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METHANE PRODUCTION IN CANADIAN MUSKEG BOGS

by

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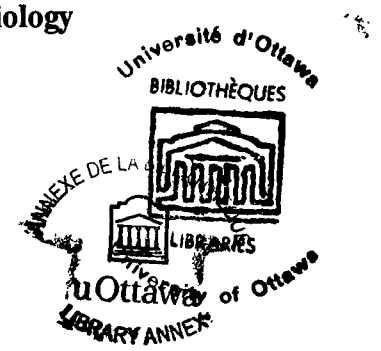
degree in Biology



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Since a number of people were involved in this work, it is appropriate for me to state that I did all the analyses reported except those of the elemental analysis of the peat soils, which were performed by Agriculture Canada, and the enumerations of microbial aerobic physiological groups, which were done by Dr. Kauri. The development of the methane samplers was a joint project by myself and members of LRRC at Agriculture Canada.

ABSTRACT

Aspects of methane production in muskeg bogs are studied as part of research into acid leaching from sulphidic mine tailings. A self-renewing, anaerobic ombrotrophic bog capping to a tailings dump would prevent the entry of oxygen, and hence the microbial oxidation of sulphides; this measure could be economically viable if based on material transported from local wetlands. For this study, 30 organic soils from the mining areas of the southern Canadian Shield were sampled and analysed for 28 organic and inorganic characteristics. Statistical methods were used to differentiate the soils. Principal component analysis shows the most promise, and should prove suitable also as a general method for classification. A method for measuring cellulose, a major component of plant biomass, from peat is described, and in these samples shows correlation with respiration and rubbed fibre content, measures of peat biodegradability. Cellulose also exhibits a reduction in quantity with depth. Sampling tubes have been developed to measure the *in situ* methane. The methane entrapped within a 350 m^3 volume of Mer Bleue, a local Ottawa, ombrotrophic bog was measured at three depths, and found to be irregularly distributed. The total amount of methane extracted was over 130 mmoles 75 sites. If the whole of this bog contains similar amounts of methane, then the total calculated amount it could contain is 1.7 Gg of methane. If similar amounts of methane are trapped within all such temperate wetlands, there is the potential, when the peat is harvested, for it to be released to the atmosphere, and this could be one of the sources of the increasing amounts of fossil (^{14}C depleted) atmospheric methane. It is suggested that this occluded methane may block the pore spaces in the peat matrix and prevent water flow, causing the low hydraulic conductivity found in ombrotropic bogs. Production of methane from laboratory peat incubations show correlation with the methane extracted from the same sites in Mer Bleue. An acidophilic methane-producing enrichment culture has been obtained from these samples.

THE PEAT BOG SOLDIERS

Far away as the eye can wander
Peat and bog are everywhere.
Not a bird sings out to greet us,
Oaks are standing gaunt and bare.

Chorus: We are the peat-bog soldiers
Marching with our spades,
To the moor.

Up and down the guards are pacing
No one, no one can get through.
Flight would mean a sure death facing,
Guns and barbed wire greet our view.

Chorus:

But for us there's no complaining
Winter will in time be past.
One day we will cry rejoicing,
'Homeland dear, you're mine at last!'

Chorus: Then will the peat-bog soldier
March no more with their spades
To the moor.

Courtesy of DJK's memory of a song he learned, more or less, 50 years ago.

1. INTRODUCTION

This investigation was initially undertaken to explore the possibility of preventing acid leaching from pyritic mine tailings by covering them with a self-renewing peat blanket. This aspect of the study had to be curtailed due to the exigencies of government funding. However, research on muskeg bogs was continued. Mer Bleue, an ombrotrophic bog, is convenient to Ottawa and, with permission from the National Capital Commission, was the site of some of the work.

Peatlands are very diverse, and may be investigated from the point of view of history, classification, geology, ecology, botany, microbiology, water chemistry, economic potential, and conservation. I will comment on all these themes, while some will be explored in detail. Figure 1.1 shows how I see the interconnecting links, which will, I hope, guide the reader through the morass of the mires.

The production of methane in muskeg encompasses the total environment of these bogs: this includes not only the characterization of the bogs, but also the determination of the primary substrate, cellulose from the plant biomass; the bacteria, which at the end of the anaerobic degradation chain, produce the methane; and the distribution of the final product, methane within the peat matrix.

The first part of the dissertation, **Peat Development**, is a survey of all aspects of the development of peat in the bog ecosystem. The history of bog investigation is outlined, first in Europe and then in Canada. The distribution and economic potential of bogs is considered. The water source and climate, on which bog evolution is dependant, is explained

in terms of the hydrological model and the water chemistry. The wetland succession is determined by the water source and the level of the water table, which in turn controls the vegetation and thus the type of bog which develops. The maintenance of a high water table in ombrotrophic bogs is also discussed, and the effect that methane production has on reducing the hydraulic conductivity in the deeper layers of the bog is explored. The factors causing erosion, and the requirements for the conservation of peatlands are studied. The formation of peat from the orginal plant biomass is described, including humus formation and diagenesis to coal. The history of methanogens in the Archaebacterial phylogenic group is discussed. The anaerobic microbial degradation of plant carbohydrates is set out, and the activated acetic acid pathway for the formation of methane is described.

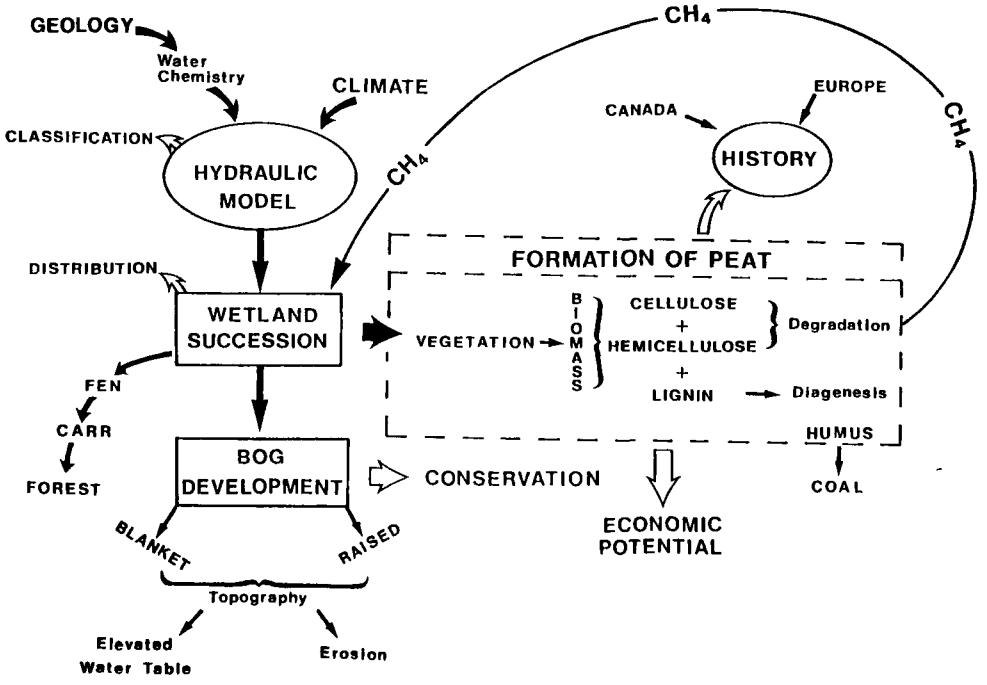


Figure 1.1 Interplay of different factors in peat development.

The second part of the thesis, **Acid Tailings**, is a discussion of the acid leaching from mine tailings, and the proposed method for preventing acid formation by covering the tailings with a layer of peat.

The third part of the thesis, **Bog Environment**, contains a description of the work undertaken, and how the results obtained increase our knowledge of the environment and the metabolic processes of muskeg bogs. To investigate the posibility of using these bogs as a cover for acid-leaching tailings, 30 bogs from the mining areas of the southern Canadian Shield were collected and analysed. Numerical methods were used to differentiate between the bogs. This is followed by an analysis of the cellulose component of the peat which is the primary substrate for methane production. Then the physiological groups of aerobic bacteria of muskeg bogs are reported, followed by a description and the requirements for growth of an acidophilic, methane-producing culture from Mer Bleue. Sampling tubes for the extraction of methane trapped within peat bogs were devised after a pilot study, and the measurement of methane extracted from Mer Bleue by these tubes, is reported. The correlation of the extracted methane with the methane produced in laboratory incubations is demonstrated.

The conclusion completes the thesis, detailing the advances made, the possible use of numerical methods to classify organic soils, potential of an acidophilic methanogen, and the effect that methane trapped within peatlands could have both on the hydraulic conductivity of wetlands and the impact of releasing this trapped methane into the environment. Various papers generated by this research are listed in Appendix 1.

2. PEAT DEVELOPMENT

2.1 HISTORICAL ASPECTS OF BOG ECOLOGY

2.1.1 EUROPE

For a long time people in Europe have differentiated between types of wetlands and the names in use such as marsh, fen, moor, carr and mire, are of ancient origin and can be traced back to Old Norse or Old German. The first accounts of the formation of bogs and peat were written in Europe in the sixteenth century, but in North America there is no literature on bogs until the end of the last century.

Many of the basic concepts of bog ecology were established several centuries ago, based on the careful observations and accurate interpretations of amateur naturalists (as described by Gorham 1953, 1957). One of the earliest English accounts of peatland is in the 'Itinerary of John Leland', written between 1535 and 1543; the writer describes different bogs and marshes, and is particularly interested in the occurrence of trees and stumps found in the peat being cut for fuel. The first classification of wetlands appears to be that of Samuel Hartlib in 1652, and there is a striking similarity between this 300-year old account and current ideas. In 1685 William King described the organic nature of peat, and the succession of plants found in raised bogs. Linnaeus, in 1751, produced lists of characteristic plants from Swedish bog hummocks.

It was not until 1743 that Maxwell mentions drainage by stream diversion to convert bogs into pasture, and this modification is discussed further by Young in 1780. By the nineteenth century agricultural expansion was becoming necessary in Europe, and the need to bring bogs under cultivation greatly increased the interest in them, especially through an

understanding of their origin and development which would assist in their utilization.

The actual formation of peat was rightly ascribed to the inhibition of decomposition by waterlogging, and in 1810 Rennie considered these factors to be important: absence of oxygen, low temperature, the stability of these two factors, and an antiseptic principle derived from some plants. The evolution of bogs was also clearly appreciated at this time, for in the same year De Luc described the transformation of a lake into wet peaty meadowland in northeastern Germany. In the next year, in England, Aiton related the development of raised bogs once the peat reached above the level of the water surface.

The influence of mineral soil water, compared to that of pure rain water, on the course of bog development was also realized (Shotyk 1987), and in the late eighteenth and nineteenth centuries the geochemistry of peatland waters was investigated. Much of this work was done in the countries of Northern Europe, where bogs account for a considerable part of the land area. Organic acids were extracted by distillation from peat by Home in 1762, and quantitative studies of mineral matter were carried out by Achard in 1786, while the chemical constituents of peatland water were analysed by Liebig in 1847.

The escape of combustible gas from the earth's surface was known to Pliny, but it is thought that Volta in 1776 was the first to describe 'combustible air', which he found to be generated from decomposing vegetation in bodies of water (Barker 1956). He realized that there was a relationship between the amount of plant material and the quantity of gas produced. Bechamp in 1867 was the first to describe methane production from a microbial fermentation (Pine 1971), and in 1887 Hoppe-Seyer quantitatively measured methane and other gases obtained from bogs. It was not until 1940 that Barker was able to show, using

radioactive carbon, that methanogens (methane forming bacteria) could reduce carbon dioxide with hydrogen to form methane.

The analysis of minerals in several German mires in 1895 by Ramann of the Royal Bavarian Institute, led to an account of the fundamental geochemical factors in ombrotrophic (rain-fed) bog development. In 1927 Kotilainen put forward the thesis that it was the dissolved oxygen rather than the electrolyte content that was the decisive factor controlling the distribution of plant species, particularly sphagnum moss, in minerotrophic (stream or groundwater fed) mires. In Holland, in 1934, Baas Becking and Nicolai compared the chemical composition of bog surface water and rainwater; the similarity of their compositions showed the studied bog probably did not communicate with the ground-water, indicating that the mineral composition depended entirely on precipitation. Kivinen in 1936 investigated many sites in Finland, measuring the electrical conductivity and hydrogen ion concentration and showed that open and dwarf shrub bogs are poor in dissolved ions, while brown moss fens have a much higher degree of mineral nutrition.

Thus, by the 1930s, the basic concepts of ombrotrophic bogs had been outlined; the peatland waters in these bogs have a low ionic strength and contain about as much dissolved calcium as rain-water, while those in fens contain abundant dissolved salts that are dependant on the mineralogy of the local rocks and water flow.

Godwin (1981) was one of the first to study English bogs in this century and he has recounted that in the 1930s, it was the Scandinavians Osvald and Jessen who were the source of the current knowledge. At this time, before the days of radiocarbon dating, pollen analysis was used for a relative chronological scale, and this had shown that the climatic

changes, since the Würm ice age (about 10,000 years ago) could be correlated throughout northern Europe. The remains of prehistoric man and his artefacts found in the bogs were an unexpected bonus in this exploration.

2.1.2 CANADA

Muskeg is a particularly Canadian word, from the Cree meaning quaking ground; it is used throughout the Canadian Shield to describe waterlogged soil. One of the first descriptions of wetlands was of the New Brunswick raised peat-bogs by Ganong (1897). At that time, he complained the American literature on peat bogs was extremely scanty compared to that of Europe, and consisted mainly of geological reports and papers on sphagnum taxonomy. He suggested that this was because, in America 'the best types occur far from the botanical centres', and were not being utilized, while in Europe 'great bogs occur within easy reach of the botanists of Germany, Switzerland and Scandinavia', where the bogs had an economic value.

Ganong (1887) described several bogs found near the coast, of which he listed the vegetation, showed the profiles of the bogs (determined by sounding), measured the temperature at different depths, and noted that the level of each bog varied with time, due possibly to changes in temperature or of barometric pressure. He seemed to be familiar with raised bogs, but believed that they are formed by 'Sphagnum growing upward and carrying the water by capillary with it'. He was, however, much intrigued by the standing water that he found near the surface in the higher parts of the bog; he could not understand 'what prevents it from flowing out from the great spongy structure by its own weight down to its proper level'. The only answer he was able to suggest was that 'the Sphagnum is able, by capillarity, to raise water to about 12 to 13 feet (4 m) above the water level of the basin

over which the bog is growing'. He could hardly believe that it can stand there indefinitely 'for what would hold it up?'. He noted the 'comparatively homogeneous structure of these bogs from top to above the bottom, makes it plain that they have been exposed to no such alternations of climate as have left traces in many of those of Europe.'

The next description is by Nichols (1918) who wrote a survey on the soil types of Cape Breton Island. At this time bogs were thought to be largely confined to Newfoundland and to parts of eastern Canada and Maine near the sea coast. Although he was familiar with raised bogs, he describes a sequence of the bogs in this area starting from a pioneer stage, changing to a bog meadow, which is then superseded by a wet bog which in turn gives way to a dry bog. He assumes that these changes depend on which particular type of sphagnum gains dominance.

Riggs (1925), described some 78 bogs of the north Pacific Coast, 13 of which were in British Columbia. Due to the complicated conditions that existed during the retreat of the glaciers there, he found that the bogs rested on a variety of surfaces, glacial till, sand, blue clay, gravelly outwash, soil and rock, also that they were deep, no bottom being found at 7 m in some cases. The bogs formed either in swamps or on lakes and ponds, and there was some evidence that some of them might have formed on a forest base, sphagnum having overcome an open growth of trees. He established that sphagnum overcomes swamp plants either by advancing over the bog, where the sphagnum plants could be 30 cm higher than those of the swamp, or by forming floating mats on the surface of water and gradually infilling the lake by vegetative growth. Dead conifers found on the margins of bogs showed the encroachment of bog on the forest, where possibly the trees were killed by a rise in the water level.

Riggs affirmed that the initial condition of the bogs was important and that this exerted a selective influence on their flora. He discussed which factors are important, from the temperature of both the bog and the air, the lack of aeration, deficiency of available nitrogen, water holding power of the peat, toxic substances, pH, to the lack of mineral constituents in the substratum. When the bogs pass maturity and grow old there is a further change in their plant composition, the most conspicuous of which is the appearance of trees, especially conifers.

Finally there is a description by Auer (1930), a Finn, who wrote a Canadian Geological Survey Memoir; but unfortunately only an abridgement of his report was published. He looked at 34 peat bogs in southeastern Canada, from Toronto in the west, down the St. Lawrence River, into New Brunswick and Nova Scotia in the east. Each bog was sampled with a Hillier type drill along a section including the deepest part (these cross-sections are included in the publication).

Auer describes the peat bogs as originating on surfaces usually covered with inorganic ooze overlain by various types of organic ooze. The water basins were generally first filled by carex vegetation, typical of swamps, and later by sphagnum moss, definitive for bogs. The younger bog surfaces conformed to the topography of the surface on which the bog grew, in contrast to the older carex bogs of the western inland district, whereas the sphagnum bogs of the eastern maritimes had upwardly convex surfaces. Auer found from pollen analysis that there had been alternating dry and wet periods and suggested these were synchronous over a wide stretch of country. He also suggested that these periods in Canada were correlated with those of post-glacial Europe, a view more recently corroborated by Larsen (1980).

2.2 DISTRIBUTION

The large peat deposits in northern Europe, Asia and North America were formed at the end of the last ice age, about 10,000 years ago. Most of these deposits rest on sand, clay or rock and probably accumulated in lakes formed in front of the receding glaciers. Peat consists of the unconsolidated residues of aquatic plants such as mosses, reeds and sedges, and to a lesser degree, of woody plants, which have accumulated in water-saturated environments over thousands of years. In cool, moist maritime climates peatlands can develop on almost any surface, even on hill slopes, but there must be a positive water balance, for the formation of peat requires the accumulation of organic material at a greater rate than its degradation. Therefore, as the ratio of precipitation to evaporation and run-off decreases, so does the organic debris accumulation, and peatland development gradually becomes confined to lowland areas and local depressions.

The habitat for blanket peat bog formation is extremely localized, and is almost entirely restricted to oceanic coasts with a high rainfall, between latitudes 45° and 65° (Figure 2.1). In northwest Europe the Westerlies carry a constant supply of moisture, which, due to the moderating influence of the Gulf Stream, falls mainly as rain. This climate, together with a geology of hard, acidic crystalline and metamorphic rocks, produces a landscape dominated by blanket peat bogs, typified by the bogs of Scotland and Ireland (Lindsay 1987).

In the tropics, peat is also found where it is wet enough for accumulation, and has been recorded from the three main rainforest regions, south-east Asia, tropical America and tropical Africa (Anderson 1964). The wetlands of Asia have been investigated most, and in Sumatra where the high humidity promotes rain forest growth, subsequent burial in a wet

anaerobic substrate (unusual for a tropical forest) preserves the accumulation from complete decay (Cameron 1987). The rate of accumulation may be about twice that found in cool temperate regions (up to 15 m) but is basically similar: that is, the rate of decay of the biomass is slower than its production thus allowing peat accumulation. The Ruwenzori Mountains in Central Africa maintain deep peat deposits, and even though they are distant from the ocean, they are high enough to receive sufficient moisture as mist for peat formation.

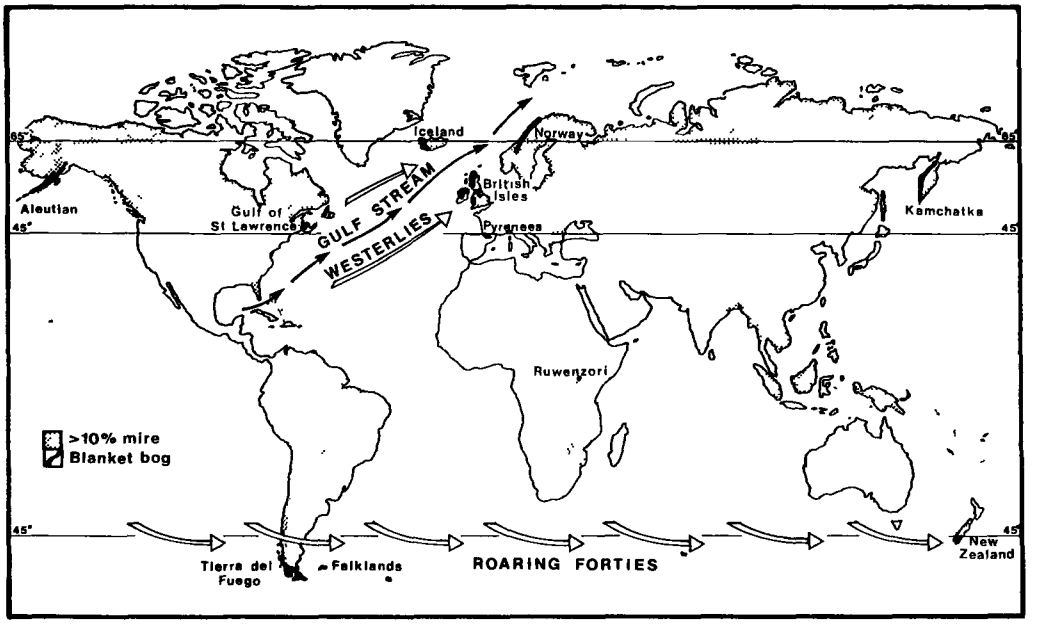


Figure 2.1 Wetlands of the world (after Lindsay 1987).

Most of Canada was covered with ice until about 12,000 years ago when the glaciers began to melt, but it was not until 7,000 years ago that all of Canada was free of ice (Larsen 1980). In eastern Canada peatland development began soon after the ice melted, but in the

central region the warm, dry climate produced spruce forest, which was replaced by grassland between 11,000 and 9,500 years ago. These periods were not favourable to peat development, and most basal peat deposits in northern Ontario and Manitoba are less than 5000 years old. Farther north however, the basal peat deposits are 7,000 to 10,000 years old, showing that peat formed soon after the ice melted. This peat accumulated in a warmer climate than that of today, without the permafrost, which developed only 3,000 to 4,000 years ago. In the Arctic little peat is being formed today, but peat formation there was common 8,500 to 9,000 years ago. Larsen has suggested that the restricted bog vegetation found between the arctic and boreal species in the Canadian Shield could be due to synchronous fires which swept the area for 200 years about 3500 years ago, which were followed by a period of cool, dry summers that was also inimical to bog plant diversity. Thus the climate appears to have varied considerably since the first post-glacial Canadian peatlands were formed (Zoltai and Pollett 1983).

It is estimated there are over 100 Mha of peatland in Canada, which is 12% of the total land area (Tarnocai 1985). The greatest concentration of wetlands in this country occurs in a broad belt extending from central Labrador, passing south of Hudson Bay, and extending northwest across the Prairie provinces to the MacKenzie Valley. It has been calculated that these lands contain approximately 335 Gtonne of dry peat.

2.3 ECONOMIC USES OF PEAT

The first recorded attempt in Canada to manufacture peat fuel dates back to the middle of the last century, when in 1864 a contract was made with the Grand Trunk Railway to supply fuel for locomotives, but it was found impossible to fulfil and the operation was halted. Later on, after more experimental work, about 13,000 tons of fuel peat were produced. There are records of at least 40 companies formed before the First World War to produce peat fuel, but all ended in failure (Swinnerton 1954).

There were serious fuel shortages in Canada in 1917, 1943 and 1982, and peat was seen as an answer to the problem. However, each time, in spite of funding for research, the fuel situation improved before a viable means of using peat had been developed. In Canada there has been much interest in using peat as an alternative energy source, and the Peat (for Energy and Chemicals) Program was the most recent attempt to find a use for Canadian peat (Tibbetts 1986).

The major problem in using peat as a fuel is the energy required to dewater it so that it may be burnt. These problems can be overcome, and in Russia, Finland and Ireland peat is used to generate electricity, although it has a low calorific value, and is less efficient than other fuels. Unfortunately, peat is not a renewable resource in the short term, and the more accessible bogs in these countries are being mined out. The microbiological production of methane (biomethanation) from peat, which does not require dewatering, has been accomplished on a laboratory scale, but has not yet proved viable industrially (Ghosh 1981).

Less humified peat is used extensively as horticultural peat moss, and in Canada it is mined across the country, with an annual production of 750 ktonnes. Peat can also be a

source of chemicals, particularly waxes, but up to the present only the USSR has commercial peat chemical production (Fuchsman 1980). The antiseptic qualities of peat have long been recognised in Europe, and peat baths are used there for therapeutic treatments, particularly in Czechoslovakia (Maltby 1986). Peat can also concentrate metals, as described in a copper swamp in New Brunswick (Boyle 1977), possibly this could be of use in metal mining. Finally we have suggested that peat from bogs could be used to ameliorate acid mine leaching (Section 3).

2.4 WETLAND SUCCESSION

Wetlands afford a diverse variety of water and soil conditions, where the biological communities are interactive and dynamic and respond constantly to changes in water and nutrient levels. Freshwater wetlands can range from high-energy rivers and freshwater tidal marshes, through sedge meadows to the raised and blanket bogs of the Canadian Shield or northern Europe. These wetlands can produce three to four times as much vegetation as grass fields and support a rich diversity of plants with abundant animal life, or they may be muskeg bogs which have low productivity and accumulate biomass slowly as peat. What causes this difference?

Terrestrial environments may culminate in 'climax' ecosystems which usually contain sufficient herbivores, carnivores and litter decomposers to use all the energy fixed by plant photosynthesis. But this balance is not achieved in peatlands, which are less stable than other habitats, and instead a 'succession' state ensues where the excess energy accumulates as peat (Moore 1987a).

A theory of wetland succession was put forward by van der Valk (1981). He replaced the concept of terrestrial climax with a model in which the presence and abundance of each species depends on its previous history, and its adaptation during that time to the environment of a particular site. Each plant type thus has a unique set of characteristics and hence a specific response to environmental factors such as water level changes. These factors constitute an 'environmental sieve' and determine which species will survive changes in the environment.

2.4.1 HYDROLOGICAL MODEL

The generally accepted model for wetlands is a hydrologic one, based on the interaction of climate, geology, hydrology and biology (Gosselink and Turner 1978). This model describes the wetland ecological niche, and the characteristic water flow, dissolved oxygen, pH and nutrient flux. The velocity and the turbulence of the flow determines the oxygen concentration and the ability of the water to carry suspended particulate matter, while the source of the water determines the ionic composition and nutrients.

Wetland systems are generally net producers so that primary production exceeds consumption. This production is enhanced by water flow from a minerotrophic source, but it is depressed by stagnant conditions with only an ombrotrophic (rain-fed) water source (Moore and Bellamy 1974). The organic accumulation is controlled by the hydrology, so that in highly flushed marine salt marshes the organic export may be nearly half of the net primary production, but in raised bogs the major part of the primary production accumulates as peat (Mitsh and Gosselink 1986), which may be deep (up to 15 m) (Anderson 1964).

2.4.2 WATER CHEMISTRY

The high ratio of organic matter to minerals found in fen and bog peats has a profound effect. The acidity of unpolluted bog waters is due to natural organic acids, and since these are coloured yellow/brown it is highly likely that they are products of plant decomposition (Gorham 1967, Gorham et al 1985). The ability of soils to take up ions is known as the exchange capacity, and most soils are saturated with metal cations (Na^+, K^+, Ca^{++}, Mg^{++}), but as the organic content of the soil increases, so does the exchangeable hydrogen ions which are adsorbed by the polygalacturonic acids of the cell walls of

Sphagnum. The transition from fen to bog occurs when 80% to 90% of the soil is organic matter. As the organic matter increases to this level, the pH drops from 6 to 5, and when the cation balance moves towards hydrogen and away from metals the pH drops further to nearly 3. The mineral content of the soil influences the ionic status even when the major portion of the metals may be adsorbed on the surface of the organic detritus (Heinselman 1970).

The water source determines the type of peatland, as the ionic balance and cation content influence the productivity and the vegetation type, which, in turn, affect the balance between peat accumulation and decay (Heinselman 1970). The type of vegetation and the local topographic evolution of the peatland determines the direction of water movement. There is no steady trend towards a climax vegetation, but instead a ceaseless, and almost random, change of the wetland vegetation over the centuries.

2.4.3 MIRE DEVELOPMENT

2.4.3.1 Minerotrophic Fen

Bog development can begin on the shores of a lake. Reeds (*Phragmites* sp.) and cattails (*Typha* sp.) fringe the shore and advance into shallow water. Behind this reed swamp there may be a band of sedge (*Carex* sp.) meadow with some reed grasses (Figure 2.2). These fen meadows will receive silt during spring floods, building the alluvium above the water table, and, as the soil becomes drier it is encroached by alder and willow bushes, which begin to form a continuous cover called a 'carr'. Dense root and underground runner systems of shrubs stabilize the soil surface, but may also impede the drainage systems and cause the wetlands to advance uphill. Alternatively, a new drainage channel may be cut which will slowly erode the alluvial silt, and in this case the water table will be lowered. The

drier soil will now support good tree growth and the shrubby carr changes to forested swamp (Heinselman 1970, Sparling 1979).

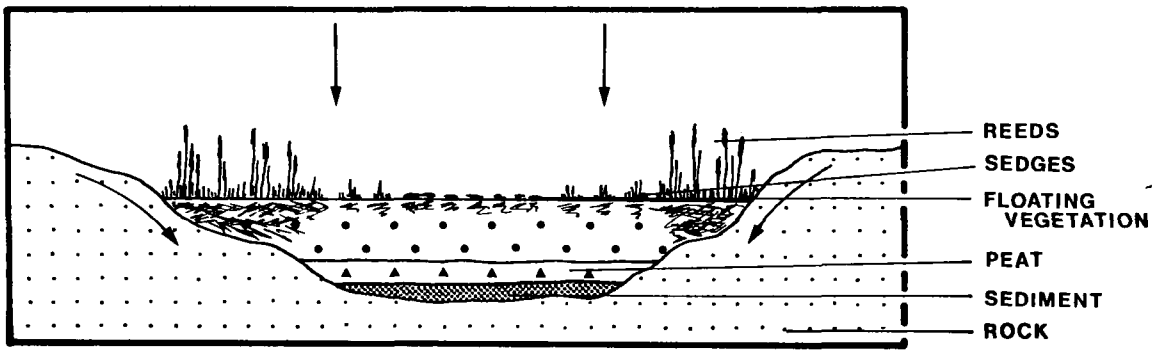


Figure 2.2 Diagram of a fen.

2.4.3.2 Ombrotrophic Bog

The succession from open water to wooded communities will take a different route when the organic material is not degraded but left to accumulate in the marsh. In a wet climate, sphagnum moss will invade, and the trees will gradually die off. If the atmosphere is sufficiently humid Sphagnum will grow until the bog is above the level of the water table to form a 'raised bog'. In the heavily glaciated crystalline rock terraine of northeastern North America, the paucity of soil nutrients allows Sphagnum bogs to be established directly in wet rock hollows. The peat so produced has little zonation, unlike the layers formed when the bog develops from a lake margin succession as described above.

True ombrotrophic bogs are found only where rainfall is the sole source of mineral nutrition. In the early stages of development a mire is often fed by ground water, but as the peat accumulates, it gradually lifts the surface vegetation above this drainage until, eventually, precipitation is the only source of water. The result is acidic peat. These

ombrotrophic mires can be divided into 'raised' and 'blanket' bogs.

2.4.3.3 Raised Bogs

Energy-rich organic matter continues to build up for thousands of years as peat, accumulating, not in the tissues of living plants (as seen in the rain forest), but as dead plant litter. The most essential phenomenon of peat formation seems to be that it is produced in a given surface layer by incomplete aerobic degradation of plant biomass (Kurbatov 1968). Once this layer becomes waterlogged and buried it becomes anaerobic, and the decomposition rate is drastically reduced since anaerobic microbial degradation is very much slower than aerobic. The end result is that over the course of time, the surface of the mire is gradually elevated above the surrounding countryside due to the accumulation of peat. This forms a 'raised bog', above the local level of the ground water (Figure 2.3); these are also known as 'domed' bogs from their typically upwardly convex shape.

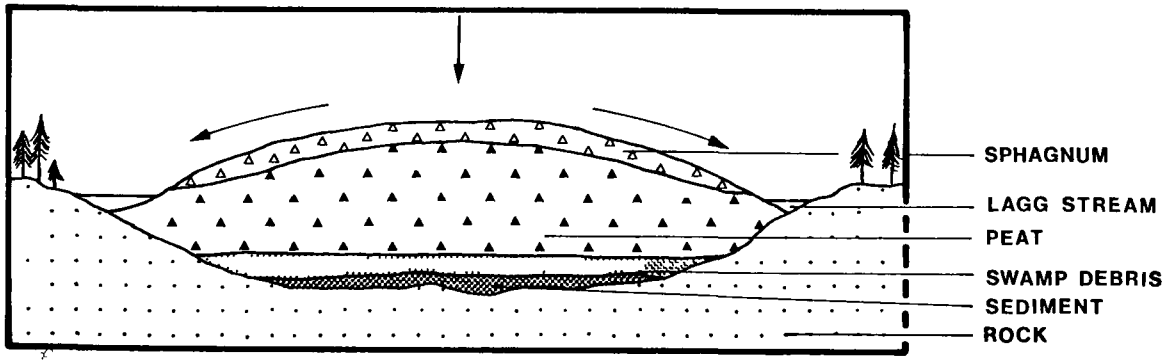


Figure 2.3 Diagram of a raised bog.

2.4.3.4 Blanket Bogs

In regions of high rainfall and with an extremely moisture-laden atmosphere (such as the windward shore of the North Atlantic Ocean), there is sufficient humidity for

complete waterlogging of the soil, and peat will form outside drainage basins. In extreme conditions peat can even accumulate over sloping hill sides, covering the landscape with a 'blanket' bog (see Section 2.2). The slopes of these blanket bogs can be quite steep; 1.5 m of peat has been recorded on slopes of 20° (Gorham 1957).

2.4.4 OFFICIAL CLASSIFICATION

Canadian wetlands have been classified according to their botanical composition. The great variation in climate and geomorphology in Canada has produced a wide range of plant types. The National Wetlands Working Group has recently published the Canadian Wetland Classification System (1987), in which a wetland is defined as land that is saturated with water long enough to promote wetland or aquatic processes. The classification has three hierarchical levels: class, form and type. The five wetland, or mire, 'classes' are based on the generic ecosystems of bog, fen, marsh, swamp and shallow water. Morphology differentiates seventy wetland 'forms', while vegetational physiognomy classifies the wetland 'types'. The bog 'class' is defined as a peatland, generally with the water table at, or near, the surface, and this again is separated into eighteen different 'forms' (Tibbetts 1986). Maps of the Distribution of Wetlands and Wetland Regions have been published by Environment Canada (Canadian Committee on Ecological Land Classification 1986). The classification of wetlands by botanical species is logical since the distribution of wetland plants is determined by the water regime.

2.4.5 RAISED WATER TABLE

How do the raised bogs maintain their water table several metres above that of the surrounding area? This phenomenon has caused much discussion; does the bog act as a massive 'sponge', or is it an impenetrable block of waterlogged peat which retains the

rainfall on the surface? In the early part of this century Scandinavian scientists believed that the bogs acted as a massive reservoir, soaking up water from their surroundings through a capillary complex. After careful observation they concluded that bogs 'breathed', and suggested that the entire bog mass expanded and contracted according to the amount of water they contained (the balance between supply and loss) (Moore 1987a). Granlund (1932) was able to show a distinct correlation between bog convexity and annual rainfall.

At the end of last century, Ganong (1897) observed a similar variation in height and suggested that it was possibly due to a change in barometric pressure, since the variation was observed just before a storm broke. More recently, surveying by laser has confirmed that bogs do indeed expand and contract, but in this particular study the variation appeared to be due to artesian pressure from ground water under the bog which forced the mass of peat upwards (Almendinger et al 1986).

Soviet hydrologists have suggested that the lower layers of peat are decomposed and compacted (catotelm) preventing water movement, and only the more open top layers of the bog (acrotelm) allow water movement (Moore 1987a). In Ireland the steady state flow in peat was measured to determine the best method of draining it. The tests showed that drainage ditches here should not be dug more than a metre deep because of the low permeability of the deeper layers. It was suggested that this was due to entrapped air which was released from solution as the water table, and the hydrostatic pressure, was lowered (Galvin and Hanrahan 1967). Another set of field trials, in Scotland, showed that water in peat flows with the expected hydraulic conductivity only in the upper layers. The lower conductivity at depth was attributed to the greater humification of the peat, which physically constricted the water flow (Rycroft et al 1975). Laboratory tests have established that

subjecting moderately humified peat to a hydraulic gradient causes a non-linear variation of the seepage velocity. Two explanations have been suggested: either that the water is structured in the pore spaces, or that there is a mechanical effect from the pressure difference on pore sizes (Waine et al 1985).

This anomalous behaviour of water movement in the deeper layers of peat needs to be explained (George 1975). It is known that the lower hydraulic conductivity at depth can be due to a significantly increased degree of decomposition causing a restriction in the pore spaces. However, Mathur and Lévesque (1985) observed that although the saturated hydraulic conductivity decreased sharply as the thickness of the peat increased, the degree of decomposition did not change significantly within this soil depth. They proposed therefore, that methane, produced by anaerobic degradation of the plant biomass in the deeper layers of the peat, can occlude pore spaces in the soil and thus curtail water movement. This will be discussed further in section 2.7.5.

2.4.5.1 PEAT BOG VEGETATION

The chief bog plant is *Sphagnum*, a bryophyte (moss), which typically grows at a pH near 3, and with nutrients derived only from rain, wind-borne dust, and its own detritus. There are many species of sphagnum moss, about 30 species in the British Isles alone, and 17 have been described in Mer Bleue (Joyal 1970), each species has its own habitat requirements. Some require more water than others, and some prefer a nutrient inflow from ground water, but most prefer an acid environment with a poor supply of nutrients. All have the capacity to hold tenaciously onto any useful inorganic ions, extracting them efficiently and storing them in their infinitely extensible stems and branches. The stems stretch down into the depths of the peat, suggesting great antiquity (Moore 1987a). Sphagnum also has

remarkable powers of water retention, due to large water storage cells that produce a high water holding capacity and low hydraulic conductivity. This helps to produce the raised water table.

Higher plants include the cotton sedges (*Eriophorum* sp.) and shrubby angosperms such as the *Ericaceae* family, blueberry and cranberry (*Vaccinium* sp.), and bog rosemary (*Andromeda* sp). Trees which are able to survive waterlogged conditions include black spruce (*Picea mariana*) and larch or tamarack (*Larix laricina*). Possibly, however, the insectivorous plants of the bog habitat are the most distinctive; these supplement the low nitrogen and phosphorus levels in rainfall by entrapping invertebrates on the bog surface. The sundew (*Drosera rotundifolia*) catches passing insects by sticky glandular hairs, while the pitcher plant (*Sarracenia purpurea*) acts as a pitfall to flies and wasps hunting nectar. In bog pools, bladderwort (*Utricularia* sp.) uses its bladder-like leaves to suck in unsuspecting microscopic copepods (Fernald 1950). Nitrogen may also be added to bog ecosystems by nitrogen fixation, notably by blue-green algae (Dickenson 1983).

Wetlands are frequently flooded, so that the roots of the emergent vegetation must be able to exist temporarily in an anoxic environment. Water supply is generally the limiting factor for plant growth, but in wetlands, where by definition water is always present, the limiting parameter is the availability of oxygen. When water flows rapidly it is turbulent and saturated with oxygen, but when it is stagnant the oxygen is soon depleted. This stresses the plants not only from the anoxia but also from the increased availability of nutrients and toxins which become more soluble in these conditions. In water-saturated conditions the depletion of oxygen means that only those plants which have specialized methods for preventing toxicity from anoxia can flourish. This depends on two factors: first the plant

must be able to transfer oxygen for metabolism from above-ground structures, such as leaves, to their roots; and second there must be some metabolic adaptation to the low oxygen tension in the tissues. In anaerobic conditions a non-adapted plant will build up acetaldehyde and ethanol, the end products of glycolysis, to toxic levels in the cells, while in flood-adapted plants (those which are able to grow in wetlands) the glycolytic pathway is diverted to oxaloacetate and malate which are not toxic (McManmon and Crawford 1971).

2.4.5.2 PEATLAND TOPOGRAPHY

The mire surfaces are very complex topographically and biologically. Aiton was the first to recognize the cyclical alternation, with time, of dry hummocks with wet hollows (Gorham 1957). The bog pools are colonized by algae and plants with different growth habitats. Some form carpets while others produce hummocks, this creates a cycle of growth that alternates from pool to carpet community to hummock, and back again to pool, as the peat slowly accumulates. It is this regeneration complex that is the principal means of establishing thick peat deposits.

The hummocks can be seen from the air to be linked together in long parallel strings that are invariably across the direction of water flow, which itself is controlled by the almost imperceptible slope of the peat surface. In northerly maritime regions, such as Labrador, the strings may stand 15 to 20 cm above open-water pools, which may be more than 20 cm deep. The development of such landscape patterns appears to be highly complex and dynamic, involving initial accumulation of peat, subsequent development of linear patterns, followed by enlargement of pools by active microbial decomposition in an oxygenated environment, together with various erosional processes (Foster et al 1983).

2.4.5.3 EROSION OF PEAT

In spite of the millenia during which peat has accumulated, it has long been known that bog erosion itself is part of natural evolution. In 1811 Aiton (Gorham 1953) described the formation of hags (isolated peat remnants) and streams which drain the layers of moss, slowly cutting into the peat, and reducing its thickness. Bog bursts also appear to be natural, and have been known for some time since Ganong (1897) mentions them; in this case the amount of water in a bog can build up to such an extent that even the ability of the sphagnum to adsorb it is exceeded. Godwin (1981) describes an Irish bog burst where exceptional rain had overfilled a small lake and saturated the surrounding peat; the increased pressure ruptured the surface crust of vegetation thus allowing the slurry of peat to pour out and fill a small valley to a depth of 5 m.

Much of the recent erosion has been due to human activity, for any development that lowers the water table stops the active growth of peat. In particular, draining, ploughing and fertilizing peat soils for forestry and agriculture have destroyed bogs that have taken thousands of years to accumulate (Thompson 1987). In much of the British Isles poisoning from acid rain carrying excess sulphates and nitrates has weakened and reduced the growth of sphagnum, which can then no longer maintain the high water table. In consequence the peat erodes to form blackened gullies, hags, and sheets of bare peat. Once the water table is lowered, the soil also dries out, permitting aerobic degradation to proceed. In some areas, such as the English Fens, the consequent loss of peat has lowered the surface of the land to such an extent that the water level in the drainage ditches must be contained behind dykes.

Other than man, the beaver, especially in Canada, has had the most influence on peat communities. In many areas the normal drainage patterns set by topography have been

drastically altered by beaver dams, producing a change in the vegetation (Gorham 1957). They may also flood ombrotrophic bogs (personal observation), and beaver dams have also been shown to reduce the flow in river catchments with concomittant increase in methane production (Ford and Naiman 1988, Nisbet 1989).

2.4.5.4 CONSERVATION

Wetlands have long been considered uneconomic wastelands whose drainage or conversion to another use seems highly desirable. The productivity of wetlands, however, is demonstrated by the large amount of wildlife dependant upon them. Commercially important fur-bearing animals include muskrat, nutria, mink, beaver and otter. Waterfowl support a large recreational hunting industry, while many fish and shellfish require wetlands for food and for spawning. Wetlands also have a value for flood mitigation, by preventing storm runoff and erosion. Coastal wetlands act as giant buffers against sea gales. The wetland hydrology allows groundwater recharge, while the organic matter is able to remove organic and inorganic nutrients and toxic materials from water that flows across them (Mitch and Gosselink 1986). It is this last attribute which has prompted many mines with acid-producing tailings to construct wetlands to solve their pollution problems. Finally wetlands have an aesthetic appeal, many visitors use hunting and fishing as an excuse to experience their wilderness and solitude. Drainage and clearing of wetlands, such as the Florida Everglades, have often been subsidized by governments without sufficient evaluation to ensure economic and ecological soundness.

The re-establishment of moorland vegetation on bare eroded peats is difficult, for the plants have failed to recolonize the surface naturally. Ironically, it has been found in England, that a complex treatment of applying fertilizer to bent grass seedlings enables them

to grow sufficiently fast to hold the peat. This grass, although native to Britain, is not a moorland species but does tolerate the poor conditions so long as it is fertilised. Native plants can then grow protected by the grass until it dies out in a few years, when the added nutrients are exhausted (Lightowlers 1988).

2.5 FORMATION OF PEAT

Peat is formed from the incomplete microbial degradation of the plant biomass. The plant carbohydrates are metabolised primarily to carbon dioxide and methane, but while the lignin is recalcitrant to biological decomposition it is chemically altered, first to humus, and then with increasing time and pressure to coal.

2.5.1 INITIAL BIOMASS COMPOSITION

The biomass of vascular plants comprises three interlinking polymers, approximately one half is cellulose, and one quarter each are hemicellulose and lignin. Cellulose is a linear homopolymer of D-glucopyranosyl residues, joined exclusively in the ß1-4 configuration. The cellulose chains are grouped together in microfibrils, which, in the majority of cell walls of algae and plants, consist of two or more discrete layers of parallel fibres (fibrils). These fibrils grow spirally in successive layers, and are laid over each other to give a net-like character to the cell wall (Swain and Cooper-Driver 1981). A matrix of lignin, and of polysaccharides, the latter collectively called hemicellulose, envelops these microfibrils. The hemicellulose is a heteropolymer made up largely of xylose units, but with varying amounts of other saccharides such as arabinose, several hexoses, glucuronic acid, and pectin, which is a partially methoxylated galacturonic acid.

Lignin, an integral part of the cell walls of all vascular plants, is a randomly linked network of phenylpropanoid units. The units are similar, except for their degree of methoxylation, but they are joined by many different chemical bonds, which resist biodegradation (Brown 1985). Non-vascular plants, such as mosses, do not contain lignin, but they do contain polyphenols which make them also recalcitrant to degradation, and inhibitory to many microorganisms.

2.5.2 DEGRADATION OF BIOMASS

Normally in terrestrial soils the plant litter is efficiently degraded by aerobic microorganisms, so that after a year over 70% of the original carbon residue may be released as carbon dioxide (Martin et al 1980). The polysaccharide portion of plant biomass is the main source of mineral carbon, since it is much more easily metabolized than is the lignin carbon. In anaerobic waterlogged soils rapid aerobic degradation does not occur.

Lignin is the most abundant renewable aromatic material on earth and is central to the earth's carbon cycle. Since not only is lignin second only in abundance to cellulose in biomass, but as well, it physically protects the cellulose from microbial hydrolysis. It is now thought that lignin is degraded biologically by a unique enzymatic 'combustion' that is, by a nonspecific enzyme-catalyzed oxidation (Kirk and Farrell 1987). In the white-rot fungi (such as *Phanerochaete chrysosporium*) the key enzyme for this reaction is ligninase (lignin peroxidase), and while many bacteria and other fungi are known to degrade lignocellulose, their reactions have yet to be elucidated.

Due to the size, heterogeneity and molecular complexity of lignin, the initial biodegradation has to be mediated by an extracellular system, which is both oxidative and nonspecific in the chemical bonds it breaks. Thermodynamically the conversion of lignin to carbon dioxide and water should be preferred, but in fact depolymerization is kinetically favoured because ligninase oxidizes the substrates by a one electron transfer, and the unstable intermediates thus produced are able to react further.

Aromatic structures are broken down in aerobic conditions by oxygenative ring cleavage, but in anaerobic conditions the microorganisms must first reduce the ring structure

before the ring can be cleaved to form aliphatic acids (Evans 1977). It appears to be easier to break the aromatic rings of the lignin than to digest the encompassing network that is partly made up of strongly bonded ether linkages. Although there have been several reports of anaerobic metabolism of lignin and lignin type compounds (Balba et al 1981, Colberg and Young 1982, Benner et al 1984), there is no convincing evidence that polymeric lignin can be biodegraded under anaerobic conditions (Kirk and Farrell 1987).

2.5.3 HUMUS FORMATION

Peats accumulate where the water table is maintained at or near the surface, and where pH is low and Eh negative. Proteins, carbohydrates and lipids are rapidly degraded by aerobic microorganisms in sediments, but when the oxygen content in the environment is minimal, organic matter is preserved as anaerobic microbial processes are slow and incomplete. Lignin may be degraded biologically only slowly, but it is susceptible to geochemical reactions. The aromatic units of lignin are chemically condensed and randomly recombined to form irregularly organized geopolymers known as humic compounds, which include humic and fulvic acids, humins and kerogen (Barnes et al 1984, Mathur and Farnham 1985).

2.5.4 DIAGENESIS

As lignin is so resistant to degradation, both aerobically and anaerobically, this polymer is available for diagenesis (post-depositional change) in peat deposits and ultimately forms a major organic carbon resource in the form of peat, lignite and coal (Figure 2.4). Since the latter part of the eighteenth century people have believed that coal is formed from plant debris that has accumulated in peat swamps, which are later overlain by inorganic sediments. In this environment the peat undergoes a series of chemical changes with

increasing burial producing a sequence of increasingly metamorphosed coals. In the early part of this century it was also realised that as cellulose is relatively easy to decompose it is not an important component in coal formation, while lignin, which is resistant to degradation, is concentrated by the coalification. Thus the major condition required for formation of coal is the incomplete degradation of the plant biomass, such as is found in waterlogged bogs (Hendricks 1945).

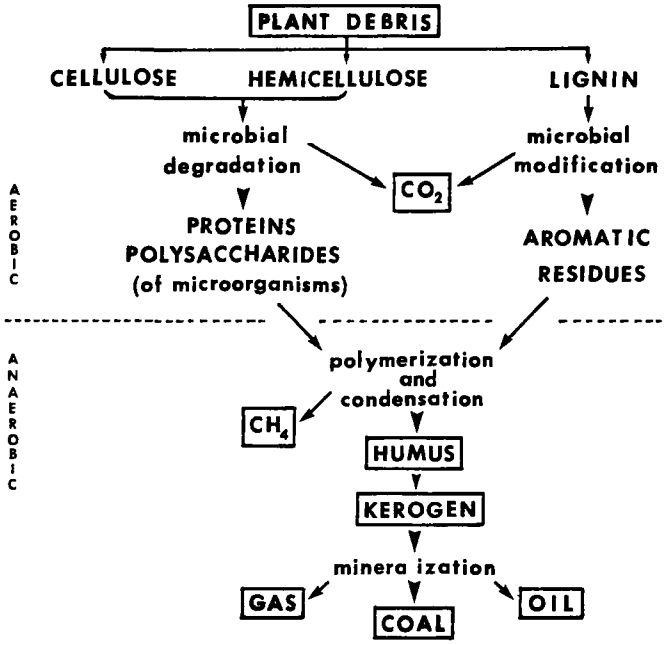


Figure 2.4 Biomass degradation

The thermal history of buried peat plays a significant role in the formation of the higher-volatile bituminous coals, for without a period of increased temperature the biomass would remain as peat or lignite. Initially the undegraded organic components become physically compacted with increased burial time, forming kerogen, an irregular geopolymer

with variable structure produced by random chemical recombination. As diagenesis proceeds to metamorphism the coal progressively increases in rank from peat to lignite through bituminous coals to anthracite. The most noticeable changes during this process are the loss of moisture, oxygen and hydrogen, and the corresponding increase in fixed carbon (Barnes et al 1984).

Humic acids do not appear to be major intermediates in the formation of coal from lignin, but clay minerals, which are major inorganic constituents of coals, play an important catalytic role in the thermal alteration of lignin. In fact, recently, coal has been made artificially by heating lignin at 150°C for several months in the absence of oxygen and in the presence of clay minerals (Hayatsu et al 1984).

2.6 MICROBIAL REACTIONS

2.6.1 SOIL ENVIRONMENT

Soil microbial populations possess a remarkabe spectrum of metabolic properties which makes them resilient to many types of disturbance. Microbes are most active on the surface of soil particles, particularly on the colloid-sized clay and humic materials. The ionic properties of these materials generally attract cations; they also have a high affinity for water molecules so that the microorganisms, substrates, enzymes, metabolites and inorganic nutrients tend to be concentrated at the clay/water or humate/water interfaces. The other principal soil particulates, sand and silt, do not retain water or accumulate organic material as well as clay; they do, however, promote porosity and hence gas and solvent diffusion. The soil environment is thus much more complex than that to which the microorganisms are exposed in traditional laboratory conditions (Burns 1983).

2.6.2 THE NATURAL pH AND pϵ (Eh) ENVIRONMENT

Redox chemistry for the dissolved species in aqueous terrestrial and aquatic systems is best described by pH and pϵ (Eh) parameters which relate the free energies of reactions to galvanic half cells. These parameters are a measure of the densities of the protons and electrons in a solute (using the potential of a theoretical cell). pH is the negative log value of the proton (H^+) activity, while pϵ is the electron activity at equilibrium, and measures the relative tendency of a solution to accept or transfer electrons. In a highly reducing solution the reactions tend to donate electrons to the solution, and cause the pϵ to be negative. pϵ is related to the equilibrium redox potential, Eh (volts, hydrogen scale) by pϵ = Eh/2.3RTF, (Garrels and Christ 1965, Stumm and Morgan 1981). Unfortunately Eh is difficult to measure in the field since it is impossible to prevent the access of oxygen to the measurement site, particularly since the platinum electrode (the usual probe) is very slow

to equilibrate; however, some indication of the redox conditions can be obtained.

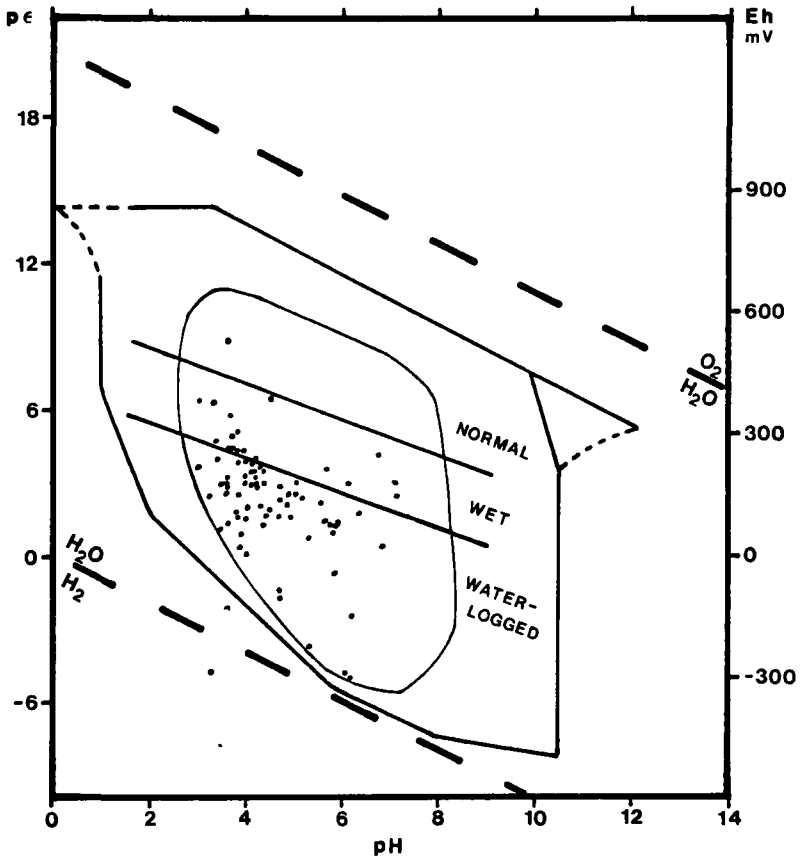


Figure 2.5 Stability field of natural environments;

approximate area of peat bogs from Baas Becking et al (1960);

showing own field measurements

There are now many thermodynamic data available for reactions at, or near NTP (25°C and 1 atmosphere), which can be used to write the pertinant free energy equations and calculate the activites. pH and pϵ outline the stability fields of chemical species and so define natural environments. Baas Becking et al (1960), used some two thousand published

Eh-pH field measurements and several thousand of their own, to outline such natural environments (Figure 2.5). The limits he found for peat bogs are shown by the solid line in this diagram, and the area of his 'waterlogged soils' is confined to the lower part. To show that these limits are valid I have added my field measurements to the diagram (more diagrams of these readings are in Appendix 8). All but two readings, out of a total of 75, are within Baas Becking's outline, and one of these appears to be incorrect.

pϵ-pH diagrams can show which chemical species theoretically will predominate under any given conditions of pϵ and pH. The redox stability field of water is shown between the two dashed lines in Figures 2.5 and 2.6. Above the upper one water becomes an effective reductant (producing oxygen), and below, it is an effective oxidant (producing hydrogen), while water is barely dissociated between these boundaries. The overall reaction can be considered as a reduction of carbon dioxide to methane, and at a pH of 7 and an Eh of -250 mV, which is the expected environment for methanogens (Zehnder and Stumm

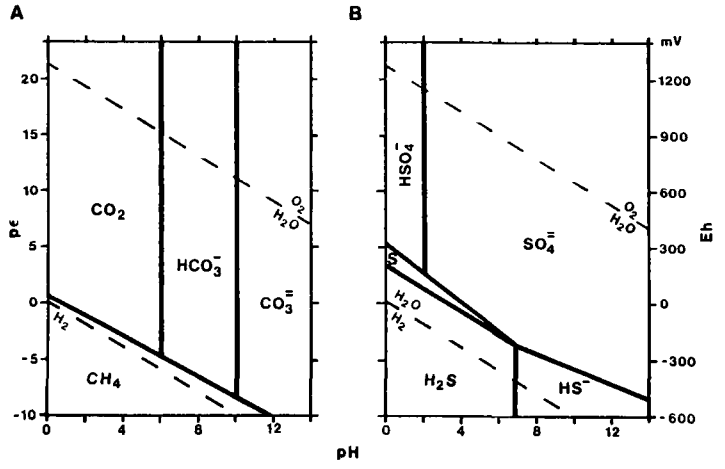


Figure 2.6 pϵ-pH diagram of: A - carbon species; and B - sulphur species (from Stumm and Morgan 1981).

1988), this reaction will occur at the phase boundary between carbon dioxide and methane. The partitioning of the different carbon species in pϵ-pH space is shown in Figure 2.6A. To maintain the environment for methanogenesis at a pH of 4 (Section 4.3.2), would require the pϵ to become less reducing to prevent moving into the area of water instability. The partitioning of the sulphur species (Figure 2.6B) shows the sulphide in the medium will be as HS^- at pH 7 but as soluble H_2S at pH 4.

2.6.3 DEGRADATION OF POLYSACCHARIDES

Biomass is complex and polymeric (see Section 2.5.1) so that the first step in its degradation must be the breakdown into soluble molecules. The main metabolic pathways in peat are shown in Figure 2.7, where the microbial degradation is principally of the carbohydrate portion of plant biomass, cellulose and hemicellulose. The organisms thus involved can be grouped first into the hydrogen-producing bacteria, which are of three types: hydrolytic bacteria which degrade complex carbon compounds (such as cellulose) to sugars, organic acids (primarily acetate), alcohols, carbon dioxide and hydrogen; secondary bacteria which consume these sugars and ferment them to volatile fatty acids or simple carbohydrates, and to carbon dioxide and hydrogen gases, this increases the overall reaction as it prevents the accumulation of sugars which inhibit the hydrolytic bacteria; and thirdly, ancillary bacteria which convert the longer-chain acids and alcohols produced from the previous fermentations to acetate, carbon dioxide and hydrogen. Various eubacterial organisms carry out different steps of this breakdown.

The release of these substances from the original biopolymers provides the substrates for the archaebacterial methanogens, which are the main hydrogen-consuming bacteria of the second group. They produce methane from carbon dioxide and hydrogen,

or from acetate. When sulphate-reducing bacteria are present, they also function as hydrogen-consumers and reduce sulphate to hydrogen sulphide (Zeikus 1983, Ljungdahl and Eriksson 1985).

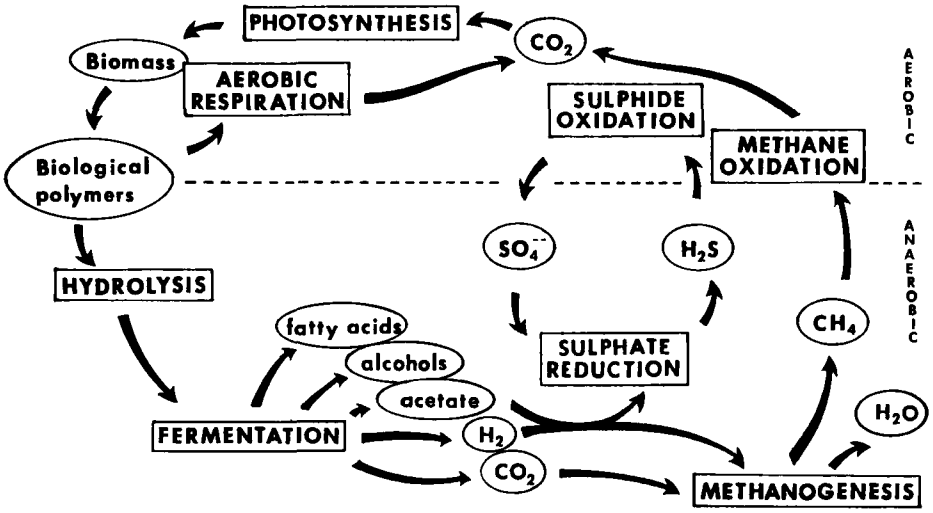


Figure 2.7 Metabolic pathways in peat.

This consortium of microorganisms is thus able to achieve the complete conversion of polymeric cellulose to gaseous methane and water (Frea 1984). The first part of the process can be portrayed as the oxidation of cellulose carbon to carbon dioxide and the reduction of protons to hydrogen. While the second part is the production of methane by the oxidation of hydrogen and the reduction of carbon dioxide and acetate. Hyrogen thus plays an essential role in anaerobic degradation: in the first step the bacteria produce the hydrogen and in the second they consume it. This is known as **interspecies hydrogen transfer**, and is essential for continuing the biodegradation as hydrogen is inhibitory to the cellulose-metabolising organisms.

Interspecies hydrogen transfer represents a classical food chain. Hydrolytic bacteria produce hydrogen from substrates that provide them with energy, and this hydrogen in turn becomes an energy substrate for the hydrogen-consumers. The special feature of this transfer is that the consumption of hydrogen prevents inhibition of the hydrolytic bacteria. One of the most sensitive steps in the accumulation of hydrogen is the oxidation of reduced nicotinamide adenine dinucleotide to its oxidized form and hydrogen, since many of the hydrogen-producing species have enzymes that are inhibited by hydrogen (Wolin and Miller 1982). As the role of hydrogen is so important in anaerobic degradation, Conrad et al (1985) measured the hydrogen flux in sewage sludge and lake sediments. The hydrogen they found accounted for only about 5% of the methane production instead of the 30% theoretically expected. They suggested therefore, that the hydrogen is transferred directly, without forming free gas, between syntrophic associations of hydrogen producers and methanogens, where the microorganisms are juxtaposed within a consortium.

2.7 METHANOGENESIS

Living organisms are now divided into three lineages: the archaebacteria, the eubacteria and the eukaryotes (Postgate 1989). The eukaryotes are all those organisms which contain a true membrane-bound nucleus, and encompasses all the multicellular organisms and unicellular organisms such as yeasts. The eubacteria, or true bacteria, comprise most of the commonly recognized species, while the archaebacteria are a group of bacteria made up of the methanogens, the extreme halophiles and sulphur-dependent thermophiles (Woese 1987).

2.7.1 ARCHAEBACTERIA

The archaebacteria are quite distinct from the eubacteria although there is a morphological resemblance, and so far they have been found only in extreme habitats. The common characteristics of the archaebacteria were derived from sequencing 16S-RNA oligomers, the absence of eubacterial peptidoglycan in the cell wall, and the occurrence of ether-linked lipids sythesized from phytanyl chains (Woese et al 1978). The ancestor of the archaebacteria is unknown, but the fact that three of the five known lines are methanogenic suggests methanogenesis was part of the ancestral phenotype's 'repertoire' (Balch et al 1979). They show rather greater divergences among their 16S-RNA catalogues than the eubacteria, indicating an older, longer family tree (Postgate 1989).

2.7.2 METHANOGENIC BACTERIA

For many years it was not believed that microorganisms were involved in bog decomposition, but finally Waksman and Stevens (1929) were able to find and to enumerate these bacteria. Since anaerobic degradation requires a consortium of several different physiological groups of bacteria, each component of which is accountable for only one part

of the whole chain, the methanogenic bacteria must be in close proximity to the eubacteria which break down the biopolymers. The metabolic diversity of anaerobic bacteria far exceeds that of aerobic organisms, for, in lieu of oxygen, they are able to couple dehydrogenative carbon transformation reactions to hydrogenation of diverse electron acceptors, such as carbon dioxide, various organic compounds, nitrate, sulphate and ferric ions (Zeikus 1983, Lovley and Phillips 1987).

Strict anaerobes must obtain their energy without using oxygen as a terminal electron acceptor, and at the same time they must also avoid being poisoned by it. Aerobes scavenge superoxide anion, the active species, with superoxide dismutase, but although this enzyme has been found in anaerobes, it does not necessarily seem to prevent oxygen sensativity (Kirby et al 1981).

The principal source of biological methane is the anaerobic fermentation of carbohydrates, which occurs in the digestive tracts of ruminants and insects, in the anaerobic layers of peat and sediments in wetlands, and in sewage digestors and landfills. The methanogenic organisms responsible for this fermentation are ubiquitous in nature, and play a major role in the cycling of carbon, for not only is methane production quantitatively significant, but in many cases it is the key product in organic turnover. Formation of methane carries out two functions, the conversion of organic carbon to inorganic gaseous carbon (carbon dioxide), and the removal of reducing equivalents by oxidation of hydrogen. Although anaerobic energy-producing fermentations are much less efficient than aerobic respiration, without these reactions the accumulation of small reduced organic compounds would inhibit carbon metabolism (Morris 1975).

2.7.3 PRODUCTION OF METHANE

Most methanogens are autotrophs which are able to obtain energy from the formation of methane from carbon dioxide and hydrogen:

$$CO_2 + 4H_4 = CH_4 + 2H_2O$$

Carbon dioxide plays a dual role in methanogenic metabolism since it is also the carbon source for autotrophic growth. Methanogens cannot fix carbon dioxide by the reductive pentose cycle as they lack these enzymes, but they do posses the nickel containing enzyme, carbon monoxide dehydrogenase, the key enzyme of the activated acetic acid pathway. The pathway for these reactions, deduced from Fuchs and Stupperich (1986) and Wood et al (1986), is shown in Figure 2.8.

The synthesis of acetyl-CoA requires that two molecules of carbon dioxide are reduced by an 8-electron transfer from hydrogen. The methyl of the acetate is derived from the reaction path which is common to both methane formation and to the assimilation of carbon. The enzymes of this pathway have not yet been characterized and not all the steps determined, but it is known to contain several unique cofactors: the first, methanofuran (MF), at the level of reduction of carbon dioxide to formate, and the second, tetrahydromethanopterin (THMP), at the methyl level. There appears to be a branch here, for about 97% of the methyl group reacts with Coenzyme M (CoM) to form methane, while the remaining 3% is transferred, first to a corrinoid enzyme ([Co]E), and then to the carbon monoxide dehydrogenase enzyme complex (/\/\/\).

The mechanism of formation of the carboxyl group of the acetate has been deduced from work on *Clostridium thermoaceticum* (Wood et al 1986). The carboxyl is formed from the second molecule of carbon dioxide, which is both reduced and attached to the reaction

site by the carbon monoxide dehydrogenase. The methyl group is transferred from Coenzyme E (not known to be present in methanogens) first to another reaction site on the enzyme and then added to the carbonyl group. The final step is the release of an activated acetate molecule by thiolysis with Coenzyme A (CoA). Cells grown on acetate are also able to catalyse the formation of methane and carbon dioxide from the acetate. The acetate reacts with ATP to form acetyl-phosphate, which then forms acetyl-CoA before being cleaved. The methyl group reacts with the corrinoid enzyme before being transferred to

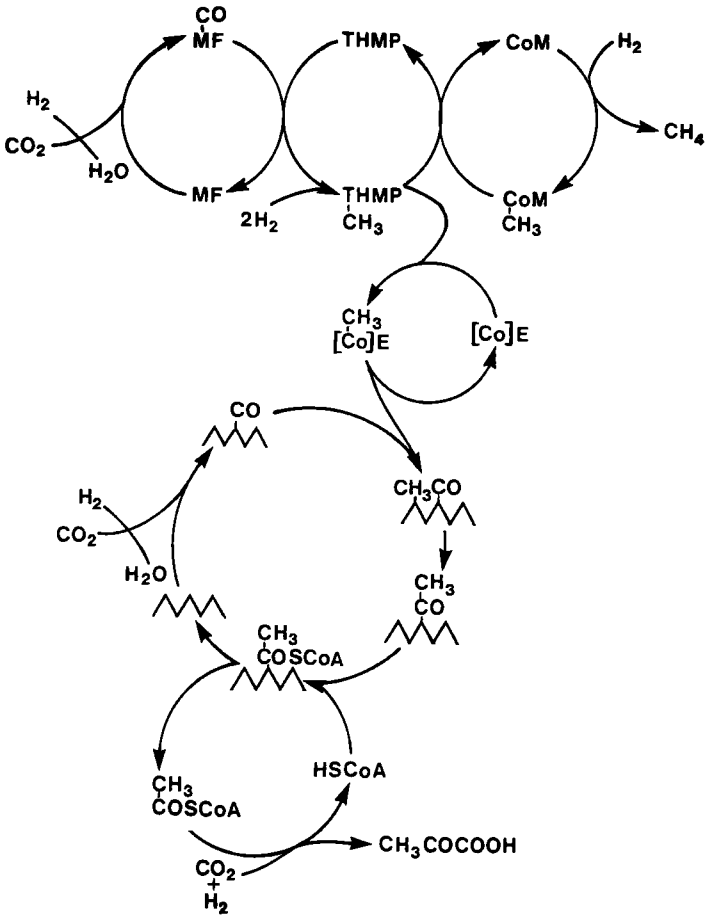


Figure 2.8 Proposed activated acetic acid pathway.

Symbols described in text. Deduced from Stupperich (1986) and Wood et al (1986).

[Co]E and CoM and reduced to form methane. The reducing equivalents for this reaction are provided by the CO-group which is oxidized to carbon dioxide (Laufer et al 1986).

The activated acetic acid cycle was studied for many years in *C. thermoaceticum* before the complete pathway was elucidated. Initially it was believed that, in this bacterium, pyruvate was required to donate the carboxyl group, and that the final product was acetate (Andreesen et al 1973). Recently it has been shown that carbon monoxide dehydrogenase not only reduces carbon dioxide to carbon monoxide but is also probably the site of the carbon-carbon addition (Ljungdahl and Wood 1982). This pathway must be either a particularly early one, and have evolved before the Eubacteria and Archaebacteria separated, or gene transfer has taken place, for this enzyme is found in both eubacterial Clostridia and sulphate reducers and in archaebacterial methanogens, as well as *Sulfolobus* and *Thermoproteus* (Fuchs and Stupperich 1986).

2.7.4 INHIBITION OF METHANOGENESIS

Acid ombrotrophic bogs are a major environmental source of methane, but characterized methanogens prefer a neutral pH. There has been only one report (but not deposition in a culture collection) of a methanogenic bacterium which is able to produce methane at pH 3.1, but unable to grow below pH 5.3 (Williams and Crawford 1985). The reduction in methanogenesis with depth has been reported by Koyama (1976) in lake sediments, and in peatlands by Williams and Crawford (1984) and by Yavitt et al (1987). There has been much discussion on the cause of this loss of activity, whether it is a buildup of metabolic products, solid or gaseous, particularly in view of the age of some of the lower levels of the peat, or whether it is lack of some particular nutrient, which is present in the near-surface layers but not at depth.

There is no evidence that methane itself is inhibitory to methanogens, although the peat bog environment has always been considered to be inhibitory to microorganisms, and it was for this reason that sphagnum was used as a wound dressing in the First World War. Low pH and low temperature reduce the rate of methanogenesis, while additions, such as glucose, do not enhance the activity of methanogenic cultures, which indeed would not be expected, since glucose is not a direct substrate for methanogenesis. Acetate is inhibitory, unless it is neutralized, for at low pH organic acids are undissociated and can penetrate the cell membrane. Neither peat nor yeast extract seem to enhance activity. Ethylene is known to be produced in some soils in sufficient quantity to cause crop damage (van Cleemput and El-Sebaay 1985), and several methanogenic bacteria can produce ethane, ethylene and acetylene from halogenated hydrocarbons (Belay and Daniels 1987), but acetylene is the only gas so far known to inhibit methane production (Sprott et al 1982).

2.7.5 EFFECT OF METHANE ON THE WATER TABLE

The restriction of water movement in the deep layers of peat has not so far been explained satisfactorily (George 1975). Mathur and Lévesque (1985) have demonstrated that the saturated hydraulic conductivity can decrease sharply as the depth of the peat increases, even when the degree of decomposition does not necessarily increase with depth. These authors suggest that some of the methane produced in the deeper layers remains where it is generated, and, since it is relatively insoluble, the gas could occlude connective soil pore spaces, curtailing both water and gas movement.

When methane is trapped in a bog it must, in part, be in the gaseous state, even when under the considerable hydrostatic pressure from the overlying water, for it is usually found in quantities above the 4% that can be held in saturation at NTP (25°C and 1

atmosphere), (Dean 1979). Since methane is less dense than air, when a gas permeable pathway is formed, or when methane-saturated water interfaces with air at the fringe of a bog, the methane will be outgassed and driven to the surface through the pore cavities. Gas bubbles can occlude nonuniform capillary tubes, which may be equated with the pore spaces found in peat bogs, and thus prevent the movement of both water and gas (Jamin 1860). As gas bubbles collect at a constriction in a capillary tube, the bubble is distorted and the surface energy is increased at the interface between gas and liquid at the small radius end of the bubble. The pressure at the broad end of the bubble then has to exceed that at the narrow end for the bubble to be able to advance through the constriction (Wyckoff and Botset 1936). This occlusion will prevent not only the movement of water with its attendant dissolved minerals, but also the release of methane and the penetration of oxygen (Mathur and Lévesque 1985).

2.7.6 ATMOSPHERIC METHANE

The magnitude of methanogenic activity is not generally appreciated; about half of the organic carbon degraded by anaerobes is eventually converted to methane. The methane that is released into the atmosphere is only a small part of what is actually generated, as much of the methane is oxidized before it ever reaches the atmosphere. Nevertheless the methane that does reach the atmosphere is equivalent to 0.5% of the annual biological production of dry organic matter, and thus makes methane one of the most abundant organic compounds on earth (Frea 1984). When this gas enters the troposphere it reacts with hydroxyl radicals to form carbon monoxide and water, while closer to the earth's surface the methane is oxidized to carbon dioxide and reenters the carbon cycle, to which it makes a small, but significant contribution (Ehhalt 1976).

The presence of methane in the Earth's atmosphere was only discovered relatively recently (Migeotte 1948). In the biosphere today the principal production of methane which can be vented to the atmosphere is from the anaerobic fermentation of carbohydrates (Lowe et al 1988). Northern peatlands produce much of this methane, and the global emission from this source is calculated to be 66 Tg (grams x 10^{12}) annually (Matthews and Fung 1987). Measurements of air bubbles in ice cores has shown that 200 years ago the concentration of atmospheric methane was only about 0.65 parts per million by volume (ppmv) (Stauffer et al 1988), while today its concentration is between 1 and 2 ppmv except in urban areas where it may be twice this (Graedel et al 1986). The current rate of increase is thought to be around 1% a year (Rasmussen and Khalil 1981, Bolle et al 1986, Steel et al 1987, Harriss et al 1988).

Although this increase would appear to be anthropogenic, as it has occurred since the industrial revolution and consequent escalation in the world population, it does not seem to be all directly produced by human activity. Radiocarbon dating of atmospheric methane indicates that a third of it is over 5000 years old and must therefore be fossil or come from fossil sources of carbon (Lowe et al 1988). The emmissions of unburnt methane by the petroleum industry do not appear to account for this increase now found in the atmosphere (Gold 1988). Other sources could be from oil drilling, and from combustion of fossil fuels (Steel et al 1987), but northern wetlands may also play a role, for they cover some 2.7 Tm^2, and contain decomposing organic matter which has accumulated over thousands of years. Many peat bogs are today being ploughed up for farming, and, on an increasing scale, excavated for domestic and industrial fuel. This disruption of the peat could allow the entrapped, and possily fossil, methane to escape. Although methane is present in the atmosphere at much lower concentrations than carbon dioxide, its influence per molecule

on the greenhouse effect is considerably greater, for its infrared absorption falls into a wavelength range that is not strongly absorbed by the other trace atmospheric gases (Blake and Rowland 1988).

The measurement of methane emission rates from ombrotrophic bogs varies considerably. Values have been reported to range from 0.16 mg CH_4 m^{-2} h^{-1} in tundra peat (Svensson 1976) and 4.67 mg CH_4 m^{-2} h^{-1} in sub-artic fens (Moore and Knowles 1987), through 4.2 to 16.7 mg CH_4 m^{-2} h^{-1} from the northern peatlands of the United States (Harriss et al 1985) to 100 to 500 mg CH_4 m^{-2} h^{-1} from the Okefenokee Swamp in the southeastern USA (King et al 1981). The production of methane from laboratory peat incubations has been investigated in Minnesota peatland by Williams and Crawford (1984) who obtained 219.6 nmol g^{-1} h^{-1} at the surface and 2.0 nmol g^{-1} h^{-1} at 210 cm; the peat above 40 cm depth produced 5-fold more methane than that at 90 cm, and below this depth the methane production decreased rapidly. Peat from Crystal Bog in Wisconsin produced 6.2 nmol g^{-1} h^{-1} methane (Goodwin and Zeikus 1987); and Big Run Bog in West Virginia produced 650 nmol g^{-1} h^{-1} at surface, and 4.4 nmol g^{-1} h^{-1} at 45 cm (Yavitt et al 1987, 1988).

The production rates were dependent on pH and temperature, and were stimulated by the addition of glucose and hydrogen/carbon dioxide, and inhibited by the addition of acetate. The acidic bog sediments from Crystal Bog were in fact from a poor fen and were overlain by 2.5 m of anoxic water. In this case the anaerobic digestion was found to be optimally adapted to function at low pH, and, at low carbon flux rates, neither acetate nor hydrogen were found to stimulate the production of methane. The Big Run Bog is also a minerotrophic fen, and raising the temperature here increased, but did not overcome, the seasonal variation in methane production; glucose, acetate and hydrogen did not stimulate methanogenesis in surface peat but glucose, acetate and hydrogen directly stimulated

methane production in subsurface peat.

3. ACID TAILINGS

3.1 PEAT COVER TO PREVENT ACID-LEACHING OF MINE TAILINGS

3.1.1 INTRODUCTION

The Canadian mining industry is an $18 billion a year enterprise, the third largest in the world after those of Russia and United States (Soganich 1985). Ore minerals are commonly disseminated throughout the rock or in narrow veins. To extract ore many tons of waste material have to be processed. The rock is crushed by milling, and when the economic minerals have been recovered the waste material, known as tailings, is transported as a slurry to a temporary pond. This pond is gradually drained, leaving beds or piles of fine sand and silt which contain waste sulphides. There are now some 500 Mtons of acid tailings in Canada, covering an area of 9 kha (Monenco Report 1984). The problem is vast, especially when it is realised that many of these tailings have been deposited on porous ground because the initial aim was to drain the tails of their liquid, as it was thought to be easier to reclaim the dried material. In addition, the old dams which once held back the tailings slurry, are now broken and incompetent.

3.1.2 FORMATION OF PYRITIC TAILINGS

Iron sulphides, such as pyrite are formed in sediments by *Desulfovibrio* species which reduce sulphate in solution to hydrogen sulphide, about 90% of which diffuses up to the oxic zone. The rest is precipitated by iron initially to form mackinawite, which is then converted to greigite and afterwards to pyrite (Figure 3.1). Pyrrhotite is formed at higher temperatures than pyrite. The sulphur is thus continuously cycled through sedimentation, metamorphism, erosion and weathering, and over time, pyrite may be locally concentrated. When these pyritic and pyrrhotitic formations come in contact with oxygen, as they do when the

sediment is eroded, they form the substrate for the chemolithic sulphur oxidizing microorganisms, notably *Thiobacillus ferrooxidans.*

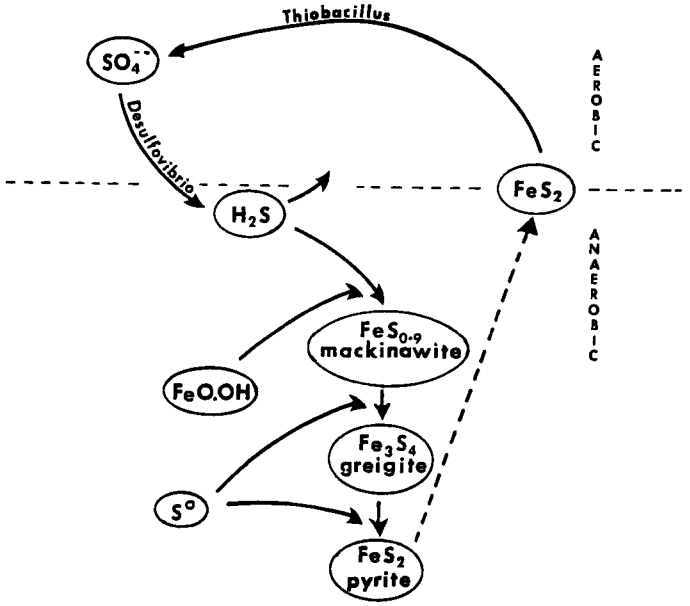


Figure 3.1 The pyrite cycle.

3.1.3 HISTORY

As early as 1918 the US Bureau of Mines was looking for a method to treat acid drainage from coal mines, known to be caused by oxidation of iron sulphides (Tracy 1921); and by the 1930s it was realised that exclusion of oxygen prevented this acid formation and the precipitation of iron (Leitch and Yant 1930); but already acid mine drainage threatened to be a problem of national importance as coal resources were increasingly developed. Even at this date the process of oxidation was being questioned, as it seemed to be too rapid to be due solely to chemical reactions, and it was suggested that it might be caused by organisms similar to *Thiobacillus thiooxidans* (Carpenter and Herndon 1933).

It was not until 1947 however, that microorganisms were generally recognized to be the cause of the breakdown of pyritic minerals to sulphuric acid (Colmer and Hinkle 1947). Although sulphide minerals are found associated both with coal and with metal mines, generation of acid from base metal mine tailings was not perceived as a problem until after 1960, when several uranium mines in Canada were shut down for economic reasons (Hester 1984).

The initial impetus for this current work was the need to find a method to ameliorate acid-leaching from mine mill wastes. Many ores are found in association with sulphides which are susceptible to bacterial oxidation and the concomitant production of sulphuric acid. The microorganisms utilize the energy from this reaction for growth. There is insufficient oxygen in stagnant water for the oxidation to take place, so flooding the tailings or depositing them under water will prevent acid production. Unfortunately this is not practical for most of the old tailings. Many mine mill wastes form vast hills, such as Waite Amulet in the Noranda mining camp, which is a relatively small tailings dump covering about 40 ha rising 12 m above the surrounding countryside. It is extremely difficult to find a method to prevent the penetration of oxygen into tailings accumulations like this. The present method is to neutralize the run-off with lime, an expensive and never-ending process.

Detailed sampling of old tailings from a mine in New Brunswick (Boorman and Watson 1976) has shown the presence of three zones within the dump (Figure 3.2): the top zone is the oxidation layer and extends from the surface to a maximum depth of 50 cm; below this there is a hard pan zone which may be up to 15 cm thick; and the rest of the dump is the reduction zone of unaltered tailings. The depth of the oxidation layer is

dependent upon the availability of oxygen for the bacteria, and this is controlled by the permeability of the tailings. Oxygen is consumed in the top oxidation layer until the concentration is inadequate to support generation of ferric ions. When the oxygen is depleted, the ferric ion is rapidly reduced by pyrite to the ferrous state, and iron oxides and/or hydroxides are then precipitated to form a well defined hardpan. This zoning was recently confirmed by Petruk and Pinard (1986) in the Waite Amulet tailings at Noranda. Here, there is a high concentration of sulphides in the base, a sharp change in concentration at the water table, and essentially no sulphides near the surface.

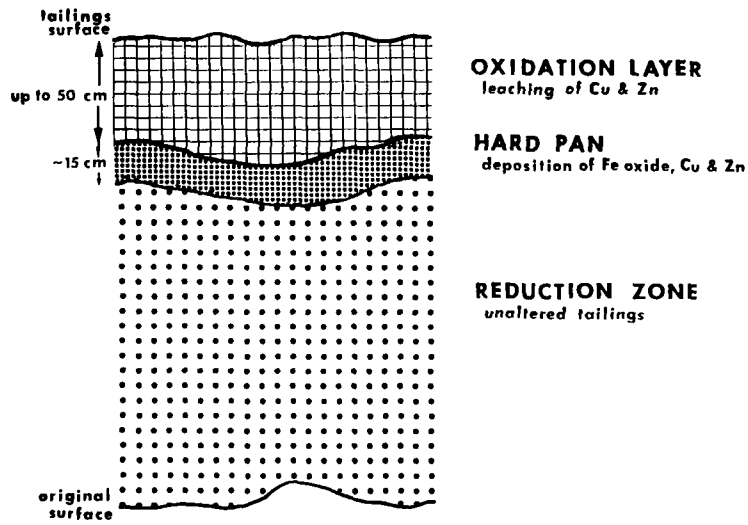


Figure 3.2 Zones of a tailings dump.

3.1.4 ACID LEACHING

Thiobacillus species are the most important group in the transformation of reduced inorganic sulphur to oxidized compounds, and hence in acid production. These organisms

require for their growth the presence, both of reduced sulphur compounds and of oxygen, which means that there is only a very narrow zone in which they can live (Fenchel and Blackburn 1979, Kuenen and Tuovinen 1981, Jorgensen 1983).

Thiobacillus ferrooxidans is able to derive energy from either the direct oxidation of inorganic sulphur compounds

$$2S^\circ + 3O_2 + 2H_2O = 2H_2SO_4$$

or from ferrous iron compounds such as pyrite, FeS_2

$$2FeS_2 + 2H_2O + 7O_2 = 2FeSO_4 + 2H_2SO_4$$

However the key characteristic of this organism is the ability to further oxidize the ferrous ion to the ferric ion provided there is sufficient oxygen available (Brierley & Brierly 1982, Taylor et al 1984)

$$4FeSO_4 + O_2 + 2H_2SO_4 = 2Fe_2(SO_4)_3 + 2H_2O$$

It is this ferric ion, either alone or in combination, that is the most important species involved in the indirect attack on sulphide minerals (Lungren & Silver 1980, Taylor et al 1984).

$$Fe_2(SO_4)_3 + FeS_2 = 3FeSO_4 + 2S^\circ$$

or, in the presence of ferric sulphate, to oxidize other metal sulphides and oxides such as chalcocite, Cu_2S

$$Cu_2S + 2Fe_2(SO_4)_3 = 2CuSO_4 + 4FeSO_4 + S^\circ$$

which, either alone or in combination with sulphate, is involved in the indirect attack on sulphide minerals. *Thiobacillus* sp. are quite resistance to heavy metals found in their environment (Wong et al 1982).

3.1.5 ENERGY METABOLISM

T. ferrooxidans is able to derive energy from the oxidation of acidic ferrous iron as well as inorganic sulphur compounds but these reactions are inefficient as they have a low energy yield, which explains why so much acid is generated during growth. The organisms grow optimally at pH 2.0, yet the internal pH is close to neutral, producing a pH gradient of 4 to 5 units across the plasma membrane. All the energy required for ATP synthesis can be obtained from such a pH gradient. But in order to maintain electro-neutrality, there must be an inwardly directed flow of protons associated with the respiratory chain so that for every four electrons available from ferrous iron oxidation, one proton enters the cytoplasm through the ATPase enzyme and three protons enter through the respiratory chain. This means that *T. ferroxidans* growing on pyrite iron would utilize four moles of ferrous iron for each mole of oxygen consumed (Cox and Brand 1984).

3.1.6 PRESENT METHODS OF AMELIORATION

There is at present no method to prevent acid-leaching, although it can be neutralized by adding lime to the leachate. When tailings contain little pyritic material, it is possible to revegetate them successfully, but on old sulphide tailings dumps, such as those of Waite Amulet, and Nickel Rim near Sudbury, the vegetation is very fragile (Michelutti 1978). There has been a heavy expenditure on lime and fertilizer, but the acid seepage continues and slowly percolates up into the covering vegetation and kills not only the plants but also the soil microorganisms. So, although revegetation, which allows oxygen penetration, may seem at first to be successful, it is actually only a temporary cosmetic measure, because it has little or no effect on the acid leaching and the long term reclamation of tailings dumps. As the acidity reasserts itself, the phytotoxic heavy metals are more easily taken up by the plants and thus cause vegetation die-off. It should be noted also

that, unlike mineral soil microorganisms which are very susceptible to metal toxification, peat microorganisms are protected by the ability of peat humus to chelate the metal ions (Mathur and Farnham 1985).

Lysimeters (containers of soil and/or tailings, designed so water draining through them may be measured) have been used to study long term effects of acid leaching. Silver and Ritcey (1982, 1985) investigated uranium tailings in lysimeters, using a simulated rain cycle, 9-fold of normal, to measure leaching time equivalent to 25 years. Vegetation established on the surface of the material in the lysimeters, reduced but did not eliminate, sulphuric acid generation, but a 30 cm layer of compost on top of submerged tailings was able to prevent acid generation.

3.1.7 SUGGESTED ALTERNATIVE METHOD

Most of the old tailings have been drained of the original slurry water long ago, and both this drainage and later rainfall have caused internal channelling, so that any water applied to the surface quickly runs through the tailings. This rapid flowthrough does not occur in bogs, where the water saturation of the bog and the gas pressure of the methane within the bog, prevents water penetration and causes the rain to run off the surface layer. If a raised bog can be established on the tailings it should ensure that the tailings remain anaerobic by the production of methane, and prevent oxygen penetration through the rise in the level of the water table. A diagramatic representation of this is shown in Figure 3.3.

An examination of several different types of mires (wetlands) in Europe (Moore 1987b), has shown that in fact many were caused by man. The clearing of the natural forest for agriculture made a major impact on the hydrology of the landscape by reducing the rates

of drainage, and leading to widespread waterlogging. This allowed the formation of small areas of mire, which then further affected the drainage until the peat cover became general. Bogs can be formed by man and beaver if the conditions are right, so it should be possible to construct bogs intentionally where they are desired. A start has been made at Falconbridge Nickel Mines, Ontario, to saturate and cover a pyrrhotite tailings dump with organic matter in order to build up an anaerobic soil layer (R.E. Michelutti personal communication). Constructed wetlands are already being used to prevent acid leaching from old coal mines in the northeastern States (Mine Drainage and Surface Mine Reclamation Conference 1988), and long term ecological engineering is being investigated by Kalin and Everdingen (1988).

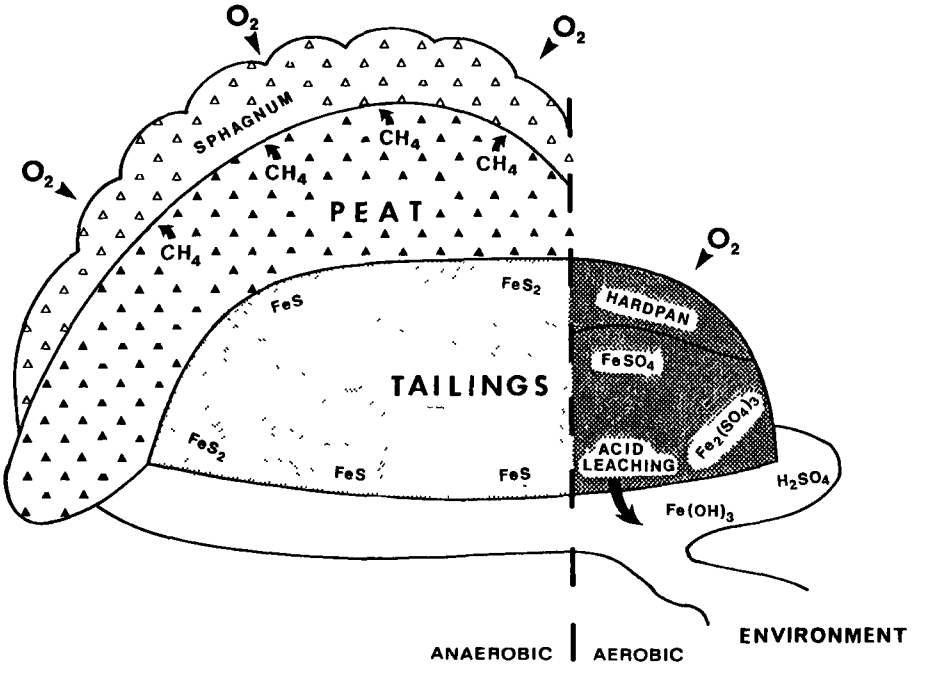


Figure 3.3 Diagram of bog cover for mine tailings.

There are at least two problems to establishing a peat cover on tailings: one is the actual application of the organic soil, and the other is raising the water table sufficiently to maintain anaerobiosis during the establishment of the bog. The formation of a bog will initially require some added organic matter, such as some type of cellulose, for the anaerobic microbiological degradation consortium to use as substrate. Tuttle et al (1969) fortuitously discovered that acid mine water temporaly trapped behind a dam of wood dust from a saw mill reduced the acidity and precipitated the sulphate as iron sulphide by bacteria using organic nutrients from the dam. In the laboratory, column experiments show the addition of paper sludge markedly enhances the production of methane (Appendix 10). However, once the metabolic cycle has been set up, the slow degradation of the bog plants on senescence should maintain the bog indefinitely, provided the rainfall remains adequate.

Unfortunately field trials could not be run, although a few laboratory test have been done. In consequence I have investigated only one aspect of the study: the environment of methanogenesis in muskeg bogs. This includes a statistical study of 30 bogs from the mining areas of Ontario and Quebec, a methane-producing microbial consortium, the field environment, and the actual production of methane, both in situ and in the laboratory. This work is described in the next section.

4. BOG ENVIRONMENT

In this section different aspects of the bog environment are investigated. First, there is an overall look with a statistical analysis of the characteristics of 30 bogs of the southern Canadian Shield; then a method to analyse the main degradable substrate, cellulose, in peat is described. The microorganisms of muskeg bogs are surveyed, first the aerobic physiological groups from the Noranda bogs, and then an enriched acidophilic culture from Mer Bleue. Finally the main product of anaerobic degradation, methane, is investigated, the method of sampling it at different depths in the peat, and the actual amounts found in Mer Bleue are reported. The effect that methane may have on the hydraulic conductivity of peat, and the possibility of atmospheric pollution from this source are considered.

4.1 RELATIONSHIPS BETWEEN ORGANIC SOILS OF THE SOUTHERN CANADIAN SHIELD

4.1.1 INTRODUCTION

In this study I was interested in distinguishing those bogs in the Canadian Shield that could best produce methane, and thus ensure an anaerobic environment if they are used to cover acid mine tailings. Different numerical methods used in the analysis of the data are discussed, and the various relationships they show between the organic soil samples are examined with view to finding a useful method of distinguishing between organic soils.

Samples were collected from bogs in the mining districts of Elliot Lake, Sudbury, Timmins and Noranda, and from one bog away from mining activity, near Ottawa, for comparison. Characteristics of 28 variables were measured, which may be divided into three associations. The inorganic elements, incuding smelter metal contamination; organic

characteristics such as cellulose and respiration which measure the carbohydrate content, and various spectroscopic measurements of the humic content of the peat; and the physical characteristics such as the water holding capacity (WHC), and the cation exchange capacity (CEC).

Numerical methods have been used extensively for classification in biology, for example Sneath and Sokal (1973) and Sokal and Rohlf (1981). In biological taxonomy, present-day relationships often reflect evolutionary descent, and although the development of soils has nothing in common with this, the techniques of numerical taxonomy are potentially able to detect patterns of similarity in soil data. Quantitative and numerical methods for soil classification have been surveyed by Webster (1977).

In reviewing statistical methods for soil classification Arkley (1976) found cluster analysis to be the most effective when the number of distinct soils or soil groups is limited to within a relatively small land area. However, in large areas, similar soils are unlikely to be contiguous, and in these cases a coordinate system, based upon pre-defined centroids, produces a more effective method of classification.

Moore et al (1972) showed that numerical procedures are capable of detecting relationships from data routinely obtained in soil surveying; they used several methods of cluster analysis based on the grouping of soils in relation to geomorphic units. Webster and Burrough (1974) found multiple linear discriminant analysis to be an effective way to distinguish between soil classes based on more than one characteristic; also that a better classification could be obtained using representative soil profiles than could be obtained by the soil surveyor's inspection alone. When classes are defined by several, not necessarily

diagnostic, characteristics, a discriminant analysis provides linear functions (whose minimum number is the number of characteristics being studied, which must be at least one less than the number of populations) that account for decreasing amounts of the variation between class centroids in terms of the generalized (Mahalanobis) distances between them.

The soils of the Lanoraie Delta, Quebec, have been analysed by Lamontagne and Camire (1987) using 18, mainly morphological, soil descriptions. A principal coordinate analysis of these produced the dominant factors governing soil distribution. Although the first two axes represented only 22% of the total variance, Lamontagne and Camire were able to show that the soils were distributed along gradients of the water regime and of soil colour. The soils were then grouped by cluster analysis.

Organic soils are traditionally analysed for many characteristics (Risi et al 1950, Levesque et al 1980, Keys and Henderson 1983, Riley 1987), but there is little agreement on which of these characteristics best define soil type (Mathur and Farnham 1985). Lowe et al (1987) used canonical variate analysis based on 26 organic and inorganic properties to discriminate among 6 upland and wetland soil horizons. The results provide support for the present soil usage of field morphology in the Canadian System of Soil Classification (1978).

In this work I attempt to determine the characteristics that best describe a methane-producing muskeg bog, and I discuss different numerical methods used in the analysis of the data, and the various relationships they show between the organic soil samples.

Figure 4.1 Locations of sampled bogs: A - General; B - Mer Bleue;

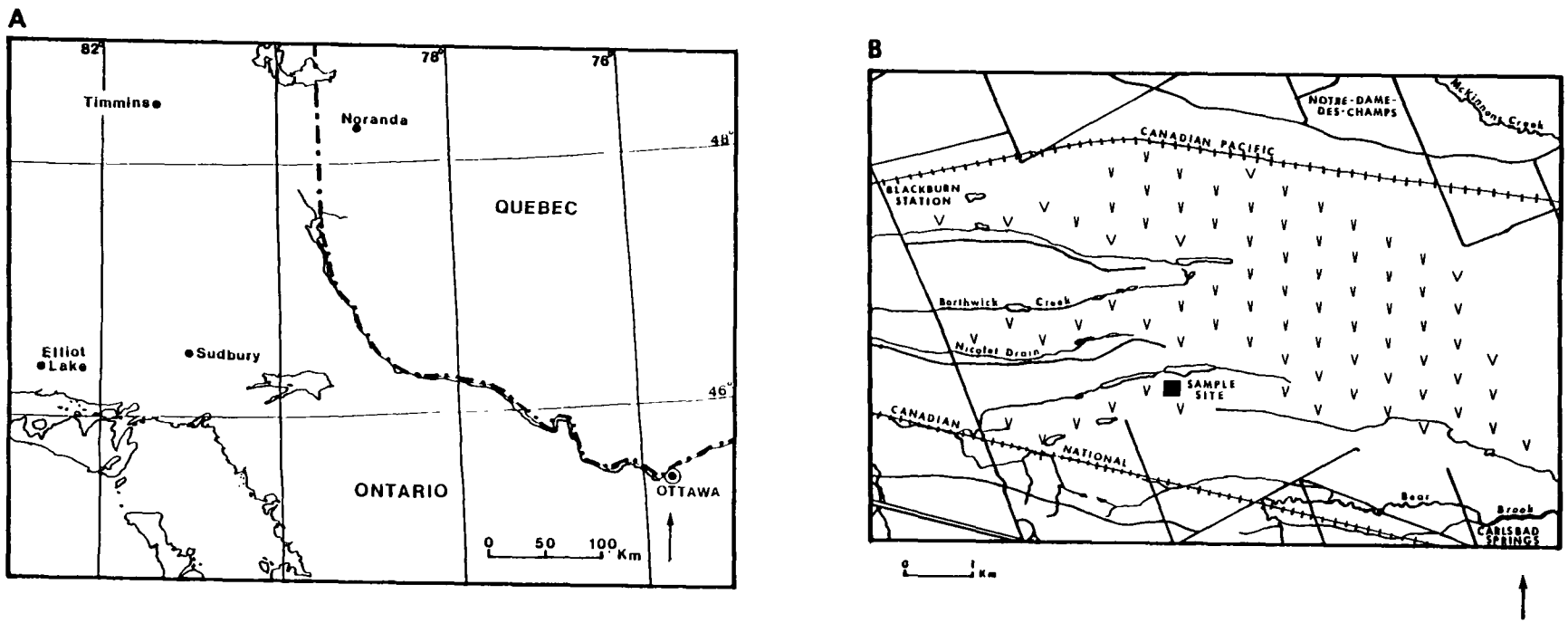
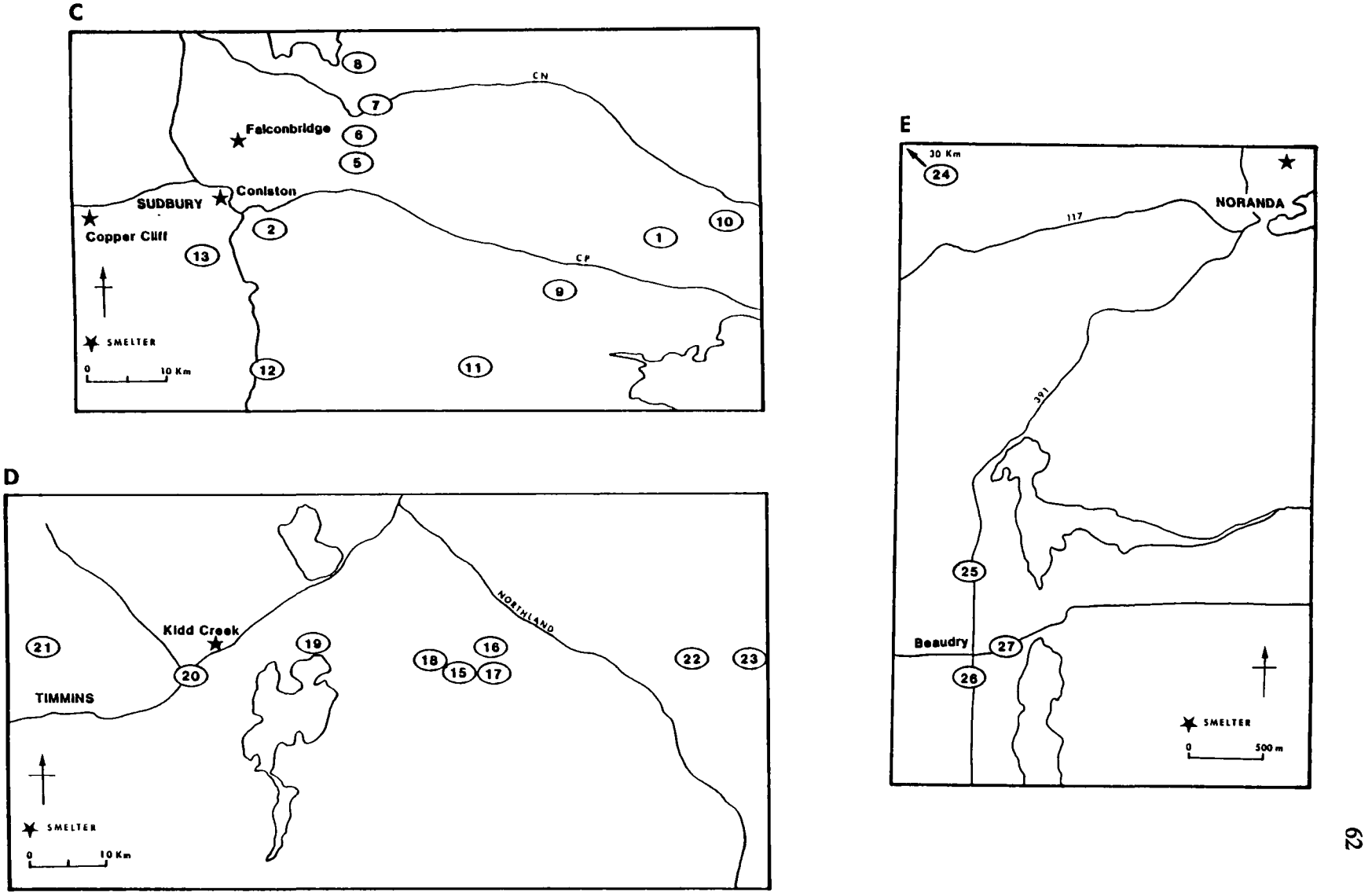


Figure 4.1 Locations of sampled bogs: C - Sudbury; D - Timmins; and E - Noranda.



4.1.2 MATERIALS AND METHODS

Samples were collected at 0 to 50 cm depth from thirty bogs in the mining districts of Elliot Lake (uranium), Sudbury (nickel, copper), Timmins (zinc, copper, lead and gold) and Noranda (copper and gold) and one sample from Mer Bleue, Ottawa (Figure 4.1) in the fall of 1985. Tables 4.IA and B show the designate number of the samples, and the data used for the analyses. The samples numbered from 1, 2 and 5 to 13 were from Sudbury, 15 to 23 from Timmins, 24 to 27 from Noranda, 28 from Mer Bleue, and 29 to 33 from Elliot Lake. I am indebted to Marvin Silver for the samples from Elliot Lake. Descriptions and pictures of some of the bogs are in Appendix 2.

These samples have been assayed for 28 characteristics using standard soil methods (Sheldrick 1984) for laboratory measurements of pH, Eh, cation exchange capacity (CEC) by barium acetate method, water holding capacity (WHC), fibre content (unrubbed and rubbed), and ash. The methods of Mathur and Rayment (1977) were used to measure respiration, that of Kaïla (1956) for the pyrophosphate (PP) index and E4:E6 ratio, and that of Tabatabai & Bremner (1969) for the enzyme phosphatase. Carbon, nitrogen, hydrogen and sulphur were measured with a Perkin Elmer elemental analyser at Agriculture Canada. Metals and other elements were extracted by digestion with a mixture of nitric, perchloric and hydrofluoric acids, and assayed by atomic absorption using certified standards by Agriculture Canada. Cellulose, acid-soluble lignin (ASL), and residue were assayed by the method of Brown et al (1988) (Section 4.2.2). Detailed descriptions of the methods are to be found in Appendix 3, together with a table showing the assay method and the determinant property for each characteristic.

TABLE 4.1. Soil characteristics: A - Physical and organic.

Sample #	pH	Eh mV	Moisture %	Ash %	CEC meq $100g^{-1}$	Pyro-phosphate OD	E4:E6 ratio	Phosph-atase $\mu molg^{-1}h^{-1}$	Unrubbed fibre %	Rubbed fibre %	Respir-ation $\mu gC/day$	Cellu-lose %	ASL %	Residue %	Methane μmol
1	4.49	370	307.7	14.1	166.1	38.4	5.7	5.6	63.3	26.0	14.7	23.5	2.3	74.3	5462
2	4.61	288	141.4	58.1	70.1	44.4	5.0	6.4	60.7	25.3	4.2	20.9	1.6	59.0	-
5	4.10	351	292.8	48.8	40.6	13.9	6.0	8.8	70.0	56.7	42.3	29.4	2.0	42.5	2336
6	4.25	337	300.2	42.2	68.3	44.6	5.2	8.0	54.0	43.3	31.1	20.9	2.4	68.4	265
7	3.54	358	372.3	6.7	112.7	21.4	7.0	8.8	66.7	50.7	29.7	29.4	2.0	66.5	247
8	3.99	370	303.8	11.5	103.4	39.9	7.2	13.0	86.7	60.0	54.8	29.6	2.7	52.9	-
9	3.60	346	659.0	21.9	71.0	10.6	5.5	19.6	82.7	78.7	117.3	37.7	3.3	23.8	-
10	4.12	347	325.7	25.6	100.5	32.1	6.8	13.0	64.7	34.7	31.0	27.2	3.1	64.6	67
11	4.55	328	253.7	49.0	75.7	30.8	7.4	7.2	68.0	31.3	12.8	18.4	2.0	67.6	-
12	4.67	422	233.9	30.9	90.9	65.3	5.9	4.0	64.0	33.3	18.5	22.2	2.7	61.2	39
13	5.24	357	219.8	45.7	83.3	36.6	5.6	7.6	68.7	44.0	8.9	20.0	1.8	64.1	-
15	5.40	321	305.3	11.3	148.3	39.7	6.5	5.4	61.3	28.7	27.2	26.1	2.3	74.5	-
16	4.85	330	310.6	8.9	142.6	11.6	6.0	1.6	63.3	32.0	10.4	26.7	1.2	84.4	187
17	5.64	313	242.7	28.7	126.6	49.5	6.8	1.8	62.0	44.7	7.2	18.2	2.2	71.7	93
18	4.82	336	369.3	8.2	144.7	11.1	5.6	0.8	67.3	38.7	6.9	24.8	1.6	61.6	77
19	5.34	327	159.2	41.7	111.1	51.0	6.6	0.8	63.3	38.0	3.9	21.9	2.0	79.2	363
20	5.48	325	224.8	32.2	149.3	81.1	6.4	2.0	87.3	25.3	10.5	25.1	3.1	69.5	-
21	6.20	309	289.2	10.4	125.2	15.9	6.5	2.4	63.3	54.0	41.0	21.8	1.7	75.7	-
22	3.33	364	379.1	7.4	121.1	81.1	7.5	3.2	77.3	20.7	17.7	26.5	2.9	78.9	32
23	4.87	340	326.0	10.1	123.9	52.9	6.5	0.6	70.7	43.3	24.6	24.4	2.5	78.5	493
24	4.82	346	305.6	11.6	120.9	51.2	7.4	3.4	78.0	40.0	30.2	25.7	2.6	75.6	104
25	4.84	357	222.5	31.4	115.1	85.4	6.6	4.0	50.0	14.7	4.9	19.6	2.6	59.0	221
26	3.26	386	633.0	3.3	98.8	15.4	7.7	3.2	85.3	41.3	49.0	35.7	3.1	47.7	201
27	3.64	388	439.7	7.9	101.5	37.2	7.6	6.6	70.0	35.3	40.7	34.6	2.9	67.9	180
28	3.29	398	678.30	2.68	107.57	16.0	7.2	0.2	68.7	30.7	16.0	38.1	2.9	70.7	864
29	4.15	394	381.91	44.31	64.29	34.1	5.8	6.2	67.3	46.7	13.7	21.4	1.7	69.3	-
30	4.33	389	127.07	72.26	38.33	16.1	8.1	5.6	56.0	22.0	1.4	15.7	3.0	64.5	-
31	4.47	392	16.09	55.22	84.19	137.9	4.4	1.0	31.3	8.7	2.0	15.6	2.8	67.9	-
32	3.85	377	1033.81	5.23	85.14	9.4	5.1	19.4	82.0	78.0	77.3	46.7	5.2	27.5	-
33	4.42	389	85.62	83.26	21.13	9.8	6.8	1.2	48.7	41.3	0.9	14.9	1.1	78.5	-

TABLE 4.1. Soil characteristics: B - Inorganic.

Sample #	Ca $\mu g\ g^{-1}$	Mg $\mu g\ g^{-1}$	K $\mu g\ g^{-1}$	Al $\mu g\ g^{-1}$	Fe $\mu g\ g^{-1}$	Mn $\mu g\ g^{-1}$	Cu $\mu g\ g^{-1}$	Ni $\mu g\ g^{-1}$	Pb $\mu g\ g^{-1}$	Zn $\mu g\ g^{-1}$	C $\mu g\ g^{-1}$	N $\mu g\ g^{-1}$	H $\mu g\ g^{-1}$	S $\mu g\ g^{-1}$
1	736	2986	683	3408	4336	175	27	29	24	34	50.6	1.9	4.6	0.17
2	7243	4107	11961	70307	44378	146	849	943	117	96	55.6	2.0	2.5	0.12
5	5090	2489	12591	41015	17407	157	163	189	64	57	50.4	1.2	3.1	0.23
6	4422	707	3037	17124	5931	52	88	108	22	26	49.3	2.2	3.0	0.29
7	3827	688	710	2419	3371	38	149	176	42	49	50.5	1.6	4.7	0.24
8	4863	958	1108	4798	17241	41	305	302	209	42	53.5	1.5	5.0	0.50
9	3652	546	2832	9919	2526	54	53	63	56	40	60.8	0.1	4.4	0.20
10	9860	1334	1776	10891	5479	67	53	42	49	69	50.8	2.4	3.3	0.48
11	9558	3497	9285	39056	18413	177	52	54	22	62	52.7	1.2	2.4	0.27
12	7304	1354	4460	34778	12794	62	249	288	36	107	58.5	1.9	2.4	0.49
13	11214	3359	11772	43929	18368	113	570	711	70	68	54.7	1.5	2.7	0.42
15	21965	1891	527	4214	3966	247	25	12	15	30	53.3	1.5	3.6	0.16
16	15047	1894	393	2418	1698	54	21	10	12	18	53.5	1.0	3.7	0.10
17	18762	2396	7020	17092	5868	209	19	10	15	33	53.7	1.3	3.3	0.16
18	15085	1727	382	1912	1504	91	12	10	7	13	54.1	1.2	4.2	0.16
19	18320	2256	10108	26167	6888	116	21	15	17	73	56.2	1.0	2.6	0.13
20	21507	3258	6371	24065	11408	425	29	44	22	50	50.9	1.2	3.1	0.15
21	18105	2254	486	2142	7471	1649	17	10	20	67	54.2	0.6	4.1	0.10
22	2394	403	800	5770	2222	20	18	12	5	24	53.5	1.6	5.0	0.14
23	11485	1387	612	6380	5175	332	24	17	10	24	52.6	1.7	4.5	0.25
24	13716	1311	1163	6561	5692	769	32	10	24	84	52.2	1.2	4.2	0.23
25	8792	1800	11348	40194	9129	79	36	27	19	46	50.9	1.1	2.5	0.16
26	1226	270	438	1576	951	21	32	10	7	41	48.4	1.7	5.2	0.15
27	1713	365	727	7567	2856	36	52	15	10	33	59.5	2.2	5.4	0.16
28	1319	808	276	1136	555	10	14	5	0	28	49.2	1.4	4.9	0.10
29	6964	1655	11648	29868	19863	902	41	25	29	84	62.7	1.9	3.4	0.62
30	5403	3710	15680	49079	107831	598	63	39	178	71	47.6	0.2	1.3	0.26
31	2511	632	10064	75711	9270	55	55	27	30	116	52.7	1.0	2.3	0.15
32	3625	868	2525	1633	3965	119	28	10	36	57	50.2	0.1	4.4	0.13
33	4738	1718	19416	65252	19199	161	21	15	29	48	73.5	0.1	1.1	0.05

Methane was extracted with specially constructed samplers (Section 4.4.2 and 4.4.3), and measured either on a 5720A Hewlett-Packard gas chromatograph, or on a PM-2 series Analytical Development Companies battery powered portable infrared gas analyser. Methane was not sampled in the field until late summer 1988 as it took several years to develop an acceptable method. The methane extractions are shown in Appendix 9.

4.1.3 STATISTICAL METHODS

I used the Statistical Package for the Social Sciences (SPSS version 8.0, 1979) for cluster analysis, and the SAS Institute Inc. package (1984, 1986) for principal component and canonical discriminant analysis. The method of Lefkovitch (1976, 1987) was used for hierarchical clustering. Methods for statistical analyses of the results are discussed in order of increasing complexity.

4.1.3.1 Probability Graphs

These graphs, described by Sinclair (1981), are plotted on cumulative frequency probability paper, which has the percentage probability for a Gaussian distribution along one axis, and either an arithmetic or logarithmic scale for the cumulative frequency of the variable on the other. The nature of the probability scale means that the cumulative figures for a normally distributed population will plot as a straight line, which is an effective method of estimating the mean, the standard deviation, and the degree of fit to such a distribution for the different variables. It is important to note that many statistical procedures are valid only for normally distributed populations. In this work the characteristics with normal frequency distributions are rubbed and unrubbed fibre, E4:E6 ratio, ash, CEC, ASL, residue, nitrogen and hydrogen, all the rest are lognormally distributed, and the data in these cases have been transformed where necessary in the calculations.

4.1.3.2 Bivariate Relationships

The relationship between two variables can be represented by graphing pairs of values for each soil sample on mutually perpendicular axes; the resulting plot is called a scattergram. When there is no correlation between the two variables, the points will be randomly scattered, and, assuming sufficient points (and normal distributions at the same representative scale) the scattergram will be roughly circular. The resulting pattern becomes increasingly elliptical with increasing correlation (Sokal and Rohlf 1981).

4.1.3.3. Canonical Variate Analysis

If the means of two characteristics are different but their distributions overlap it is impossible to differentiate the samples using just the one characteristic. However, between populations, possibility of separation with several variables becomes more likely. When there are two or more groups of individuals, which have several characters measured on every member, they may be envisaged as ellipsoids in character space, one ellipsoid for each group. The distance between points, based on the possibly correlated variates, is known as their Mahalanobis distance, when allowance is made for their correlation.

4.1.3.4 Ordination by Principal Component Analysis

The objective of ordination by principal components is the representation of the data points from a multidimensional scattergram on the principal axes of the ellipsoid formed by them. The principal axes are defined by choosing the next direction in space having maximum variance subject to the condition that it is perpendicular to all those previously chosen. The total number of axes is equal to the number of variables, and the axes are ranked for ordination from large to small. The first few axes generally represent a large

portion of the total variance and the justification for discarding the rest is that they represent random variation. The coordinates of each individual projected on to the chosen axes represents the population scatter on the plane (for two axes) of the data.

4.1.3.5 Cluster Analysis

Cluster analysis, routinely used in biological taxonomy, can also be suitable for the classification of soils. In the nearest neighbour strategy clusters are formed by amalgamating the two most proximate clusters until all cases are assimilated into one. The history of the joining process is then represented by a dendrogram or cluster triangle. A dendrogram can also be obtained directly from projections of the data on the components, that is, the principal coordinate matrix, based on the signs of the elements (Lefkovitch 1976, 1987).

4.1.4 DISCUSSION OF RESULTS

4.1.4.1 Probability Graphs

The frequency distributions of each characteristic have been determined by cumulative probability graphs, those not discussed here will be found in Appendix 4. A normal distribution is shown for CEC values (Figure 4.2A) and a lognormal probability graph for zinc (Figure 4.2.B). In some of the probability plots more than one population can be recognized; these can be distinguished by an inflection in the graph. Both copper and nickel demonstrate this clearly. The copper values shown in Figure 4.3, have two distinct populations; one, which contains samples from all areas and where the metal concentration is below 70 μg ml^{-1}, and another above the inflection point (arrow), which is a second population with a much higher concentration of copper, all of whose members come from Sudbury. This latter population is from bogs close to the smelting centres by which they apparently have been contaminated; as can be seen from the location numbers of the

sampled bogs (Figure 4.1C).

Some of the cumulative probability plots of other characteristics also suggest two populations (as shown in the secondary populations in Figure 4.4A, B and C), but in many

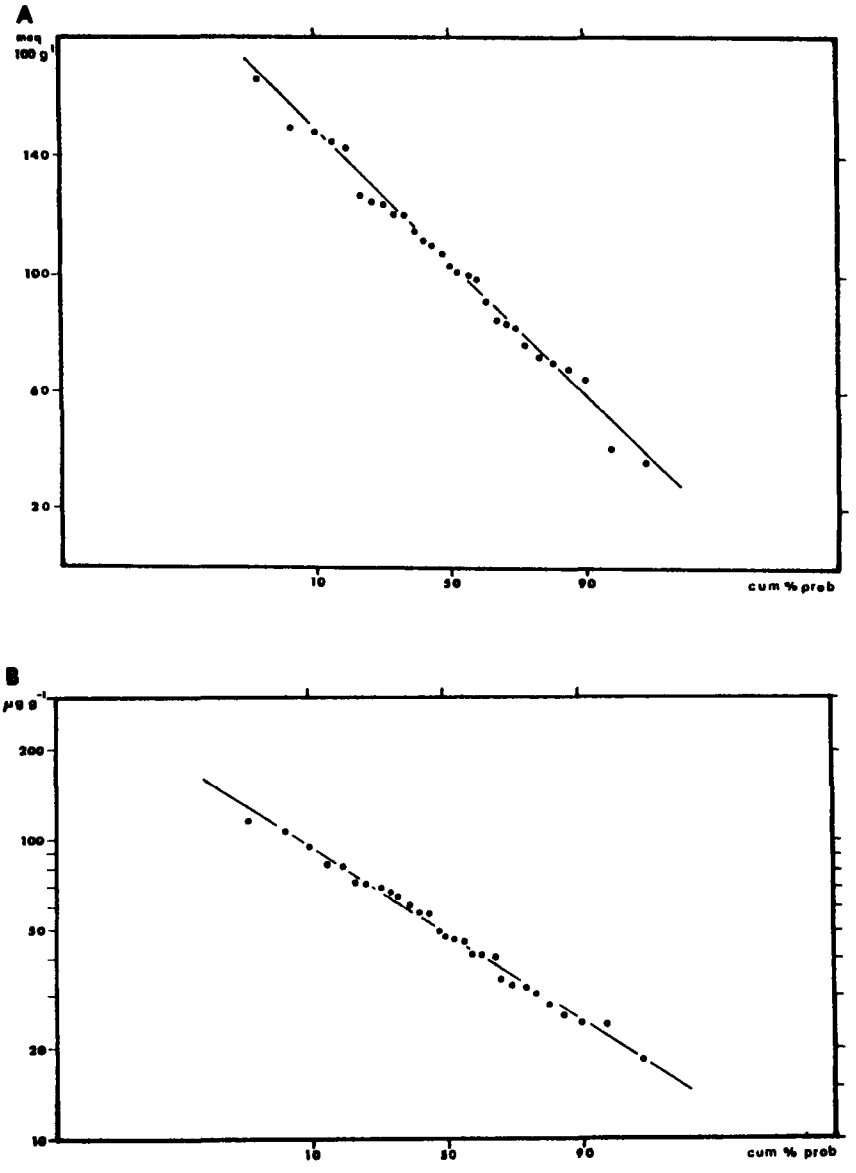


Figure 4.2 Cumulative percentage probability plots from 30 bogs showing: A - CEC with normal distribution; and B - Zinc with lognormal distribution.

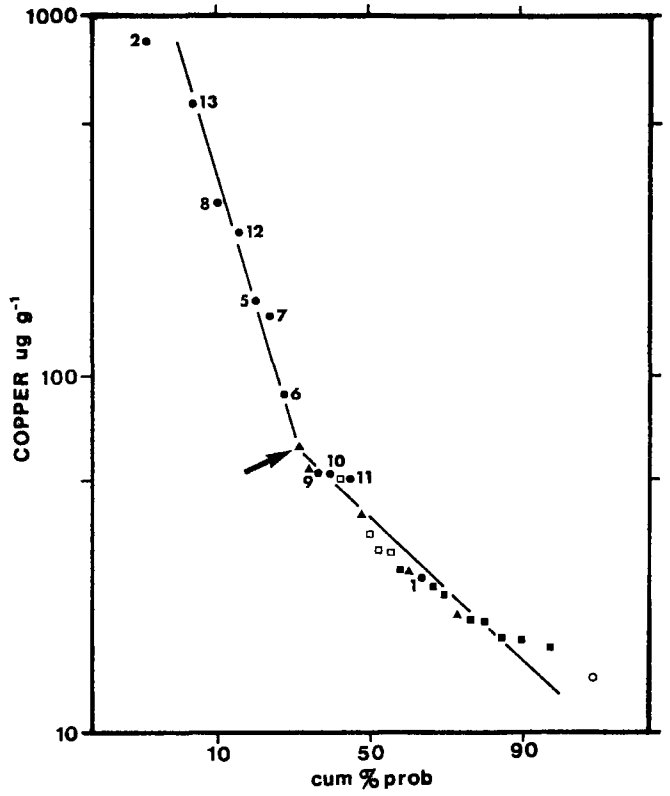


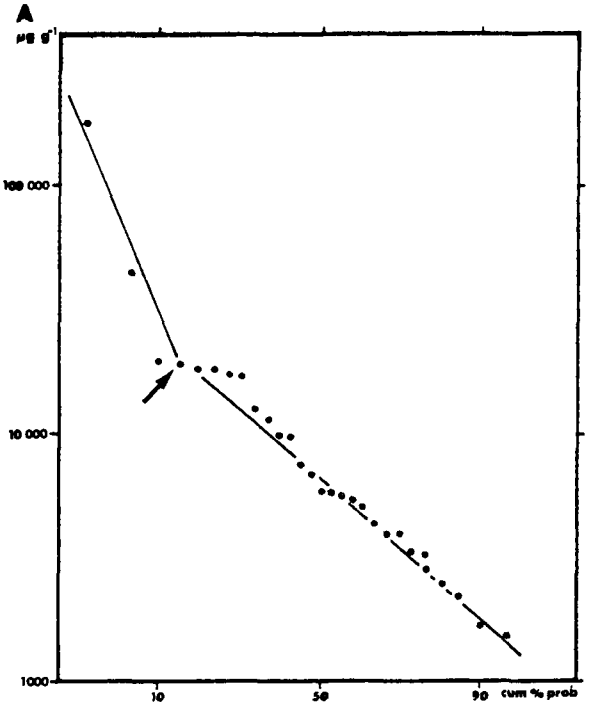
Figure 4.3 Cumulative percentage probability plot of 30 copper analyses showing smelter contamination above arrow:

● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.

cases the second one is small, often only three or four samples; and most are the same for several characteristics. This is the case for samples 2 and 13 from Sudbury, which are heavily contaminated with most metals, and also samples 30, 31, 33 (and for some elements sample 29) from Elliot Lake. It is possible these are really outliers. The cumulative percentage probability plot for iron (Figure 4.4A) shows this type of population. Samples 5, 19 and 25, which are high in both aluminum and potassium are possibly contaminated with clay, probably from periodic flooding.

Figure 4.4 Cumulative probability plots from 30 bogs of analyses of:

A - Iron; B - Calcium;



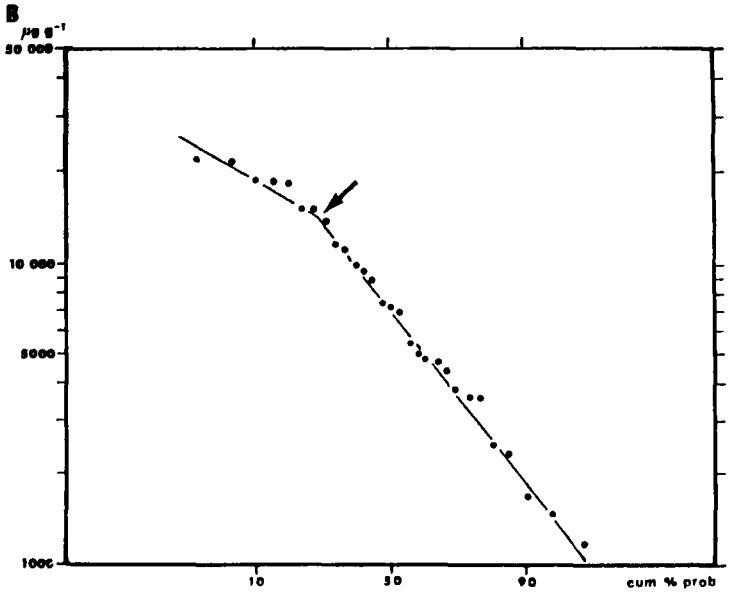
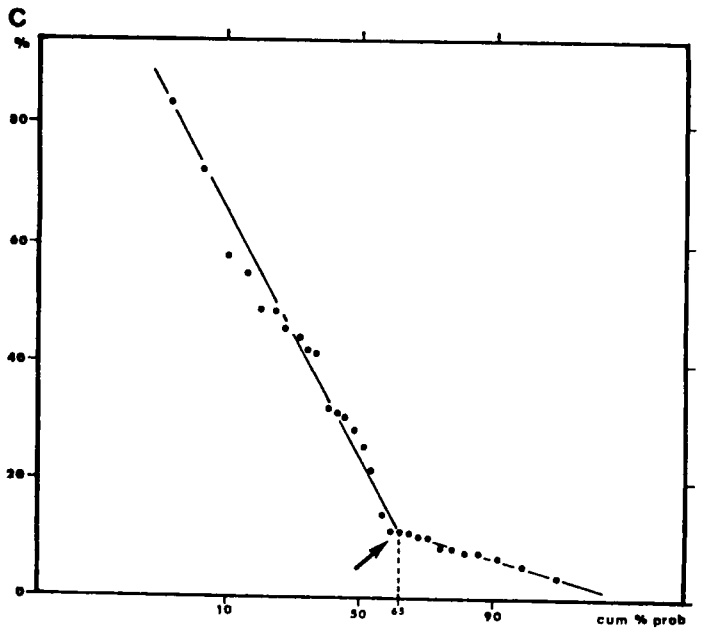
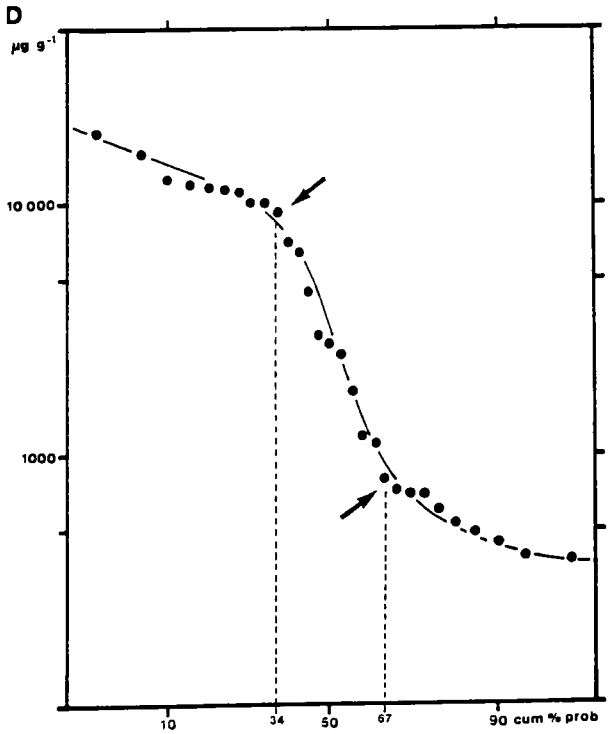


Figure 4.4 Cumulative probability plots from 30 bogs of analyses of: C - Ash; and D - Potassium.





All the Timmins samples (except sample 22 which is from an area high in carbonaceous sediments) are rich in calcium, probably derived from the underlying ultramafic and mafic volcanics and intrusives characteristic of this area. These Timmins samples, together with sample 24 which is on a similar formation and is the nearest Noranda sample, form a second geologically defined population for calcium (Figure 4.4B).

Probability graphs for ash, potassium and phosphatase all suggest two populations, and the percentage of the total for each population can be calculated (Sinclair 1981). For

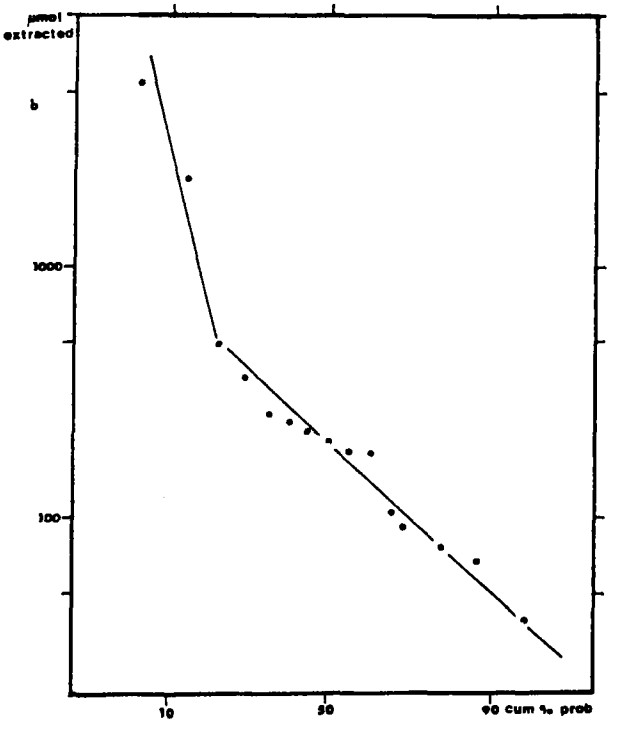


Figure 4.5 Cumulative percentage probability plot of extracted methane from 16 bogs.

instance a high ash content (>12%) is found in 60% of the population (Figure 4.4C), while the potassium concentration shows a typical S-shaped curve for two populations which overlap, with 34% of the total having a concentration greater than 10,000 $\mu g\ g^{-1}$, and 33% with less than 1000 $\mu g\ g^{-1}$ (Figure 4.4D).

The methane cumulative probability plot (Figure 4.5) has a distinct inflection, which could imply two populations, but is more likely to be caused by insufficient data points at the higher values.

4.1.4.2 Bivariate Relationships

In this study some of the measured characteristics show no significant bivariate correlation when the data are treated as a single population, but do show association when the values are grouped by geographical area. Five scatterplot matrices are shown in Appendix 5, where the bivariate correlations can be studied in more detail. The scattergram for CEC against WHC (Figure 4.6A) shows the samples clustering into the geographical areas of Sudbury and Elliot Lake and Timmins, while Noranda is separated into two groups.

Several variables exhibit good linear bivariate relationships. In the group of economic metals, copper and nickel (Figure 4.6B) are well correlated (Pearson coefficient r=0.9540), but lead and zinc (not shown) are less well correlated (r=0.3076). A good correlation also exists between aluminum and potassium (r=0.9248) (Figure 4.7A), and between the sum of these metals and ash (r=0.9100) (Figure 4.7B). The bogs high in aluminum (and potassium) may thus have had clay introduced during flooding, and in many cases the high aluminum correlates with high iron. The bogs contaminated by economic metals are also high in ash, which is particularly evident in those samples taken near the smelters in Sudbury (2, 5, 6, 11, 12 and 13), while the ash component in Elliot Lake samples

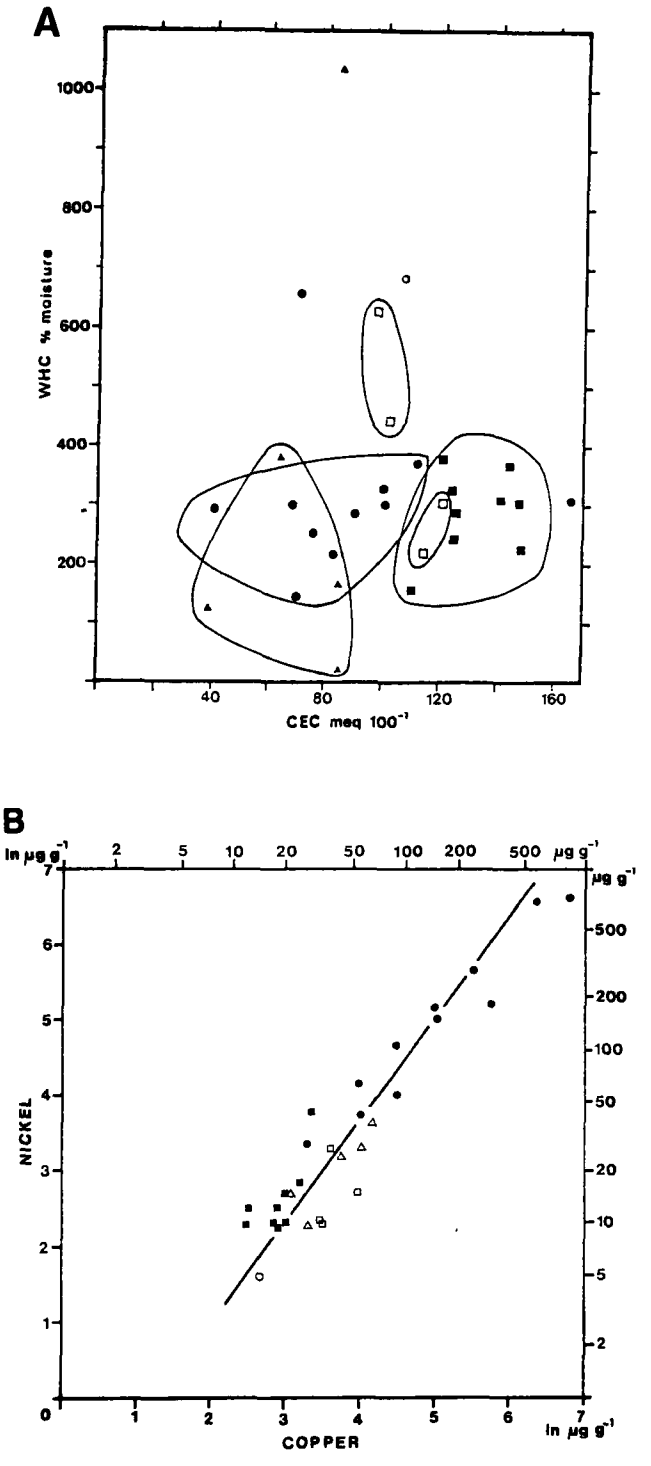


Figure 4.6 Bivariate correlation between: A - WHC and CEC; and B - Copper and Nickel.

● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.

(29, 30, 31, 33) appears to be due to contaminating clay elements.

The relationship between calcium and magnesium for all samples has a correlation coefficient of only r=0.5774, but, when the samples are divided into two geographical groups, considerably better correlation values are obtained. The Timmins/Noranda area then has a correlation of r=0.9720 (Figure 4.8A), and the Sudbury/Elliot Lake area one of r=0.7117 (without sample 1, an anomalous bog type) (Figure 4.8B). There is also a good correlation between the sum of the concentrations of calcium and magnesium (the two alkaline earth metals) and pH (r=0.8714), showing that pH in these bogs is largely controlled by the availability of these metals (Figure 4.8C). This is particularly so at Timmins, where both the alkaline earth metal concentration and pH is higher than in the other mining districts (except for sample 22 which is underlain by a different rock type). The mining districts are all characterized by distinctive pH values, which decrease from Timmins (calcium-rich) through Sudbury to Elliot Lake and Noranda (two groups) to Mer Bleue (the most typical ombrotrophic bog).

The major index of an organic soil is the content of organic matter, which can be estimated from the total carbon, but all soil organic matter is not the same. The major part of the peat biomass is derived from plants, which are composed of three polymers, cellulose, hemicellulose and lignin (Brown 1985). The two carbohydrate polymers are degraded biologically (the hemicellulose at an early stage), both aerobically and anaerobically, but lignin is biologically difficult to degrade aerobically, and, as far as is known, impossible to degrade anaerobically (Kirk and Farrell 1987); lignin is, however, altered chemically in soil and, over time, loses oxygen and hydrogen.

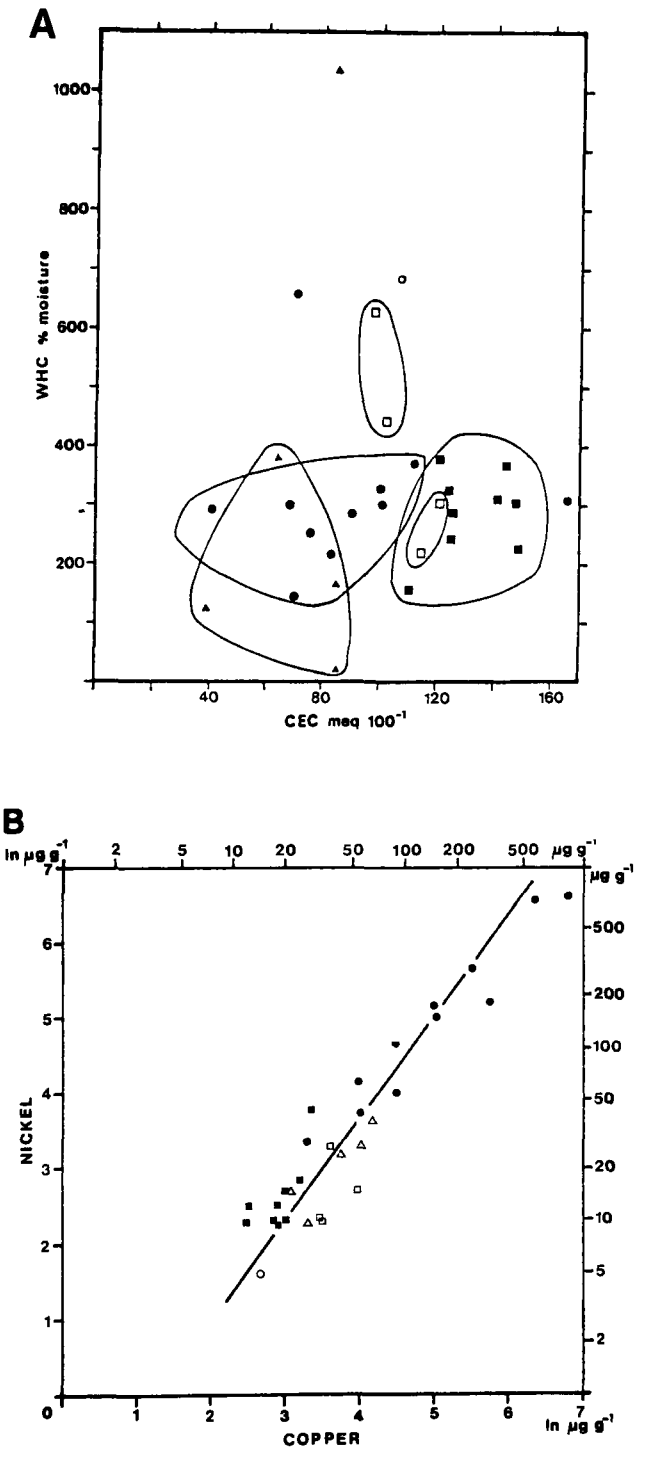


Figure 4.6 Bivariate correlation between: A - WHC and CEC; and B - Copper and Nickel.

● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.

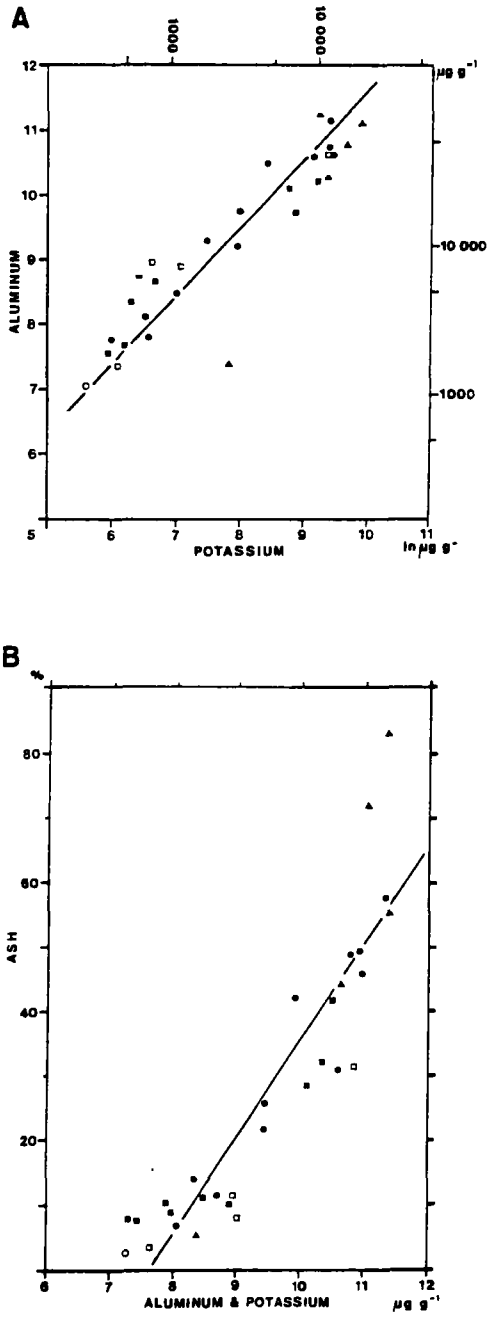


Figure 4.7 Bivariate correlation between: A - Aluminum and potassium; and B - Sum of aluminum and potassium against ash.

● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.

(29, 30, 31, 33) appears to be due to contaminating clay elements.

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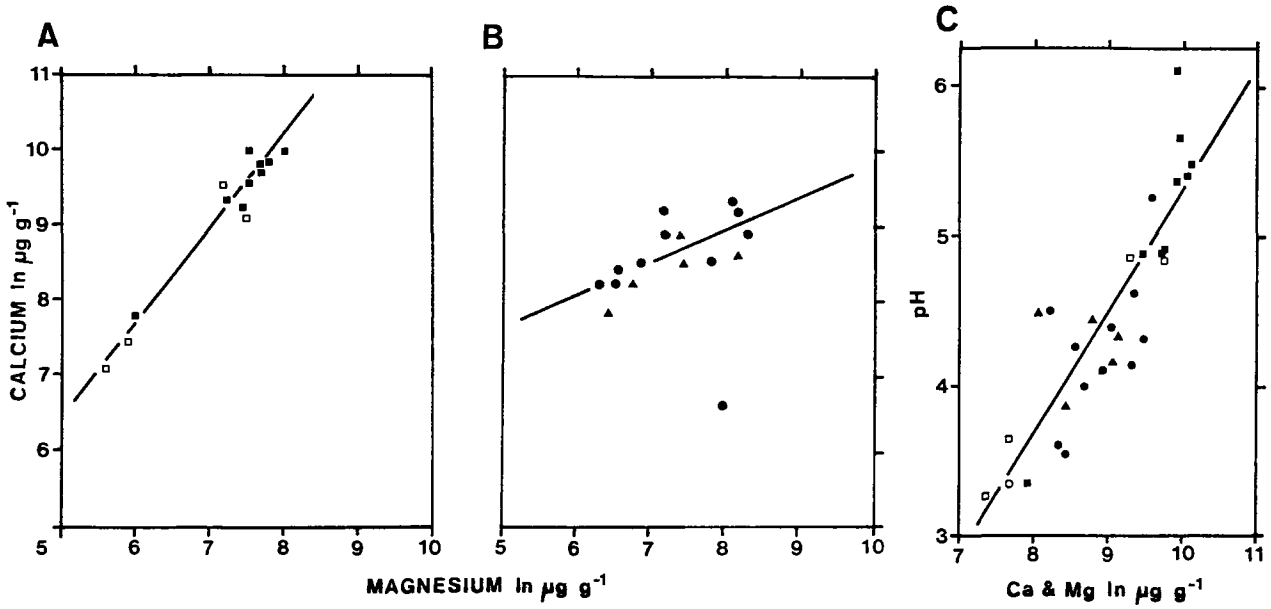
Figure 4.8 Correlation between calcium and magnesium: A - Timmins/Noranda; B - Sudbury/Elliot Lake; and C - correlation between sum of calcium and magnesium against pH in all areas.
● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.



Traditionally, organic soils have been classified by their potential for biodegradation. This potential can be measured either by aerobic respiration, which is slow, or indirectly by the more subjective test of rubbed fibre content. I have recently shown that cellulose content correlates with these two parameters (Section 4.2). Within the group of organic characteristics, rubbed and unrubbed fibre values show a good correlation for all areas except that of Timmins. The carbohydrate component of the peat, as measured either by respiration, by rubbed fibre or by cellulose, is not correlated with carbon (whose values in all samples are too similar to be well differentiated), but it is correlated with total hydrogen. Cellulose also correlates well with WHC, but residue and hydrogen do not.

A substantial part of peat is humus, a recalcitrant, aromatic amorphous component derived mainly from plant lignin, but, since humus is intractable, measurement is difficult. Traditionally the PP index and the E4:E6 ratio are used as measures of this humus content (Kaïla 1956). However the pyrophosphate does not specifically dissolve all humus, and the E4:E6 ratio has been found to be mainly governed by the particle size and not directly related to the concentration of the condensed aromatic rings of humus (Chen et al. 1977). It is not therefore surprising that there is little correlation between these two measurements and either the ASL, or the residue from the cellulose extraction which is the acid-insoluble, and thus the non-cellulosic, component of the peat.

Some other characteristics are more revealing, particularly the CEC which is principally a measure of the dissociable hydroxyl groups that are the site for many reactions, whether associated with the cellulose or with the lignin. This parameter exhibits good geographical grouping, albeit overlapping, when plotted against WHC (Figure 4.6A), cellulose, residue, and pH (data not shown).

4.1.4.3 Canonical Variate Analysis

The SAS Institute program for canonical variate analysis was used to process 25 characteristics, and showed a tight and well-separated grouping into four geographical areas (Figure 4.9A). The Mahalanobis distances between these groups are shown in Table 4.2, where it can be seen that the two closest groups are Noranda and Timmins, followed by Noranda and Elliot Lake, while Sudbury is the most distant.

Table 4.2 Mahalanobis distances between classes in canonical variate analysis with 25 variables.

Region	ELLIOT L.	NORANDA	SUDBURY	TIMMINS
ELLIOT L.	*			
NORANDA	18.51	*		
SUDBURY	48.35	42.00	*	
TIMMINS	27.16	12.61	33.05	*

Various combinations of the different characteristics were tried to determine whether acceptable separation could be obtained using fewer characteristics. It did not seem important which variables were chosen, but, with fewer variables involved in the analysis, the Mahalanobis distance between the groups was reduced until, with only three characteristics, calcium, nickel and CEC, three geographical groups were obtained: Sudbury, Timmins and Elliot Lake, with Noranda divided between the last two (Figure 4.9B). This separation is rather better than that produced by just two characteristics, CEC and WHC (Figure 4.6A). It is suggested that the calcium content indirectly separates the bogs by pH, the CEC determines the reactivity of the peat, while the nickel segregates those bogs which have been contaminated by smelter metals (here, exclusively those of the Sudbury area).

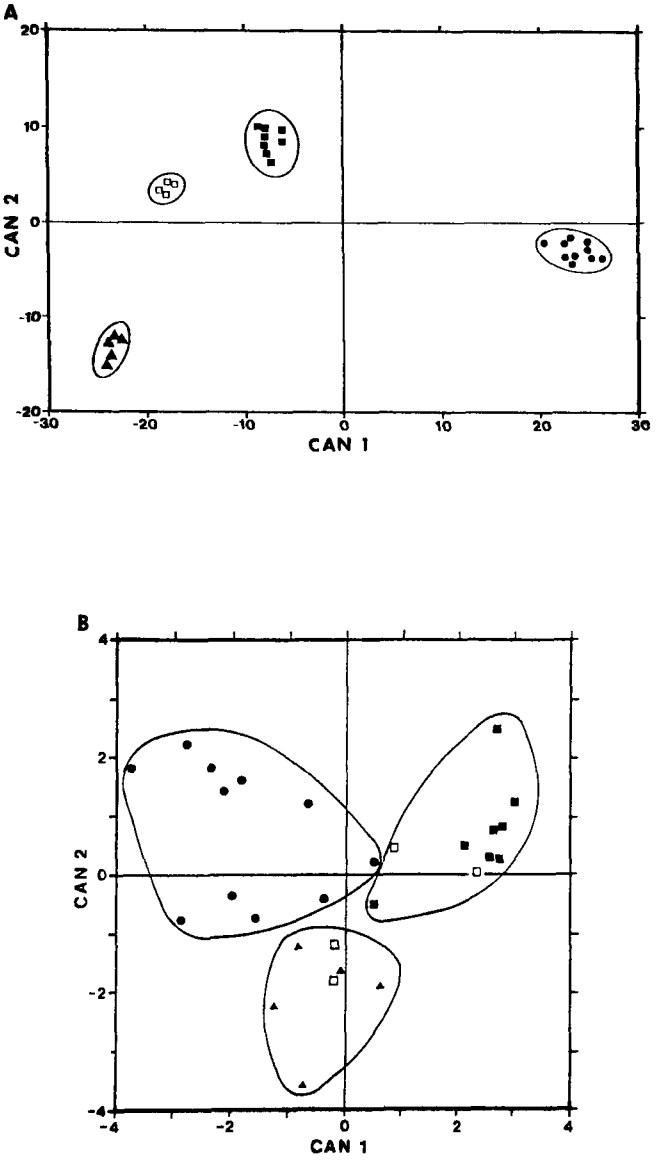


Figure 4.9 Canonical variate analysis of 30 bogs:

A - With 25 variables; and B - With 3 variables

● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.

Of all the methods employed, only the canonical discriminant analysis definitively separated the samples into distinct geographical areas, and this separation was better with the greater number of variables included in the analysis.

4.1.4.4 Principal Component Analysis

Multivariate correlation matrices were explored by principal component analysis. When the 28 variables measured from the 30 soil sites were analysed by this method, the largest eigenvalue of the correlation matrix was found to account for a third of the total variance, and, when the second component was added, over half the variance was accounted for. Table 4.3 shows the principal components of the different variates, and from this it is possible to give a tentative interpretation of the axes from the variables with the highest loadings.

The first principal axis is related in one direction to inorganic components of ash, aluminum, iron, and potassium, probably introduced into the bogs by flooding with mineral soil water; and in the other, it is associated with the carbohydrate component as represented by WHC, hydrogen, cellulose and respiration vectors. The second principal axis is related in one direction to metal contamination, with high weightings for copper, lead and nickel, but this group is not the exclusive source, since phosphatase and respiration are also represented; and it is associated in the opposite direction with the humus component, as represented by residue, CEC, and pH. Since many of the characteristics in this study are reported as percentages the logit transformation was used to spread them into approximate normality; however, this did not significantly alter the results.

Table 4.3 First three vectors for correlation matrix for 28 variables from 30 bogs.

	PRIN 1	PRIN 2	PRIN 3
Variance	32.8%	19.3%	10.8%
Cumulative	32.8%	52.1%	62.9%
pH	0.176353	-0.207431	0.260877
Eh	-0.057440	0.121524	-0.281021
WHC	-0.301076	0.088690	0.057744
Unrub. fibre	-0.195642	0.121653	0.161578
Rub. fibre	-0.126863	0.240475	0.329226
PP	0.107908	-0.095537	-0.298255
E4:E6	-0.065040	-0.082059	-0.063914
CEC	-0.137830	-0.274083	0.032241
Phosphatase	-0.044272	0.353380	0.031140
Respiration	-0.200191	0.261157	0.204492
Cellulose	-0.285140	0.149072	0.085887
ASL	-0.161898	0.169392	0.015488
Residue	0.096261	-0.345108	-0.144627
Ash	0.293369	0.092823	0.021916
C	0.096571	0.027712	0.054434
S	-0.031441	0.162367	-0.221165
N	-0.038427	-0.035066	-0.390794
H	-0.299402	-0.016398	-0.029660
Ca	0.142594	-0.135234	0.313314
Mg	0.231517	-0.072596	0.221171
K	0.269319	0.156716	0.013278
Fe	0.283172	0.153455	0.045879
Al	0.291497	0.103573	-0.102273
Cu	0.122997	0.296202	-0.167589
Pb	0.175954	0.291217	0.099380
Zn	0.181734	0.190811	-0.133421
Ni	0.143898	0.274862	-0.134004
Mn	0.167186	-0.059089	0.340714

The sample sites are projected on to the plan of the first two principal components in Figure 4.10A, where the samples from the same geographical areas are outlined. The samples in the top right quadrant of the graph are contaminated most heavily with both metal and clay elements; samples in the top left quadrant are contaminated with metals only and not with clay; while those in the bottom right quadrant are only contaminated with clay;

finally, samples in the bottom left quadrant are those with the least inorganic contamination and with the highest organic content of both carbohydrate and humus.

Many of the known differences within the geographic areas are reflected in the principal component analysis. The two sphagnum bogs (9, 32), are high in cellulose and low in humus, as expected from bryophyte-based bogs. They are also contaminated with metals but not by clays, showing these bogs are not regularly flooded. The overlap of the Sudbury and Elliot Lake samples, and their fairly low carbohydrate content, indicate that many of them are contaminated by clay elements, suggesting flooding by oxygenated, alluvium-laden, water. Noranda samples are spread along a line showing a moderate variation in carbohydrate content and clay element contamination, but a similarity in metal contamination and humus content. Sample 1 from Sudbury is anomalous, in that it has much less contamination and greater organic content than the other Sudbury samples. All the Timmins samples have a relatively high humus content, and little metal contamination, but sample 22 shows much less inorganic contamination than the other Timmins samples, consistent with its much lower concentration of calcium and magnesium. Mer Bleue, as expected for an ombrotrophic bog distant from mining centres, has the least inorganic contamination and the highest organic content for both carbohydrate and humus.

When the sample sites are plotted in an isometric projection (Figure 4.10B) using the first three principal components, slightly different groupings are seen. It is difficult, though, to identify any particular aspect of the bogs from the third component which plots for manganese, rubbed fibre and calcium in the upwards direction and nitrogen and pyrophosphate index in the downwards. Sample 21 (presumably due to the high manganese and rubbed fibre content) joins the two sphagnum bogs at the top of the graph; on the right-

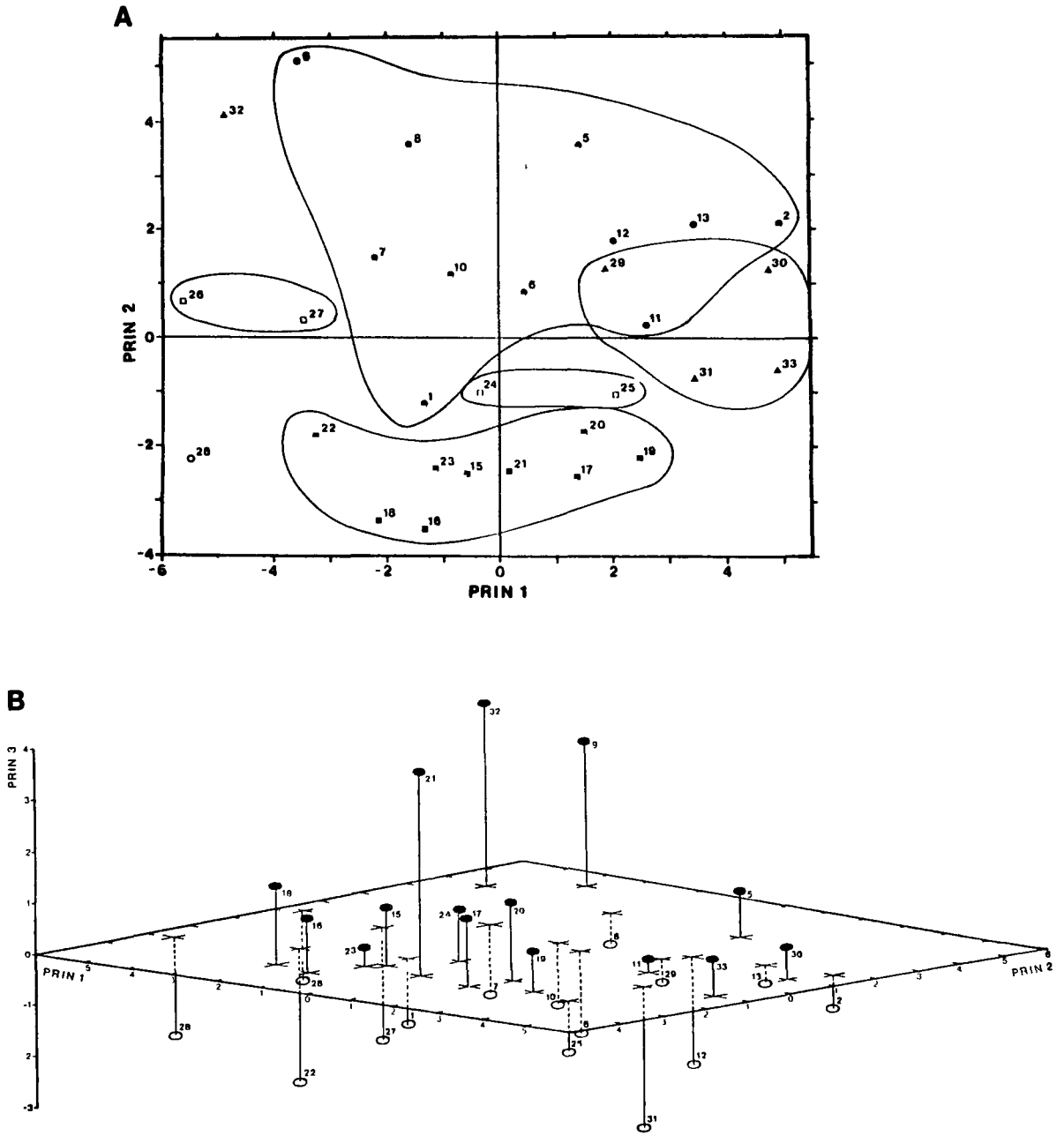


Figure 4.10 Principal component analysis of the 30 bogs projected on to:

A - the plane of the first two principal components grouped into geographical areas.

B - an isometric view of the first three principal components

● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.

hand side there is a fairly wide group, spanning the plane of the graph, which contains those bogs most heavily contaminated with clay minerals (2, 5, 11, 12, 13, 29, 30, 31 and 33); in the centre of the graph there is a group, below the plane, of Sudbury samples (6, 7, 8, 10) with 25 from Noranda; and above it of Timmins bogs (15, 16, 17, 18, 19, 20, 23) and 24 from Noranda; the final group is at the left of the graph where sample 1 from Sudbury joins the four most acidic bogs of cluster C of the nearest-neighbour cluster analysis. Although this grouping further splits the geographical areas it does not appear to give any better division between the sampled bogs.

4.1.4.5 Cluster Analysis

The data were separated into an organic, inorganic, and all-variables groups, before being examined by single linkage cluster analysis. Distances computed for the all-variables group are illustrated as a cluster triangle (Figure 4.11A). This shows that the two most extensively sampled areas Timmins (9 samples) and Sudbury (11 samples), labelled A and B respectively, have internally strong affinities. The largest cluster, A1/A2, contains eight out of the nine Timmmins samples, while cluster B contains eight of the Sudbury bogs and no others. Three Sudbury samples (1, 9, 11) and one Timmins sample (22) plot outside their major geographical clusters. The four Noranda samples are split into two clusters, one (24, 25) plots with Timmins in A1, and the other, also containing Mer Bleue (26, 27, 28) forms, with 22 from Timmins, the ill-defined cluster C comprising the four most acidic bogs. Elliot Lake is heterogeneous: one sample (29) plots with the Timmins cluster in A1, while the rest form the poorly represented cluster D, which can be divided in two: three clay contaminated samples (30, 31, 33), and two sphagnum samples (9, 32).

The inorganic metal variables give the most distinct grouping (Appendix 6), and the analysis again separates two major clusters, cluster A1/A2, which contains eight out of the nine Timmins samples, and cluster B1/B2, which is composed of nine Sudbury samples. The same type of clustering is exhibited when the organic variables alone are plotted (data not shown), but while the geographic affinity is still apparent, the clusters are much less area specific, but when carbon is omitted, clustering into geographical areas is again much stronger.

Cluster analysis appears to differentiate these soils generally into geographic locations, but when the clusters are superimposed onto the principal component analysis (Figure 4.11B), it is clear why samples 1, 11, 24, 25 and 29 are placed in cluster A1 with the Timmins samples. Clusters B and C form clear groups on the principal component graph, while cluster D, which is poorly represented, is divided into two: the sphagnum samples, and the clay-contaminated Elliot Lake samples.

Cluster analysis based on principal coordinates divides the bogs into four clusters, which is shown in the dendrogram in Figure 4.12A. There are two main divisions, cluster I which contains all the most highly contaminated bogs, which can be grouped in one main cluster R, containing four smelter-contaminated Sudbury bogs, three from Timmins, again near the smelter, 29 from Elliot Lake, and 25 from Noranda; and the remaining four bogs (2, 30, 31, 33) which are less closely associated, and cannot be said to form a cluster. Cluster II contains the least contaminated bogs, and can be divided into three: group S which contains the two sphagnum bogs (9, 32) and the four most acid ones (22, 26, 27, 28); group T comprising five of the Timmins bogs and 24 from Noranda; and group U containing the five least contaminated Sudbury bogs.

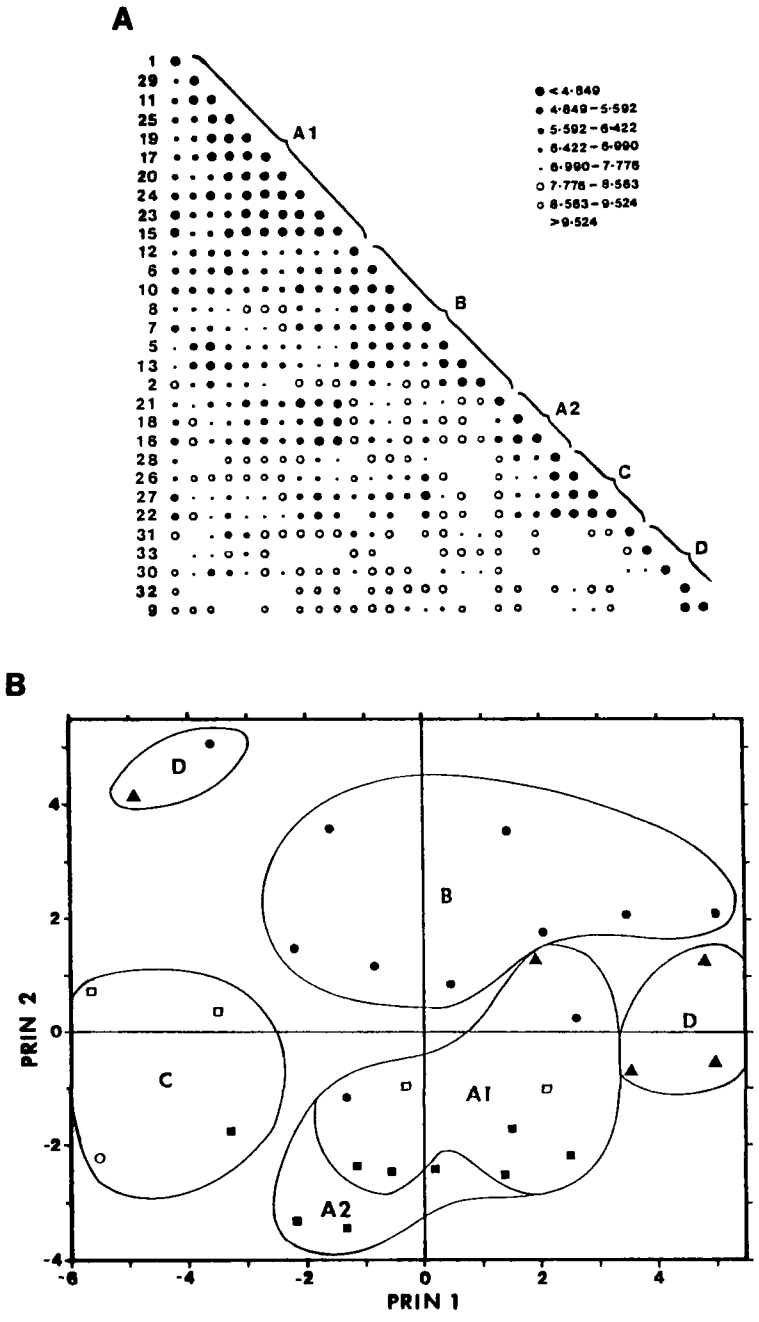


Figure 4.11 Nearest-neighbour clustering: A - Cluster triangle of all variables of 30 bogs; and B - Clusters from A superimposed on principal components from Figure 4.10.

● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.

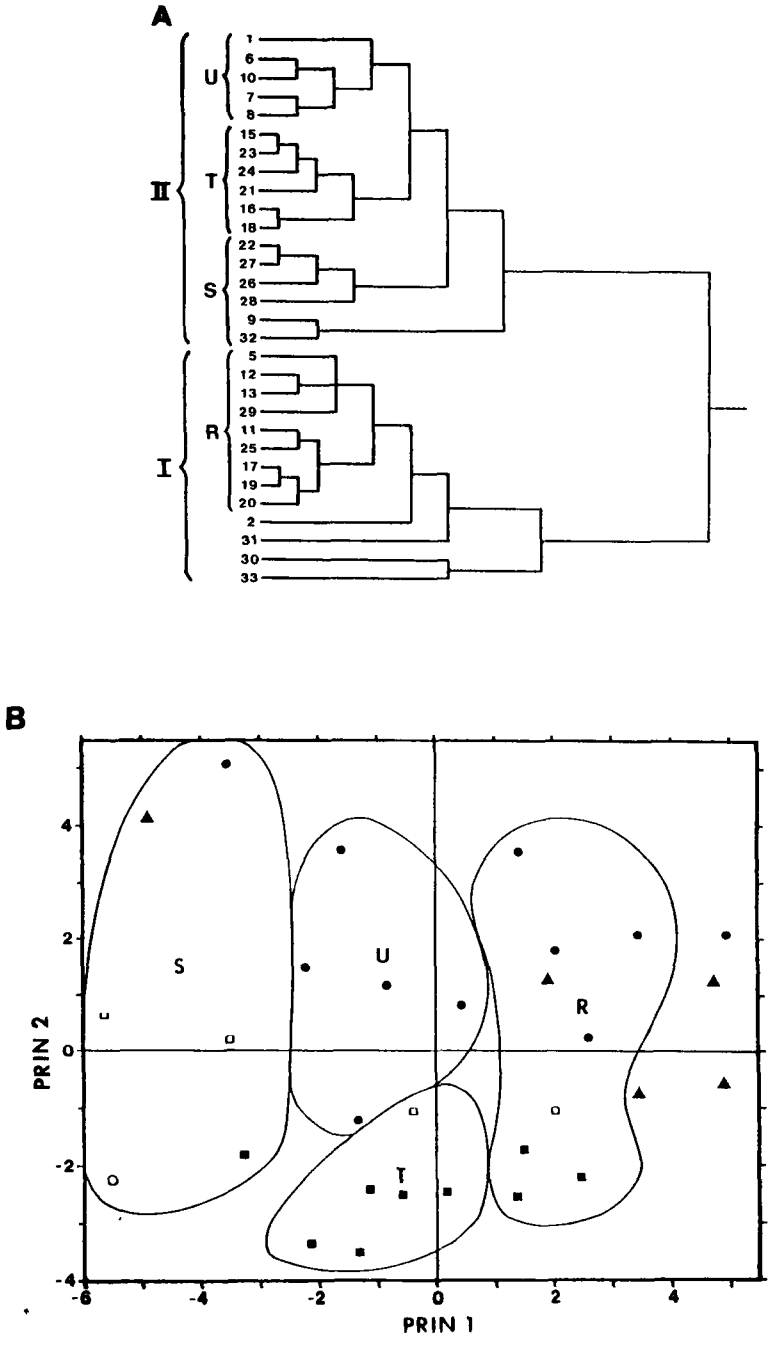


Figure 4.12 Clustering by principal coordinate matrix: A - Dendrogram of 30 bogs; and B - Clusters from A superimposed on principal components from Figure 4.10.

● Sudbury, ■ Timmins, □ Noranda, ○ Mer Bleue, and ▲ Elliot Lake.

When this clustering is projected on to the principal component analysis (Figure 4.12B), cluster I includes all the bogs on the right side of the figure while cluster II contains the rest of the bogs to the left. The subdivisions R to U plot as contiguous groups.

These results show that, with these bog samples, the principal component analysis and the two different methods of cluster analysis are consistent. The bogs appear to form a continuum, and the precise division depends on how the characteristics are chosen for clustering.

4.1.4.6 Multiple Regression

To determine which characteristics need to be measured to estimate methane production, a stepwise regression analysis was performed. Since methane was measured in only 17 bogs, data were scarce. Logit transformations were performed on those characteristics which were reported as proportions (percentages).

The three significant partial regression coefficients (those whose ratio to their standard errors, the Student t-ratio, was more than 2.0) were calcium, magnesium and rubbed fibre (Table 4.4). The sign is negative for calcium, and positive for magnesium, and although intuitively a relationship might be expected to exist with pH, there does not appear to be sufficient information to support this. As shown in Figure 6C, it is the sum of calcium and magnesium which correlates with pH. While the calcium content can be a measure of the minerotrophic contamination of the bogs, this is true also for magnesium. The rubbed fibre is also positive, this characteristic is a measure of the potential of biomass for degradation, and for methane production, so that the correlation between rubbed fibre and methane is logical. Because of the small amount of data, these three characteristics cannot

yet be accepted as sufficient for determining the methane production of bogs.

Table 4.4 Regression coefficients of methane reduction from 17 bogs.

	estimate	standard error	T (Students)
Constant	5.25	2.07	2.54
calcium	-1.19	0.22	-5.36
magnesium	1.55	0.317	4.89
rubbed fibre	0.88	0.41	2.17

4.1.5 CONCLUSION

The statistical methods used here to study organic soil samples provide an insight into the organic soils of these mining areas. The initial difficulty was to determine criteria for differentiation among the samples, for although locality plays a part in the differences among the bogs, it was unimportant for my purpose of characterizing methane-producing bogs. The samples were originally intended to be mainly from ombrotrophic bogs, that is, those bogs whose water and mineral supply is received wholly from the atmosphere. Since it was not always possible to find true ombrotrophic bogs near the mines where the tailings have been produced, the actual samples are from a wider range of organic soil types.

The various numerical methods discussed here show that it is possible to differentiate between the organic soils of bogs. Cumulative probability graphs are useful for determining the distribution of the populations of different characteristics, while the bivariate analyses show which characteristics are interdependant and so might be eliminated. The canonical variate analysis groups the bogs into definite geographical areas, and the cluster analysis groups the bogs mainly by locality, while the principal component analysis

is able to separate the samples both by their considerable contamination, and by their organic components.

The differentiation of bogs by locality could be due to environmental differences, as the areas are fairly far apart (several hundred kilometres), and Elliot Lake and Sudbury are two degrees of latitude further south than Timmins and Noranda. It is more likely, however, that the difference is geologic, as Elliot Lake and Sudbury samples are mainly in gneissic granitoid rocks, while Timmins and Noranda are in volcanic and intrusive rocks. However, for our purpose, that of finding those bogs which would produce most methane, the geographic location of the bogs is not important.

The principal component analysis differentiates the samples mainly by their inorganic contaminants, either water-borne clays from flooding, or mining pollution which is possibly wind-borne. Dustfall has been shown to increase the calcium and magnesium content of organic soil (Gorham et al 1985), and in these mining areas dust from the tailings and smelters may well have caused contamination. There are 14 characteristics each for the organic and inorganic variables, but the inorganic characteristics seem to contribute more heavily to the principal components, possibly because many of these characteristics are indicative of the same property, such as the two metals likely to be associated with clays, aluminum and potassium. If so, the number of inorganic characteristics possibly could safely be reduced, and at the same time, the definition and measurement of the organic characteristics ought to be improved, particularly those for the humic component.

In general the bogs of the mining areas are shallower (average 65 cm), with an average depth of extraction of only 45 cm, compared to depths of 30 cm to 120 cm used in

Mer Bleue. This led to extraction of much lower concentrations of methane in all but two of the seventeen bogs tested for methane. All produced more than 25 μmol of methane, while Samples 1 and 5 produced methane in excess of 2 mmol. These two bogs were both extremely wet, at sample 1 site the subsurface peat was a slurry rather than a solid, and was in excess of 3 m deep, and at sample 5 site, although the bog was shallow (65 cm), at the time of sampling the water table was well above the surface. It seems likely, therefore, that provided degradable organic matter is present in sufficient quantities, methane will be produced in anaerobic conditions, but that the amount of methane depends upon the hydrological regime present in the individual bog. Stepwise regression analysis isolated calcium, magnesium and rubbed fibre as the characteristics determining the methane production within this limited population. This study shows that all the samples of muskeg organic peat soils tested were able to produce methane, and hence maintain an anaerobic environment. Thus convenient organic soils near to the mine tailings may be used as an oxygen-excluding cover. However more work will be required to discover the parameters necessary to establish such a self-renewing, constructed bog on top of tailings. This exploration of numerical methods indicates that principal component analysis has potential also as a general method for differentiation between organic soils.

4.2 MEASUREMENT AND SIGNIFICANCE OF CELLULOSE IN PEAT SOILS

4.2.1 INTRODUCTION

The most important characteristic of a peat soil is the degree of decomposition of its plant biomass. The cellulose component that can still be metabolized by microorganisms determines the potential for further biodegradation of the peat. A physical measure of this decomposition can be obtained from the rubbed fibre content, while respiration indirectly shows the carbohydrate content of the peat remaining by the aerobic degradation to carbon dioxide (which is measured). Chemical analysis of the cellulose however, directly measures the main microbial substrate. Measurements of rubbed fibre content and respiration have been shown to correlate well with the potential biodegradability of peat (Lévesque & Mathur 1979).

Peat, as already described in section 2.5.1, is a matrix of carbohydrate and phenolic components. Cellulose is the major component of plants, and it is much more readily metabolized than the phenolic lignin component, so that it is the main source of energy for the soil degradation organisms. It is difficult to distinguish physically between cellulose fibres and amorphous degraded plant material, as they are closely associated and have to be separated by rubbing. Since this separation involves manual manipulation it is subjective and prone to operator error. Measurement of respiration, on the other hand, is time consuming, and it is also influenced by soil heterogenieties which make it difficult to obtain reproducible results.

Lévesque et al (1981) have shown that cellulose extracted from organic soils is similar to plant cellulose; they found it possible, from the cellulose content, to assess the proportion of undecomposed plant material in the peat, and hence the degree of

decomposition. Thus a method which is able to extract cellulose from organic peat soils would be suitable for the estimation of soil biodegradability.

Fractionation of cellulose from soils is a tedious operation (Risi et al 1950), but the cellulose content of wood pulp is routinely determined by acid hydrolysis and measurement of the glucose produced (Saeman et al 1954). The applicability of this method to organic soils was studied to determine whether the results could be correlated with the respiration rate and the rubbed fibre content, to determine if such a cellulose assay might be used as a more objective measure of the degree of peat decomposition.

4.2.2 MATERIALS AND METHODS

Representative samples from 50 cm depth were collected from northern Ontario and Quebec peatlands, as described in section 4.1.2. The fibre content, on a volume basis, was determined by the method described by Lévesque and Dinel (1977). The steady state respiration was measured in samples with water added to give two-thirds of the water-holding capacity, and incubated at 20°C in biometer flasks for 4 weeks, as described by Mathur and Rayment (1977). Output of carbon dioxide was determined at half-weekly intervals by titration.

The adaptation by the Western Research Centre (#953) of a TAPPI (Technical Association of the Pulp and Paper Industry) method (Saeman 1954) was found to be suitable for the extraction of hydrolysed cellulose from peat soils. One-tenth of a gram of air-dried and ground (Wiley mill 20 mesh) peat was used, and this was swollen for an hour at 35°C in 72% sulphuric acid, and then diluted to 3% sulphuric acid and hydrolysed at 121°C for one hour in sealed vials, further details of the method are to be found in

Appendix 3. Filtration through weighed sintered glass funnels, which were dried overnight, gave the acid-insoluble residue. The acid-soluble lignin in the filtrate was measured spectroscopically (TAPPI T222 os-74, and TAPPI useful method 250).

The sugars in the filtrate were assayed spectroscopically using the method of Dubois et al (1956). Whatman CF11 cellulose was used as standard, both alone as a control, and in spiked samples to ensure there was no interference in the assay from other components of the peat. All assays were done in duplicate.

Hydrolysates of three different peat soil types (histosol, mesisol and fibrisol, Section 4.3.1.1) were used for determination of the sugars. The hydrolysate was neutralized with saturated barium hydroxide (Léger et al 1987), the supernatant was evaporated to dryness and the sugars acetylated. They were analysed on a Hewlett Packard 5730A gas chromatograph, using a 100/120 Supelcoport column (4 mm x 200 cm) run at 230°C.

4.2.3 RESULTS AND DISCUSSION

The analysis of the samples are shown in Table 4.5; more detailed respiration results are to be found in Appendix 7. On average, the sum of the cellulose, acid-soluble lignin and insoluble residue was over 90% by weight adjusted for ash content. This indicates that less than 10% of the total is unaccounted for by this method, part of which may be acid-soluble mineral matter. The cellulose content varies from 14.9% to 46.7% (mean 25.7%) of the total. The major portion of the peat, measured in this way, is acid-insoluble residue which varies from 23.8% to 84.4% (mean 64.9%), while acid-soluble lignin is only a small portion of the total, 1.1% to 5.2% (mean 2.4%). The respiration varies from less than 1 to over 117 $\mu g\ g^{-1}$ carbon respired per day; however this latter value seems anomalously high and

TABLE 4.5 Distribution of organic carbon, respiration rate, and rubbed fibre content in 30 bogs.

Sample	Peat Type	Cellulose %	Residue %	ASL %	Total %	Respiration $\mu g\ g^{-1}C/day$	Rubbed Fibre %
1	M	23.5	74.3	2.3	100.1	14.7	26.0
2	M	20.9	59.0	1.6	81.5	4.2	25.3
5	H	29.4	42.5	2.0	73.9	42.3	56.7
6	H	20.9	68.4	2.4	91.7	31.1	43.3
7	M	29.4	66.5	2.0	97.9	29.7	50.7
8	H	29.6	52.9	2.7	85.2	54.8	60.0
9	H	37.7	23.8	3.3	64.8	117.3	78.7
10	M	27.2	64.6	3.1	94.9	31.0	34.7
11	H	18.4	67.6	2.0	88.0	12.8	31.3
12	M	22.2	61.2	2.7	86.1	18.5	33.3
13	H	20.0	64.1	1.8	85.9	8.9	44.0
15	H	26.1	74.5	2.3	102.9	27.2	28.7
16	M	26.7	84.4	1.2	112.3	10.4	32.0
17	F	18.2	71.7	2.2	92.1	7.2	44.7
18	M	24.8	61.6	1.6	88.0	6.9	38.7
19	M	21.9	79.2	2.0	103.1	3.9	38.0
20	F	25.1	69.5	3.1	97.7	10.5	25.3
21	H	21.8	75.7	1.7	99.2	41.0	54.0
22	M	26.5	78.9	2.9	108.3	17.7	20.7
23	F	24.4	78.5	2.5	105.4	24.6	43.3
24	M	25.7	75.6	2.6	103.9	30.2	40.0
25	H	19.6	59.0	2.6	81.2	4.9	14.7
26	F	35.7	47.7	3.1	86.5	49.0	41.3
27	M	34.6	67.9	2.9	105.4	40.7	35.3
28	H	38.1	70.7	2.9	111.7	16.0	30.7
29	F	21.4	69.3	1.7	92.4	13.7	46.7
30	M	15.7	64.5	3.0	83.2	1.4	22.0
31	H	15.6	67.9	2.8	86.3	2.0	8.7
32	F	46.7	27.5	5.2	79.4	77.3	78.0
33	F	14.9	78.5	1.1	94.5	0.9	41.3
AVG		25.4	64.9	2.4	92.8	25.0	38.9
STD		7.2	14.0	0.8	11.2	24.8	15.7
MAX		46.7	84.4	5.2	112.3	117.3	78.7
MIN		14.9	23.8	1.1	64.8	0.9	8.7

H - humisol
M - mesisol
F - fibrisol

has not been used in the correlation. The next highest value, 77.3 $\mu g\ g^{-1}$ carbon per day, has been taken as the maximum, giving a mean of 21.8 $\mu g\ g^{-1}$ carbon. The rubbed fibre varies from 8.7% to 78.7% (mean 38.9%).

The sugars found in the neutralized hydrolysates from the three different soil types, averaged 93% (±2.5%) glucose; four peaks of other sugars were visible on the gas chromatograph, but were not identified. Extracts of the original peat, by either hot or cold water contained less than 10 $\mu g\ ml^{-1}$ of sugar (corresponding to less than 2% cellulose). Therefore, it appears justifiable to conclude that the major portion of the sugar involved in the Dubois reaction is from glucose produced by the hydrolysis of cellulose.

Inspection of the frequency histograms show that while both the cellulose and the rubbed fibre have normal distributions, this is not necessarily so for the respiration; which seems to follow a log normal distribution to about 30 $\mu g\ g^{-1}$ carbon per day, and after this value there is a break in the slope of the line. Since, overall the line appears to fit a normal distribution reasonably well the data have not been transformed for this correlation study. To assess the use of this method in determining the degree of degradation of the peat, covarient relationships between cellulose and respiration, and between cellulose and rubbed fibre have been plotted (Figure 4.13A and B).

The measured cellulose correlates well with the respiration, with a Pearson correlation coefficient of $r=0.7728$ (without Sample 9). The correlation of cellulose with rubbed fibre is less good, with an r value of 0.5663. However, the standard error of estimate using all samples, at the 66% confidence level, includes nearly two thirds of all the sample

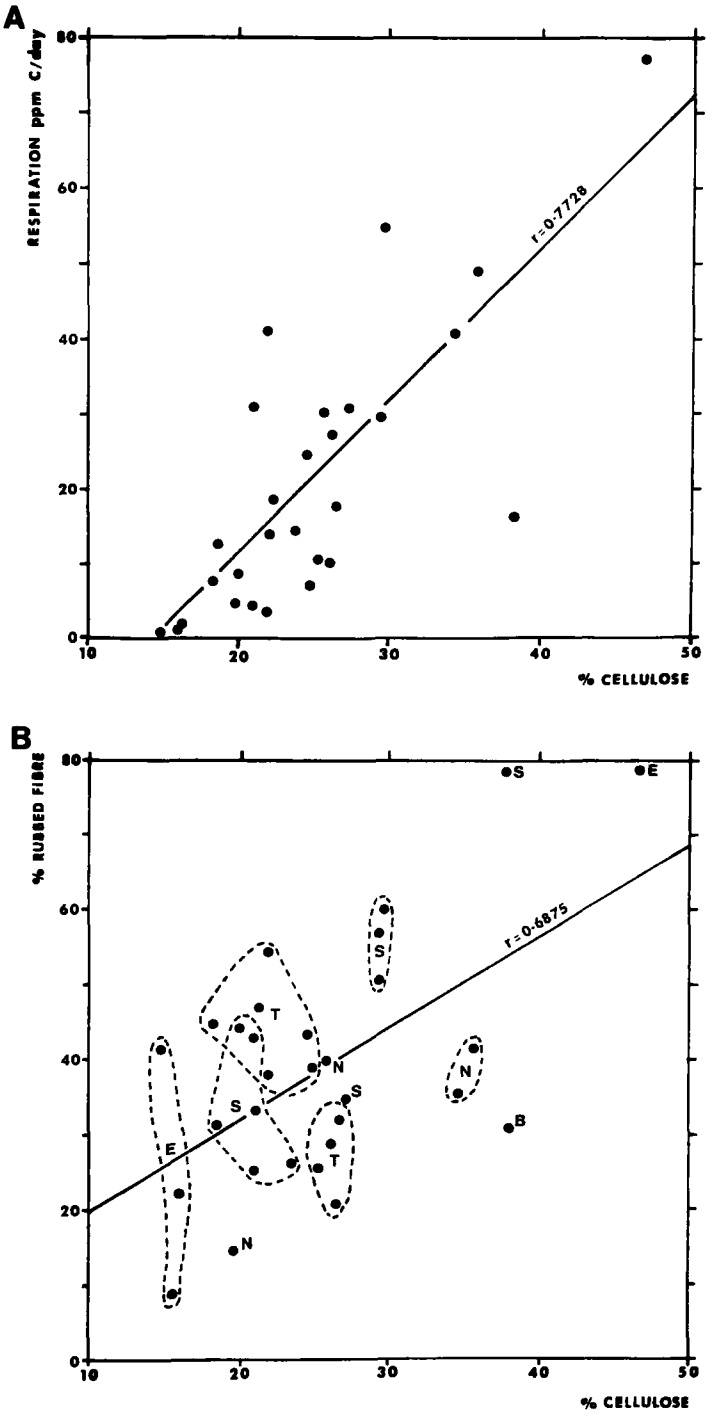


Figure 4.13 Correlation of cellulose with: A - Respiration; and B - Rubbed fibre, grouped into geographical areas:

B-Mer Bleue, E-Elliot Lake, N-Noranda, S-Sudbury, T-Timmins

values, and the slope of the regression line is within the 80% confidence level. But the correlation is not simple, as the values are clustered by geographical location (Figure 4.13B). Much of the variation is from the rubbed fibre (a subjective assay) rather than the cellulose component, as can be seen from the elongate shape of the groups. If the means of these groups are calculated, and used with the outliers for the correlation, then $r = 0.6875$, and the 66% confidence level includes all but the four furthest outliers.

4.2.4 CONCLUSION

The study shows that the measurement of cellulose by hydrolysis is much faster and less subjective than by either respiration or rubbed fibre. Overall the correlation between these methods is close, which suggests that they are interchangable, and that the measurement of cellulose is an acceptable method for measuring the degree of degradability of peat.

4.3 MICROBIAL STUDIES OF MUSKEG BOGS

Two studies were done on the microbiology of the muskeg environment: the physiological groups of aerobic bacteria were enumerated (by T. Kauri) from three of the bogs sampled at Noranda and an enriched culture was obtained of an acidophilic methanogen from Mer Bleue.

4.3.1 PHYSIOLOGICAL GROUPS OF BACTERIA IN MUSKEG BOGS

4.3.1.1. INTRODUCTION

Samples from three bogs (25, 26, 27) from the Noranda area were chosen for the study of aerobic bacterial populations. The samples were all taken within a kilometre of each other (Figure 4.1E), and they represent three soils at different stages of degradation: 25 is a humisol (well degraded), 27 a mesisol (moderately degraded) and 26 a fibrisol (little degraded). When the population studies were begun, we did not know that 25 had been subjected to flooding although we did know that the inorganic content was higher than in the other two bogs.

4.3.1.2 MATERIALS AND METHODS

Samples were collected both from the surface (10 cm depth) and at approximately 45 cm depth. Soil samples were suspended in Winogradsky's standard salt solution and homogenized in a ATO-mix for 3 min. A dilution series was prepared using the same salt solution. These dilutions were used for plating onto soil-extract agar and for most probable number analysis. Details of the different media used are in Appendix 3.

The viable populations of different physiological groups were enumerated by Dr. T. Kauri after culturing aerobically on agar plates containing starch, pectin, chitin, cellulose,

cellobiose, arabinose and xylose. Incubation periods at room temperature varied between one and eight weeks. Total viable counts were determined by dilution plate method on soil extract agar.

4.3.1.3 RESULTS AND DISCUSSION

There is a relatively high population of aerobic bacteria, in the peat soil surface layer. The numbers are fewer at the greater depth (45 cm), but still show a fair population, both of total bacteria, and of bacteria in different physiological groups (Figure 4.14). Spore forming bacteria are a very small part of the total population, particularly at depth, which is unexpected for soil microorganisms. The highest bacterial counts are found in the humisol, followed by the mesisol, and then the fibrisol, both in the surface layer and at depth. The biggest physiological group of bacteria, in all soils at both depths, is the cellulose degraders, followed closely by starch degraders; the next most numerous groups are the pectin and chitin degraders, with a surprisingly high number of ammonifiers. The least abundant groups are the degraders of the di- and monosaccharides, cellobiose, lactose, galactose, and arabinose.

The presence of a physiological group does not necessarily mean these organisms are active, only that under favourable conditions they could become so. The soils have pHs ranging from 3.26 to 4.84, and the microorganisms have remained viable (without forming spores) in these conditions. It is significant that the most numerous groups are those that degrade complex carbohydrates, and, in the case of the ammonifiers, complex amino acids, rather than the simpler sugars, because the main substrates in this environment are plant biomass polymers. These peat soils seem to have the capacity to preserve a specific aerobic microflora in an otherwise anaerobic environment, so that when the soils are drained there

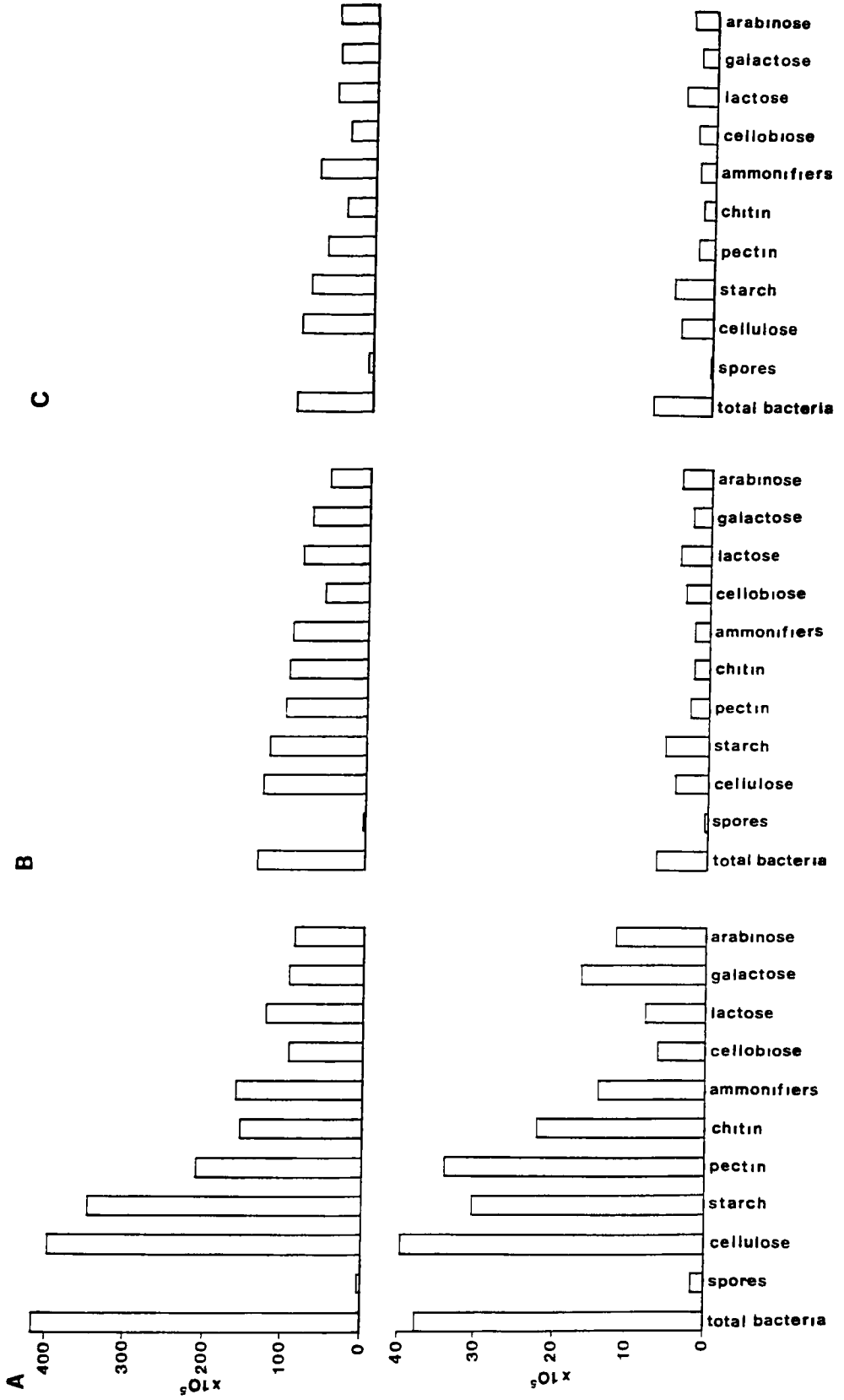


Figure 4.14 Mean numbers and spores in different physiological groups, at the peat surface (upper figure) and at 45 cm depth (lower figure). A - Histosol, B - Mesisol; and C - Fibrisol.

is a rapid aerobic metabolism of the peat biomass. This rapid loss of organic matter has often been observed in newly drained wetlands.

4.3.2. AN ACIDOPHILIC METHANE-PRODUCING ENRICHMENT CULTURE

4.3.2.1 INTRODUCTION

Although the involvement of anaerobic microorganisms in the decomposition of plant biomass in peat was established by Waksman and collaborators in the 1920's (Waksman and Stevens 1929), there has still been little success in isolating acidophilic methanogens. Only one brief report is known of a methanogenic isolate capable of producing methane at pH 3.1, but it is unable to grow below pH 5.3 (Williams and Crawford 1984). More recently, a methanogen isolated from a putative peat bog (Zellner et al 1989), actually had an optimal pH for growth of 7.0, a pH characteristic of a fen.

Mer Bleue is an ombrotrophic bog with an average field pH of 3.8, from which methane has been extracted in considerable quantities, and laboratory incubations of peat taken from the same sites have also produced methane (Section 4.4.3). Some of these incubations were quite active and were used as the source for an acidophilic methane-producing enrichment culture.

4.3.2.2 MATERIALS AND METHODS

These initial methanogenic enrichment culture incubations were similar to those described in Section 4.4.2.2, except that 10 g of wet peat was put into litre flasks adapted to take Wheaton anaerobic closures, and the deoxygenated water was increased to 100 ml. Culture medium. This is tabulated in Table 4.6, and is based on the media described by Patel et al (1976). To provide buffering capacity in the range of pH 4, part of the phosphate

buffer was replaced with citric acid. The two stock solutions were made up and added in the quantities indicated, trace minerals, vitamins and 0.2% ferrous sulphate were added together with molybdate, tungstate, nickel and selenite. This solution was brought to the boil, and flushed for 15 minutes under a stream of 20:80 carbon dioxide/hydrogen, before being anaerobically dispensed into Wheaton vials, sealed and autoclaved. Before inoculation the vials were reduced with 0.1 ml per 20 ml media of titanium citrate (1.5% solution) as described by Zehnder and Wuhrmann (1976), 1 mM sodium sulphide was added and then filter-sterilized antibiotics, at 0.1 mg ml^{-1}. The inoculum was either 0.5% or 1.0%. The vials were repressurized with the gas mixture to a pressure of 70 kPa (above gauge), and incubated while shaking at 25°C.

Plating methods. Gelright, a gellum gum K9A40 from Kelco, a division of Merck & Co. Inc. was used at 1% concentration with 1 g l^{-1} of added magnesium sulphate, to provide a gel which would set at pH 4. The media was made up as described above, the Gelright was added after the media had been flushed and it was reheated until dissolved. After autoclaving, the vials were reduced with titanium citrate, and sulphide and antibiotics added; the vials were immediately put into the anaerobic chamber and poured into pre-reduced petri dishes. After streaking with culture the plates were incubated in an adapted pressure cooker in a pressurized atmosphere (70 kPa) of carbon dioxide/hydrogen.

Gas chromatography. Methane was measured by flame ionization chromatography on a Hewlett-Packard model 5720A.

4.3.2.3. RESULTS AND DISCUSSION

Since little is known about acidophilic methanogens, it was necessary to experiment with the growth conditions. Initially methane evolution was measured from 10 g of wet peat in 10 ml of deoxygenated water, but by diluting the peat with 100 ml of deoxygenated water

Table 4.6 Medium for an acidophilic methanogen.

			final g l^{-1}
SOLUTION 1	citric acid	4.2 g	0.672
make up to 1 litre	$NaCl_2$	0.3 g	0.048
	$(NH_4)_2SO_4$	3.0 g	0.480
	$MgSO_4.7H_2O$	0.6 g	0.096
	$CaCl_2.2H_2O$	0.4 g	0.064
SOLUTION 2	K_2HPO_4	3.5 g	0.613
make up to 1 litre			
For 1 litre media take:	Soln. 1	160 ml	
	Soln. 2	175 ml	
add:	Trace minerals	10 ml	
	Vitamins	10 ml	
	$FeSO_4$ soln.	5 ml	0.0200
	$Na_2MoO_4.2H_2O$	10 µl	0.0240
	$NaWO_4$	10 µl	0.0165
	$NiCl_2$	10 µl	0.0119
	Na_2SeO_3	10 µl	0.1500
pH to 3.4			
Bring to boil under 20:80 CO_2/H_2			
Flush 15 min			
Dispense anaerobically into vials			
Autoclave			
To 20 ml media add:	Ti(III) citrate	0.1 ml	1.5%
	Na_2S	0.1 ml	1.0 mM
	Kanomycin	0.1 ml	0.1 mg ml^{-1}
	Penicillin	0.1 ml	0.1 mg ml^{-1}
Final pH about 4.0			

greater quantities of methane could be produced. The water was then replaced with 1:10 dilution of a chemically defined medium (Patel et al 1976), which was later increased to full strength. While there was still some peat left in the culture the pH of 3.8 was maintained, but once the peat substrate had been diluted out, its buffering capacity was replaced by the phosphate/citrate medium (Table 4.6). However to poise the redox potential once the peat

was eliminated, it was found necessary to reduce this medium, rather than just boiling it under reducing conditions. A mixture of sodium sulphide and cysteine is generally used to reduce anaerobic media, but the amount of sulphide needed for this method proved to be inhibitory to the methanogen. Instead, the medium was reduced, after autoclaving, with titanium citrate (Zehnder and Wuhrmann 1976), to which 1 mM sodium sulphide was added. Finally, in an attempt to eliminate eubacteria, filter-sterilized penicillin and kanamycin were added at 0.1 mg ml^{-1} concentration (after several antibiotics had been investigated). Figure 4.15 shows the increase in methane production of the enrichment culture during the 30 weeks that the growth medium was developed.

The methane-producing consortium initially consisted of four morphologically different organisms. Continual subculturing has now reduced these to two. Plating on Gelright was unsuccessful, for although some colonies were produced, when they were pricked and cultured in media they did not produce methane. The predominant type of organism is a medium-sized rod, which in later stages of growth can form long chains and exceptionally, bundles, similar to those of *Methanothrix soehngenii* (Oremland 1988). There are some extremely small cocci, which, although they have not been seen to be connected to the rods, appear to be of similar size to those which are released from *Methanobacterium uliginosum* as described by König (1984). There are a few extremely fat rods which appear to contain some type of occlusion, such as gas vacuoles. The final member of the consortium is a motile, sigmoidal rod, which is possibly a sulphate reducer. Of the two organisms in the last enrichment culture, by far the most numerous is the medium rod, while the motile rod is much less common. Sometimes very small spheroids are seen which could be spheroplasts formed during lysis of the bacteria.

Vancomycin has recently been reported to be successful in isolating a methanogen from an accompanying eubacterium (Zinder et al 1987); but, while this antibiotic does appear to prevent the growth of the remaining eubacteria, it appears also to interfere with the production of methane.

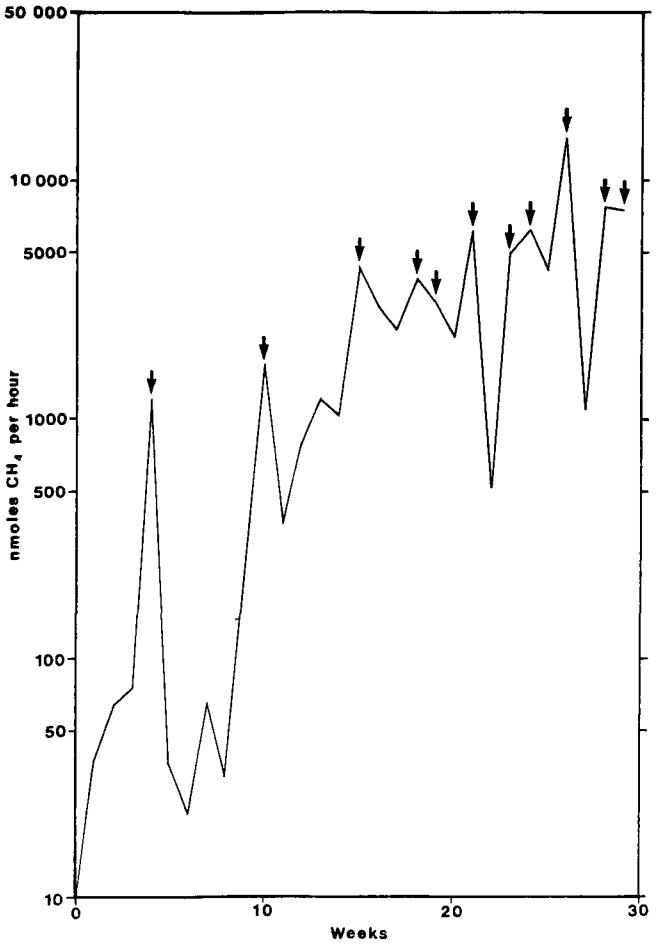


Figure 4.15 Methane production by an enrichment culture of the acidophilic methanogen. Arrows indicate time of transfer.

At a pH of 4 most of the sulphide in the medium will be in the gaseous phase (Section 2.6.2), so that if the vials are flushed sulphide will be lost, but without flushing, inhibitory metabolites appear to build up and prevent methane formation. Most methanogens require sulphide for growth as they are unable to reduce sulphate themselves. In this culture the methanogen(s) appear to be quite sensitive to the amount of sulphide in the incubation vial, so that, if there is a sufficient concentration of sulphide to reduce the medium, it inhibits the growth of the methanogen. Titanium citrate can reduce the medium sufficiently for growth to commence, but it seems unable to poise the medium at a sufficiently low redox (Patel et al 1982). Attempts to replace the sulphide, after flushing, with different sulphide sources have so far proved unsuccessful, possibly as it is not sufficiently dilute. However, the addition of trace amounts of Coenzyme M (2-mercaptoethanesulphonic acid) shows promise. Yeast extract, which has been used to enhance reducing conditions in media (Patel et al 1982), is inhibitory to this culture. So far no other method of maintaining the requisite low redox potential has been discovered.

The enriched methane-producing consortium appears to exhibit some inter-dependancy, for without the continual provision of sulphide at low concentrations by the putative sulphate-reducing organism, the methanogen either lacks a source of soluble sulphide at this pH, or the redox cannot be maintained at a sufficiently negative level for growth. The best methane production has so far been found to occur between pH 4.0 and 5.5, and at 25°C.

4.3.2.4. CONCLUSION

Acidophilic methanogens do exist in bog environments. The main reason they have not yet been isolated appears to be the difficulty of providing a suitable medium for their

growth, as they require a very low reducing potential (about -250 mV, Zehnder and Stumm 1988), but are inhibited by more than small amounts of sulphide. The optimum growth of this culture occurs at pH 4 and, at a sufficiently negative pϵ to place the conditions right on the boundary of the stability of water where, thermodynamically, hydrogen is spontaneously liberated (Figure 2.6). When such methanogens are finally isolated their energy metabolism should prove to be interesting, since it is likely that a large pH gradient across the cytoplasmic membrane would be generated in regulating the cytoplasmic pH. This large proton potential may represent a major storage of energy which can be utilized by the organism in a manner similar to that found in *T. ferrooxidans* (Section 3.1.5).

4.4 METHANE PRODUCTION IN MUSKEG BOGS

4.4.1 EXTRACTION OF METHANE FROM PEATLANDS

4.4.1.1 INTRODUCTION

It has been known for nearly 200 years that decaying organic matter evolves methane (Dalton 1804, in Roscoe and Harden 1970), yet very little is known about the size of the methane pool (Armentano and Menges 1986). Peat bogs produce measurable amounts of methane and carbon dioxide, but the flux of gas varies during the diurnal and annual cycles, and methane especially is subject to sudden and irregular surges (Clymo 1983). These are probably due to ebullition, possibly caused by changes in atmospheric pressure. The uncertainity of the pool size is partly due to the lack of suitable techniques to sample peatland waters and gases at specific depths without exposure to the atmosphere, and partly to low *in situ* methane production in peatlands due to suboptimal pH and temperature (Stafford et al 1980, van Cleemput and El-Sabaay 1985).

The production of methane has been discussed in Section 2.7.2, but its measurement has proved difficult. It is generally estimated either from laboratory incubations of peat samples taken at various depths, or through measurements of methane released from peatland surfaces (King and Wiebe 1978, Sikora and Keeney 1983, Williams and Crawford 1984, Svensson 1984, Harriss et al 1985, Moore and Knowles 1987). However, the potential methane production, when measured by either of these methods may not correctly depict either the methane pool size or its flux. Peat may contain random pockets of occluded methane, and the concentration of dissolved methane may increase with depth, similar to that found in lake sediments (Reeburg 1969).

Methane is nonpolar and, unlike carbon dioxide, is poorly soluble in water, although an increase in solubility is seen with depth due to the hydrostatic pressure of the overlying water (Stafford et al 1980, Stolzy and Jury 1982). As discussed in section 2.7.5, Mathur and Lévesque (1985) have proposed that the occlusion of interstitial pores in the peat by bubbles of methane will retard the flow of water at the peripheries of peatlands, around auger holes, and in ditches and drains. If gaseous methane is occluded within the peat, a large part could be released sporadically, influenced both by changes in the hydrostatic and atmospheric pressure, and by the temperature of the surface and subsurface waters, making any observation difficult to interpret.

Field measurements are difficult to obtain and understand, and some of the present methods allow exposure and contamination by the atmosphere, as in the cintered bronze cup of Dowdell et al (1972), or do not provide sufficient volume for the collection of all the occluded gases in the deep layers, as in the case of Tackett (1968) who used capillary tubes of 25 cm length. Thus, to provide better information on the pool and flux of methane within the bog environment, and their effects on the hydraulic conductivity in bogs, and methane concentration in the atmosphere, it has been necessary to devise a new method of collection.

4.4.1.2 PILOT STUDIES

The initial work was done in cooperation with Dr. S.P. Mathur of Agriculture Canada, and various sites in four bogs close to Ottawa were investigated; these bogs are Alfred, Albion Road, Gatineau Park, and Mer Bleue. In the first study a hole was made with an augur and the water that collected in the hole was sampled with an evacuated bottle; but the stopcocks of the bottles are narrow, and, as the water carries suspended matter, the bottles were continuously being blocked preventing collection of adequate

samples. We then found, that if the original hole was bailed out, the water that refilled it was relatively free of suspended matter but contained little methane, probably as most of the water came from the surface. After testing various methods and equipment, a sampler made from small bore copper tube (water pipe) was successful, and these samplers were used for the pilot studies. The design was then duplicated in stainless steel, which is less effected by the bog environment.

4.4.1.2 METHODS

The two main study sites were the Gatineau Park bog and Mer Bleue (described in Appendix 2). Twelve tubes were fabricated in stainless steel, and were of three different lengths, 60 cm, 90 cm, and 120 cm so the gas could be sampled at different depths. The sampling equipment consists of a stainless steel tubular probe, a vacuum gauge (used in the initial trials only) and an evacuated polypropylene bottle (265 ml) for sample collection, obtained from Sargent Welch Co. The gauge and bottles were joined to the probe with tygon flexible tubing. The probe (Figure 4.16) is composed of an outer tube and a inner plunger, both with 1.2 mm thick walls. The plunger is closed at the bottom end. The outer tube has 1.9 cm inside diameter and a 20 cm side opening to allow fluid to enter the bottom reservoir from the sampled soil. The plunger is fitted with an oil-lubricated O-ring which acts as a seal to prevent atmospheric contamination during sample collection through the side arm into the pre-evacuated bottle. A strap wrench was used as necessary, to help move the tubes up and down in the peat.

The sampling of methane at three different depths was achieved by introducing probes of three different lengths, 50 cm apart, all perpendicular to the peat surface at each station. The probes were pushed into the peat steadily to minimize disturbance, and to

ensure a tight fit between the peat and the tubes. When the marker on the outside of the tube reached the peat surface, the vacuum gauge and an evacuated gas bottle were connected to the sidearm of the probe, with both stopcocks A and B closed. The plunger was then pulled up until the marker on it was flush with the top of the jacket. After 30 seconds, stopcock B of the bottle was opened to measure the vacuum in the bottle, and then stopcock A was opened to collect the gas and water in the bottle. About a minute was

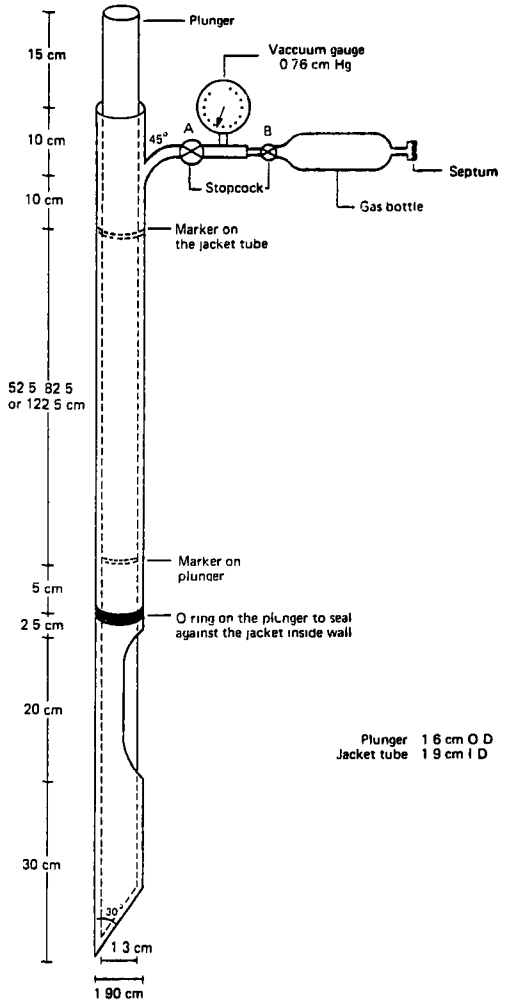


Figure 4.16 Diagram of the methane sampler.

generally required to replace the vacuum in the bottle with the collected water and gas. The evacuated bottles had a negative pressure of approximately 60 cm Hg, while pressure in the charged bottle was approximately atmospheric. Stopcocks A and B were closed after the collection and the filled bottle was replaced with an evacuated one. This sampling procedure was repeated sequentially at each location.

In later work the vacuum gauge (and stopcock A) was removed as it reduced the amount of vacuum in the bottle and the extra joins in the tubing were a source of blockage during extraction, leaving only stopcock B on the bottle; the tygon tubing was pinched by hand to close the system when changing the bottles.

4.4.2.3 RESULTS AND DISCUSSION

During the period of field measurements in the late summer of 1986, the temperature of water 40 cm to 135 cm below the surface of the peatlands was 10 ± 2°C, while the water table was within 2 cm above or below the surface. The pH of the sampled water layers ranged from 3.0 to 3.2 at the Gatineau Park site, and from 3.2 to 3.7 at Mer Bleue. No methane was detected in the peatland surface water collected for up to 30 minutes by the inverted funnel method (Williams and Crawford 1984), unless gas was released by disturbing the peat underneath.

Many simultaneous measurements are needed to find how uniform the methane production and concentration is in a bog, both over time, and also spatially in the vertical and horizontal sense. The results reported here are an average of two stations each in the Gatineau bog and in Mer Bleue (Table 4.7A, and complete measurements in Appendix 8). Most methane was evolved from the 120 cm depth, and the least from 60 cm depth, both

in the initial 15 minutes and over the total 2 hour sampling period. Approximately 75% of the total methane was in fact released during the first 30 minutes (Figure 4.17A). Carbon dioxide collection generally followed the same pattern as that for methane (Figure 4.17B). These trends, when tested in the Gatineau bog, for both time and depth, on different days and locations, were reproducible; although the actual values varied, particularly at the lowest depth.

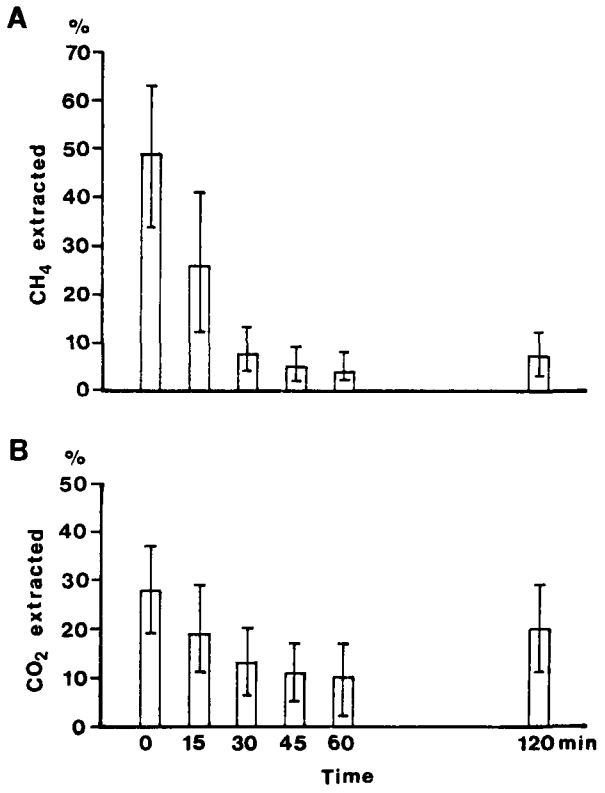


Figure 4.17 Percentage of gas extracted over time from four bog stations: A - methane; B - carbon dioxide.

The average quantity of methane collected in the sampling tubes was, with one exception (60 cm at Station 2 in Mer Bleue), far above the capacity of the collected water to hold in solution at 10°C to 20°C and atmospheric pressure. This was not so for the carbon dioxide. The methane must have been collected therefore, either from occluded gas bubbles in the peat, and/or from water in the sampling tube, and/or from water in the area surrounding the side opening of the probe. The out gassing was greatest during the first 15 minutes, notably from the deepest levels (Table 4.7B).

Little water was sucked into the bottles during the first 30 minutes, since the tube first had to be filled, while about 75% of the gas was released during this time. Indeed, the samples from the deepest depth, at 15 and 30 minutes, when there was little water in the probe, contained considerably more methane than subsequent samples (Table 4.7A). The variation in the amount of methane and carbon dioxide evolved at different times and from different depths, were consistent with the postulated presence of pockets of occluded gaseous methane, and saturation of the subsurface waters with methane (Mathur and Lévesque 1985). The flow of water and gas from the adjoining soil may have been impeded by the size of the side opening, or possibly by the absence of cracks and fissures in the peat such as those found in aerobic soils (Tackett 1968). The organic detritus can provide a matrix in which the methane gas bubbles can form and be trapped, as has been shown in organic-rich lake sediments (Reeburgh 1969).

The ratio of evolved methane to carbon dioxide, on both molar and volume bases, increases with depth. A ratio of 1.5 to 2.33 is typically found between methane and carbon dioxide in anaerobic decomposition of natural organic materials (Stafford et al 1980). This is similar to that found at the 120 cm level; at the shallower depths the ratios become

Table 4.7 Extractions from Gatineau Park and Mer Bleue, at 3 depths:
A - Amount of water, methane and carbon dioxide; and
B - Average masses of the gases collected,
(expressed as 100 ml water).

A	Depth	60 cm			90 cm			120 cm		
	Time min	H_2O ml	CH_4 mg	CO_2 mg	H_2O ml	CH_4 mg	CO_2 mg	H_2O ml	CH_4 mg	CO_2 mg
GATINEAU										
Station 1	15	0	32.0	80.6	0	45.9	29.6	0	60.8	25.3
	30	106	5.6	14.2	112	28.2	19.3	0	105.8	45.8
	45	150	5.6	13.9	66	10.4	40.5	0	40.6	16.7
	60	36	7.0	17.7	40	2.9	14.1	70	38.2	24.7
	75	74	1.8	4.4	0	-	-	78	19.8	14.7
	120	144	4.8	11.7	132	3.8	50.7	152	14.9	17.4
Total		510	56.8	142.0	350	91.2	154.2	300	280.1	144.6
Station 2	15	52	40.5	30.1	92	14.2	16.3	12	122.6	55.9
	30	172	5.8	15.5	190	5.9	13.5	0	53.8	17.2
	45	172	6.7	14.2	180	1.9	5.0	150	32.2	28.6
	60	174	6.2	27.7	194	2.2	5.6	196	11.0	18.5
	75	176	4.3	10.9	190	5.2	17.2	196	10.6	19.6
	120	168	9.6	17.9	188	6.9	24.9	192	16.3	66.7
Total		914	73.1	116.3	1034	36.3	82.5	746	246.5	206.5
MER BLEUE										
Station 1	15	4	12.3	10.3	1	32.8	20.9	0	79.7	33.4
	30	65	10.4	9.3	33	38.7	25.8	0	79.7	41.9
	45	69	1.0	7.0	45	4.3	6.3	74	26.9	15.0
	60	40	-	0.4	40	1.0	7.3	135	8.5	10.1
	75	135	0.5	2.3	73	4.0	6.5	176	4.0	12.2
	120	106	0.6	10.6	113	3.5	15.1	179	4.5	16.0
Total		419	24.8	39.9	305	84.3	81.9	564	203.3	128.6
Station 2	15	5	11.5	12.6	12	29.3	20.0	8	70.6	32.3
	30	56	2.1	10.5	39	31.0	21.1	-		-
	45	57	0.5	7.4	82	3.0	23.2			
	60	74	0.3	5.1	47	2.1	9.8	194	2.7	10.6
	75	103	0.5	9.8	47	0.5	5.8	155	5.0	13.7
	120	177	0.3	5.1	109	1.3	12.8	182	10.2	24.8
Total		472	15.2	50.5	336	67.2	92.7	-		-
B										
GATINEAU										
Station 1			7.7	19.4		12.7	21.4		35.2	18.2
Station 2			5.6	8.7		11.9	23.7		15.9	15.8
MER BLEUE										
Station 1			3.8	5.9		12.5	14.5		16.4	12.8
Station 2			1.9	6.1		9.5	15.6		-	-

smaller, and at the 60 cm depth are similar to those observed by Moore and Knowles (1987) from the surface of four subarctic fens during summer.

These results show the feasibility of collecting representative water and gas samples from different depths in peatlands with the samplers described. The equipment proved to be good for sampling specific layers of peatlands, and for measuring the relative concentrations of gases in these layers. However, the results were obtained from isolated stations in the two bogs investigated, and show considerable variation in the quantity of the extracted gases. The next step therefore, was to investigate the amount of methane occluded in a specific area of a bog. Mer Bleue, with the permission of the National Capital Commission, was chosen for this further study.

4.4.2.2 MATERIALS AND METHODS

Study site. At Mer Bleue, an ombrotrophic bog near Ottawa, Ontario, Canada (75°30'W, 45°24'N), a 25-station, grid square (5 x 5) was laid out at 6 m spacing. At each station, sampling sites were at 60 cm, 90 cm and 120 cm depths below surface. Four randomly chosen sites were also sampled at 30 cm.

Methane extraction. The methane was sampled with the stainless steel tubes and evacuated bottles as described in Section 4.4.1.3. At each station each site (individual depth) was extracted with 5 evacuated polypropylene bottles (265 ml) for a total fluid volume of 1 l. Each bottle was extracted for 1 minute, separated by 15 minute intervals.

Gas chromatography. The pressure in the bottles was equilibrated with the atmosphere, and the methane content was measured by flame ionization chromatography on a Hewlett-Packard model 5720A.

Methane incubations. Peat for the laboratory incubations was collected with a McCauley sampler from all depths at the same stations as the 30 cm *in situ* methane samples were extracted. These samples were immediately placed in polythene bags, air expelled, sealed, returned to the laboratory, and stored at 4°C. The incubations were set up within 24 hours of sample collection in 160 ml anaerobic serum vials with Wheaton closures, using 10 g of wet peat (approximately equivalent to l g dry weight) and 10 ml of deoxygenated water. The vials were mixed by vortexing, flushed with a 20:80 mixture of carbon dioxide and hydrogen for 5 minutes, and brought to 70 kPa (over gauge) pressure with the same gas. They were incubated for one week at 25°C before measuring methane evolution. All incubations were in triplicate, with autoclaved controls.

Mixed incubations. Mixed incubations were set up as above but using 5 g of wet peat from the 30 cm depth and 5 g of wet peat from the 60 cm depth. Controls included autoclaved 60 cm depth peat and Whatman CF11 cellulose.

Peat analysis. Material from each field sample was analysed for cellulose, acid-soluble lignin (ASL) and residue (Section 4.2.2); for bulk-density determinations (van Lierop 1981), for pH measurement in 0.015M $CaCl_2$, and for ash at 550°C, and dry weight was obtained by heating at 105°C to constant weight. The actual weight of peat used in the incubations and percentage compositions was calculated from the dry weight and ash figures. All analyses were in duplicate.

4.4.2.3 RESULTS

Initially methane was extracted *in situ* from three depths, 60 cm, 90 cm, and 120 cm at 25 stations, 75 sites in all. As we had previously found that 75% of the methane was extracted within the first 30 minutes and little more was extracted after one hour (Section 4.4.2.3), in this study each sample tube was extracted for a period of 75 minutes. However in some cases the bottles still retained negative pressure on removal from the sampling tube, showing the vacuum was insufficient to extract either water or gas from the peat. This was due in some instances to blocking of the apparatus by peat slurry; in other cases the final bottles contained a substantial quantity of methane, implying that not all the available methane could be extracted in the time allowed. In this part of Mer Bleue we found the peat to be extremely impermeable, and in several cases the suction from the evacuated bottle was sufficient to retract the central rod of the sampling tube.

The evacuated bottles commonly remove water as well as gas from the peat, but, in general, methane is drawn off in the earlier bottles before large volumes of water. Since the methane was in some cases difficult to extract, it was suspected that channelling in the peat might allow more methane to collect in the bottles which contained water. However the data do not appear to show any correlation between the quantities of water and the methane

extracted.

The peat appears to be very heterogeneous, with methane trapped in irregular pockets. The amount extracted at any one site varied from 0.014 to 8.26 mmol (into 1 fluid litre), with an average of 1.75 mmol. At the 60 cm depth the average accumulation was 0.864 mmol, at 90 cm 2.45 mmol, and at 120 cm 1.94 mmol; the total extracted for the

Station	60 cm	90 cm	120 cm	Total	Average
I	1.36	1.93	2.23	5.52	1.84
II	1.92	4.11	3.08	9.12	3.04
III	0.53	2.30	2.43	5.26	1.75
IV	1.00	1.80	2.37	5.12	1.72
V	0.53	0.02	1.64	2.19	0.73
VI	1.60	8.26	0.60	10.46	3.49
VII	0.06	2.49	1.91	4.46	1.49
VIII	0.65	3.05	4.50	8.19	2.73
IX	0.09	0.46	0.58	1.14	0.38
X	0.18	6.04	0.40	6.62	2.21
XI	1.16	2.51	1.18	4.85	1.62
XII	0.67	1.03	1.43	3.13	1.04
XIII	3.19	3.57	2.10	8.85	2.95
XIV	0.99	3.80	2.76	7.54	2.51
XV	0.20	1.88	0.04	2.13	0.71
XVI	0.53	0.98	0.80	2.31	0.77
XVII	1.42	2.18	3.34	6.94	2.31
XVIII	0.54	1.77	1.86	4.17	1.39
XIX	1.29	2.05	2.59	5.93	1.98
XX	0.01	0.38	1.19	1.59	0.53
XXI	0.38	0.79	1.41	2.58	0.86
XXII	1.72	2.75	3.06	7.53	2.51
XXIII	0.47	2.19	0.72	3.38	1.13
XXIV	0.68	0.67	1.32	2.67	0.89
XXV	0.43	4.33	4.88	9.64	3.21
Total	21.59	61.35	48.43	131.37	43.79
Average	0.86	2.45	1.94	5.25	1.75
Standard Deviation	0.71	1.82	1.20	2.69	0.90

Table 4.8 Amount of methane in mmoles extracted from 25 stations at 3 depths in Mer Bleue.

whole grid was 131 mmol (Table 4.8, Appendix 9). The contoured quantity of methane extracted at each station for each depth, and for the average of these three depths, are presented in Figure 4.18. These contours show that there are sites in each horizon which have higher concentrations of methane, but that such concentrations do not generally continue through to other levels (sites) at that station.

Probability graphic analysis (Sinclair 1981) of all subsurface methane extracted shows that the values are from one population with a log normal frequency distribution (Figure 4.19). The sub-group values for each depth show more variation than the total population, but also approximate to a log normal distribution. Anova analysis shows there is more variation within the three groups at different depths (80%), than between these groups (20%) (calculations in Appendix 12). However, the average of extracted methane is different for each level, and it therefore appears that the conditions for methanogenesis were similar throughout a horizontal plane, and were probably determined at the time of burial when the material became waterlogged.

Incubations of peat from four of these sampling stations taken at three subsurface levels (60 cm, 90 cm and 120 cm) produced an average of 2.68 nmol of methane g^{-1} (dry weight) h^{-1} (range 0.80 to 5.28 nmol g^{-1} h^{-1}). Again, the 90 cm level produced more methane than the other two, 3.49 nmol g^{-1} h^{-1} compared to 1.75 nmol g^{-1} h^{-1} from the 60 cm depth and 2.82 nmol g^{-1} h^{-1} from the 120 cm depth (Table 4.9). The linear regression coefficient between the incubated methane and the extracted methane is 0.66 when one obviously incorrect outlier is omitted (Figure 4.20); with this number of samples the regression coefficient falls within the 95% confidence limits.

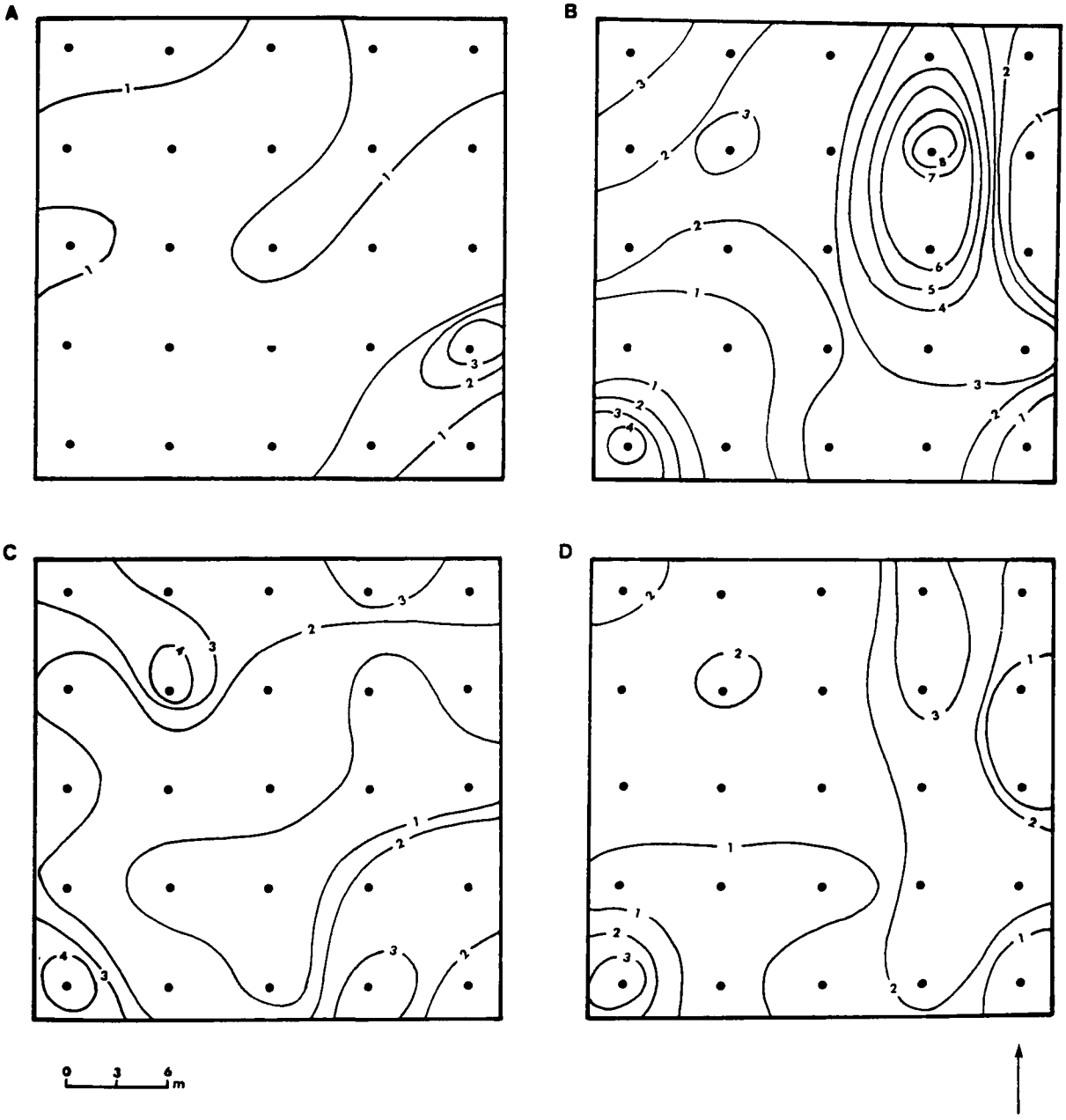


Figure 4.18 Amount of methane, in mmoles, extracted during 75 mins from 25 stations in Mer Bleue: A - 60 cm depth; B - 90 cm depth; C - 120 cm; and D - total methane (average).

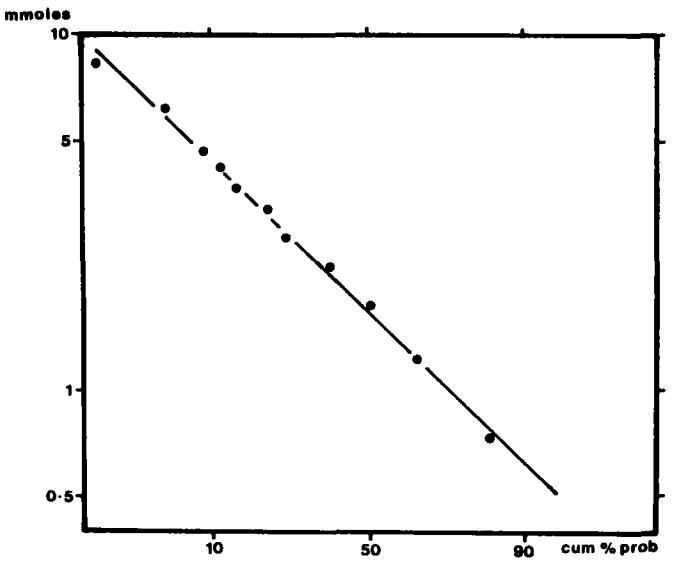


Figure 4.19 Cumulative probability plot of 75 analyses (classed) of extracted methane from Mer Bleue.

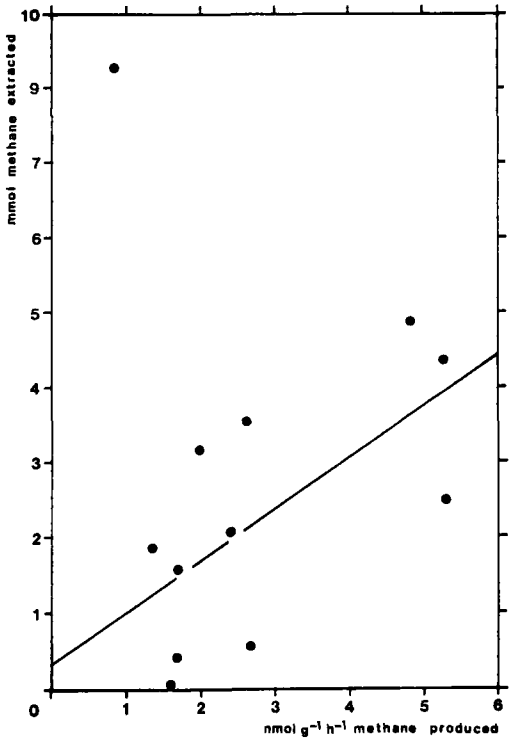


Figure 4.20 Correlation between the amount of methane extracted and the amount of methane produced in laboratory incubations from 12 sites in Mer Bleue.

TABLE 4.9. Methane production and substrate analysis in 16 sites from Mer Bleue.

Sample No.	Methane Extracted mmol	Methane Incubated nmol g^{-1} h^{-1}	Cellulose %	Acid-soluble Residue %	Lignin %	Total %	Bulk Density g ml^{-1}	Ash %	Ph
VI 30	0.032	2.02	20.27	61.05	2.35	83.67	0.0888	2.36	3.19
VI 60	1.601	1.72	21.54	64.03	1.77	87.34	0.0880	2.74	3.59
VI 90	8.262	0.80	10.05	85.99	1.19	97.23	0.1054	4.61	4.30
VI 120	0.602	2.69	9.25	86.00	0.38	95.63	0.1058	6.06	4.63
VII 30	0.089	62.68	36.70	55.23	2.31	94.25	0.1097	4.16	3.04
VII 60	0.061	1.61	31.63	63.03	2.03	96.69	0.0962	2.45	3.09
VII 90	2.493	5.28	37.28	52.66	2.24	92.18	0.0722	1.84	3.36
VII 120	1.910	1.36	18.54	83.87	0.62	103.03	0.0863	3.90	3.91
XIII 30	0.029	35.86	21.41	61.15	2.47	85.03	0.1115	3.92	3.08
XIII 60	3.189	1.97	29.30	58.72	2.98	91.00	0.0857	3.66	3.43
XIII 90	3.566	2.60	19.21	78.05	0.94	98.20	0.0916	2.60	4.27
XIII 120	2.096	2.40	9.52	82.67	0.77	92.97	0.1108	3.12	4.61
XXV 30	0.007	59.09	30.10	57.24	2.70	90.04	0.1047	3.34	3.08
XXV 60	0.429	1.69	25.35	64.01	1.37	90.73	0.0919	2.09	3.38
XXV 90	4.330	5.28	14.32	74.28	1.10	89.69	0.0977	3.49	3.77
XXV 120	4.884	4.81	11.00	94.53	0.55	106.08	0.1085	4.91	4.44
30 average	0.039	39.91	27.12	58.67	2.46	88.25	0.1037	3.44	3.10
60 average	1.320	1.75	26.96	62.45	2.04	91.44	0.0904	2.74	3.37
90 average	4.663	3.49	20.22	72.74	1.36	94.32	0.0917	3.14	3.93
120 average	2.373	2.82	12.08	86.77	0.58	99.43	0.1028	4.50	4.40

Previously, incubations of surface peat in our laboratory have produced an order of magnitude more methane than that obtained from subsurface peat, so further sampling at Mer Bleue was carried out at the same four stations mentioned above at 30 cm, the shallowest depth thought to ensure an anaerobic environment. In the field much less methane was extracted from 30 cm than at the greater depths, averaging only 0.039 mmol l^{-1} (range 0.007 to 0.089 mmol l^{-1}), but the laboratory incubations produced greater amounts of methane from 30 cm level samples than from samples of the deeper subsurface levels, which averaged 39.9 nmol g^{-1} h^{-1} (range 2.02 to 62.7 nmol g^{-1} h^{-1}).

The correlations between methanogenesis and several other parameters were investigated. Cellulose is the primary substrate for methane production (Section 2.7.3), and while the average percentage of cellulose is similar for the top two levels (30 cm and 60 cm), 27.1% and 26.9% respectively, it decreases with depth to 20.2% at 90 cm and 12.1% at 120 cm. Anova analysis (Appendix 12) shows that all these cellulose values are from one population, but while there is little correlation between the cellulose and either the incubated or extracted methane at each site, when the values for each characteristic are averaged for each horizon, the means show some dependance.

My method of cellulose extraction also allows measurement of the acid-soluble lignin, and the acid-insoluble residue which is probably mainly polymeric lignin or humus. As the cellulose decreases with depth the concentration of the recalcitrant aromatic residue and ASL increase. The relative changes in these three parameters do not quite balance, for the summed total actually ranges from an average of 88.3% at 30 cm to 99.4% at 120 cm. The portion unaccounted for, which is presumably an acid-soluble, non-lignin component, is nearly 12% at 30 cm, but practically disappears with depth (and hence with geological

age) to 0.6% of the total at 120 cm (Figure 4.21).

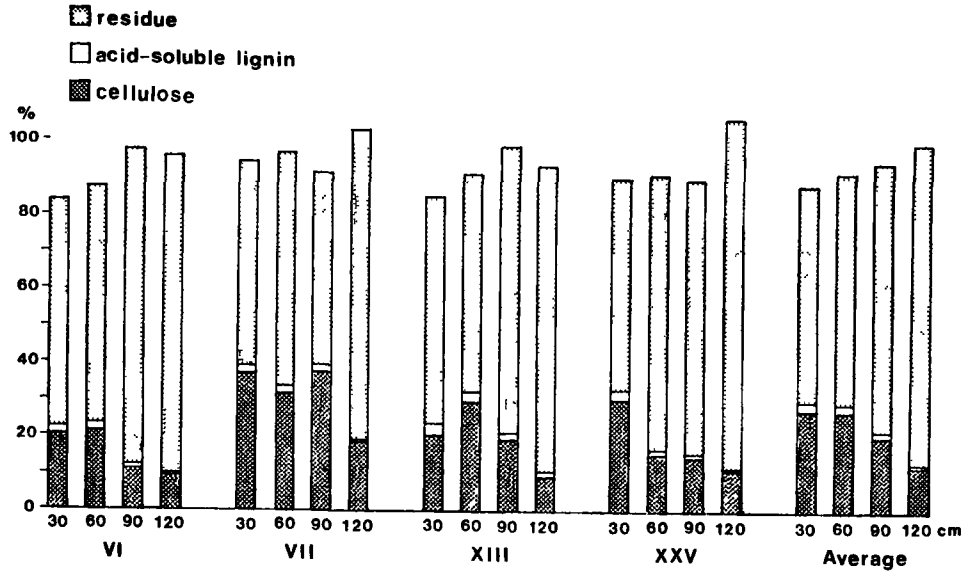


Figure 4.21 Percentage composition of cellulose, acid-solubile lignin and residue of 4 stations at 4 depths from Mer Bleue.

At all four stations the pH increases steadily with depth, averageing 3.1 at 30 cm to 4.4 at 120 cm (Table 4.9). The ash content varies more, although the trend is towards an increase in the relative amount of ash with depth as the organic component is reduced, from 2.74% at 60 cm to 4.5% at 120 cm. The greater ash content at the surface (3.45%) probably shows contemporary wind-borne contamination. There appears to be no particular trend or correlation with other factors in the reconstituted bulk density values (ratio of mass of dried soil to its volume *in situ*) which range from 0.072 to 0.112 g ml^{-1}.

4.4.2.4 DISCUSSION

This study shows there is a moderately good correlation, for samples taken at 60 cm and deeper in the peat profile, between the amount of methane collected *in situ* from the bog, and the rate of methane production from samples taken from the same sites and incubated in the laboratory. Although there is a great difference between the amount of methane extracted *in situ* and that produced during one hour's incubation, calculation of the amount that could be produced in 100 years using the laboratory production rate, gives figures of similar magnitude to that actually found in the peat (Appendix 12).

The near-surface samples, taken at 30 cm depth, have a much higher rate of methane production but lower total *in situ* accumulation than the subsurface samples. This suggests that the surface layers of the bog may have a relatively high gas conductivity, as has been reported from other bogs (Galvin and Hanrahan 1967, Rycroft et al 1975), so that methane produced here is readily vented to the atmosphere and relatively little is retained within the peat. Alternatively, much of the methane dissolved in the pore water, may be microbially oxidized (Yavitt et al 1988) and released to the atmosphere as carbon dioxide. Methane produced at deeper levels, however, may not be able to escape and therefore remains trapped indefinitely within the peat at the location where it is formed in an anoxic environment. These bogs have been accumulating since the end of the last ice age (Section 2.1.2), and the continued production of methane from fossil substrate means that the gas will be depleted in ^{14}C. The average amount of methane I found in the peat at 60 cm is nearly two orders of magnitude greater than that at 30 cm, although the greatest quantity of methane was extracted from the 90 cm level, followed by the 120 cm and then the 60 cm levels.

The production of methane in the laboratory incubations is much greater at the 30 cm than the 60 cm depth. The reduction in methanogenesis with depth has been reported previously in lake sediments by Koyama (1976), and in peatlands, by Williams and Crawford (1984) and by Yavitt et al (1987). The low methane production cannot be due to lack of substrate, for in my samples the relative amount of cellulose was similar at both the 30 cm and 60 cm depths, and over time, the 60 cm level has been found to be able to produce as much methane as the 30 cm peat. This difference in methanogenesis needs to be investigated. Preliminary results of mixed incubations of 30 and 60 cm peat (Appendix 11) suggest that methanogenesis in the lower levels of peat is inhibited by some volatile substance, for the inhibition can be overcome by autoclaving the 60 cm peat, or by flushing the samples. However as discussed in (Section 2.4.7), the only gas known to be inhibitory is acetylene (Sprott et al 1982).

If the area I have sampled is representative of Mer Bleue, I have calculated the amount of methane that could be occluded in the whole of the bog to be 107 Mmoles or 1.7 Gg (Appendix 12). If I am correct in this estimation of the amount of methane encompassed within Mer Bleue, and if other peat bogs hold similar amounts, then the global quantity of methane could be considerably greater than that already recognized. The total global area of wetlands has been estimated to be approximately 5.3 Tm^2 (Matthews and Fung 1987), and even if the average depth occluding methane is taken to be only 1 m, the amount of methane that could be occluded within this volume would be 144 Tg.

The recent increase in atmospheric fossil carbon methane suggests that it is being anthropogenically released from ancient sources. The emissions of unburnt methane by the petroleum industry do not appear to account for the increase now found in the atmosphere

(Gold 1988). However, the peatlands of the northern temperate latitudes have been accumulating since the last ice age (Larsen 1980), during which time the anaerobic metabolism of the peat biomass has been slowly producing methane, much of which, it appears, has remained within the wetlands. The current increased exploitation of northern peatlands, for farming and mining (Thompson 1987), may now be releasing considerable quantities of this trapped methane into the atmosphere, which could account for the current increase in fossil carbon methane.

5. GENERAL CONCLUSION

The initial impetus for this work was to find a method to ameliorate acid leaching from mine mill tailings. Microorganisms oxidize the iron sulphides that are found together with many ores, and use the energy thus obtained for growth, unfortunately with the concomitant production of sulphuric acid. If oxygen is unavailable the sulphides cannot be oxidized, the organisms cannot grow, and so acid is not produced. But it is extremely difficult to prevent the penetration of oxygen into many of the vast tailings piles. There is insufficient oxygen in stagnant water for this oxidation to take place, so that flooding the tailings or depositing them under water prevents acid production. However, this is impractical for most of the old tailings dumps, and the present method of amelioration is to neutralize the run-off with lime. This is very expensive, and needs to be continued indefinitely after the mines are closed.

Ombrotrophic bogs are able to maintain a watertable above that of the surrounding countryside, and also to preserve an internal anaerobic environment by the production of methane. Thus if it were possible to create a peat bog on top of acid-producing tailings, it would both stop acid leaching by preventing the penetration of oxygen, and once established, it would be self-sustaining and not require further maintenance. The main problem appears to be in the initial excavation and transportation of the peat bog to the tailings, and re-establishment of an active bog thereon. Since the production of methane was an important part of the original project, when funding by the Ministry of Energy Mines and Resources was cancelled during general cutbacks, research was continued into the factors determining methane production and distribution in ombrotrophic bogs, but plans to study bog growth were abandoned.

The research that I have completed gives some understanding of the environment for methane production in muskeg bogs. The 'quaking ground' of the Cree Indians is found across Canada. These are areas of wetland containing organic material, derived from plant biomass, that have been buried and waterlogged before aerobic microbial degradation has been completed. Once waterlogged, the cellulose part of the biomass can be further degraded microbiologically to methane, but the lignin portion is biologically recalcitrant, although it can be chemically altered first to humus and then to lignite and coal.

All the northern bogs investigated from areas around Elliot Lake, Sudbury, Timmins, and Noranda are quite similar. The amount of organic material varies over a fairly narrow range, with an average cellulose content of 25.4%, and all but two samples were within one and a half standard deviations of this. It is regrettable that as yet there is no adequate measure for the humus portion of peat, which can only be estimated from the acid-insoluble residue and the pyrophosphate index. The residue shows a comparable range of values for all samples but the two sphagnum bogs (9, 32).

Unexpectedly, the samples are differentiated better by the inorganic contaminants than by the organic content. Since the samples were taken from mining areas, pollution by ore metals was to be expected, and those closest to smelters in Sudbury are indeed contaminated with nickel and copper. But one of the more surprising results is the number of bogs which have high aluminum and potassium concentrations which suggests contamination by clay minerals during periodic flooding. The most notable example of this is in the Noranda area, where sample 25, contains 10 times as much aluminum and potassium as do samples 26 and 27 which are all within 1 km of each other. However, 25 is a grass/carr bog type rather than the rain-fed type of the other two.

The statistical methods used to differentiate the bogs show that canonical variate analysis is able to bunch the bogs into tight geographical groups with 25 characteristics, and even when only three characteristics are used there is still a distinct geographical grouping. Principal component analysis proved to be the best method of distinguishing these bogs, both within and between the geographical groups, since it discriminates the bogs both by their considerable contamination, as well as by the carbohydrate and humus biomass components of the organic soil. The two different methods of cluster analysis used were generally able to classify the bogs by locality, and when projected on to the plan of the principal component axis they were consistent with this analysis also. Although the bogs are different, they form a continuum so a precise division depends on the qualities considered to be important. Locality plays an important part in the differences between the bogs, probably due to the geology of the bedrock.

The inorganic content proved to be the main discriminating feature of the bogs, rather than the organic content, and this is due in part, to the inorganic elements being easier to measure than the organic components. Although there are 14 parameters for each, the inorganic component seems to be more heavily weighted, possibly because many of the parameters are indicative of one property, such as the clay elements. The organic characteristics, on the other hand, are all composed of carbon, hydrogen and oxygen which are difficult to differentiate into carbohydrate and humus. Moreover, it is the organic components of the peat which are important for microbial activity, as they provide the substrate.

Multivariate stepwise regression was able to identify three characteristics, calcium, magnesium and rubbed fibre, which were important in the production of methane in the

bogs we tested. But the data base is too small to place much reliance on this initial result. It does show however, this method is feasible and could be used when more data are available to find which characteristics are important for methane production. This will be important in estimating regional methane emissions from a small number of measuring sites. It will also assist in determining the role of wetlands in the increase of atmospheric methane.

The ability to measure the cellulose content is an advance in the characterization of peat, since cellulose is the major carbohydrate and microbial substrate of the plant biomass. Cellulose is demonstratably reduced with time and depth in the peat, showing that it is gradually metabolized. The present method of measurement of the humus portion of the peat is unsatisfactory, and the traditional methods of measuring the humic component are ambiguous. The nature of humus with its chemical derivation from the lignin and polyphenols of the plant biomass makes it very difficult to characterize, so that new procedures, such as critical carbon dioxide extraction (Chuaqui, personal communication), need to be investigated.

The microbial ecology of muskeg peat bogs is little known, but there is a relatively high population of aerobic bacteria, in the surface layers of the peat, and a surprising number of inactive aerobic bacteria are still to be found in the deeper layers. Peat soils seem to have the ability to preserve a specific aerobic microflora, without necessarily forming spores, in an otherwise anaerobic environment.

All methanogenic bacteria that have been characterized to date require neutral pH for growth, but peat becomes acidic from the accumulation of acidic metabolites during

burial of plant biomass and its degradation. Since ombrotrophic peat bogs do not have the capacity to neutralize this acidity, the pH is between 3 and 4. I have now obtained a vigorous acidophilic, methane-producing culture from Mer Bleue, but so far it has proved impossible to obtain colonies or to isolate a single organism.

Methane is freely produced in laboratory incubations from field samples of the top layers of peat, but in incubations of peat from deeper levels the production of methane is inhibited. This inhibition does not appear to be permanent for, if the peat is kept and periodically flushed for long enough (sometimes for over 12 weeks), an equivalent amount of methane to that from the surface samples, can be obtained. The removal of the inhibition is promoted by autoclaving peat from deeper layers in mixed incubations, and by flushing the vials, which suggest some volatile component(s) is removed. Analysis by gas chromatography has not so far been able to identify this compound(s).

The near-surface layers of the peat produce methane at a fairly rapid rate (40 nmol g^{-1} h^{-1}). Part of this methane may be bacterially oxidized in the surface layers, and part is probably vented unaltered to the atmosphere, but some of this methane appears to remain buried within the peat itself. Methane is also slowly produced in the lower layers (2.5 nmol g^{-1} h^{-1}) where it accumulates *in situ* until in time one extracted litre of mixed water and gas can contain over 8 mmol methane. The amount that was extracted from the grid in Mer Bleue varied considerably from site to site, indicating that the peat structure and composition is very heterogeneous. Where the peat is deep enough, the methane is unable to escape to the atmosphere and appears to remain where it is produced. Most of this methane must be in the gaseous state since it is only sparingly soluble in water. In consequence it is likely that gas bubbles block the pore spaces in the peat and prevent the

movement of both gas and water. The results support this suggestion that the accumulation of methane is one of the main causes of the low hydraulic conductivity of peat, so that once the peat becomes waterlogged, the presence of this methane renders the peat impermeable, initiating the formation of an ombrotrophic bog.

The effect that this occluded methane could have on the atmosphere is considerable. I have calculated that 1.7 Gg or 28 g m^{-3} is trapped in Mer Bleue. If similar amounts are found in all such wetlands, then 144 Tg of methane could be entrapped world-wide. If the peat is ploughed up for farming or is mined for fuel, as is increasingly happening today, this entrapped methane will be released into the atmosphere. Even though the atmospheric concentration of methane is only 0.5% of that of carbon dioxide, it has a much greater influence, per molecule, on the greenhouse effect, as it aborbs at an infrared wavelength that is not adsorbed by other atmospheric gases. Methane is known to be increasing by about 1% per year, a third of which is fossil methane (depleted in ^{14}C). This entrapped methane in the temperate peatlands has been accumulating for a long time, so that its release today may be the source of much of the atmospheric fossil methane. Prevention of further destruction of wetlands would not only conserve them but could also significantly reduce one of the gasses causing the greenhouse effect.

The major accomplishments of this thesis have been:

1. A method of analysis of the cellulose content of peat, which can be used as a measure of its potential biodegradability.
2. The differentiation of wetland organic soils by statistical methodology, which could have important practical applications in the classification of these soils, and in determining the characteristics important to methane emissions from

different wetlands.

3. A part in the design and testing of sampling tubes to measure methane within peat bogs.
4. Identification and description of the heterogeneity of a large pool of methane within a local bog, and information on the effect this could have both on the hydraulic conductivity of ombrotophic bogs, and if released, to the atmosphere.
5. The inter-correlation of remnant cellulose content, field methane measurements and laboratory methane incubations.
6. The growth of a unique enrichment culture of an active acidophilic methanogen.

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APPENDICES

1. Publications from the thesis
2. Descriptions of some of the bogs
3. Methods of analysis
4. Probability graphs
5. Scatterplot matrices
6. Cluster analysis of inorganic characteristics
7. Respiration measurements
8. Redox measurements
9. Methane extractions
10. Methane incubations
11. Mixed incubations
12. Calculations

APPENDIX 1

PUBLICATIONS

1. D.J. KUSHNER, A. BROWN and T. KAURI
Microbial control of acid leaching from mine tailings: report submitted to CANMET, Energy Mines and Resources, Ottawa, pp38 with appendices, September 1986.

2. TIIU KAURI, ANN BROWN and DONN J. KUSHNER
Physiological groups of bacteria in muskeg bogs: presented at ASM Annual Meeting, Atlanta, Georgia, 1-6 March 1987.

3. ANN BROWN, D.J. KUSHNER and S.P. MATHUR
Amelioration of mine tailings with local peat soils in southern Canadian Shield: presented at the Twelfth Annual Meeting, Canadian Land Reclamation Association, Laurentian University, Sudbury, Ontario, 179-188, 7-11 June 1987.

4. A. BROWN, D.J. KUSHNER and S.P. MATHUR
Feasibility of assessing metal pollution in Canadian Shield mining areas through analysis of peat soils: presented at Symposium '87 Wetlands/Peatlands, Edmonton, Alberta, 299-305, 23-27 August 1987.

5. D.J. KUSHNER, T. KAURI and D.A. BROWN
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11. ANN BROWN, S.P. MATHUR, ANTON BROWN and D.J. KUSHNER
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An acidophilic methane-producing enrichment culture: presented at CSM Annual Meeting, Laval, Quebec, 11-15 June 1989.

13. S.P. MATHUR, A. BROWN, H. DINEL, A. BUTTLER and M. LÉVESQUE
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APPENDIX 2

DESCRIPTIONS OF BOGS

Table 1 shows the types and locations of the bogs investigated in this thesis, photographs and descriptions of nine of the muskeg bogs have been appended, arranged from the most minerotrophic fen to the most ombrotrophic bog.

Bog number 8 is a shore fen located at the edge of a small lake, and is subject to flooding. The well humified peat is shallow, only 20 cm deep, over glacial-fluvial sand on top of clay. The vegetation is blueberry and sedge with a few old large stumps.

Bog number 5 is a horizontal fen with a flat surface in a shallow depression between sandy ridges, the humified peat is 65 cm deep on top of gravel. The vegetation is blueberry and sphagnum hummocks with alder interspersed with grasses and reeds. It was flooded when visited.

Bog number 25 is a flat bog subject to flooding, with 70 cm of well decomposed peat. Vegetation is very high grass, some sphagnum with swamp maple and aspen.

Bog number 7 is a flat bog not subject to flooding, with peat deeper than 50 cm. The vegetation is typical of this type of bog with sphagnum in hummocks, cotton grass and blueberry, and with an open arborial vegetation of white pine, black spruce and tamarack.

Bog number 16 is similar to number 7 but with a much great cover of arborial vegetation of spruce, tamarack, balsam and cherry, the understory is of sphagnum, labrador tea and blueberry. The peat is well rotted and woody to a depth of 80 cm depth.

Bog number 10 is a basin bog with a 30 cm top layer of moderately degraded peat, this is underlain by alternating organic and sediment layers for a further 40 cm, below which there is a clay layer containing fibres. The peat is a mixture of grass reed and sphagnum with old tamarack stumps.

The Gatineau Park bog is a 14 ha basin bog of up to 9 m depth with a flow of water under the peat from the surrounding hills and is possibly floating. The arboreal vegetation of the Gatineau Park bog (75°47'W, 45°27'N) consists mainly of *Picea mariana* (Mill.) BSP., *Pinus strobus* L. and *Larix laricina* (Du Roi) Koch. The shrub vegetation is composed of *Ledum groenlandicum* Retsius, *Chamaedaphne calyculata* (L.) Moench, *Kalmia angustifolia* L., and *Aronia melanocarpa* (Michx.) Ell., *Sphagnum* spp., *Sarracenia purpurea* L., *Vaccinium oxycoccos* L. and *V. macrocarpon* Ait. constitute the ground vegetation (Lévesque et al 1980). The peat type is mainly undecomposed *Sphagnum* spp. mixed with ericaceous material and some sedge remnants.

Bog number 1 is a true floating bog with moderately humified peat of greater then 3 m, the lower layer of which is loose peat. Tamarack and spruce are growing to greater than 20 m, with a sphagnum, brown moss, and grass under story.

Bog number 9 is a mound bog with isolated mounds of sphagnum and blueberry in a fen, which at the time of the last visit was flooded to such an extent it was impossible to cross the lagg stream. It appeared to be flooded due to beaver activity. The peat is a sphagnum peat.

The Mer Bleue deposit is a typical ombrotrophic domed bog and has an open arboreal vegetation, composed of *Larix laricina* (Du Roi) Koch., *Picea mariana* (Mill.) BSP and *Betula pumila* L. The shrub vegetation is dominated by ericaceous spp. such as *Chamaedaphne calyculata* (L.) Moench., *Vaccinium angustifolium* Ait., *Ledum groenlandicum* Retzius and *Andromeda glaucophylla* Link. The ground vegetation consists mainly of *Eriophorum* spp. and other Cyperaceae; *Sphagnum* spp., *Vaccinium oxycoccos* L. and *Sarracenia purpurea* L. The peat material here is a moderately decomposed mixture of sedge and wood in a more decomposed matrix. This is a large deposit of about 2500 ha with a maximum depth of 7 m.

Table 1. Locations and vegetation of sampled bogs.

MUSKEG TYPES

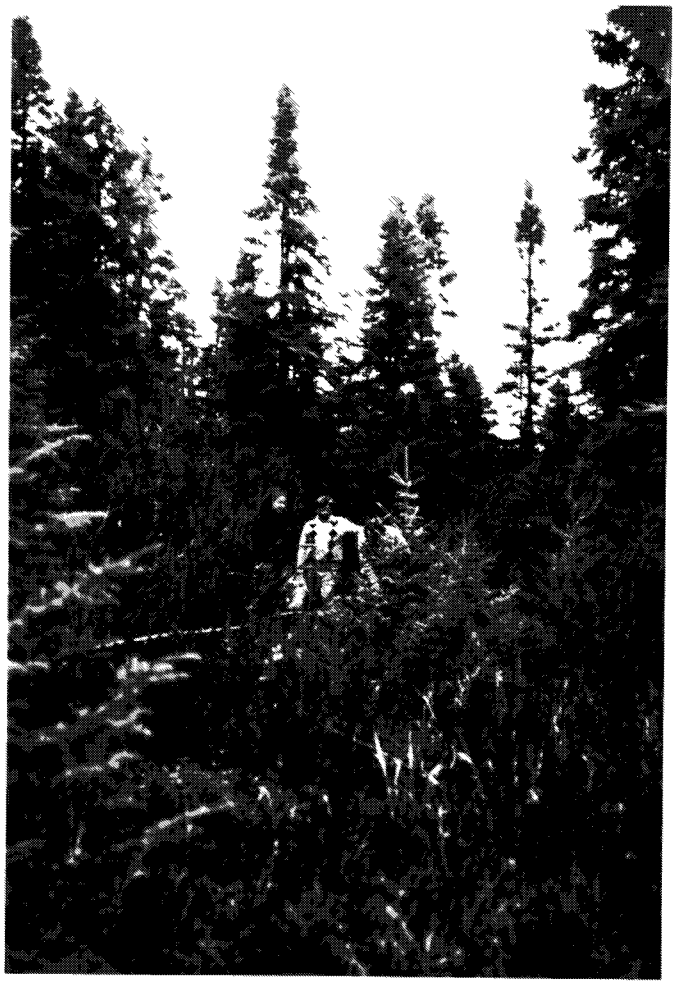
Sample #	Meridinal Coordinates	Soil type	Depth	Vegetation							
				sphagnum	moss	sedge	reed	cotton grass	labrador tea	tamaracl	other
1	80° 07' 15" W 46° 27' 45" N	typic mesisol	<160cm		+	+					
2	80° 46' 0" W 46° 27' 45" N	terric mesisol	40-160cm		+	+					
4	80° 49' 0" W 46° 34' 15" N			+				+			cattail
5	80° 39' 0" W 46° 32' 45" N	terric humisol	40-160cm		+	+					wood
6	80° 39' 0" W 46° 34' 30" N	terric humisol	90-160cm	+							grass
7	80° 36' 15" W 46° 37' 15" N	terric mesisol	40-160cm			+			+	+	
8	80° 37' 15" W 46° 40' 15" N	terric humisol	40-160cm	+			+	+	+	+	wood
9	80° 19' 30" W 46° 23' 15" N	terric humisol	40-160cm	+			+		+		wood
10	80° 0' 30" W 46° 28' 0" N	typic mesisol	>160cm	+			+	+			wood
11	80° 26' 15" W 46° 18' 30" N	terric humisol	40-160cm		+	+					
12	80° 47' 15" W 46° 17' 45" N	typic mesisol	160cm		+	+		+			
13	80° 53' 30" W 46° 25' 45" N	terric humisol	40-160cm		+	+	+				
15	80° 40' 45" W 46° 31' 50" N	typic humisol	>150cm	+					+		spruce
16	80° 38' 30" W 46° 32' 30" N	typic mesisol	40-160cm								
17	80° 38' 15" W 46° 31' 15" N	terric fibrisol	40-160cm								
18	80° 43' 30" W 46° 32' 0" N	fibric mesisol	>160cm	+							woody
19	80° 55' 15" W 46° 32' 45" N	terric mesisol	40-160cm								woody
20	81° 08' 0" W 48° 31' 15" N	mesic fibrisol	<60cm								
21	81° 22' 15" W 48° 33' 0" N	typic humisol	>160cm								
22	80° 18' 30" W 48° 32' 0" N	typic mesisol	>160cm	+					+		lichen
23	80° 13' 10" W 48° 31' 50" N	typic fibrisol	>160cm								woody
24	79° 0' 0" W 48° 32' 0" N	mesisol						+		+	woody
25	79° 09' 30" W 46° 09' 15" N	humisol	40-160cm	+				+			swamp maple
26	79° 09' 30" W 48° 06' 15" N	fibrisol	>160cm	+					+		spruce
27	79° 08' 30" W 48° 06' 45" N	mesisol	>160cm	+					+	+	spruce
28	75° 30' 0" W 45° 28' 0" N	humisol	>160cm	+					+	+	bog rosemary
29						+					
30											
31											grass
32				+							
33											weed

BOG NUMBER 8

BOG NUMBER 5

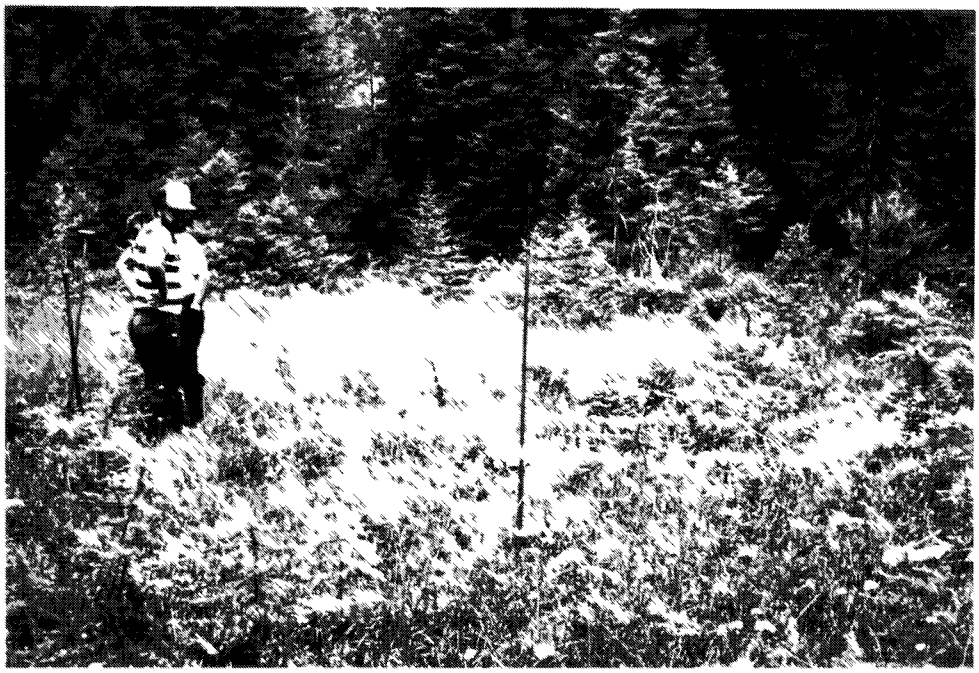
BOG NUMBER 25

BOG NUMBER 7

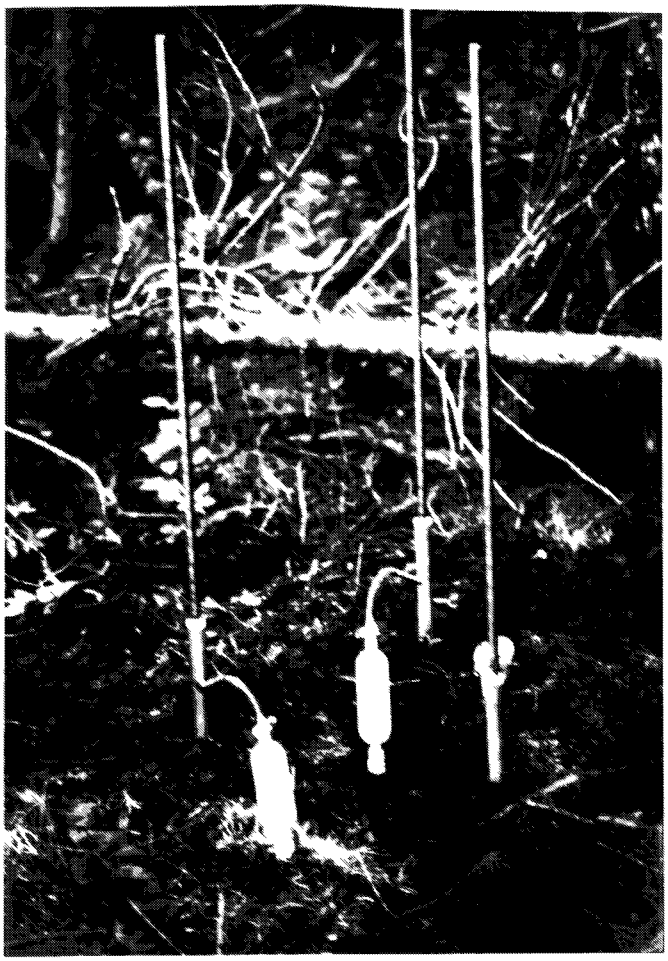


BOG NUMBER 16

BOG NUMBER 10



GATINEAU PARK



BOG NUMBER 1

BOG NUMBER 9

MER BLEUE

APPENDIX 3

METHODS OF ANALYSIS

The different characteristics of muskeg soils can be divided into physical, metals or inorganic, organic aromatic and organic carbohydrate groups, this division is shown in Table 1. The following methods are mainly from 'Analytical Methods Manual 1984': B.H. Sheldrick, Editor; Research Branch, Agriculture Canada.

pH. One gram of sample is mixed in duplicate with 2 ml of 0.01M calcium chloride, allowed to equilibrate for half an hour then measured with a pH electrode.

Eh. Similar to above except the electrode was a platinum type combination redox electrode (Fisher).

Moisture. This has been determined in both air-dried samples which have then been ground to mesh #20 in a Whiley mill, and samples dried only to a moist state. Drying to two different states is necessary for different tests: where a finely ground material is required, and the physical state is not important, then air-dry samples are used; when the physical state is important, such as the water holding capacity, then the moist samples are used. Samples were dried in weighed metal weighing tins, with lids, overnight at 105°C, and cooled in a desicator to constant weight. Reported results are an average of duplicate samples. All percentages are calculated from the dry weight of the soil.

Water Holding Capacity. Soils are placed in plastic Buchner-style funnels and saturated with water overnight. They are then placed over a 3 bar suction for 20 minutes, the resultant moist soil is then transferred to metal weighing tins, weighed and dried as above. The

Table 1. Methods of analysis for different bog characteristics.

	CHARACTER	METHOD	USE
PHYSICAL	pH	meter	acidity
	Eh	meter	redox
	% moisture	oven dry	calculation of dry weight
	% water holding capacity	dry by suction	adsorption of water
	cation exchange capacity	barium acetate	adsorption of cations
	phosphatase	p-nitrophenol phosphate	abiotic enzymes
METALS	Cu, Ni, Pb, Zn	atomic absorption	smelter contamination
	Ca, Mg, Fe, Mn, Al, K	atomic absorption	sediment and groundwater contamination
AROMATIC	pyrophosphate index	spectrophotometric	humification
	E4:E6 ratio	spectrophotometric	aromaticity
	acid soluble lignin	hydrolysis	lignin
CARBOHYDRATE	C, N, H, S	catalytic	organic matter
	% ash	muffle furnace	organic matter
	unrubbed fibre	by hand	amorphous material
	rubbed fibre	by hand	degradation potential
	respiration	titration	rate of degradation
	cellulose	hydrolysis	substrate

results are again an average of duplicate samples. Since bogs are normally water-logged, it is important to know how much water the sample can hold when wetting for respiration experiments.

Ash. A suitable amount of ground air-dry sample, in duplicate, is weighed into a tared porcelain crucible. Dried overnight at 105°C and reweighed, then put into a muffle furnace at 550°C, again overnight. When the crucibles are removed, they are put into a desiccator, cooled and reweighed.

Carbon, Nitrogen and Hydrogen. Using Perkin Elmer elemental analyser, model 240. Metal and transition elements The soils were extracted by digestion with nitric, perchloric and hyrofluoric acids, and then assayed by atomic absorption using certified standards for calcium, magnesium, potassium, iron, aluminium, copper, lead, zinc, nickel and manganese.

Fibre Content. This is determined using a modified 5 ml plastic hypodermic syringe, made by cutting away half of the cylinder wall, in a longitudinal direction between the zero and the 5 ml marks, the rest is not altered. The moist sample is cut into about 5 mm lengths, and then packed into the modified syringe so the surface is level with the cut edge, pressing hard enough to expel the air but not the water. All the soil material from the syringe is transfered to a 100 mesh sieve. The sample is washed under running water until it runs clear; then colleced together and dried with a paper towel, it is then repacked into the modified syringe and the plunger pushed down to the smallest volume levelling the surface with a spatula. This volume is the unrubbed fibre. This process is repeated, but this time the fibre is rubbed between finger and thumb under the water. The volume in the repacked syringe is the rubbed fibre. All these tests were done in triplicate.

Cation Exchange Capacity. Half a gram of soil is weighed into a 250 ml Erlenmeyer flask, 50 ml of 0.5N hydrochloric acid added and shaken for 30 minutes. Material is spun down in a centrifuge, and the precipitate washed with 100 ml portions of water until there is no precipitate when 1% silver nitrate is added to the supernatant, showing there is no more free chloride ion. The filtrate is discarded. The precipitate is treated with 100 ml 0.5N barium acetate and shaken for 15 minutes. The samples are again centrifuged and washed with three lots of 100 ml of water. The washings are titrated against 0.1N sodium hydroxide using phenolthalein as indicator. The sodium hydroxide was standardized using 10 ml of acid potassium phthalate solution. Triplicate samples were used for this assay.

Pyrophosphate Index. A quarter of a gram of the dry soil is weighed into screwtop plastic tubes and extracted with 0.025M sodium pyrophosphate by shaking overnight, it is then spun down, the supernatant diluted 5 times and the absorption read at 550 nm.

E4:E6 Ratio. The above extraction is measured at 465 nm and 665 nm, and the ratio calculated between these two readings.

Phosphatase. Weigh out 25 mg of air-dryed, ground soil in quadruplicate, and place in a 50 ml Erlenmeyer flask.

Add 4 ml of modified universal buffer (MUB), and 1 ml toluene.

Heat in a bath at 37°C for 10 mins.

Add 1 ml of Sigma 104 p-nitrophenyl phosphate (2.2 g in 50 ml MUB) to 3 out of 4 flasks.

Incubate with shaking 1 hour.

Stop the reaction with 1 ml calcium chloride (0.5 M) and 4 ml sodium hydroxide (1.5 M).

Add 1 ml Sigma 104 after incubation to the fourth flask.

Filter through a Whatman #41 paper into a 25 ml volumetric flask.

Rinse, wash and make up to volume.

Read the colour at 420 nm. and calculate the p-nitrophenyl content from a standard curve.

Standard curve: use p-nitrophenol standard solution containing 0.1 to 0.8 μmoles ml^{-1}. Add calcium chloride and sodium hydroxide as above and read.

Controls: run 6 , all without soil; 3 incubated with Sigma 104, and 3 with Sigma 104 added after sodium hydroxide. Add the difference between these to the blank incubated without Sigma 104 but with sample.

MUB stock solution:

Tris base	12.1 g
Maleic acid	11.6 g
Citric acid	14.0 g
Boric acid	6.28 g
NaOH (1:1)	26 ml
Distilled water	1000 ml pH 6.5, dilute 1:5 for use.

(Tabatabai and Bremner 1969).

Cellulose Assay. Air-dried peat ground to 20 mesh on a Wiley mill was used for this assay; 0.1 g was weighed into a small test tube, and 2 ml 72% sulphuric acid added, the tube was vortexed, then another 1 ml of the same sulphuric acid added to wash the sides of the tube, these were incubated at 35°C for one hour. The contents of the tubes were then washed into 160 ml anaerobic Wheaton vials with 84 ml distilled water, to give 3% sulphuric acid, capped and autoclaved for one hour at 121°C. When cool, the contents of the vials were filtered under suction through weighed medium sintered glass funnels and economically washed with distilled water, the filtrate and the washings were made up to 100ml in volumetric flasks. These assays were done in duplicate.

Acid-insoluble Residue: The funnels were dried overnight at 60°C, cooled and reweighed.

This gave the acid-sinsoluble residue.

Acid-soluble Lignin. The filtrate was measured without delay spectroscopically at 205 nm, and the acid-soluble lignin is determined by the absorbance divided by 110, times the dilution if any.

Sugar Assay. The Dubois assay was used to determine the sugars present in the filtrate, this generally required 1:10 dilution.

Aerobic Microbial Samples. Soil samples were suspended in Winogradsky's standard salt solution and homogenized in a ATO-mix for 3 min. A dilution series ($1x10^{-1}$, $1x10^{-2}$, $2x10^{-3}$, $1x10^{-3}$, $2x10^{-4}$, $1x10^{-4}$) was prepared using the same salt solution. These dilutions were used for plating on to soil extract-agar and for the most probable number technique.

Winogradsky's standard salt solution:

K_2HPO_4	0.25 g
$MgSO_4$	0.125 g
$NaCl_2$	0.125 g
$Fe_2(SO_4)_3$	0.0025 g
$MnSO_4$	0.0025 g
Distilled water to	1000 ml, pH 6.7

Soil-extract agar

Glucose	1.0 g
Peptone	1.0 g
Yeast extract	1.0 g
K_2HPO_4	10.0 g
Agar	16.0 g
Soil extract	400 ml
Distilled water	600 ml, pH 6.5-6.7
Cycloheximide	50 μg ml^{-1} (filter sterilized)

The soil extract is prepared by boiling 1 kg soil with 1 l distilled water for 45 min and filtering until clear.

The soil extract agar plates are used for plate counts. Five replicates were prepared for each dilution and incubated at 20°C for 2 weeks.

Chitin hydrolysis

KH_2PO_4	0.03 g
KNO_3	4.0 g
$MgSO_4.7H_2O$	0.6 g
$FeCl_2.6H_2O$	0.05 g
$ZnSO_4.7H_2O$	0.03 g
Purified chitin	3.0 g
Agar	16.0 g
Distilled water	1000 ml, pH 7
Cycloheximide	50 μg ml^{-1} (filter sterilized)

The chitin agar was poured on to a thin layer of water agar in petri dishes. The chitin caused a marked opalescence in the plates. After incubation at 20°C in plastic bags, the plates were screened for clear zones around colonies.

Cellulose degradation

NH_4NO_3	2.0 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
$CaCl_2.2H_2O$	0.02 g
$FeSO_4.7H_2O$	0.02 g
$MnSO_4.2H_2O$	0.02 g
Agar	15.0 g
Distilled water	1000 ml, pH 7.3-7.5
Cellulose (acid-swollen)	5.0 g
Cycloheximide	50 μg ml^{-1} (filter sterilized)

Degradation of cellulose is determined in a 2-layer medium. The overlay has the addition of cellulose and cycloheximide to the basic agar.

Starch hydrolysis

K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
$(NH_4)_2SO_4$	0.5 g
Soluble starch	2.0 g
Agar	12.0 g
Distilled water	1000 ml, pH 7
Cyclohemimide	50 μg ml^{-1} (filter sterilized)

After incubation at 20°C the plates are flooded with Lugol's iodine solution. Starch hydrolysis shows colonies in clear zones against a blue background.

Pectin hydrolysis

K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
$(NH_4)_2SO_4$	0.5 g
Pectin	4.0 g

Agar	15.0 g
Distilled water	1000 ml
Cycloheximide	50 μg ml^{-1} (filter sterilized)

After incubation at 20°C the plates are flooded with a water solution of 2% cetavlone (N-acetyl-N,N,N-trimethylammonium-bromide).

Ammonification

Tryptone	2.0 g
K_2HPO_4	0.3 g
$NaCl_2$	5.0 g
Bromthymol blue	0.03 g
Agar	12.0 g
Distilled water	1000 ml, pH 7.1
Cycloheximide	50 μg ml^{-1} (filter sterilized)

Ammonification is shown by a dark blue colour after incubation at 20°C for 5 to 7 days.

Acid production from sugars

Peptone	2.0 g
$NaCl_2$	5.0 g
K_2HPO_4	0.3 g
Bromthymol blue	0.03 g
Sugar*	10.0 g (filter sterilized)
Agar	12.0 g
Distilled water	1000 ml
Cycloheximide	50 μg ml^{-1} (filter sterilized)

* Any of the following sugars may be used: arabinose, cellobiose, galactose, lactose, maltose, mannose, rhamnose, sucrose, and xylose. Acid production is shown by a change of colour by the indicator to yellow, after incubation at 20°C for 5 to 7 days.

APPENDIX 4

PROBABILITY GRAPHS

The cumulative probability graphs (Sinclair 1981) of the characteristics which were not included in the main body of the thesis are shown here. These include inorganic characteristics of nickel, lead, aluminum, and manganese (Figure 1); carbohydrate organic characteristics of unrubbed and rubbed fibre, cellulose, and respiration (Figure 2); aromatic organic characteristics of PP, E4:E6, ASL and residue (Figure 3); and WHC, phosphatase and magnesium in Figure 4.

Figure 1. Probability graphs: A - Nickel, B - Lead, C - Aluminium, and D - Manganese.

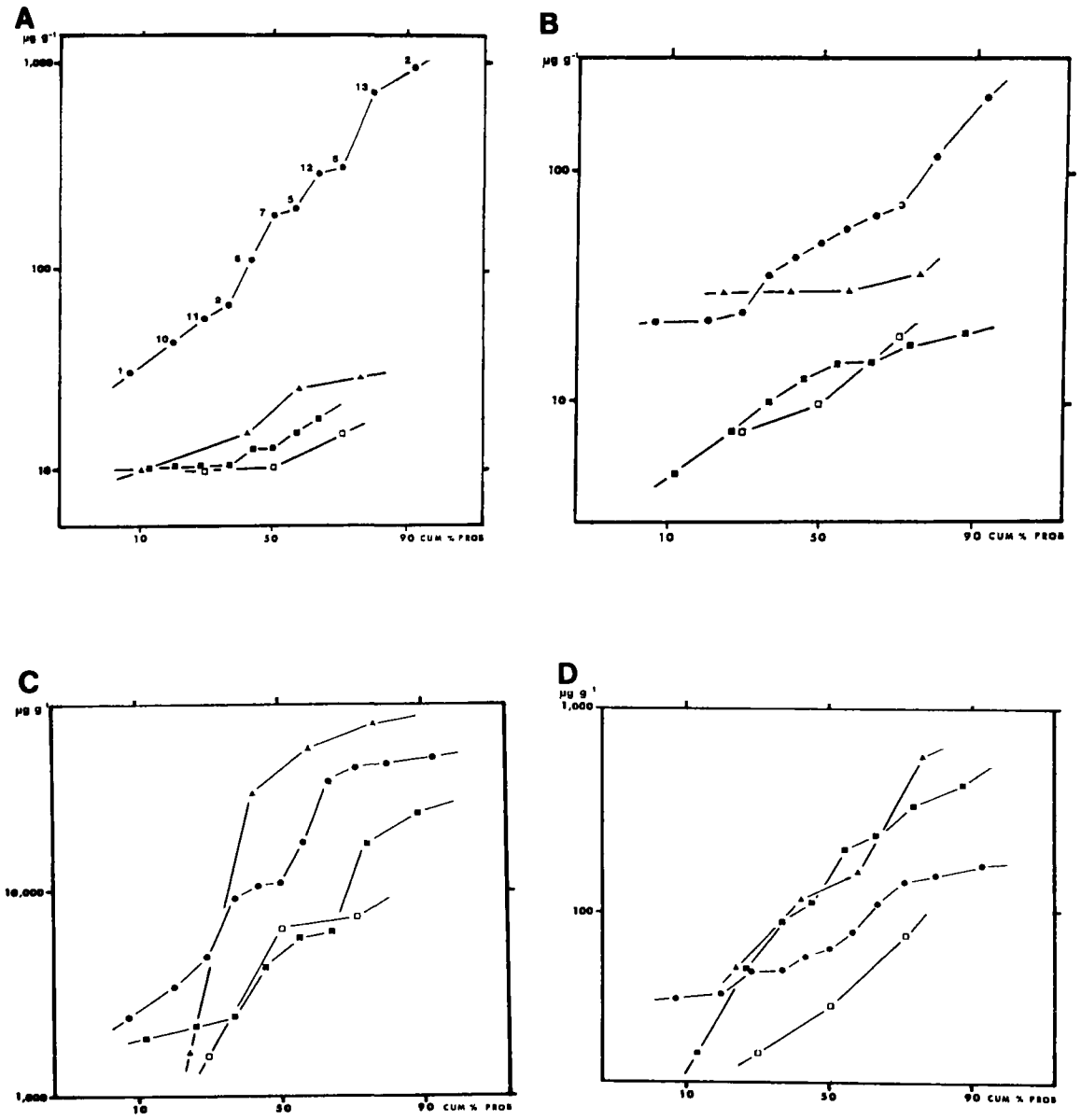


Figure 2. Probability graphs: A - Unrubbed fibre, B - Rubbed fibre, C - Respiration, and D - Cellulose.

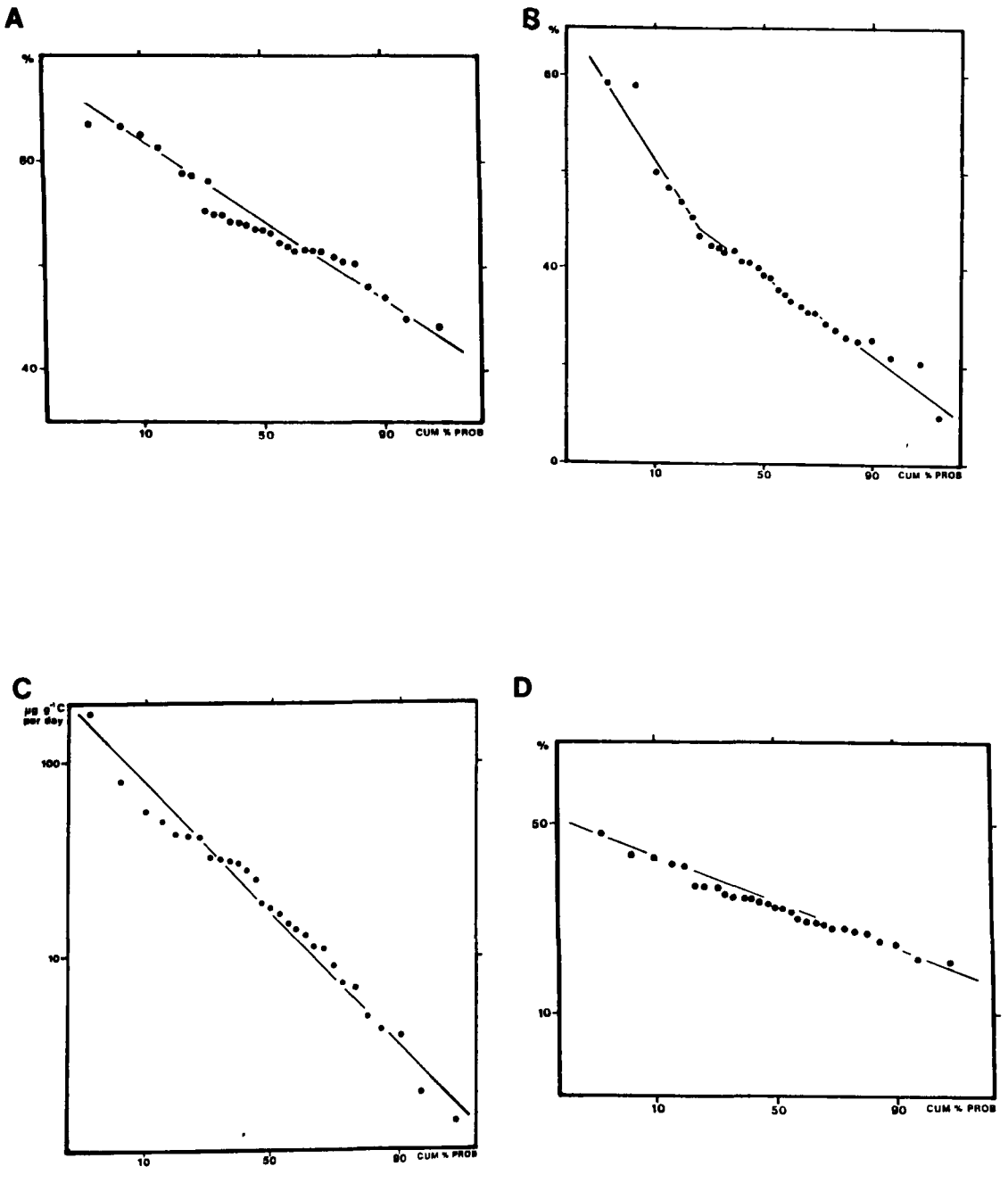


Figure 3. Probability graphs: A - Pyrophosphate, B - E4:E6 ratio, C - Acid-soluble lignin, and D - Residue.

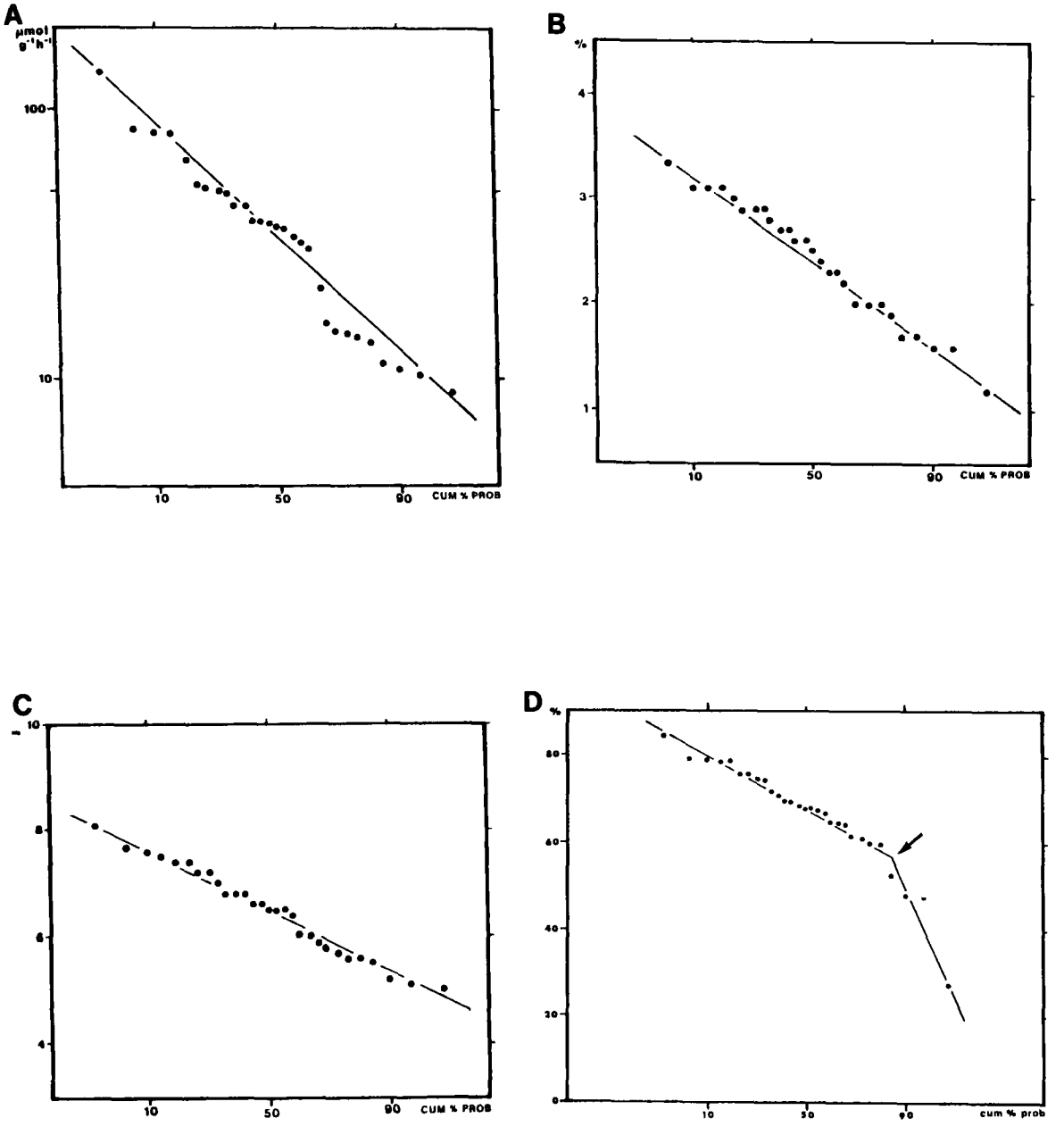
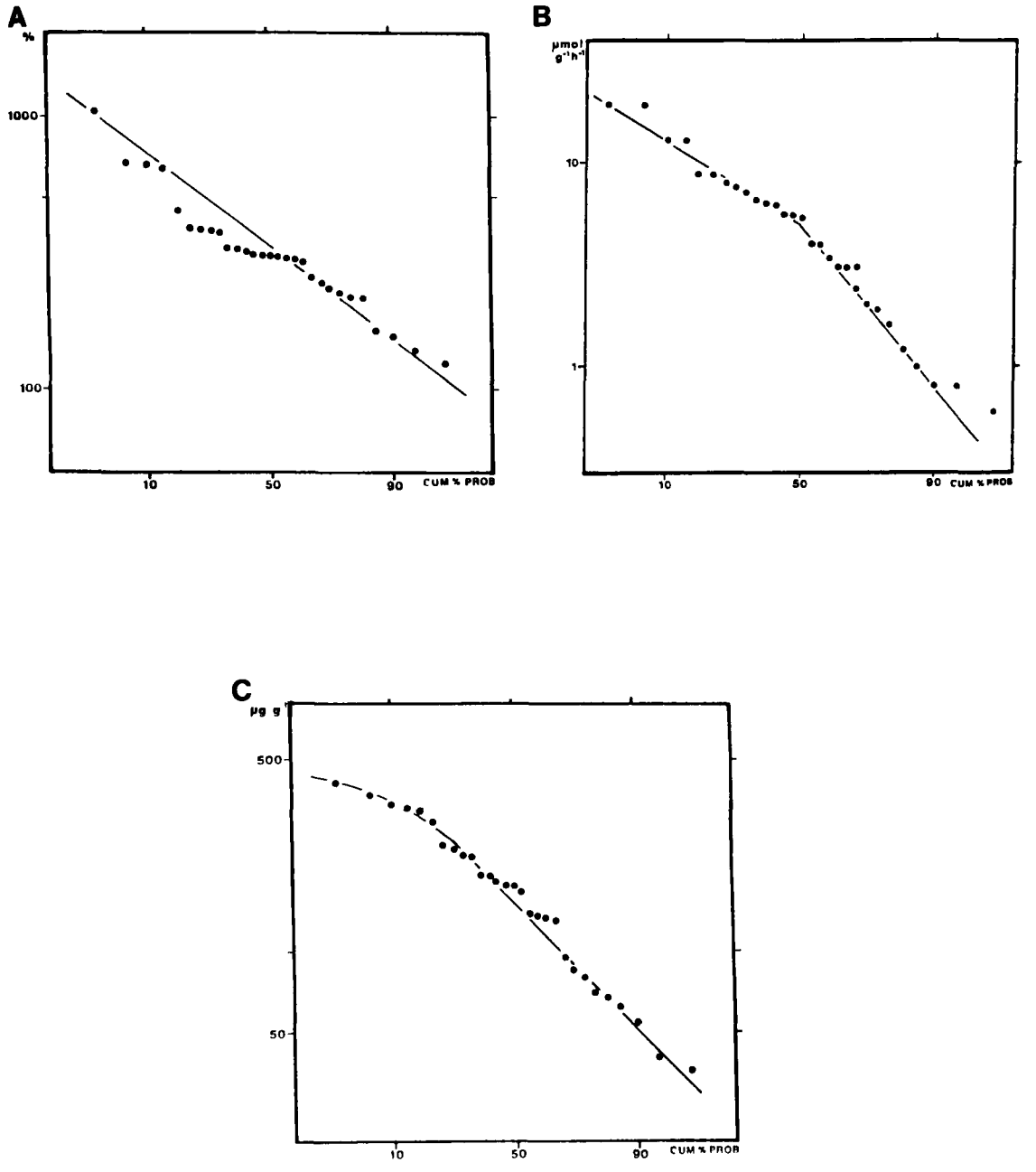


Figure 4. Probability graphs: A - Water-holding capacity, B - Phosphatase, and C - Magnesium.



APPENDIX 5

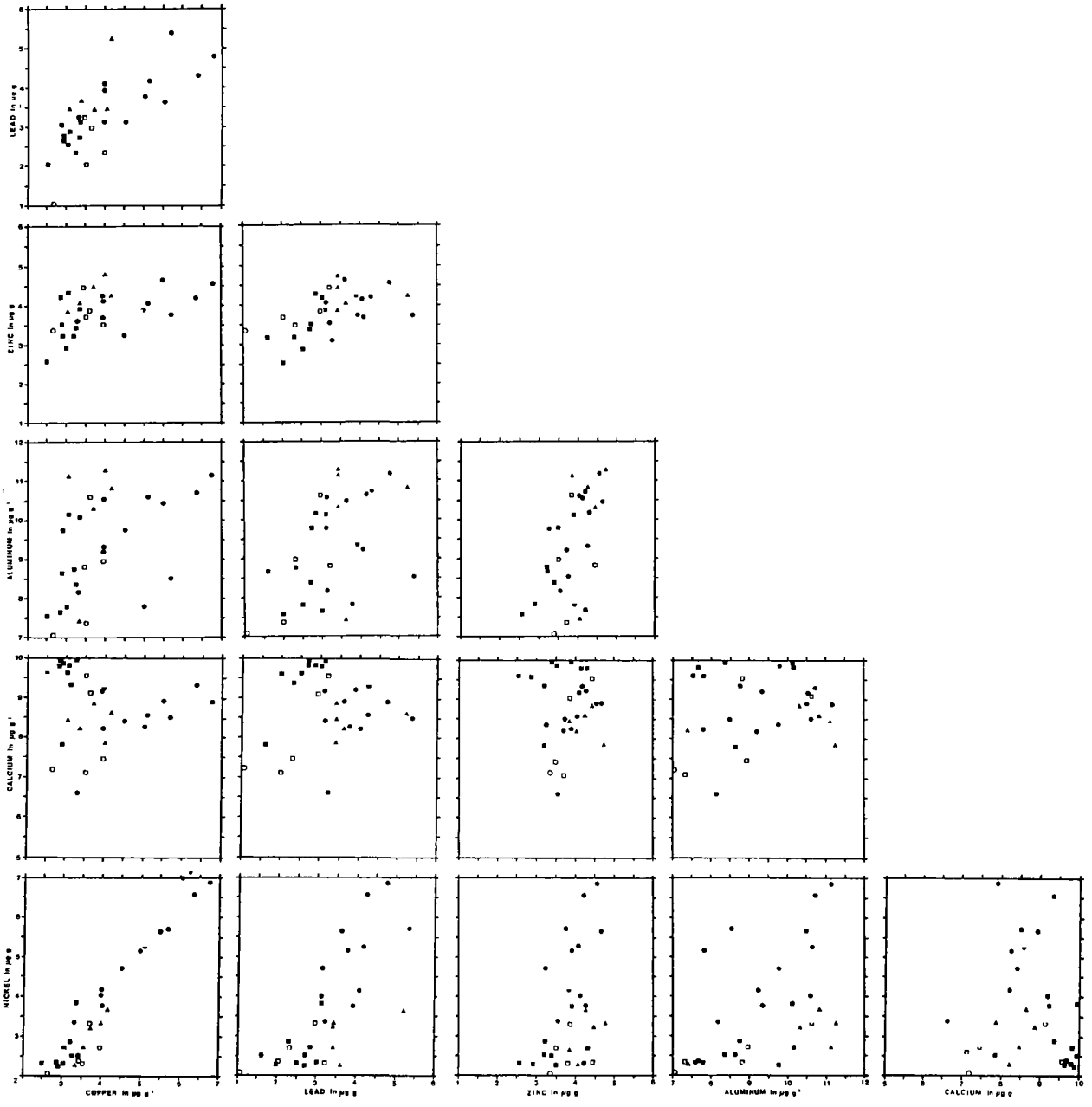
SCATTERPLOT MATRICES

Scatterplots are arranged in a matrix with shared scales so that each variable can be compared with all the other variables plotted in that matrix. There are five matrices shown here, which may be roughly divided into: 1. commercial metal contaminants; 2. other inorganic, mainly clay, contaminants; 3. the cellulose component of peat; 4. the humus component of peat; and a final matrix 5. including the different groups, of copper (commercial metal), aluminum (clay), calcium (pH), cellulose (substrate), CEC (active site) and phosphatase.

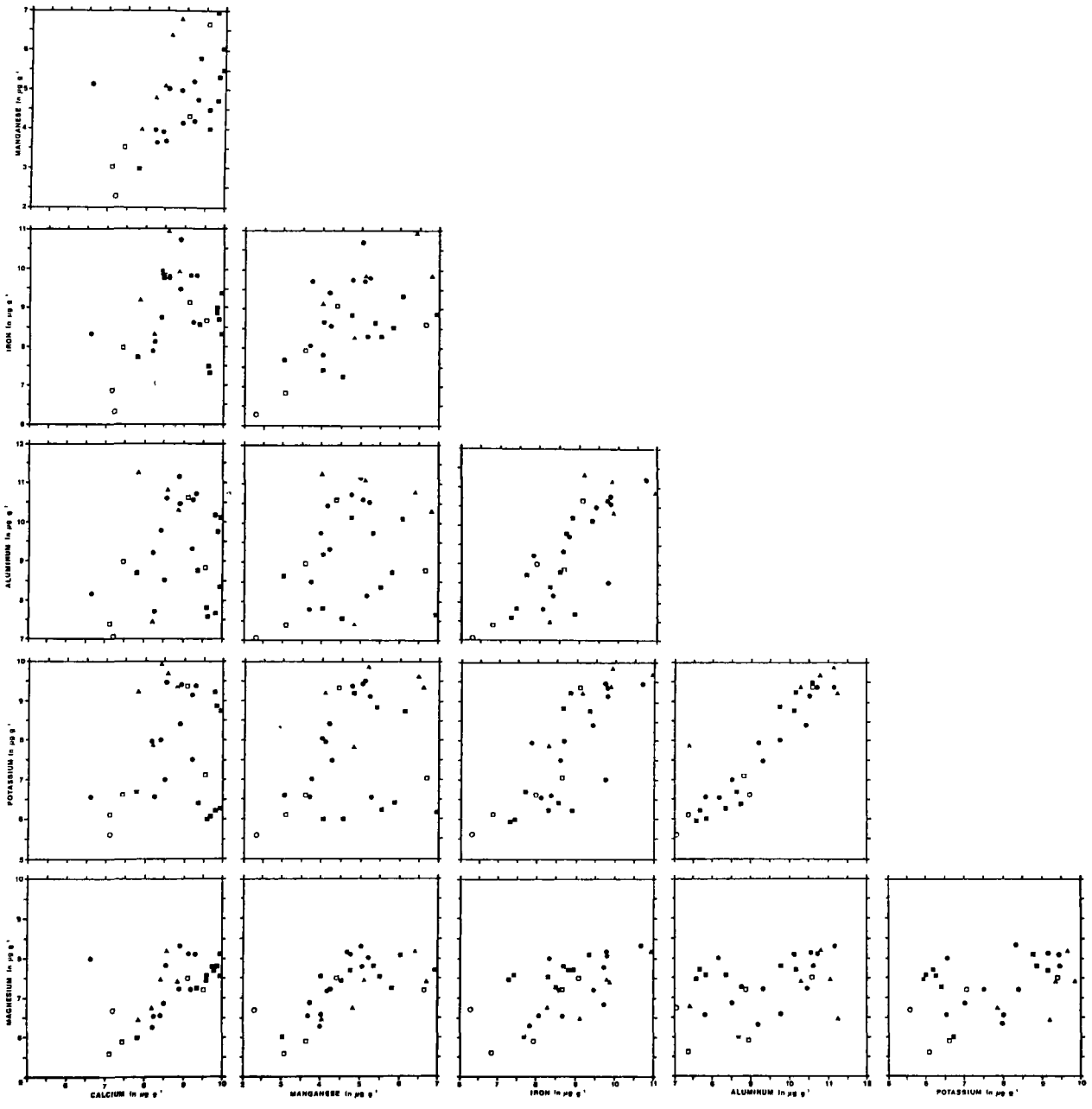
Some of the characteristics have strong bivariate relationships, such as copper and nickel, calcium and magnesium, cellulose and respiration and CEC and residue. Others have a less strong correlation such as lead and zinc, iron and manganese, hydrogen with unrubbed fibre and copper and aluminum. Hydrogen shows two lines of correlation with both CEC and E4:E6, and the relationship between copper and phosphatase is not linear. There are also less close relationships between aluminum and zinc and aluminum and iron, hydrogen with unrubbed fibre but not rubbed, and CEC and aluminum and CEC and calcium.

The correlation coefficients of these bivariate plots are used in the various statistical programs, but it is not possible to decipher from the correlation matrix the various relationships of each individual character. The scatterplots show these in a much clearer way.

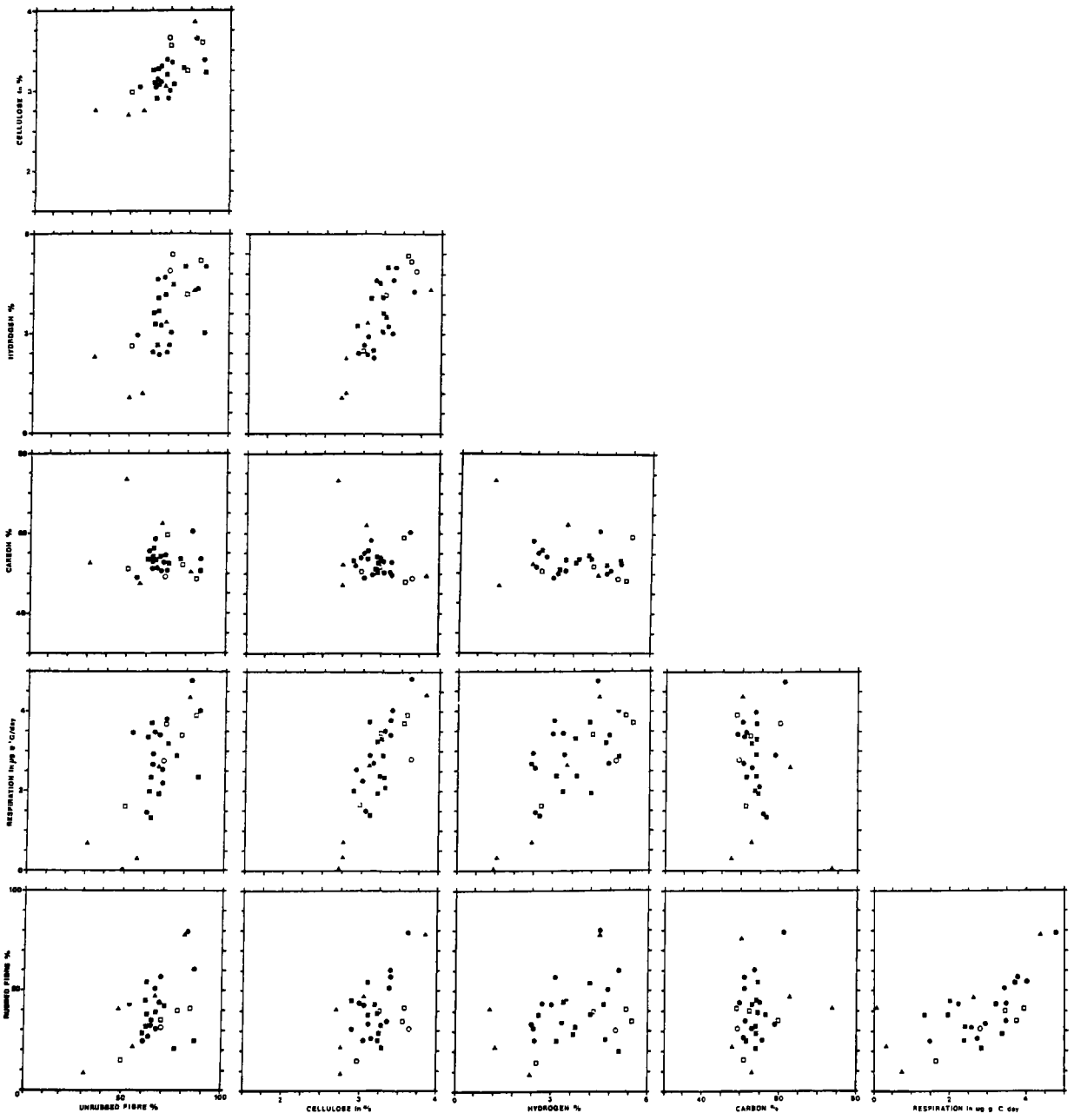
Scatterplot 1. Ore metals.



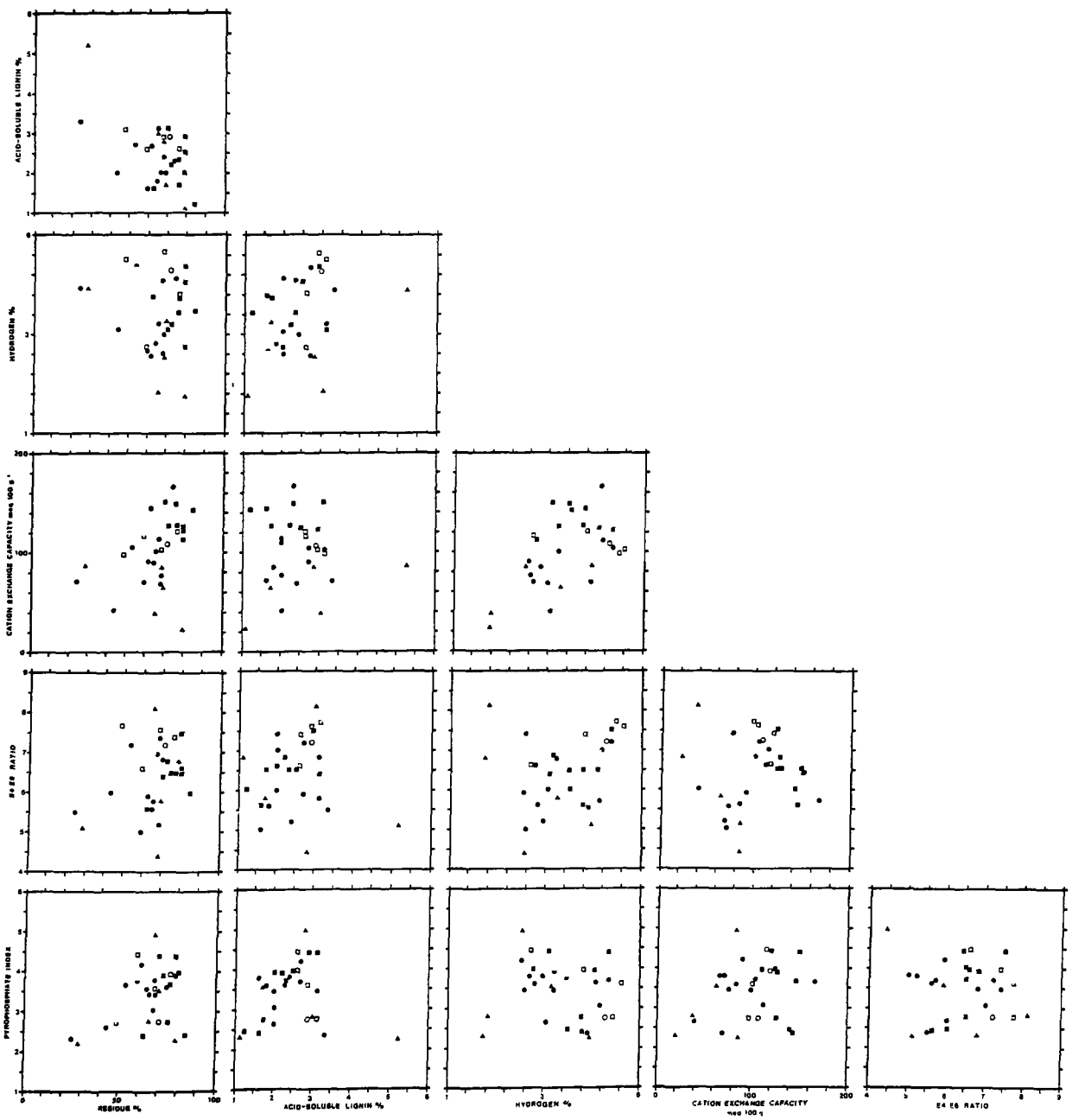
Scatterplot 2. Inorganic characteristics.



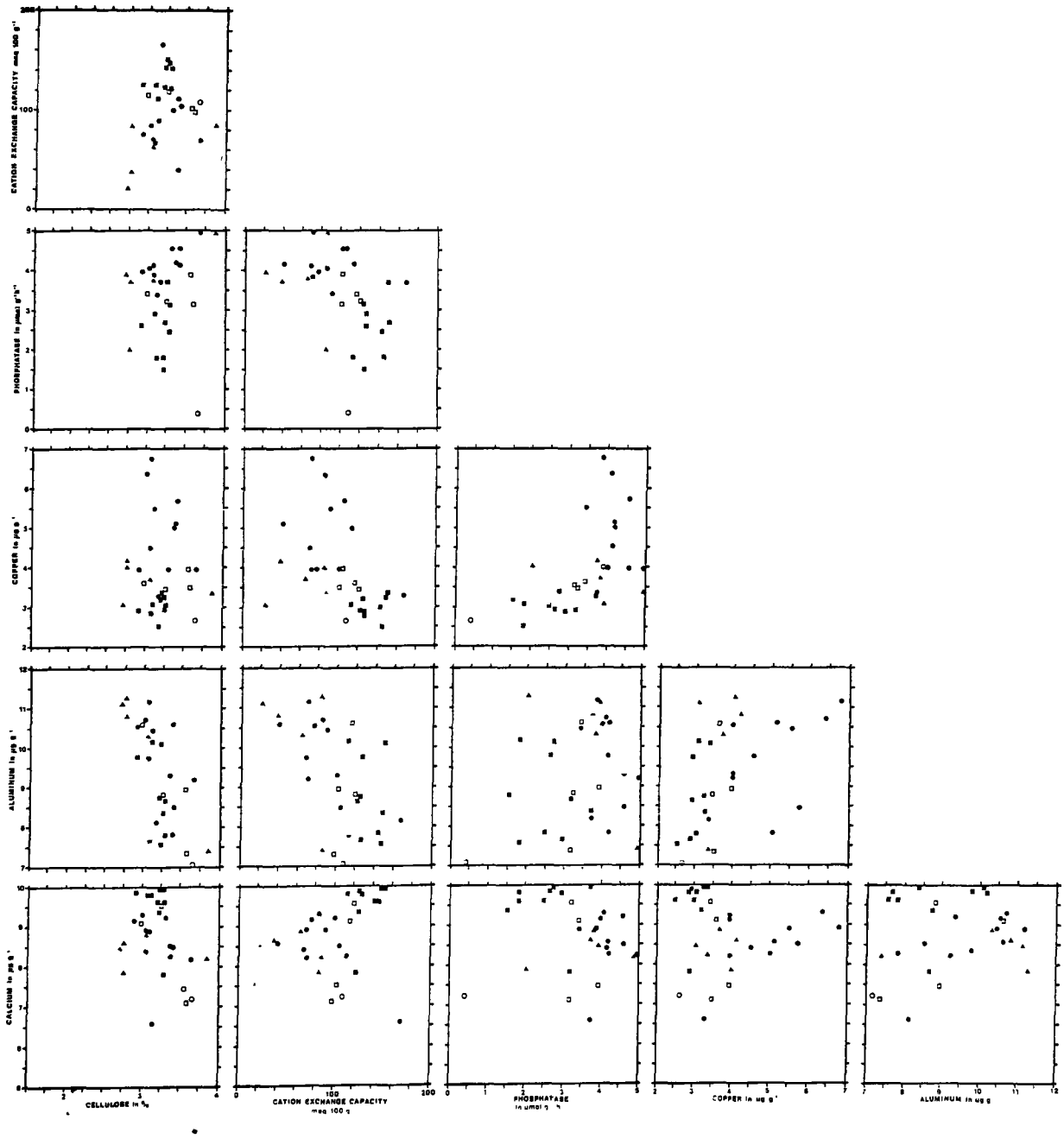
Scatterplot 3. Peat cellulosic component.



Scatterplot 4. Peat humic component.



Scatterplot 5. Mixed characteristics.



APPENDIX 6

CLUSTER ANALYSIS OF INORGANIC CHARACTERISTICS

The metal values were analysed by case cluster analysis using a SPSS Computer Program (1979), where clusters are iteratively processed from an initial state in which each case is ascribed to its own 'cluster'. At each iteration the two most proximate clusters are amalgamated, until all cases are assimilated into one cluster. The two most extensively sampled areas, Timmins and Sudbury show strong affinities, and are labelled A and B respectively (Figure 1). The largest group, A1 and A2, contains eight out of the nine Timmins samples, and could be designated 'Timmins-type' bog. Groups B1 and B2 are more defined and have an even stronger regional composition. Out of the eleven cluster B members all but two are Sudbury bogs, and so could be called the 'Sudbury-type'.

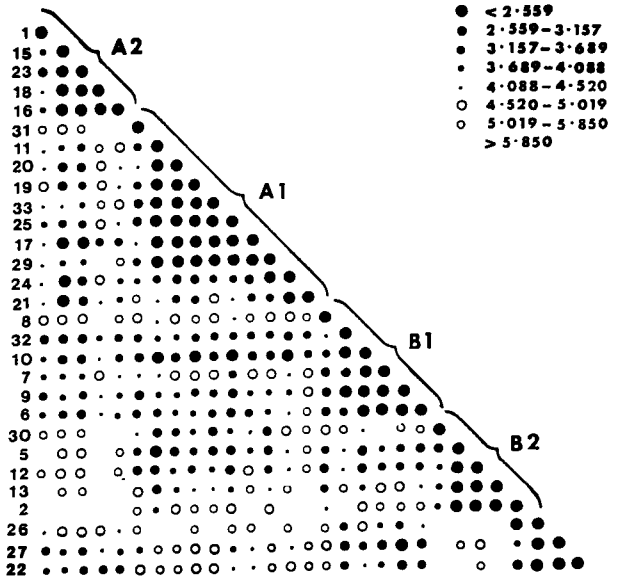


Figure 1. Cluster triangle; the circles represent distances as listed.

APPENDIX 7

RESPIRATION MEASUREMENTS

The organic soil is moistened to two thirds water holding capacity and is placed in the main chamber of the Belco glass flask (Figure 1). Five ml of 0.1N potassium hydroxide, which adsorbs the carbon dioxide evolved, is placed in the side chamber. Every few days the alkali is removed by suction, air is drawn through the Ascarite filter mounted on top of the flask to replace that removed, and the alkalie replaced. The alkali is titrated against 0.05N hydrochloric acid using phenolphthalein as indicator (Bartha and Pramer 1965). The assays are done in duplicate, but it requires several weeks for the respiration to become stable, and even then there is a considerable variation in the rates.

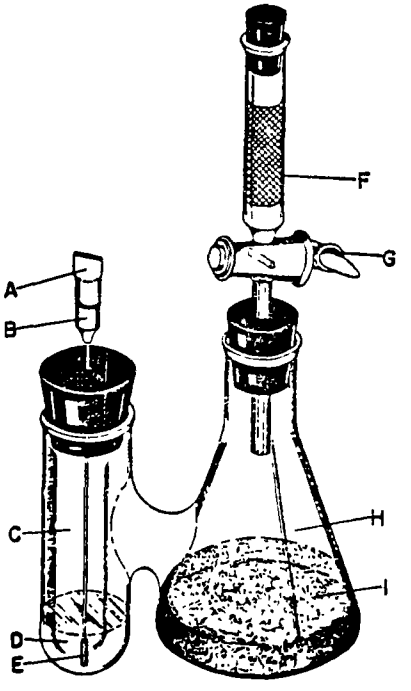


Figure 1. Flask for measuring the production of carbon dioxide during aerobic respiration.

Table 1 shows the respirations obtained during 4 weeks, while the individual samples are graphed in Figure 2.

Table 1. Soil respiration rates from sampled bogs.

Sample	C/day ppm Apr 11	C/day ppm Apr 15	C/day ppm Apr 18	C/day ppm Apr 22	C/day ppm Apr 25	C/day ppm Apr 29	C/day ppm May 2	C/day ppm May 6	C/day ppm May 9	AVG	MAX	MIN	STD	VAR	SAMPLE AVG	SAMPLE STD
1	11 74	11 70	13 92	14 81	18 63	18 11	16 77	10 40	12 30	14 27	18 63	10 40	2 827	7 930		
	12 64	13 47	16 16	15 15	18 63	18 11	16 32	12 07	13 64	15 13	18 63	12 07	2 215	4 908	14 78	2 576
2	1 73	2 52	2 89	3 25	4 93	3 77	3 71	2 52	5 15	3 38	5 15	1 73	1 068	1 141		
	3 17	3 07	4 57	5 05	5 41	8 08	4 43	6 29	5 63	5 08	8 08	3 07	1 464	2 143	4 23	1 536
4	8 37	10 20	11 33	13 03	13 79	15 08	12 98	8 47	11 10	11 71	16 08	8 37	2 387	5 700		
	10 25	10 40	12 09	12 75	13 79	16 37	13 36	11 01	9 59	12 19	16 37	9 59	2 013	4 054	11 95	2 221
5	26 89	29 55	32 75	33 15	59 11	37 99	41 11	30 63	32 22	35 94	59 11	26 89	9 131	83 366		
	34 87	35 35	53 52	49 12	33 55	38 79	75 32	60 27	56 63	48 60	75 32	33 55	13 443	180 710	42 27	13 120
6	18 26	22 50	29 71	32 21	36 91	47 68	43 07	53 40	65 48	38 80	65 48	18 26	14 296	204 377		
	16 58	18 09	22 65	22 50	23 38	29 44	26 81	24 83	26 22	23 38	29 44	16 58	3 879	15 043	31 09	13 006
7	18 53	20 78	29 65	27 34	27 95	34 14	29 78	22 88	24 45	26 17	34 14	18 53	4 646	21 589		
	27 26	31 72	35 97	30 99	36 78	41 40	35 59	30 58	28 81	33 21	41 40	27 26	4 239	17 966	29 69	5 674
8	51 74	51 25	58 58	55 28	47 79	66 37	56 16	48 87	50 33	54 04	66 37	47 79	5 491	30 152		
	50 77	51 62	59 06	56 01	56 14	64 18	60 05	51 06	50 81	55 52	64 18	50 77	4 579	20 966	54 78	5 110
9	110 58	95 22	130 88	105 88	103 68	131 11	129 40	109 58	119 88	116 01	131 11	95 22	12 377	153 198		
	112 63	97 06	139 22	103 68	102 21	141 00	131 35	114 63	124 76	118 58	141 00	97 06	15 399	237 115	117 25	14 026
10	28 58	28 96	28 48	27 79	34 68	28 17	23 02	22 73	22 73	27 24	34 68	22 73	3 656	13 363		
	37 45	37 45	39 01	35 11	40 22	33 61	29 14	29 34	29 34	34 66	40 22	29 14	3 959	15 751	30 95	5 322
11	11 97	13 53	13 88	13 95	14 57	16 38	14 52	11 20	10 92	13 44	16 38	10 92	1 670	2 789		
	10 58	12 08	11 94	11 45	12 35	15 76	12 58	10 58	11 48	12 09	15 76	10 58	1 459	2 129	12 76	1 707
12	12 15	15 32	16 68	16 89	19 39	24 30	19 73	15 89	16 82	17 46	24 30	12 15	3 200	10 241		
	15 48	17 51	21 26	20 01	19 80	25 85	21 39	19 31	15 99	19 62	25 85	15 48	2 976	8 859	18 54	3 274
13	12 60	11 95	12 87	11 49	7 82	13 74	10 23	8 93	9 31	10 99	13 74	7 82	1 903	3 623		
	4 96	6 90	7 97	6 21	6 90	8 01	8 70	6 41	5 95	6 89	8 70	4 96	1 107	1 225	8 94	2 576
15	21 05	24 53	27 02	25 63	26 60	39 02	32 34	27 72	29 90	20 29	39 02	21 05	4 844	23 469		
	20 07	23 80	20 31	26 36	26 60	35 37	26 50	24 07	23 58	26 07	35 37	20 07	3 933	15 942	27 18	4 575
16	7 12	7 92	9 05	6 03	11 31	11 26	7 13	8 26	10 26	12 01	17 34	9 63	2 592	6 716		
	9 63	9 80	10 05	10 93	17 34	13 52	15 27	11 26	7 28	7 62	10 04	4 42	1 534	2 353	10 41	2 780
17	4 42	6 52	9 09	8 30	8 10	10 04	8 07	6 79	7 28	6 82	8 10	4 42	1 322	1 749		
	4 42	6 23	7 51	4 74	8 10	7 97	8 07	7 09	7 28	6 82	8 10	4 42	1 322	1 749	7 22	1 487
18	3 17	5 76	6 65	6 14	8 96	8 41	8 92	6 88	5 35	6 69	8 96	3 17	1 779	3 166		
	5 21	6 53	7 17	6 53	8 45	8 03	7 90	6 88	6 88	7 06	8 45	5 21	0 921	0 848	6 88	1 429
19		3 01	5 08	1 52	4 23	3 29	3 54	3 29	2 53	3 03	5 08	0 00	1 428	2 038		
	3 45	4 06	2 03	4 06	7 62	6 32	5 90	4 55	4 21	4 69	7 62	2 03	1 576	2 483	3 86	1 717
20	6 82	9 61	8 90	10 41	12 28	12 50	11 53	7 71	9 75	9 95	12 50	6 82	1 847	3 410		
	8 60	11 75	11 39	9 08	12 28	15 69	11 53	10 64	8 69	11 07	15 69	8 60	2 096	4 394	10 51	2 054
21	35 14	34 23	38 12	34 98	39 37	41 59	37 22	33 72	30 72	37 01	41 59	33 72	2 514	6 322		
	36 64	40 63	47 65	45 52	52 41	53 21	45 21	41 59	41 72	44 95	53 21	36 64	5 175	26 776	40 98	5 605
22	12 85	18 01	17 37	10 01	19 67	23 28	18 06	14 12	13 48	17 21	23 28	12 05	3 116	9 707		
	12 34	18 39	19 41	19 16	22 22	24 43	22 64	16 30	14 50	18 16	24 43	10 30	4 564	20 828	17 68	3 936
23	21 53	23 00	26 85	25 26	28 56	31 73	24 07	19 69	20 67	24 68	31 73	19 69	3 678	13 529		
	20 56	21 23	26 85	26 36	27 58	31 00	24 56	21 88	20 67	24 52	31 00	20 56	3 471	12 040	24 60	3 577
24	30 01	29 97	34 73	32 11	35 92	40 52	30 09	25 30	24 88	31 62	40 52	24 88	4 582	20 993		
	26 21	28 19	30 92	29 62	33 06	35 19	28 67	23 81	23 46	28 79	35 19	23 46	3 722	13 851	30 20	4 406
25	1 87	4 05	4 68	4 59	4 08	6 46	4 04	1 61	2 33	3 92	6 46	1 61	1 537	2 364		
	4 03	5 94	5 04	6 21	7 74	8 34	4 84	5 30	5 56	5 90	8 34	4 03	1 300	1 690	4 91	1 733
26	30 66	43 83	45 40	50 35	52 40	49 07	47 39	42 58	44 98	46 07	52 40	30 66	3 905	15 802		
	47 34	49 26	53 61	51 97	59 16	56 29	50 28	40 71	49 31	51 99	59 16	47 34	3 748	14 051	49 03	4 071
27	44 14	37 75	36 76	37 33	41 57	41 41	32 96	29 58	32 96	37 16	44 14	29 58	4 467	19 952		
	40 75	43 69	46 94	47 93	46 66	52 81	42 53	30 03	39 15	44 28	52 81	38 03	4 474	20 013	40.72	5 714
28	5 53	16 31	15 82	17 05	17 55	17 36	16 50	13 66	11 00	14 54	17 55	5 53	3 740	13 991		
	16 38	19 65	17 30	16 88	19 53	20 31	16 99	16 25	14 53	17 51	20 31	14 53	1 003	3 251	16 03	3 291
29		11 00	10 08	10 81	12 07	12 38	9 15	8 88	7 35	9 09	12 38	0 00	3 547	12 580		
	16 61	17 56	19 09	17 03	23 23	23 68	8 79	19 10	19 55	18 38	23 68	8 79	4 084	16 678	13 74	6 019
30	0 00	1 74	1 50	1 63	2 38	1 73	1 03	1 02	0 75	1 40	2 38	0 00	0 665	0 442		
	0 16	1 12	1 50	1 63	1 84	1 53	1 15	2 14	1 15	1 36	2 14	0 16	0 530	0 281	1 30	0 681
31	0 00	1 28	2 44	3 11	3 84	1 46	4 25	2 37	1 58	2 17	4 25	0 00	1 175	1 300		
	0 00	0 91	2 44	2 19	2 07	2 37	2 79	2 18	1 33	1 81	2 79	0 00	0 838	0 702	1 99	1 036
32	83 94	76 12	95 30	85 84	100 95	98 35	75 36	65 69	71 20	83 64	100 95	65 69	11 920	139 898		
	63 44	64 35	71 23	66 32	85 72	133 59	72 42	58 72	23 24	71 00	133 59	23 24	27 265	743 404	77 32	21 945
33	0 00	0 95	0 30	0 95	1 59	0 28	0 95	0 00	0 44	0 62	1 59	0 00	0 498	0 248		
	0 66	1 52	1 14	1 33	1 08	1 61	1 33	1 33	0 95	1.22	1 61	0 66	0 278	0.077	0.92	0 583

Figure 2. Graphs of soil respiration rates from sampled bogs.

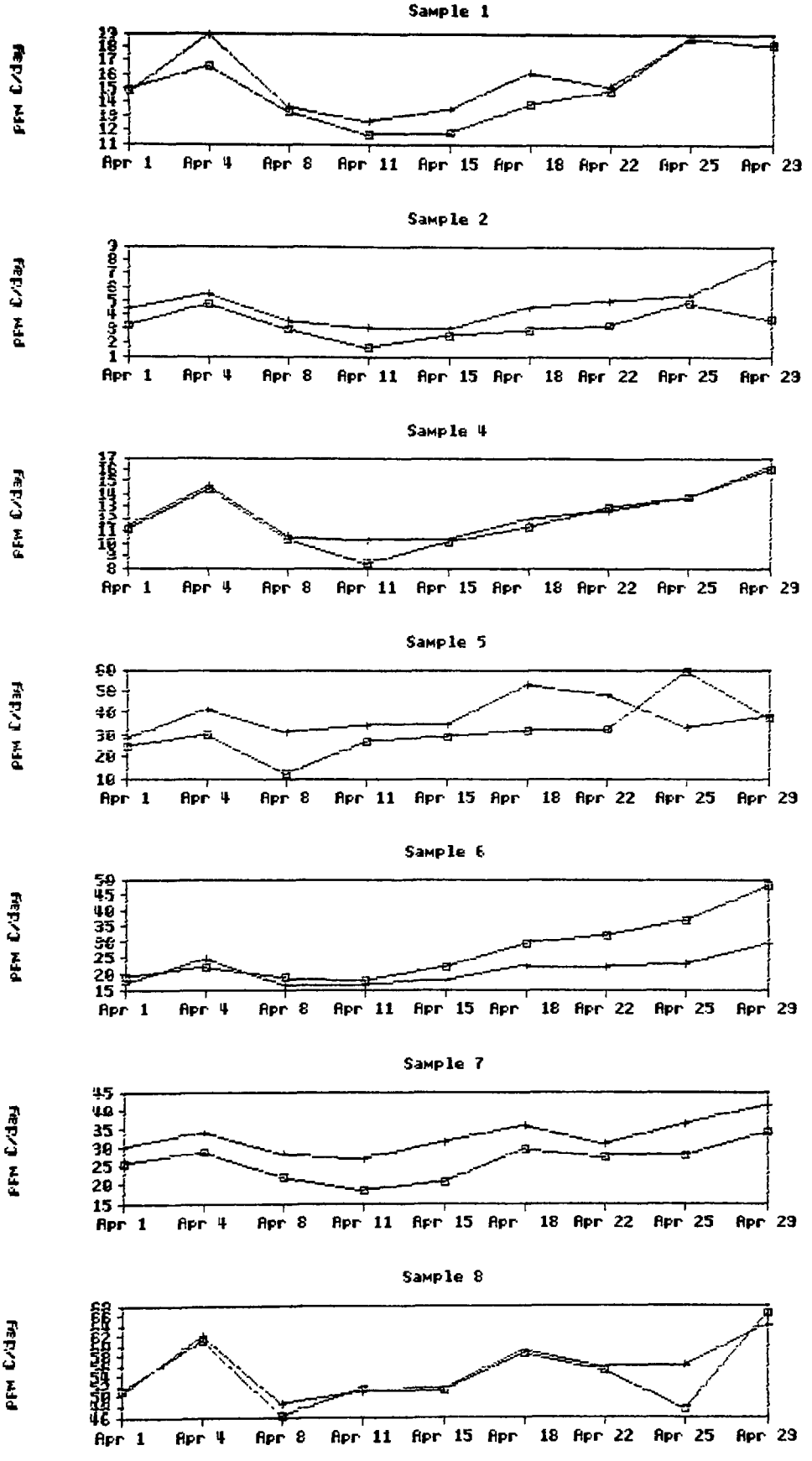


Figure 2 continued.

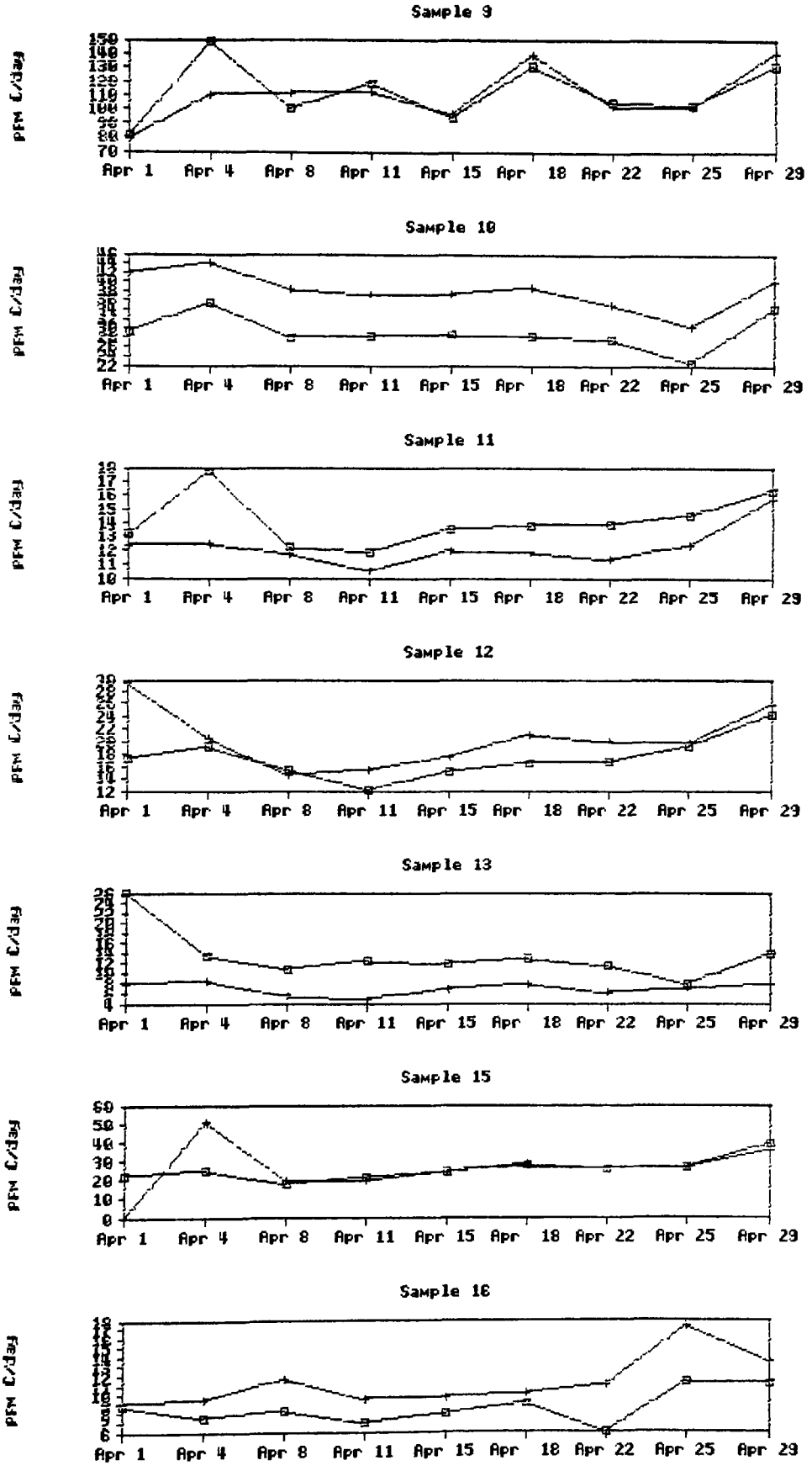


Figure 2 continued.

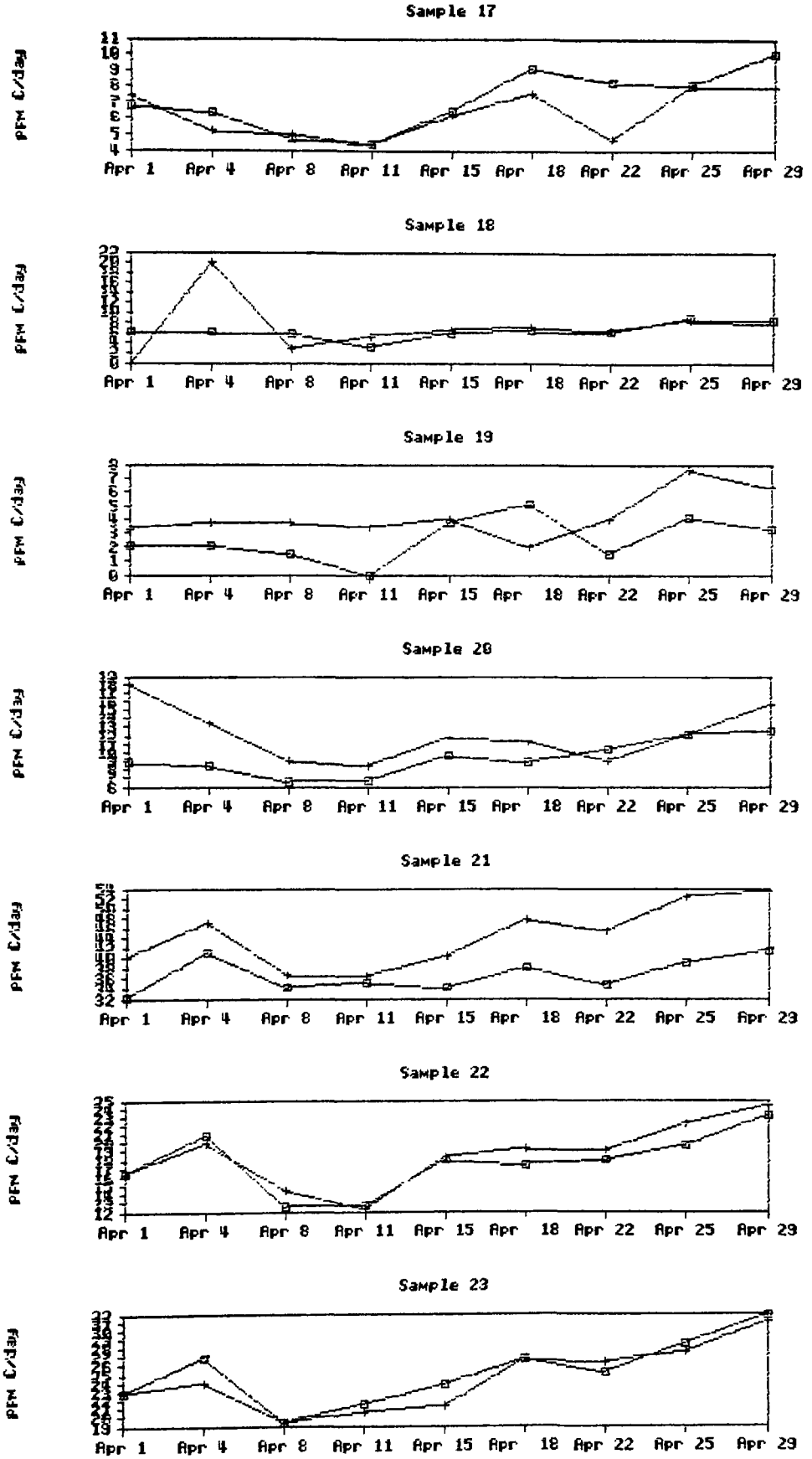


Figure 2 continued.

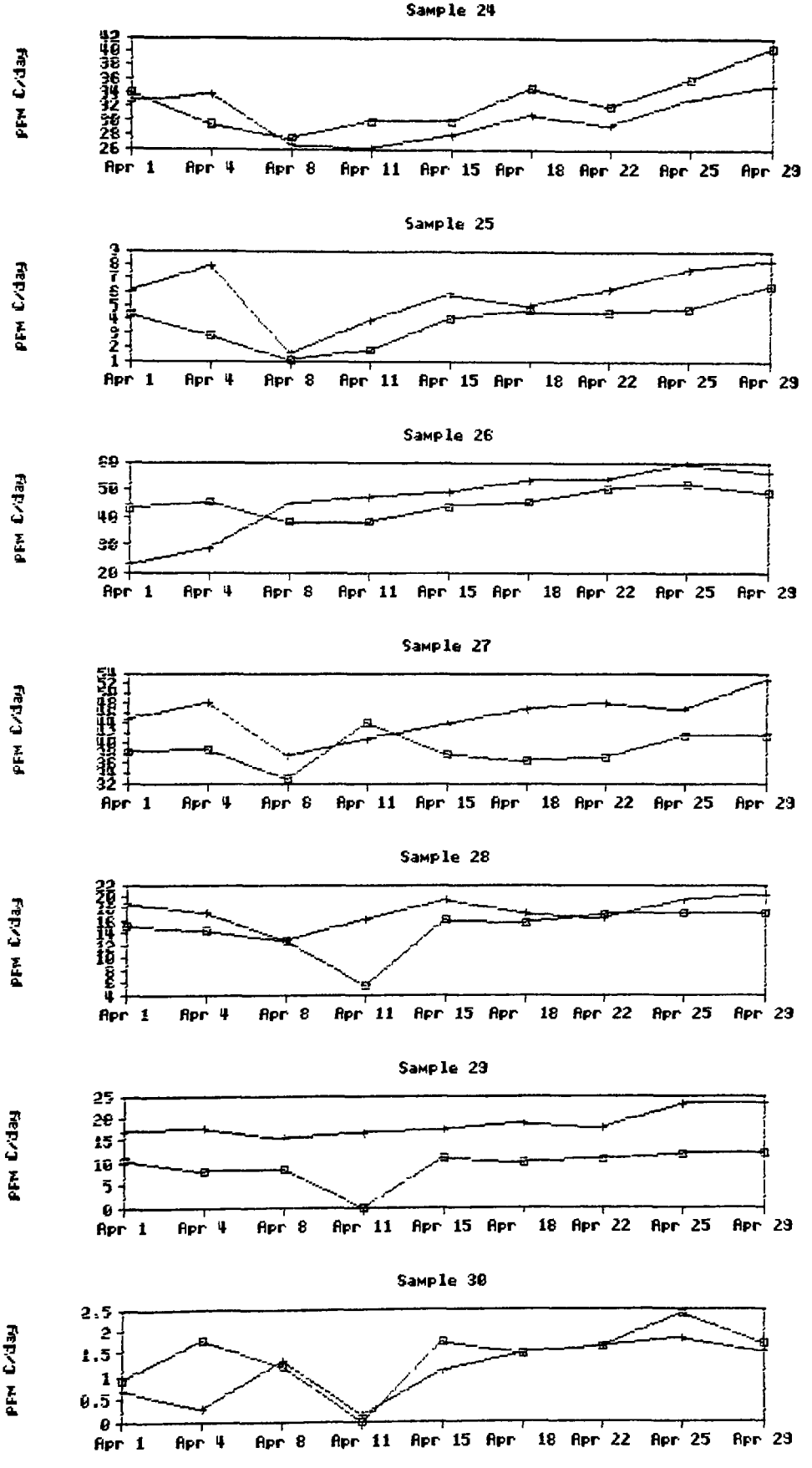
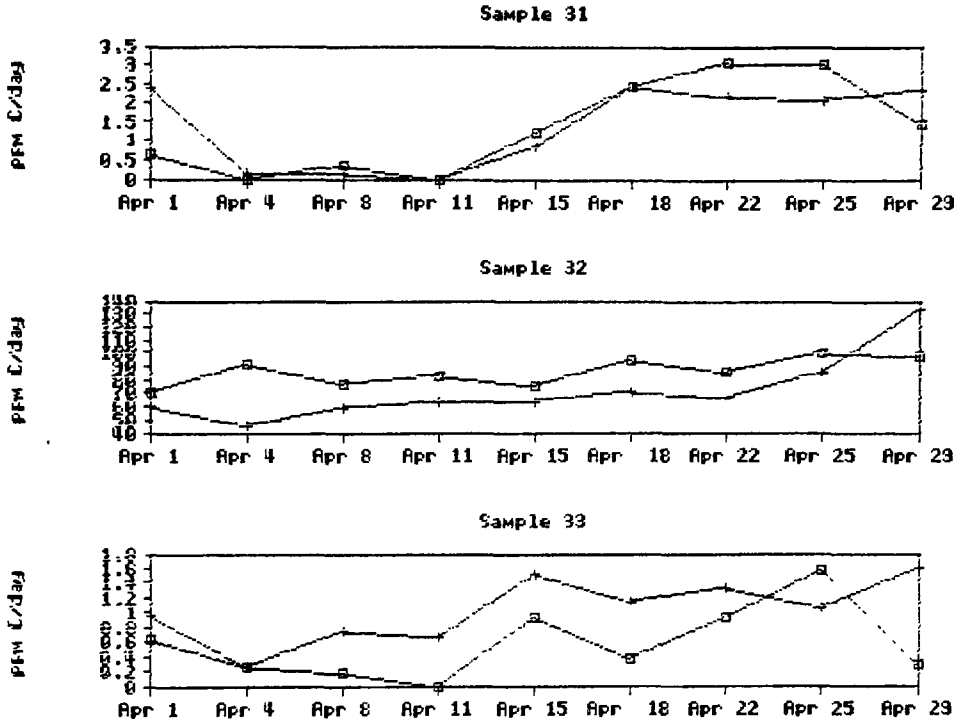


Figure 2 continued.



APPENDIX 8

REDOX MEASUREMENTS

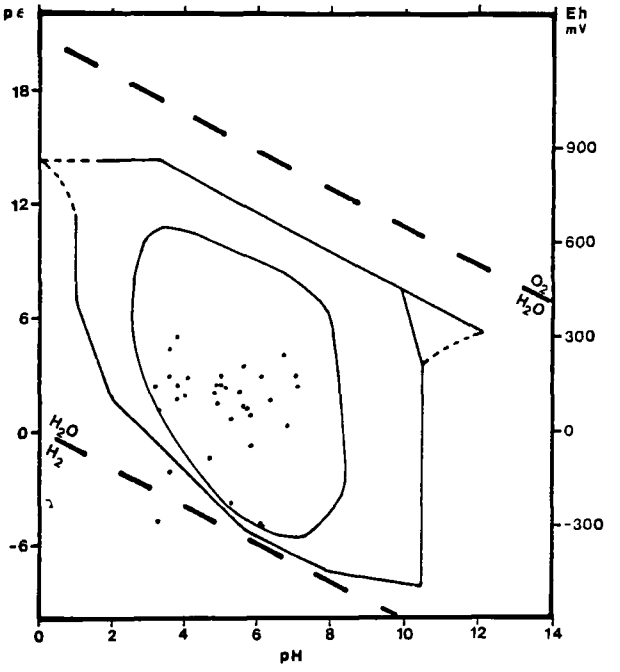
The plots of some 70 pH and Eh readings obtained from three different bogs in the Ottawa area and from bogs in the northern mining areas are shown in Figure 1. The readings are plotted onto the environmental limits calculated by Baas Becking et al (1960), and all but two of the readings fall within his outline for peat bogs.

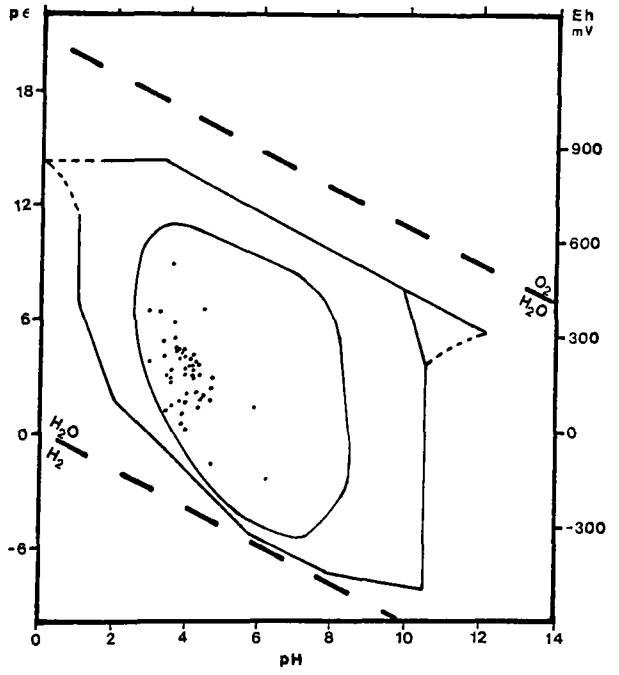
The readings from the Ottawa bogs are clustered within one group, with only five readings not included; these readings are at the extreme acid range, but span the soil type from 'normal', through 'wet', to 'waterlogged'. The readings from the northern bogs cluster into three groups with three outliers; all are in the 'wet' to 'waterlogged' zones. The most acid group are those from ombrotrophic bogs, while the group with neutral pH are from more minerotrophic ones. However the highly reduced group is the most interesting, for it shows that it is possible to measure in the field an Eh value close to that expected for a methane-producing environment.

Figure 1. pH and Eh environment of peat bogs:

A - Readings from northern mining areas; and

B - Readings from bogs near Ottawa.





APPENDIX 9

METHANE EXTRACTIONS

Figure 1 (6 pages) shows bar charts of methane extraction from 25 sites at 60 cm (short), 90 cm (medium) and 120 cm (long) depths in Mer Bleue from Table 2.

Table 1. The first season's extractions with prototype samplers from Gatineau Park, Mer Bleue and Albion Road, described in Section 4.4.1. All times in minutes.

Table 2 (2 pages). The second season extractions with custom made samplers from a 24 m square grid in Mer Bleue, described in Section 4.4.2.

Table 3. The third season extractions of methane from 16 northern muskeg bogs, described in Section 4.1.

Figure 1. Methane extraction from Mer Bleue.

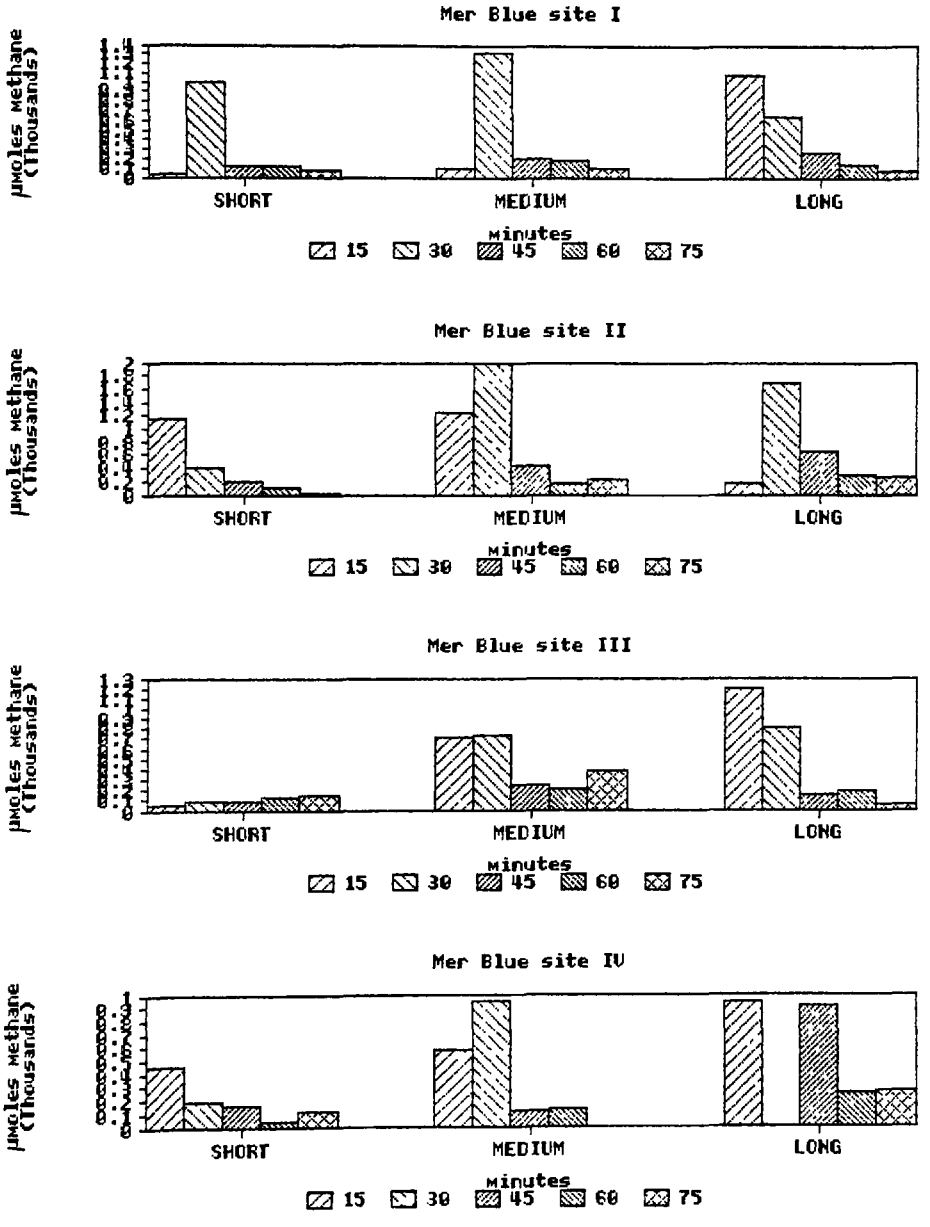


Figure 1 continued.

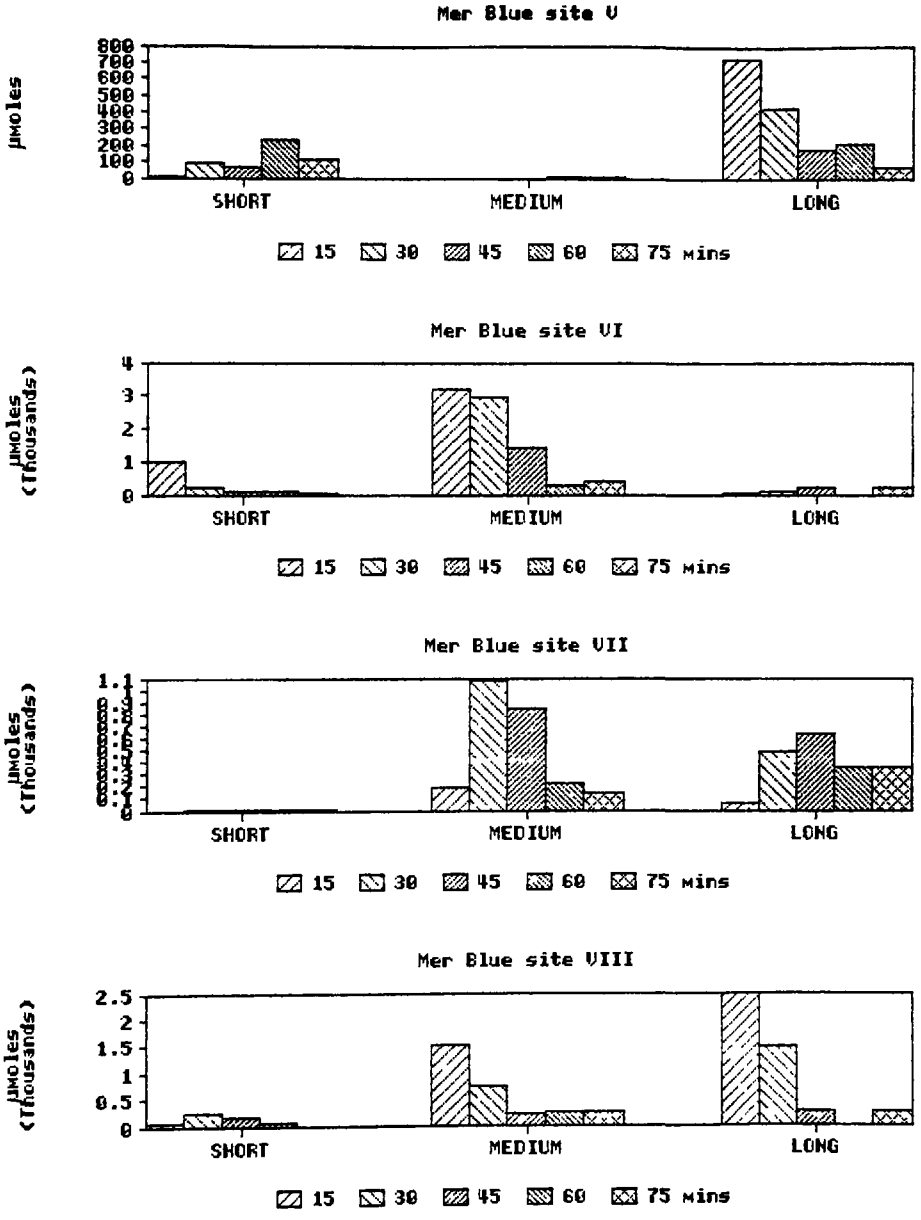


Figure 1 continued.

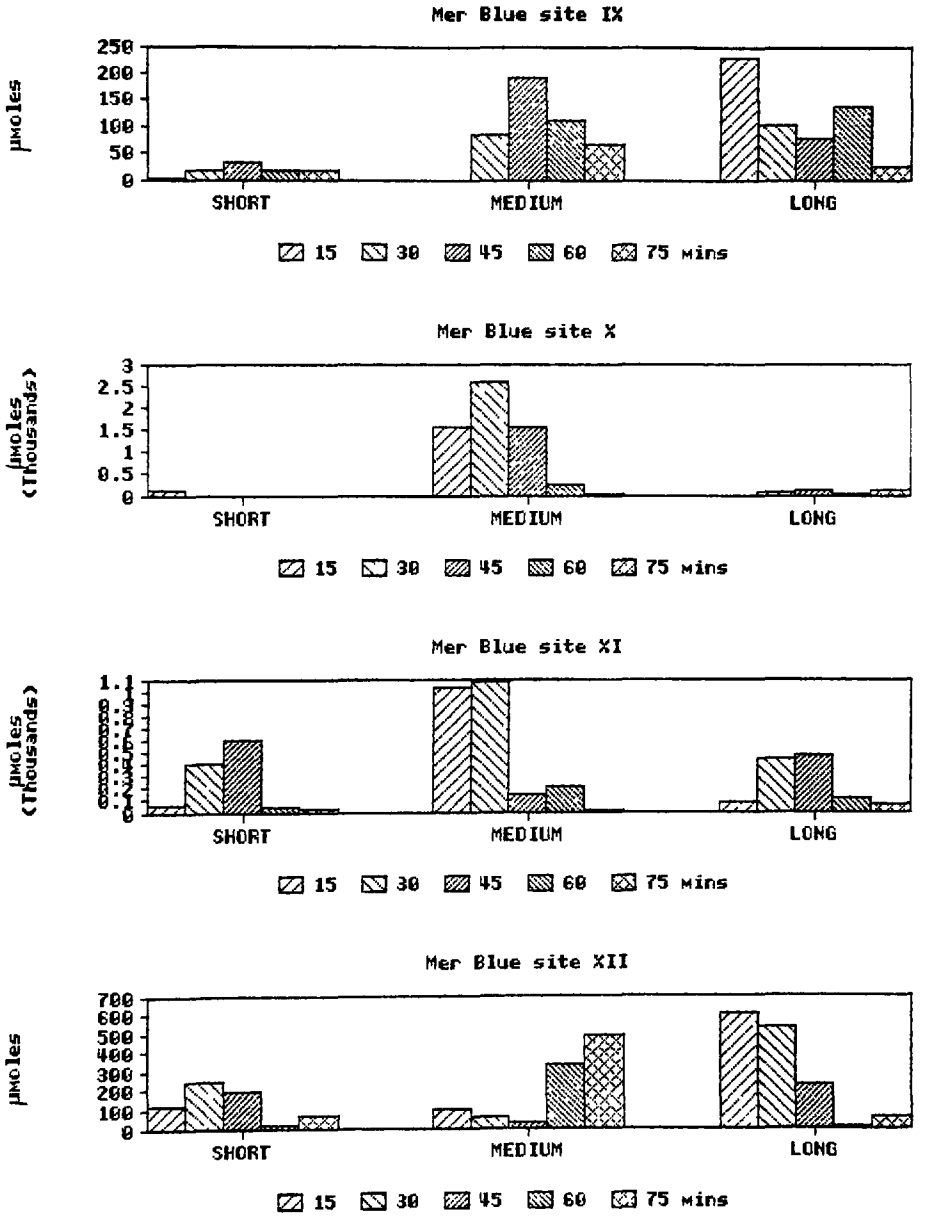


Figure 1 continued.

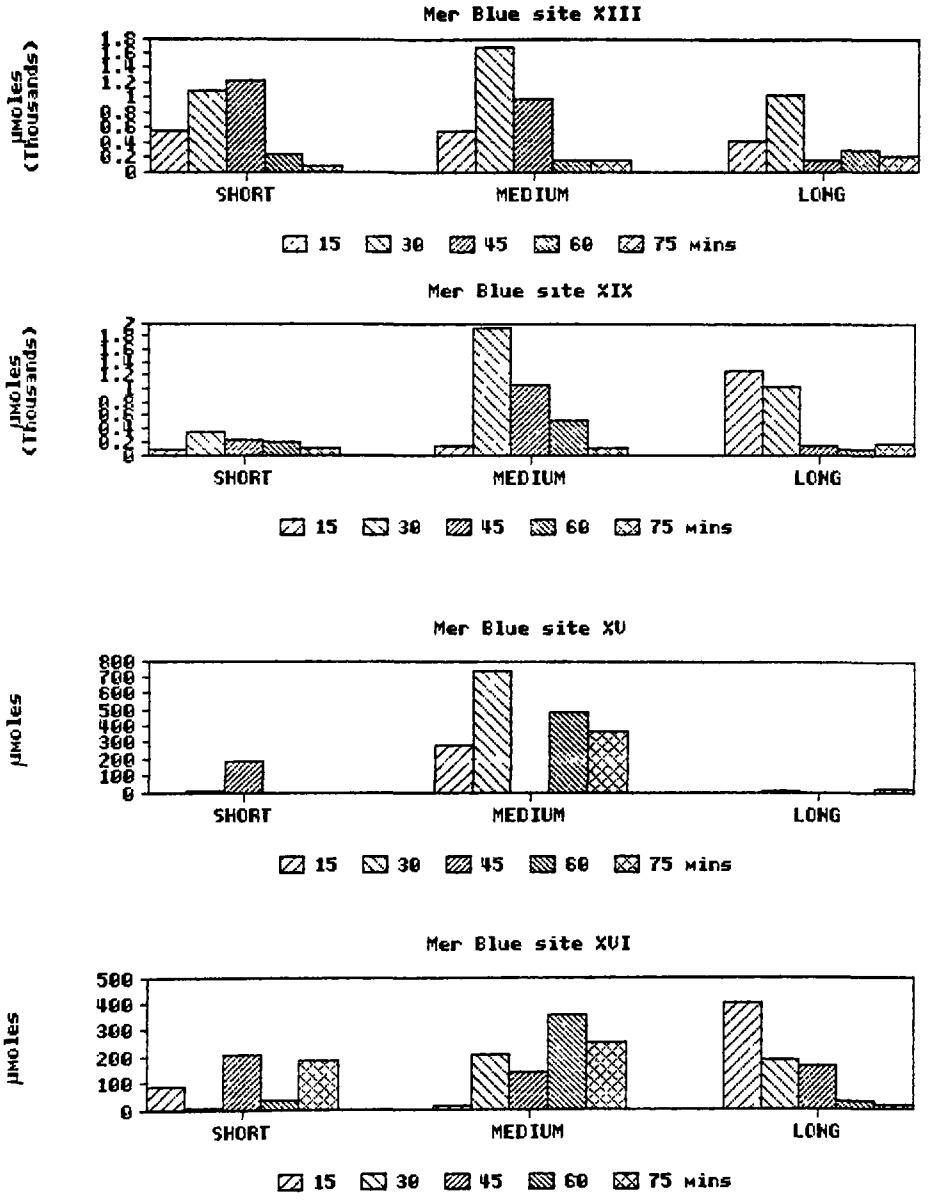


Figure 1 continued.

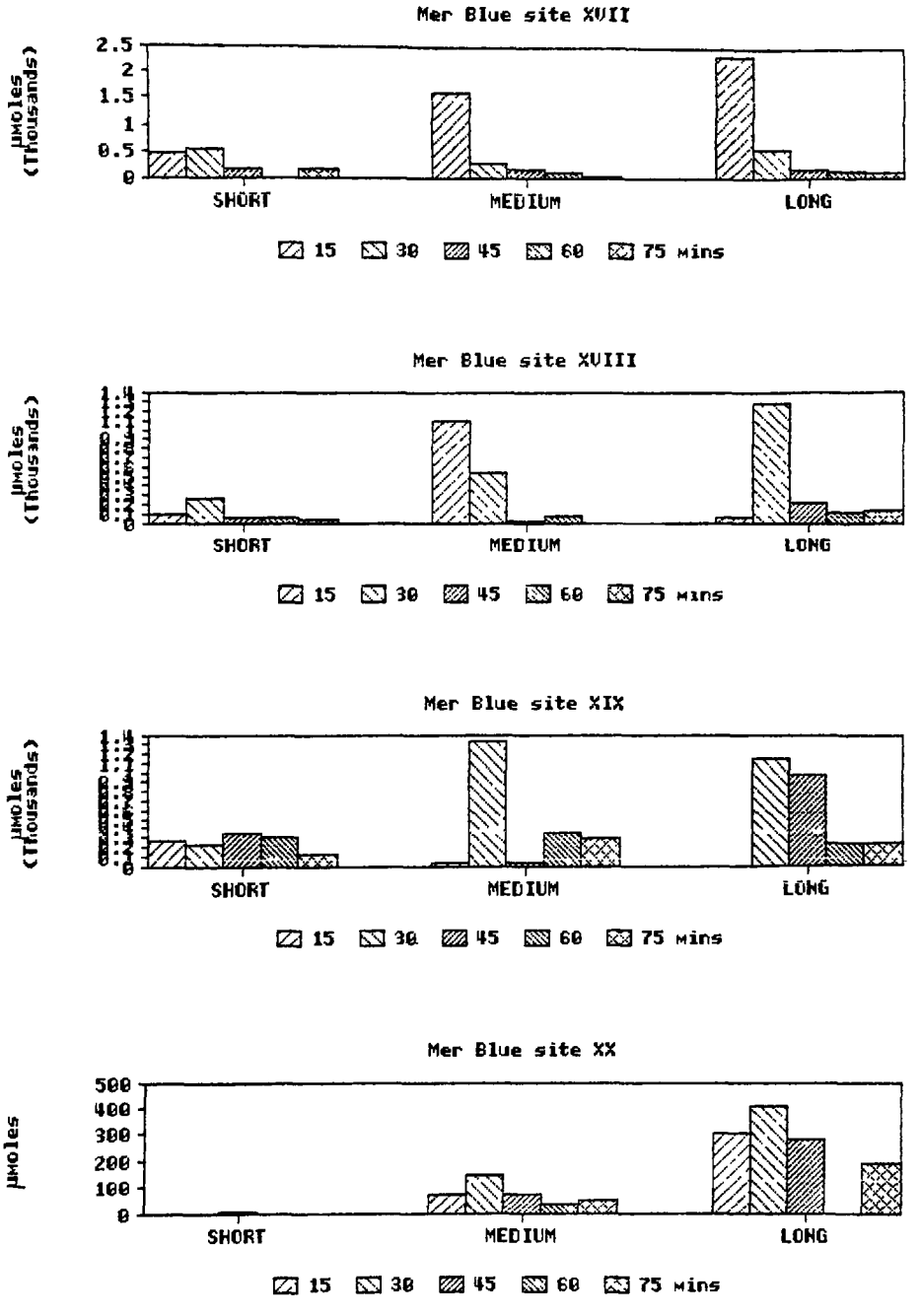


Figure 1 continued.

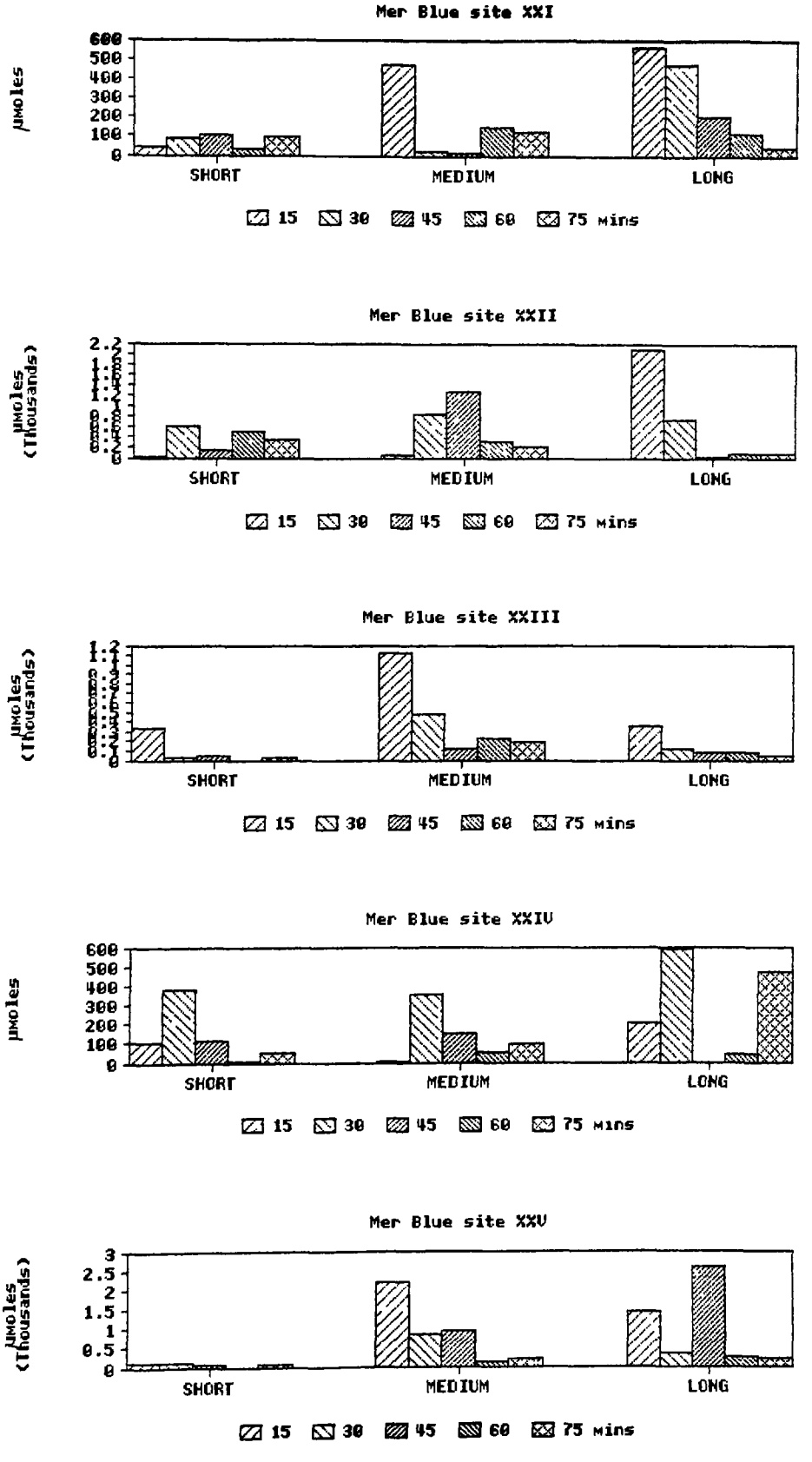


Table 1. Methane extractions from Ottawa bogs.

	I				II				III				Total	umoles avg CH4/ml
	% CH4	ml water	umole CH4		% CH4	ml water	umole CH4		% CH4	ml water	umole CH4			
#1	16 0	2	1972 4		12 5	0	1470 7		27.5	0	3253 1			
80cm	37 3	94	2847.3		10 3	4	1208 1		23 5	160	1101 5			
	11.9	194	377 2		30 4	89	2300 4		2 0	120	129 5			
	0 6	17	66 4		3 5	89	275 0		8 5	178	258 2			
	0 0	172	365 3		16 9	176	871 4							
		846 0	5620 6			967 0	5813 6			604 0	4742 3		5461 5	6 91
#5	23 4	69	2047 4		5 2	11	589 6		16 4	42	1632 6			
50-ml	8 0	10	0 0		14 8	67	1237 4		2 4	138	136 1			
	5 3	107	184 5		10 5	172	435 9		0 0	70	69 6			
	2 3	159	129 4		0 7	174	353 4		3 4	186	119 9			
	1 0	175	48 2											
	0 9	185	32 1											
		570 0	2433 6			636 0	2616 4			624 0	1950 2		2336 0	3 84
#6	0 6	70	50 1		0 6	73	51 4		1 2	126	74 5			
60cm	1 1	1	129 6		0 6	105	42 9		1 4	131	83 7			
	0 5	25	53 6		0 1	20	10 9		1 5	164	67 6			
	0 3	43	29 7		0 0	126	49 6		1 8	197	40 6			
	0 9	170	30 2		0 6	70	52 2							
					0 1	15	11 2							
		1008 0	301 2			1181 0	218 2			442 0	274 4		264 6	0 37
#7	0 3	17	33 2		0 5	34	51 6		0 4	28	42 7			
45cm	0 9	192	29 3		0 6	43	79 3		1 7	155	93 5			
	1 0	200	25 4		0 9	102	65 6		0 9	203	24 8			
	1 0	202	26 1		0 8	14	89 6		0 6	3	58 6			
					0 8	98	59 6		0 5	90	39 1			
					1 0	199	29 5							
		441 0	116 1			1100 0	375 1			840 0	248 6		246 6	0 30
#10	0 3	7	34 6		0 1	29	48 5		0 1	0	11 0			
40cm	0 1	95	7 6		0 5	33	51 0		0 1	38	10 1			
	0 2	151	10 2		0 2	45	19 6		0 3	49	28 9			
	0 0	184	0 0		0 1	50	9 8		0 1	99	7 4			
									0 0	29	0 0			
									0 1	99	7 4			
		542 0	52 3			981 0	91 6			1040	58 3		67 4	0 08
#12	0 3	2	35 2		0 1	0	11 0		0 0	4	0 0			
42cm	0 1	2	11 7		0 0	0	0 0		0 1	132	5 9			
	0 1	185	3 6		0 3	196	9 2		0 0	202	0 0			
	0 4	192	13 0		0 1	100	3 4		0 3	202	0 4			
	0 2	125	12 5		0 0	60	0 0							
					0 0	0	0 0							
					0 1	200	2 9							
		819 0	76 1			476 0	27 4			195.0	14 4		39 3	0 07
#16	0 3	48	29 3		0 2	0			1 0	18	110 3			
30cm	1 0	196	30 0		0 1	0			1 5	196	48 2			
	1 5	203	41 5		0 1	0			1 3	206	34 2			
	1 2	206	31 6		0 1	0			1 7	208	48 3			
				leaking										
		489 0	133 3							440 0	240 0		186 6	0 44
#17	0 4	38	40 5		0 2	0			0 0	3	0 0			
30cm	0 6	190	17 9		0 2	0			0 3	90	23 4			
	0 5	176	19 9		0 0	0			0 5	174	20 3			
	0 7	201	20 0		0 1	0			0 7	194	22 2			
				leaking					0 0	200	20 4			
		447 0	98 3							394 0	86 3		92 3	0 22
#18	0 4	25	42 9		0 0	0	0 0		0 4	19	43 9			
40cm	0 7	197	21 2		0 2	44	19 7		0 5	193	15 1			
	0 7	204	19 1		0 4	202	11 2		0 5	203	13 0			
	0 1	105	7 1		0 4	205	10 7		0 6	207	15 5			
					0 4	204	10 9							
		529 0	90 3			405 0	52 6			438 0	89 4		77 4	0 17
#19	1 7	10	193 5		0 2	48	19 4		0 1	3	11 7			
32cm	2 0	8	231 2		1 7	75	144 2		0 0	14	89 8			
	0 7	39	70 6		1 0	200	52 2		1 9	142	104 3			
	1 4	202	39 4		1 3	207	33 7		0 1	7	11 5			
					1 5	204	40 0		1 3	183	47 5			
		803 0	534 7			591 0	290 3			976 0	264 0		383 3	0 48
#22	0 2	45	10 6		0 0	20	0 0		0 1	30	10 6			
30cm	0 1	44	9 9		0 1	100	7 4		0 2	132	11 9			
	0 1	60	0 0		0 2	122	12 8		0 3	206	7 9			
	0 1	125	6 2											
		770 0	44 6			388 0	20 1			427 0	30 3		31 6	0 06
#23	0 7	5	81.2		1 6	5	105 7		2 4	80	189 6			
40cm	1 2	5	139 3		1 9	60	168 0		2 6	92	200 0			
	0 9	2	105 7		0 6	84	49 5		2 4	162	110 4			
	1 1	5	127 7		0 5	54	47 1		1 7	160	73 8			
		1043.0	453 9			851 0	450 1			550 0	574 4		492 0	0 67
#24	1 3	0	153 0		0 0	2	0 0		0 2	180	7 6			
40cm	0 1	0	11 0		0 4	38	40 5		0 1	10	11 4			
	0 3	32	31 2		0 4	80	33 0		0 1	200	2 9			
	0 1	72	0 6		0 0	0	0 0		0 1	12	11 3			
		956 0	205 4			412 0	73 6			550 0	33 2		184 1	0 15
#25	3 0	0	354 0		0 2	99	14 8		0 4	170	17 0			
80cm	0 0	10	0 0		0 3	105	10 7		0 0	202	0 0			
	1 0	70	87 0		0 3	204	0 2		0 4	201	11 4			
	0 4	93	32 5		0 0	26	0 0		0 4	203	11 1			
	0 0	0	0 0		0 1	187	3 5		1 1	209	27 6			
	1.0	86	79 9		0 1	187	3 5							
		821.0	554 3			463 0	40 7			277 0	67 0		220 7	0 33
#26	0 1	0	11 0		1 0	36	104 0		0 1	86	0 9			
80cm	0 2	0	23 7		0 2	40	20 1		0 7	170	29 7			
	0 4	0	47 3		1 0	190	53 0		1 1	187	38 3			
	0 2	0	23 7		1 7	199	50 1		0 1	41	10 0			
	0 1	10	11 4		1 0	203	27 7		1 0	199	29 5			
									1 2	203	33 2			
		1315 0	117.0			649 0	335 7			724 0	149 5		201 0	0 27
#27	0 0	0			0 4	20	42 3		0 2	152	10 1			
50cm	0 0	0			2 1	122	134 1		0 6	148	31 3			
	0 0	0			0 0	26	0 0		0 0	184	36 1			
	0 0	0			1 1	205	29 5		0 1	0	11 0			
	0 1	0			0 3	202	0 4		0 3	143	16 3			
	leaking				0 9	195	28 1							
					0 2	134	11 7							
						704 0	254 1			710 0	105 7		179 9	0 25

Table 2. Methane extraction from Mer Bleue.

Site	time	SHORT ml water	SHORT CH4 peak	SHORT umoles	MEDIUM ml water	MEDIUM CH4 peak	MEDIUM umoles	LONG ml water	LONG CH4 peak	LONG umoles	total
I	15	0	0 0291230	35 6	0	0 0926050	113 1	0	0 8938100	1091 5	
29.7 87	30	3	0 8367500	1010 3	0	1 0726000	1309 9	0	0 5228600	638 5	
	45	38	0 1190800	124.6	90	0 2649400	213 7	0	0 2243000	273 9	
	60	140	0 2001500	115 3	94	0 2456200	193 6	0	0 1169000	142 8	
	75	36	0 0697180	73 6	72	0 1123800	100 0	0	0 0680320	83 1	
		217		1359.3	256		1930 1	0		2229 8	5519 2
II	15	0	0 9563800	1167 9	0	1 0296000	1257 3	0	0 1421800	173 6	
	30	0	0 3315400	404 9	0	1 6375000	1999 7	0	1 3900000	1697 5	
	45	104	0 2642200	196 0	78	0 5114100	440 7	0	0 5401500	659 6	
	60	186	0 3533600	128 6	178	0 4165800	167 0	0	0 2378000	290 4	
	75	118	0 0394300	26 7	176	0 6005400	246 3	0	0 2155200	263 2	
		408		1924 2	432		4111 1	0		3084 3	9119 6
III	15	0	0 0438620	53 6	0	0 5911600	721 9	0	0 9866500	1204 9	
	30	0	0.0721360	88 1	14	0 6347400	734 2	56	0 8519200	820 5	
	45	0	0 0760830	92 9	122	0 3745100	246 8	112	0 2169800	153 0	
	60	0	0 1136300	138 8	140	0 3639900	209 7	200	0 6632300	198 7	
	75	25	0 1431100	158 3	10	0 3304500	388 3	0	0 0398040	48 6	
		25		531 6	286		2300 9	368		2425 7	5258 2
IV	15	0	0 3828400	467 5	0	0 4809500	587 3	0	0 7700200	940 3	
	30	114	0 2878200	200 3	34	0 8935500	951 2	0	0 0000000	0 0	
	45	150	0 2964800	157 1	198	0 3951900	122 0	0	0 7429800	907 3	
	60	200	0 1707100	51 1	162	0 2980300	141 5	0	0 2124500	259 4	
	75	172	0 2885900	123 7				0	0 2160900	263 9	
		636		999 7	394		1802 0	0		2371 0	5172 8 25069 7
V	15	0	0 0133850	16 3	0	0 0000000	0 0	0	0 5985300	730 9	
5 8 82	30	0	0 0766470	93 6	0	0 0023199	2 8	0	0 3509500	428.6	
	45	0	0 0625170	76 3	0	0 0039139	4 8	0	0 1507100	184 0	
	60	0	0 1918100	234 2	0	0 0058365	7 1	0	0 1795000	219 2	
	75	0	0 0934400	114 1	0	0 0055318	6 8	0	0 0595290	72 7	
		0		534 6	0		21 5	0		1635 5	2191 6
VI	15	0	0 8548400	1043 9	0	2 6212000	3201 0	0	0 0431300	52 7	
	30	142	0 4058100	230 0	0	2 4342000	2972 6	0	0 0890570	108 8	
	45	184	0 3743700	139 7	34	1 3210000	1406 2	0	0 1745700	213 2	
	60	190	0 2870500	99 2	130	0 4351600	270 7	0	0 0095230	11 6	
	75	136	0 1476000	87.7	60	0 4358300	411 7	0	0 1767400	215 8	
		652		1600 6	224		8262 3	0		602 1	10465 0
VII	15	0	0 0000000	0 0	0	0 1556600	190 1	0	0 0507660	62 0	
	30	0	0 0067810	8.3	0	0 8850800	1080 9	0	0 3967900	484 6	
	45	0	0 0161010	19 7	65	0 9228300	850 5	0	0 5282900	645.1	
	60	68	0 0256590	23 3	126	0 3526500	225 9	68	0 3946700	358 3	
	75	192	0.0274050	9 2	130	0 2340900	145 6	192	1 0693000	359 7	
		260		60 5	321		2493 0	260		1909 7	4463 2
VIII	15	0	0 0581130	71 0	0	1 2499000	1526 4	0	2 0323000	2481 8	
	30	90	0.3398300	274 1	98	0 9880500	760 4	90	1 8379000	1482 2	
	45	196	0 6446900	205 0	70	0 2404600	216 1	196	0 8100900	257 6	
	60	20	0 0797880	90 1	200	0 8823500	264 3	0	0 0000000	0 0	
	75	192	0 0156340	5 3	192	0 8292600	279 0	192	0 8292600	279 0	
		498		645 4	560		3046 1	478		4500 6	8192 0 25311 9
IX	15	0	0 0044540	5 4	0	0 0000000	0 0	0	0 1901200	232 2	
8 8 87	30	0	0 0140740	17 2	0	0 0715130	87 3	0	0 0866100	105 8	
	45	120	0 0514200	34 4	0	0 1590900	194 3	0	0 0646220	78 9	
	60	125	0 0289470	18 7	104	0 1530400	113 5	0	0 1115300	136 2	
	75	108	0 0259000	18 7	158	0 1363500	67 2	0	0 0221190	27 0	
		353		94 4	262		462 4	0		580 1	1136 9
X	15	0	0 1276800	155 9	0	1 2670000	1547 3	0	0 0026819	3 3	
	30	0	0 0018809	2 3	0	2 1442000	2618 5	0	0 0815960	99 6	
	45	0	0 0025240	3.1	62	1 6885000	1579 6	0	0 1168500	142 7	
	60	0	0 0141570	17.3	68	0 2929900	266 0	0	0 0277150	33 8	
	75	0	0 0000000	0 0	0	0 0237740	29 0	0	0 0988180	120 7	
		0		178 6	130		6040 3	0		400 1	6619 0
XI	15	0	0 0499090	60 9	0	0 8449100	1031 8	0	0 0563420	81 0	
	30	0	0 3356400	409 9	44	1 0720000	1091 8	0	0 3630600	443 4	
	45	15	0 5277000	607 9	166	0 3357300	153 2	0	0 3919000	478 6	
	60	58	0 0457650	43 7	152	0 4042700	210 5	0	0 0908030	110 9	
	75	66	0 0361140	33 1	130	0 0361140	22 5	0	0 0560740	68 5	
		139		1155 6	492		2509 7	0		1182 3	4847 6
XII	15	0	0 0986380	120 5	0	0 0845570	103 3	0	0 4968000	606 7	
	30	0	0 2047900	250 1	0	0 0504470	61 6	0	0 4363800	532 9	
	45	11	0 1673600	195 9	0	0 0259740	31 7	30	0 2083000	225 6	
	60	0	0 0197880	24 2	0	0 2747100	335 5	10	0 0052809	6 2	
	75	84	0 0934070	77.9	34	0 4546700	494 6	68	0 0677370	61 5	
		95		668 5	34		1026 7	108		1432 9	3128 1 15731 6

Table 2 continued.

Site	time	SHORT ml water	SHORT CH4 peak	SHORT umoles	MEDIUM ml water	MEDIUM CH4 peak	MEDIUM umoles	LONG ml water	LONG CH4 peak	LONG umoles	total	
XIII	15	0	0.4523200	552.4	0	0.4570700	558.2	0	0.3442700	420.4		
9.8.87	30	0	0.9019100	1101.4	0	1.3921000	1700.0	34	0.8771900	1040.2		
	45	0	1.0083000	1231.3	16	0.8662600	994 0	80	0.1737400	148.1		
	60	144	0.4200000	234.2	168	0.3577600	159.9	194	0.8726500	285.5		
	75	100	0 0920020	70.0	188	0.4325100	153.5	178	0.5028200	201.6		
		244		3189.3	372		3565.6	486		2095.9	8850.8	
XIV	15	0	0.0678460	82.9	0	0.1190200	145 3	0	1.0456000	1276.9		
	30	0	0.2913300	355.8	0	1.5912000	1943.2	40	1.0139000	1051.3		
	45	0	0.1911300	233.4	15	0.9211100	1061.2	112	0.2155200	152.0		
	60	0	0.1616000	197.3	70	0 5813400	522.4	20	0.0844700	95.4		
	75	0	0.0956410	116.8	10	0.1084200	127.4	182	0.4701500	179.8		
		0		986.2	95		3799.5	354		2755.3	7541.0	
XV	15	0	0.0023099	2.8	0	0.2336700	285.4	0	0.0026604	3.2		
	30	0	0.0059999	7.3	0	0.6019100	735.1	0	0.0062817	7.7		
	45	0	0.1559000	190.4	0	0 0041567	5.1	0	0.0045436	5.5		
	60	0	0.0023442	2.9	0	0.3980500	486.1	0	0.0035552	4.3		
	75	0	0 0000000	0.0	105	0.5086600	372.7	0	0.0159720	19.5		
		0		203.4	106		1884.3	0		40.3	2128.0	
XVI	15	0	0.0735230	89.8	0	0.0131460	16.1	0	0.3307400	403.9		
	30	0	0 0036254	4.4	0	0.1740900	212.8	0	0.1519100	185.5		
	45	46	0.2046500	206 5	0	0.1171700	143.1	50	0.1651000	163.6		
	60	0	0.0315560	38 5	30	0.3338600	361.6	40	0.0299750	31.1		
	75	0	0.1521800	185.8	132	0.4093000	250.9	16	0.0104070	11.9		
		46		525.1	162		984.2	106		796.0	2305 3	20825.0
XVII	15	56	0.5179700	498.9	18	1.3982000	1591.5	14	1.9986000	2311.7		
15.9.87	30	84	0.6512700	543.2	140	0.4973200	286.5	153	1.0653000	549.8		
	45	188	0.5315800	188.6	154	0.3419500	174.9	36	0.1802400	190.2		
	60	46	0.0152360	15.4	172	0.2505100	107.4	190	0.4718800	163.1		
	75	188	0.4806300	170.5	120	0.0292110	19.5	128	0.2004500	126.6		
		562		1416 6	604		2179.8	521		3341.4	6937.8	
XVIII	15	24	0 1013600	112.6		0.9124500	1114.3		0.0455210	55.6		
	30	70	0.3002200	269 8		0.4434700	541.6		1.0675000	1303.6		
	45		0.0485440	59.3	100	0.0310910	23.6	170	0.5260000	230.6		
	60	32	0.0562660	60.4		0.0661140	80.7	134	0.1992600	120.3		
	75	192	0.1036300	34.9		0.0076238	9.3	194	0.4568200	149.5		
		318		536.9	100		1769.5	498		1859.6	4166.1	
XIX	15		0.2186900	267.1	58	0.0379450	36.2			0 0		
	30		0.1896850	231 6	26	1.2155000	1338.7	100	1.5109000	1148.8		
	45	120	0.5170900	345.5	14	0.0349020	40.4	150	1.0465000	978.6		
	60	176	0.7657100	314.0	158	0.7112200	350.7	196	0.7350000	233 7		
	75		0.1097300	134.0	133	0.4692500	285.4		0.1869700	228.3		
		296		1292.3	389		2051.4	446		2589.4	5933.2	
XX	15	60	0.0027275	2.6	74	0.0841790	74.1		0.2511250	306 7		
	30		0.0016784	2 0	120	0.2196100	146.7		0.3358800	410.2		
	45	16	0 0052156	6.0	170	0.1667200	73 0		0.2316500	282 9		
	60	24	0.0011127	1.2	86	0.0454970	37.5			0.0		
	75		0.0020039	2.4	174	0.1166800	48.9		0.1595400	194.8		
		100		14.3	624		380.3	0		1194.6	1589.1	18626.2
XXI	15		0.0342280	41.8	112	0.6712900	473.3	92	0.7055500	562.5		
17.9.87	30		0.0760410	92.9	30	0.0256440	27.8	100	0.6271100	476.8		
	45		0.0869340	106.2	24	0.0159200	17.7	66	0.2292100	210.2		
	60		0.0310570	37.9	194	0.4545400	148 7	152	0.2483100	117.9		
	75		0.0812910	99.3	202	0.4361400	126.6	196	0.1327100	42.2		
		0		378.0	562		794.1	616		1409.6	2581.7	
XXII	15		0 0205210	25.1		0 0594210	72.6	66	2.2966000	2106 1		
	30	74	0 7243100	637 5	20	0.7541400	851.4	166	1.6266000	742.1		
	45		0.1273000	155.5	24	1.1396000	1265.6	198	0.1412300	43.6		
	60	96	0.6955400	541.7	132	0.5362100	328 6	36	0.0786420	83.0		
	75	122	0.5390700	355.2	192	0.6832400	229.8	16	0.0763410	87.6		
		292		1715.0	358		2748.1	482		3062.4	7525.5	
XXIII	15	114	0.4786800	333.1	14	0.9771800	1130.3	112	0.5088900	358.8		
	30		0.0307510	37.6	25	0.4424800	489.4	162	0.2536700	120.4		
	45	178	0 1553800	62 3	12	0.1132600	132.0	174	0.2230100	93.5		
	60	146	0.0159130	8.7	120	0.3555900	237.6	198	0.3133000	96.7		
	75		0.0257200	31.4	154	0.3036300	196 2	194	0.1647800	53.9		
		438		473.1	325		2185.6	840		723.4	3382.0	
XXIV	15	74	0.1246500	109.7	74	0.0090975	8.0		0.1703800	208.1		
	30	92	0.4885800	389.5	142	0.6341100	359.4	52	0.6065500	595.4		
	45	154	0.2287700	117.0	174	0.3601700	151.0		0.0020132	2.5		
	60		0.0050446	6.2	68	0.0625150	56.8		0.0333150	40.7		
	75	56	0.0571080	55.0	194	0.2991200	97.9	112	0.6756900	476.4		
		376		677.4	652		673.1	164		1323.0	2673.5	
XXV	15	82	0.1611700	135.9		1.8055000	2204.9		1.1664000	1424.4		
8.10.87	30	132	0.2243700	137.5		0.7094500	866.4		0.3035600	370 7		
	45	102	0.1944700	74.4	124 0	1 4336000	931.5	10	2.2217000	2610.8		
	60	5	0.0115700	13.9	35 0	0.1150300	121.9	198	0.8926600	275.6		
	75	176	0.1641300	67.3	194.0	0.5499400	205.3	186	0.5574400	202.9		
		577		429.0	343		4330.0	394		4884.4	9643.4	131370.5

Table 3. Methane extractions from Northern bogs.

	I			II			III				
	% CH4	ml water	umole CH4	% CH4	ml water	umole CH4	% CH4	ml water	umole CH4	Total avg	umoles CH4/ml
#1	16.0	2	1972.4	12.5	0	1478.7	27.5	0	3253.1		
80cm	37.3	94	2847.3	10.3	4	1200.1	23.5	180	1101.5		
	11.9	194	377.2	30.4	89	2388.4	2.0	120	129.5		
	0.5	17	88.4	3.5	89	275.0	0.5	178	258.2		
	0.0	172	365.3	16.9	176	871.4					
		846.0	5620.6		967.0	6013.6		694.0	4742.3	5461.5	6.91
#5	23.4	69	2047.4	5.2	11	589.6	16.4	42	1632.6		
50cm	0.0	10	0.0	14.0	67	1237.4	2.4	138	136.1		
	5.3	107	104.5	10.5	172	435.9	0.8	70	69.6		
	2.3	139	129.4	0.7	174	353.4	3.4	186	119.9		
	1.0	175	40.2								
	0.9	185	32.1								
		570.0	2433.6		636.0	2616.4		624.0	1958.2	2336.0	3.64
#6	0.6	78	50.1	0.6	73	51.4	1.2	126	74.5		
60cm	1.1	1	129.6	0.6	185	42.9	1.4	131	83.7		
	0.5	25	53.6	0.1	28	10.9	1.5	164	67.6		
	0.3	43	29.7	0.0	126	49.6	1.6	197	46.6		
	0.9	170	38.2	0.6	70	52.2					
				0.1	15	11.2					
		1088.0	381.2		1101.0	218.2		442.0	274.4	264.6	0.37
#7	0.3	17	33.2	0.5	34	51.6	0.4	26	42.7		
45cm	0.9	192	29.3	0.8	43	79.3	1.7	155	83.5		
	1.0	200	25.4	0.9	102	65.5	0.9	203	24.8		
	1.0	202	20.1	0.8	14	89.6	0.5	3	58.5		
				0.8	98	59.6	0.5	90	39.1		
				1.0	199	29.5					
		441.0	116.1		1100.0	375.1		840.0	240.8	246.6	0.30
#10	0.3	7	34.6	0.1	29	10.5	0.1	0	11.0		
48cm	0.1	95	7.6	0.5	33	51.0	0.1	30	10.1		
	0.2	151	10.2	0.2	45	19.6	0.3	49	20.9		
	0.0	184	0.0	0.1	50	9.6	0.1	99	7.4		
							0.0	29	0.0		
							0.1	99	7.4		
		542.0	52.3		303.0	91.6		1048	50.3	67.4	0.08
#12	0.3	2	35.2	0.1	0	11.0	0.0	4	0.0		
42cm	0.1	2	11.7	0.0	0	0.0	0.1	132	5.9		
	0.1	185	3.6	0.3	196	9.2	0.0	202	0.0		
	0.4	192	13.8	0.1	188	3.4	0.3	202	0.4		
	0.2	125	12.5	0.0	60	0.0					
				0.0	0	0.0					
				0.1	200	2.9					
		818.0	76.1		476.0	27.4		196.0	14.4	39.3	0.07
#16	0.7	46	22.3	0.2	0		1.0	16	110.3		
20cm	1.0	195	34.8	0.1	0		1.5	195	46.2		
	1.5	203	41.5	0.1	0		1.3	206	34.2		
	1.2	208	31.6	0.1	0		1.7	208	49.3		
				leaking							
		469.0	133.3					440.0	240.0	186.6	0.44
#17	0.4	38	48.5	0.2	0		0.0	3	0.0		
38cm	0.6	198	17.9	0.2	0		0.3	98	23.4		
	0.5	176	19.9	0.0	0		0.5	174	20.3		
	0.7	201	20.0	0.1	0		0.7	194	27.2		
				leaking			0.8	208	20.4		
		447.0	98.3					394.0	86.3	92.3	0.22
#18	0.4	25	42.9	0.0	0	0.0	0.4	19	43.9		
40cm	0.7	197	21.2	0.2	44	19.7	0.5	193	16.1		
	0.7	204	19.1	0.4	202	11.2	0.5	203	13.8		
	0.1	185	7.1	0.4	205	10.7	0.6	207	15.5		
				0.4	204	10.9					
		529.0	90.3		485.0	52.6		438.0	89.4	77.4	0.17
#19	1.7	10	193.5	0.2	48	19.4	0.1	3	11.7		
32cm	2.0	6	231.2	1.7	75	144.2	0.0	14	89.5		
	0.7	38	70.6	1.8	200	52.2	1.9	142	104.3		
	1.4	202	39.4	1.3	207	33.7	0.1	7	11.5		
				1.5	204	48.8	1.3	183	47.6		
		883.0	534.7		591.0	298.3		976.0	264.8	363.3	0.48
#22	0.2	45	19.6	0.0	28	0.0	0.1	38	10.5		
38cm	0.1	44	9.9	0.1	188	7.4	0.2	132	11.9		
	0.1	68	0.0	0.2	122	12.8	0.3	208	7.9		
	0.1	125	6.2								
		778.0	44.6		388.0	28.1		427.0	38.3	31.6	0.06
#23	0.7	5	81.2	1.6	5	185.7	2.4	88	189.6		
40cm	1.2	5	139.3	1.9	66	150.0	2.6	92	208.0		
	0.9	2	185.7	0.6	84	49.5	2.4	162	110.4		
	1.1	5	127.7	0.5	54	47.1	1.7	158	73.6		
		1043.0	453.9		851.0	458.1		550.0	574.4	492.0	0.67
#24	1.3	0	153.0	0.0	2	0.0	0.2	188	7.6		
40cm	0.1	0	11.0	0.4	38	40.5	0.1	18	11.4		
	0.3	32	31.2	0.4	88	33.0	0.1	208	2.9		
	0.1	72	0.6	0.0	0	0.0	0.1	12	11.3		
		956.0	205.4		412.0	73.6		658.0	33.2	104.1	0.15
#25	3.0	0	354.9	0.2	99	14.0	0.4	178	17.0		
60cm	0.0	10	0.0	0.3	185	10.7	0.0	202	0.0		
	1.0	78	87.0	0.3	204	8.2	0.4	201	11.4		
	0.4	83	32.5	0.0	26	0.0	0.4	203	11.1		
	0.0	0	0.0	0.1	187	3.5	1.1	208	27.5		
	1.0	88	79.9	0.1	187	3.5					
		821.0	554.3		463.0	46.7		277.0	67.0	228.7	0.33
#26	0.1	0	11.0	1.0	36	184.0	0.1	66	0.5		
60cm	0.2	0	23.7	0.2	48	32.1	0.7	178	29.7		
	0.4	0	47.3	1.0	158	53.0	1.1	187	38.3		
	0.2	0	23.7	1.7	199	58.1	0.1	41	10.0		
	0.1	18	11.4	1.0	203	27.7	1.0	199	29.5		
							1.2	203	33.2		
		1315.0	117.0		649.0	335.7		724.0	149.5	201.0	0.27
#27	0.0	0		0.4	28	42.3	0.2	152	10.1		
60cm	0.0	0		2.1	122	134.1	0.6	148	31.3		
	0.0	0		0.0	25	0.0	0.0	164	36.1		
	0.3	0		1.1	205	29.5	0.1	0	11.0		
	0.1	0		0.3	202	0.4	0.3	143	18.3		
	leaking			0.9	195	28.1					
				0.2	134	11.7					
					704.0	254.1		718.0	105.7	179.9	0.25

APPENDIX 10

METHANE INCUBATIONS

Details of three methane incubation experiments are given in this Appendix together with some initial results of incubations in columns.

Experiment 1. 10g of wet peat was incubated, in triplicate, with 10 ml of different deoxygenated media to assess the best method of growth. The peat extract was prepared by autoclaving for 30 min 500 g of wet peat with 750 ml distilled water; filtered through cheesecloth, centrifuged and finally filtered through #3 Whatman paper; brought to the boil under nitrogen, anaerobically dispensed into vials, capped and autoclaved.

Sample A contained 10 ml peat extract

Sample B contained 5 ml peat extract and 5 ml methanogen medium

Sample C contained 5 ml peat extract and 5 ml water

Sample D contained 10 ml medium

Sample E contained 10 ml water

Sample F contained 10 ml water and was autoclaved 30 min as control.

The vials were incubated in the dark at 25°C without shaking. Figure 1 shows the integrated methane peaks from the gas chromatograph, during one week incubation, and Table 1 shows the calculation of the amount of methane from these peaks. Methane accumulated during the week, even when its production was calculated per hour. The best media appears from this experiment to be water, followed by peat extract, the methanogen media produced the least methane both with and without peat extract.

Experiment 2. The incubations of four sites from four different stations in Mer Bleue, is described in Section 4.4.2. The cumulative probability graph plotted in Figure 2 shows the

typical S-shape of two populations. Calculations (Sinclair 1981) divide these populations into 45% for the high production of methane and 55% for the low. The theoretical curve for this division of the population is shown by the open triangles. Table 2 shows the readings and calculations, over two weeks, from these incubations.

Experiment 3. Table 3 shows the methane produced from incubations of commercially available peat. Sphagnum and peat samples came from Rivière du Loup, Quebec, and peat samples from Moose Creek, Alfred bog, and Caladonia Springs, in Ontario. Samples were taken from both the top and from newly turned peat at Caladonia Springs, and it is interesting that the sample from the deeper layer took much longer to produce methane. Over time all the samples were good methane producers except the sphagnum peat from Rivière du Loup.

Experiment 4. The experiment was set up with 90 x 300 mm Ace glass columns, with screw-in sintered glass bases with a central drain. This was covered first by 50 g washed quartz chips, and then with a thin layer of glass wool, and finally No.1 Whatman filter paper. The columns were charged with test mixtures as follows:

1. 50 mm tailings
2. 50 mm tailings + 130 mm peat
3. 50 mm tailings + 130 mm 50:50 mix peat/paper sludge
4. 50 mm tailings + 65 mm paper sludge + 65 mm peat
5. 130 mm peat
6. 130 mm 50:50 mix peat/paper sludge

These were saturated and covered with water to the surface. The columns were maintained at room temperature for 46 days with no throughput of water. At the end of this

time a tube with compressed air was attached to the bottom of the column to blow out the entrapped gas. The top of the column was closed with #15 rubber bung, adapted to take a butyl rubber stopper through which the gas phase could be sampled. The methane was calculated assuming that all the entrapped gas was displaced into the top of the column and that it was homogeneously mixed.

The results are shown in Table 4. The peat and paper sludge produced much the most methane, particularly when mixed. Peat by itself produced little methane during this time. This result is encouraging as it shows that it is possible to produce measurable amounts of methane in a layered system in columns in a reasonable time, and that it may be possible to achieve similar results in lysimeters and in field situations.

Figure 1. Gas chromatograph plots from Experiment 1.

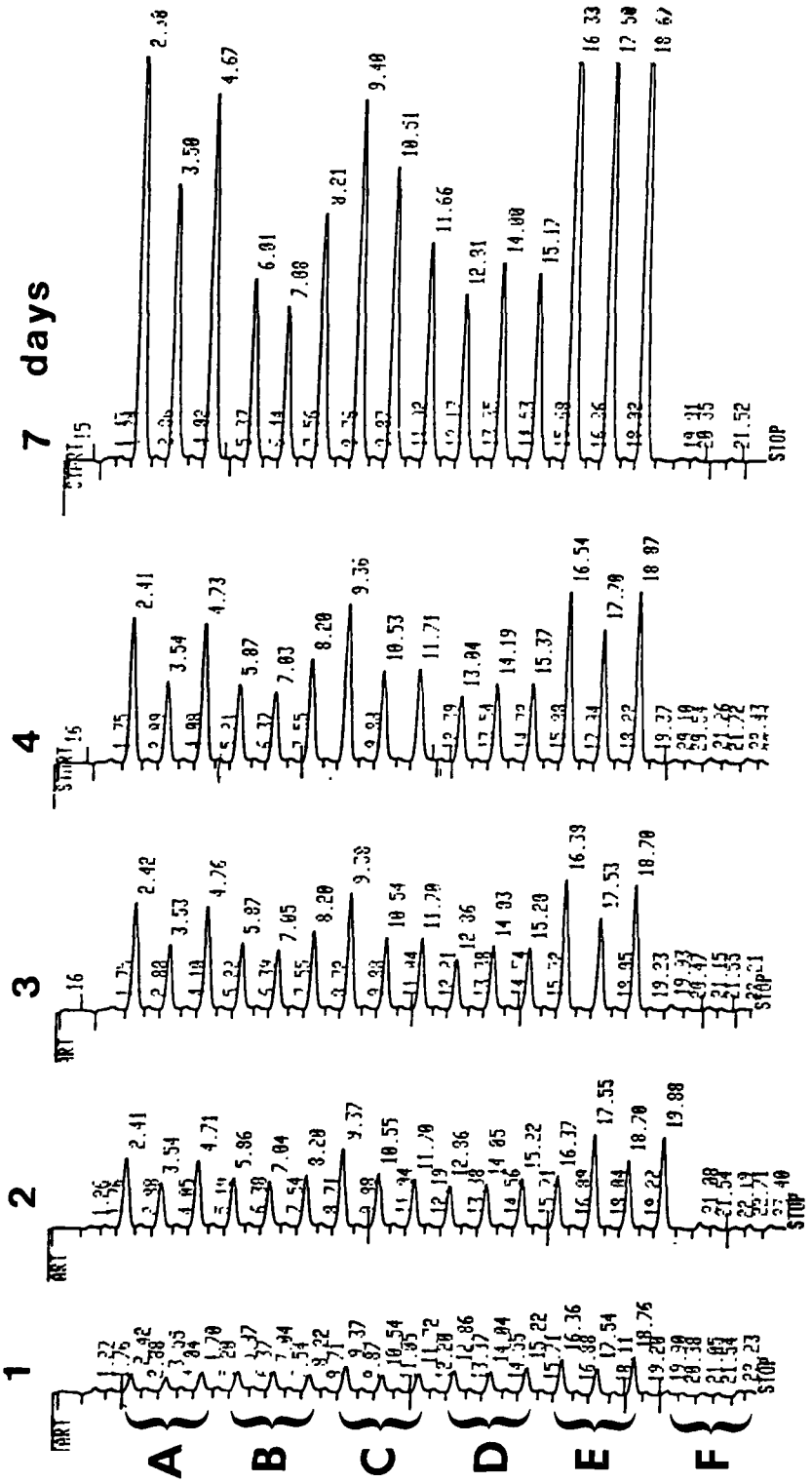


Table 1. Methane incubations from Experiment 1.

Sample		24 11 87 area	µmoles	nmoles /g/hr	25 11 87 area	µmoles	nmoles /g/hr	26 11 87 area	µmoles	nmoles /g/hr	27 11 87 area	µmoles	nmoles /g/hr	30.11.87 area	µmoles	nmoles /g/hr
Peatext	1	0 0015140	0 165		0 0061322	0 505		0 0101850	0 574		0 0132060	0 825		0 0388850	3 260	
	2	0 0013542	0 150		0 0041051	0 299		0 0066151	0 403		0 0076306	0 392		0 0241500	2 168	
	3	0 0017919	0 198		0 0060942	0 465		0 0095276	0 554		0 0124510	0 766		0 0318200	2 622	
			0 171	6.835		0 423	16 910		0 510	20.414		0 661	26 441		2 683	35 776
Peatext + media	1	0 0019124	0 216		0 0047661	0 300		0 0061649	0 388		0 0073257	0 382		0 0155280	1 264	
	2	0 0019429	0 215		0 0043203	0 255		0 0057295	0 334		0 0065306	0 352		0 0136120	1 084	
	3	0 0017479	0 197		0 0048635	0 338		0 0074435	0 457		0 0095627	0 556		0 0215900	1 732	
			0 209	8 375		0 298	11 914		0 393	15 724		0 430	17 202		1 360	18 136
Peatext + water	1	0 0025456	0 283		0 0070330	0 485		0 0106140	0 644		0 0143100	0 879		0 0311500	2 408	
	2	0 0016486	0 184		0 0048288	0 348		0 0065920	0 357		0 0083768	0 535		0 0257890	2 018	
	3	0 0018683	0 209		0 0044904	0 281		0 0065207	0 413		0 0083977	0 477		0 0193480	1 574	
			0 225	9 010		0 372	14 861		0 471	18 356		0 630	25 213		2 000	26 667
Media	1	0 0022121	0 245		0 0039523	0 185		0 0047326	0 314		0 0061429	0 648		0 0148230	0 896	
	2	0 0020963	0 232		0 0045210	0 258		0 0058778	0 365		0 0073042	0 402		0 0176520	1 461	
	3	0 0025728	0 285		0 0047573	0 235		0 0056282	0 362		0 0072890	0 404		0 0167420	1 355	
			0 254	10 147		0 226	9 039		0 347	13 880		0 485	19 397		1 237	16 493
Water	1	0 0032890	0 367		0 0083802	0 552		0 0117390	0 686		0 0153660	0 921		0 0497020	4 049	
	2	0 0024871	0 279		0 0062218	0 401		0 0084745	0 435		0 0120610	0 838		0 0391720	3 314	
	3	0 0033299	0 370		0 0082828	0 539		0 0112200	0 656		0 0153180	0 954		0 0503800	4 224	
			0 338	13 535		0 497	19 893		0 592	23 693		0 904	36 169		3 862	51 496

Figure 2. Cumulative probability plot of methane incubations from Mer Bleue peat from Experiment 2.

——— • ——— Best fit curve to measured points.

- - - - - △ - - - - Theoretical curve for 45% high and 55% low populations.

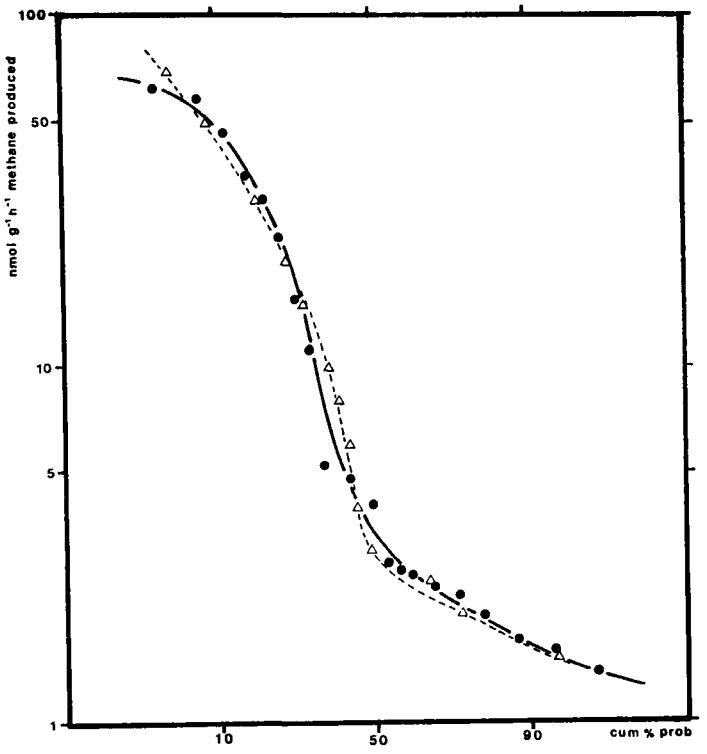


Table 2. Methane incubations of Mer Bleue peat from Experiment 2.

Station	area	umoles	nmoles /g/hr	area	umoles	nmoles /g/hr	Station	area	umoles	nmoles /g/hr	area	umoles	nmoles /g/hr
VII 20cm	0.1864400	12.028		0.4722500	30.468		XIII 20cm	0.3822800	24.663		0.8352300	53.886	
	0.1178400	7.603		0.4616200	29.782			0.4147600	26.759		0.7535500	48.616	
	0.2723500	17.571		1.0346000	66.748			0.5460700	35.230		1.2597000	81.271	
		12.401	58.54		42.333	124.90			28.884	131.66		61.258	174.52
60cm	0.0048928	0.316		0.0243850	1.573		60cm	0.0086143	0.556		0.0106570	0.688	
	0.0043479	0.281		0.0404780	2.611			0.0094134	0.607		0.0094721	0.611	
	0.0022468	0.145		0.0290600	1.875			0.0076212	0.492		0.0111900	0.722	
		0.247	1.72		2.020	3.00			0.552	1.97		0.674	1.50
90cm	0.0017835	0.115		0.0024582	0.159		90cm	0.0104800	0.676		0.0084473	0.545	
		0.000			0.000			0.0106800	0.689		0.0099543	0.642	
		0.000			0.000			0.0091222	0.589		0.0090294	0.583	
		0.115	0.80		0.159	0.24			0.651	2.60		0.590	1.47
120cm	0.0047279	0.305		0.0040424	0.261		120cm	0.0058854	0.380		0.0063203	0.408	
	0.0062008	0.400		0.0057072	0.368			0.0076171	0.491		0.0086299	0.557	
	0.0071290	0.460		0.0058931	0.380			0.0113400	0.732		0.0139990	0.903	
		0.388	2.69		0.336	0.54			0.534	2.40		0.623	1.75
VI 20cm	0.2825400	18.228		1.4582000	94.077		XXV 20cm	0.0616810	3.979		0.2818200	18.182	
	0.2083500	13.442		0.9999600	64.514			0.1703700	10.992		0.9366300	60.428	
	0.2822200	18.208		1.4338000	92.503			0.0515140	3.323		0.2252000	14.529	
		16.626	77.24		83.698	243.03			6.098	28.25		31.046	89.90
60cm	0.0102350	0.660		0.0264160	1.704		60cm	0.0081884	0.528		0.0084957	0.548	
	0.0052865	0.341		0.0072407	0.467			0.0082273	0.531		0.0079359	0.512	
	0.0065728	0.424		0.0074762	0.482			0.0065823	0.425		0.0089437	0.577	
		0.475	1.61		0.885	1.88			0.495	1.69		0.546	1.16
90cm	0.0150790	0.973		0.0105070	0.678		90cm	0.0146820	0.947		0.0127360	0.822	
	0.0175070	1.129		0.0138110	0.891			0.0207310	1.337		0.0215860	1.393	
	0.0188430	1.216		0.0208420	1.345			0.0213740	1.379		0.0229150	1.478	
		1.106	5.28		0.971	2.90			1.221	5.28		1.231	3.33
120cm	0.0053957	0.348		0.0057895	0.374		120cm	0.0167270	1.079		0.0142000	0.916	
	0.0045049	0.291		0.0064673	0.417			0.0167840	1.083		0.0186690	1.204	
	0.0040709	0.263		0.0035321	0.228			0.0195360	1.260		0.0143970	0.929	
		0.300	1.36		0.340	0.96			1.141	4.81		1.016	2.69

Table 3. Methane incubations from commercial peat in Experiment 3.

Sample		22.11.88 area	umoles	nmol/g//h	5.12.88 area	umoles	nmol/g//h	13.12.88 area	umoles	nmol/g//h	9.1.89 area	umoles	nmol/g//h
R du L sphag.	1	0.0017849	0.115		0.0088951	0.574		0.0046321	0.299		0.0124430	0.803	
	2	0.0013141	0.085		0.0133760	0.863		0.0097481	0.629		0.0445710	2.876	
	3	0.0011126	0.072		0.0114340	0.738		0.0102640	0.662		0.0318060	2.052	
			0.091	0.281		0.725	2.247		0.530	1.643		1.910	5.922
R du L peat	4	0.0012910	0.083		0.0037187	0.240		0.0348860	2.251		0.1583200	10.214	8.172
	5	0.0011997	0.077		0.0045890	0.296		0.0160510	1.036		0.1584000	10.219	8.176
	6	0.0032261	0.208		0.0361980	2.335		0.0686200	4.427		0.2066200	13.330	10.665
			0.123	0.459		0.957	3.573		2.571	9.599		11.255	42.020
Moose C	1	0.0010645	0.069		0.0437080	2.820		0.3390600	21.875				
	2				0.0145710	0.940		0.1302500	8.403				
	3	0.0013326	0.086		0.0509150	3.285		0.6396500	41.268				
			0.077	0.176		2.348	5.347		23.849	54.300			
Alfred	1	0.0011122	0.072		0.0096780	0.624		0.0911450	5.880				
	2	0.0013105	0.085		0.1078300	6.957		2.2487000	145.077				
	3	0.0011312	0.073		0.1111900	7.174		2.8564000	184.284				
			0.078	0.203		4.918	12.744		111.747	289.581			
Cal Sp top	1				0.2233600	14.410		2.8458000	183.600				
	2	0.0016256	0.105		0.1610100	10.388		2.0990000	135.419				
	3				0.0589590	3.804		0.9800000	63.226				
				0.284		9.534	25.863		127.415	345.635			
Cal Sp bottom	1	0.0010863	0.070					0.0033605	0.217		0.2511900	16.206	
	2							0.0014103	0.091		1.1852000	76.465	
	3							0.0013507	0.087		0.0327700	2.114	
				0.235					0.132	0.442		31.595	47.016

Table 4. Methane production in laboratory columns.

Columns	I	II	III	IV	V	VI
Packing	tailings	tailings + peat	tailings + peat/sludge	tailings + sludge + peat	peat	peat/sludge
gas vol ml	1463	573	445	509	700	700
μmol CH_4	1	14	427	200	2.5	353

APPENDIX 11

MIXED METHANE INCUBATIONS

The deeper layers of the peat produce considerably less methane than the upper layers, and also air-dried peat and sphagnum seem to exhibit an inhibitory effect on methane production. Several mixed incubations were set up to determine whether this inhibition was due to the physical alteration of the peat on drying, or whether it was due to some inhibitory substance in the peat itself.

Experiment 1. Two peat samples (3 and 4) from Mer Bleue were used, 10 g of wet peat was incubated in 10 ml of deoxygenated water under an atmosphere of hydrogen/carbon dioxide. 30 cm and 60 cm peat were incubated separately, a mixture of 5 g each from the two peat depths were incubated together; and as controls, 5 g of the 30 cm depth peat was incubated with autoclaved 60 cm peat and a refined cellulose, Whatman CF11.
Sample 3 was more active than sample 4, but the 30 cm peat produced much more methane than the 60 cm peat. The mixed incubations produced little more methane than the 60 cm alone. Surprisingly however, the 30 cm peat with the autoclaved 60 cm peat was the next most productive after the 30 cm peat alone, followed by 30 cm with CF11. From these results it appears that there is a definite inhibitor in the 60 cm peat, and this cannot only be removed by autoclaving, but with sample 4 autoclaving actually enhanced the activity of the 30 cm peat.

Experiment 2. This was a repeat of the previous experiment using Mer Bleue samples 5, 6, 7 and 8, without the CF11 control. These samples generally were not very active, but the results confirm those of Experiment 1.

Experiment 3. Mer Bleue samples 7 and 8 were used, and these were incubated alone and with horticultural sphagnum and with air-dried peat. Initially both the dried sphagnum and peat were inhibitory, however after eight weeks the methane production had increased in the incubations of sample 7 with sphagnum, and of sample 8 with the dried peat. This result possibly shows that since the substrates are natural products they are very heterogeneous, and that even triplicate incubations are not sufficient to ensure consistent results.

Table 1. Mixed peat incubations from Experiment 1.

Sample		9.8.88 area	µmoles	nmoles/g/h	16.8.88 area	µmoles	nmoles/g/h	30.8.88 area	µmoles	nmoles/g/h	13.9.88 area	µmoles	nmoles/g/h
3030	1	0.1391100	8.975	53.422	0.6467600	41.726		0.9615600	62.036		0.5837900	37.664	
	2	0.0783690	5.056	30.096	0.3152000	20.335		0.5414800	34.934		1.4200000	91.613	
	3	0.0497580	3.210	19.108	0.2684300	17.318		0.4201300	27.105		0.2991700	19.301	
			5.747	30.647		26.460	70.552		41.358	110.277		49.526	132.054
3060	1	0.0035333	0.234		0.0038964	0.258		0.0022921	0.148		0.0062517	0.403	
	2	0.0079797	0.515		0.0065256	0.421		0.0030655	0 198		0.0135250	0.873	
	3	0.0086278	0.557		0.0110400	0.712		0.0071849	0.464		0.0274250	1.769	
			0.435	2.775		0.464	1.478		0.270	0.860		1.015	3.236
3030+601		0.0253300	1.689		0.0527600	3.404		0.0291480	1.816		0.0187440	1.209	
	2	0.0091711	0.592		0.0150280	0.970		0.0078902	0.509		0.0075372	0.486	
	3	0.0195580	1.262		0.0455210	2.937		0.0357570	2.307		0.0303770	1.960	
			1.184	6.876		2.437	7.075		1.544	4.483		1.218	3.538
3030	1	0.0751600	4.849		0.2475400	15.970		0.3953900	25.541		0 4728400	30.506	
+60auto	2	0.0228280	1.473		0.0344350	2.222		0.0327920	2 115		0.0528140	3.407	
	3	0.0143340	0.925		0.0240180	1.550		0 0291730	1.882		0.0428670	2.766	
			2.416	14.027		6.580	19.107		9.846	28.589		12.226	35.500
3030	1	0.0035756	0.231		0.0034743	0.224		0.0580450	3.745		0.3439900	22.193	
+CF11	2	0.0059242	0.382		0.0205600	1.326		0.2454000	15.832		0.7521700	48.527	
	3	0.0076861	0.496		0.0228570	1.475		0.0630930	4.071		0.0658390	4.248	
			0.370	2.079		1.308	3 936		7.883	22.172		24.989	70.289
4030	1	0.0301330	1 944		0.0985940	6.361		0.1510400	9.745		0.1680400	10.841	
	2	0.0292950	1.890		0.0563570	3.636		0.0420560	2.713		0.0549320	3.544	
	3	0.0294570	1.900		0.0726140	4.685		0.0439300	2.834		0.0280450	1.809	
			1.912	10.325		4.894	13.217		5.097	13.786		5.398	14.579
4060	1	0.0078839	0.509		0.0091129	0.588		0.0099323	0.641		0.0073732	0.476	
	2	0.0045390	0.293		0.0064685	0.417		0.0087014	0.561		0.0073483	0.474	
	3	0.0052344	0.338		0.0067604	0.436		0.0082445	0.532		0.0080192	0.517	
			0.380	2.442		0.480	1.545		0.578	1.859		0.489	1.572
4030+601		0.0146310	0.944		0.0410380	2.648		0.0581130	3.749		0.0325380	2.099	
	2	0.0261740	1.689		0.0649000	4.187		0.0540500	3.487		0.0361210	2.330	
	3	0.0376430	2.429		0.0841840	5.431		0.0766730	4.947		0.0439320	2.834	
			1.687	9.905		4.089	12.003		4.061	11.922		2.421	7.108
4030	1	0.0233530	1.507		0.0462250	2.982		0.1197900	7.728		0.2500600	16.133	
+60auto	2	0.0381980	2.464		0.0862070	5.562		0.0808810	5.218		0.1427500	9.210	
	3	0.0200410	1.293		0.0493150	3.182		0.0674410	4.351		0.0730630	4.714	
			1.755	10.302		3.909	11.474		5.766	16.927		10.019	29.412
4030	1	0.0064070	0.413		0.0280670	1.811		0.1133000	7.310		0.0988050	6.375	
+CF11	2	0.0106320	0.686		0.0402700	2.598		0.0770660	4.972		0.0902780	5.824	
	3	0.0032331	0.209		0.0108740	0.702		0.0156090	1.007		0.0121940	0.787	
			0.436	2.469		1.703	4.824		4.430	12.544		4.329	12.257

Table 2. Mixed peat depth incubations from Experiment 2.

Sample		18.10.88 area	µmoles	nmol/g/h	25.10.88 area	µmoles	nmol/g/h
5@30	1	0.0110130	0.711		0.0473380	3.054	
	2	0.0112200	0.724		0.0599600	3.868	
	3	0.0059855	0.386		0.0287130	1.852	
			0.607	4.762		2.925	10.594
5@60	1	0.0098564	0.636		0.0284130	1.833	
	2	0.0114870	0.741		0.0349940	2.258	
	3	0.0086179	0.556		0.0209430	1.351	
			0.644	4.072		1.814	5.291
5@30+60	1	0.0072894	0.470		0.0143790	0.928	
	2	0.0088068	0.568		0.0206230	1.331	
	3	0.0083127	0.536		0.0242370	1.564	
			0.525	3.718		1.274	4.165
5@30 +60auto	1	0.0059401	0.383		0.0171160	1.104	
	2	0.0106290	0.686		0.0171750	1.108	
	3	0.0230090	1.484		0.0629370	4.060	
			0.851	6.029		2.091	6.836
6@30	1	0.0257250	1.660		0.0848770	5.476	
	2	0.0323440	2.087		0.0680500	4.390	
	3	0.0366230	2.363		0.0748930	4.832	
			2.036	15.273		4.899	16.959
6@60	1	0.0057432	0.371		0.0065302	0.421	
	2	0.0051943	0.335		0.0045734	0.295	
	3	0.0072263	0.466		0.0139760	0.902	
			0.391	2.387		0.539	1.521
6@30+60	1	0.0109860	0.709		0.0200780	1.295	
	2	0.0070422	0.454		0.0075020	0.484	
	3	0.0124330	0.802		0.0181030	1.168	
			0.655	4.435		0.982	3.070
6@30 +60auto	1	0.0169300	1.092		0.0162320	1.047	
	2	0.0191560	1.238		0.0310230	2.001	
	3	0.0194670	1.256		0.0387550	2.500	
			1.195	8.089		1.850	5.780

Sample		18.10.88 area	µmoles	nmol/g/h	25.10.88 area	µmoles	nmol/g/h
7@30	1	0.0943460	6.087		0.2210800	14.263	
	2	0.1062400	6.854		0.2857000	18.432	
	3	0.0952820	6.147		0.3183000	20.535	
			6.363	47.721		17.744	61.420
7@60	1	0.0093865	0.606		0.0065265	0.421	
	2	0.0048027	0.310		0.0048330	0.312	
	3	0.0167400	1.080		0.0109520	0.707	
			0.665	4.065		0.480	1.353
7@30+60	1	0.0130030	0.839		0.0177450	1.145	
	2	0.0126180	0.814		0.0257730	1.663	
	3	0.0149870	0.967		0.0352640	2.275	
			0.873	5.943		1.694	5.322
7@30 +60auto	1	0.0193490	1.248		0.0334270	2.157	
	2	0.0174960	1.129		0.0299870	1.935	
	3	0.0199970	1.290		0.0389990	2.516	
			1.222	8.319		2.202	6.918
8@30	1	0.0476330	3.073		0.1758800	11.347	
	2	0.0340110	2.194		0.0707320	4.563	
	3	0.0538860	3.477		0.2013800	12.992	
			2.915	23.884		9.634	36.437
8@60	1	0.0053681	0.346		0.0053949	0.348	
	2	0.0045661	0.295		0.0050323	0.325	
	3	0.0045094	0.291		0.0035661	0.230	
			0.311	2.286		0.301	1.022
8@30+60	1	0.0168740	1.089		0.0351240	2.266	
	2	0.0518600	3.346		0.1126500	7.268	
	3	0.0127470	0.822		0.0371010	2.394	
			1.752	13.629		3.976	14.272
8@30 +60auto	1	0.0352150	2.272		0.1751000	11.297	
	2	0.0190980	1.232		0.0826920	5.335	
	3	0.0080325	0.518		0.0346690	2.237	
			1.341	10.428		6.289	22.578

Table 3. Methane incubations from commercial sphagnum and peat from Experiment 3.

Sample	25.10.88 area	µmoles	nmol/g/h	8.11.88 area	µmoles	nmol/g/h	22.11.88 area	µmoles	nmol/g/h	5.12.88 area	µmoles	nmol/g/h	13.12.88 area	µmoles	nmol/g/h
#7 1	0.0258410	1.667		0.1257800	8.115		0.0444600	2.868		0.0348700	2.250		0.0233890	1.509	
2	0.0159180	1.027		0.4355100	28.104		0.2609000	16.832		0.4592100	29.626		0.3303500	21.313	
3	0.0207010	1.336		1.5393000	99.310		3.4146000	220.297		3.5041000	226.071	724.586	2.1848000	140.955	
		1.343	9.329		45.176	134.453		79.999	238.093		85.982	275.585		54.592	284.3
#7sphag1	0.0011652	0.075		0.0073073	0.471		0.0306470	1.977		0.2441200	15.750	50.480	0.7285800	47.005	
2	0.0015889	0.103		0.0020235	0.131		0.0036178	0.233		0.0062354	0.402		0.0156840	1.012	
3	0.0010757	0.069		0.0032132	0.207		0.0041380	0.267		0.0136780	0.882		0.0105070	0.678	
		0.082	0.572		0.270	0.803		0.826	2.458		5.678	18.199		16.232	84.5
#7dr vpt1	0.0018907	0.122		0.0037298	0.241		0.0019055	0.123		0.0029159	0.188		0.0034190	0.221	
2	0.0015377	0.099		0.0044048	0.284		0.0025749	0.166		0.0031930	0.206		0.0040904	0.264	
3				0.0022469	0.145		0.0019262	0.124		0.0029398	0.190		0.0031802	0.205	
		0.111	0.768		0.223	0.664		0.138	0.410		0.195	0.624		0.230	1.2
#8 1	0.0267160	1.724		1.0208000	65.858		1.7895000	115.452		1.1807000	76.174		0.9792400	63.177	
2	0.0213110	1.375		0.3399800	21.934		0.0968330	6.251		0.0310720	2.005		0.0137960	0.890	
3	0.0157690	1.017		0.1866400	12.041		0.3067000	19.797		0.1198900	7.735		0.0573020	3.697	
		1.372	9.527		33.278	99.041		47.163	140.367		28.638	91.788		22.588	117.6
#8sphag1										0.0014688	0.095		0.0016634	0.107	
2	0.0016919	0.109		0.0020437	0.132		0.0018861	0.122		0.0025182	0.162		0.0041649	0.269	
3	0.0017392	0.112		0.0020884	0.135		0.0088223	0.569		0.0387650	2.501		0.0728630	4.701	
		0.111	0.769		0.133	0.397		0.345	1.028		0.919	2.947		1.692	8.8
#8drypt1				0.0022991	0.148		0.0054091	0.349		0.0236140	1.523		0.1872800	12.083	
2	0.0020519	0.132		0.0065346	0.422		0.0254700	1.643		0.1996300	12.879		1.0443000	67.374	
3	0.0024398	0.157		0.0109340	0.705		0.0633040	4.084		0.6423600	41.443		3.6140000	233.161	
		0.145	1.006		0.425	1.265		2.025	6.029		18.615	59.664		104.206	542.7

APPENDIX 12

CALCULATIONS

ANOVA for Methane Extractions from Mer Bleue

Method from Biometry: R.R. Sokal and F.J. Rohlf, W.H. Freeman and Co., New York, 1981. Boxes 9.3 and 9.4.

Data from Table 4.8.

a = 3
n = 75

1. Grand total = 524.776
2. Sm of squared observations = 3781.467
3. Sum of the squared group totals divided by n = 3690.32
4. Grand total squared and divided by total sample size = 3671.868
5. Sum of squares, total = 109.599
6. Sum of squares, groups = 18.452
7. Sum of squares, within = 91.147

ANOVA table

Source of variation	df	SS	MD	F
Among groups	2	18.452	9.226	7.288
Within groups	72	91.147	1.266	
Total	74	109.599		

$F_{.005} = 5.52$, $F_{.001} = 7.55$

The three different levels of methane extraction are significantly different, and from these calculations do not form one population. However according to the graphical method of Sinclair (1981) the methane extraction is a single population (Figure 4.19). The varience component can be calculated from the above ANOVA:

Varience component $\frac{9.226 - 1.266}{25} = 0.3187$

Variation between groups $\frac{0.3187 \times 100}{1.266 + 0.3187} = 20.1\%$

Variation among groups $\frac{1.266 \times 100}{1.266 + 0.3187} = 79.9\%$

The major part of the variation is therefore, within the methane extraction at each level, and not between the different levels.

ANOVA for Cellulose Component in Mer Bleue Samples.

Data from Table 4.9.

a = 4
n = 16

1. Grand total = 337.5
2. Sum of squared observations = 8573.19
3. Sum of the squared group totals divided by n = 1927.299
4. Grand total squared and divided by total sample size = 1779.785
5. Sum of squares, total = 6793.405
6. Sum of squares, groups = 147.514
7. Sum of squares, within = 6645.891

ANOVA table

Source of variation	df	SS	MD	F
Among groups	3	147.514	49.171	0.466
Within groups	63	6645.891	105.490	
Total	66	6793.405		

$F_{.75} = .405$, $F_{.50} = .798$

The variance is not significant, thus the cellulose component of Mer Bleue peat is from one population.

Long term methane production in Mer Bleue.

The average methane production during laboratory incubations from the 120 cm level of Mer Bleue is 2.82 nmol g^{-1} h^{-1}. In one year, at this rate, 24.70 μmol of methane would accumulate, and in 100 years this would amount to 2.5 mmol. The 75 sites in Mer Bleue produced 130 mmol, or 1.7 mmol per litre, so the 2.5 mmol of methane could be accommodated within 1.5 of the extracted litres.

Amount of Methane in Mer Bleue.

The area around the sampling tube from which methane can be extracted is not known, but the maximum amount of water that can be collected by an evacuated bottle is 200 ml, i.e. a total of 1000 ml or $(10\ cm)^3$ for each site.

There are 75 sites, therefore the total volume that could have been extracted was 0.075 m^3.

From this volume we actually accumulated 130 mmol of methane.

So 1 m^3 would produce 1.7 mol methane.

The total area of Mer Bleue is 2050 ha, if an average depth of 3 m is taken, the total volume would be 61.5 Mm^3.

If the methane values we obtained can be taken as representative of the bog as a whole, the calculated amount of methane it could contain is 107 Mmol or 1.7 Gg methane.