

CONTROL OF CATION CONCENTRATIONS, AND IN PARTICULAR  
OF pH, IN *SPHAGNUM* DOMINATED COMMUNITIES

by

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*Abstract*

The concentration of organic acid anions in water samples from *Sphagnum* dominated bog communities is found to be far from stoicheometrical equivalence with the H<sup>+</sup> in these samples. Reasons are reviewed for supposing that the main source of H<sup>+</sup> is indeed from the plants, but that the equivalent organic acid anions remain in complex structural polymers in the plants.

Most of the members of the genus *Sphagnum* (bog-mosses) are only semi-aquatic, but shallow lakes in regions of rocks resistant to weathering may be colonised by these plants and they are, in general, abundant in Boreal regions (Sjörs 1961).

The present contribution is concerned mainly with one aspect of the control of their environment by *Sphagnum* spp., that is with the concentrations of cations in the water around the plants, and particularly with the pH. It is well known that in areas dominated by *Sphagnum* the pH of the water round the plants is usually below 4.5, and sometimes below 3.0 (POORE and WALKER, 1959), especially on hummocks.

The most plausible explanations of the continuing acidity of *Sphagnum* dominated areas are:

1. Excretion of whole organic acids from the plants into the external solution — an explanation most recently supported by RAMAUT (1955 a and b). ("Whole organic acid" is intended to indicate stoicheometrically equivalent amounts of H<sup>+</sup> and organic acid anion).
2. Cation exchange for H<sup>+</sup> on new exchange sites in the plants, formed as a result of growth at the plant apex.
3. Activities of sulphur metabolising bacteria which are known to be active at least in parts of the anaerobic zone of bogs (for example, BENDA 1957, BURGEFF, 1961, CLYMO, 1965).

Some importance has been ascribed to interconversion of sulphur compounds by GORHAM (1956), but it is difficult to assess how much they contribute to bog acidity and they will not be considered further here.

A fourth explanation — that the low pH is mainly due to high CO<sub>2</sub> concentration in the water (VILLERET, 1951) — seems unlikely on general mass conservation grounds. This is because where there is a net accumulation of

organic matter (as there would be on a growing bog surface) one would expect the  $\text{CO}_2$  concentration in the immediate environment of the plants, considered over periods of months or years, to be on average less than the concentration found in water which is in equilibrium with air. There are also detailed physico-chemical objections to such an explanation.

In some cases a fifth source — industrial pollutants — may be important (for example GORHAM 1958), but this too will not be considered here.

The importance of whole organic acids excreted by the *Sphagna* in determining the acidity of their environment has been stressed most recently by RAMAUT (1955 a and b). He found that *Sphagnum recurvum* plants contained an organic acid, which, after a lengthy isolation procedure, he identified as succinic acid (or a polymer thereof). The amounts isolated seem rather small (about 2 mg/kg of plants) but it could be argued that the plants make organic acid(s) and then excrete them almost at once. MAAS & CRAIGIE (1964) have also examined the free organic acids in various species of *Sphagnum* and comment that the amounts are small compared with higher plants. If the excretion process were quantitatively important one would expect to be able to detect the organic acids (or their anions) in the immediate aquatic environments of the plants though Ramaut did not describe any attempt to do this. Two such attempts to find organic acids are described below; the first by ordinary chemical methods, the second by  $^{14}\text{C}$  labelling.

In the first attempt samples of water of 20 litres volume were collected from the environment of *Sphagnum* plants, and the pH adjusted to 7 to convert any free acids to the salt form. The samples were filtered, using the technique described by SHAPIRO (1961) to increase efficiency, and concentrated in a climbing film evaporator at a temperature less than  $25^\circ\text{C}$ .

Part of the concentrate was passed through a cation exchange resin (Zeocarb 225 in the  $\text{H}^+$  form) to convert organic acids to the free acid form. The exchanger was put through 20 cycles before use and no appreciable contamination could be attributed to it in a control with added sugars. The column effluent was subsequently titrated with  $\text{Ba}(\text{OH})_2$  in 95% ethanol through which  $\text{N}_2$  was bubbled (to remove any  $\text{CO}_2$  and  $\text{HCO}_3^-$ ) and pH changes were measured. With known mixtures of sulphuric and acetic acids the pK values are sufficiently different to separate the equivalence points of each (fig. 1). For bog water concentrates when change of pH is plotted against amount of  $\text{Ba}(\text{OH})_2$  (first derivative plot) the curve also shows two distinct equivalence points. The first corresponds fairly closely to the total strong acid salts determined by the method of MACKERETH (1963), and the second may be due to weak organic acids. There are some organic acids (for example oxalic acid  $\text{pK}_1 = 1.23$ ) which could confuse this simple picture. Attempts to isolate organic acids either by organic solvent extraction (SHAPIRO, 1957) or directly by paper chromatography have not been successful.

The infrared spectrum of vacuum freeze-dried material moulded with KBr does show a small peak at about  $1715\text{ cm}^{-1}$  in acid conditions which is reduced in alkaline conditions. At the same time there is an increase in absorption at  $1600\text{ cm}^{-1}$  (fig. 2), though the complete spectrum is highly complex and probably due to a mixture of compounds. This shift is

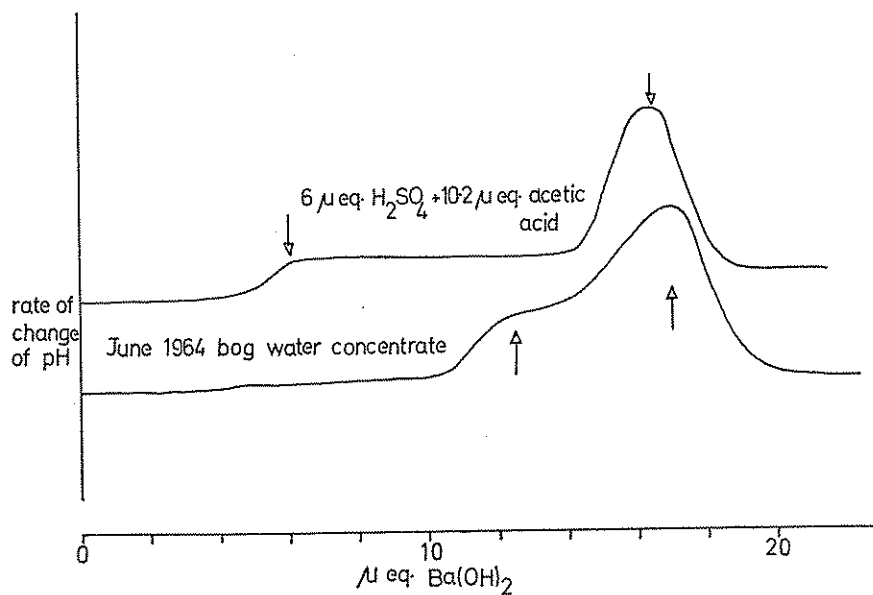


Fig. 1. First derivative pH titration curves of a known mixture, and of a concentrate of bog water after passage over a cation exchange resin in the  $H^+$  form to ensure that acids are present in the free acid form. Arrows on the known mixture curve show expected equivalence points; on the bog water curve they show positions taken as equivalence points.

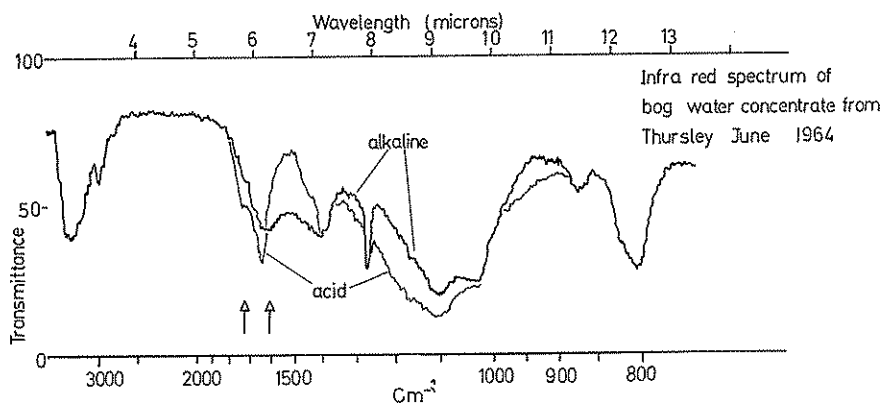


Fig. 2. Infrared spectra of vacuum freeze-dried bog water concentrate, initially in acid or alkaline solution. Only those parts of the acid spectrum between 850 and 1800  $cm^{-1}$  are shown.

characteristic of carboxylic acids (BELLAMY 1958). A very crude estimate of  $>C=O$  concentration can also be obtained by comparing the extinction at 1715  $cm^{-1}$  with that of standards as is done with spectrophotometric methods used on solutions. Very little reliance can be placed on this estimate (because the extinction coefficient of all organic acids is not the same), and it is only of interest as it leads to comparable results with the titrimetric method (table 1).

TABLE 1  
Concentration of acids in bog water measured by different methods, & pH values. All values are mean of duplicate results.

| Sample     | Date     | $\mu$ eq/l<br>Total strong<br>acids.<br>(Mackerth's<br>method). | $\mu$ eq/l<br>Strong acids<br>(pH titration) | $\mu$ eq/l<br>Weak acids<br>(pH titration) | > C = 0<br>(infra-red<br>O.D.) | Actual<br>pH | pH if all weak<br>acids produced<br>equivalent H <sup>+</sup> . | pH assuming<br>40% loss |
|------------|----------|-----------------------------------------------------------------|----------------------------------------------|--------------------------------------------|--------------------------------|--------------|-----------------------------------------------------------------|-------------------------|
| Moor House | Mar 1964 | 38(1)                                                           | 39(7)                                        | 39                                         | 29                             | 3.4          | 4.4                                                             | (4.2)                   |
| Thursley   | Aug 1963 | 42(7)                                                           | 41(4)                                        | 30                                         | 20                             | 3.7          | 4.5                                                             | (4.3)                   |
|            | Dec 1963 | 29(2)                                                           | 28(0)                                        | 39                                         | 18                             | 4.2          | 4.4                                                             | (4.2)                   |
|            | Feb 1964 | 41(5)                                                           | 41(4)                                        | 35                                         | 15                             | 4.1          | 4.5                                                             | (4.2)                   |
|            | May 1964 | 35(7)                                                           | 34(4)                                        | 40                                         | 53                             | 3.7          | 4.4                                                             | (4.2)                   |
|            | Jun 1964 | 36(9)                                                           | 35(0)                                        | 44                                         | 37                             | 3.5          | 4.4                                                             | (4.1)                   |

Ultimate recovery of organic acids added to the original bog water samples ranged from 58% to 82%. The data in table 1 do not, unfortunately, provide an unequivocal indication that free organic acids excreted by *Sphagna* are, or are not, responsible for a major part of the observed acidity. On the one hand there could be some strong organic acid present; on the other hand the infrared spectrum does not correspond at all closely with that of any of the common strong organic acids (for example oxalate, even in a mixture in amounts sufficient to give the observed  $1610\text{ cm}^{-1}$  peak in alkaline conditions would also produce a discernible peak at about  $1300\text{ cm}^{-1}$ ). It is also at least possible that the organic acid(s) observed arise in breakdown processes (or are released from the plants after breakdown) with equivalent amounts of cations other than  $\text{H}^+$  and would not, therefore, contribute to bog acidity. On the simplest interpretation of the data in table 1, without allowing for any ionisation effects, it appears that in four cases more than 70% of the  $\text{H}^+$  is not accounted for by simple organic acid anions. This conclusion is supported by the results of experiments in which live *Sphagnum papillosum* plants were sprayed with a solution approximating to rainwater in composition, in which the  $\text{CO}_2$  and  $\text{HCO}_3^-$  were labelled with  $^{14}\text{C}$ . The amounts of  $^{14}\text{C}$  in various parts of the system were measured by methods essentially the same as those used by WATT (1966), applied to the 70% alcohol extract as well as the solution.  $^{14}\text{C}$  in the alcohol insoluble fraction was determined as  $\text{BaCO}_3$  after wet combustion. Results are shown in table 2. Over 24 hours the ratio of  $^{14}\text{C}$

TABLE 2

Distribution of  $^{14}\text{C}$  in and around plants of *S. papillosum* 24 hours after first spraying with an artificial rain in which the  $\text{CO}_2$  and  $\text{HCO}_3^-$  were present partly as  $^{14}\text{CO}_2$  and  $\text{H}^{14}\text{CO}_3^-$ . Units are  $10^4$  c.p.m.

| Extracellular solution    |        |       | Particulate.<br>(removed on $0.5\ \mu$ Millipore filter) |
|---------------------------|--------|-------|----------------------------------------------------------|
| Anion + neutral           | Cation | (pH)  |                                                          |
| 0.96                      | 0.05   | (4.6) | 0.37                                                     |
| Alcohol soluble in plant. |        |       | Alcohol insoluble in plant.                              |
| Anion + neutral           | Cation |       |                                                          |
| 693                       | 88     |       | 543                                                      |

incorporated into the alcohol insoluble fraction of the plants to that appearing in the aquatic environment was 521 : 1. If the new molecules are of the same average composition (about 20% uronic acids) as those in the rest of the plant, the ratio of C incorporated into  $-\text{COOH}$  in the insoluble fraction to C appearing in organic form outside the plants is about 13 : 1.

Radioautograms of concentrates of the aquatic environment showed that the major part of the  $^{14}\text{C}$  was in sugars (glucose and some fructose) rather than in organic acids. The possible importance of long term breakdown of plant constituents to give free acids which are then released is still being examined.

At present then, it does not seem that there is much evidence that release

of whole organic acids is an important source of acidity in the aquatic environment of the *Sphagna*.

The last possible source to be considered here of continuing high  $H^+$  concentrations in *Sphagnum* bogs is  $H^+$  on  $-COOH$  freshly produced in new growth of the plants and then exchanged for some of the cations in rain or groundwater which flows over the plants.

There is a good deal of evidence to show that in common with many plant materials *Sphagnum* spp. can behave as cation exchangers, even when dead (ANSCHUTZ and GESSNER 1954, THEANDER 1954, PUUSTJARVI 1955, RAMAUT 1955 a and b, KNIGHT, CROOKE and INKSON 1961, and others) and that these exchange properties are owed to long chain polymers containing unesterified uronic acids, which are similar to sugars but with C-6 part of a carboxyl group (THEANDER 1954, CLYMO 1963, CRAIGIE and MAAS 1966). These long chain molecules may be mixed molecules containing both sugars and uronic acids (THEANDER 1954). The *Sphagna* are unusual among non-marine plants in the large amounts of these molecules which they contain. Up to about 30% of the dry weight of the plants may be present as uronic acid residues (CRAIGIE and MAAS 1966), though about 20% seems more usual (CLYMO 1963, CRAIGIE and MAAS, 1966).

In order to predict the effects which the plants may have on their chemical environment some sort of model must be used. The simplest is to treat the plant and environment as a closed system in which a Donnan equilibrium is set up between the plant (which contains indiffusible carboxyl groups) and the aquatic environment. Using this model it has been shown (CLYMO 1963) that the exchange sites in live or dead *Sphagnum papillosum* behave *chemically* as if they were at an average concentration of about 1.0 equiv./l. (This is not the same as the average concentration by *volume* of *plant*, which is about 1.6 equiv./l.) Part of the reason for this difference is presumably that the exchange sites are not uniformly distributed through the whole volume of the plant. The maximum exchange ability when most of the carboxyl groups are dissociated into the anionic form is about 1.0 meq/g dry weight — about a tenth to a third that of synthetic carboxylic cation exchangers.

If a solution containing cations (other than  $H^+$ ) is allowed to flow past a *Sphagnum* plant, in which most of the exchange sites are in the  $-COOH$  form, the pH at first drops sharply in the course of a few minutes but subsequently rises slowly (fig. 3). The precise timing depends on flow rate, plant size, previous history, solution composition, and temperature, amongst other variables but the general features are constant. (That the pH of the outflow is eventually higher than that of the inflow is probably due to removal of  $CO_2$  by the plants). Similar results have been found by RAMAUT (1955a) using a batch technique, and it seems clear that the process is equivalent to the gradual exhaustion of synthetic ion exchange resins (HELFFERICH 1962). A familiar practical case of this is in water softening devices, which need regeneration after some use. In natural conditions exhaustion of the  $H^+$  supply would occur due to leaching by rain or groundwater. In the case of *Sphagnum* there is at present no clear evidence that the existing exchange sites are actively regenerated. If such a process does

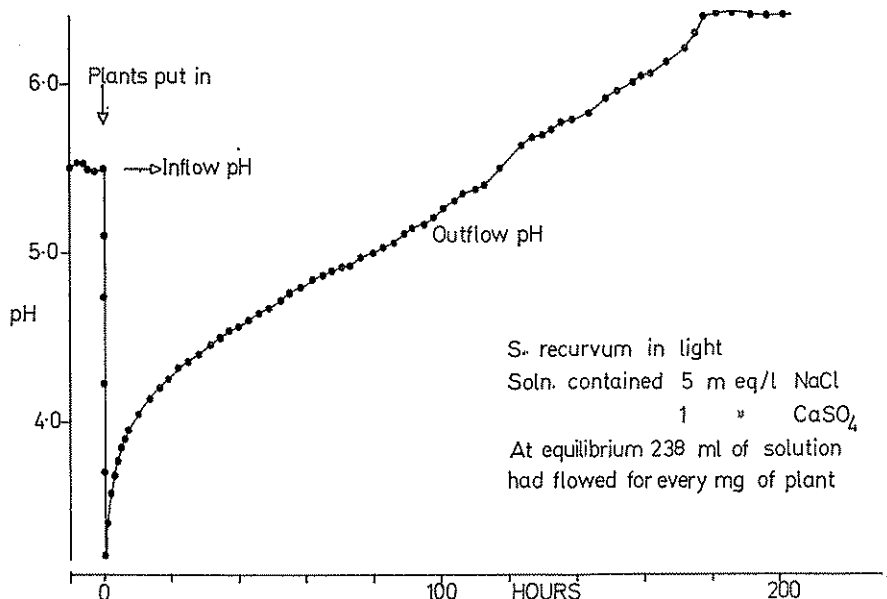


Fig. 3. pH of solution flowing past plants of *S. recurvum* in fluorescent light (about 0.05 cal/cm<sup>2</sup>/min), at about 1 ml/min.

occur it would only be possible on a *continuing* basis if the plants were able to "generate" hydrogen ions (as they cannot produce any other cation as far as is known).

New exchange sites are however being continually produced by growth at the apex of the plant. Assuming that these originate in the H form (and general considerations of charge equality would seem to support such an assumption taken over a long period) it is possible to calculate the eventual distribution of cations between plant and environment. The same simple model as before is used: one part — the new growth — containing the indiffusible carboxyl ions reaching equilibrium with another part — the rain or groundwater which forms the aquatic environment of the plants.

It can be shown that the eventual pH around the plants =  $-\log_{10}(h)$  is given by the solution of

$$(1) \quad \eta - h + \alpha[1 - 1/(1 + PR)] + \beta[1 - 1/(1 + PR^2)] + \gamma[1 - 1/(1 + PR^3)] = 0$$

where

$$(2) \quad R = \{(\eta - h - PKa) + [(\eta - h - PKa)^2 + 4PKa(PU_{max} + \eta - h)]^{0.5}\} / 2hP$$

and

$$(3) \quad P = DZ / 176 U_{max} V$$

Where D = g dry wt of new growth on an area,

Z = proportion of this as uronic acid (residual equiv.wt. = 176)

V = volume (l) of (precipitation or groundwater — evaporation)

U<sub>max</sub> = chemical concentration of exchange sites when fully dissociated

$K_a$  = dissociation coefficient of exchanges sites  
 $\eta, \alpha, \beta, \gamma$  = initial concentrations of respectively hydrogen ions, other monovalent cations, divalent cations and trivalent cations  
 $h, a, b, c,$  = final concentration of these cations

It is perhaps worth emphasizing that this apparently complicated expression arises from the simple premises already outlined. It is in general alien to the ecologist to trust himself to the frail raft of physical chemistry but one argument in favour of this trust is that it allows one to make quantitative predictions which can in principle be checked by observation.

There are so many variables in these equations that it is difficult to present the solutions in a manner which is easily followed. There is one simplification — growth and rainfall always appear together in the equations as the ratio  $D/V$ , although the biological relations of these two quantities are much more complex. This is most usefully expressed as  $\log_{10}$  (growth in metric tonnes/ha/yr) / (net water supply in cm/yr). Thus if growth were 5 t/ha/yr, and precipitation-evaporation were 50 cm/yr,  $\log D/V$  would be  $-1$ .

Some predictions are shown in fig. 4, with ions of different valency separated. In Southern England for example, taking the growth rate as 10 t/ha/yr, effective (precipitation-evaporation) as 20 cm/yr, and rain after evaporation losses containing 0.2 meq/l of cations one would expect from fig. 4 an average pH of about 4.0-4.2 to be maintained in bog systems. Locally in time and space — for example during summer (when growth is at maximum) and on hummocks of *S. acutifolium* (which has a polyuronic acid content of about 24% — CLYMO, 1963), one might expect lower values. Much of the rain probably runs down the plants rapidly before equilibrium is reached so that the removal of  $H^+$  from the upper parts of the plants is less important than might be expected. Later, evaporation concentrates the remaining water, and it is at least plausible that pH values lower than 3.5 may result. It is indeed generally found that hummocks are the most acid parts of bogs (for example, CLYMO 1963). When one leaves the equilibrium state, however, and attempts to deal with the kinetic state the whole matter becomes very much more complicated. No reliable information is at present available concerning the movement of water about *Sphagnum* plants in natural conditions.

There is a final consequence of the cation exchange properties which is perhaps worth a brief mention. This is the buffering effect on external cation concentrations.

The simplest way of showing this effect is to examine the equilibrium states reached for different concentrations of the same solution. These can be found from equations (1), (2) and (3). An example with 4 concentrations of the same mixture of cations is shown in table 3. It is apparent that in likely real situations the amount of cations held exchangeably in the plants is equal to or considerably greater than that in their aquatic environment and that a tenfold change in the concentration of a cation supplied to the plants results finally in a much smaller change in the environment. This effect is more marked as the valence of the ion increases.



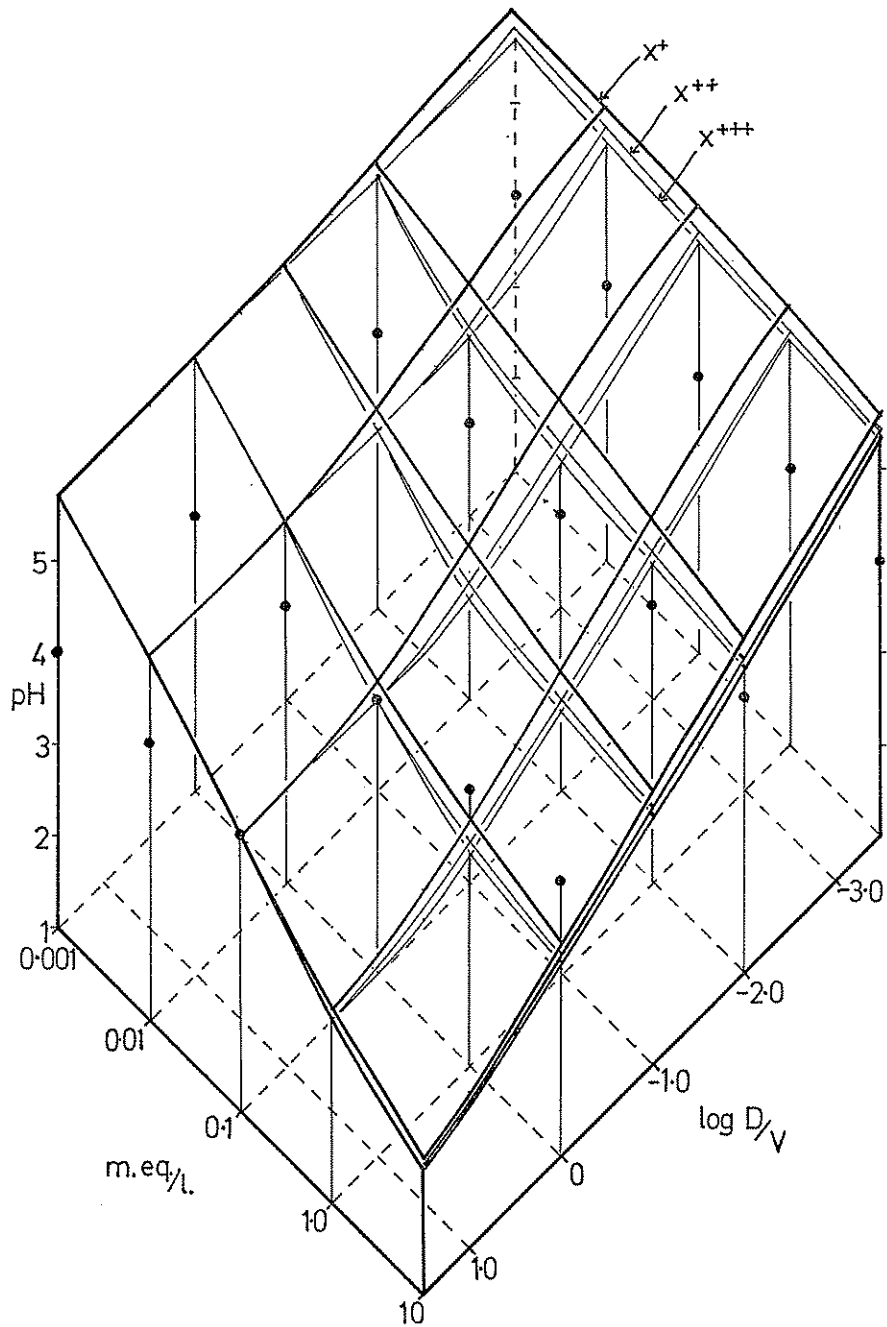


Fig. 4. Predicted pH values in relation to cation type, cation concentration, and ratio of growth to net precipitation (see text). The filled circles define a plane of  $pH = 4$ . The data refer to plants with 20% uronic acid content,  $K_a = 1.0 \times 10^{-4}$ , maximum exchange site concentration 1000 m.eq./l.

TABLE 3

Calculated concentrations of cations in four solutions containing a mixture of cations. Values are shown for the solution as supplied (underlined) and when equilibrium is established, together with % of total cations initially in the system which are finally in exchangeable form in plants. The plants are presumed to have the exchange sites initially all in the H<sup>+</sup> form. The calculations are for 20% uronic acid content, 1000 meq/l maximum concentration of exchange sites,  $K_a = 1.0 \times 10^{-4}$ , and growth (t/ha/yr) equal to one tenth of net precipitation (cm/yr) - for example 5 - t/ha/yr and 50 cm net precipitation.

| No. | Total original cations meq/l |          | H <sup>+</sup>            |              | Monovalent cations (other than H <sup>+</sup> ) meq/l |            |              | Divalent cations meq/l |            |               | Trivalent cations meq/l |               |  |
|-----|------------------------------|----------|---------------------------|--------------|-------------------------------------------------------|------------|--------------|------------------------|------------|---------------|-------------------------|---------------|--|
|     | Original pH                  | Final pH | % H <sup>+</sup> in plant | Original     | Final                                                 | % in plant | Original     | Final                  | % in plant | Original      | Final                   | % in plant    |  |
| 1   | <u>6.00</u>                  | 5.27     | 99.6                      | <u>0.005</u> | 0.0018                                                | 63         | <u>0.001</u> | 10 <sup>-9</sup>       | 99.9       | <u>0.0002</u> | 10 <sup>-10</sup>       | virtually all |  |
| 2   | <u>6.00</u>                  | 4.66     | 98.2                      | <u>0.05</u>  | 0.04                                                  | 18         | <u>0.01</u>  | 10 <sup>-8</sup>       | 97.7       | <u>0.002</u>  | 10 <sup>-6</sup>        | "             |  |
| 3   | <u>6.00</u>                  | 4.16     | 94.1                      | <u>0.5</u>   | 0.48                                                  | 2.5        | <u>0.1</u>   | 0.06                   | 36.5       | <u>0.02</u>   | 0.001                   | 92.8          |  |
| 4   | <u>6.00</u>                  | 3.90     | 89.0                      | <u>5.0</u>   | 4.96                                                  | 0.7        | <u>1.0</u>   | 0.96                   | 4.4        | <u>0.2</u>    | 0.15                    | 22.6          |  |

### Conclusion

Much of the acidity of the aquatic environment of *Sphagna* could be explained by cation exchange, though other sources may also be of some importance.

### ACKNOWLEDGMENTS

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### DISCUSSION

Name of questioner: J. Shapiro

Question: Is it possible to regenerate the exchange sites with dilute acid?

Answer: Yes.

### REFERENCES

- ANSCHUTZ, I., and F. GESSNER, Der Ionenaustausch bei Torfmoosen (*Sphagnum*). Flora Jena, **141**, 178-236 (1954).
- BELLAMY, L. J., The infra-red spectra of complex molecules. Methuen, London (1958).
- BENDA, I., Mikrobiologische Untersuchungen über das Auftreten von Schwefelwasserstoff in den anaeroben Zonen des Hochmoores. Arch. Mikrobiol. **27**, 337-74 (1957).
- BURGEFF, H., Mikrobiologie des Hochmoores. Fischer, Stuttgart 1961.
- CLYMO, R. S., Ion exchange in *Sphagnum* and its relation to bog ecology. Ann. Bot. Lond. N.S. **27**, 309-24 (1963).
- CLYMO, R. S., Experiments on breakdown of *Sphagnum* in two bogs. J. Ecol. **53**, 747-58 (1965).
- CRAIGIE, J. S., and W. S. MAAS, The cation exchanger in *Sphagnum* spp. Ann. Bot. Lond. N.S. **30**, 153-4 (1966).
- GORHAM, E., On the chemical composition of some waters from the Moor House Nature Reserve. J. Ecol. **44**, 375-82 (1956).
- GORHAM, E., Free acid in British soils. Nature **181**, 106 (1958).
- HELFFERICH, F., Ion exchange. McGraw-Hill, New York (1962).
- KNIGHT, A. H., W. M. CROOKE and R. H. E. INKSON, Cation exchange capacities of tissues of higher and lower plants and their related uronic acid contents. Nature **192**, 142-3 (1961).
- MAAS, W. S. G. and J. S. CRAIGIE, Examination of some soluble constituents of *Sphagnum* gametophytes. Canad. J. Bot. **42**, 805-813 (1964).
- MACKERETH, F. J. H., Some methods of water analysis for limnologists. Freshwater Biological Association Scientific Publication No. 21 (1963).
- POORE, M. E. D., and D. WALKER, Wybunbury Moss, Cheshire. Mem. and Proc. Manchester Lit. and Philosoph. Soc. **101**, 1-24 (1959).
- PUUSTJARVI, V., On the colloidal nature of peat forming mosses. Arch. Soc. Zool. - Bot. Fenn. Vanamo **9**, 257-72 (1955).
- RAMAUT, J., Étude de l'origine de l'acidité naturelle des tourbières acides de la Baraque - Michel. Bull. de l'Acad. Roy. Belgique (Cl. des Sciences) 5th ser. **41**, 1037-52 (1955a).
- RAMAUT, J., Extraction et purification de l'un des produits responsables de l'acidité des eaux des hautes tourbières et secrété par *Sphagnum*. Bull. de l'Acad. Roy. Belgique (Cl. des Sciences) 5th ser. **41**, 1168-99 (1955b).

- SHAPIRO, J., Chemical and biological studies on the yellow organic acids of lake water. *Limnol. and Oceanogr.* **2**, 161-79 (1957).
- SHAPIRO, J., High rate laboratory filtration with Buchner funnels. *Science* **133**, 1828-9 (1961).
- SJÖRS, H., Surface patterns in Boreal peat land. *Endeavour* **20**, 217-24 (1961).
- THEANDER, O., Studies on *Sphagnum* peat. 3. A quantitative study of the carbohydrate constituents of *Sphagnum* mosses and *Sphagnum* peat. *Acta Chem. Scand.* **8**, 989-1000 (1954).
- VILLERET, S., Recherches sur le rôle du CO<sub>2</sub> dans l'acidité des eaux des tourbières à Sphaignes. *C. R. Acad. Sci. Paris* **232**, 1583-5 (1951).
- WATT, W. D., Release of dissolved organic material from the cells of phytoplankton populations. *Proc. Roy. Soc. B.* **164**, 521-51 (1966).