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BIOGEOCHEMICAL INVESTIGATIONS IN THE
PERCH LAKE AREA, CHALK RIVER, ONTARIO

(Report, 5 figures and 8 tables)

E. H. W. Hornbrook



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ABSTRACT

Biogeochemical investigations were conducted at a sample plot near Chalk River, Ontario, in 1966, primarily to determine the plant sampling density required to evaluate the heavy metal distribution in background areas. Secondary objectives were to test the performance of two mobile trailer laboratory units during transportation and while operating under field conditions and to examine the feasibility of conducting a biogeochemical and geochemical dispersion experiment at the sample plot site.

Plant and humus ash and mineral soil were leached with hot nitric acid and Pb, Cu, Zn, Mn, Ag, Ni, and Co were determined by atomic absorption spectrophotometry.

Heavy metal content is uniformly distributed, within acceptable limits, among like organ samples collected at 15 stations in the 100- by 120-foot plot. Plant organ samples collected on 100-foot station intervals will provide satisfactory data to evaluate heavy metal distribution in a background area.

The performance of the mobile trailer laboratories was entirely satisfactory and the execution of biogeochemical and geochemical dispersion investigations was found to be feasible.

BIOGEOCHEMICAL INVESTIGATIONS IN THE PERCH LAKE AREA, CHALK RIVER, ONTARIO

INTRODUCTION

Biogeochemical investigations were carried out to determine the density of sample stations required at a given location in a background area to obtain a representative value for the heavy metal concentration in plant organs. At a selected location near Perch Lake, 15 stations were established in a 100- by 120-foot plot, and in 1966 samples of the A, B and C soil horizons as well as bark, twig, petiole and leaf organs of trembling aspen were collected and processed. Analytical determinations for Cu, Pb, Zn, Mn, Ag, Co and Ni were completed in 1968.

Secondary objectives were (1) For the first time to test laboratory operations and equipment reliability in two new modified laboratory house trailers under field operating conditions, and the effect of transportation to and from the field on the equipment; and (2) To examine the feasibility of a biogeochemical and geochemical dispersion experiment in which certain elements would be placed in a trench on the uphill section of the sample plot and allowed to disperse downhill through the plot for several years. After dispersion, plant organs and samples of the soil horizons would be collected from the plot, analyzed, and the results compared to the original results to obtain information on the mobility of the elements examined.

Access to the sample plot is by private road, eastward from Highway 17 at Chalk River, Ontario, to the Atomic Energy of Canada Ltd. (AECL), plant on the west bank of the Ottawa River, and thence via fire trail roads B-2 and B-4 to the plot, a total distance of about 6 miles (Fig. 1). The sample preparation and analytical trailer laboratories were set up at the Petawawa Forest Experiment Station shown on Figure 1.

The sample plot (see Fig. 2) is near the Perch Lake basin, which was first investigated in 1955 in co-operation with the AECL as a possible site for the disposal of radioactive waste materials from the nuclear reactor. The porous, fractured bedrock, and the nature and distribution of the surficial sediments in the vicinity of the Chalk River plant of AECL were investigated by Gadd (1959). This area was chosen for the location of the sample plot because it is remote from any known sources of heavy metals and should have a low uniform element distribution characteristic of background areas.

Both trailers were prepared for transport and hauled 120 miles to the field site by standard tow trucks, unpacked, and equipment set up for routine operations in a three-day period.

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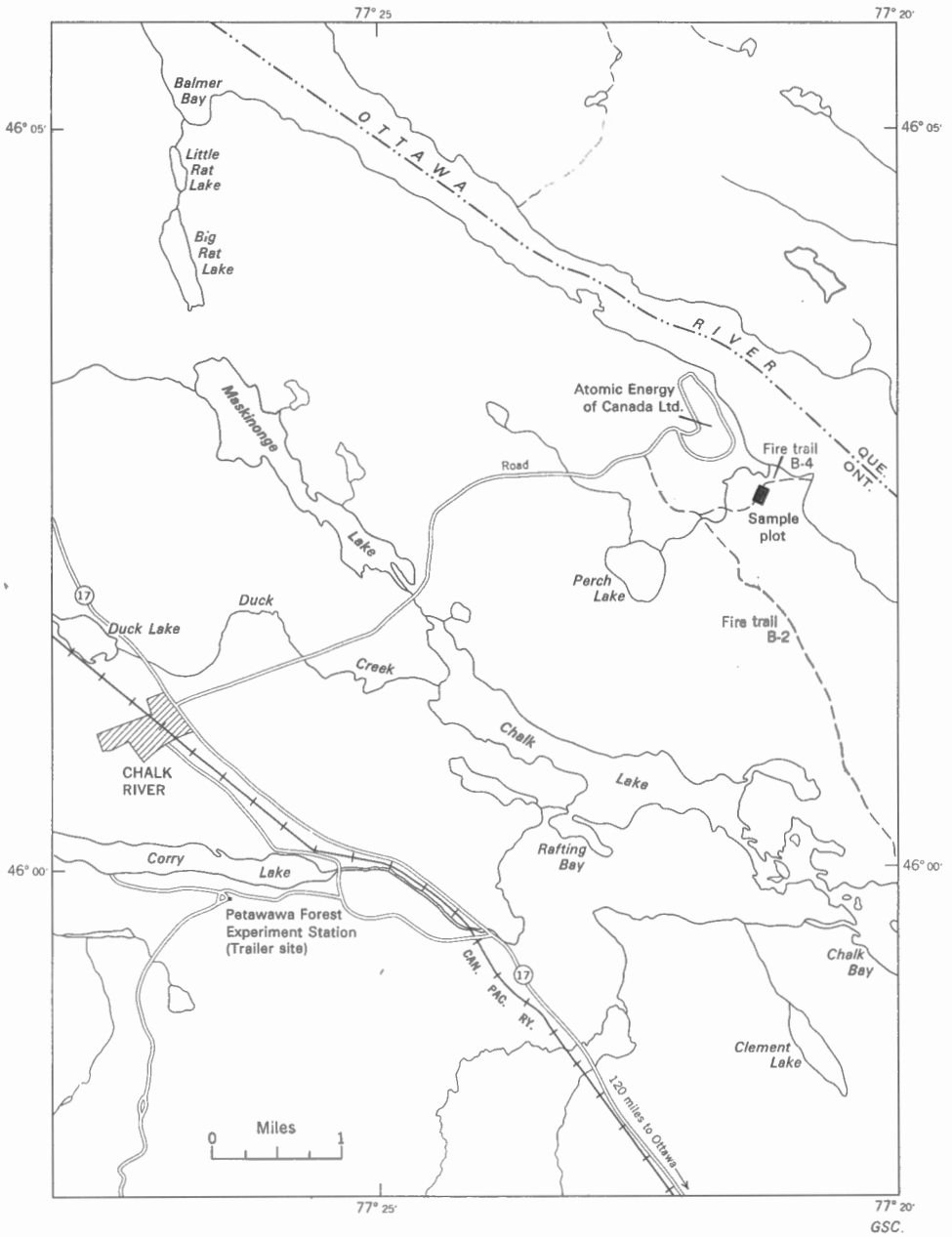


Figure 1. Location and access map showing the sample plot and trailer laboratory site at the Petawawa Forest Experiment Station.

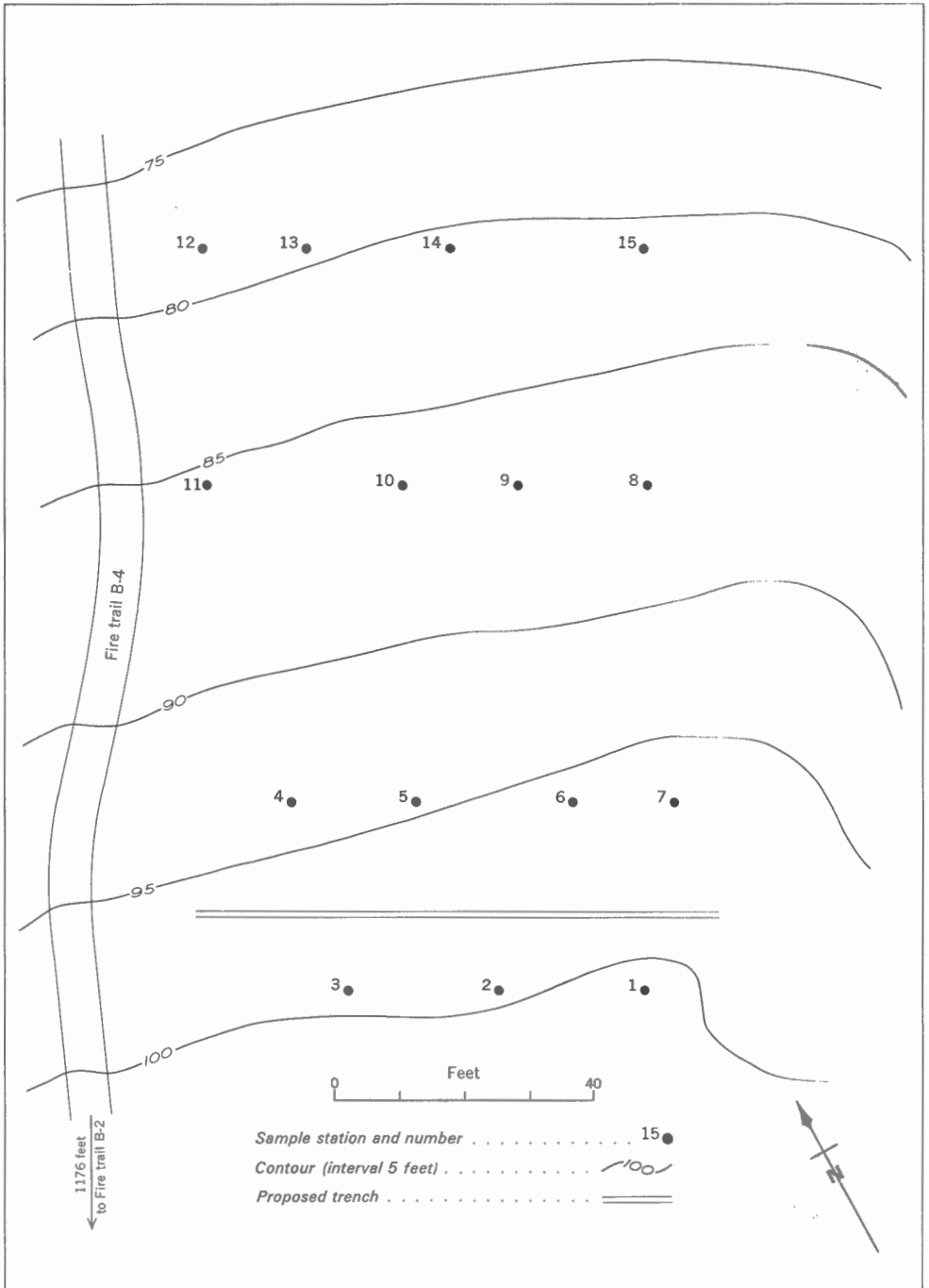


Figure 2. Plan of the sample plot.

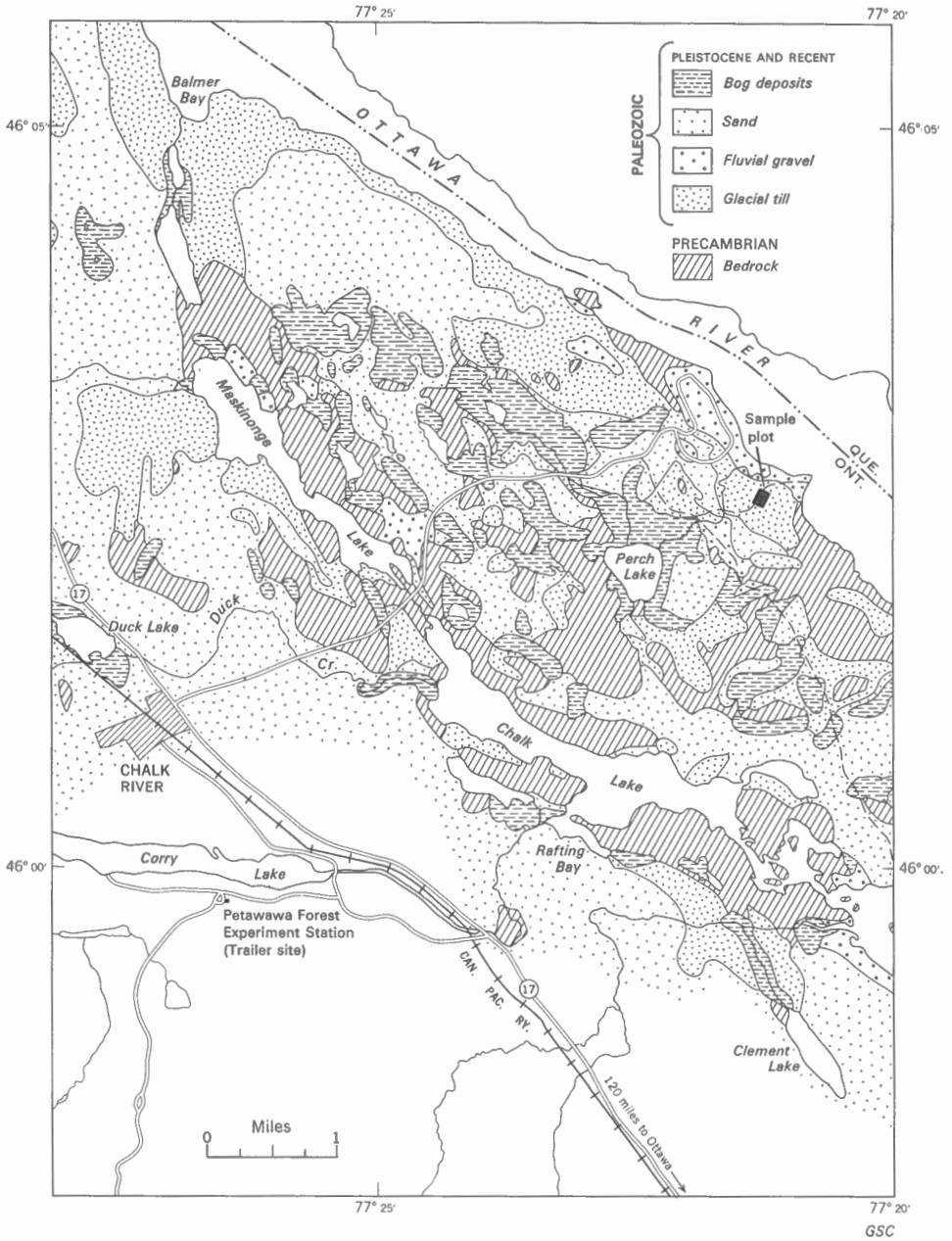


Figure 3. Surficial geology of the Chalk River area (after Gadd, 1963).

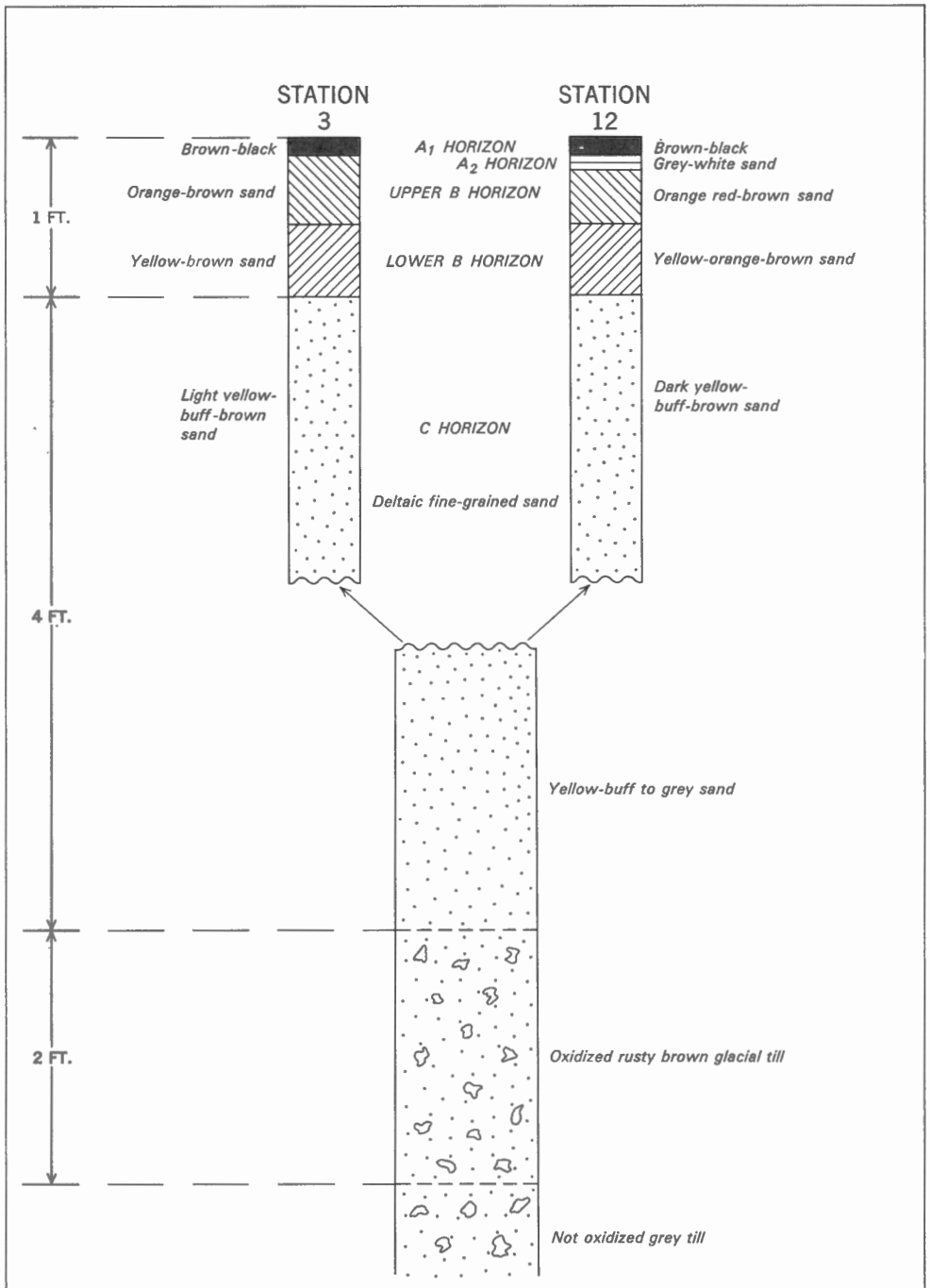


Figure 4. Typical soil development and variation of the sample plot.

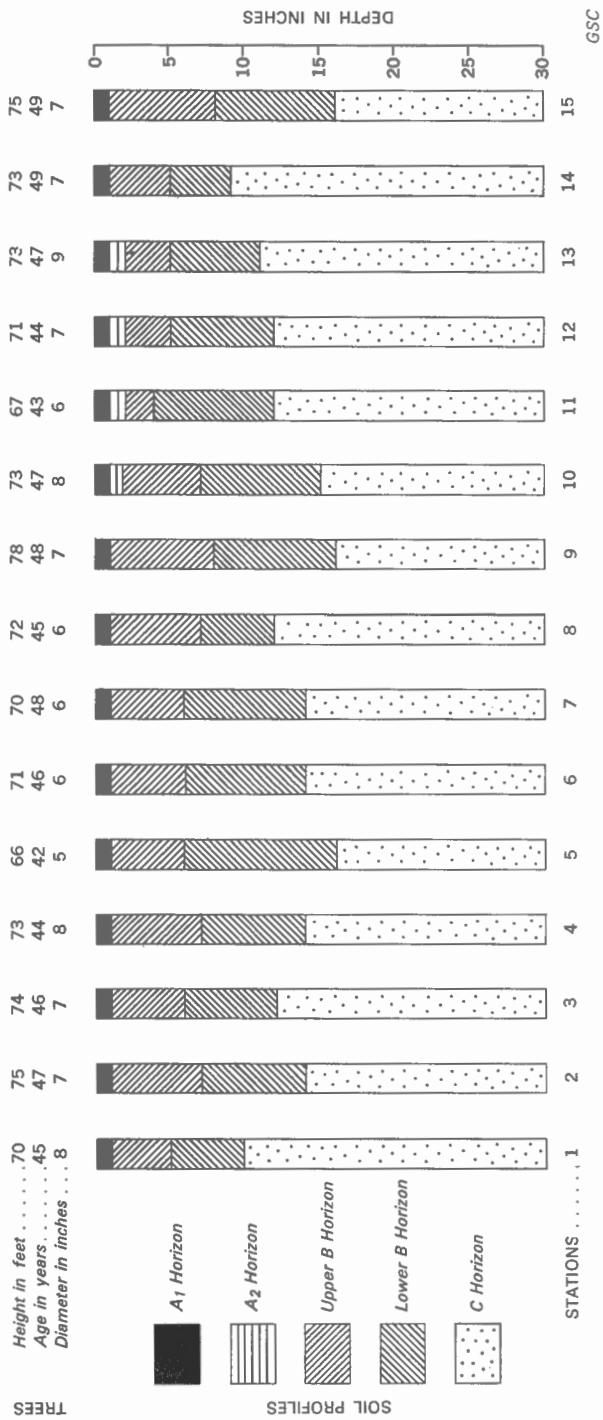


Figure 5. Soil profiles and age, height and diameter of trembling aspen sample trees at 15 stations.

Acknowledgments

The writer was aided in his field work and initial laboratory analyses by E. A. Debock and K. D. Wollin to whom he is indebted for their able assistance and excellent companionship. Thanks are due to Mr. Cullen and staff of the Petawawa Forest Experiment Station for their help and co-operation in providing the site and services for the trailer laboratories. The writer would also like to express his appreciation to J. A. C. Fortescue for his suggestion of the area for a biogeochemical investigation.

General Description of the Landscape

Surficial and bedrock geology of the Chalk River area (see Fig. 3), has been described by Gadd (1963) as follows: "Much of the area is underlain by pink and grey Precambrian gneisses, but a few outcrops of granite, pegmatite, amphibolite and Grenville-type metasedimentary gneisses occur in the area". There are no known metalliferous deposits in the vicinity of the plot.

Surficial material covering the area is composed of glacial till, gravel, or other glacially derived material, and/or younger sediments produced by postglacial erosion. The plot is situated on a well drained deltaic deposit of uniform, fine-grained, yellow-buff sand which is usually about 4 feet thick. Glacial till underlying the sand is composed of material from silt to boulder size in a heterogeneous mixture. A lower, undisturbed compact till is overlain by a softer oxidized layer, about 2 feet thick, of loose rubble composed of poorly sorted gravel and boulders derived from the reworking of glacial till with the removal of the fine material (see Fig. 4). Although the last regional ice movement was southwestward Gadd (1959), the local movement in the plot area was southeastward down the Ottawa River valley.

A brown podzol soil has developed throughout most of the area and two soil profiles illustrating the variations observed at the sample plot are shown in Figure 4. Individual soil profiles observed at each station in the sample plot are shown in Figure 5. The soil development is relatively uniform. The A₂ horizon is less than 1/4 inch thick at all stations except 10, 11, 12 and 13 where it is approximately 1 inch thick. Parent material of the developed soil horizons is the sand layer described previously.

Irregularity of the topography is shown by the variation from flat sand plains to a rolling hill type of upland terrain. The plot is on the northeast-facing slope of a former large island in the Ottawa River. This island was formed by a bedrock ridge elongated in a northwesterly direction and is presently covered with glacial deposits. The slope of the hillside at the plot is approximately 10 degrees to the east and the drainage off the hillside is northeastward to the Ottawa River.

Rowe (1959) has described the forest vegetation as being in the middle Ottawa section of the Great Lakes - St. Lawrence Region, as follows:

"The area is roughly U-shaped, comprising in the main a hard-rock upland which encloses the Palaeozoic lowlands of the upper St. Lawrence on all but the eastern side. The adjoining Section on the north and west (L. 4b) has a more boreal character, seen in the prominence there of balsam fir (Abies balsamea) and

white spruce (Picea glauca), while the Section at the extreme south (L. 1) differs in its pronounced affinities to the Deciduous Forest.

"The usual constituents of the upland forests are sugar maple (Acer saccharum), beech (Fagus grandifolia), yellow birch (Betula lutea), red maple (Acer rubrum), and hemlock (Tsuga canadensis), almost always accompanied by white pine (Pinus strobus) and red pine (P. resinosa). The last two species also characterize dry ridges and sand flats in association with jack pine (P. banksiana). Varying amounts of white spruce, balsam fir, aspen, (Populus tremuloides), white birch (Betula papyrifera), red oak (Quercus rubra) and basswood (Tilia americana) are present throughout. Rather common are hardwood and mixedwood swamps in which eastern cedar (Thuja occidentalis), tamarack (Larix laricina), black spruce (Picea mariana), black ash (Fraxinus nigra), red maple and elm (Ulmus americana) appear. A number of more southerly species, not characteristic but scattered here and there, are butternut (Juglans cinerea), bur oak (Quercus macrocarpa), white ash (Fraxinus americana) and black cherry (Prunus scrotina).

"The topography is irregular, varying from lowland flats to a strongly rolling type of upland terrain. The underlying bedrock of Precambrian granites, gneisses, schists and crystalline limestones is covered with glacial deposits, shallowly on the hills and more deeply in the valleys. Some of the landforms are drumlins, till-capped rock ridges and lacustrine plains, the latter resulting from post-glacial Lake Algonquin. Along the boundary north of the Champlain Sea re-entrant, marine silts and clays are spread in and out among the hills. One extensive area of marine modification is the Gatineau River valley as far north as Maniwaki. Brown podzolic and podzol soils are usual, with some development of brown forest profiles on calcareous materials."

Locally, trembling aspen (Populus tremuloides), is predominant on the northeast slope in the vicinity of the plot with an occasional white birch (Betula papyrifera) and red maple (Acer rubrum). Thin underbrush about the plot is mostly alder or young maple. Old burnt stumps, charcoal fragments in the humus and A₂ horizons, and the extremely uniform age of the aspen stand indicate that a forest fire burnt through the area at least 50 years ago. Figure 5 shows the age, height and breast height diameter of the trembling aspen sample trees.

The hillside containing the plot is completely undisturbed and there is little possibility of contamination from external sources.

DISCUSSION OF METHODS

Methods of Establishing the Sample Plot

The plot had to be situated in a landscape that had certain environmental features suitable for the requirements of this investigation including the possibility of a dispersion experiment. The location for the plot satisfied these requirements (1) Uniform vegetation with respect to species, age, height and foliage growth; (2) Sufficient density to permit resampling in the future; (3) Little and/or simple undergrowth plant community; (4) Gently

sloping daylight surface that is essentially planar; (5) Large enough area to permit a plot size of at least 100 by 100 feet; (6) Uniform soil development in a lateral as well as vertical sense. Further, it was necessary for the plot to be remote from any known sources of metalliferous contamination so that (1) values obtained for heavy metal concentrations would be of regional background content and (2) to obtain, as far as possible a uniform element distribution in plant and soil material.

After a satisfactory location was selected for the plot, boundaries were established by rope tied to convenient trees. Fifteen stations were selected in the plot and sample trees identified by numbers 1 through to 15 in preparation for sample collection. A plan of the sample plot is shown in Figure 2.

Methods of Collection and Preparation of Sample Material

The following materials were collected and prepared for analyses: leaves; petioles; first, second and third year twigs; lower, middle and upper bark (breast height, 1/3 and 2/3 of the main stem respectively); A_h horizon; upper and lower B horizon; and C horizon. C horizon was not sampled at stations 1 through to 7. Operational techniques used in the sample collection and preparation methods as described by Hornbrook (1969a) were modified and are reproduced in Appendix A. Ashing techniques and associated problems have been discussed in some detail by Fortescue and Hornbrook (1967).

Methods of Sample Analyses

Plant material was initially analyzed by the scan spectrographic method in the mobile trailer laboratories in the field at Petawawa and the soil material analyzed by colorimetric techniques in the Geological Survey laboratories at Ottawa in 1966. These techniques are described in Fortescue and Hornbrook (1967). Because the scan spectrographic method is a semiquantitative method the data were not entirely satisfactory for the purposes of this investigation. Therefore, in 1968 all samples were analyzed again by atomic absorption methods. Plant ash samples were analyzed in the field atomic absorption laboratories and soils were analyzed in the atomic absorption laboratories at the Geological Survey in Ottawa.

Briefly, the atomic absorption method of analyses involves the following operational procedures:

- 1) 200 mg of plant ash or soil are placed in a test tube and 3 ml of HNO₃ added.
- 2) Each tube is shaken and placed in a water bath at 90° C. The sample material is leached for one hour and the tubes are shaken every 10 minutes.
- 3) Tubes are removed and the sample solution diluted up to 10 ml with metal-free water. The tubes are again shaken, and the solution is allowed to stand for about three hours to permit suspended particles to settle producing a clear solution.
- 4) Sample solution is aspirated into the flame of the atomic absorption unit and percentage absorption is recorded.

- 5) Element concentration in ppm was obtained from percentage absorbance by use of a percentage absorbance against concentration in μ /ml standard curve and a conversion factor determined from the sample solution volume and original sample weight.

RESULTS

Presentation of Results

Data for element concentrations in plant and humus ash were converted to ppm and recorded on an oven dry basis. Ash percentages for each sample are shown in Table 1. Oven dry plant and humus data together with B and C horizon data are tabulated in ppm separately by element in Tables 2 to 8. This format was chosen to facilitate the comparison of each element's distribution among sample materials analyzed at each station.

Unfortunately Co, Pb, Ag and Ni content in B and C horizon samples was below the detection limit of the method used; hence concentration for each element is tabulated as less than the detection limit.

General Observations

An examination of, and a comparison among, element distribution in all sample materials warrants the following observations:

- 1) The humus horizon is enriched in all elements relative to other sample materials particularly for Pb, Co, Ni, and Mn. This type of enrichment was also observed by Hornbrook (1970) for Mo, Cu, Pb, Zn and Ni in British Columbia. Enrichment of the humus horizon relative to only the B horizon was observed at Cobalt, Ontario by Boyle and Dass (1967) for Pb, Zn, Co, As, Mo, Ag, Cu and Mn and by Warren and Delavault (1960) for Pb at numerous localities in Canada.
- 2) The concentration of all elements is consistently higher in leaves than in petioles.
- 3) Leaf organs preferentially concentrate Mn, Co, and Ni relative to other plant organs.
- 4) Pb is progressively concentrated in twigs; the maximum concentration being in third year twigs. Hornbrook (1969b) also found lead concentrated in twigs of black spruce Picea mariana and balsam fir Abies balsamea.
- 5) At a given sample station, the concentration of an element in different types of samples of the same tree organ is usually similar: for example, in Table 8, Ni concentration is similar in lower, middle and upper bark; and in Table 7 Co concentration is similar in first, second and third year twigs. However, for some elements such as Pb, the concentration is not similar and it shows an increase directly related to an increasing age of the twig sample as shown in Table 3. This emphasizes the fact that for some elements the concentration in a single sample tree

species not only varies among bark, twig, leaf organs etc. but also varies in different ages or types of the same plant organ. Therefore, it is important to collect exactly the same plant organ sample from the same sample species at every station to prevent false variation of element distribution among adjacent stations.

- 6) Occasional extremely high concentrations of an element, for example Pb in humus at station 7 or Mn in upper bark at station 2 in Tables 3 and 5 respectively, are probably erratic analytical determinations or a result of contamination from external sources.
- 7) Erratic behaviour of element distribution in humus can be attributed to the higher degree of variation in ash percentage from sample to sample as compared to plant sample ash percentage (see Table 1), but for some elements, such as Co, the distribution is relatively stable.
- 8) There is a suggestion of a possible trend, most obvious in Zn and Mn data, Tables 4 and 5 respectively, toward an increase in element concentration in those samples collected from stations situated in the lower downhill part of the plot. The suggested trend may be related to the change in the B horizon iron content that is manifested by the increased intensity of red and orange soil colours at stations 11 to 15 compared to uphill stations 1 to 10.
- 9) The ratio between the lowest and the highest element concentration for any 15 like samples is usually less than, and seldom exceeds 2:1. Where the ratio equals or exceeds 2:1, one, or at the most two or three, of the results for the 15 like samples will be high or low and the remainder relatively uniform. Otherwise results for all 15 like samples are relatively uniform. For example in Table 2, Cu distribution in upper bark shows high concentrations at stations 1 and 15 and uniform concentrations for the remaining stations; whereas in middle bark, Cu concentrations at all 15 stations are uniform by comparison. A somewhat unique development in high concentrations is demonstrated in Table 5 by the Mn distribution in all sample materials. A high Mn concentration persists for samples collected at adjoining stations 5 and 10 for all sample materials. No reason is suggested to explain the high Mn at these two stations. For some elements, such as copper in Table 2, the distribution in petioles is erratic but is uniform in all other materials.

Table 1. Ash percentages, plant and humus ash samples

Material	Station														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Leaves	4.55	5.68	5.41	6.94	5.85	6.86	5.54	5.76	6.04	7.32	5.66	7.04	7.07	6.18	5.61
Petioles	3.53	5.01	5.14	4.39	4.61	6.63	4.17	5.32	5.07	4.75	4.46	5.01	5.19	5.99	4.80
First Year Twigs	4.45	5.41	5.15	5.57	5.82	5.71	4.69	4.90	4.83	5.66	4.53	6.33	6.13	5.06	5.19
Second Year Twigs	3.77	4.73	4.61	4.73	4.82	4.20	3.76	4.10	3.93	5.07	3.76	5.28	5.31	4.77	4.29
Third Year Twigs	3.96	4.99	4.71	5.62	4.90	4.74	4.17	3.97	4.03	4.94	4.01	5.24	5.29	5.05	4.44
Upper Bark	6.22	4.02	3.95	4.85	5.46	4.03	4.23	3.92	3.62	4.24	5.30	4.65	4.73	4.96	6.29
Middle Bark	3.05	3.35	3.54	5.15	4.99	3.45	3.24	3.48	3.02	4.34	3.22	4.61	4.39	4.53	4.50
Lower Bark	3.48	3.25	2.66	5.39	4.69	2.69	3.02	3.28	3.25	4.14	3.40	4.91	4.66	5.01	4.78
Humus	61.35	77.42	21.84	43.80	32.51	34.38	69.40	27.57	33.45	25.49	35.06	43.19	49.83	36.55	42.12

Table 2. Copper determinations in ppm, soils, plants and humus

Element - Copper																	
Material	Station							Number									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Leaves	5	6	6	6	6	8	6	6	6	10	7	10	6	5	6		
Petioles	6	7	7	4	10	7	6	8	10	11	7	14	14	13	14		
First Year Twigs	7	8	7	10	10	8	7	8	8	9	6	9	10	8	10		
Second Year Twigs	6	7	7	10	8	7	6	7	7	7	5	6	8	7	7		
Third Year Twigs	6	7	7	9	6	7	6	7	7	7	5	6	8	7	6		
Upper Bark	2.1	1.8	1.3	1.6	1.3	1.5	1.2	1.6	1.5	1.4	1.8	1.4	1.5	1.5	2.2		
Middle Bark	1.7	1.3	1.2	1.6	1.8	1.2	1.4	1.3	1.3	1.3	1.4	1.4	1.3	1.6	1.6		
Lower Bark	3.3	3.4	4.0	2.8	3.5	5.1	3.9	2.6	2.6	3.3	2.8	2.7	3.5	2.9	2.7		
Humus	6.7	5.0	14.0	9.4	8.1	12.4	5.9	13.4	10.2	16.0	8.2	21.2	10.2	9.9	11.4		
Upper B Horizon	2.0	1.0	1.5	2.0	2.5	1.5	2.5	2.0	2.5	2.5	3.0	1.5	2.5	3.0	2.0		
Lower B Horizon	3.5	2.0	2.5	3.0	2.5	2.0	2.0	2.0	2.0	2.0	2.0	1.5	2.0	2.0	3.0		
C Horizon								3.5	2.5	3.0	3.0	3.5	3.0	3.5	5.5		

Table 3. Lead determinations in ppm, soils, plants and humus

[illegible]

Table 4. Zinc determinations in ppm, soils, plants and humus

Material	Element - Zinc														
	Station					Number									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Leaves	69	108	86	135	91	103	77	88	122	117	98	116	110	102	90
Petioles	60	75	70	134	61	67	60	63	82	84	46	94	99	104	84
First Year Twigs	74	85	74	117	108	94	71	68	82	100	74	100	95	92	96
Second Year Twigs	62	69	62	102	88	71	58	58	67	85	56	84	81	85	76
Third Year Twigs	69	81	72	103	86	84	70	67	77	96	73	88	93	101	80
Upper Bark	184	107	104	134	131	108	100	97	109	115	128	114	125	158	173
Middle Bark	99	85	82	158	107	97	81	84	92	100	84	108	122	124	104
Lower Bark	84	65	48	132	86	56	57	72	71	78	66	84	86	113	107
Humus	123	66	247	117	125	227	111	193	167	318	170	242	189	209	240
Upper B Horizon	19	12	19	11	17	25	17	21	21	22	23	17	16	14	28
Lower B Horizon	25	23	22	22	19	38	24	17	22	29	17	18	22	22	13
C Horizon								7	12	7	16	14	10	9	12

Table 5. Manganese determinations in ppm, soils, plants and humus

Material	Element - Manganese														
	Station					Number									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Leaves	166	202	184	240	428	295	197	230	305	473	195	255	380	346	290
Petioles	81	104	105	126	225	138	106	131	160	197	100	123	186	215	189
First Year Twigs	45	46	44	101	143	72	54	68	78	127	56	64	104	101	100
Second Year Twigs	38	44	41	87	120	63	52	56	64	107	38	57	92	81	72
Third Year Twigs	42	50	42	109	121	71	56	62	70	124	47	56	94	90	77
Upper Bark	72	41	41	50	108	55	48	52	53	84	55	40	71	90	111
Middle Bark	41	36	35	55	92	53	41	47	53	87	38	38	77	79	61
Lower Bark	35	31	20	45	75	32	30	36	39	66	29	32	58	68	62
Humus	760	700	1920	1430	960	1480	970	1470	1120	2200	1010	1420	2300	1550	1610
Upper B Horizon	160	350	55	80	130	140	60	75	75	240	85	130	70	135	180
Lower B Horizon	45	85	45	40	85	60	45	60	70	65	40	60	30	50	35
C Horizon								55	50	60	50	85	45	60	75

Table 7. Cobalt determinations in ppm, soils, plants and humus

[illegible]

MOBILE TRAILER OPERATIONS UNDER FIELD CONDITIONS

Information on the design, construction and the analytical equipment installed in both mobile trailer laboratories is given in Fortescue and Hornbrook (1967).

In 1965 the sample preparation trailer, with some of its facilities installed, was used in the field at Fraserdale, Ontario. The analytical laboratory trailer remained in Ottawa.

In 1966, for the first time, as a mobile unit, both trailers were transported to Petawawa, Ontario and set up at the Department of Forestry, Petawawa Forest Experiment Station. Both trailers were hauled by commercial tow trucks to the site, about 120 miles from Ottawa. At Petawawa, the trailers were jacked up, levelled, and supported at 8 positions on the frame by concrete blocks for stability during operations. In one day, power, water and drainage services were installed, all equipment unpacked and the analytical facilities prepared for routine operations. Holman and Durham (1967), have described problems in transporting and setting up a similar mobile laboratory.

No breakage or loss of equipment occurred during transportation to and from the field and sample preparation and analytical facilities performed satisfactorily under field operating conditions.

BIOGEOCHEMICAL AND GEOCHEMICAL DISPERSION EXPERIMENT

The feasibility of conducting, in the future, an experiment on the relative mobility of certain elements was examined during this investigation. Some features of the environment at the plot were required only because of the possibility of future sampling for this experiment.

The suggested procedure would include the following stages: A 4-foot-deep trench would be excavated parallel to and upslope from stations 4, 5, 6 and 7 as shown in Figure 2. The contact between the sand layer and the oxidized till (see Fig. 4), would be the bottom of the trench. Material containing known amounts of selected elements would be distributed along the trench floor at a uniform thickness. After the trench was filled in the plot would remain undisturbed for a period up to several years. Throughout this period, parameters affecting dispersion in the plot would be recorded. During the undisturbed period migration of elements would take place downhill in solution in the soils and with nutrient uptake in the trees. At the end of the period, a repeat sampling program would be carried out at the plot and the analytical results would be compared to those of the present investigation. The comparison would provide information on element migration in soils and plants and lead to a better understanding of geochemical and biogeochemical dispersion.

The sample plot appears to be suitable for the experiment because of the previously described features of the environment and the relative uniformity of the element content of various materials.

SUMMARY AND CONCLUSIONS

Element distribution throughout the plot is essentially uniform, considering the general observations listed previously and that the degree of variation in element concentrations among 15 like samples is within an acceptable range. Element concentrations determined in samples collected from any one station in the plot are representative in almost all cases of similar samples collected from the remaining stations. Therefore plant organ samples collected on 100-foot station intervals will provide representative data to evaluate the heavy metal distribution in this and similar background areas.

Although this study was carried out in a background area with low element concentrations, the assumption can be made that a similar situation exists in an area where anomalous element concentrations are present, except that the effective area represented by the samples from one station would probably be reduced.

The performance of the mobile laboratory trailer units was satisfactory during transportation to and from the field and during sample processing and analytical operations under field conditions.

The possibility of conducting a dispersion experiment at the sample plot was examined and found to be feasible. The experiment may be carried out in the future.

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APPENDIX A

Sample Collection and Preparation Methods

At each station the A_h horizon was sampled for a humus sample first to avoid any possible contamination by the mineral soil horizons. Next the sample tree was cut down and the foliage and bark collected. Wooden discs, from which the bark samples are removed, were cut from the main stem at breast height, 1/3 and 2/3 of its length. Foliage was systematically collected from all parts of the tree, as branches of combined third, second and current growth twigs and leaves. A clean cut, wooden disc from the main stem at breast height was retained for growth ring count to determine the age of the tree. The soil pit was dug with a mattock where the humus was sampled as close to the sample tree as possible. Representative samples of the upper and lower B horizon and C horizon were collected using hand tools. All samples were put in paper bags having a high wet strength in the event of high sample moisture content.

In the sample preparation laboratory, foliage, bark and humus were cabinet dried at 50°C (for up to three days in the case of wet humus). The samples were then subsampled: foliage was separated into first, second and third year twigs and leaves and petioles; bark was reduced to small fragments; they were then oven dried at 80°C. B and C horizon samples were cabinet dried as long as necessary and then sieved to obtain the minus 80 mesh portion for analyses. The plus 80 minus 10 mesh portion of the humus samples was retained in sealed plastic vials.

A 10-gram amount of each sample of plant material or humus was dry ashed in a muffle furnace on a time-temperature controlled cycle in which the temperature slowly increases to 435°C and then is maintained at that temperature for the remainder of the 10-hour cycle. The ash of each sample was thoroughly mixed and stored in sealed plastic vials.