.
2. Grind vegetation to pass through a 20-mesh sieve.
3. Weigh 1 g (or 105 C dry weight equivalent) of ground plant material into a styrene cup.
4. Add 50 ml of 0.1 M HClO₄ and stir for 30 minutes on a magnetic stirrer.
5. Dispense 50 ml buffer solution into the cup.
6. Initially standardize the electrode system with appropriate fresh standard solutions (usually 10 ppm and 100 ppm F⁻) and check response periodically throughout the day. Standards and unknowns must be stirred slowly on a magnetic stirrer during determination. Rinse electrodes with distilled water and wipe dry between solutions.
7. Immerse electrodes in unknown solution and record millivolt output when reading becomes constant for at least 30 seconds.
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Eastern Ohio Resource Development Center, Caldwell, Noble County: 2053 acres
Green Springs Crops Research Unit, Green Springs, Sandusky County: 26 acres

Jackson Branch, Jackson, Jackson County: 502 acres
Mahoning County Farm, Canfield: 275 acres
Muck Crops Branch, Willard, Huron County: 15 acres
North Appalachian Experimental Watershed, Coshocton, Coshocton County: 1047 acres (Cooperative with Agricultural Research Service, U. S. Dept. of Agriculture)
Northwestern Branch, Hoytville, Wood County: 247 acres
Pomerene Forest Laboratory, Coshocton County: 227 acres
Southern Branch, Ripley, Brown County: 275 acres
Western Branch, South Charleston, Clark County: 428 acres