DETERMINATION OF LEAD CONCENTRATIONS IN LEAVES OF THE AMERICAN ELM, *ULMUS AMERICANA L.*

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CHAPTER I

INTRODUCTION

At present, there is an increasing concern over the quality of the environment. The atmosphere is one large component of the environment which is being contaminated by various kinds of atmospheric emissions.

One important component of atmospheric contamination is lead. Lead is released into the atmosphere from the combustion of leaded gasolines from motor vehicles. Precipitation may carry this lead into waterways and soil. Mining, smelting and commercial uses of lead also contribute lead to the environment (11).

The increase of lead in the environment is documented from the chronologic layers of snow strata found in the Greenland ice cap. Between 1750 and 1940 there was a 400 percent increase and from 1940 to 1967, it rose another 300 percent (15).

Several studies have shown a definite relationship between lead contamination of vegetation and soils and proximity to motor vehicular traffic. The overall general trend from these studies reveals that lead content of vegetation and soil increases with traffic volume and decreases with increasing distance from the highways (8, 9, 12, 29, 30, 35).

Evidence exists for lead poisoning in animals. It has been disclosed that a large proportion of the animals at the Staten Island Zoo suffer from lead poisoning. It appears that the major source of lead is from the atmosphere, although paint from the cages may be a contributing source. Analysis of brain, blood and fecal samples revealed that many animals were contaminated with lead, often in concentrations far exceeding the level considered toxic to man. Obviously, these findings raise questions relating to human health and lead in the environment (4).

Another study revealed that young horses, feeding on forage near a smelter complex, suffered from lead poisoning. The high levels of lead in the forage were related to the presence of lead in the soil surface due to accumulation from emissions of the smelter (34).

Lead poisoning of humans is a matter of increasing concern. At present, trace levels of lead are not believed to have any biological function in the human body. Yet due to the pervasiveness of lead in a modern industrial state, we all have some lead in our systems (24). Acute lead poisoning symptoms in humans include loss of appetite, weakness, awkwardness, apathy and miscarriage. It can also cause lesions of the neuromuscular system, circulation system, brain and gastrointestinal tract. An important facet of the lead poisoning question is the symptoms of long term, low level exposure to lead. We do not know what these symptoms are (15).

Even in areas of low human population density and non-industrialized areas, lead contamination may be a serious problem (29). With this in mind the following research project had four objectives:

1. To determine the lead concentration in leaves of the American elm, *Ulmus americana L.*, in four areas of eastern South Dakota.

2. To compare the lead content in leaves collected from the four different areas (1) rural, Lake County, South Dakota; (2) small town,

population 6,315;[1] (3) town, population 13,717;[1] and (4) a city, population 72,488;[1] and then to determine if there is a relationship between lead content of the leaves of elm trees and human population densities (Figure 1).

3. To determine the extent of relationship between the lead content in leaves and traffic flow patterns.

4. To determine if American elm leaves could be a reliable indicator of lead contamination in the environment.

The American elm is a common tree throughout the eastern half of the United States. It is found in farm groves and along farm roads. It is also found in town and city yards and along streets and avenues. It is the most extensively planted ornamental tree in the Upper Midwest region (31). Since elm trees grow in close association with human beings, lead content in their leaves could be an indicator of lead contamination in the environment.

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[1] 1970 Census Data

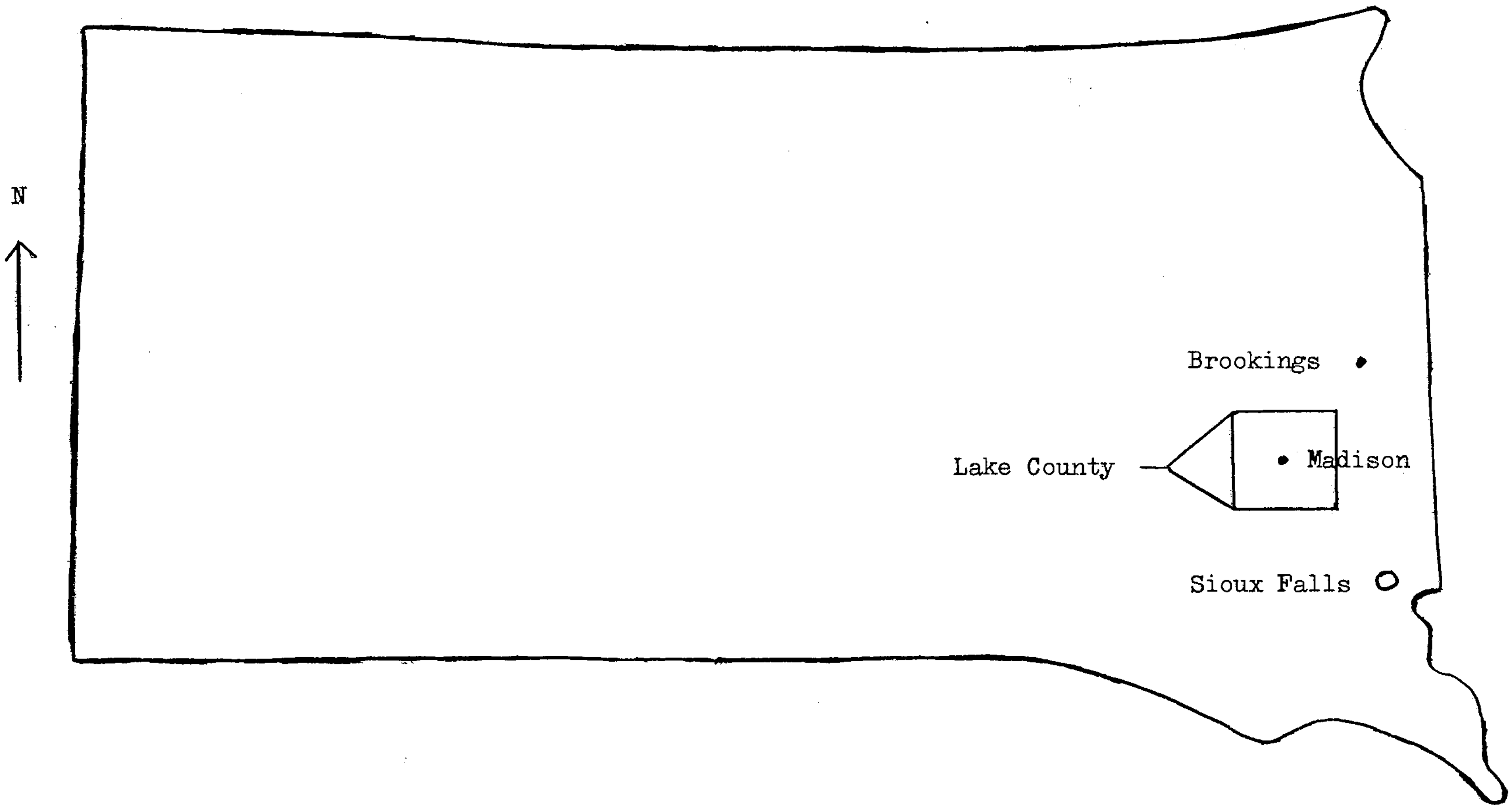

Figure 1 Map of the State of South Dakota Showing the Locations of the Four Areas of Study

1 inch equals approximately 50 miles

CHAPTER II

THE STUDY AREA

The study area was located in east central South Dakota. A map depicting the location of the sampling areas is presented in Figure 1.

Madison, South Dakota is a small town with a population of 6,315.[1] It has an area of approximately 2 square miles. Its economy is based on the surrounding farmland, a small state college, and several small industries, the largest being a firm that produces mobile homes.

The part of Lake County, South Dakota that leaves were sampled from, covered about 70 square miles. The human population in this area is approximately 900.[1] This part of Lake County is gently rolling hills, that are farmed extensively. The major crops are corn, flax, small grains, soybeans and alfalfa. Feeding of livestock occurs on many farms. Groves of trees are associated with most farm buildings. Shelter belts oriented in an east-west direction are common in the county. County and township gravel and dirt roads criss-cross the area every mile. Lake Madison and Lake Herman are two natural prairie lakes located in the study area.

Brookings, South Dakota is a town with a population of 13,717.[1] It has an area of approximately 3.2 square miles. South Dakota State University is located here, with an approximate student population of

[1] 1970 Census Data

6,000. Besides the University the economy is based on the surrounding farmland and some small industries.

Sioux Falls, South Dakota is a city with a population of 72,488.[1] It has an area of approximately 17 square miles. Its economy is based in part on a large surrounding area that includes numerous farms and small towns within a radius of about 50 miles. Manufacturing is an important segment of the economy with the largest being food processing plants. Sioux Falls has three colleges, is served by four airlines, is the home of the Great Plains Zoo, contains 20 city parks and numerous other recreational facilities.

Topography

East central South Dakota is a rolling prairie land studded with numerous lakes and marshes. The native vegetation was tall grass prairie before the development of agriculture (33). Elevations range from 1440 feet above sea level at Sioux Falls to 1669 feet at Madison. Most of the soils of eastern South Dakota are a clay loam soil formed from the glacial drift. The major soil group is chernozem (33).

Climate

East central South Dakota has a temperature continental climate with frequent extremes in weather conditions. The average annual temperature is about 46^{o} F. The annual precipitation averages from 20-24 inches with two-thirds of this falling during the growing season of April through September (39).

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[1]1970 Census Data

CHAPTER III

MATERIALS AND METHODS

Collection of Leaves

A small cardboard box, filled about one-third full with wood shavings, was used to carry ten numbered Erlenmeyer flasks in the car when elm tree leaves were collected. Each day that leaves were collected, the 125 ml Erlenmeyer flasks had about 100 ml of deionized water placed in each and were stoppered with rubber stoppers.

Two-hundred and forty leaves of the American elm tree were collected from 120 different trees in four different areas during the period of July 2, 1972 to August 9, 1972. The leaves from each area were numbered from one to sixty. Every two leaves came from the same tree, for example, 1 and 2, 3 and 4, 5 and 6,.....59 and 60. The areas (Figure 1) were:

1. Madison, South Dakota
2. The rural area of Lake County, South Dakota surrounding Madison within a range of 1/2 to 7 miles
3. Sioux Falls, South Dakota
4. Brookings, South Dakota

Leaves were collected once a week from each area for six weeks. Leaves were collected on Sundays in Madison, Mondays in Lake County, Tuesdays in Sioux Falls, and Wednesdays in Brookings. Thirty trees in each area, were arbitrarily selected for leaf sampling. Five trees were selected each week and these sites were recorded on a map for each area. By looking at the map each week, trees were selected in such a way as to distribute the sampling sites more or less uniformly over the geographic area involved.

Ten leaves were collected each day. The leaves were never higher than 2.5 meters from the ground. Records were kept of the site that leaves were collected from in each area (See Appendix A). When the leaves were removed from the tree they were rolled into a cylinder in order to place them in the Erlenmeyer flask. When all ten leaves had been collected they were taken to the laboratory.

Laboratory Analysis of Leaves

In the laboratory the flasks were attached to a Burrell wrist action shaker. The shaker was on a time clock which turned on at 12:30 A.M. and off at 8:30 A.M. The flasks were shaken for 8 hours. The leaves were in water for approximately 15 hours. This washing was for removing some of the inorganic contaminants. Around 8:30 A.M. leaves were removed from the flasks and each leaf was placed individually on a watch glass. Watch glasses and leaves were placed in an oven at 110° C for 30 minutes (25). After drying, each dry leaf was ground in a separate coors mortar with pestle for about 30 seconds (20). Then each ground leaf was transferred from the mortar to a weighing bottle. The weighing bottle was capped with a ground glass lid.

A Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer (A.A.S.) set for standard conditions for lead was used for leaf analysis. The sampling boat(TM) attachment, which greatly improves sensitivity, was used (22). The wave length was 283.3 nanometers. The slot setting was four, the fuel was acetylene, and the oxidant was air. A noise suppression of one was used and the scale expansion was one (3, 24). The instrument was warmed up for a minimum of 30 minutes before use.

Each weighing bottle with a ground leaf and a metal spatula was weighed on a Mettler balance, model HIOT, to the nearest ten thousandth of a gram. The weighing bottle was handled with a test tube holder with the wire loops that touched the bottle wrapped in aluminum foil. The handle of the metal spatula was wrapped in aluminum foil to prevent residues from the fingers from affecting the weight. The aluminum foil was slid over the handle of the spatula, and was removed for weighing. The same spatula was used in each weighing bottle. It was wiped clean with tissue paper before being placed in a different weighing bottle.

A new sampling boat was used each day. Before use, each boat was inserted into the flame to remove contaminants (3). Also, a 10% w/v solution of $Mg(NO_3)_2 \cdot 6H_2O$ was used as an ashing-aid (13). Two portions of 0.2 ml of the ashing-aid were pipetted into the boat and dried and burned to build up a residue on the boat before analysis started. A small amount of the ground leaf was placed in the sampling boat using the metal spatula. After the leaf was in the boat, the burner was lit and then 0.2 ml of $Mg(NO_3)_2$ solution was pipetted into the boat. The boat was moved close to the flame for drying. A standard distance of 14 mm from the flame for all samples was used (24).

The sample was dried for exactly two minutes. During these two minute intervals, the weighing bottle was picked up with the test tube holder with the spatula in the bottle, but the aluminum foil cover on the handle was removed. The weighing bottle was placed on the Mettler balance and weighed. This weight was recorded. The weighing bottle was picked up again with the test tube holder and returned to the A.A.S. where the aluminum foil was again placed over the handle of the spatula in the weighing bottle. After two minutes, the chart was turned on at a speed of 120 mm per minute. The boat was inserted into the flame. When the recording pen had returned to the base line the boat was removed from the flame and the flame was turned off. This was necessary because the sample of ground leaf could not be placed in the sampling boat with the flame on.

Two samples of each leaf were analyzed. These were averaged to obtain the lead concentration for that sample (24). The difference in the weights of the weighing bottle after each weighing was subtracted from the previous one to give the weight of the leaf placed in the sampling boat. The location that the leaf sample was collected from was also recorded on the chart.

Two blanks of 0.2 ml of the ashing-aid were burned between samples 2 and 3, 6 and 7, and after 10. A lead standard of one ppm was also burned between samples 2 and 3, 6 and 7, and after sample 10 (24). Between these same samples a sample of leaf was burned at the non-absorbing lead line of 280.0 nanometers. This was necessary in order to be able to distinguish the responses of the recorder due to lead or responses that may be due to smoke or some other interference.

The lead standard was prepared by dissolving 1.598 gms of oven dried lead nitrate, $Pb(NO_3)_2$ in one liter of 1% (v/v HNO_3) and placed in a polyethylene bottle. This gave a stock standard of 1,000 ppm lead (3). A 10 ppm lead standard was prepared by pipetting 5 ml of the 1,000 ppm stock standard into a 500 ml volumetric flask with 1%

(v/v HNO_3). This was shaken and allowed to stand for about 20 minutes. Then 10 ml of the 10 ppm standard were transferred to a 100 ml volumetric flask with 1% (v/v HNO_3) and filled to volume. This gave a one ppm standard which was burned in the A.A.S. The 10 ppm and 1 ppm standards were made fresh each day (3).

Care of Glassware

After use, the 125 ml Erlenmeyer flasks were washed with a brush and rinsed twice in deionized water and allowed to dry before using again. The watch glasses were rinsed and dried after each use. The weighing bottles were rinsed four times with deionized water and air dried. After drying and before each use, the weighing bottles were wiped on the inside with tissue paper. The mortars and pestles were allowed to stand over night in 20%, triple distilled, HNO_3 solution. Just before use they were rinsed twice with deionized water and wiped dry with two separate pieces of tissue paper. All glassware was numbered from one to ten.

Procedure for Determining Lead Concentrations

On the chart paper each peak was measured to the nearest 1/10th of a millimeter. An average of the two blanks was subtracted from the measurement of the samples. Two portions of the lead standard were burned and the two were averaged for the peak height of the lead standard (24).

The formula for determining lead in µg/g is as follows (25):

$$\frac{\text{Sample Leaf Peak Height} \left(\frac{\mu g \text{ Pb}}{\text{Lead Standard Peak Height}} \right)}{\text{Sample Weight in Grams}}$$

μg Pb = 0.2 ml x 1 ppm = 0.2 μg Pb

Analysis of Results

For comparison of the data from the four different areas simple one-way analysis of variance was used. The formula for F is (5, 32):

$$F = \frac{msb}{msw} \qquad msb = \frac{SSb}{DFb} \qquad msw = \frac{SSw}{DFw} \qquad DFb = k - 1 \qquad DFw = N_{tot} - k$$

$$SSb = \frac{(\Sigma Y_1)^2}{N_1} + \frac{(\Sigma Y_2)^2}{N_2} + \frac{(\Sigma Y_3)^2}{N_3} + \frac{(\Sigma Y_4)^2}{N_4} - \frac{(\Sigma Y_{tot})^2}{N_{tot}}$$

$$SSw = \Sigma Y_1^2 - \frac{(\Sigma Y_1)^2}{N_1} + \Sigma Y_2^2 - \frac{(\Sigma Y_2)^2}{N_2} + \Sigma Y_3^2 - \frac{(\Sigma Y_3)^2}{N_3} + \Sigma Y_4^2 - \frac{(\Sigma Y_4)^2}{N_4}$$

$$SS_{tot} = \Sigma Y_{tot}^2 - \frac{(\Sigma Y_{tot})^2}{N_{tot}}$$

F = F ratio

msb = mean squares between groups

msw = mean squares within groups

SSb = sum of squares between groups

SSw = sum of squares within groups

SS_{tot} = Sum of squares total

DFb = Degrees of Freedom between groups

DFw = Degrees of Freedom within groups

k = number of groups

N_{tot} = total number of samples

N_1, N_2, N_3, N_4, = number of samples in each group

Y = values for lead variable

Σ = the sum of

Y_1, Y_2, Y_3, Y_4, = values for lead variable in each group

If the analysis of variance showed a significant difference at the .05 level then Duncan's New Multiple Range Test was used to determine where the difference exists between the four sampling areas.

The procedure for the Duncan's Test is (5):

Step 1. Determine the mean lead value for each area: $\frac{\Sigma Y_1}{N_1}$ $\frac{\Sigma Y_2}{N_2}$ $\frac{\Sigma Y_3}{N_3}$ $\frac{\Sigma Y_4}{N_4}$

Step 2. The means determined in step one are ranked from lowest to highest.

Step 3. Determine the standard error of the means:

$$SEm = \sqrt{\frac{msw}{N \text{ (per group)}}}$$

Step 4. From DUNCAN'S MULTIPLE RANGE TABLE obtain the "significant studentized ranges."

Step 5. Then multiply each "studentized range" value by the standard error of the means, (Step 3). This results in the minimum mean difference symbolized by R for a given range of comparisons.

Step 6. If the difference between any two means is larger than the minimum mean difference (R) for that range, it is considered to be significant.

The .05 level of significance was used.

The following criteria was used to determine if the American elm could be used as an indicator of lead contamination in the environment.

Since no information currently exists on the actual amounts of lead in the four areas, a direct comparison for evaluating elm trees as an indicator of lead contamination cannot be used. Hence, indirect comparisons were made. One was a comparison of the lead content in leaves in the four areas using analysis of variance. The second was relationship between lead content in leaves and traffic flow patterns.

Two precedures using analysis of variance were used to determine if elm leaf lead could be an indicator of lead in the environment. One was the same procedure as described above for the comparisons of the four areas.

Significant differences at the .05 level between areas indicates that elm leaf lead concentrations are related to area differences, such as population differences, and hence, indirectly to the lead concentration in the environment.

The second was a two factor Factorial Design which will determine the relationship between time, area and time-area interaction.

The procedure for the two factor Factorial Design is (5):

Step 1. Sum all lead values for each week, within each area, this results in 24 different measures, 6 weeks x 4 areas.

Step 2. Square all lead values and add together.

Step 3. Add sums of all 24 measures (Step one) to get a grand sum. Square this figure and divide by N = 240. This yields the correction term.

Step 4. SS_{tot} = Step 2 - Step 3 (Correction term)

Step 5. Add the sums of the four areas for each week. This gives six measures. Square each one of the six and divide by N = 40, add the answers together.

Step 6. SS_{time} = Step 5 - Step 3.

Step 7. Add the sums of the six different weeks for each area. This gives four measures. Square each one and divide by N = 60, add the answers together.

Step 8. SS_{area} = Step 7 - Step 3.

Step 9. Take the sums of each of the 24 measures (Step one) and square and divide by N = 10. This gives 24 measures add these together.

Step 10. $SS_{time\ x\ area}$ = Step 9 − (Step 3 + Step 6 + Step 8).

Step 11. SS_{error} = Step 9 − (Step 6 + Step 8 + Step 10).

Step 12. $SS_{leaves} = \sum \frac{(Y_1 - Y_2)^2}{2}$

Step 13. $SS_{trees} = SS_{error} - SS_{leaves}$

Step 14. Determination of the Degrees of Freedom

DF for SS_{tot} = total number of leaves minus 1, 240 − 1 = 239

DF for SS_{time} = the number of weeks minus 1, 6 − 1 = 5

DF for SS_{area} = the number of areas minus 1, 4 − 1 = 3

DF for $SS_{time\ x\ area}$ = DF for SS_{time} x DF for SS_{area},

5 x 3 = 15

DF for SS_{error} = DF for SS_{tot} minus the DF for SS_{time} + SS_{area} + $SS_{time\ x\ area}$, 239 − (5 + 3 + 15) = 216

DF for $SS_{leaves} = \frac{N}{2} = \frac{240}{2} = 120$

DF for $SS_{trees} = DF_{error} - DF_{leaves}$, 216 − 120 = 96

Step 15. The mean squares are computed as $\frac{SS}{DF}$:

$$\frac{SS_{time}}{DF_{time}} = ms_{time} \quad \frac{SS_{area}}{DF_{area}} = ms_{area} \quad \frac{SS_{time\ x\ area}}{DF_{time\ x\ area}} = ms_{time\ x\ area}$$

$$\frac{SS_{error}}{DF_{error}} = ms_{error} \quad \frac{SS_{leaves}}{DF_{leaves}} = ms_{leaves} \quad \frac{SS_{trees}}{DF_{trees}} = ms_{trees}$$

Step 16. The several F-ratios are then computed as:

$$\frac{ms_{time}}{ms_{trees}} \quad \frac{ms_{area}}{ms_{trees}} \quad \frac{ms_{time\ x\ area}}{ms_{trees}}$$

SS = sums of squares

ms = mean squares

DF = Degrees of Freedom

If no significant difference at the .05 level exists for time or for time-area interaction, this would be evidence for accepting elm tree leaves as a reliable indicator of lead contamination.

For the relationship between lead content in leaves and traffic volume, the following procedures were employed:

1. Traffic flow information was obtained from traffic flow maps provided by the South Dakota Highway Department, Pierre, South Dakota.

2. Scatter diagrams were constructed for traffic flow versus lead content in leaves.

3. Pearson Product Moment Correlation Coefficients were calculated. The formula is (32):

$$r = \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}}{\sqrt{\left[\Sigma X^2 - \frac{(\Sigma X)^2}{N}\right]\left[\Sigma Y^2 - \frac{(\Sigma Y)^2}{N}\right]}}$$

Y = values for lead variable

X = traffic count

N = the number of traffic counts

4. Linear regression lines were used. The formula is (32, 38):

$$Y = a + bX$$

5. The linear regression lines were checked for lack of fit, which is a test to determine if the variability around the regression line is random or not. The formulas involved are (38):

$$F \text{ ratio} = \frac{ms_{\text{lack of fit}}}{ms_{\text{error}}} \qquad DF = N - 2,\ N$$

$$S^2 = ms_{\text{error}} = \frac{SS_{\text{error}}}{DF} \qquad DF = N$$

$$ms_{\text{lack of fit}} = \frac{SS_{\text{lack of fit}}}{DF} \quad DF = N - 2$$

$$SS_{\text{error}} = \sum \frac{(Y_1 - Y_2)^2}{2}$$

$$SS_{\text{lack of fit}} = \left(\sum \bar{Y}^2 - \frac{(\sum Y)^2}{N}\right) - SS_{\text{regression}}$$

$$SS_{\text{regression}} = \frac{\left(\sum X\bar{Y} - \frac{(\sum X)(\sum \bar{Y})}{N}\right)^2}{\sum X^2 - \frac{(\sum X)^2}{N}}$$

If the lack of fit of the linear regression line is not significant at the .05 level, this would be evidence for accepting elm tree leaves as indicators of lead contamination.

6. A "t" test for the presence of a significant relationship was made. The formula is (38): $"t" = \frac{b}{S_b}$ DF = N

If the "t" test is significant at the .05 level, this indicates that the elm leaf lead concentration is related to the traffic count, and hence, indirectly to the lead concentration in the environment.

Y = the dependent variable lead in a certain leaf at a certain location.

X = the independent variable traffic count at a certain location

$$a = \bar{\bar{Y}} - b\bar{X}$$

$$b = \frac{\sum X\bar{Y} - \frac{(\sum X)(\sum \bar{Y})}{N}}{\sum X^2 - \frac{(\sum X)^2}{N}}$$

N = number of traffic counts

$$\bar{Y} = \frac{Y_1 + Y_2}{2}$$

Y_1 and Y_2 = two lead concentrations in two leaves from the same tree at a certain location

$\bar{\bar{Y}} = \frac{\sum Y}{N}$ N = number of leaves

$$\bar{X} = \frac{\Sigma X}{N}$$

$$S_b = \frac{ms_{error}}{\sqrt{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}}$$

$SS_{regression}$ = Sums of squares for regression

$SS_{lack\ of\ fit}$ = Sums of squares for lack of fit

SS_{error} = Sums of squares for error

$ms_{lack\ of\ fit}$ = mean squares for lack of fit

ms_{error} = mean squares for error

DF = Degrees of Freedom

CHAPTER IV

PRESENTATION AND ANALYSIS OF RESULTS

Table I presents the lead concentrations in µg/g or ppm by area and week.

The hypothesis tested was: There would be no significant differences between the means of the lead in leaves taken from the four areas. To test this hypothesis a one-way analysis of variance was used. The F-table for this analysis is presented in Table II. The analysis showed a significant difference with a calculated F value of 42.55, while the tabular F value was 2.65 at the .05 level, with 3 and 236 degrees of freedom.

Because the one-way analysis of variance showed a significant difference, Duncan's New Multiple Range Test was used to determine which specific areas differed significantly from each other. The results of this test are presented in Table III. The results show that all four areas were significantly different from each other. Hence the above stated hypotheses was rejected, and a relationship between lead in the environment and human population densities was established.

Criteria for Determining if Elm Tree Leaves Can Be Used as Indicators of Lead Contamination

1. Simple one-way analysis of variance and Duncan's Multiple Range Test were used (same procedure as described above). Results are

TABLE I

LEAD CONCENTRATION IN LEAVES IN μg/g OR ppm
BY AREA AND WEEK

Date	Sample (Leaf) Number	Madison	Lake County	Sioux Falls	Brookings
	1	4.02	4.00	5.58	3.19
	2	7.38	4.69	7.18	5.36
	3	5.26	2.68	12.30	7.01
	4	6.05	3.65	18.17	6.94
July 2 to	5	5.59	4.22	7.69	6.00
	6	5.13	5.69	3.82	5.33
6, 1972	7	6.78	5.43	10.29	5.47
	8	5.42	4.60	7.87	7.28
	9	5.55	4.79	6.29	2.83
	10	7.48	6.83	9.32	4.38
	11	3.71	1.57	17.56	7.84
	12	3.31	2.34	11.76	6.71
	13	6.47	2.64	20.06	13.36
	14	5.02	4.56	25.65	20.89
July 9 to	15	4.73	5.78	4.84	7.80
	16	4.30	4.10	5.53	6.54
13, 1972	17	5.99	3.50	10.21	3.88
	18	4.84	2.58	13.05	5.06
	19	2.31	3.69	9.50	5.38
	20	2.73	4.67	9.24	10.06
	21	7.62	4.65	9.33	4.29
	22	7.58	3.38	7.13	7.20
	23	3.85	3.22	7.47	4.67
	24	4.29	2.26	15.20	3.81
July 16 to	25	5.33	2.31	12.95	4.68
	26	3.21	3.68	7.06	5.03
20, 1972	27	5.55	2.77	10.49	6.43
	28	4.40	3.04	7.72	7.33
	29	5.98	5.84	6.04	15.02
	30	6.00	6.84	5.03	8.50
	31	7.16	4.00	20.11	8.36
	32	5.55	3.52	9.23	17.30
	33	5.41	1.25	9.50	11.59
	34	4.07	1.65	8.87	12.27
July 23 to	35	3.94	2.40	17.36	6.08
	36	4.64	1.96	14.68	6.60
27, 1972	37	6.27	2.52	14.14	13.86
	38	7.12	2.57	14.39	12.72
	39	9.52	11.08	36.68	6.76
	40	3.78	7.81	24.10	6.04

TABLE I (Continued)

Date	Sample (Leaf) Number	Madison	Lake County	Sioux Falls	Brookings
	41	2.82	11.13	6.67	4.99
	42	7.20	6.94	15.71	5.15
	43	12.17	2.39	22.92	6.00
	44	9.11	3.61	15.05	5.16
July 30 to	45	5.94	3.46	18.66	9.99
	46	5.24	7.23	11.94	8.19
August 3,	47	3.90	3.06	3.38	7.65
	48	3.97	3.40	6.06	6.28
1972	49	5.55	3.36	9.90	16.68
	50	4.25	4.91	10.05	9.16
	51	5.29	11.16	9.31	10.14
	52	6.17	6.07	13.54	6.58
	53	3.89	2.01	6.42	7.12
	54	3.87	1.81	7.21	6.33
August 6 to	55	16.68	4.70	20.55	10.13
	56	14.97	5.78	12.82	11.66
10, 1972	57	12.11	4.35	8.02	4.70
	58	6.07	6.24	20.53	3.39
	59	5.84	3.88	15.20	6.89
	60	8.40	5.26	14.43	5.39

TABLE II

F-TABLE RESULTS FOR ONE-WAY ANALYSIS OF VARIANCE OF LEAD CONCENTRATION IN LEAVES

Source	Sums of squares	DF	ms	F	P @ .05	Significance
Total	5647.48	239	-	-	-	
Between groups	1982.67	3	660.89	42.55	2.65	significant
Within groups	3665.43	236	15.53	-	-	

TABLE III

RESULTS FROM DUNCAN'S TEST

Means for Lead		Means ranked	SR	SEm	(SR)(SEm)
Madison	5.95	4.33 (1)	SR-2 = 2.772	$\frac{15.53}{60}$ = .507	SR-2 x .507 = 1.405 = R-2
Lake Count	4.33	5.95 (2)	SR-3 = 2.918		SR-3 x .507 = 1.479 = R-3
Sioux Falls	12.03	7.69 (3)	SR-4 = 3.017		SR-4 x .507 = 1.530 = R-4
Brookings	7.69	12.03 (4)			

Testing the difference between the various means at the .05 level

Sioux Falls vs. Lake County	(R-4 = 1.530)[1]	12.03 - 4.33 = 7.70 (significant)
Brookings vs. Lake County	(R-3 = 1.479)[1]	7.69 - 4.33 = 3.37 (significant)
Madison vs. Lake County	(R-2 = 1.405)[1]	5.95 - 4.33 = 1.62 (significant)
Sioux Falls vs. Madison	(R-3 = 1.479)[1]	12.03 - 5.95 = 6.08 (significant)
Brookings vs. Madison	(R-2 = 1.405)[1]	7.69 - 5.95 = 1.74 (significant)
Sioux Falls vs. Brookings	(R-2 = 1.405)[1]	12.03 - 7.69 = 4.34 (significant)

SR = studentized ranges (from tabulated tables (5))

SEm = Standard Error of the Means

[1]If the difference between means is greater than the R value for that range it is considered to be significant at the .05 level.

presented in Tables II and III. Significant differences at the .05 level between areas indicates that elm leaf lead concentrations are related to area differences, such as population differences, and hence, indirectly to the lead concentration in the environment. The results show that all four areas were significantly different from each other. This can be used as evidence for considering lead in elm tree leaves as indicators of lead contamination.

2. An analysis of variance using the two factor Factorial Design was used, and the results are presented in Table IV. The hypothesis tested was: There would be no significant differences between time periods (weeks) or for time-area interaction at the .05 level. If no significant difference at the .05 level exists for time or for time-area interaction, this would be evidence for accepting elm tree leaves as indicators of lead contamination. The results show a significant difference for time. This can be used as evidence for rejecting lead in elm tree leaves as an indicator of lead contamination. There was no significant difference for time-area interaction. This can be used as evidence for accepting lead in elm tree leaves as indicators of lead contamination.

Because the traffic counts for Lake County were taken every three to five miles the data for Lake County could not be used. In the other three areas only certain traffic count locations corresponded to locations where elm tree leaves were collected. There were 18 such locations in Madison, 19 in Brookings, and 14 in Sioux Falls out of a possible 30 in each area. The following results are based on the lead content in leaves at the locations where traffic count data were available. The traffic count information, expressed in average number of

TABLE IV

F-TABLE RESULTS FOR ANALYSIS OF VARIANCE USING
TWO FACTOR FACTORIAL DISIGN

Source	Sums of Squares	DF	ms	F	P @ .05	Significance
Total	5648.70	239	-	-	-	-
Time	284.96	5	56.99	2.48	2.30	significant
Area	1982.12	3	660.71	28.74	2.70	significant
Time x Area	414.29	15	27.62	1.20	1.79	ns
Error	2967.33	216	13.74	-	-	-
Trees	2206.78	96	22.99	-	-	-
Leaves	760.55	120	6.34	-	-	-

ns = not significant

vehicles per day, along with their corresponding leave sample numbers is presented in Appendix B.

3. Scatter diagrams are presented in Figures 2, 3, 4, and 5 for all three areas and then for all the areas combined.

4. Pearson r's were compiled for all three areas and for all the areas combined, and the results are presented in Table V.

5. Linear regression lines were computed for all three areas and then for all the areas combined, the results are presented in Table V.

6. The linear regression lines were checked for lack of fit, which is a test to determine if the variability around the regression line is random or not. The results are presented in Table VI. The hypothesis tested was: That the lack of fit for any of the four regression lines is not significant at the .05 level. If the lack of fit of the linear regression line is not significant at the .05 level, this could be evidence for accepting elm tree leaves as indicators of lead contamination. The results show the lack of fit is not significant for any of the regression lines for the areas taken separately. This can be used as evidence for accepting lead in elm tree leaves as indicators of lead contamination. It was significant for all three areas combined. This can be used as evidence for rejecting lead in elm tree leaves as indicators of lead contamination.

7. A "t" test for the presence of a significant relationship between the angle formed between the linear regression line and a horizontal line was made. This was done for each of the three areas and for all three areas combined. The results are presented in Table VII. The hypothesis texted was: That the "t" test for the four regression lines would not be significant at the .05 level. If the "t"

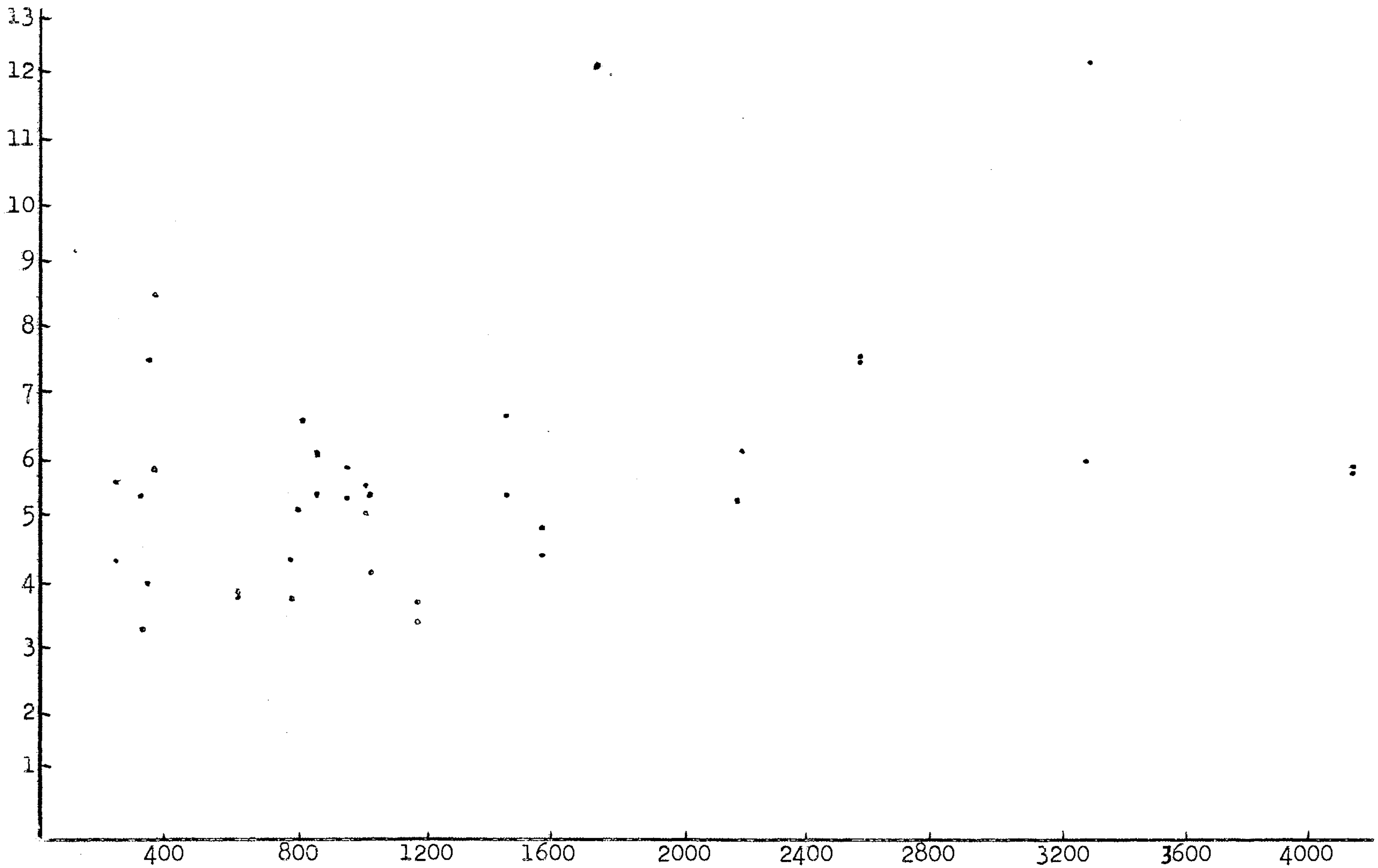

Figure 2. Scatter Diagram for Lead Content in ppm (vertical axis) and Traffic Count (horizontal axis) for Madison

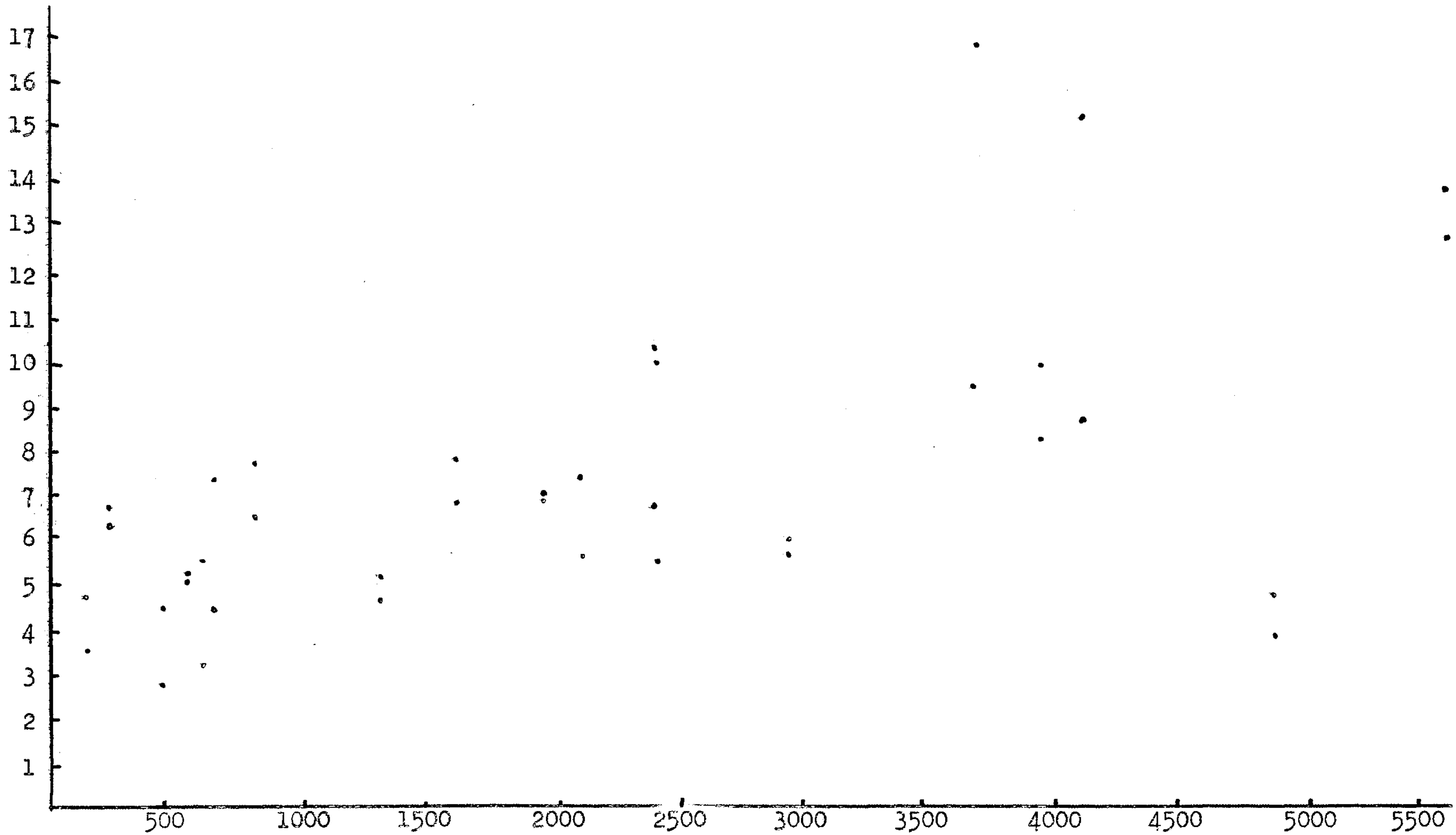

Figure 3. Scatter Diagram for Lead Content in ppm (vertical axis) and Traffic Count (horizontal axis) for Brookings

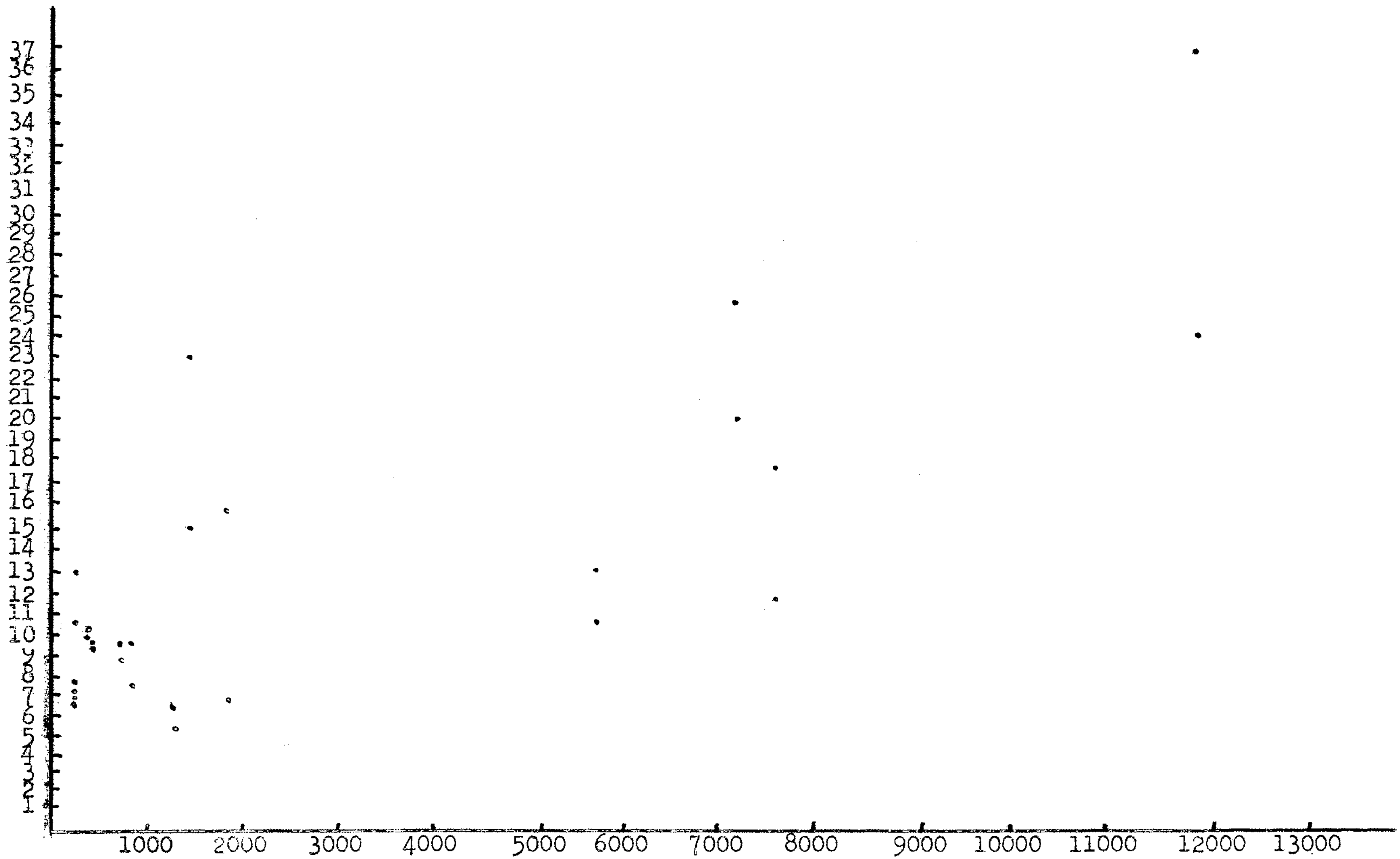

Figure 4. Scatter Diagram for Lead Content in ppm (vertical axis) and Traffic Count (horizontal axis) for Sioux Falls

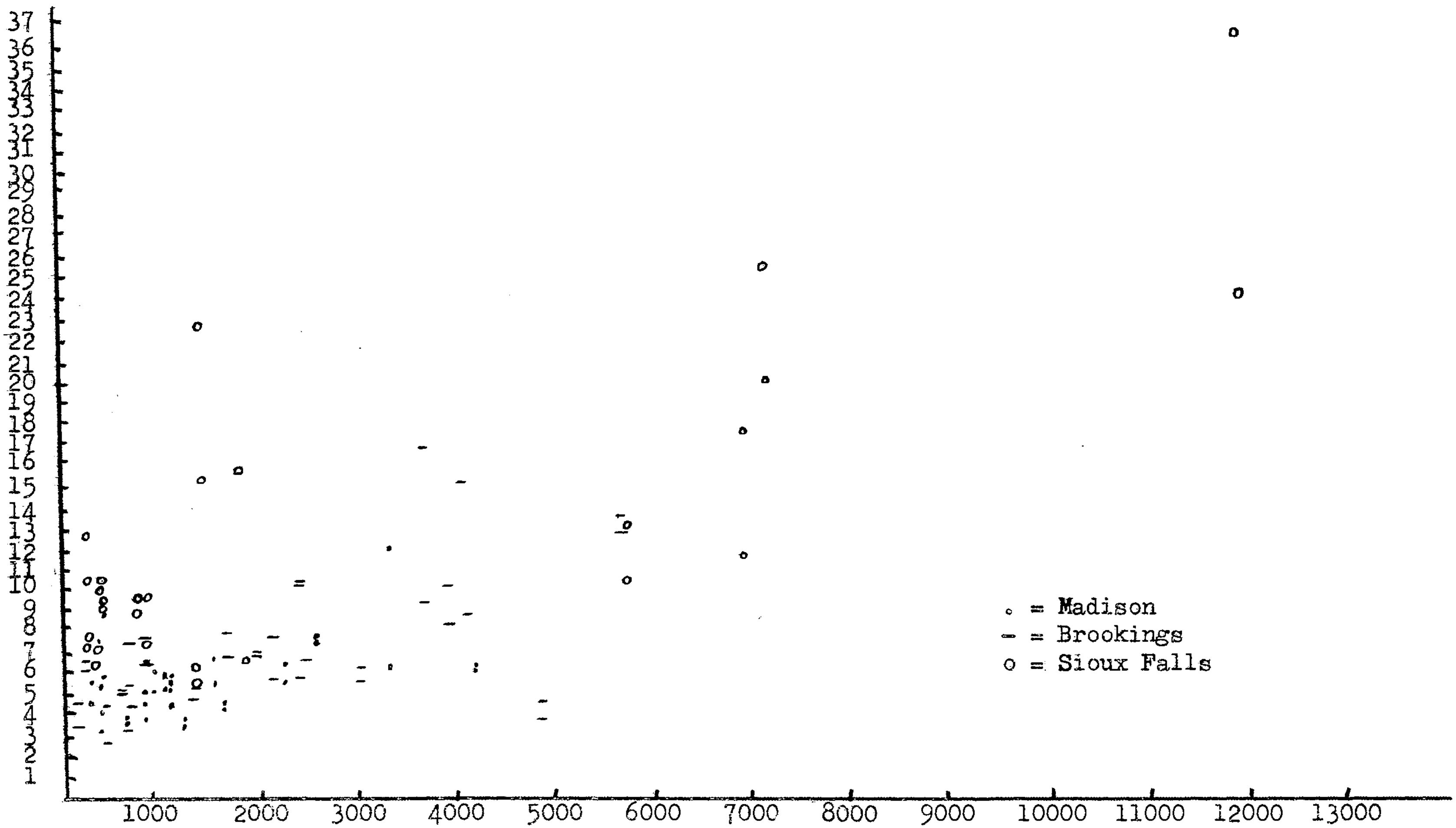

Figure 5. Scatter Diagram for Lead Content in ppm (vertical axis) and Traffic Count (horizontal axis) for All Three Areas Combined

TABLE V

VALUES FOR PEARSONS r AND LINEAR REGRESSION LINES

Madison	Brookings	Sioux Falls	All Three Combined
r = .43	r = .61	r = .77	r = .71
a = 4.64	a = 7.08	a = 8.28	a = 4.77
b = .000677	b = .001190	b = .001560	b = .001629
S^2 = 1.91	S^2 = 4.31	S^2 = 15.15	S^2 = 6.44

TABLE VI

F-TABLE FOR CHECK ON LACK OF FIT AROUND THE REGRESSION LINE

Source	Sums of Squares	DF	ms	F	P @ .05	Significance
			Madison			
Regression	9.08	18	–	–	–	–
Lack of Fit	23.68	16	1.48	0.77	2.25	ns
Error	34.45	18	1.91	–	–	–
			Brookings			
Regression	71.66	19	–	–	–	–
Lack of Fit	80.01	17	4.71	1.09	2.21	ns
Error	81.88	19	4.31	–	–	–
			Sioux Falls			
Regression	432.85	14	–	–	–	–
Lack of Fit	186.98	12	15.58	1.03	2.53	ns
Error	212.10	14	15.15	–	–	–
			All Three Combined			
Regression	707.66	51	–	–	–	–
Lack of Fit	532.33	49	10.86	1.69	1.60	significant
Error	328.43	51	6.44	–	–	–

ns = not significant

TABLE VII

"t"-TEST RESULTS FOR A SIGNIFICANT RELATIONSHIP OF THE LINEAR REGRESSION LINE

b	S_b	DF	"t"	P @ .05	Significance
		Madison			
.000677	.000429	18	1.58	2.01	ns
		Brookings			
.001190	.000605	19	1.97	2.09	ns
		Sioux Falls			
.001560	.001136	14	1.37	2.15	ns
		All Three Combined			
.001629	.000394	51	4.13	2.01	significant

ns = not significant

test is significant at the .05 level, this indicates that the elm leaf lead concentration is related to the traffic count, and hence, indirectly to the lead concentration in the environment. The results show that the three regression lines for each separate area are not significant. This can be used as evidence for rejecting lead in elm tree leaves as indicators of lead contamination. The regression line for all three areas combined is significant. This can be used as evidence for accepting lead in elm tree leaves as indicators of lead contamination in the environment.

CHAPTER V

DISCUSSION AND RECOMMENDATIONS FOR FUTURE STUDY

The technique of analyzing plant and animal tissues directly, without ashing or chemical digestion, for micro-concentrations of elements by Atomic Absorption Spectroscopy (A.A.S.) is a relatively recent development. It offers several advantages over techniques which require that the tissue be put into solution before an analysis can be made. The tissue is analyzed directly which substantially reduces the possibility of contamination either from glassware or chemicals. Direct analysis shortens the time needed to prepare samples for analysis because no ashing or chemical digestion is involved. Direct analysis also eliminates the possibility of losses by vaporization or adherence to glassware for the element being analyzed. The direct analysis of tissues holds promise for a quick, relatively easy, and inexpensive method of analyzing various biological components of the environment for certain types of contaminants.

One aspect of the direct analysis of leaves that needs further study is the washing and washing time, 15 hours in water and 8 hours on the shaker in this study. If the washing could be eliminated, or reduced in time, this would greatly reduce the time involved in leaf preparation, and speed up the analysis process and make it possible to analyze more leaves in shorter period of time.

Possible changes in leaf lead content through time and the lead content in leaves at different locations on the same tree are two other areas that need to be studied. Although the analysis of variance in this study indicated that time was only a minor factor, a more intense and detailed study needs to be made, to determine the extent of lead build up in elm tree leaves through time. If lead builds up substantially in elm tree leaves over a period of time this could reduce its reliability as a possible indicator of lead contamination, because leaves collected at different times would not be directly comparable.

The lead content in leaves at different locations on the same tree needs to be studied. It is my feeling that the lead concentration in leaves may vary substantially in leaves on the same tree, either by height above the ground or by the relative position of the leaves in relation to roads, traffic, buildings or wind direction.

One of the recommendations of the Committee on Biologic Effects of Atmospheric Pollutants (11) was for expanded studies on lead chemistry in nature. Direct analysis of certain tissues by A.A.S. may be an easy and useful way to obtain some of this information. As examples, the feathers of birds or the hair of mammals may be dried and ground and analyzed in a similar manner as the elm leaves. Also bone, which may contain over 90% of the total lead of the body, (11) might be dried and ground and analyzed in a similar manner as the elm leaves. The chitin of insects or other arthropods may be another possibility for direct analysis. If the above suggestions proved feasible then the tracing of quantities of lead along biologic pathways may be possible.

If feathers, hair, bone or chitin could be analyzed directly it would improve the possibilities of comparing present contaminants in biological materials with those from the past, like those that are preserved as museum specimens, or as study or research collections. Since direct analysis of tissues requires only a small amount of the tissue, the museum specimens should not be destroyed or damaged.

The analysis of 240 leaves from the four areas has established a base line for lead content in elm tree leaves, for the summer of 1972. This may be a rather critical general time period as non-leaded gasolines for cars has been recently introduced. Also air pollution control standards are being implemented, for motor vehicles and industry. Hence, some study in the future may be undertaken to assess the effects of these measures on the lead contamination in the environment and comparisions made to this time period.

The analysis of variance showed a significant difference between lead concentrations in elm tree leaves, and the four areas from which the leaves were collected. This shows a definite relationship between lead contamination and human population densities. When the means of the lead concentrations were ranked from lowest to highest, the lowest mean corresponded to the area with the lowest human population, the next lowest mean to the next lowest population and so forth, for the other two areas. Whether the lead concentration in the environment in any of these areas is a human health hazard is not known, because the effects of low levels of lead in the human body are not well known (15). However, an index to the lead contamination in the various areas has been established which may be of use in the future in relation to human health and lead in these four areas.

The analysis of variance using the two factor Factorial Design revealed that time was playing a role in the amount of lead in elm tree leaves, although a minor one when compared to area differences. The calculated F-ratio for time was 2.48, the tabulated F-ratio was 2.30 at the .05 level, while the calculated F-ratio for area was 28.74 and the tabulated value was 2.70 at the .05 level.

The relationship between lead in elm leaves and traffic counts was a weak one. The three regression lines for the three separate areas did not show a significant relationship between the angle formed by the regression line and a horizontal line. A significant relationship is necessary to demonstrate a relationship between lead in elm leaves and traffic flow patterns. The scatter diagrams, Figures 2, 3, 4, and 5, also show weak relationships between traffic counts and lead concentrations. These findings are in contrast to others who have found stronger relationships between traffic counts and lead in vegetation and/or soils along highways (8,9,12,29,30,35). Some possible reasons for this weak relationship might include the following:

1. In towns and cities traffic from neighboring streets may contribute to the lead concentration where leaves were sampled.

2. No effort was made to determine such parameters as traffic speed, prevailing wind direction, stop signs or stop lights and their proximity and relationship to the trees that were sampled.

3. The air in towns and cities may be mixed by moving vehicles and/or deflection of wind by buildings and trees in such a way as to confound the relationship between lead in leaves and traffic counts. However, since the regression line for all three areas combined, did show a significant relationship, a larger range of lead concentration values and traffic counts may be needed before a relationship can be demonstrated.

Evidence for determining if the lead in elm tree leaves could be used as an indicator of lead in the environment was present, but not conclusive. Considerable evidence from the two analysis of variance tests indicates that elm leaf lead would be a good indicator of lead contaminzation in the environment. However, this is tempered by the analysis of elm leaf lead and traffic counts, which revealed a weak relationship. When all evidence is taken into account it may be stated that the concentration of lead in elm leaves shown considerable promise as an indicator of lead contamination, but without further study, cannot at this time, be firmly established as a reliable indicator of lead contamination in the environment.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Direct analysis of the lead content in elm tree leaves was carried out using Atomic Absorption Spectroscopy. Two-hundred and forty leaves collected from 120 trees in four different areas in eastern South Dakota were analyzed. The information collected was analyzed using analysis of variance and regression. Recommendations for further study were made.

The following conclusions are based on analysis of the data collected.

1. A base line for the lead content in elm tree leaves for the summer of 1972 has been established for four areas of eastern South Dakota.

2. The lead content in elm tree leaves differs significantly between the four areas. These differences are related to population density, with the lowest lead concentration in the area with the lowest human population and the highest lead concentration in the area with the highest human population.

3. The relationship between traffic counts and lead concentration was weak.

4. Evidence for using elm tree leaf lead as an indicator of lead contamination in the environment was present, but not conclusive, hence, elm leaf lead as an indicator of lead contamination shows

promise, but cannot be firmly established at this time. Further study is needed in order to accept or reject elm leaf lead as a reliable indicator of lead contamination in the environment.

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APPENDIX A

ADDRESS OR SITE OF COLLECTION OF LEAVES FROM THE FOUR AREAS

ADDRESS OR SITE OF COLLECTION OF LEAVES IN MADISON

Sample No.	Address or Site
1 and 2	Northeast corner of Fifth Street and Lee Avenue
3 and 4	Northwest corner of Fifth Street and Lincoln Avenue
5 and 6	Just west of creek on East Center Street
7 and 8	In front of the Junior High School on West Center Street
9 and 10	Across the street from 416 Northwest Sixth Street
11 and 12	Garfield school playground Northwest area, between Harth and Lee Avenues
13 and 14	Southwest Fourth Street between Union and Liberty Avenues North side of the street
15 and 16	Southwest First Street between Olive and West Avenues South side of the street 801 Southwest First Street
17 and 18	North Chicago Avenue between Seventh and Eighth Street West side of the Avenue
19 and 20	Dakota State College campus lawn South side of East Hall
21 and 22	Northwest corner of Harth Avenue and Northeast First Street
23 and 24	Southeast corner of Liberty Avenue and Northwest First Street about 50 feet east of corner
25 and 26	912 Northwest Third Street
27 and 28	506 North Josephine Avenue
29 and 30	510 North Egan Avenue
31 and 32	204 Northwest Ninth Street First elm tree East of the creek
33 and 34	Northeast Ninth Street in shelter belt south of the high school about midway between east and west ends of shelter belt
35 and 36	Near Southwest corner of Ashmont Road and Fifth Street across Fifth Street from 916 Northeast Fifth Street

Madison (Continued)

37 and 38	East Highway 34 Northeast corner of Larry Green's yard
39 and 40	Southwest corner of South Garfield Avenue and Southeast Third Street
41 and 42	612 Northwest Fifth Street
43 and 44	219 South Union Avenue
45 and 46	Across Egan Avenue from 802 South Egan Avenue at entrance to Flynn Field
47 and 48	717 Southeast First Street
49 and 50	Northeast corner of Northeast Seventh Street and North Summit Avenue
51 and 52	Southeast corner of Northeast Ninth Street and Washington Avenue second tree south on east side
53 and 54	319 North Liberty Avenue
55 and 56	Southwest corner of Southwest Third Street and South Van Eps Avenue
57 and 58	822 Northeast Third Street along highway 34 north side
59 and 60	1/2 block west on Seventh Street from Egan Avenue north side

SITE OF COLLECTION OF LEAVES IN LAKE COUNTY

Sample No.	Site
1 and 2	1/4 of a mile east of the first corner north of The Dakota State College Field House
3 and 4	2 and 1/2 miles east of the first corner north of The Dakota State College Field House
5 and 6	3 miles east of the junction of highways 34 and 19 in Madison, 1 mile south and 1/2 mile east
7 and 8	2 miles south of Madison on highway 19, 2 and 1/2 miles east
9 and 10	4 miles south of Madison on highway 19 and gravel road 1 mile east
11 and 12	Lamonte Weise farm 1 mile west of the junction of highways 81 and 34 in Madison and 2 miles north
13 and 14	Graydon Rorhor farm 4 miles west of the junction of highways 81 and 34 in Madison and 2 miles north
15 and 16	Paul J. Hoff farm 4 miles west of the junction of highways 81 and 34 in Madison and 1 mile north
17 and 18	5 miles west of the junction of highways 81 and 34 in Madison and 2 miles south large abandon farm grove
19 and 20	3 miles west of the junction of highways 81 and 34 in Madison and 2 miles south and 1/4 mile west
21 and 22	1 mile west of the junction of highways 81 and 34 in Madison and 1 mile south, southwest corner of the intersection
23 and 24	Shelter belt extreme south end of Lake Herman State Park
25 and 26	M. Breuer farm 1 mile south of south end of Lake Herman State Park
27 and 28	2 miles of the junction of highways 19 and 34 in Madison 1/2 mile west, west end of shelter belt
29 and 30	1 mile east of the junction of highways 19 and 34 in Madison and 1 and 1/2 miles south
31 and 32	2 miles north of the junction of highways 81 and 34 in Madison and 1 and 3/4 mile east tree on north side of road

Lake County (Continued)

33 and 34	Elden Blase farm 3 miles north of the junction of highways 81 and 34 in Madison, 2 miles east and 2/5 south
35 and 36	3 miles north of the junction of highways 81 and 34 in Madison and 2 and 1/2 miles east shelter belt north side of the road
37 and 38	James Schrepel farm 3 miles north on first oiled road east of Madison and 1 and 1/5 miles east
39 and 40	3 miles east of the junction of highways 19 and 34 in Madison at intersection of graveled road and highway 34 Northwest corner
41 and 42	1 and 3/5 miles north of the junction of highways 81 and 34 in Madison on highway 81 farm east side of highway
43 and 44	Carl Meinert farm 3 miles north of the junction of highways 81 and 34 in Madison and 3/5 mile west
45 and 46	P. and G. Bohl farm 3 miles north of the junction of highways 81 and 34 in Madison 1 mile west and 1/10 mile north
47 and 48	Okke DeBoer farm 1 mile north of the junction of highways 81 and 34 in Madison and 2 and 3/10 miles west
49 and 50	George Krueger farm 1 mile north of the junction of highways 81 and 34 in Madison and 2/5 mile west elm tree on south side of the road
51 and 52	3 miles west of the junction of highways 81 and 34 in Madison and 1 mile south shelter best east side of the road
53 and 54	4 miles west of the junction of highways 81 and 34 in Madison and 7/10 mile south large grove west side of the road
55 and 56	2 and 3/10 miles west of the junction of highways 81 and 34 in Madison Along highways 81 and 34 north side
57 and 58	1 mile east of the junction of highways 19 and 34 in Madison and south about 100 yards southwest corner of farm grove
59 and 60	1 mile east of the junction of highways 19 and 34 in Madison and 1/2 mile north Northeast most tree in Graceland Cemetry

ADDRESS OR SITE OF COLLECTION OF

LEAVES IN SIOUX FALLS

Sample No.	Address or Site
1 and 2	2024 South Summit Avenue
3 and 4	515 West Twenty Ninth Street
5 and 6	1912 South Main Avenue
7 and 8	820 West Second Street
9 and 10	1015 South Summit Avenue
11 and 12	Across the street from 716 South Kiwanis Avenue Northwest corner of the Great Plains Zoo parking lot
13 and 14	Southwest corner of Twenty Sixth Street and Western Avenue about 100 feet south of corner west side
15 and 16	2705 South Holly Avenue corner of Holly Avenue and Larkin Drive
17 and 18	305 East Forty First Street
19 and 20	Southeast corner of Twenty Eighth Street and Sherman Avenue
21 and 22	712 South Covell Avenue
23 and 24	926 West Ninth Street
25 and 26	East Austin Street between Wayland and Blauvelt Avenues South side eastern most tree
27 and 28	Southeast corner of Thirteenth Street and Conkline Avenue
29 and 30	Northwest corner of Eighteenth Street and Van Eps Avenue
31 and 32	1215 South Willow Avenue
33 and 34	Southwest corner of Thirty Seventh Street and Hawthorne Avenue about 100 feet west of the corner
35 and 36	Tenth Street between Holly and Elmwood Avenues large tree south side about in the middle of the block
37 and 38	Between 506 and 510 North Duluth Avenue

Sioux Falls (Continued)

39 and 40	Eleventh Street between Dakota and Main Avenues largest tree on the north side of Washington High School
41 and 42	913 First Avenue South
43 and 44	1709 Seventh Avenue South
45 and 46	Southwest corner of Twelfth Street and Sneve Avenue
47 and 48	1308 South Tabbert Circle
49 and 50	Northeast corner of Thirteenth Street and Blauvelt Avenue About 100 feet east of the corner
51 and 52	Twenty Fourth Street and Menlo Avenue Twenty Fourth Street ends tree in middle of the end of the street east side
53 and 54	2316 South Third Avenue
55 and 56	Northeast corner of Fifteenth Street and Sixth Avenue tree about 100 feet north of corner on east side
57 and 58	Southwest corner of Fourth Street and Sherman Avenue tree 30 feet west on south side
59 and 60	Sherman Avenue between Walnut and Mulberry Streets tree in middle of the block east side

ADDRESS OR SITE OF COLLECTION OF LEAVES IN BROOKINGS

Sample No.	Address or Site
1 and 2	1363 Fifth Street
3 and 4	1017 First Street
5 and 6	Southeast corner of Forest Street and Medary Avenue
7 and 8	610 Seventh Avenue
9 and 10	1022 Eighth Avenue
11 and 12	Southwest corner of Fourth Street and Ninth Avenue
13 and 14	Northeast corner of Third Street and Fifth Avenue
15 and 16	317 Second Avenue
17 and 18	202 Eighth Street
19 and 20	1/2 block west of Medary Avenue on Eleventh Street south side Northwest corner of faculty parking lot
21 and 22	1211 Seventh Street
23 and 24	Southeast corner of Third Street and Twelfth Avenue
25 and 26	118 Sixteenth Avenue across Sixteenth Avenue from the west end of Olwien Street
27 and 28	Southwest corner of State Avenue and Ohio Drive
29 and 30	818 Eighth Street
31 and 32	Sixth Street across street from Ford Farm Equipment dealer
33 and 34	515 Fifth Street
35 and 36	223 South Seventh Avenue
37 and 38	Northwest corner of Fifth Street South and Main Avenue

Brookings (Continued)

39 and 40	Southeast corner of First Avenue South and Folsom Street
41 and 42	Southwest corner of Lincoln Lane and Olwien Street Second tree west
43 and 44	527 Twentieth Avenue
45 and 46	1405 Third Street
47 and 48	506 Eleventh Avenue
49 and 50	620 Main Avenue
51 and 52	Southwest corner of Second Street South and Fifth Avenue South
53 and 54	Eighth Avenue between Second and First Streets middle of the block west side
55 and 56	Eleventh Avenue first tree south of the sidewalk on east side of the avenue sidewalk going into United Ministries Building on the campus of South Dakota State University
57 and 58	Tenth Street and Circle Drive east side tree closest to fire plug
59 and 60	About 100 yards Northwest of the corner of Tenth Street and First Avenue west side of gravel road two metal fence posts near tree

APPENDIX B

TRAFFIC COUNT INFORMATION EXPRESSED IN

AVERAGE DAILY TRAFFIC

TRAFFIC COUNT INFORMATION FOR MADISON

Sample No.	Traffic Count
1 and 2	341
3 and 4	876
5 and 6	1029
7 and 8	1470
11 and 12	1190
13 and 14	820
15 and 16	1578
21 and 22	2575
23 and 24	790
25 and 26	324
29 and 30	4132
33 and 34	1033
45 and 46	956
49 and 50	235
51 and 52	2195
53 and 54	615
57 and 58	3284
59 and 60	354

TRAFFIC COUNT INFORMATION FOR BROOKINGS

Sample No.	Traffic Count
1 and 2	605
3 and 4	1963
5 and 6	2918
7 and 8	2107
9 and 10	444
11 and 12	1608
19 and 20	2394
21 and 22	646
23 and 24	4834
25 and 26	1289
29 and 30	4079
37 and 38	5700
39 and 40	216
41 and 42	520
45 and 46	3911
47 and 48	790
49 and 50	3658
51 and 52	2388
57 and 58	126

TRAFFIC COUNT INFORMATION FOR SIOUX FALLS

Sample No.	Traffic Count
11 and 12	7562
13 and 14	7134
17 and 18	5668
19 and 20	404
21 and 22	807
25 and 26	226
27 and 28	238
29 and 30	1256
33 and 34	686
39 and 40	11910
41 and 42	1826
43 and 44	1416
49 and 50	345
53 and 54	250

VITA

Robert Dale Buckman

Candidate for the Degree of

Doctor of Education

Thesis: DETERMINATION OF LEAD CONCENTRATIONS IN LEAVES OF THE AMERICAN ELM, ULMUS AMERICANA L.

Major Field: Higher Education Minor Field: Zoology

Biographical:

Personal Data: Born at Nampa, Idaho, December 22, 1935, the son of Ralph I. and Myrtle M. Buckman. Married to Norma Sue Gould, on December 22, 1971.

Education: Attended grade school at South Side Boulivard near Nampa, Idaho, went one year to Nampa Junior High School and one year to Nampa Senior High School. Graduated from Gayville High School, Gayville, South Dakota in 1953. Attended the University of South Dakota and graduated with a B. S. Ed. in 1963 and an M. S. in 1965. Graduate study at the University of South Dakota and the University of Minnesota. Completed the requirements for the Doctor of Education degree at Oklahoma State University in July, 1973, with a major in Higher Education and a minor in zoology.

Professional Experience: Taught high school biology and general science at Minneota, Minnesota 1963-1964, was a graduate assistant in zoology 1965-1966 at the University of South Dakota. From 1967 to 1972 have taught General Biology, General Botany, General Ecology, Conservation, Ornithology, Embryology, Cytoloty, and Special Methods of Teaching Biology in Secondary Schools at Dakota State College, Madison, South Dakota.

Professional Organizations: South Dakota Higher Education Faculty Association, National Education Association, South Dakota Academy of Science, Creation Research Society, South Dakota Ornithologists Union, Inland Bird Banding Association.