EFFECT OF SULFUR DIOXIDE
ON
EPIPHYTIC LICHENS AND BRYOPHYTES

by

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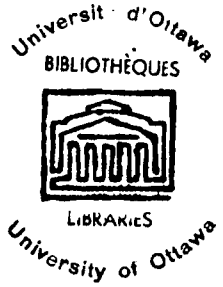

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ABSTRACT

The effect of sulfur dioxide on epiphytes was investigated by methods comprising field studies, transplantation and laboratory experiments.

In the field studies, an inventory of the epiphytic flora for the region of Wawa, Ontario, was made on the basis of the investigation of 48 sites. A total of 110 epiphytic species (71 lichens, 29 mosses and 10 liverworts) were recorded. The distribution of sulfur dioxide emitted by an iron-sintering plant in the area, as well as the pH and sulfate content of surface-water, soil and vegetation, were determined. The fallout pattern of SO_2 and the chemical indices (pH & SO_4^{--}) of water and soil were correlated with the epiphytic flora of the region. With a rise in the concentration of SO_2 in air and of SO_4^{--} in water and soil, a marked reduction in the epiphytes, both as to the number of species and their abundance, was noted. There was also a significant decrease in the sulfur content of the vegetation with an increase in the distance from the center of pollution. According to the pattern of pollution, the investigated region can be delineated into five zones, corresponding with the epiphytic flora. From this survey it has become apparent that there are various degrees of toxiphoby among epiphytes (from highly toxitolerant to highly toxisensitive species), and that there are several species of lichens and mosses, the absence of which can serve as indicators of SO_2-pollution.

In the transplant experiments, forty-two circular discs (diam. 4.8 cm), bearing 19 species of mosses and lichens, were cut from the bark of trees growing in unpolluted areas near Ottawa, Ontario. These discs were transplanted onto trees in the region of Sudbury, Ontario, where the atmosphere is heavily polluted by sulfur dioxide. After one year, the majority of the epiphytes growing on these transplanted discs were either dead or seriously damaged, and only a few species such as Bacidia chlorococca and Parmelia sulcata appeared to be toxitolerant. Thalli of Parmelia caperata and P. sulcata, collected from such discs, showed abnormal features on microscopic examination such as: (i) marked reduction in the thickness of the thallus; (ii) formation of a thin layer of a whitish, crystalline, water-insoluble and acetone-soluble substance on the upper surface of the thallus; (iii) plasmolysis, chloroplast damage, and formation of oil-globules in Trebouxia cells, the algal symbiont; and (iv) formation of chlamydospore-like bodies by the fungal symbiont, especially in the lower cortex of the thallus. From these observations it is evident that the epiphytes are affected to various degrees, both externally and internally, by SO_2-pollution.

In the laboratory experiments, thalli of Xanthoria fallax, X. parietina, Parmelia caperata and Physcia millegrana were exposed to 5 ppm sulfur dioxide for 24 hours under various conditions of humidity. Abnormalities such as bleaching of the chlorophyll, permanent plasmolysis, and the formation of sporadic brown spots on the chloroplasts were

observed in the algal cells. Sulfurous acid and Mg^{++} were detected in the extracts of the SO_2-exposed thalli. Sulfate concentration increased in thalli exposed to SO_2 in increased humidity. The absorption spectrum of chlorophyll extracted from SO_2-exposed thalli showed a maximum absorption at 667 mμ characteristic of phaeophytin-a, thus indicating the degradation of chlorophyll-a into phaeophytin-a under the influence of SO_2. These observations are of interest with regard to the well known sensitivity of lichens to atmospheric pollution.

RESUME

Au cours des études sur le terrain, nous avons fait l'inventaire de la flore de la région de Wawa, Ontario. Au total nous avons récolté 110 espèces (71 lichens et 39 bryophytes). Nous avons déterminé le pH et la teneur en sulfate des eaux de surface, du sol, et de la végétation; nous avons également essayé d'établir une corrélation entre ces données et le taux de SO_2 présent dans l'atmosphère. Nous avons subdivisé la région de Wawa en 5 zones, ou degrés de pollution, en nous basant sur l'intensité des dommages faits à la végétation, sur le nombre d'épiphytes présents et sur la quantité de sulfate accumulé dans le sol. Nous avons également établi une liste des épiphytes d'après leur degré de toxiphobie.

Nous avons transplanté des échantillons d'écorce sur lesquels poussaient des épiphytes dans la région de Sudbury où l'atmosphère est très polluée par le SO_2. Après un an la majorité des mousses et lichens étaient morts ou avaient subi des dommages considérables. Un examen au microscope du thalle de certains lichens nous a révélé des changements importants aussi bien chez l'algue-gonidie que chez les hyphes du champignon.

Au cours d'expériences de laboratoire nous avons soumis des thalles de lichens épiphytiques à l'action de 5 ppm de SO_2 durant 24 heures et à des degrés variés d'humidité. Des analyses chimiques et des examens au microscope nous ont révélé des changements morphologiques et physiologiques causés par le SO_2 au thalle des lichens.

GENERAL INTRODUCTION

Lichenologists have known of the detrimental influence of cities on lichen growth for almost a century. Already in 1866 Nylander commented on the sparse lichen vegetation on the trees in Paris thus: "Les lichens donnent à leur manière la mesure de la salubrité de l'air et constituent une sorte d'hygiomètre très sensible". Arnold (1891-1901) made the same observation in Munich. Sernander (1926) notes that large parts of central Stockholm are completely devoid of lichens. Erichsen (1928 ; Hambourg), Haugsja (1930; Oslo); Vaarna (1934; Helsinki), Bouly de Lesdain (1948; Paris), Sauberer (1951; Vienna), Jones (1952; Birmingham); Vareshi (1953; Caracas), Steiner and Schulze-Horn (1955; Bonn), Beschel (1958; Innsbruck), Brightman (1959; Belfast), Magdefrau (1960 Munich), LeBlanc (1961; Montreal), DeSloover (1962; La Dendre, Belgium), Skye (1964; Stockholm), Gilbert (1965; The Tyne Valley, England), Brodo (1965; Long Island, N.Y.), and many other workers have contributed to our knowledge of the effect of towns upon lichen distribution. In most cases the studies have been limited to the epiphytic species of lichens and mosses because they seem to be much more sensitive than the terricolous species.

Two major explanations have been offered for this extreme sensitivity of epiphytes to urban areas. Some authors as Rydzak (1959) ascribe it to the climatic dryness resulting from the efficient drainage system in towns, but

most people like Jones (1952), Skye (1958), Fenton (1960), Gilbert (1965), and many others attribute it to atmospheric pollution. Barkman (1958) has critically reviewed these two hypotheses, drought or toxic gas and concludes that the harmful effect of drought upon epiphytes is undeniable but that of air pollution seems to be much more important. We will see that there is a direct relationship between the damaging effects of sulfur dioxide on the lichen thallus and the relative humidity at which the thallus had been exposed to the gas. It appears, therefore, that the "xeric" conditions, found in cities, would lessen, to some extent, the harmful influence of the toxic gas. The scarcity of lichens in wide areas around cities, particularly where humidity is fairly high, suggests dryness to be only a secondary cause. LeBlanc (1961) pointed out that in the center of the epiphyte "desert" of Montreal there is a small uninhabited island where the forest vegetation has been well preserved, a beautiful Acereto-Ulmetum laurentianum (Ulmus americana, Acer rubrum, A. saccharinum, and Fraxinus nigra). The water table remains high even in summer and, as a result, the soil keeps either permanently wet or humid. Because of the density of the vegetation and the hygric condition of the soil, the relative humidity is usually high and the island should be an ideal habitat for the growth of epiphytic mosses and lichens. Nevertheless, the 64 trees examined in a 200-square-meter quadrat were devoid of epiphytes, except for the presence of a crustose lichen (Bacidia chlorococca) and an unicellular green alga

(Protococcus viridis), which are apparently quite toxitolerant. In this case, it seems evident that air pollution and not dryness is the limiting factor. Moreover, it is believed that lichens and mosses accumulate pollutants, because they have a tendency to accumulate substances, e.g., minerals, very efficiently (Gorham 1959; Smith 1960). If such is the case, large quantities of toxic substances can accumulate, over a long period of time, in these perennial organisms.

The pollution hypothesis is supported by laboratory investigations. Pearson and Skye (1965) reported a normal pattern of photosynthesis in lichens which had been dried for 4.5 to 6 months in the laboratory. To observe the long term effect of desiccation, we tested the viability of the algal symbiont of a thallus of Xanthoria parietina which had been kept dry for one and one-half years under continuous illumination and then for the same length of time under continuous darkness. When a small portion of this thallus was grown on agar medium with Bristol's solution (pH 5.6) in the light, we observed after eight days the formation of aplanospores. The ability to produce spores indicated that even after three years of drying the algal symbiont was still alive.

The various studies made on epiphytes in relation to pollution have enabled us to understand the distribution pattern of epiphytes, especially lichens, in the polluted areas and also to appreciate their value as indicators of air pollution. Why epiphytes are so sensitive to pollution is still

unknown and the aim of this study is to obtain some information in this direction.

Atmospheric pollution has many components. Two major components are solid matter, or soot, and gaseous sulfur dioxide. For lichens there is a considerable body of evidence that SO_2 may be the main harmful component (Skye 1958; Brightman 1959). Consequently, sulfur dioxide has been singled out as the toxic agent for the purpose of the present investigation, which comprises the following three approaches:

i) field studies: to determine the distribution pattern of epiphytes in an area polluted from a single source and to correlate this distribution with the amount of SO_2 found at various distances from the source of pollution in order to find species which can serve as biological indicators of atmospheric pollution,

ii) transplant experiments: to observe the macro- and microscopic changes produced in epiphytes, especially lichen thalli, transplanted from an unpolluted area to an industrial area having a constant source of SO_2-pollution, and

iii) laboratory experiments: to determine the effect of SO_2 on the algal symbiont of lichen thalli exposed to a known concentration of the gas under controlled moisture conditions.

Also, an attempt was made to investigate the effect of sulfur dioxide on lichen substances which are special organic compounds encrusted on the surface of the hyphae of the fungal symbiont in the lichen thallus (Hale 1961). It has been suggested that these substances help lichens to obtain essential metallic elements from their substrates (Schatz 1963), increase the cell permeability of the algal symbiont (Follmann 1960), and inhibit bacterial or mold decomposition of the thalli (Harder and Uebelmesser 1958). In view of the above suggestions it is evident that a degradation of the lichen substances can upset lichen symbiosis. We compared color reaction and fluorescence under ultraviolet irradiation of some of the lichen substances, e.g., atranorin, usnic acid and parietin, isolated from extracts of the normal and the SO_2-exposed thalli, but we did not find any appreciable difference between the two conditions. Our investigations in this connection, however, led us to suggest a possible role of atranorin in the utilization of low light intensities and of usnic acid and parietin in the quenching of high light intensities in the lichen thallus. These findings, which do not relate to the effect of SO_2, have been included in appendix.

PART 1

EFFECT OF SULFUR DIOXIDE ON EPIPHYTIC LICHENS AND BRYOPHYTES: FIELD STUDIES IN WAWA

An investigation of polluted areas for the presence of factors, such as sulfur dioxide, should provide information to support or negate the presumption that the scarcity of epiphytes in cities and industrial areas is caused by some chemical factor in the environment to which these organisms are sensitive. If it could be possible to select an area in which the degree of pollution gradually decreases along a line transect from a single source of pollution and if transitions in epiphytic vegetation occur along this transect, then one could perhaps demonstrate a direct correlation between the degree of pollution and the disappearance of epiphytic vegetation.

Such an area is found in Wawa, where air-pollution from a single iron sintering plant has affected the vegetation primarily in one direction, i.e., north east from the source of pollution under the influence of the prevailing SW winds.

Wawa, 192 km north of Sault Sainte-Marie, is situated at latitude 48° N and longitude 84° 48' W, at an elevation of 210 m above sea level. The country is an unbroken succession of rocky hills and ridges with numerous flats along the river sides and lake shores. The underlying

granitic rocks are of Precambrian age (Collins et al. 1926). The area is drained by the Magpie river into Lake Superior situated at a distance of nearly 10 km south of the sinter plant. As regards the natural vegetation, it is basically part of the Boreal Forest but it also contains certain species from the Great Lakes - St. Lawrence Forest Region either as scattered individuals or as more or less isolated patches (Rowe 1959). The dominant forest association is mixed in character, consisting of Abies balsamea, Picea mariana and Betula papyrifera with scattered individuals of Picea glauca and Populus tremuloides. Sand terraces along the rivers are colonized by Pinus banksiana, which also associates with Picea mariana on poor, rocky soils.

The sinter plant operations were begun in 1939 and the plant was greatly expanded in 1949. It concentrates nearly 2,804,480,000 kg (2,800,000 long tons) of iron carbonate ($FeCO_3$) and pyrite (FeS_2) ore per annum to produce nearly 1,001,600,000 kg (1,000,000 long tons) of sinter product for blast furnace feed. During the sintering process the pyrite is burnt and its sulfur content is reduced from 4.5% to 0.09%; thus about 100,160,000 kg (100,000 long tons) of sulfur per year are emitted from the sinter plant (The Algoma Steel Corporation, Limited, Bull. 1964). Most of the sulfur is converted to sulfur dioxide and a small percentage is oxidized further to the trioxide stage by the following reactions:

$$4FeS_2 + 11O_2 \longrightarrow 2Fe_2O_3 + 8SO_2$$

$$2SO_2 + O_2 \longrightarrow 2SO_3$$

The formation of sulfur trioxide may be increased by using excess oxygen and by the catalytic action of ash constituents, especially iron oxides (Engdahl 1962). Sulfur dioxide can also be oxidized directly into SO_3 by a photochemical reaction, which is now known to be of the first order with respect to SO_2 in an excess of O_2 in intense natural sunlight (Gerhard and Johnstone 1955).

The oxides of sulfur are emitted into the atmosphere through two stacks 76 m tall with inside diameters of 4.7 m and 4.9 m at the top. As the smoke plume leaves the stacks, fallout of pollutants begins in the area. Depending on the prevailing meteorological conditions and turbulence, diffusion usually reduces considerably the sulfur dioxide concentration to a low level at some distance from the source. In the presence of moisture sulfur dioxide forms sulfurous acid, a strong reducing agent which is slowly oxidized to sulfuric acid, resulting in an irritant mist which is often responsible for the bluish-white plumes emitted in industrial and power-plant operations. The rate of conversion of SO_2 to SO_3 or to H_2SO_4 is of special interest, because the toxicity of sulfuric acid is about 20 times that of sulfur dioxide (Gartrell et al. 1963). Sulfuric acid is soon converted to sulfates such as ammonium, and especially, calcium sulfate (Johnston and Coughanowr 1958).

Since sulfur dioxide is eventually oxidized to sulfate, it is the fallout of the dissolved sulfate that was determined to find out the distribution of SO_2 in the Wawa area. Gordon and Gorham (1963) observed that the concentration of soluble

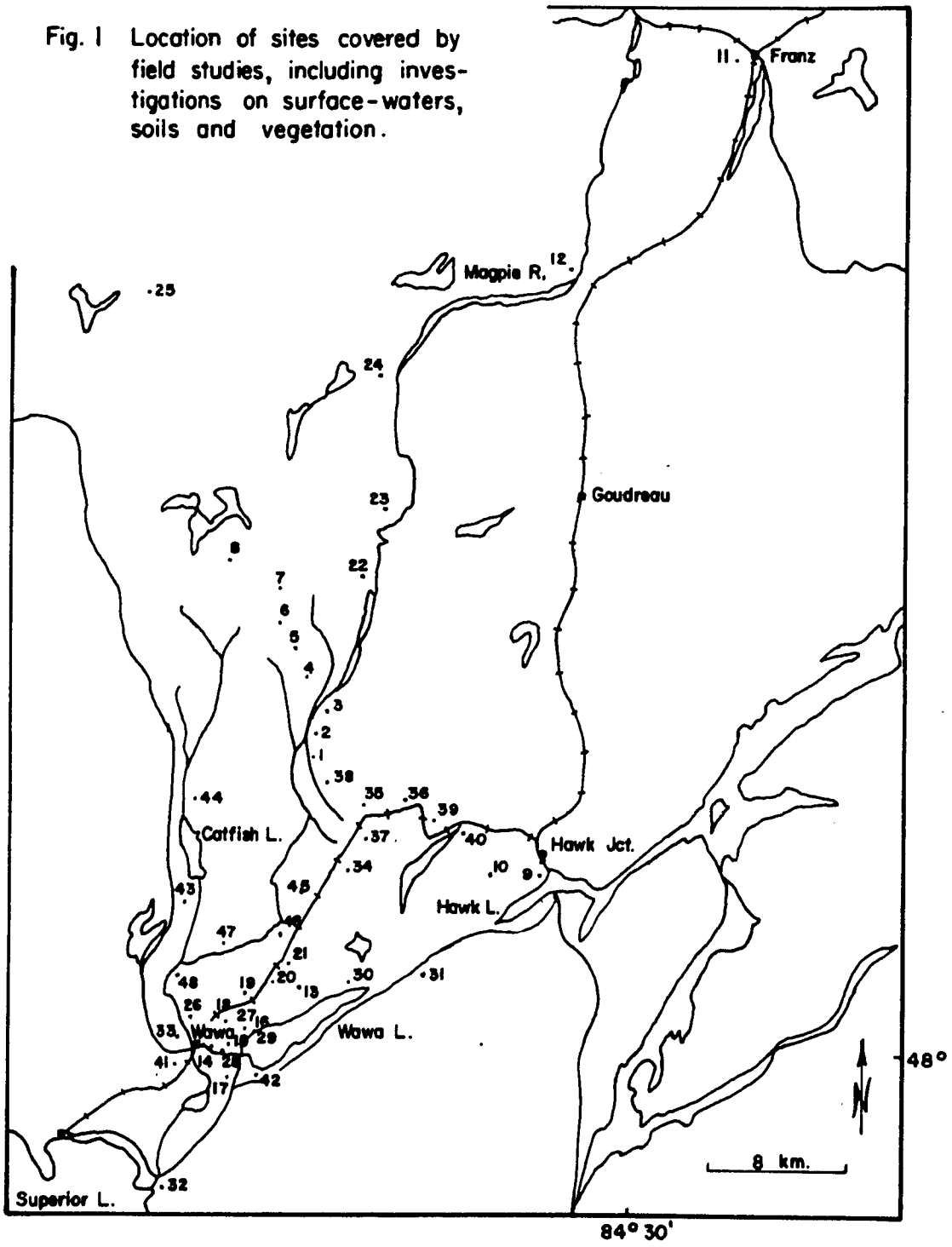
sulfate in water and soil samples was a good indicator of sulfur dioxide pollution.

MATERIALS AND METHODS

During the summer of 1965, we selected 48 sites in all directions from the sinter plant (Fig. 1) for a study of the epiphytic flora, and for chemical investigation of water, soil and plant materials. At each site we tried to analyse the epiphytic flora of Betula papyrifera, Populus tremuloides, Picea mariana, Abies balsamea, Pyrus decora, Alnus rugosa and Salix humilis, which are the most common trees and shrubs in the region. One or two (rarely 3) of the large-sized trees of each of the above species were chosen at random, except, of course, in the heavily polluted areas where the living trees were of small size only. Circular quadrats 30 cm in height were traced around the base and at breast height (ca. 1.5 m) around the trunk of each tree, and a tally of all the epiphytes therein was made. A complete list of epiphytes (lichens and bryophytes) was drawn, and their zonation on the tree (base/trunk) was recorded. At each site where there were no epiphytes, an inventory, as complete as possible, was made of terricolous and saxicolous lichens and bryophytes.

Fallout patterns, i.e., the dispersion of sulfur dioxide with distance, were determined by comparing the concentrations of the atmospheric sulfur dioxide and the soil sulfate at various sites. Correlation between these patterns and the transitions in epiphytic vegetation was sought in the hopes

Fig. 1 Location of sites covered by field studies, including investigations on surface-waters, soils and vegetation.



of finding useful pollution-indicator species. In areas where no epiphytes were present, the terricolous and saxicolous species were used in an assessment of tolerance to pollution.

Water, soil and plant samples

For chemical investigations, samples from the surface waters of lakes having restricted drainage were taken in 125 ml polyethylene bottles. Five drops of a saturated solution of mercuric chloride were added to each bottle to stop the activities of micro-organisms (Hellwig 1964) and the samples were stored for later determinations of sulfate concentration, specific conductance and total hardness as $CaCO_3$. The pH was determined on site. Samples of surface-soil, collected in 4" x 3" polyethylene bags, were air-dried and stored for later determination of soluble-sulfate concentration. The pH was determined immediately in the field by taking a 1:2.5 soil-deionized water suspension. Samples of epiphytes (lichens and bryophytes), leaves of Betula papyrifera along with specimens of Pohlia nutans and a Cladonia species from epiphyte-free areas, were air-dried and stored in paper envelopes for pH and sulfate determination.

Determination of pH, sulfate content, specific conductance and total hardness as $CaCO_3$

Hydrogen ion concentration was determined with a portable, direct reading pH Meter manufactured by Aagaard Nielsen and Schroder, Copenhagen, Denmark. A glass electrode

type G202A and a calomel electrode, type K401 were used. All determinations were made at a temperature of 20°C.

Sulfate content was determined by a turbidimetric method (Tonnies and Bakay 1953). The following reagents were used:

1. Ethanol-glycol mixture (40%). 450 ml of absolute ethanol were mixed with 550 ml of dipropylene glycol. To obtain a 40% solution, 400 ml of this mixture was mixed with 500 ml of sulfate-free water. After adding 9.5 ml of HNO_3 (sp. gr. 1.42), the mixture was cooled and diluted to a final volume of 1,000 ml with sulfate-free water.

2. Barium chloride solution. 1.34 M Barium chloride solution was prepared with sulfate-free water. After standing for at least three hours, this solution was filtered through a fine sintered-glass filter. Six ml of the filtrate were diluted to 120 ml in a graduated cylinder with sulfate-free water and finally made up to 200 ml with 40% ethanol-glycol mixture.

A calibration curve for sulfate was prepared (Fig. 2) by taking 1, 2, 3, 4, and 5 ml aliquots of a standard sulfate solution (100 mg $(NH_4)_2SO_4$ in 100 ml of reagent No. 1) in a series of tubes. Reagent No. 1 was added to bring the volumes in each tube to 5 ml. Then 0.2 ml of 6 N HCl and 5 ml of $BaCl_2$ reagent were added to each tube. The contents were mixed immediately and allowed to stand for 15 minutes. The optical density (O.D.) was measured at 420 mμ in a Coleman Model 6A Junior Spectrophotometer using 19 x 150 mm round cuvettes,

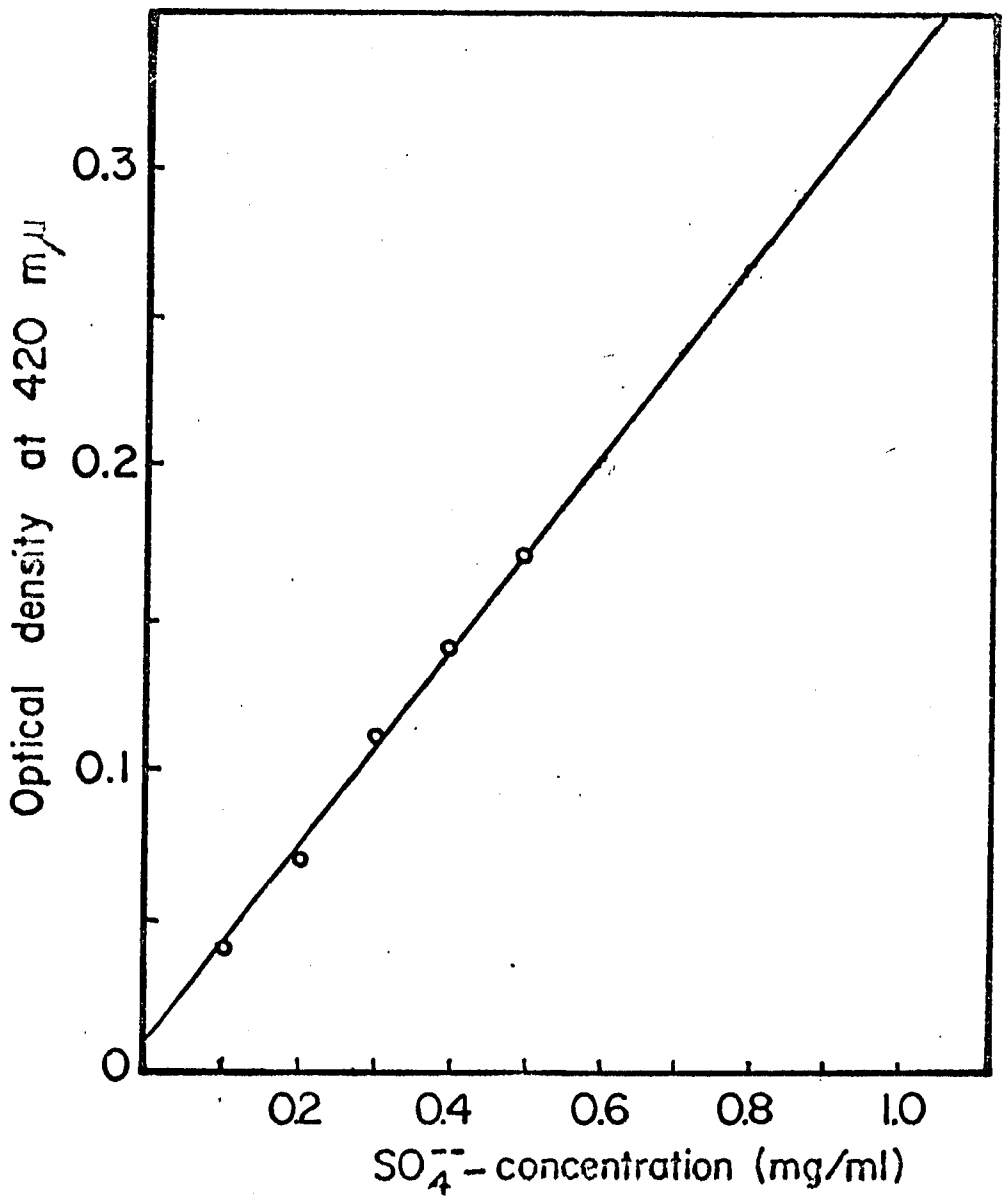


Fig. 2. Calibration curve for sulfate

against a blank formed by mixing 5 ml of 40% ethanol-glycol mixture, o.2 ml of 6 N HCl and 5 ml of $BaCl_2$ reagent. The O.D. was plotted against mg SO_4^{--} and a Conversion Factor to yield meq SO_4^{--}, having a value of 2.95/ 48 (=0.0615), was determined.

Specific conductance was measured with a 60-cycle current, 115-volt enclosed-switch-type wheatstone bridge, a pointer-type a.c. galvanometer, an insulating transformer and a pipette-type conductivity cell.

Total hardness as ppm $CaCO_3$ was obtained by titration with a standard solution of ethylenediaminetetraacetic acid (EDTA) using Erichrome Black T as a visual end point indicator.

These two latter determinations were made by the Industrial Water Section, Department of Mines and Technical Surveys, Ottawa.

Sulfate determination of water and soil samples

The water samples were freed of any suspended matter by centrifugation in a 15 ml teflon tube for 15 minutes at 2340 x g in a PR_2 International refrigerated centrifuge using head No. 811. Samples of 2.5 ml were used for sulfate analysis in the standard procedure already described. Sulfate concentration, expressed in meq/l, was calculated in the following way:

$$O.D. \times 0.0615 \times 400$$

The soil samples were pulverized, sieved through a No. 60 mesh strainer, dried in an oven at 100° C for two

hours and stored in a desiccator. 500 mg of dried soil were added to a 15 ml teflon centrifuge-tube containing 2.5 ml of sulfate-free water and 2.5 ml of 40% ethanol-glycol mixture. The contents were stirred and allowed to stand overnight. Then the mixture was decolorized with activated charcoal and filtered. The filtrate was diluted to 5 ml with 40% ethanol-glycol mixture and tested for sulfate by the standard procedure. Sulfate concentration, expressed in meq/100 g, was calculated in the following way:

$$\text{O.D.} \times 0.0615 \times 200$$

pH and total sulfur (as sulfate) determination of plant samples

For pH determination, 300 mg of air-dried plant material were ground to a paste with water in a mortar and made to a final volume of 15 ml with deionized water. The pH of this suspension was measured.

For sulfate determination, the plant material was oxidized to convert the sulfur compounds to sulfate by a wet-ashing technique (Patterson 1958). The soil-free plant samples were dried for two hours in an oven at 100°C. One hundred mg of the dried material were digested with 2 ml of concentrated HNO_3 in a 30 ml micro-Kjeldahl flask on a microburner for 30 minutes. One ml of 60% perchloric acid was then added and the digestion continued for at least one hour after the fumes of perchloric acid appeared. After cooling, 2 ml of 6 N HCl were added and the mixture was reheated until the fumes of perchloric acid reappeared. It

was then cooled, and if colored, treated with activated charcoal. The sample was then diluted to 5 ml with ethanol-glycol mixture for the standard sulfate procedure. SO_4^{--}-concentration, expressed in meq / 100 g was calculated in the following way:

$$0.D. \times 0.0615 \times 1000$$

Concentration of sulfur dioxide in the atmosphere

Data for the relative concentrations of sulfur dioxide in the atmosphere, at distances of 16.0, 22.4, 33.6, 38.4, 48.0 and 54.4 km NE, 8.3 km SW, and 8.0 km NW of the sinter plant (Fig.3) were obtained through the courtesy of Mr. B.R. Dreisinger, Sulfur Fumes Arbitrator, Ontario Department of Mines, Sudbury. These determinations were made by means of lead peroxide candles. The absorption of SO_2 by lead peroxide (PbO_2) to form lead sulfate ($PbSO_4$) provides the basis of an empirical method for comparing the concentrations of sulfur dioxide in the air at different places and at different times. For such a determination,a porcelain cylinder, covered by a cotton cloth coated with PbO_2, is exposed to the air for a period of time, usually one month. The lead sulfate thus formed is then dissolved by immersing the cloth in a warm 4% aqueous solution of sodium bicarbonate and its amount then determined by a gravimetric or colorimetric method. The results are expressed as mg $SO_3/dm^2/day$, where dm refers to area of the cylinder.

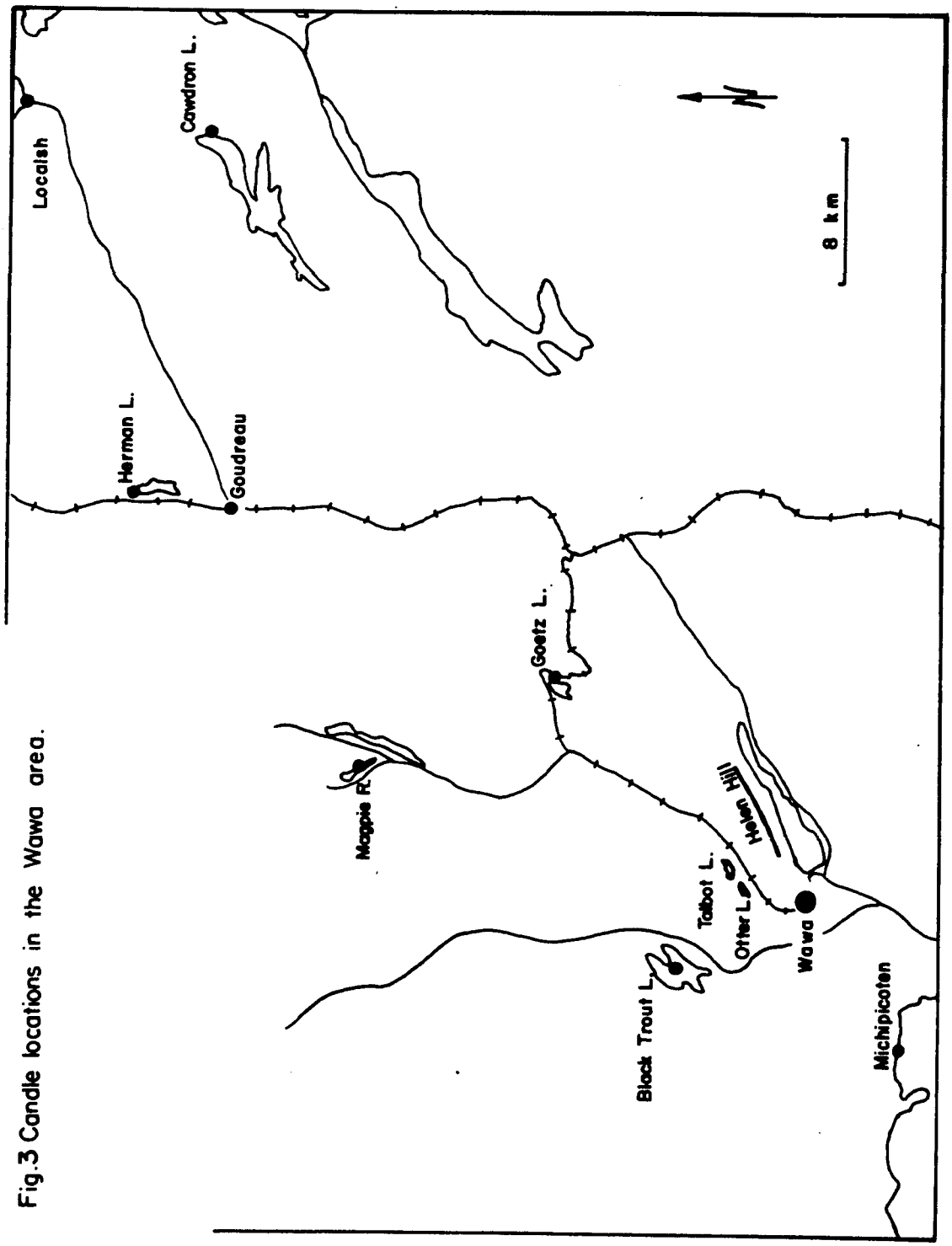


Fig.3 Candle locations in the Wawa area.

RESULTS

The vegetation damage due to the operation of the sinter plant is mainly concentrated in the northeast direction in which no trees are present for a distance of 12.8 km. Beyond this zone of severe damage, trees start coming up and gradually their number and crown density increase until the vegetation becomes characteristic of the region at a distance of 56.4 km. To the north, south, east and west of the sinter plant, the vegetation is not so severely affected.

No epiphytes were recorded within 16.1 km NE and 2.5 km N, NNE, ENE and ESE of the sinter plant, but 0.5 km SE in the town of Wawa three toxitolerant species, Bacidia chlorococca, Cladonia coniocraea and Parmelia sulcata, were growing on Populus balsamifera. These epiphytes were more or less depauperate and scarce. Beyond the epiphyte "desert", corticolous lichens and bryophytes gradually appeared. Nearest to the sinter plant Bacidia chlorococca and Cladonia coniocraea were the only species to appear, and then only on the bases of trees, expecially Betula papyrifera and Populus tremuloides. At a greater distance in more favorable habitats they gradually appeared on the trunk (Fig. 4). Further away still, the epiphytic flora gradually increased in richness in all directions (Table I). For example, on the north of the sinter plant the number of epiphytic species present were 0, 3, 13, 15 and 16 on Betula papyrifera and 0, 1, 3, 3, and 11 on Pyrus decora at

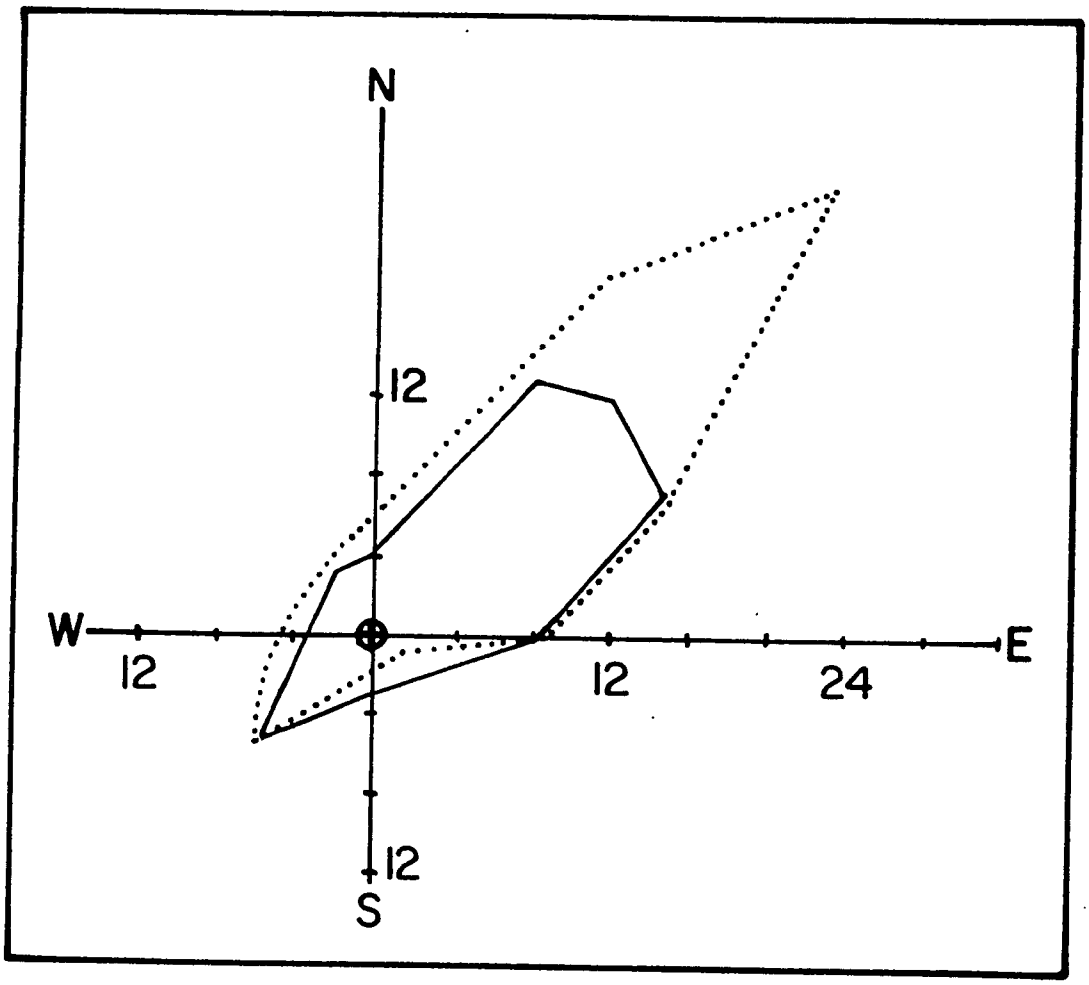


Fig. 4. Distances (km) showing the appearance of Cladonia coniocraea as an epiphyte around the sinter plant (⊕), ___ on tree-base; .... on tree-trunk.

TABLE I

NUMBER OF EPIPHYTES RECORDED AT VARIOUS DISTANCES AND

km from the sinter plant	0.5	0.8	1.6	2.5	3.2	4.0	4.8	5.6	6.5	8.0	8.9	11.2	12.8	14.5
Direction														
NE		x			x	x	x	x		x		x	x	0
ENE		0												3
NNE														4
E			0		1			1			8		25	
ESE			0		0									
SE	3				7									
N		0				3			15			16		
NW					12									
W			1											
SW				2						14				
S					15									

N.B.: x - sites without trees and epiphytes

0 - sites with trees but no epiphytes

TABLE I

AT VARIOUS DISTANCES AND IN DIFFERENT DIRECTIONS FROM THE SINTER PLANT

5.5	8.0	8.9	11.2	12.8	14.5	15.0	16.1	17.7	19.3	20.9	21.7	22.5	23.4	24.2
	x		x	x	0		0							
					3	3	19		19					
					4		13	5	11	16	22	17	30	16
		8		25										
15			16											27
	14													

D IN DIFFERENT DIRECTIONS FROM THE SINTER PLANT

.5	15.0	16.1	17.7	19.3	20.9	21.7	22.5	23.4	24.2	29.1	35.4	43.5	56.4
0		0										35	47
3	3	19		19									
4		13	5	11	16	22	17	30	16	30	17		
									27				
											49		

distances of 0.8, 4.0, 6.5, 11.2 and 24.2 km respectively.

In all, 110 epiphytic species (71 lichens, 29 mosses and 10 liverworts) were collected from 256 trees of 16 different species (Table II). The epiphytic species on Betula papyrifera (47 trees) totaled 54 and the maximum number of species found on one tree (43.5 km NE) was 22, similarly on Populus tremuloides (39 trees) the total number of species was 64 and the maximum number on one tree (35.4 km NW) was 20. It was found that the maximum number of epiphytes per tree (ignoring the species) was found at 43.5 km NE, 35.4 km NW, 24.2 km N and 12.8 km E of the sinter plant. At these distances, even smaller trees (diam. 3 cm) were rich in epiphytes; for example, 23 species in all were found on Alnus rugosa with 11 species on a single tree. On Salix humilis there were 22 species in all with 12 on one tree.

The terricolous and saxicolous species of lichens and bryophytes, collected in the epiphyte-free zone, are given below in a sequence to indicate their presence in areas increasingly distant from the stacks:

Dicranella heteromalla, Pohlia nutans, Cladonia coniocraea, C. deformis, C. floerkaena, Lepraria aeruginosa, Cladonia nemoxyna, Bryum bimum, Ceratodon purpureus, Funaria hygrometrica, Cladonia fimbriata, C. pyxidata, Bryum caespiticium, Polytrichum juniperinum, P. piliferum, Reboulia hemispherica, Distichium capillaceum, Cladonia bacillaris and C. chasmariae.

NUMBER (

Site number	1	2	3	4	5	6	7	9	10	11	12
Direction	NNE	NNE	NNE	NNE	NNE	NNE	NNE	ENE	ENE	NE	NE
km from the stacks	16.1	17.7	19.3	20.9	21.7	22.5	23.4	19.3	16.1	56.4	43.
No. of epiphytic species recorded	13	5	11	16	22	17	30	19	19	47	35
Betula papyrifera	5		9	7	9		13		2	26	26
Populus tremuloides	6	5	7	9		8	16	9	5	11	14
Picea mariana	0	0	0	0			9	9			10
Abies balsamea						6		7	7		
Pyrus decora		0	2	0	4		5		11		
Alnus rugosa	0	0		0	2		7		6		8
Salix humilis	0		0	0	9			8			
Thuja occidentalis					14	12			9	10	
Populus balsamifera										18	
Pinus banksiana										15	
Prunus pensylvanica	0	0	0					11			
Fraxinus nigra	10										
Acer spicatum	0									9	
Picea glauca										8	
Larix laricina											
Acer rubrum											
No. of tree species examined	8	5	6	6	5	3	5	5	6	7	4

TABLE II

NUMBER OF EPIPHYTES RECORDED ON DIFFERENT TREE SPECIES AT VARIOUS COLLECT

5	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
E	NE	NE	E	SE	E	E	S	NE	NE	NE	NE	NNE	NNE	NNE	NW	N	ENE
.1	56.4	43.5	5.6	0.5	1.6	3.2	3.2	0.8	3.2	4.0	4.8	24.2	29.1	35.4	35.4	0.8	0.8
9	47	35	1	3	0	1	15	x	x	x	x	16	30	17	49	0	0
2	26	26	0	1	0	0						11	12		18	0	0
5	11	14	0	1								6	12	4	32		
		10	0	1	0		1					5	10		14	0	
7												4	17		14		
1			0	1	0	0										0	0
5		8				0	2						6	9	13	0	
			0	1	0											0	
9	10		1														
	18			3			14										
	15						1					6		11			
																0	
	9				0	0											
	8					1											
							2										
																0	
5	7	4	6	6	5	5	5	x	x	x	x	5	5	3	5	7	2

N.B. x - Sites without trees
0 - Sites with trees

VARIOUS COLLECTION SITES IN THE WAWA AREA

24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
NNE	NW	N	ENE	ESE	ESE	E	E	SW	W	NE	NE	NE	NE	NNE	ENE	EN
5.4	35.4	0.8	0.8	1.6	3.2	8.9	12.8	8.0	1.6	11.2	14.5	16.1	12.8	14.5	15.0	14.
17	49	0	0	0	0	8	25	14	1	x	0	0	x	4	3	3
	18	0	0	0	0	5	18	1	1		0	0		0	2	1
4	32			0			7	12	1					3	3	
	14	0			0		12		1					1	0	0
	14				0	4	12	0	1						0	0
		0	0	0	0	7	4	2						0	0	3
9	13	0														0
		0		0			12	5	1							
					0											1
11														0		
		0		0										1	0	
					0			0								
		0						0								
3	5	7	2	5	6	3	6	7	5	x	1	1	x	6	6	6

34	35	36	37	38	39	40	41	42	43	44	45	45	47	48
NE	NE	NE	NE	NNE	ENE	ENE	SW	SE	N	N	NE	NE	N	NW
11.2	14.5	16.1	12.8	14.5	15.0	14.5	2.5	3.2	6.5	11.2	8.0	5.6	4.0	3.2
x	0	0	x	4	3	3	2	7	15	16	x	x	3	12

	0	0		0	2	1	2		13	15			3	9
				3	3									
				1	0	0	1	1	7	7				5
					0	0	1			2				6
				0	0	3	1	1	3	3			1	
						0								
							1	7						
						1							1	
				0										
				1	0									

x	1	1	x	6	6	6	5	3	3	4	x	x	3	3

The amount of SO_2 was greater close to the stacks than at more distant locations. More SO_2 was recorded during summer than winter for all the six locations situated between 16 to 54.4 km NE of the sinter plant. For a SW location, (8.4 km distant), however, the amount of SO_2 was more in winter than summer, and for a NW location (8.0 km distant) the amount of SO_2 was small and without any seasonal quantitative differences (Table III). A gradual decrease in SO_2-concentration at increasing distances along a NE-transect-line, provides evidence of more and more dilution of the pollutant in its onward journey and thereby less and less chances of pollution ahead. For this reason the concentration of SO_2 from Goetz Lake to Localsh (Fig. 3) is noticeably different and various pollution zones between 16.0 to 54.4 km can be easily distinguished.

For surface-waters (Table IV), in the areas immediately adjacent to the sinter plant, a low pH was accompanied by a high sulfate-concentration, specific conductance and total hardness; these properties were especially marked in the NE direction. At increasing distances on the NE-transect-line a gradual increase in pH and decrease in other factors back to the unpolluted levels, as indicated by the results from Magpie River, was noted(Fig. 5). The sulfate-concentration in surface-waters of various lakes usually ranged from 2.7 to 0.18 meq/l, but in the case of Otter and Talbot Lakes it was as high as 13.03 and 17.7 meq/l respectively (Fig. 6). The high values obtained for acidity and other factors for

TABLE III

WAWA PbO_2 CANDLE RESULTS EXPRESSED AS mgm $SO_3/dm^2/DAY$

Station (km and direction from Wawa)	Year	May-June	June-July	July-Aug.	Aug.-Sept.	Sept.-Oct.	Mean	Winter Oct.-May
Goetz L. (16.0 NE)	1961	-	3.56	2.04	1.37	0.85	1.96	0.73
	1962	-	1.72	1.51	0.79	0.92	1.24	0.79
	1963	1.63	1.70	0.41	2.02	2.47	1.65	-
	1964	-	1.43	1.05	0.96	0.91	1.09	
Magpie R. (22.4 NE)	1961	-	1.46	0.89	0.50	0.40	0.81	0.56
	1962	0.82	0.99	0.26	0.24	0.65	0.59	0.62
	1963	1.19	1.23	1.65	0.26	0.41	0.95	-
	1964	0.66	0.58	0.44	0.44	0.53	0.53	
Goudreau (33.6 NE)	1961	-	1.20	0.45	0.47	0.51	0.66	0.35
	1962	0.45	0.78	0.41	0.46	0.30	0.48	0.35
	1963	0.54	0.93	0.47	0.61	0.83	0.68	0.29
	1964	0.61	0.56	0.30	0.21	0.89	0.51	
Herman L. (38.4 NE)	1961	-	0.88	0.37	0.36	0.43	0.51	0.29
	1962	0.41	0.79	0.32	0.41	0.14	0.41	0.32
	1963	0.45	0.67	0.39	0.49	0.50	0.50	0.35
	1964	0.58	0.58	0.65	0.71	0.54	0.61	
Cawdron L. (48.0 NE)	1961	-	0.65	0.37	0.25	0.07	0.34	0.13
	1962	0.14	0.28	0.15	0.20	0.24	0.21	0.19
	1963	0.29	0.42	0.33	0.34	0.39	0.35	0.14
	1964	0.34	0.26	0.39	0.15	0.28	0.28	
Localsh (54.4 NE)	1961	-	0.80	0.35	0.20	0.16	0.38	0.11
	1962	0.17	0.28	0.20	0.16	0.09	0.18	0.17
	1963	0.17	0.29	0.20	0.34	0.51	0.30	0.12
	1964	0.27	0.22	0.52	0.35	0.92	0.46	
Michipicoten (8.3 SW)	1961	-	-	0.16	0.08	0.12	0.12	0.68
	1962	0.25	0.21	0.14	0.02	0.27	0.18	0.33
	1963	0.38	0.12	0.03	0.20	0.17	0.18	0.23
	1964	0.26	0.05	0.37	0.74	0.41	0.37	
Bl. Trout L. (8.0 NW)	1961	-	0.13	0.15	0.07	0.06	0.10	0.13
	1962	0.11	0.11	0.08	0.08	0.17	0.11	-
	1963	0.16	0.10	0.02	0.05	-	0.08	0.08
	1964	0.12	0.14	0.46	0.53	0.63	0.38	

TABLE IV

REPRESENTATIVE ANALYSES OF WATERS FROM THE WAWA AREA

Source	km and direction from the sinter plant	pH	Sulfate meq/l	Sp. cond. micromhos at 25° C	Total hardness ppm as $CaCO_3$
Lake Soulier	1.5 NE	3.8	2.15	245	81
" Otter	2.5 NE	2.6	13.03	1,794	536
" Talbot	3.2 NE	2.5	17.7	2,219	639
" Lagarde	4.0 NE	3.4	2.7	292	60
" Blueberry	11.2 NE	3.6	2.33	283	52
" Josephine	14.5 NE	6.2	1.23	160	66
" Goetz	15.3 NE	6.7	0.86	147	59
" Eva	21.8 NE	6.3	0.49	133	55
" Alice	22.5 NE	6.8	0.37	134	56
" Reynolds	23.4 NE	6.8	0.25	75	34
" Sausage	43.5 NE	6.7	0.18	144	60
" Rosa	56.4 NE	7.5	0.23	89.5	37
" Moran	1.6 ENE	6.3	2.95	298	129
" Spud	4.5 ENE	6.5	5.33	555	134
" Leg	15.2 ENE	6.4	0.61	145	62
" Cuthbertson	15.5 ENE	6.1	0.86	123	52
" Ireland	23.8 ENE	5.5	0.21	67	25
" Catfish	8.9 NNW	5.8	0.26	92	35
" Swallow	4.9 E	5.8	2.33	31	137
" Wawa	0.8 SE	6.8	0.53	172	71
" Twin	12.1 SE	7.4	0.34	178	75.5
" Hawk	16.1 E	6.6	0.40	127	54
" Superior	10.0 SW	6.8	0.10	121	50
Magpie River		6.8	0.18	130	54

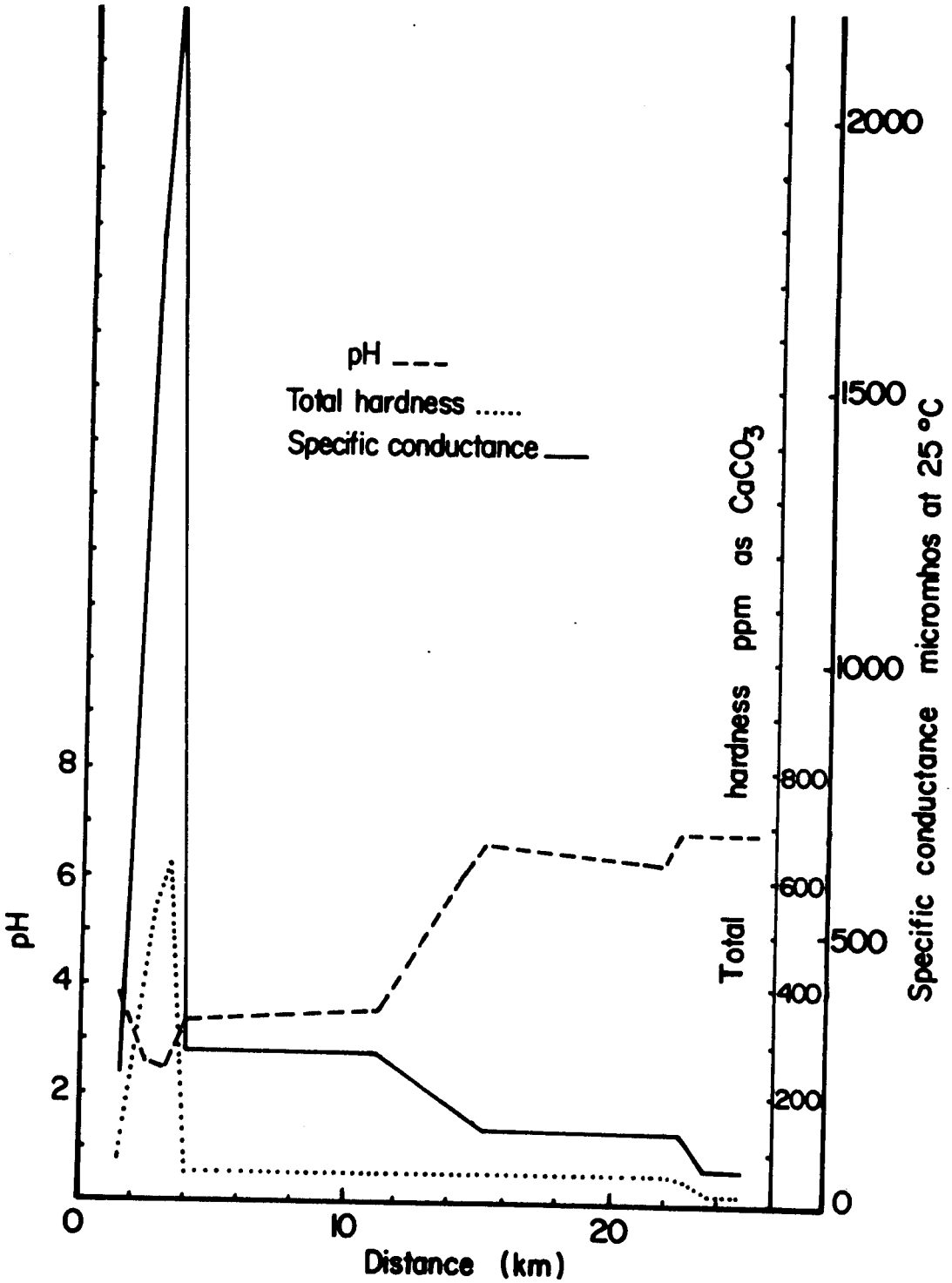


Fig. 5. pH, total hardness ppm as $CaCO_3$, and specific conductance of surface-waters of lakes NE of the sinter plant at Wawa.

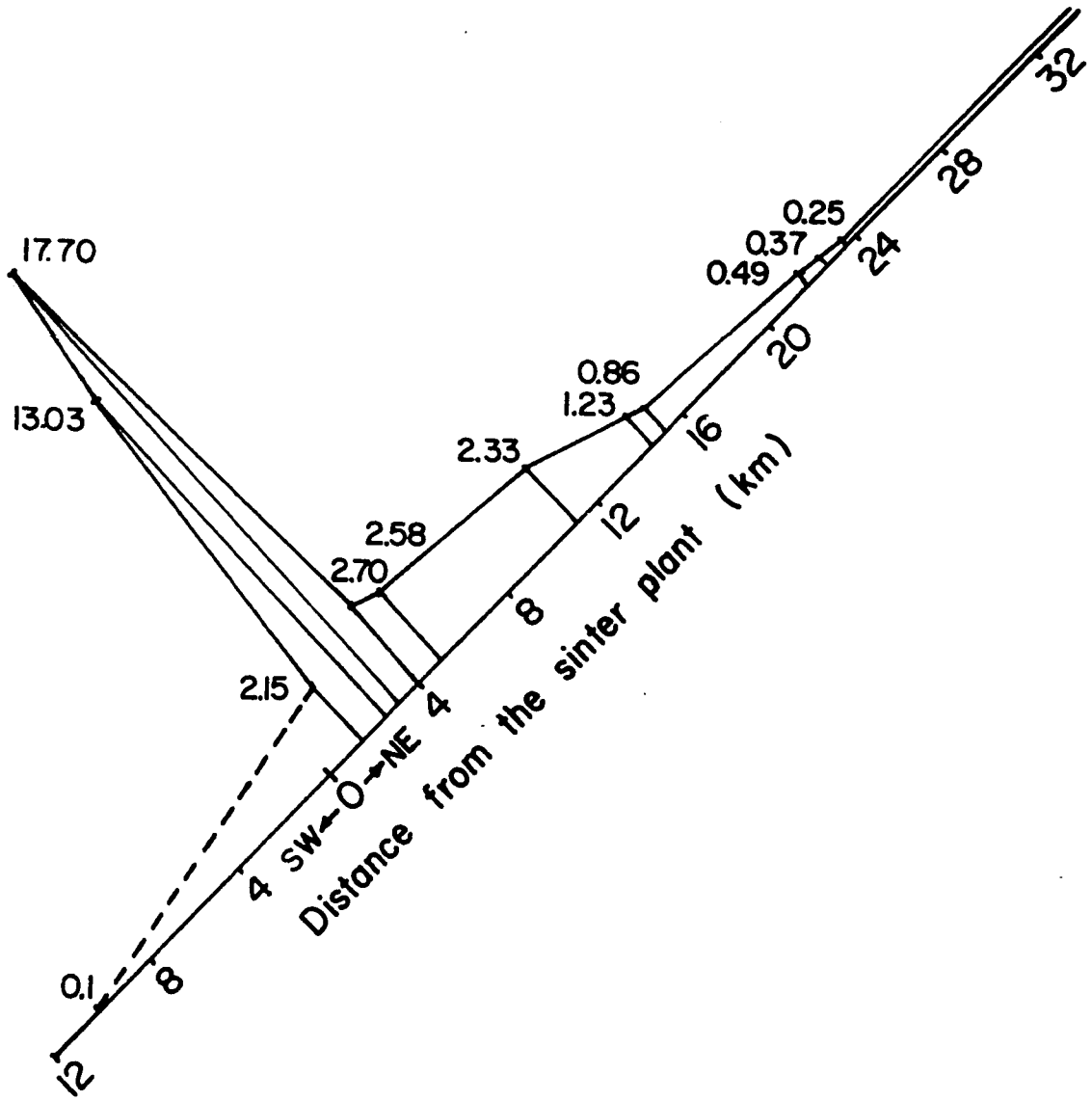


Fig. 6. SO_4^{--} (meq/l) in surface-waters of lakes situated on the transect SW-NE. "O" indicates the location of the sinter plant.

the water samples of these two lakes are perhaps due to the bacterial action in the sulfide-contaminated mine-water flowing into them. It has been demonstrated that the presence of bacteria belonging to the Ferrobacillus - Thiobacillus group causes rapid oxidation of sulfide minerals to form sulfuric acid and iron (Hoffert 1947).

The acidity and the sulfate concentration of surface soils were usually higher near the sinter plant and gradually decreased with increasing distances from the stacks (Fig. 7).

The acidity as well as the sulfate concentration of extracts from the plant materials gradually decreased as the distance from the source of sulfur dioxide increased (Table V). Only a small number of analyses were made of specimens of Pohlia nutans and Cladonia species, but from the data that we have, it is evident that close to the stacks the plant materials had a low pH and a high sulfate concentration. For example, in Pohlia nutans, 0.8 km NE of the stacks the pH was 4.2 and the sulfate concentration was 24.1 meq/100 g.

DISCUSSION AND CONCLUSIONS

Near the sinter plant there is a severe damage to vegetation, the presence of an epiphyte "desert", a higher concentration of sulfur dioxide in the atmosphere, and an increase in the acidity and the sulfate concentration of surface-waters, soils and plant materials. On moving

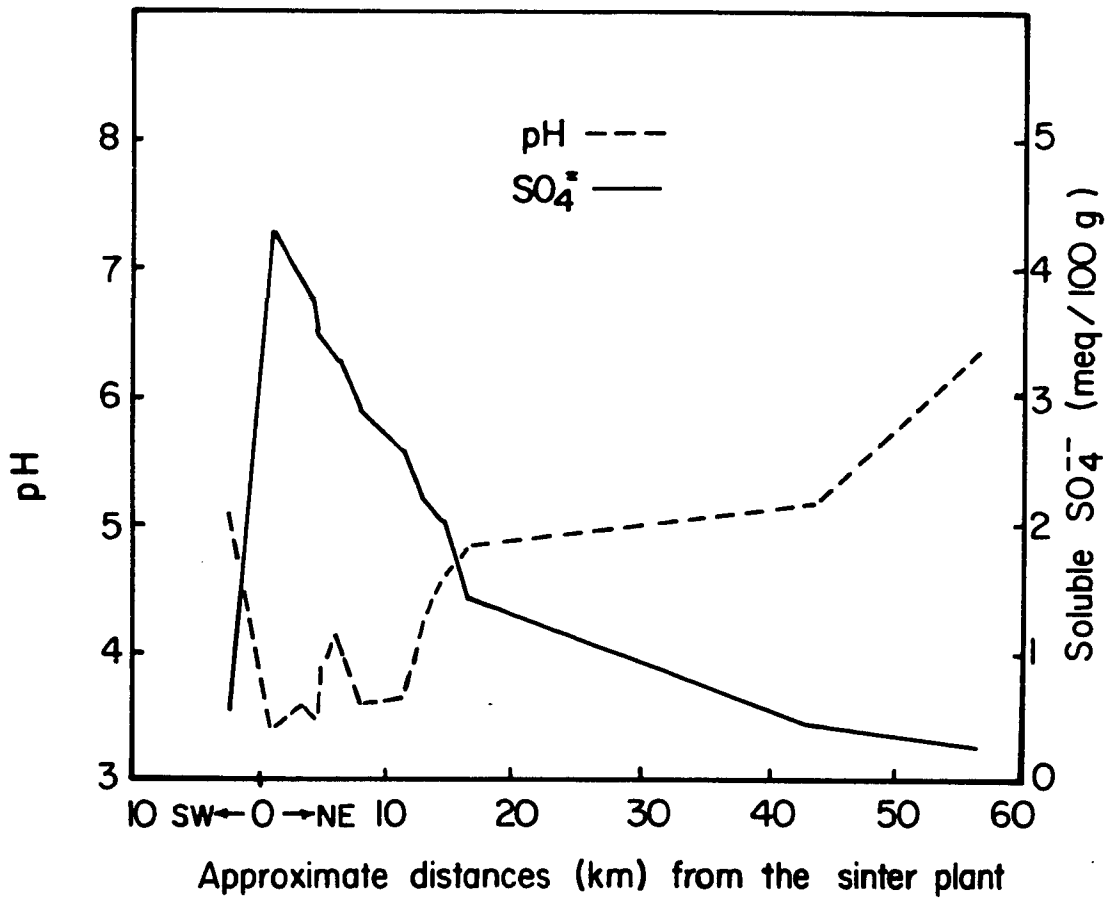


Fig. 7. pH and soluble SO_4^{--} of surface soils collected on the transect SW-NE. "0" indicates the location of the sinter plant.

TABLE V

HYDROGEN ION CONCENTRATION & SO_4^{--}-CONTENT (meq/100 g)

OF SOIL, LEAVES OF

BETULA PAPYRIFERA, POHLIA NUTANS AND CLADONIA SP.

Collection site km and direction from the sinter plant		Soil samples		B.papyrifera		P.nutans		Cladonia sp.	
		pH	SO_4	pH	SO_4	pH	SO_4	pH	SO_4
NE	0.8	3.4	4.3			4.2	24.1		
"	3.2	3.6	3.9				22.6		
"	4.0	3.5	3.8				20.3		
"	4.8	3.8	3.5				19.9		
"	5.6	4.2	3.2					4.8	7.3
"	8.0	3.6	2.9						
"	11.2	3.7	2.6				18.4		
"	12.8	4.3	2.2	4.4	13.8	4.5	14.7	4.7	4.6
"	14.5	4.6	2.0	4.8	12.7	4.9	14.1	4.5	4.3
"	16.1	4.8	1.4	5.2	10.4	5.3	13.4	5.0	3.5
"	43.5	5.2	0.43						
"	56.4	6.3	0.25						
NNE	14.5	4.8	0.86				12.9	4.5	3.5
"	16.1	6.6	0.80				12.2	4.7	3.3
"	17.7	6.6	0.78						
"	20.9	6.9	0.70						
"	21.7	7.0	0.43				11.4		
"	23.4	6.5	0.30						
"	24.2	5.2	0.25						
"	29.1	5.5	0.12						
"	35.4	5.4	0.12						
N"	0.8	5.8	1.5				19.05		
"	2.5	5.0	0.98						
"	4.0	5.6	0.79				17.21		
"	11.2	5.5	0.67						
"	24.2	7.0	0.18						
E	1.6	4.6	0.86	5.3	10.8	5.2	19.1	5.3	5.1
"	3.2	4.5	0.43	5.0	10.4	5.1	15.3	5.2	5.9
"	5.6	5.6	0.36				12.9	4.8	4.5
"	8.9	5.5	0.43						
"	12.8	5.8	0.30						
ENE	0.8	4.5	1.7	5.0	10.4		21.5		
"	14.5	4.8	0.86					4.5	3.9
"	16.1	6.1	0.12					5.2	3.2
"	19.3	6.0	0.06						
ESE	1.6	5.0	0.98						
"	3.2	5.7	0.86				15.3	5.6	3.6
SE	2.5	5.0	0.78						
"	3.2	5.2	0.46					4.8	3.4
W	1.6	5.1	0.62					5.0	2.5
SW	2.5	5.1	0.58				14.7		
NW	3.2	5.3	1.2						
"	35.4	5.5	0.36						

away from the stacks there is a gradual improvement in epiphytic vegetation (both in coverage and frequency of species), a decrease in the concentration of sulfur dioxide in the atmosphere, and a decrease in the acidity and the sulfate concentration of surface-waters, soils and plant materials. Thus, there exists a definite correlation between the growth of epiphytes, the concentration of SO_2 in the air, and the chemical factors (pH and SO_4^{--}) of water, soil and vegetation. The results indicate that the SO_2-concentration in air is directly related to the acidity and the SO_4^{--}-content in various samples but it is inversely related to the growth of epiphytes. Considering the SO_2-concentration gradient in the area, it appears that the effects on epiphytic growth and pH and sulfate concentration are mainly governed by the fallout pattern of the pollutant.

Dispersion of pollutants

Pollutants emitted into the atmosphere are normally dispersed in two ways, laterally by surface winds and vertically by thermals. When the air is calm, the pollutants will remain in the vicinity of the source for hours. Meteorologists (Niemyer 1960) have established that the concentration of contaminants is generally inversely proportional to wind speed. Doubling the wind speed, other conditions being equal, doubles the amount of air into which the pollutants are emitted, thereby diluting the concentration by one-half. It follows that the chances for pollution are greater when the winds are light. The

pollutants emitted at Wawa are carried away from the point of origin by mostly SW winds. These are the prevailing winds which over the years have created a semi-permanent pattern of pollution downwind from the sinter plant. The high level of SO_2 in the NE-direction during summer is due to the prevailing SW winds. However, in winter the winds frequently come from the NE-direction and so create a high concentration of SO_2 during this season in a SW-direction. There is also a daily fluctuation in wind velocity. During the night and the early morning hours a light wind regime persists (Table VI) and consequently pollution will be most severe at this time.

Vertical dispersion is brought about by air currents which depend upon the air temperatures being inversely proportional to the altitudes. The height to which the air rises, known as its mixing depth, depends on the extent of the temperature differential from low altitudes to high altitudes. This mixing depth changes from one season to another, and even from daytime to nighttime. In the summer, temperatures normally drop sharply with height, while in winter, especially when there is snow cover on the ground, a temperature inversion frequently occurs, i.e., the coldest temperature is at the ground level. Under this condition, there is no vertical air movement and pollutants remain near the ground. A similar inversion normally occurs during the night and early morning hours when the ground cools off. During the late morning and afternoon hours the ground is warmed by the

TABLE VI

MEAN HOURLY WIND SPEEDS FOR EACH MONTH

AT WAWA, ONTARIO FOR PART OF 1964 AND 1965

Hour of Day E.D.S.T.	1964 Oct.	1964 Nov.	1964 Dec.	1965 Jan.	1965 Feb.	1965 March	1965 April	1965 May	1965 June	1965 July	1965 Aug.	1965 Sept.	1965 Oct.
1	6	9	9	9	8	8	7	5	4	5	3	6	6
2	6	9	9	9	8	7	7	5	4	5	2	7	5
3	6	9	9	9	8	8	6	5	5	5	3	7	6
4	7	9	9	9	7	8	6	4	4	5	3	7	6
5	8	10	9	8	7	8	7	4	5	5	3	7	6
6	8	10	9	8	7	8	6	5	5	5	3	7	6
7	9	10	9	8	7	8	6	5	4	5	3	7	5
8	9	8	9	8	7	8	7	5	5	5	3	7	5
9	9	10	9	8	6	8	7	6	5	6	4	8	5
10	9	10	9	7	7	8	8	7	7	6	4	7	6
11	9	10	9	8	8	9	9	9	8	7	5	9	8
12	10	11	9	9	10	10	10	11	9	9	6	10	10
13	10	12	9	8	9	10	11	12	9	8	7	10	11
14	11	12	8	9	11	12	11	12	10	9	7	11	11
15	12	11	8	8	11	12	12	13	10	10	7	12	12
16	10	11	8	8	11	12	12	13	10	10	7	12	12
17	9	9	8	8	11	11	11	13	9	10	6	11	11
18	9	9	7	8	10	10	10	11	9	9	6	10	10
19	8	9	7	8	8	9	8	10	8	8	5	9	8
20	8	9	7	8	8	8	7	9	7	7	4	8	6
21	7	10	8	8	8	8	6	7	5	6	3	7	6
22	8	10	9	9	8	8	6	5	5	5	2	6	5
23	7	9	10	10	9	9	5	5	4	4	2	6	6
24	7	10	9	9	8	8	6	5	4	5	2	7	5

sun (Crow 1964) and air will rise upward. Hence, pollution is more severe during winter and during the night and early morning hours.

Topographic features, fog, humidity, temperature and light intensity

The topographical barrier, provided by Helen Hill (Fig.3) extending ENE of the sinter plant and standing nearly 100 m higher than the stacks, should confine the spread of the pollutants to the downwind area, which is really a channel-like depression with numerous lakes. This barrier may also create stagnating high pressure systems, in which condition the temperature inversions become very persistent. Conditions of fog, a common feature in this area, also contribute to the persistence of the temperature inversions by preventing the sun from heating the ground level. An inversion accompanied by low winds, gradually builds up a layer of highly polluted air over an extended area. Thus the micro-climatic conditions and topography of the area combine to enhance considerably the concentration of pollutants and their damaging effect on the vegetation at Wawa.

It has been noted that plants are injured by SO_2 most severely when the gas-visitation coincides with a period of high humidity (Katz and Ledingham 1939). The high humidity conditions are favorable to a high rate of SO_2 oxidation (Meetham 1952). During the growing season at Wawa the relative humidity is 70% or more as a result of

the moisture-laden SW winds blowing off of Lake Superior. This combined together with frequent fog provides a moisture condition which is conducive to a high rate of SO_2 oxidation and accordingly, serious damage to vegetation.

It has been observed that higher plants are more liable to injury during daylight than at night and the toxicity of the gas increases with the rise in temperature and light intensity (Katz and Ledingham 1939). The effect of light is interrelated with that of other factors which govern the movement of the stomata. In general the thesis has been that in darkness the stomata are closed and therefore, pollutants cannot enter the cuticularized leaves and cause damage (Bobrov 1955; Juhren et al. 1957). In the case of lichens and bryophytes, however, there is no such protection and besides this they are evergreen perennials. Hence, they are always exposed to the pollutants.

Fallout of SO_2 and its effect on water, soil and vegetation

Sulfur dioxide as well as sulfur trioxide may be absorbed at the surfaces of water and soil directly, or may reach them indirectly through absorption by vegetation or by solution in rainfall. When absorbed directly these eventually become H_2SO_4, increasing the acidity. The acid then forms sulfates of calcium, magnesium and other elements. For surface-waters the increase in sulfate concentration, specific conductance (mineral content) and total hardness are in parallel relationship and are accompanied by a decrease in pH (Fig. 5). Since the patterns in pH and SO_4^{--}-concen-

tration of surface-waters and soils are similar to the pattern of the concentration of atmospheric SO_2, it is concluded that is is possible to use these chemical factors for the assessment or comparison of the degree of SO_2-pollution in different areas.

Sulfate concentrations in Betula papyrifera, Pohlia nutans and Cladonia species from many sites (Table V) suggest that a definite correlation exists between the sulfate content of vegetation and the SO_2-concentration in the atmosphere. Plants in areas of high pollution usually had a high sulfate concentration and vice versa. It is interesting to note that at the same site the concentration was higher in Pohlia nutans and Betula papyrifera than in Cladonia species. With respect to pH and SO_4^{--}-concentration there was no apparent similarity between different plant materials. At a site 1.6 km E of the sinter plant, the sulfate concentration in Pohlia nutans, Betula papyrifera and Cladonia species were respectively 19.1, 10.8 and 5.1 meq/100 g although the pH of their extract was about 5.2. From field observations it is evident that the following terrestrial plant species are most tolerant to sulfur dioxide:

Trees: Betula papyrifera, Populus tremuloides and Prunus pensylvanica.

Shrubs: Sambucus pubens, Pyrus decora, Salix humilis and Alnus rugosa.

Herbs: Polygonum cilinode, Maianthemum canadense and

Aster macrophyllum.

Mosses: Dicranella heteromalla, Pohlia nutans and Ceratodon purpureus.

Lichens: Cladonia coniocraea, C. deformis, C. floerkaena and Lepraria aeruginosa.

Although Betula papyrifera and Populus tremuloides are very sensitive to SO_2 and their leaves will show necrosis and bleaching of margins or intercostal areas, they are, nevertheless, quite hardy and will resist longer than most other trees, especially the conifers.

Terricolous lichens and mosses and their tolerance to pollution

Dicranella heteromalla and Pohlia nutans were found growing on soils with a low pH (3.4) and containing a high concentration of sulfate (4.3 meq/100 g); (the normal level for SO_4^{--} in the area was about 0.2 meq/100 g). Cladonia coniocraea, C. deformis, C. floerkaena and Lepraria aeruginosa were collected from soils with SO_4^{--} concentration up to 4.0 meq/100 g. The majority of terricolous lichens and bryophytes, however, inhabited only those sites where the sulfate in soils did not exceed 1.0 meq/100 g (Table VII). The occurrence of the above species in areas near the sinter plant and on soils having a high sulfate concentration suggests that the terricolous lichens and mosses are tolerant to pollution. In order to explain this behavior of the terricolous species, factors such as snow cover (LeBlanc 1961), shelter from the wind (Geiger

TABLE VII

RELATION BETWEEN FLORISTIC VARIETY OF TERRICOLOUS LICHENS & BRYOPHYTES AND SULFATE CONTENT OF SURFACE SOILS AROUND THE SINTER PLANT

	Sulfate content (meq/100 g)					
	>4.0	3.0–4.0	2.0–3.0	1.0–2.0	1.0–0.5	<0.5
Dicranella heteromalla	+	+		+	+	
Pohlia nutans	+	+	+	+	+	+
Cladonia coniocraea		+	+		+	
Cladonia deformis		+	+		+	+
Cladonia floerkaena		+	+			
Lepraria aeruginosa		+				
Cladonia nemoxyna			+			
Bryum bimum			+			
Ceratodon purpureus			+		+	+
Funaria hygrometrica			+	+		
Cladonia fimbriata				+		+
Cladonia pyxidata				+		
Bryum caespiticium				+	+	
Polytrichum juniperinum				+	+	+
Polytrichum piliferum				+	+	+
Reboulia hemispherica				+		
Distichium capillaceum				+		
Cladonia bacillaris					+	
Cladonia chasmariae					+	+
Cladonia cristatella					+	+
Catillaria sp. a					+	
Lecidea cinereotra					+	
Parmelia stenophylla					+	
Stereocaulon saxatile					+	
Bartramia pomiformis					+	
Bryum capillare					+	
Ditrichum pusillum					+	+
Leptodictyum riparium					+	
Mnium affine					+	+
Polytrichum commune					+	+
Cephalozia sp.					+	
Lophozia sp.					+	+
Cladonia verticillata						+
Amblystegium varium						+
Atrichum angustatum						+
Blepharostoma trichophyllum						+
Fissidens cristatus						+
Trematodon ambiguus						+
Cladonia rangiferina						+
Pleurozium schreberi						+
Dicranum polysetum						+
Dicranum bonjeanii						+
Plagiothecium sylvaticum						+
Thuidium recognitum						+
Clapodiella fluitans						+

1965) and the buffering action of soil (Russel 1932), which might reduce and modify the effects of the pollutant, should be taken into consideration.

Brightman (1959) and Gilbert (1965) in their respective studies of the lichen flora in the southeast of the city of London and in the Tyne Valley, England, observed that a high base (e.g., $CaCO_3$) content of the substrate and a consequent power of neutralizing acidic atmospheric pollutants, favored lichen growth. Whether or not the chemical composition of the soil influences the bark of trees directly, viz., by the uptake of minerals through the root is not known, but probably this influence, if it exists at all, is a minor one (Barkman 1958), and consequently, the corticolous species have no such protection against the acidic pollutants present in the atmosphere.

Epiphytes as indicators of atmospheric pollution

The epiphytic vegetation, both qualitatively and quantitatively, is influenced by the microclimatic variations occurring at different regions (base to crown) of the phorophyte (Barkman 1958). Outside the "desert" zone we noticed the epiphytes first at the bases of trees; probably a less intensive contact with the polluted air and a blanket of snow during winter at the tree-base make it a more favorable habitat than the trunk. So far, little is known about the problems of epiphytes in relation to their environment and it is rather difficult to tell whether or not the absence of an epiphyte in a given habitat is due to unfavo-

rable conditions. Nevertheless, in a polluted area where climate and other ecological factors are otherwise unchanged, the transitions in epiphytic flora could possibly be associated with the pollution. Mrose (1941) observed that the specimens of Usnea once present at a locality near a mining and industrial area in Germany had subsequently entirely disappeared. Barkman (1963) in a study of epiphytes of the Belgian province of Limburg observed the poorest epiphytic flora around mines and larger factories.
De Sloover (1964) developed an interesting method to map the phenomenon of pollution and to determine the "index of atmospheric purity" through the study of corticolous epiphytes, which cannot, apparently, tolerate the toxicity of the environment. Sernander (1926), LeBlanc (1961), Pisut (1962), and many others, from their studies of epiphytes, have distinguished three distinct zones in and around cities, namely: (1) "desert" zone without epiphytes; (11) "struggle" zone with depauperate epiphytes; and (111) "normal" zone with healthy epiphytes. These zones, when considered in terms of pollution, correspond respectively to most, less and least polluted areas. At Wawa, from a point source of pollution and with a constant pattern of dispersion, there is a gradual transition in the degree of pollution. In our survey of epiphytes we have seen that the degree of pollution has a direct influence on the epiphytic population (Fig.8). For example, at various sites on the transect-line N of the sinter plant, the number of epiphytes as well as their coverage is inversely

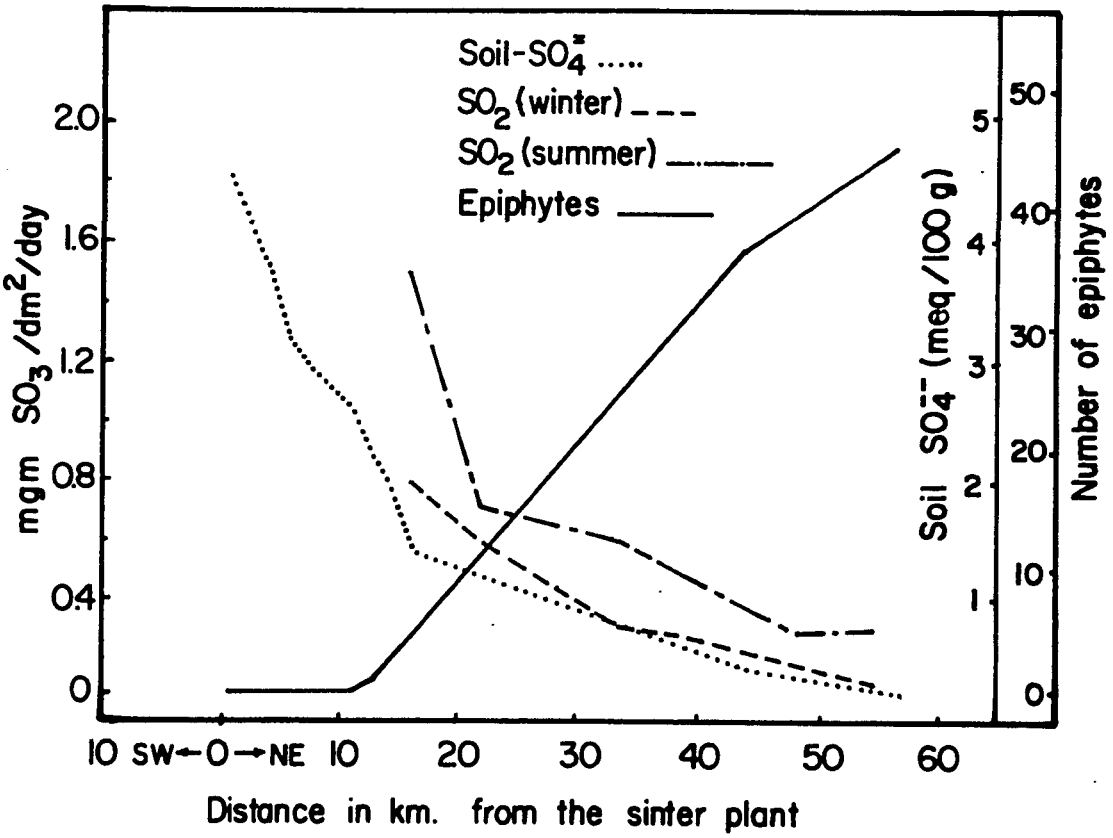


Fig. 8. Comparison of soil-$SO_4^=$, atmospheric-SO_2 and number of epiphytes at varying distances NE of the sinter plant.

proportional to the degree of pollution, expressed in terms of soil-sulfate concentration (TableVIII). At Wawa, therefore, it is possible to circumscribe not only three but five zones, to delimit areas differing in the degrees of pollution as well as in the epiphytic population (Fig.9):

Zone 1. Sulfate concentration more than 1.4 meq/100g, epiphytes absent.

Zone 2. Sulfate concentration between 0.9 to 1.4 meq/ 100 g, the number of epiphytic species at any site ranging from 1 to 5. Only the following nine species (six lichens, two mosses and one liverwort) were present in this zone:

Bacidia chlorococca, Cladonia coniocraea, C. decorticata, Dicranum flagellare, Drepanocladus uncinatus, Parmelia physodes, P. sulcata, Mycoblastus sanguinarius and Ptilidium pulcherrimum.

Zone 3. Sulfate concentration between 0.9 to 0.7 meq/100 g, the number of epiphytic species at any site ranging from 5 to 15. The epiphytes recorded in zone two were present along with the following 52 species (31 lichens, 16 mosses and five liverworts) which appeared for the first time in this zone:

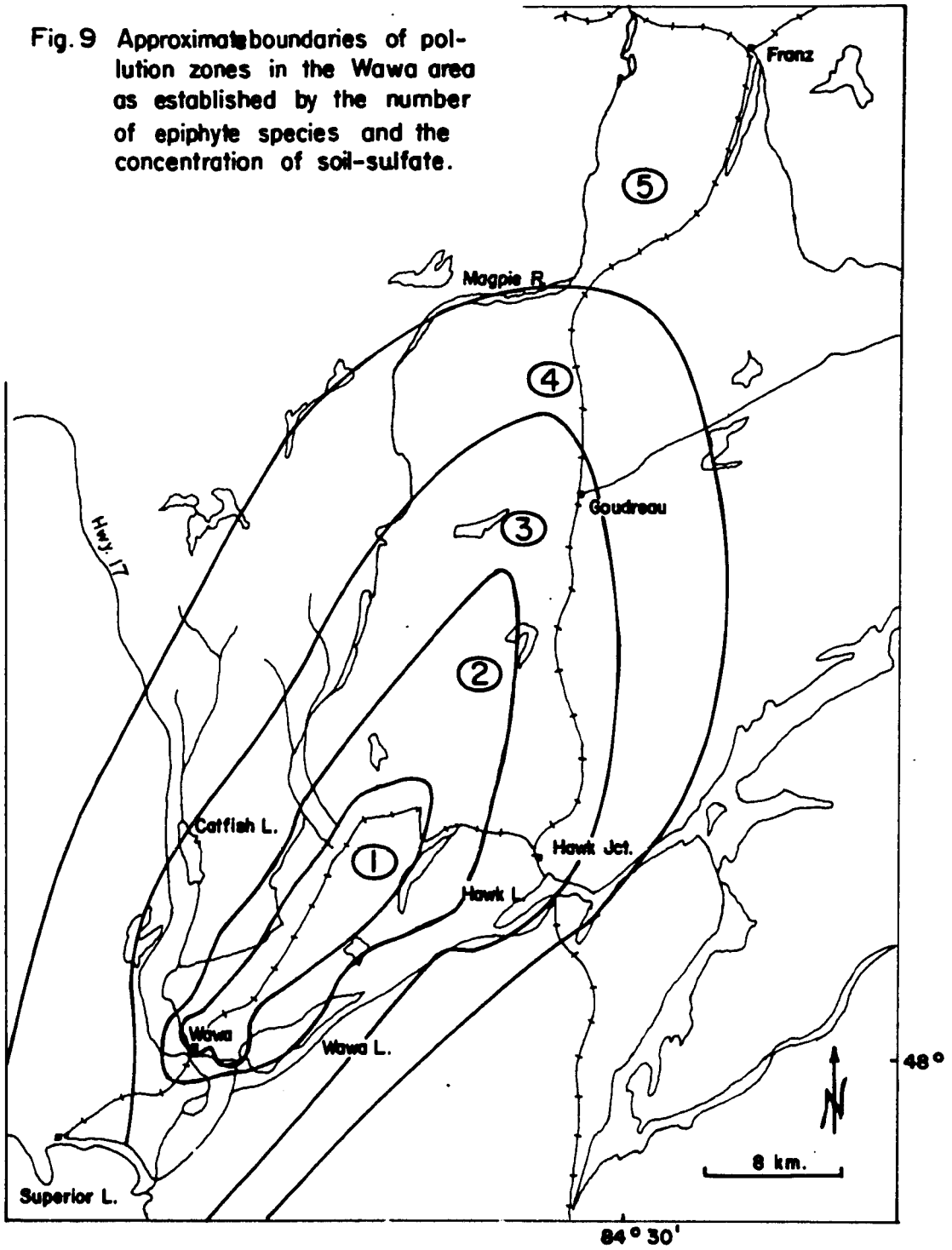
Alectoria canadensis, A. glabra, A. nidulifera, Blepharostoma trichophyllum, Brachythecium oxycladon, B. reflexum, B. salebrosum, Caloplaca sp., C. cerina, Campylium chrysophyllum, Candelariella vitellina, Cetraria ciliaris,

TABLE VIII

EPIPHYTES PRESENT ON THE LINE-TRANSECT N OF THE SINTER PLANT

Collection site No.	8	44	43	47	26
km from the sinter plant	24.2	11.2	6.5	4.0	0.8
EPIPHYTES					
Alectoria canadensis	+		+		
A. glabra		+	+		
A. nidulifera	+		+		
Bacidia chlorococca	+	+	+	+	
Blepharostoma trichophyllum	+				
Cetraria ciliaris	+		+		
C. oakesiana		+			
C. pinastri	+	+	+		
Cladonia chlorophaea	+		+		
C. coniocraea	+	+	+	+	
C. verticillata		+	+		
Dicranum flagellare	+	+			
D. fulvum			+		
D. montanum	+	+			
D. polysetum	+				
Drepanocladus uncinatus	+				
Evernia mesomorpha	+	+			
Frullania eboracensis	+	+			
Jamesoniella autumnalis		+			
Lecanora chlarona	+				
Mnium cuspidatum	+				
M. drummondii	+				
Mycoblastus sanguinarius			+	+	
Parmelia physodes	+	+	+		
P. olivacea	+				
P. sulcata	+				
Parmeliopsis aleurites		+			
P. ambigua	+				
P. hyperopta	+	+			
Pertusaria sp.			+		
Pleurozium schreberi	+				
Ptilidium pulcherrimum	+	+	+		
Pylaisiella polyantha	+				
Usnea subfusca	+				
U. variolosa	+	+	+		
Total No. of epiphytes	27	16	15	3	0
meq SO_4/100 g of soil	0.18	0.69		0.79	1.5

Fig. 9 Approximate boundaries of pollution zones in the Wawa area as established by the number of epiphyte species and the concentration of soil-sulfate.



C. pinastri, C. saepincola, Cladonia bacillaris, C. chlorophaea, C. fimbriata, C. verticillata, Cladonia spp. (squamules), Dicranum fulvum, D. montanum, Evernia mesomorpha, Fissidens cristatus, Frullania eboracensis, Heterophyllium haldanianum, Hypnum pallescens, Jamesoniella autumnalis, Lecanora sp., Lecanora symmicta, Lecidea helvola, Leptogium crenatellum, Lophocolea heterophylla, Orthotrichum obtusifolium, Parmelia exasperatula, P. olivacea, Parmeliopsis ambigua, P. hyperopta, Pertusaria sp., Physcia aipolia, P. grisea, Plagiochila asplenioides, Plagiothecium denticulatum, P. sylvaticum, Platygyrium repens, Pohlia nutans, Pylaisiella polyantha, Rinodina orchea, Tetraphis pellucida, Usnea hirta, U. variolosa, Xanthoria polycarpa and Physcia stellaris.

Zone 4. Sulfate concentration between 0.4 to 0.7 meq/100 g, the number of epiphytic species at any site ranging from 15 to 30. The epiphytes recorded in zones two and three were present along with the following additional 27 species (20 lichens, six mosses and one liverwort):

Alectoria subcana, Amblystegium serpens, Anaptychia obscurata, A. pseudospeciosa, A. speciosa, Barbilophozia barbata, Caloplaca crysophthalma, Cetraria oakesiana, Cladonia

squamosa, Dicranum polysetum, D. scoparium, Lecanora chlarona, Cladonia cristatella, Lepraria aeruginosa, Lobaria pulmonaria, Mnium cuspidatum, M. drummondii, Ochrolechia rosella, Parmelia subaurifera, P. saxatilis, P. septentrionalis, P. trabeculata, Parmeliopsis aleurites, Pleurozium schreberi, Ramalina fastigiata, Usnea scabiosa and U. subfusca.

Zone 5. Sulfate concentration less than 0.4 meq/100g, the number of epiphytic species at any site more than 30. The epiphytes recorded in zones two, three and four were present along with the following 22 species (14 lichens, five mosses and three liverworts) which appeared only in this zone:

Bacidia sphaeroides, Buellia stillingiana, Calicium populneum, Cetraria aurescens, C. glauca, Cladonia cenotea, Collema nigrescens, C. subfurvum, Eurhynchium pulchellum, Lecanora chlarotera, Lecidea vernalis, Lepidozia reptans, Lophocolea minor, Oncophorus wahlenbergii, Orthotrichum sp., Paraleucobryum longifolium, Parmelia caperata, Pertusaria multipuncta, Radula complanata, Ulota crispa, Usnea cavernosa and U. dasypoga.

Gordon and Gorham (1963) in a study of the ecological aspect of the air pollution from the iron sintering plant at Wawa have recognized: 1, very severe; 2, severe; 3, considerable; 4, moderate; and 5, not obvious categories of

cover damage for higher vegetation. The approximate boundaries of these categories have been established by aerial survey of vegetation. We find that the five zones delineated on the basis of soil sulfate concentration and epiphytic vegetation are quite similar to those for the cover damage of vegetation.

In any epiphytic population the sensitive species have to be considered mainly for the assessment of pollution. The toxitolerant species are of little value in this connection as they are equally present in polluted and non-polluted areas. From the fact that different species of epiphytes appear at different distances from the source of pollution, it may be inferred that there are various degrees of toxitolerance or toxiphoby among them. The order of toxiphoby or poleophoby[1] (Erichsen 1928) in some epiphytes has been indicated by Barkman (1958; Netherlands), Jones (1952; Birmingham), Haugsja (1930; Oslo), and many other workers.

It is not easy to place the epiphytes in a strict order of tolerance to SO_2-pollution, because at long distances from the source of pollution minor habitat variations can override the effects of decreasing pollution. However, in more or less uniform habitat conditions, the conspicuous absence or presence of certain species could provide a general picture of their sensitivity to pollution.

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[1] To designate the sensitivity of epiphytes to city-atmosphere the term "poleophoby" is generally used.

For this purpose, the species appearing for the first time on the trunk of trees (not on the base) on the transect-line NNE of the sinter plant have been arranged in a sequence according to increasing toxiphoby (Table IX). For the purpose of comparison, 12 degrees of toxiphoby, as distinguished by Barkman in Netherlands, are considered here. The species with a high degree of toxiphoby should be considered as indicator species sensitive to pollution.

Presence of Bacidia chlorococca, Mycoblastus sanguinarius and Cladonia coniocraea nearest to the epiphyte "desert", indicates that the crustose and the squamose lichens are relatively more tolerant to pollution than the other growth forms. It is thought that the more surface area an organism exposes to the polluted air, the more susceptible it will be to pollution. It is seen that there is a sequence of decreasing tolerance or increasing sensitivity from crustose to foliose to fruticose lichens and bryophytes. Thus, in increasing air toxicity the large fruticose and foliose lichens and bryophytes will be the first to be affected, then the smaller foliose species and finally the crusts and algae. This seems to be a common phenomenon, although there may be many exceptions as is evident from the table. At Wawa, Parmelia sulcata and P. physodes seem to possess a higher degree of toxitolerance than their European counterparts.

Thus, from the survey of epiphytic flora and its correlation with the fallout pattern of SO_2 in air, water, soil and vegetation, it is evident that the epiphytes are

TABLE IX

TOXIPHOBY OF EPIPHYTIC LICHENS AND BRYOPHYTES

	Wawa Ont.	Nether-lands	Birming-	Oslo	Growth forms
Bacidia chlorococca	1				Crustose
Mycoblastus sanguinarius	2				Crustose
Cladonia coniocraea	2				Squamose
Parmelia sulcata	3	5	6	6	Foliose
P. physodes	3	6	6	6	Foliose
Ptilidium pulcherrimum	3				Liverwort
Cetraria pinastri	4				Foliose
Parmelia exasperatula	4			7.5	Foliose
Physcia grisea	4	4		7.5	Foliose
Pylaisiella polyantha	4				Moss
Caloplaca cerina	5				Crustose
Parmeliopsis ambigua	5				Foliose
Physcia aipolia	5	8		10.5	Foliose
Candelariella vitellina	6	5			Crustose
Cetraria ciliaris	6				Foliose
Evernia mesomorpha	6				Fruticose
Orthotrichum obtusifolium	6				Moss
Alectoria canadensis	7				Fruticose
A. glabra	7				Fruticose
Parmelia olivacea	7				Foliose
Physcia stellaris	7			9	Foliose
Platygyrium repens	7				Moss
Usnea subfusca	7				Fruticose
U. variolosa	7				Fruticose
Blepharostoma trichophyllum	8				Liverwort
Xanthoria polycarpa	8	7		9	Foliose
Alectoria subcana	9				Fruticose
Anaptychia obscurata	9				Foliose
Parmelia saxatilis	9	10	6		Foliose
P. septentrionalis	9				Foliose
P. subaurifera	9				Foliose
P. trabeculata	9				Foliose
Ochrolechia rosella	9				Crustose
Pleurozium schreberi	9				Moss
Ramalina fastigiata	9	8	10		Fruticose
Usnea scabiosa	9				Fruticose
Alectoria nidulifera	10				Fruticose
Parmeliopsis hyperopta	10				Foliose
Usnea hirta	10	9			Fruticose
Cetraria oakesiana	11				Foliose
Caloplaca chrysophthalma	11				Crustose
Parmeliopsis aleurites	11				Foliose
Paraleucobryum longifolium	12				Moss
Pertusaria multipuncta	12				Crustose
Ulota crispa	12				Moss
Usnea [illegible]	12	11			[illegible]

quite sensitive to SO_2 and that there are several species of corticolous lichens and bryophytes the absence of which can serve as an indicator of SO_2-pollution.

PART II

EFFECT OF SULFUR DIOXIDE ON EPIPHYTIC LICHENS AND BRYOPHYTES: FIELD STUDIES IN SUDBURY

About 71% of the world's production of nickel (Allen 1963) comes from the mines of the Sudbury region, Ontario, where the International Nickel Company of Canada and the Falconbridge Nickel Mines have exploited the rich ore deposits since 1888 (LeBourdais 1953). The annual production of both companies amounts to about 226,800,000 kg (250,000 tons) of nickel.

The ores, chiefly iron and copper pyrites containing nickel sulfide, are treated on the spot in three immense smelters, the most important one being at Copper Cliff. The ores are burnt and most of the sulfur present in them is removed as sulfur dioxide. Thus, along with the smelter fumes, considerable amounts of SO_2 are emitted into the atmosphere through large stacks up to 200 m high. The fallout of the pollutants has caused a severe damage to vegetation and any one travelling in the Sudbury region cannot help noticing the vast unforested territories and eroded lands, the results of the intense fumigations of SO_2.

Sudbury, a town of 80,000 inhabitants, is located in Ontario at 48° 30' latitude north and 81° longitude west. The topography of the region is more or less flat and the mean altitude varies between 250 and 310 meters.

A great deal of the climax vegetation has been destroyed either by fire, by human activities and/or by the toxic effects of SO_2. The actual disclimax vegetation is mainly composed of Betula papyrifera and Populus tremuloides.

In spite of the harmful consequences that the SO_2-emissions can generate in Sudbury, only three or four works have been undertaken to study this problem. McCallum (1944) tried to determine the range of damage caused to forests by SO_2. Linzon (1958) reported that white pine (Pinus strobus) was particularly sensitive to this gas and he also stated that these trees showed a reduced growth in diameter and some were rapidly dying even at distances 40 km from the source of pollution. Gorham and Gordon (1960a, 1960b) showed that the number of plant species decreased progressively with distance when one approached the smelters. Among the species most tolerant to SO_2-pollution they note mainly Acer rubrum, Quercus rubra, Sambucus pubens, and Polygonum cilinode. Dreisinger (1965), after many years of observation, noticed that the plants most sensitive to SO_2 in the Sudbury region are Fagopyrum sagittatum, Trifolium pratense, Populus tremuloides, Pinus banksiana, Pteridium aquilinum, Betula papyrifera, and Pinus strobus.

Many ecologists have noticed the extreme sensitivity of lichens and mosses, particularly epiphytic species, to atmospheric pollutants, but so far, little has been done to measure in situ the effects of the pollutants on these organisms. Arnold (1891-1901) attached epiphytic lichens to tree trunks in Munich and stated that their death occurred

shortly afterwards. Tobler (cited by Sauberer 1951) demonstrated that in towns cultivation of some lichens was possible only in filtered air. Recently, Brodo (1961) used a transplantation method to study the behavior of corticolous lichens in polluted areas. By using a cylindrical cutting blade, he removed lichen-bearing bark-discs and transplanted them on trees in the locality where the behavior of these lichens was to be studied. We have used this technique, with some modifications, to study the effect of sulfur dioxide on some corticolous lichens and mosses.

MATERIALS AND METHODS

During the summer of 1964 we cut from the bark of trees 42 circular discs, 4.8 cm in diameter, on which 19 species of lichens and mosses were growing. These samples were taken mostly from isolated trees along the roads at about 30 km from the city of Ottawa. To cut these discs and to make holes for transplantation, we used a circular saw, 4.8 cm in diameter and 5 cm in depth, powered by a nickel-cadmium battery. All the discs were photographed and the species on them were identified. We selected seven stations near Sudbury in the areas polluted by SO_2 and transplanted these discs on trees of the same genera from which they had been originally removed. While transplanting, care was taken to place them always at the same height (about one meter), on the same side of the tree and, as far as possible, in the same environmental conditions (except, of

course, for SO_2) which prevailed at the site of their origin. The cut-end of the disc and the hole made in the host-tree were first disinfected with ethyl alcohol and then the disc was tightly fixed in the hole with the help of grafting wax. In order to avoid any displacement due to the cambial activity of the stem in future, the disc was firmly fastened in position by a stainless-steel nail.

RESULTS

Our bark samples were transplanted on the host-trees in July 1964. In May 1965 we noticed that most of the lichen and moss species were more or less damaged. In October 1965 we reexamined the discs more closely with a hand lens and the results were compiled for each tree and for each station (Table X). It was quite difficult to appreciate objectively on the spot the damages caused to the lichens and the mosses. We, therefore, adopted the following numerical scale to express approximately the range of damages:

4. Mosses and lichens not damaged but not growing either; a slight loss of color in the plant body (Plate I: Figs. 1-2).
3. Mosses and lichens damaged and also showing a distinct loss of color in the plant body; only about 75% of the original covering remaining (Plate I: Figs. 3-4).
2. Mosses and lichens dead or very strongly damaged;

TABLE X

ESTIMATION OF DAMAGES CAUSED TO THE LICHENS AND MOS

Location from Copper Cliff	3.2 km ESE				3.2 km E				4.8 km E									
Epiphytes \ Trees	150	153	154	179	37	38	166	178	128	51	52	62	69	73	98	119	143	
Bacidia chlorococca				3							4		3	4				
Caloplaca cerina										0						1		
Candelaria concolor	1				3	2			3		1							
Graphis scripta															3			
Orthotrichum obtusifolium		1	1			1			2									
Parmelia caperata																		
P. rudecta																		
P. subaurifera													1					
P. sulcata													3	3				3
Physcia adscendens		2														0		
P. aipolia		2																
P. grisea			1					1	2									
P. millegrana				1			3	1										
P. orbicularis	0																0	
P. stellaris										0	0	1						
Platygyrium repens																		
Pylaisiella selwynii		1	2														2	
Ulota crispa																		
Xanthoria fallax			3	2	4	3	4	2								2		

150, 153, 154 and 179: Ulmus americana onto U. americana
37 and 38: Acer negundo onto A. negundo
166 and 178: Ulmus americana onto U. americana
128: Fraxinus pensylvanica onto F. pensylvanica
51, 62 and 69: Populus tremuloides onto P. balsamifera
52 and 73: Populus tremuloides onto P. grandidentata
98: Quercus rubra onto Q. rubra
119 and 143: Ulmus americana onto U. americana

54,
188
89 &
56,
1, 2
57,
93:

TABLE X

MAGES CAUSED TO THE LICHENS AND MOSSES TRANSPLANTED IN THE REGION OF SUDBURY, ONTARIO

4.8 km E									14.4 km E				17.6 km E				11.2 km NE									17.6 km
128	51	52	62	69	73	98	119	143	54	63	83	84	188	193	89	90	56	60	67	68	71	72	74	80	82	94	1	2	6	57	75
	0	4		3	4		1				4		2	4					3				4	3	4		3	4			
3		1																	2									3			
						3																					3	4			
2																															
																										3					
																										1		2			
				1																	1										
				3	3				3		4		1	2	1						3						2				
							0																3								
																	1				1								1		
2																	2														
																											2				
								0																			1	1			
	0	0	1							3	3	3					1		0	3	2	2	2	2	1				3	2	
								2							4	3															
															3																
							2																								

54, 63, 83 and 84: Populus tremuloides onto P. balsamifera
188 and 193: Pinus strobus onto P. resinosa
89 and 90: Quercus rubra onto Q. rubra
56, 60, 67, 68, 71, 72, 74, 80, 82 and 94: Populus tremuloides onto P. bals
1, 2 and 6: Acer saccharum onto A. saccharum
57, 75 and 87: Populus tremuloides onto P. tremuloides
93: Quercus rubra onto Q. rubra

X

MOSSES TRANSPLANTED IN THE REGION OF SUDBURY, ONTARIO

14.4 km E				17.6 km E				11.2 km NE										17.6 km SW						
54	63	83	84	188	193	89	90	56	60	67	68	71	72	74	80	82	94	1	2	6	57	75	87	93
			4	2	4					3				4	3	4		3		4				
										2									3					
																		3		4				
																			3					
																	3							2
																	1			2				
												1												
3		4		1	2	1						3		3				2				4	3	
								1				1									1			
								2																
																		2						
																		1	1					
	3	3	3					1		0	3	2	2	2	2	1					3	2		
						4	3																	2
						3																		2

54, 63, 83 and 84: Populus tremuloides onto P. balsamifera
188 and 193: Pinus strobus onto P. resinosa
89 and 90: Quercus rubra onto Q. rubra
56, 60, 67, 68, 71, 72, 74, 80, 82 and 94: Populus tremuloides onto P. balsamifera
1, 2 and 6: Acer saccharum onto A. saccharum
57, 75 and 87: Populus tremuloides onto P. tremuloides
93: Quercus rubra onto Q. rubra

dry and highly decolorized; only about 50% of the original covering remaining.

1. Mosses and lichens dead, very dry and entirely without color; only about 25% of the original oovering remaining (Plate I: Figs. 5-6).

0. Mosses and lichens dead and decayed; nothing remaining on the disc.

Thalli of Parmelia sulcata and P. caperata were collected from the surfaces of the discs for microscopic examination. The following features were noted:

a. A marked reduction in the thickness of the thallus. This was apparent from a comparison with the thickness of a thallus collected at the same site where the lichen-bearing discs were cut originally.

b. Incipient to total plasmolysis in Trebouxia cells, the algal symbiont.

c. Chloroplast injury in the algal cells which ranged from sporadic brown spots on the surface to complete disappearance of the green pigment.

d. Formation of a thin layer of a whitish, crystalline and water-insoluble substance on the surface of the thallus. This substance when dissolved in acetone and recrystallized in GE (glycerine-acetic acid, 1.3 v/v), formed colorless, irregular, plate-like crystals possibly indicating some fatty substance.

e. Presence of large oil-globules in Trebouxia cells. For testing for the presence of oil, a small portion of the dust-free thallus was soaked in water for one

PLATE I: Bark-discs bearing lichens transplanted in Sudbury.

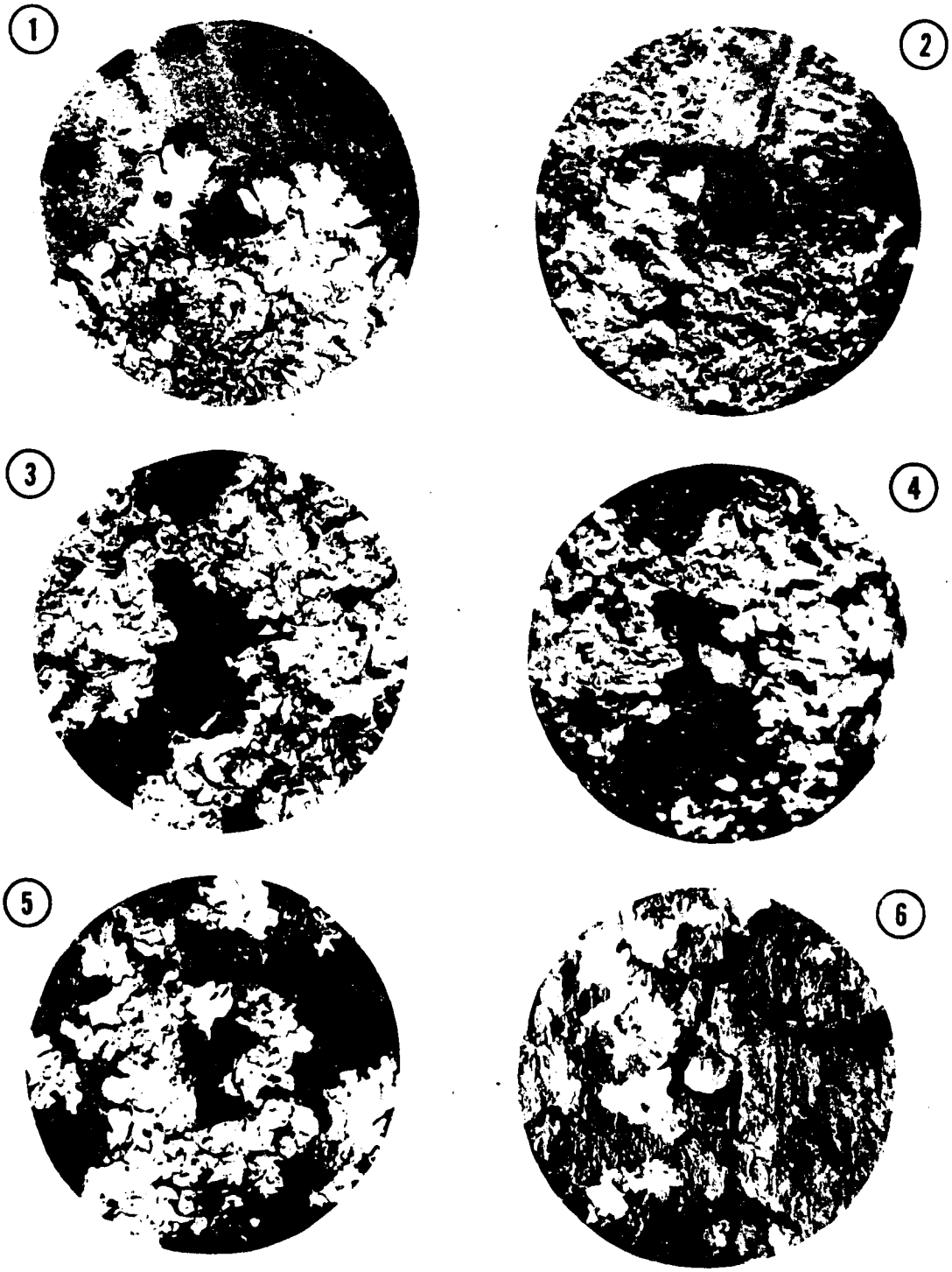
Figs. 1-2. *Parmelia sulcata* on *Populus tremuloides*.

Figs. 3-4. *Parmelia caperata* and *P. rudecta* on *Populus tremuloides*.

Figs. 5-6. *Parmelia sulcata* and *Bacidia chlorococca* on *Pinus strobus*.

Figs. 1, 3 and 5 represent samples before transplantation and Figs. 2, 4 and 6 represent the same samples 15 months after transplantation. Perforation in the center of the disc has been made by the drill of a circular saw.

PLATE I



hour. It was then transferred to a vial containing a saturated solution of Sudan III in 70% alcohol and kept overnight at room temperature. For microscopic examination it was washed in 50% alcohol and crushed under a coverslip in a drop of 30% glycerine on a slide (McLean and Cook 1963). Oil-globules which stained orange-red, were placed either separately or aggregated within the cell. The lichen thalli from non-polluted areas were mostly negative or, rarely, slightly positive to this test.

f. Formation of chlamydospore-like bodies by the fungal symbiont especially in the lower cortex.

DISCUSSION AND CONCLUSIONS

Dreisinger (1965) estimates that 0.95 ppm of SO_2 for one hour, or 0.55 ppm for two hours, or 0.35 ppm for four hours, or 0.25 ppm for eight hours at the ground level are enough to cause serious damages to many plants of economic importance. In the Sudbury region, the Ontario Department of Mines has installed ten Thomas autometers which automatically register the amount of SO_2 present in the atmosphere. One of our stations was located only a few meters from the autometer near Garson, whereas all the other stations were situated a few km E and SW from this autometer. Dreisinger (1965) has compiled the data registered by the autometer at Garson during the growing season (May-October) for the

years 1954 to 1963[1]. Of a total of 38,795 reading hours, about 20% indicated presence of SO_2 in the atmosphere. For 1735 hours, the concentration was more than 0.25 ppm; for 602 hours it was 0.50 ppm and for 85 hours it was 1.0 ppm. Concentrations of 3.06 ppm were also registered during daytime and of 1.64 ppm during night. Out of the 1657 days during which the autometer was in operation, presence of SO_2 was registered 1042 times; in 209 of these, the gas concentrations, according to Dreisinger, were higher than the critical levels for a large number of cultivated plants. One must not forget, however, that certain species are much more sensitive and they are severely affected even by small doses of SO_2. For example, a concentration of 0.5 ppm for one hour is sufficient, under high humidity conditions (Dreisinger 1965), to cause serious damages to Populus tremuloides, Pinus banksiana, Fagopyrum sagittatum or Pteridium aquilinum.

It is evident that mosses and lichens as well as the higher plants are affected to various degrees by pollution. Some species such as Bacidia chlorococca and Parmelia sulcata are quite toxitolerant and may live in the proximity of industrial cities, whereas others, such as the species of Usnea and Cetraria are extremely toxisensitive.

Out of the 19 species of lichens and mosses that we have studied, Bacidia chlorococca has resisted most the ecological conditions prevailing at Sudbury. Bacidia chlorococca is a crustose lichen growing appressed to the

1 For the years 1964-65 the data are likely to be the same as those registered between 1954-63.

bark and it is difficult to measure the extent of its thallus. It seems, however, that it did not suffer much by the pollution. Parmelia sulcata and P. caperata, two foliose lichens, were less damaged than Parmelia subaurifera which was found to be very sensitive. Out of the six species of Physcia that we studied, P. stellaris and P. aipolia were more tolerant, P. grisea and P. millegrana were less tolerant and P. adscendens and P. orbicularis were least tolerant to SO_2 pollution. Among the mosses, Orthotrichum obtusifolium, Platygyrium repens, Pylaisiella selwynii and Ulota crispa were all equally damaged and under the conditions they appeared to be more toxitolerant than the lichens.

Brodo (1965) transplanted at Long Island, New York, bark samples on which a foliose lichen, Parmelia caperata, was growing. After only four months of exposure to the polluted atmosphere of that city, this lichen had undergone considerable damages even at distances up to 60 km from the center of the city. After one year almost all the thalli were dead. Control samples, transplanted well outside the zone of pollution, not only had not been affected but indicated a slight growth in the thallus after one year. Unfortunately, we did not place controls in the Sudbury region because of the widespread pollution. One may remark that the transplanting treatment alone, rather than the atmospheric pollution, is responsible for the damages caused to the lichens and mosses. It is possible that the moisture condition of the transplanted barks is slightly unbalanced and this factor along with pollution may affect the epiphytes.

Let us say, however, that the discs were firmly pressed on the host tree and that rain water contributed to keep these barks relatively moist. Also, the samples transplanted at McCharles Lake, nearly 18 km SW of Copper Cliff (the least polluted locality) were less damaged than those transplanted at Garson, nearly 11 km NE of Copper Cliff (the most polluted locality). In the summer of 1964, we transplanted bark-discs bearing lichens on trees in a park of the city of Ottawa; these lichens still seem to be unaffected.

Plasmolysis and chloroplast damage of the algal symbiont in the transplanted thalli suggest hampering of anabolic processes and consequently, a gradual emaciation of the thallus.

The production of a fatty substance on the thallus surface may be interpreted as a reaction to an irritant. However, a thick layer of such hydrophobic substances could be a barrier in water transfer through the external surface and gradually upset the moisture conditions of the thallus, producing significant changes such as plasmolysis.

The significance of the oil-globules formed inside the algal cells of the transplanted thalli is not understood. Russell-Wells (1932) in his studies of brown algae correlates the high percentage of fats in Pelvetia libera with its exposed habitats. Perhaps the presence of oil in the algal symbiont enables the cells to resist desiccation. Fat when oxidized, gives rise to twice as much water as the other foodstuffs on account of its high content of hydrogen; this

proportionately large-scale production of metabolic water is important for organisms living under xeric conditions (Baldwin 1957).

Little is known of the exact factors inducing the formation of chlamydospore-like bodies but it is generally assumed that such spores in the free-living fungi are formed in response to the onset of unfavorable conditions, especially a limitation of nutrients. For example, in Candida albicans chlamydospores are formed when the concentration of glucose in the medium is low (Nickerson and Mankowski 1953). Also, the chlamydospores of species of Fusarium are generally found in old cultures where the medium is almost depleted of essential nutrients (Hawker 1957). Ahmadjian (1966) reports that slow drying stimulated the artificial development of fruiting structures in Cladonia cristatella; soredia and squamules were formed in response to the joint effect of slow drying and nutrient-poor conditions. Alternatively, Richards (1949) reported that sporulation in Alternaria species occurs under the influence of ozone. Perhaps the concentration of SO_2 in the atmosphere was alone sufficient to stimulate formation of spores. In the transplanted thalli, all these factors: desiccation, nutrient-poor conditions and sulfur dioxide could have simultaneously affected sporulation.

The almost complete absence of epiphytic mosses and lichens in the region of Sudbury is probably the result of the intense atmospheric pollution. It is certain that before the advent of the present conditions this region had

a rich epiphytic flora. The epiphytic species have disappeared one after the other from this region and today there is a vast epiphyte "desert" around the smelters. For the moment, it is certainly difficult to indicate the absolute concentration of sulfur dioxide that the epiphytic mosses and lichens can tolerate without being damaged. Precise autoecological studies will have to be undertaken in order to determine the limits of tolerance for each species.

PART III

EFFECT OF SULFUR DIOXIDE ON EPIPHYTIC LICHENS AND BRYOPHYTES: LABORATORY EXPERIMENTS

In vascular plants necrosis and chlorosis of the leaves indicate damage to chlorophyll caused by sulfur dioxide (Katz 1949), but no such visible symptoms can be seen in the lichens. Pearson and Skye (1965) found that discs of Parmelia sulcata kept in flasks contaminated with sulfur dioxide showed morphologic and photosynthetic abnormalities similar to those of lichens collected from industrial centers. They suggested that the scarcity of lichens in city environments may be due to atmospheric pollutants which destroy chlorophyll, but presented no experimental evidence to support this view.

In the experiments to be described here, an attempt has been made to determine the nature of the damage to the lichen thallus exposed to sulfur dioxide by investigating morphological, chemical, and other changes occurring in the chloroplast of the algal symbiont.

MATERIALS AND METHODS

Thalli of Xanthoria fallax, X. parietina, Parmelia caperata, and Physcia millegrana, all having Trebouxia as the algal symbiont, were used as experimental material.

Exposure to sulfur dioxide

The thalli were exposed to an atmosphere of 5 ppm of SO_2 for 24 hours at room temperature under a variety of moisture conditions in leak-proof desiccator jars. The gas was generated inside the jars by reacting dilute sulfuric acid with sodium bisulfite, the amounts adjusted to produce the desired gas concentration. Constant relative humidities of 20, 45, and 92% were obtained by placing uncovered saturated solutions of potassium acetate, potassium nitrite, and sodium carbonate (Na_2CO_3) respectively in the desiccators (McLean & Cook 1963). To obtain an initial condition of moisture saturation, the thallus was soaked in water prior to exposure. Control thalli, for each of the relative humidities used, were placed in jars without SO_2. In all subsequent tests both control and treated thalli were used.

Preparation of extract for testing sulfurous acid and sulfate

Three hundred mg of thallus were finely ground in a mortar with 10 ml of 80% acetone (acetone: water, 4:1 v/v). The extract was decanted and filtered and the filtrate was transferred to a separatory funnel containing 15 ml of petroleum ether. After being shaken well, the two layers were allowed to separate. The lower acetone-water layer was drawn off and stored in a stoppered vial at room temperature for use in tests for sulfurous acid and sulfate ions. The upper petroleum ether layer was discarded.

To test for the presence of sulfurous acid (Taylor 1948)

a drop of this preparation was spotted onto a piece of filter paper impregnated with a solution of potassium iodate and starch, and dried at room temperature. The development of a blue color resulting from the reduction of iodate to iodine and the reaction of the latter with starch indicates the presence of sulfurous acid. The intensity of the color increases with time but is proportional to the concentration of H_2SO_3.

The remaining acetone-water layer was transferred to a 15-ml teflon tube containing a small amount of activated charcoal for adsorption of the pigmented impurities. After being shaken well, the mixture was allowed to stand for 3-4 hours, then the clear supernatant was decanted and filtered.

In the test for sulfate (Toennies & Bakay 1953), 0.5 ml of 3% H_2O_2 was added to 2.5 ml of the decolorized acetone-water extract. The mixture was shaken well, then 2.5 ml of 40% ethanol-glycol mixture, 0.2 ml of 6 N HCl, and 5 ml of $BaCl_2$ reagent were added immediately. The resultant mixture was allowed to stand for 15 minutes. Its optical density was measured at 420 mμ in a Coleman Model 6A Junior Spectrophotometer. Round cuvettes (19 X 150 mm) were used and a blank mixture was formed by using 2.5 ml of sulfate-free water in place of the extract in the above reaction mixture. The optical density reading was multiplied by a conversion factor (2.95) to obtain the concentration of soluble sulfate (mg per 2.5 ml) in the sample.

Extraction of chlorophyll and determination of its absorption spectrum

Three hundred mg of thallus were mixed with 150 mg of sodium carbonate and rapidly ground in a mortar with 10 ml of 80% acetone (acetone: water, 4:1 v/v). The addition of sodium carbonate in the grinding mixture prevents the degradation of chlorophyll during extraction by the acid present in the thallus (Smith & Benitez 1955). The extract was filtered and transferred to a separatory funnel containing 15 ml of petroleum ether. The two layers were allowed to separate after stirring. The lower acetone-water layer was drawn off and later used for testing for the presence of magnesium in it.

The upper petroleum ether layer containing the pigments was washed twice with distilled water and concentrated under reduced pressure. For chromatography of the pigments, the concentrated extract was spotted on Whatman No. 1 filter paper. The chromatogram was developed by the ascending method in a petroleum ether solvent containing 0.5% n-propanol and was run to a height of 20 cm. The green or brownish-green spot near the solvent front of each air-dried chromatogram was eluted with ether and the absorption spectrum of the dissolved pigment was recorded in a Cary Model 14 Spectrophotometer (Applied Physics Corp., U.S.A.).

Test for magnesium

The acetone-water layer obtained during extraction of chlorophyll was decolorized by activated charcoal as

described above. To one ml of the colorless filtrate, 5 drops of 8 N NaOH and 2 drops of a reagent, prepared by dissolving 0.5% of p-nitrobenzeneazoresorcinol (a complex dye) in 1% NaOH solution were added. The presence of Mg^{++} is indicated by the formation of a blue precipitate resulting from the union of $Mg(OH)_2$ with the dye (Sorum 1960).

RESULTS

1) The microscopic appearance of algal cells teased from treated thalli revealed abnormal characteristics such as bleaching of the chlorophyll, permanent plasmolysis, and sporadic brown dots on the chloroplast. The latter were more conspicuous in specimens exposed to SO_2 at higher humidities (Plate II: Figs. 1-4). The algal cells of the control did not show any of these abnormalities (Plate II: Fig. 5).

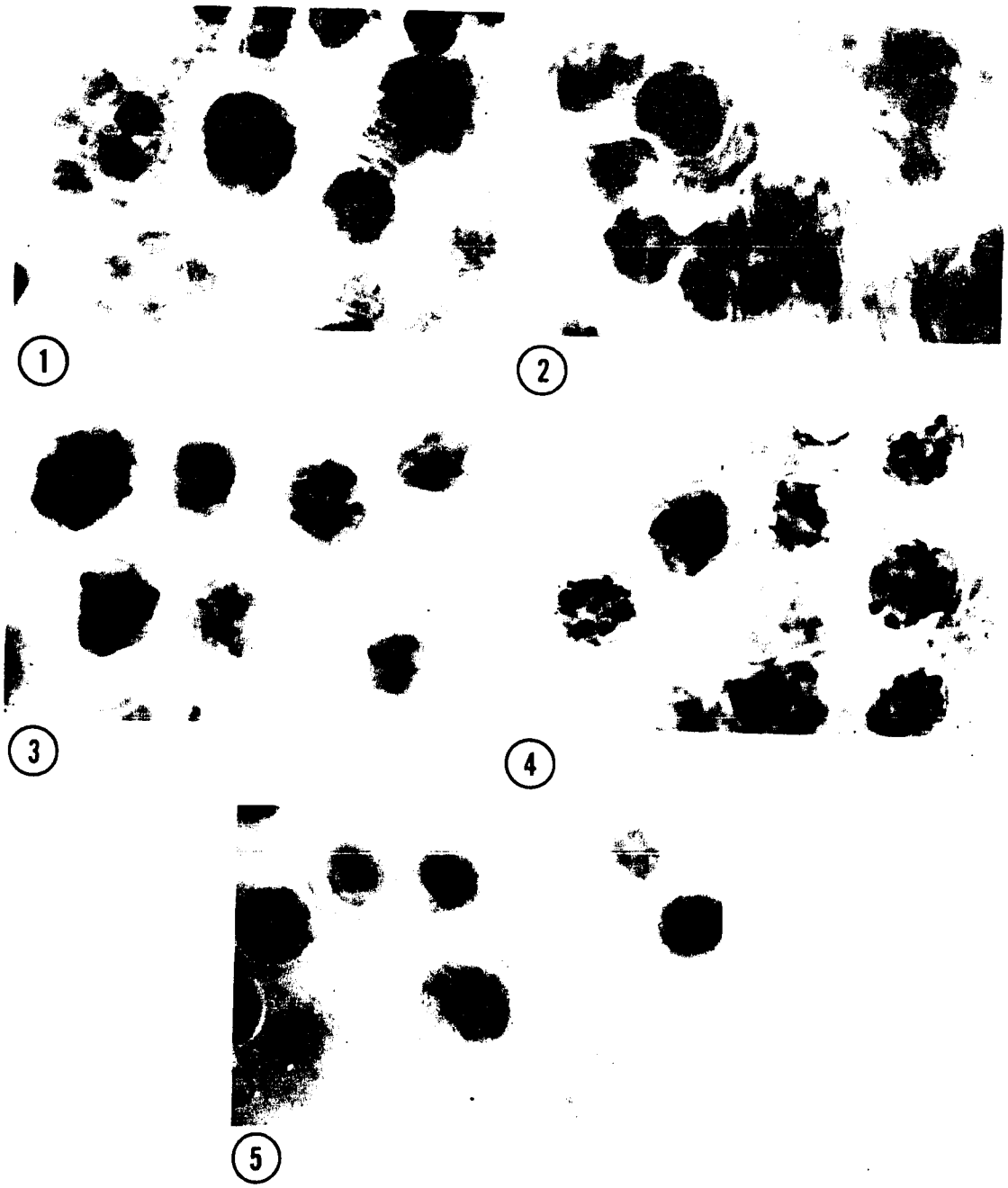
The tests for sulfurous acid, SO_4^{--} and Mg^{++} were positive in the extracts of the SO_2-exposed thalli and negative in the extracts of the normal thalli. Of these tests, only the sulfate test was quantitative. It showed that there was a direct relationship between SO_4^{--} concentration in the thallus and the relative humidity to which the thallus had been exposed. The sulfate concentration in 2.5 ml samples of extracts prepared from equal amounts of thalli of Physcia millegrana exposed to SO_2 in relative humidities of 20, 54, and 92% was found to be 0.08, 0.12, and 0.23 mg respectively; but in the initially

PLATE II: The algal symbiont Trebouxia.

Figs. 1-5: Trebouxia from Xanthoria fallax in the experiments with sulfur dioxide, x 1250.

1. The alga exposed to 5 ppm of SO_2 in 20% relative humidity.
2. The alga exposed to 5 ppm of SO_2 in 45% relative humidity.
3. The alga exposed to 5 ppm of SO_2 in 92% relative humidity.
4. The alga exposed to 5 ppm of SO_2 in the initially wet thallus.
5. The alga from an untreated thallus (control).

PLATE II



wet thallus the concentration was 0.35 mg.

3) The absorption spectra of chlorophyll from the SO_2-exposed and normal thalli were different in that the absorption peaks were at 667 mμ and 662 mμ respectively (Fig. 10). These peaks on the absorption spectra, although very close together, are well known to correspond respectively to phaeophytin-a and chlorophyll-a (Smith & Benitez 1955).

Wilhelmsen (1959) observed the presence of phaeophytin in the chlorophylls extracted from the non-desiccated thalli of Peltigera, Parmelia and Xanthoria. He suggested that it was best to desiccate the lichens prior to the extraction of chlorophyll. It takes longer for complete grinding of non-desiccated thalli and I believe that during that period the acids present in the lichen affect the chlorophyll. To avoid destruction of the chlorophyll during extraction, small quantities (300 mg) of thalli were mixed with sodium carbonate (150 mg) and ground in subdued light very rapidly, first in the dry state and then in 80% acetone. The chlorophylls thus extracted from untreated thalli did not show phaeophytin.

DISCUSSION AND CONCLUSIONS

The abnormal morphological features produced in most of the algal cells give evidence of cellular injury as a result of exposure to SO_2. This injury is presumably a result of the presence of sulfurous acid and SO_4^{--} in the thallus. These ions must have come from the SO_2 since they

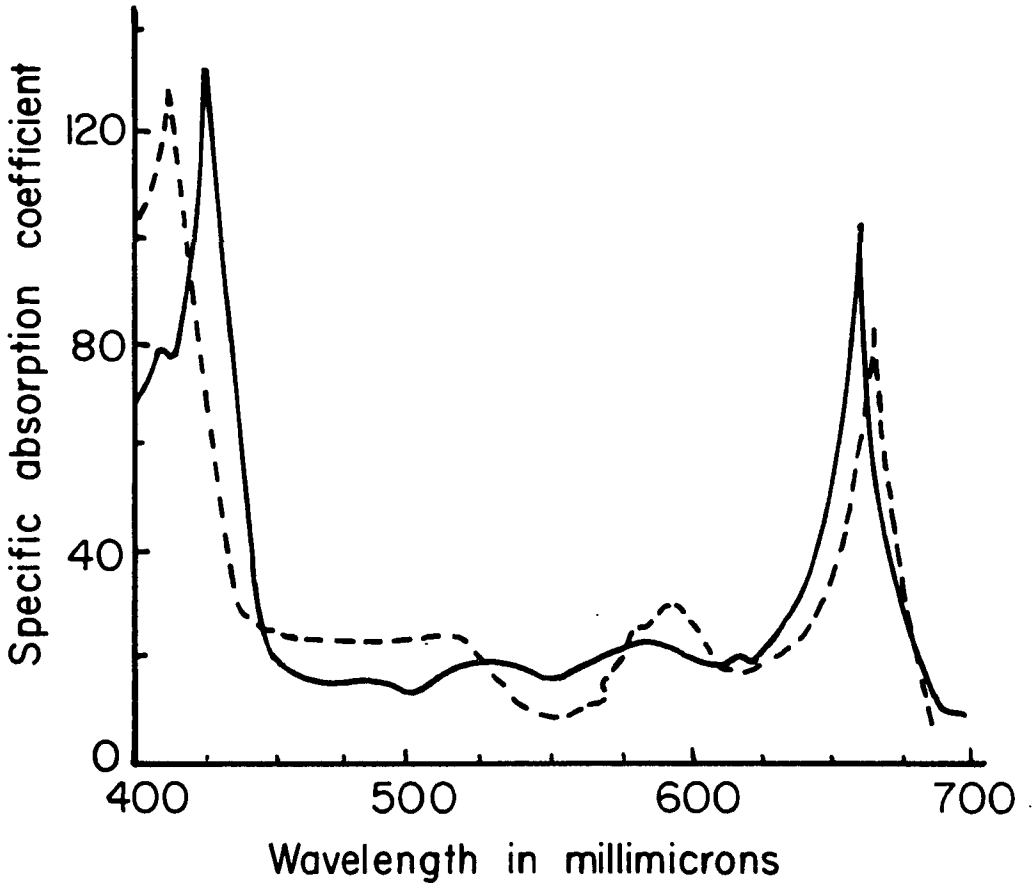


Fig. 10 Absorption spectra of chlorophyll from normal (—) and SO_2-exposed (----) thalli of Xanthoria fallax.

were not found in the extracts of the normal thallus.

Sulfur dioxide is quite soluble in water producing a solution of sulfurous acid. This acid ionizes fairly strongly (Yost & Russell 1946)

$$H_2SO_3 \rightleftharpoons HSO_3^- + H^+$$
$$HSO_3^- \rightleftharpoons SO_3^{--} + H^+$$

and has a standard redox potential (E_h^o) of +0.20 v (Heslop & Robinson 1960). It acts as an efficient bleaching and strong reducing agent. The loss of color in the algal cells could be attributed to the H_2SO_3 present in the thallus either through the formation of colorless addition compounds of SO_2 with the coloring matters or through the reduction of colors to a colorless compound, possibly by nascent hydrogen (Partington 1953).

The plasmolysis was presumably due to the presence of an external surrounding liquid of a higher osmotic value than that inside the algal cells. This may have been produced by the concentration of SO_3^{--} and SO_4^{--} in the thallus together with a possible leaching of substances from the fungal symbiont under the acid influence. The acidic ions would presumably denature the proteins of the algal cells and prevent their deplasmolysis.

The presence of Mg^{++} in the acetone-water layer and of the brown dots on the chloroplasts suggest that Mg^{++} has been removed from chlorophyll-a, converting it to phaeophytin-a (Smith & Benitez 1955). This conclusion is corroborated by the similarity of the absorption spectrum of the pigment isolated from the SO_2-treated extract to that of phaeophytin. The acid condition existing in the

SO_2-treated thallus could cause this degradation by increasing the concentration of H ions. These would displace the Mg^{++} atom from the chlorophyll molecule, transforming it to phaeophytin

$$\text{chlorophyll} + 2H^{+} \longrightarrow \text{phaeophytin} + Mg^{++}.$$

At higher humidities an increase in SO_4^{--}-concentration in the thallus implies a higher rate of absorption of SO_2 and consequently greater absorbed acidity and increased H ion concentration— a condition which would lead to progressive and ultimately irreversible alterations in cell structure and function.

In conclusion I believe that SO_2 absorbed by the lichen thallus causes degradation of chlorophyll-a. Even a slight degradation of the limited chlorophyll is likely to impair the metabolic processes in the thallus. It is thus the algal symbiont that is vulnerable to SO_2 and this probably explains why lichens rank among plants most sensitive to polluted atmospheres.

PART IV

EFFECT OF SULFUR DIOXIDE ON EPIPHYTIC LICHENS AND BRYOPHYTES: SUMMARY AND CONCLUSIONS

The sensitivity of epiphytes to atmospheric pollution has been investigated by several workers. The present investigation is an attempt to describe the effects of sulfur dioxide on epiphytes and to understand the underlying causes responsible for these effects.

A survey of the epiphytes (lichens and bryophytes), a study of the distribution of sulfur dioxide in air, water, soil and vegetation, and a correlation of the fallout pattern of the pollutant with the transitions in the epiphytic population in the Wawa area, show the following facts:

1. Appreciable SO_2-concentrations have been found in the atmosphere at points up to 40-50 km NE of the sinter plant. Frequency of SO_2-visitations in this area is governed by the topography and the persistence of the prevailing SW winds; and the incidence of high concentration of the pollutant especially during the night and early morning hours, is associated with the presence of fog, low wind velocity, high humidity and the absence of a temperature gradient.
2. Lead peroxide-candle results show a large decrease in average and maximum SO_2-concentrations with distance, in the region of 16.0 to 54.4 km NE of Wawa. The magnitude of concentrations found in the region

are noticeably different and various pollution-zones can be conveniently distinguished.

3. In areas with high concentration of SO_2, adjacent to the sinter plant, there is a marked increase in the acidity and the sulfate content of surface-waters, soil and vegetation, but these effects diminish rapidly with increasing distance from the source of pollution.

4. Vegetation damage is mainly concentrated NE of the sinter plant where no trees are present for a distance of 12.8 km, but beyond this zone of severe damage, trees start coming up and gradually their number and crown density increase until the vegetation becomes characteristic of the region at a distance of 56.4 km. The plant species most tolerant to SO_2 are:

> Betula papyrifera, Populus tremuloides, Prunus pensylvanica, Sambucus pubens, Pyrus decora, Salix humilis, Alnus rugosa, Polygonum cilinode, Maianthemum canadense and Aster macrophyllum.

5. Some terricolous lichens and bryophytes are toxitolerant, and species such as Dicranella heteromalla, Pohlia nutans, Cladonia coniocraea, C. deformis and C. floerkaena can grow on highly polluted soils (pH 3.4, SO_4^{--}-concentration 4.3 meq/100 g).

6. Epiphytes are absent within 16.1 km NE and 2.5 km N, NNE, ENE and ESE of the sinter plant. Beyond the epiphyte "desert" corticolous lichens and bryophytes

gradually appear, first on the bases of trees and then on the trunk. Nearest to the sinter plant, Bacidia chlorococca, Mycoblastus sanguinarius, Cladonia coniocraea and Parmelia sulcata are the only species to appear, and apparently, these are the most toxitolerant epiphytes. Further away from the source of pollution the epiphytic flora gradually increases in richness.

7. The number of epiphytes as well as their coverage are inversely proportional to the degree of pollution. At Wawa, five zones can be circumscribed to delimit areas differing in degrees of pollution (expressed in terms of soil sulfate concentration) as well as in the epiphytic population.

8. A comparison of the degree of toxiphoby or sensitivity to pollution of various epiphytic species appearing on the trunk of trees on the line-transect NNE of the sinter plant reveals that species, such as Usnea hirta, Cetraria oakesiana, Parmeliopsis aleurites, Paraleucobryum longifolium, Pertusaria multipuncta, Ulota crispa and Usnea dasypoga are highly toxiphobous and the absence of these species can serve as an indicator of SO_2-pollution.

A macro- and microscopic examination of the epiphytes present on bark-discs, transplanted on host-trees in the region of Sudbury and exposed to the harmful influence of SO_2 for more than one year, reveals the following changes:

9. Slight to severe damages to most of the lichen

and moss species.

10. Marked reduction in the thickness of lichen thalli (Parmelia caperata and P. sulcata).

11. Formation of a thin layer of a whitish, crystalline and water-insoluble compound (perhaps a fatty substance) on the surface of the lichen thallus. Presence of such a hydrophobic substance on the surface could be a barrier in water absorption and gradually upset the moisture conditions of the thallus.

12. Incipient to total plasmolysis in Trebouxia cells, the algal symbiont.

13. Chloroplast injury in the algal cells ranging from sporadic brown spots on the surface to complete disappearance of the green pigment. The chloroplast damage of the algal symbiont in the transplanted thalli suggests hampering of the photosynthetic activity and consequently, a gradual emaciation of the thallus is observed.

14. Presence of oil-globules in Trebouxia cells. Perhaps the presence of oil in the algal symbiont enables the cells to resist desiccation, or perhaps it is simply another manifestation of damage to the cell in an unfavorable environment.

15. Formation of chlamydospore-like bodies by the fungal symbiont, especially in the lower cortex. It is assumed that such spores in the free-living fungi are formed in response to the onset of unfavorable

conditions, such as a limitation of nutrients and desiccation. In the transplanted thalli, factors like desiccation, nutrient poor condition, and presence of SO_2 could simultaneously affect sporulation.

A morphological and chemical investigation of the algal symbiont from lichen thalli exposed to 5 ppm SO_2 under varying moisture conditions, presents the following features:

16. Abnormal characteristics such as bleaching of the chlorophyll, permanent plasmolysis, and sporadic brown dots on the chloroplast. The latter are more conspicuous in specimens exposed to SO_2 at higher humidities. These abnormal morphological features give evidence of cellular injury as a result of exposure to SO_2.

17. Presence of sulfurous acid, SO_4^{--}, and Mg^{++} in the extracts of the SO_2-exposed thalli. Sulfurous acid is an efficient bleaching agent and its presence in the thallus may be responsible for the loss of color in algal cells. Presence of SO_3^{--} and SO_4^{--} in the thallus may produce a higher osmotic concentration outside the algal cells and cause plasmolysis. The acidic ions would presumably denature proteins of the algal cells and prevent their deplasmolysis.

18. Sulfate concentration in the thallus is directly related to the relative humidity to which the thallus

had been exposed to SO_2. At higher humidities, an increase in SO_4^{--}-concentration in the thallus implies a higher rate of absorption of SO_2 and consequently, greater absorbed acidity and increased H ion concentration.

19. Presence of Mg^{++} in the thallus extract and brown dots on the chloroplast suggest that Mg^{++} has been removed from chlorophyll-a, converting it to phaeophytin-a. This conclusion is corroborated by the similarity of the absorption spectrum of chlorophyll extracted from the SO_2-exposed thalli to that of phaeophytin-a, with a maximum absorption at 667 mμ. The acid-condition existing in the thallus perhaps causes this degradation by increasing the concentration of H ions. These would displace the Mg^{++} atom from the chlorophyll molecule, transforming it to phaeophytin.

Sulfur dioxide causes significant damages, both externally and internally to the epiphytes, and thus, the epiphytic vegetation is a clear indicator of the degree of air pollution. In the case of lichens, the algal symbiont appears to be highly vulnerable to SO_2 and this probably explains why these organisms rank among plants most sensitive to polluted atmospheres. Precise autoecological and experimental studies will be necessary to determine the threshold concentration of SO_2 that the epiphytes can tolerate without being damaged, and to differentiate between the damaging effects of sulfur dioxide and ozone

which may be created in the lower atmosphere as a by-product photochemical reaction in the oxidation of SO_2 to H_2SO_4 (Blacet 1952).

APPENDIX

A POSSIBLE ROLE OF FLUORESCENT AND PIGMENTED SUBSTANCES IN THE LICHEN THALLUS

Lichens, with their limited quantity of chlorophyll--only one-fourth to one-tenth that of a leaf per unit area (Wilhelmsen 1959)--embedded in the deeper layers of the thallus, face problems uncommon to most other plant groups. The cortex absorbs 26-43% of the incident light as compared with 4-13% by the epidermis of a leaf (Wilhelmsen 1959). The quantity and location of the chlorophyll in lichens would imply that high light intensity is required in order to reach the compensation point for photosynthesis, but this does not seem to be the case.

Light generates heat and consequently produces a desiccating effect. Therefore it is necessary to consider the role of light intensity in relation to moisture because this is perhaps the most important factor limiting growth. In exposed habitats, some lichens have certain morphological structures that may represent adaptations against the damaging effects that direct sunlight or high light intensities would have on their thalli. Butin (1954) states that a lichen growing in the sun develops a thicker upper cortex and more intense pigmentation than when growing in the shade. The relation between light and rate of growth in lichens has

been studied by many workers. Des Abbayes (1951) cited that Henrici reported active assimilation of CO_2 in weak light intensities between 45 to 400 lux, lesser assimilation in medium light intensities between 2,000 and 3,500 lux, and no assimilation at very high intensities. According to him this is true for some species of Peltigera, which are heliophobous, and also for some species of Cladonia, which are heliophilous. Stålfelt (1939) showed the maximum rate of photosynthesis in lichens in western Europe to be greater in winter than in summer. Bliss and Hadley (1964), in their study of photosynthesis and respiration of alpine lichens, reported that these plants take best advantage of those periods when the thalli are wet and exposed to low light intensities and temperatures.

These studies seem to indicate that reduced intensity of light and low temperatures are favorable for the growth of lichens, primarily because under these conditions the moisture content of the thallus tends to remain high. In locations of intense light and high temperatures the depletion of moisture can be very rapid and furthermore in such habitats the pigments together with the thick cortex certainly absorb much of the solar radiations before they reach the algal layer. Therefore, regardless of where lichens grow, the photosynthetic process of the algal component must cope with low light intensities.

The question now arised whether lichens have some means of making maximum use of the low light intensities available to them. I believe that fluorescent lichen

substances suggest themselves as possible agents in this function.

Lichen substances are unique organic compounds which are insoluble in water and encrust on the outer surface of the hyphae. On the basis of their chemical structure they have been classified into different groups such as depsides, depsidones, quinones, etc. The majority of these compounds are colorless and are deposited in the deeper layers of the thallus. Some like atranorin, barbatic acid, and lobaric acid are fluorescent. Atranorin is common and appears in very many lichens. It is a depside, possibly a derivative of β-orsellic acid (Hale 1961). A few lichen compounds are yellow, orange, or red pigments, usually present in the upper cortex of the thallus. These are closely related to the anthraquinones of the free-living fungi (Hale 1961).

In the experiments to be described, the fluorescence spectrum of atranorin was studied in an attempt to determine its possible effect on the quality of the light available to the algal cells in the lichen thallus. It is our purpose to try to demonstrate that atranorin can increase the potentiality of the lichen alga to make use of the low light intensities.

EXPERIMENTAL METHODS AND RESULTS

Extraction of lichen substances

Three foliose species were used for the extraction of

the lichen substances: Parmelia caperata (L.) Ach., a heliotolerant epiphytic species; Peltigera canina (L.) Willd., a heliophobous epigaeic species; and Xanthoria fallax (Hepp) Arn., an extremely heliophilous (LeBlanc 1963) epiphytic lichen. The air-dried lichens were coarsely crushed in a mortar and 300 mg of the thalli were soaked in vials containing 5 ml of toluene for two days. The residue was then discarded and the crude toluene extracts were kept in stoppered vials at room temperature.

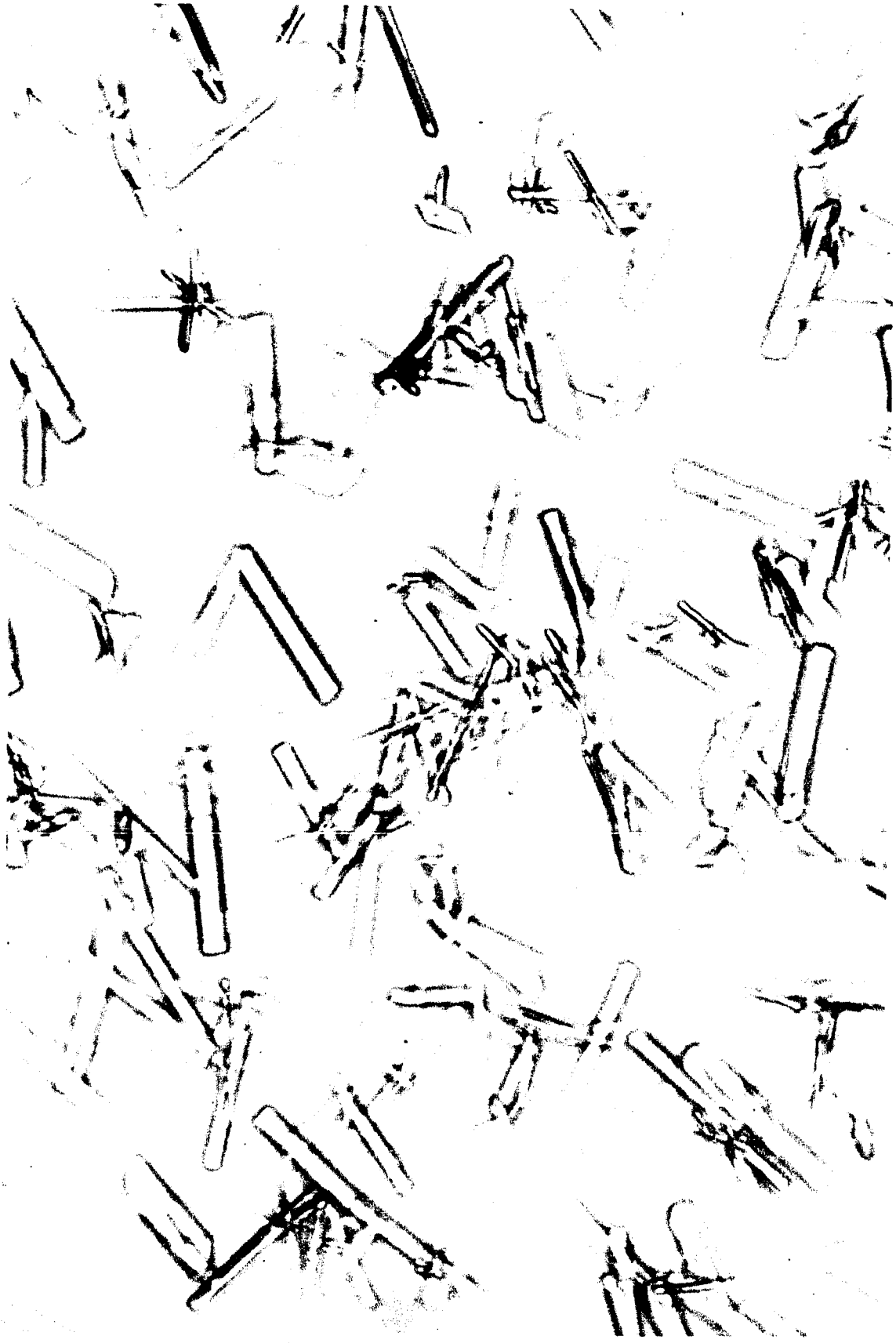
Isolation of atranorin

One ml of the extract from Parmelia was spotted 2 cm from the lower edge of a strip of Whatman No. 1 filter paper (3 x 25 cm) for chromatography by the ascending method in n-butanol saturated with ammonium hydroxide. The air-dried chromatogram was scanned in a dark room with a portable monochromatic light source emitting at 2537 Å (983-20V2 ultraviolet lamp, Fisher Scientific Co., Ltd.). One spot exhibiting yellow fluorescence was marked on the paper. Its R_f-value was 0.68, and it produced a reddish-brown coloration with alcoholic $FeCl_3$. These are characteristics of atranorin (Culberson 1963).

In a microcrystallographic test, the marked spot was eluted with toluene. A small quantity of the eluate was then evaporated with a drop of GE reagent (1 part glycerine: 3 parts acetic acid). Microscopic examination of the slide revealed the colorless crystals of atranorin (Plate III).

The results of these chemical and crystallographic tests

PLATE III



Crystals of atranorin in the GE solution X500

are taken as evidence that the yellow fluorescing material isolated from the extract was indeed atranorin. This substance was also found to be present in Xanthoria but not in Peltigera.

Fluorescence spectrum of atranorin and its comparison with the absorption spectrum of chlorophyll

We used an Aminco-Bowman Spectrophotofluorometer (American Instrument Co.) for the fluorescence assay. This instrument permits excitation of compounds and measures the resulting fluorescence throughout the ultraviolet and visible regions. The light from a xenon arc is dispersed by the exciting monochromator into monochromatic radiation which shines on the sample. Fluorescent light emitted by the sample is dispersed by a similar monochromator, scanned by a sensitive photomultiplier, amplified by a photometer and read as percent transmission of the exciting light.

First, the excitation wavelength producing the maximum fluorescence of atranorin eluted from the paper chromatogram was determined. The excitation at 400 mμ produced the maximum fluorescence at 425 mμ with 54% of the exciting energy emitted as fluorescence (Fig. 11). Then by setting the excitation monochromator at 400 mμ, the fluorescence spectrum was determined. The excitation spectrum was similarly determined by setting the fluorescence monochromator at 425 mμ. The excitation and fluorescence spectra of atranorin were characterized by their peaks at 400 mμ and 425 mμ respectively. The two spectra were identical but the fluorescence

Figs. : 11-14

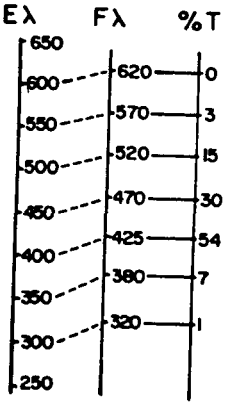
Fig. 11. Wave lengths of excitation (Eλ) and fluorescence (Fλ) in relation to maximum percentage of light transmission (%T) from atranorin eluted in toluene.

Fig. 12. Excitation (fluorescence set at 452 mμ) and fluorescence (excitation set at 400 mμ) spectra of atranorin eluted in toluene.

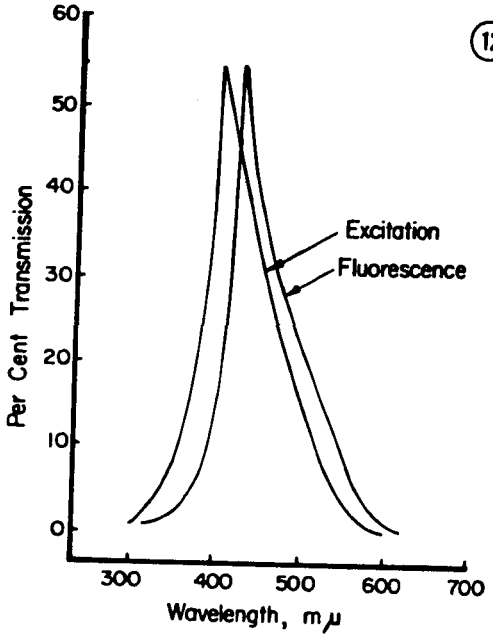
Fig. 13. Absorption spectrum of chlorophyll and fluorescence spectrum of atranorin.

Fig. 14. Fluorescence spectra of crude extracts from Peltigera canina, Parmelia caperata and Xanthoria fallax. The excitation monochromator was set at 400 mμ.

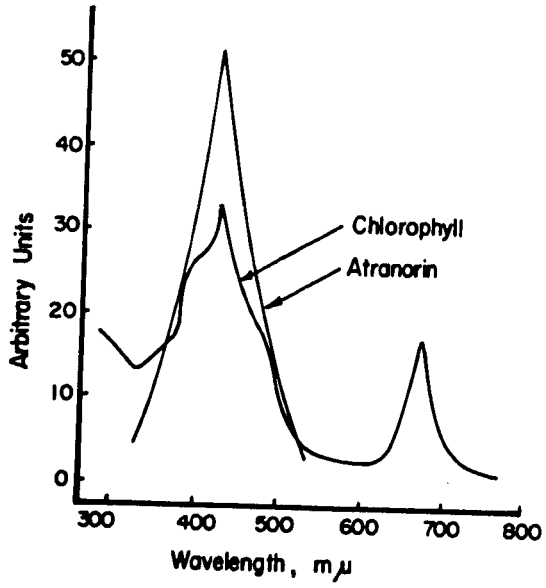
(11)


Eλ
Fλ
%T
650
600
550
500
450
400
350
300
250
620
570
520
470
425
380
320
0
3
15
30
54
7
1

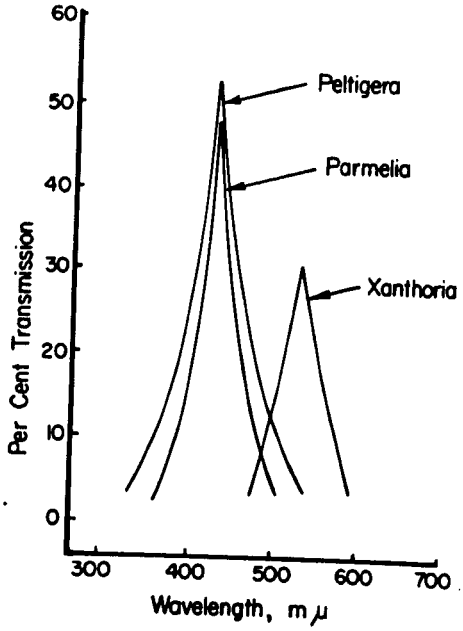
(12)


Per Cent Transmission
Wavelength, mμ
Excitation
Fluorescence
60
50
40
30
20
10
0
300
400
500
600
700

(13)


Arbitrary Units
Wavelength, mμ
Chlorophyll
Atranorin
50
40
30
20
10
0
300
400
500
600
700
800

(14)


Per Cent Transmission
Wavelength, mμ
Peltigera
Parmelia
Xanthoria
60
50
40
30
20
10
0
300
400
500
600
700

spectrum was displaced towards higher wavelengths (Fig. 12).

The absorption spectrum of chlorophyll, extracted from Parmelia caperata in petroleum ether, showed peaks at wavelengths of 425 and 662 mμ. The fluorescence spectrum of atranorin is similar to the absorption spectrum of chlorophyll in that the two spectra overlap in the region of 300 to 500 mμ, with a common peak at 425 mμ (Fig. 13).

Fluorescence spectra of the crude toluene extracts

The fluorescence spectra of each of the crude extracts, in response to an excitation wavelength of 400 mμ, were determined (Fig. 14). Extracts from Parmelia and Peltigera emitted appreciable fluorescence at 425 mμ. In these cases the fluorescence peak was the same as in atranorin with a slight reduction in the percentage of transmission. On the other hand, the extract from Xanthoria showed no fluorescence at 425 mμ and emitted only faintly at 530 mμ. This may be due to the presence of pigments in the extracts.

Fluorescent substance in Peltigera

One ml of concentrated toluene extract of Peltigera was spotted on a strip of filter paper and the chromatogram was prepared in the same way as detailed for atranorin. A light yellow spot on the air-dried chromatogram (R_f 0.97) exhibited yellow fluorescence in ultraviolet light and did not give any color with alcoholic $FeCl_3$. In a microchemical test, a few drops of toluene eluate of the fluorescent spot dried on a slide and recrystallized in GE solution on a

warming plate at 60°C produced colorless platelike crystals.

The nature of crystals and the negative reaction to $FeCl_3$ suggested a fatty acid (Hale 1961). The fluorescence spectrum of this substance was similar to that of atranorin in having the excitation and the fluorescence peaks at 400 mμ and 425 mμ respectively.

DISCUSSION AND CONCLUSIONS

When a molecule absorbs a photon of light, it is raised to a higher level of energy and is left in an excited state. The excited molecule may return to its original state by immediate emission of light at a greater wavelength (lower energy) than the light absorbed--this is fluorescence. If a nearby molecule possesses the power to absorb energy of the frequencies emitted, it will itself be excited.

The fluorescence spectrum of atranorin, with a peak at 425 mμ, coincides with the absorption spectrum of chlorophyll in the blue region and suggests the possibility that this emitted light can be used in photosynthesis. If this is so, then atranorin can play a very important role in energy relations within the lichen thallus. To establish whether such a role is actually played in vivo presents an intriguing problem.

The fluorescence spectra of crude extracts indicate a quenching of radiations emitted at 425 mμ. This is probably due to the presence of pigments which by absorbing light allow emittance of a smaller percentage of the same or

reduced energy radiations. The pigments in Parmelia, mostly usnic acid, reduced the percentage of light emission at 425 mμ without any change in the level of energy, but the quinones in Xanthoria completely quenched the radiations of 425 mμ wavelength and allowed only a small percentage of reduced energy radiations emitting at 530 mμ to pass. The reasons for such differences in the light-quenching properties of pigments are not clear but their ultimate role as light absorbers is quite evident. This suggests that these pigments can absorb light and their presence in the upper cortex may quench the incident light on the surface and thus protect the thallus against intense radiations. The fluorescence due to atranorin and such other substances in the deeper layers of the thallus is, however, not affected by these pigments.

To study the effect of light on the growth rate of lichens one should consider the thickness of the cortex, the limited quantity of the chlorophyll, the normally low moisture conditions, the light absorbing pigments, and the presence of fluorophors like atranorin. These constitute some of the most important factors in the lichen thallus which limit and modify the role of light in different ecological situations.

It is possible that atranorin and some other fluorophors may be helpful as accessory light absorbers in the lichen thallus. They may increase the ability of the lichen alga to use light of shorter wavelengths and thus permit maximum utilization of low light intensities.

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