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Chemical character and origin of organic acids in streams and seepage lakes of central Maine

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Abstract. Organic acids and inorganic chemistry were examined in seventeen seepage lakes, seven streams, and one seep in central Maine. The objectives of this analysis were to determine the quantity and quality of dissolved organic carbon (DOC), and to assess the relationship between organic and inorganic surface water chemistry. Lakes and streams sampled were dilute (average conductivity of 20.3 μS cm^{-1}) with a wide range of DOC (125–2593 μmol C L^{-1}). Organic acids in DOC were evaluated by:

—DOC fractionation (hydrophobic acids and neutrals, and hydrophilic acids, bases, and neutrals);
—DOC isolation followed by FT-IR, base titration, and chemical analyses;
—adsorption on solid phase extraction columns; and charge balance studies.

All lakes and streams were dominated by hydrophobic and hydrophilic acids (60 to 92% of DOC). Lakes and streams with low DOC had low hydrophobic to hydrophilic acid ratios (ca 1.2–1.3), regardless of pH and acid neutralizing capacities (ANC), compared to lakes and streams with moderate to high DOC concentrations (ca 1.9–2.4). Based on FT-IR spectroscopy and chemical analysis, organic acids were found to be dominated by a strong carboxylic character. Titration data of isolated DOC allowed accurate prediction of organic anions, which were strongly pH dependent (organic anions ranged from 14 to 198 μeq L^{-1}). Exchange acidity averaged 11.3, 13.6, and 8.7 μeq mg C^{-1} for lake hydrophobic acids, lake hydrophilic acids, and stream hydrophobic acids, respectively.

Overall evidence suggested that DOC and organic acid characteristics were related to their carboxylic functional group content and that the nature of these constituents was similar despite the source of origin (upland soils, wetlands, or *Sphagnum* deposits). Also, contact of soil leachates with B horizons seemed to be a controlling factor in DOC quantity and quality in the lakes and streams studied.

Introduction

Organic acids in freshwater ecosystems are poorly quantified and have not been well characterized (Thurman 1985). Although they are thought to be important for a variety of biogeochemical processes, the actual compounds present, mechanisms of formation or transformation, and their effects on other processes such as metal mobilization are poorly understood (Perdue & Gjessing 1990). Generally, DOC is the only parameter measured in terrestrial and aquatic ecosystems, and at best merely provides an estimate of the soluble organic material present (Driscoll et al. 1989).

The role of upland terrestrial ecosystems, wetlands, and autochthonous production of compounds in controlling DOC quantitiy and quality in lakes and streams is also poorly defined. In some streams of the northeastern US, adsorption of DOC solubilized in the forest floor controls surface water concentrations of DOC (McDowell & Wood 1984; McDowell & Likens 1988; Vance & David 1989; Vance et al. 1989). For other streams and lakes in the Northeast, extent of wetlands in the watershed is of greater importance in determining the level (Hemond 1990). Insufficient data exist on DOC fractions and mechanisms of production and transformation in various streams and lakes of this region. Litle is known about the character of DOC derived from upland ecosystems versus wetlands and *Sphagnum* deposits.

Concern about acidification of freshwaters in the northeastern U.S. by acidic deposition has led to several hypotheses that involve the role of natural organic acids. Krug & Frink (1983) and Driscoll et al. (1989) have suggested that inputs of strong acid result in a shift from organic acids to strong mineral acids in controlling acidification of surface waters. However, little is known about the nature of organic acids in the systems subject to such acidification, or how strong acids would interact with or alter their formation and solubility.

Organic acids are known to be important in regulating the acid-base status of many aquatic ecosystems with high DOC concentrations (Hemond 1980; Urban et al. 1987; Kortelainen & Mannio 1988). Sullivan et al. (1989) recently pointed out the influence DOC can have on ANC measurements, indicating the importance of understanding the contribution of DOC to total acidity. However, in aquatic systems with low to moderate DOC levels, effects on acid-base chemistry have not been well studied. Dissolved organic carbon has been found to exhibit a wide range of pK_a values (Thurman 1985; Perdue et al. 1984; Driscoll et al. 1989). Few measurements, however, have been made to characterize the charge properties of DOC because of the difficulty in isolating (required for concentrating DOC) and titrating the organics present.

To examine the role of organic acids in the charge balance of aquatic ecosystems, electroneutrality approaches have been used (Driscoll & Newton 1985; Driscoll et al. 1989; Kahl et al. 1989). Another method used is based on the relationship between organic charge, pH, and DOC similar to that developed by Oliver et al. (1983) or Perdue et al. (1984). Driscoll et al. (1989) used the Oliver et al. (1983) approach to calculate the charge contribution of organic anions in lakes of the eastern US, and found it to predict charge deficits rather well after parameter adjustments.

Many of these charge relationships have often been based on limited samples. For example, the widely used Oliver et al. (1983) model is based on the titrations of two aquatic samples (combined fulvic and humic acids), one each from Ontario and Nova Scotia. Clearly, further examination is needed of organic acids in aquatic ecosystems.

The objective of this study was to examine DOC and organic acids in a variety of central Maine lakes and streams to determine their importance in the overall chemistry of these surface waters. Methods used in the study of these surface waters included:

- Characterization of several inorganic and organic constituents that are believed to play important roles in surface water chemistry;
- fractionation of DOC in each of the surface waters; and
- isolation and detailed study of the dominant DOC fractions in a subset of the surface waters.

One major hypothesis to be tested was that regardless of source, DOC quantity and quality reflected the contact of forest floor leachates with B horizon soils. This hypothesis was tested by examining both DOC concentrations and characteristics in a number of surface water ecosystems.

Materials and methods

Sampling

Seventeen seepage lakes, seven streams, and one seep in central Maine were sampled (Table 1). Two of the streams, East and West Bear, are located at the Bear Brooks Watershed in Maine (BBWM), and have been intensively studied as part of the Watershed Manipulation Project supported by the Environmental Protection Agency. These paired streams were sampled biweekly above their weirs from October of 1987 through October of 1988, and monthly from that point through May of 1989 for DOC characterization.

During May of 1988, bulk samples (10 L) were collected from three streams (Baker, Rocky, and Sinclair) located near the BBWM site studied previously during 1985–1989 (Haines et al. 1989). The remaining lakes and streams were sampled (1–10 L) during June 1989 using a weighted throw bottle that collected water at a 0.5 m depth. Most of these lakes and streams were part of the Aquifer Lakes Study (ALPS) previously conducted in Maine and are "seepage input" lakes (Kahl et al. 1989). A few had outlets and are not true seepage lakes as defined by Wetzel (1983). A seep was also sampled near Pickeral and Crocker Ponds. Mean surface area of the lakes was 15 ha, with nine $\leqslant 4$ ha, seven 5–15 ha, and only one > 15 ha (Schoodic Lake, 160 ha) (Table 1). Average elevation of the lakes was 84 m, and ranged from 68–113 m. It was not known if the lakes were stratified during sampling.

Analytical methods

The following analytical methods were used for all samples except the East and West Bear Brook streams, which are discussed separately. Generally on the day of collection, air-equilibrated pH was determined on an unfiltered aliquot and the remainder of the sample filtered through a 0.7 μm Whatman GF/F filter. Following 1-4 days of storage at 3° C, each sample was shipped to the University of Illinois in insulated boxes cooled with ice-packs. Inorganic and organic analyses of the lake and stream samples were completed following the techniques listed in Table 2. All samples were stored at 3° C until analysis.

Table 1. Cluster #, location, physical characteristics, and DOC of lakes and streams from central Maine used for examining the chemical character and origin of organic acids in surface waters.

Name	Code	Cluster #	Location	Surface Area (ha)	Elevation* (m, a.s.l.)	DOC (μmol L^{-1})
Seepage Lakes						
Barren Pond	BP	5	44°50′26″N,67°52′25″W	3	82	1122
Crocker Pond	CP	3	44°58′59″N,68°28′16″W	1	76	1055
Duck Pond	DU	3	44°49′59″N,67°52′31″W	4	82	464
Gould Pond	GP	3	44°23′55″N,69°49′38″W	9	82	242
Hadley East	HE	3	44°52′20″N,67°51′35″W	14	76	345
Hadley West	HW	5	44°52′11″N,67°51′0″W	7	76	534
Hamilton Pond	HP	4	44°28′15″N,69°50′20″W	15	80	195
Joe Pond	JP	5	44°24′26″N,69°49′25″W	11	83	873
Lily Bay	LB	5	44°24′13″N,69°49′12″W	1	84	1492
Morrison Pond	MP	1	44°44′3″N,68°28′7″W	2	82	971
N. Hamilton Pond	NH	5	44°28′33″N,69°50′21″W	0.5	78	683
Pickeral Pond	PP	4	44°58′25″N,68°27′17″W	5	77	251
Pretty Pond	PR	4	44°49′38″N,67°54′10″W	12	82	212
Schoodic Lake	SL	3	44°41′45″N,67°53′35″W	160	68	132
SSEUN1	S1	4	44°53′51″N,68°6′12″W	1	105	408
SSEUN2	S2	1	44°54′22″N,68°6′32″W	0.5	105	885
Unknown Pond	UN	5	44°54′43″N,68°6′37″W	1	113	847
Streams				Stream Order		
Baker Stream	BS	1	44°54′37″N,68°4′48″W	3	122	1116
Rocky Stream	RS	1	44°53′55″N,68°5′13″W	2	113	1141
Sinclair Stream	S1	1	44°52′57″N,68°4′6″W	2	104	975
SS Duck	SS	1	44°49′45″N,67°52′17″W	3	79	833
East Bear Brook	EB	6	44°51′34″N,68°6′19″W	1	278	142
West Bear Brook	WB	6	44°51′34″N,68°6′19″W	1	280	125
SSEUN3	S3	1	44°54′0″N,68°6′2″W		98	743

*Elevation of stream was where sample was collected.

Table 2. Methods used for examining the inorganic and organic chemical characteristics of lakes and streams from central Maine.

Characteristic	Methods	References
pH	Potentiometric with glass electrode after equilibration with 330 ppm CO_2 in air	
SO_4^{2-}, NO_3^-, Cl^-	Ion chromatography	EPA 1987
NH_4^+	Wescan autoanalyzer	Scott et al. 1989
Ca^{2+}, Mg^{2+}, Na^+, K^+	Atomic absorption spectroscopy	EPA 1987
Acid neutralizing capacity (ANC)	Strong acid titration with Gran plot analysis	Gran 1952
Total F	Potentiometric with ion-selective electrode after addition of total ionic strength adjustment buffer	EPA 1987
Total Al	Colorimetric with pyrocatechol	Dougan & Wilson 1974
Total Fe	Colorimetric with 1,10 phenanthroline	APHA 1988
Specific conductance	Conductivity bridge	EPA 1987
Dissolved organic carbon (DOC)	UV-persulfate oxidation, IR detection (Dohrmann DC-80)	EPA 1987
DOC fractionation	Resin adsorption	Leenheer (1981)
DOC isolation	Resin adsorption	See text
DOC titrations	Autotitrator-Orion	See text
DOC functional groups	Resin adsorption	See text
DOC functional groups	FT-IR, Nicolet 7199 Spectrometer	See text
Monosaccharides	Colorimetric-MBTH	Johnson & Sieburth (1977)
Polyphenols	Colorimetric	APHA (1988)

For East and West Bear Brook streams, chemistry for all ions, except DOC and DOC fractionations, were from weekly samples collected during 1987. Analysis of these samples followed the methods described in EPA (1987), with the same parameters measured as described in Table 2.

Dissolved organic carbon analyses

Detailed studies were made of the DOC in each lake and stream sample. Fractionation of the DOC into hydrophobic acids and neutrals, and hydrophilic acids, bases, and neutrals followed a modified procedure of Leenheer (1981) using XAD-8, anion-exchange (Duolite A-7), and cation-exchange (AG-MP-50) resins. Samples with a DOC concentration below 400 μmol L^{-1} were flash evaporated (35–40° C) to increase concentration to 800 μmol L^{-1}, whereas samples above 1000 mmol L^{-1} were diluted to 800 μmol L^{-1}. These changes in DOC concentrations were used to standardize the fractionation procedure by having each sample at a similar DOC concentration. The dominant DOC fractions (hydrophobic acids and hydrophilic acids) from eight of the lakes and streams were isolated using the following procedure. Samples (6 to 10 L) were passed through a 250 ml column of cation-exchange resin, lowered to pH 2.0 with 6 N HCl, and pumped through columns of XAD-8 and anion-exchange (200 ml) resins that were connected in sequence. A similar k′ factor (45) was used to normalize the amount of XAD-8 resin needed for DOC fractionation and isolation methods (Leenheer 1981). Hydrophobic acids were eluted from the XAD-8 column using 0.1 N NaOH and protonated by passing through another column containing cation-exchange resin. Hydrophilic acids were eluted from the anion-exchange column with 1 N NaOH, passed through a cation-exchange column, acidified to pH 2, and readsorbed on XAD-8 using a k′ factor of approximately 0. Adsorbed hydrophilic acids were then removed following the procedure outlined for collection of hydrophobic acids.

Isolated hydrophobic and hydrophilic acids were characterized for exchange acidity, charge density, and functional group composition by potentiometric titration and Fourier Transform Infrared Spectroscopy (FT-IR). Titrations were conducted on an Orion 960 Autochemistry unit using 0.1 N KOH. Ionic strength was adjusted to 0.1 using KCl and samples bubbled with N_2 gas for the duration of the base titrations. Exchange acidity was determined from first derivative analysis of the titration data and compensating for residual anions and cations in the isolated sample. Charge density calculations derived from mass balance analysis was done as follows:

$$A^- = \frac{\text{Residuals}_{(\text{cations-anions})} + [k^+] + [H^+] - [OH^-]}{\text{mg C}} \tag{1}$$

Where A^- is the unit charge at each pH, K^+ the base added, and the denominator is mg of C in solution titrated. The FT-IR spectra were run on protonated samples (1 mg incorporated into KBr pellets) using a Nicolet 7199 FT–IR spectrometer that accumulated 100 scans and compensated for background noise.

Monosaccharide and polyphenol equivalents were determined on a majority of the surface water samples. For the monosaccharide analysis, a glucose equivalent was measured using the 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) method of Johnson & Sieburth (1977). Polyphenol equivalents were determined following the tannin and lignin method of APHA (1988) using gallic acid standards.

An investigation of DOC adsorption on solid phase extraction columns prepacked with normal phase, reverse phase, and ion-exchange sorbents (Bakerbond spe Methods Development Kit - J.T. Baker, Phillipsburg, NJ), and a small column of XAD-8, was done using two lake samples. Columns were preconditioned with methanol and rinsed thoroughly with deionized-distilled water. Cation and anion columns were also conditioned with acid or base, respectively, to saturate exchange sites. Triplicate columns of every type of sorbent were each eluted each with 70 ml of lake sample and the unretained solution collected for DOC analysis. The DOC was determined on a 20 ml sample that was flash evaporated to remove residual methanol and resolubilized in low-DOC, distilled water.

Organic anions were estimated using the titration analysis discussed above. A cation-anion charge balance was also used to examine the anion deficit which was presumed to be the contribution of organic anions:

$$\begin{aligned} A^- = {} & [H^+ + Ca^{2+} + Mg^{2+} + K^+ + Na^+ + Al^{n+} + Fe^{3+}] \\ & - [F^- + Cl^- + NO_3^- + SO_4^{2-} + HCO_3^-] \end{aligned} \qquad (2)$$

Total aluminum was speciated using ALCHEMI (Schecher & Driscoll 1987, 1988) to provide an estimate for the charge equivalents of Al. Bicarbonate was calculated using air equilibrated pH and assuming atmospheric CO_2 partial pressures of $10^{-3.5}$. Calculated pH was estimated from ANC and the atmospheric partial pressures of CO_2 listed above.

Quality assurance and cluster analysis

An extensive quality assurance/quality control program was used to ensure data integrity. Duplicate, known audit, and blanks were used in most analyses. A theoretical conductivity was calculated for each sample, and compared to measured values. Ratios of actual to theoretical conductance varied from 88.4 to 122.7%, with a mean of 106%. Differences probably reflected high concentrations of DOC, which were not accounted for in the theoretical conductivities as well as underestimates of HCO_3^- from using an atmospheric pCO_2 value.

To facilitate analysis and discussion of the lakes and streams, cluster (FASTCLUS) and canonical discriminant (CANDISC) analyses were performed to group lakes with similar characteristics using PC-SAS (SAS 1988). After extensive use of all chemical parameters and variable numbers of clusters (3–8), we found that pH, Ca^{2+}, ANC, DOC, and hydrophobic acid percentage best delineated six clusters of the lakes and streams into meaningful and logical groups (Fig. 1). Discussion of DOC and inorganic chemistry will utilize the similarities among lakes and/or streams in these clusters.

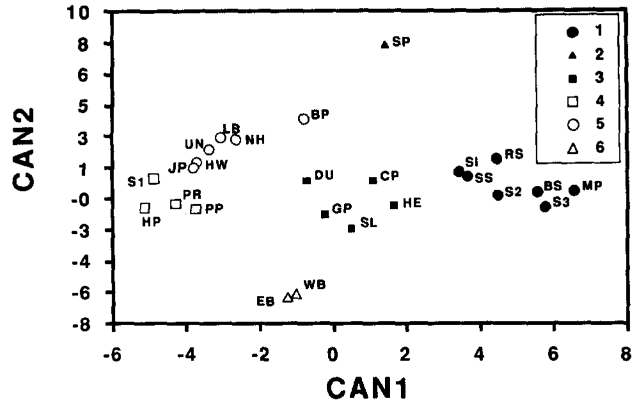


Fig. 1. Cluster analysis showing canonical variables for lakes, streams, and seep sampled in central Maine. Two letter codes identify surface waters and are listed in Table 1. Canonical variables CAN1 and CAN2 are linear combinations of the quantitative variables (pH, Ca^{2+}, ANC, DOC, and hydrophobic acid percentage) and a classification variable from the cluster analysis.

Results and discussion

Inorganic water chemistry

Mean conductivity of the lakes and streams was 20.3 $\mu S\,cm^{-1}$, indicating that these were very dilute waters with few dissolved ions (Table 3). Based on mean concentrations for base cations and anions, the order of importance was $Na^+ > Ca^{2+} > Mg^{2+} > K^+$ and $SO_4^{2-} > Cl^- > F^-$, respectively. Dominance of base cations by Na^+ was probably because of the influence of marine salts in many of the surface waters. Nitrate and NH_4^+ were generally below detection ($< 2\,\mu eq\,L^{-1}$) and unimportant in these solutions.

Table 3. Mean, coefficient of variation (CV), and range in chemical characteristics of lakes and streams from central Maine.

Characteristic	Unit	Mean	CV	Range
Conductivity	$\mu s\ cm^{-1}$	20.3	29	10.5–37.3
DOC	$\mu mol\ L^{-1}$	743	78	125–2793
pH		4.91	152	4.16–7.00
ANC	$\mu eq\ L^{-1}$	26.3	190	−78.4–97.9
Ca^{2+}	$\mu eq\ L^{-1}$	54	67	7–116
Mg^{2+}	$\mu eq\ L^{-1}$	27	43	8–54
K^+	$\mu eq\ L^{-1}$	13	72	5–43
Na^+	$\mu eq\ L^{-1}$	60	36	28–90
SO_4^{2-}	$\mu eq\ L^{-1}$	43	50	0–100
Cl^-	$\mu eq\ L^{-1}$	39	33	10–73
F^-	$\mu eq\ L^{-1}$	1.9	82	0.8–7.1
Al	$\mu mol\ L^{-1}$	2.7	58	0.9–4.8
Fe	$\mu mol\ L^{-1}$	1.3	80	0–4

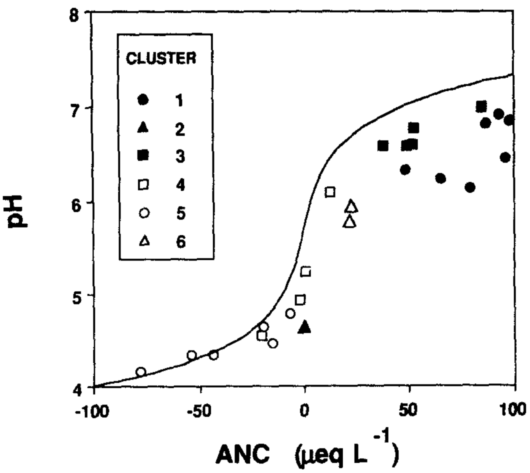


Fig. 2. Air-equlibrated (symbols) and calculated pH (solid line) versus ANC with clusters for lakes, streams, and seep sampled in central Maine.

When plotted against ANC, calculated pH based on equilibration with atmospheric CO_2 ($pCO_2 = 10^{-3.5}$atm.) gave a smooth curve and agreed well with measured pH at ANC's $< -50\,\mu eq\,L^{-1}$ (Fig. 2). At all ANC's above $-50\,\mu eq\,L^{-1}$, measured air equilibrated pH was lower than calculated. This was probably a result of organic acids depressing pH below that which is suggested on a theoretical carbonate basis only.

Solution ANC was related strongly to Ca + Mg levels, and all surface waters had Ca + Mg concentrations $< 200\,\mu eq\,L^{-1}$. ANC's were low in all lakes and streams, ranging form -78 to $98\,\mu eq\,L^{-1}$. These waters are similar in chemistry to many poorly buffered waters sampled as part of larger studies in the northeastern U.S. (Driscoll & Newton 1985; Brakke et al. 1988; Kahl & Scott 1988; Kretser et al. 1989; Kahl et al. 1990).

Lake and stream DOC

Surface waters had a wide range of DOC (Table 1, 125–2793 $\mu mol\,C\,L^{-1}$) which was found to be independent of both pH and ANC ($p > 0.05$, regression analysis). The lake and stream DOC was dominated by organic acids (hydrophobic and hydrophilic acids), with lower percentages of both neutral and the base DOC fractions. Organic acids ranged from 60 to 92% of DOC and showed no relationship with pH (Fig. 3a). However, the proportion of organic acids did increase as DOC increased (Fig. 3b), although the relationship was weak ($p > 0.05$, regression analysis) (Fig. 3b). Of the other three DOC fractions determined (hydrophobic neutrals, hydrophilic neutrals, and hydrophilic bases), hydrophilic neutrals were the largest fraction, with smaller proportions of the other two (Fig. 4).

Cluster analysis indicated there were important differences in DOC fractions among the lakes and streams allowing the grouping of similar types of surface

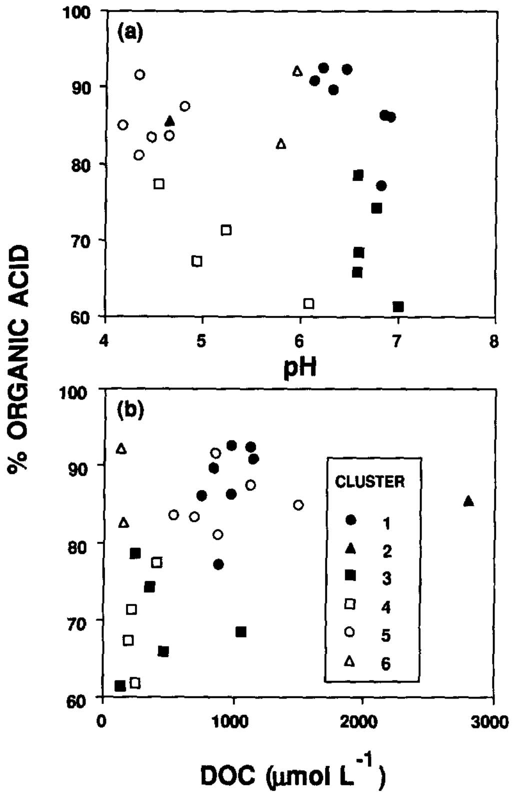


Fig. 3. Organic acids (hydrophobic + hydrophilic acids) as a percent of total DOC as related to (*a*) pH and (*b*) DOC concentrations for lakes, streams, and seep sampled in central Maine, indicating clusters.

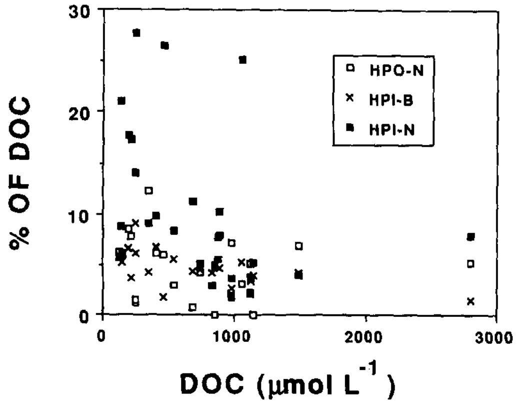


Fig. 4. Percent of DOC as hydrophobic neutrals (HPO-N), hydrophilic bases (HPI-B), and hydrophilic neutrals (HPI-N) as related to DOC concentrations for lakes, streams, and seep sampled in central Maine.

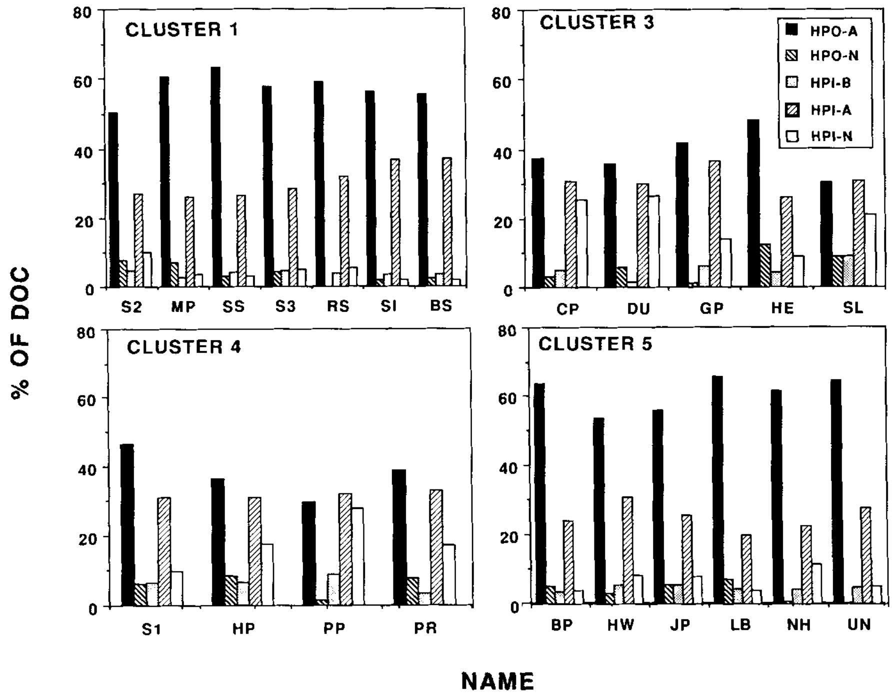


Fig. 5. Dissolved organic carbon fractions expressed as a percentage of DOC for clusters 1, 3, 4, and 5 lakes and streams sampled in central Maine. Fractions are as follows: HPO-A, hydrophobic acids; HPO-N, hydrophobic neutrals; HPI-B, hydrophilic bases; HPI-A, hydrophilic acids; and HPI-N, hydrophilic neutrals.

waters. Cluster 1 represented high pH, high ANC streams and one lake (Morrision Pond), with moderate DOC levels (mean of 950 μmol C L^{-1}) dominated by an average of 57% hydrophobic acids (Fig. 5). Hydrophilic acids averaged 30% of DOC, with small proportions of the other fractions (each < 10%). Cluster 2 was the lone seep sampled (2800 μmol C L^{-1}), and had 60 and 26% hydrophobic and hydrophilic acids, respectively. For cluster 3, high pH, high ANC lakes with low DOC (mean of 447 μmol C L^{-1}), hydrophobic acids made up a smaller proportion of the DOC (average of 39%) and hydrophilic acids greater (average of 37%), in comparison to cluster 1 (Fig. 5). Four of the lakes in this cluster had relatively low DOC (mean of 296 μmol C L^{-1}), with one lake (Crocker Pond) having a high concentration (1055 μmol C L^{-1}). Crocker Pond was the only high DOC lake or stream with a small proportion of hydrophobic acids (with a corresponding large proportion of hydrophilic acids), which caused it to be grouped in cluster 3.

Cluster 4 included lakes with low DOC (mean of 266 μmol C L^{-1}), low pH, and low ANC (Fig. 5). Percentages of hydrophobic and hydrophilic acids averaged 38 and 32% of DOC, respectively. Hydrophilic neutrals averaged 18% of DOC, with the remaining hydrophobic neutrals and hydrophilic bases making up 12%.

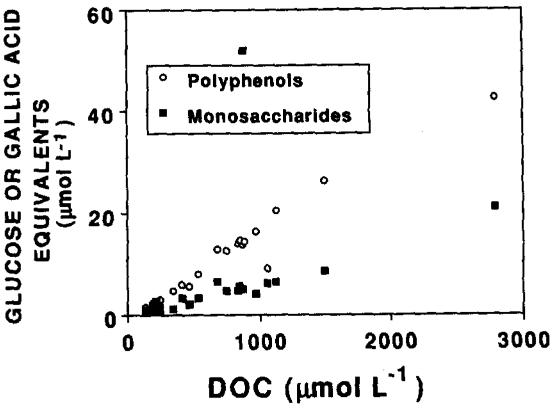


Fig. 6. Polyphenol and monosaccharide equivalents as related to DOC for lakes, streams, and seep sampled in central Maine.

Sphagnum-dominated, low pH, and low ANC lakes made up cluster 5, which had an average pH of 4.41 (Fig. 5). These lakes had high proportions of hydrophobic acids averaging 61% of DOC and the smallest fraction of hydrophilic acids (average of 25%), with high DOC (mean of 925 μmol C L^{-1}). Lakes in this cluster represent the typical brownwater acid lakes found in many areas of the northeastern and northcentral U.S (Clair & Freedman 1986; Blancher & McNicol 1987; Cook et al. 1987; Landers et al. 1988; Urban et al. 1989).

Cluster 6 was comprised of East and West Bear Brooks; high pH, low ANC and low DOC streams. These streams are low DOC ($<$ 250 μmol C L^{-1}), clearwater surface waters. Each stream drains an approximately 11 ha catchment, and had similar DOC contents and qualities suggesting similar formation processes. Organic acids dominated the DOC in the streams, with hydrophilic and hydrophobic acids representing 44 and 39% of total DOC (average of both streams), respectively. Hydrophilic bases, hydrophilic neutrals, and hydrophobic neutrals totaled 17% of DOC. The acid DOC fractions showed remarkably little variation over the two year sample period, and all fractions did not vary as a function of DOC concentration. Both of the neutral fractions showed considerable variation as a percentage of DOC, although on an absolute basis they were low in concentration.

Concentrations of polyphenols and monosaccharides were closely related to DOC concentrations, except for SSEUN 2 and Crocker Pond which deviated nificantly from the other surface waters (Fig. 6). The reason for the SSEUN 2 high monosaccharide content is unknown. Crocker Pond had a low polyphenol level compared to the other surface waters. This lake, with a relatively high DOC and low hydrophobic acid content, was also unusual in that it clustered with low DOC lakes. Therefore, the DOC in Crocker Pond is apparently quite different than the other high DOC lakes. A significant relationship was noted between polyphenol and hydrophobic acid contents (r^2 = 0.99, n = 20), suggesting hydroxyl functional groups of these acids may be similar in arrangement to gallic acid.

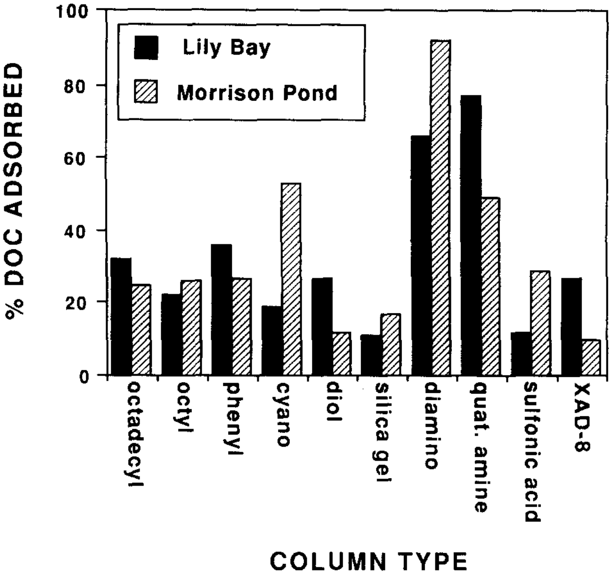


Fig. 7. Percentage of DOC in Lily Bay and Morrison Pond adsorbed by solid phase extraction columns.

DOC adsorption on solid phase extraction columns

Samples from Lily Bay (LB) and Morrison Pond (MP) were used to determine DOC retention on various types of solid phase extraction resins (Fig. 7). The amount of DOC retained by the different resins was a consequence of several mechanisms of adsorption including: ionic, hydrogen, and hydrophobic bonding, and various dipole interactions. A major factor affecting adsorption selectivity is pH. For this study, lake samples were eluted at their normal pH's in order to evaluate adsorption under natural conditions. These two samples were chosen because they represent the pH extremes of the lakes and streams sampled (pH values of 4.16 and 6.86 for LB and MP, respectively).

The percentage of DOC adsorbed on reverse phase columns, octadecyl, octyl, and phenyl was generally greater for LB (32, 22, and 36%, respectively) than MP (25, 26, and 27%, respectively). Octadecyl and phenyl resins adsorb nonpolar compounds whereas octyl resin retains moderately polar organics, indicating a higher percentage of DOC in MP may be of this latter character. Normal phase columns, cyano and diol, and silica gel resin adsorb polar compounds; 19, 27, 11% and 53, 12, 17% of LB and MP DOC, respectively. The high percentage of MP DOC adsorbed by the cyano column may be because of adsorption of polar, nonpolar, and ionic species.

Ion-exchange columns used were of two types; cation and anion exchange resins. The two anion exchange columns, diamino and quaternary amine, adsorbed a high percentage of the DOC from both lake samples; 66, 77% and 92, 49% of LB and MP DOC, respectively. The cation exchange column

Table 4. Titration analysis of the organic acid fractions isolated from lakes and streams from central Maine.

Source	C_t	pKa	b	m_1	m_2
Hydrophobic acids					
Barren Pond	10.3	4.55	− 7.97	3.63	−0.167
Lily Bay	11.6	4.26	− 7.24	3.89	−0.193
Morrison Pond	11.1	4.12	− 7.90	4.13	−0.218
N. Hamilton Pond	12.4	4.04	− 8.40	4.55	−0.240
Unknown Pond	11.3	4.33	− 8.40	4.13	−0.205
Mean	11.3	4.26	− 7.98	4.07	−0.205
Hydrophilic acids					
Barren Pond	13.2	4.22	−12.07	5.79	−0.326
Lily Bay	13.5	4.40	−11.56	5.44	−0.290
Morrison Pond	13.8	4.54	−11.89	5.20	−0.240
N. Hamilton Pond	13.2	4.52	−10.87	4.95	−0.241
Unknown Pond	14.2	4.07	− 9.60	5.18	−0.270
Mean	13.6	4.35	−11.20	5.31	−0.273
Hydrophobic acids					
Rocky Stream	8.85	4.35	− 6.95	3.37	−0.176
Baker Stream	8.60	4.45	− 6.61	3.18	−0.164
Sinclair Stream	8.69	4.28	− 6.00	3.08	−0.157
Mean	8.71	4.36	− 6.52	3.21	−0.166

C_t = Exchange acidity
b, m1, and m2 = Regression values used for the determination of pH dependent charge densities ($A^- = b + m_1pH + m_2pH^2$).

retained a much lower percentage of the DOC from both lakes. These results are consistent with naturally occurring organic compounds existing primarily as anionic constituents (Thurman 1985).

A column containing XAD-8 resin was used in conjunction with the above columns to investigate hydrophobic adsorption of lake DOC at natural pH's. In DOC fractionation procedures, DOC adsorbed at pH 7 is classified as a hydrophobic base. For MP, with a pH near 7, results suggest that possibly 10% of the DOC is hydrophobic bases. At pH 4.2, LB hydrophobic solutes adsorbed on XAD-8 (27% of DOC) could not be assigned to any specific hydrophobic fraction but rather to the hydrophobic character of LB DOC.

Acid functional group analysis by titration

Dissolved organic carbon from eight of the lakes and streams was isolated and used to determine charge properties (Table 4). Exchange acidity averaged 11.3, 13.6, and 8.71 μeq mg^{-1} C for lake hydrophobic acids, lake hydrophilic acids, and stream hydrophobic acids, respectively, with average pK_a's of 4.26, 4.35, and 4.36. These data suggest that the organic fractions are comprised of a diverse group of organic acids with a range of pK_i's (Perdue et al. 1980, 1984).

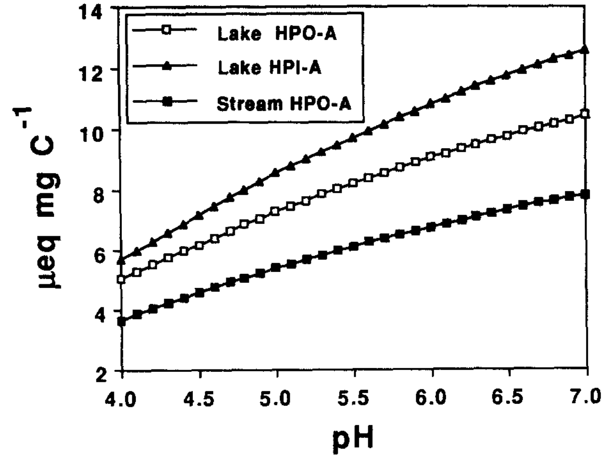


Fig. 8. Average charge equivalents per mg C for lake hydrophobic acids (HPO-A), lake hydrophilic acids (HPI-A), and stream hydrophobic acids (HPO-A) as a function of pH.

Dissociation of the organics is dependent on solution pH, as illustrated in Fig. 8. Oliver et al. (1983) presented a method for estimating pK_i's by use of an empirical expression considering pH and DOC only. A major assumption in this expression is that all organic acids from natural waters have similar acidic properties. However, Oliver et al. (1983) used only DOC that would be classified as hydrophobic acids. Our data, and that of McKnight et al. (1985), suggest the acidity of hydrophobic and hydrophilic acids are different. Therefore, it is essential that when characterizing DOC from natural waters, regardless of its origin, acidic properties should be determined on hydrophobic as well as hydrophilic acids.

Another method of quantifying organic anions is by the determination of the μeq A^- per unit C as related to pH of the sample (Fig. 9). The organic anions increased with pH across the surface waters studied, reflecting their wide range of dissociation constants. Several studies have reported values of A^- which have been estimated by a number of methods (Oliver et al. 1983; McKnight et al. 1985; Wright et al. 1988; Kramer & Davies 1988; Driscoll et al. 1989; Urban et al. 1989; Kahl et al. 1989; Lam et al. 1989). Many of these studies determined, by an indirect approach, the organic anion concentration using the charge balance deficit after considering all inorganic cations and anions. Often these results have been erroneously presented as an indication of total organic acidity (i.e., maximum μeq A^- per unit C). Generally what these authors were calculating was the charge density. Charge density is a more appropriate measure of deprotonated organic anions, and describes the organic anions charge contribution at a particular pH. Because it is pH dependent, however, charge density should always be presented with the pH of the sample. The above terms should also not be confused with the estimation of exchange acidity, which is an approximate measure of carboxyl group content obtained by titration analysis.

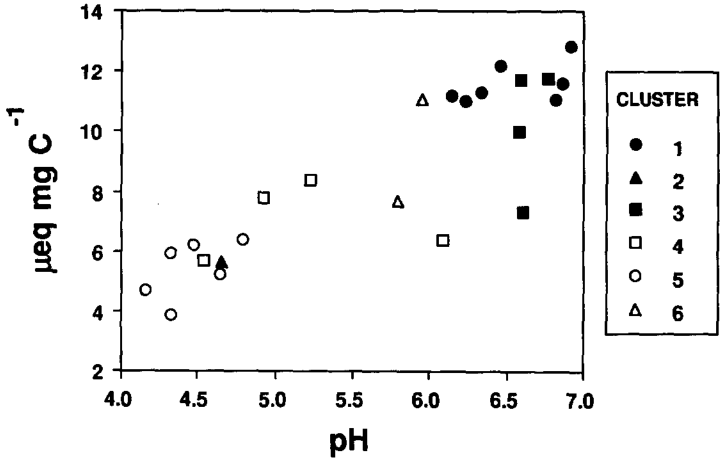


Fig. 9. Organic anion charge per unit C as related to pH for lakes, streams, and seep sampled in central Maine, indicating clusters.

Using the percentage of hydrophobic and hydrophilic acids and our measured charge density relationships (which include pH), free organic anions were calculated for each surface water (Fig. 10a). Organic anions varied from 14 to 198 μeq L^{-1}, and increased with DOC as would be expected. Organic anions compared well to the charge deficit, although in most cases they were less than the deficit (Fig. 19b). This discrepancy may have resulted from an underestimation of HCO_3^- by the use of atmospheric CO_2. Charge balances with and without inclusion of organic anions, averaged 2.8 and 27% of total charge, respectively when calculated as follows:

$$\% \text{ charge balance} = \frac{(\mathrm{K+Na+Ca+Mg+NH_4+H+Al+Fe}) - (\mathrm{Cl+NO_3+SO_4+OA})}{\text{sum of cations, anions, OA}} \times 100$$

This indicates the importance of including organic acids in any study evaluating surface water anions and acidity across a wide range of DOC concentrations.

We also used the Oliver et al. (1983) model to predict free organic anions. The Oliver et al. (1983) approach gave from 2% less to 47% more organic anions than our method, with an average of 20% greater organic anions. Therefore, for the lakes and streams evaluated here, the Oliver et al. (1983) approach generally predicted a larger amount of organic anions based on DOC, pH and their average exchange acidity of 10 μeq mg C^{-1}.

FT-IR analysis of functional groups

Isolated hydrophobic and hydrophilic acids from five lakes and four streams were also analyzed by FT-IR for characterization of structural components such

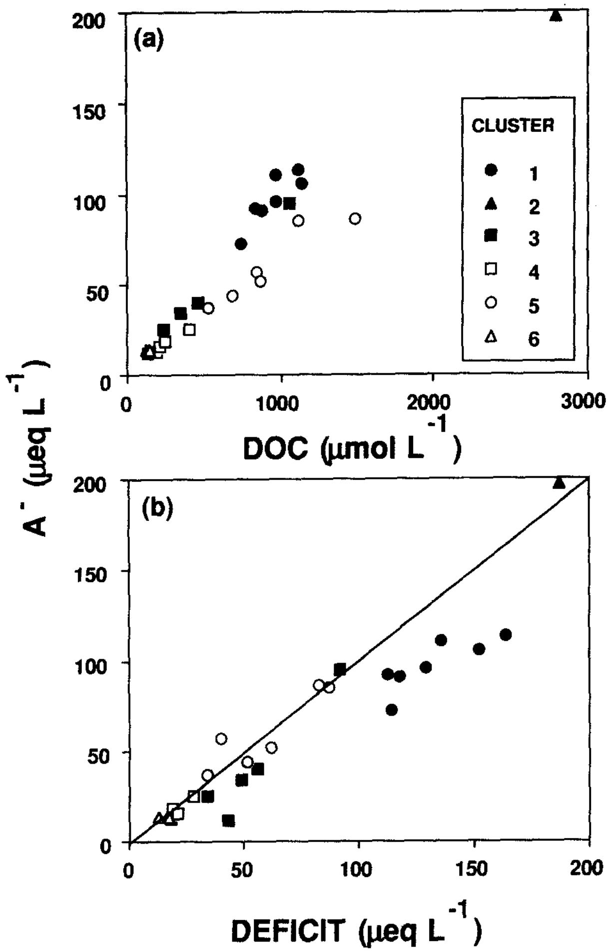


Fig. 10. Relationship between (*a*) total organic anion charge calculated using titration data and DOC and (*b*) anion deficit as related to organic anion charge for lakes, streams, and seep sampled in central Maine, indicating clusters.

as functional groups. The FT-IR spectra for lake and stream DOC isolates (Fig. 11) indicate they are primarily of a carboxylic nature, as shown by the strong COOH absorption band at 1720 cm^{-1}. Other prominent absorption bands in the hydrophobic and hydrophilic acids are a broad band in the 3200 to 3600 cm^{-1} region and bands around 2940, 2600, 1620, 1500, 1400, 1210, 1085 and 980 cm^{-1}.

The broad band in the 3200 to 3600 cm^{-1} region is due to 0–H stretching. In most cases, maximum absorption occurs near 3400 cm^{-1} suggesting H-bonding of the OH groups (Stevenson 1982). Absorption in the 2900 cm^{-1} region is due to aliphatic C–H bonds in methyl and methylene groups, which are apparently more prevalent in the stream hydrophobic acid isolates. Bands at 2600 cm^{-1} are

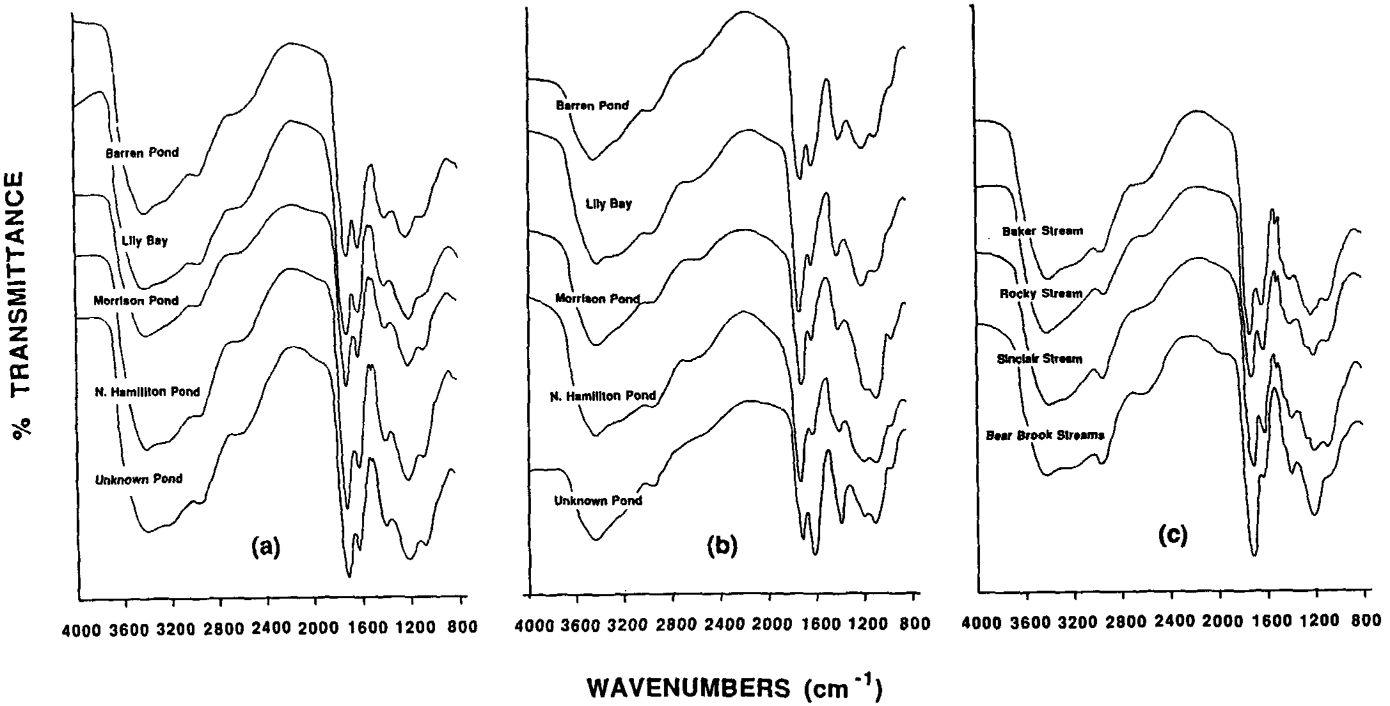


Fig. 11. FT-IR spectra of (*a*) lake hydrophobic acids, (*b*) lake hydrophilic acids and (*c*) stream hydrophobic acids.

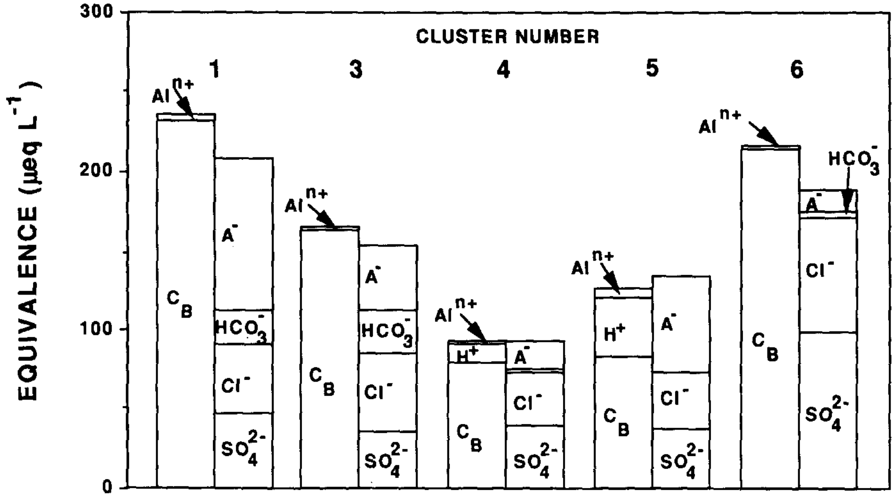
an indication of strongly H-bonded OH of COOH; metal complexation or conversion of COOH to COO^- would reduce the intensity of this band (Piccolo and Stevenson 1982). Assignment based on the $1620\,cm^{-1}$ band is often to aromatic C = C structures conjugated with C = O or COO^- groups (Schnitzer & Skinner 1968); however, other possibilities exist (Stevenson 1982). Other absorption bands occurring near $1400\,cm^{-1}$ may be due to O–H groups of alcohols or carboxylic acids, or C–O of phenolic OH groups, and bands centered around $1210\,cm^{-1}$ are related to C–O and O–H vibrations of COOH groups, which decreases with COO^- formation.

Three bands indicating differences between hydrophobic and hydrophilic acids were noted at 1500, 1085, and $980\,cm^{-1}$. Only hydrophobic acid spectra contain the band at $1500\,cm^{-1}$ which is believed to be due to simple aromatic acids; simple aromatic acids have been found to be almost completely adsorbed on XAD-8 resin (David et al. 1989). Hydrophilic acids, however, generally show greater absorption at $1085\,cm^{-1}$, and occasionally have bands around $980\,cm^{-1}$, in comparison to hydrophobic acid spectra. These bands suggest the hydrohilic acids contain more carbohydrate material than do hydrophobic acids. Overall, only slight variations were noted between lake and stream hydrophobic acids, suggesting the composition of these organic acids, as determined by FT-IR analysis, is very similar.

Importance of organic anions

Equivalence diagrams can be used to examine the relative proportion of cations and anions in any solution. Chemistry for each of the clusters, except cluster 2, is presented in Fig. 12, showing the relative importance of organic anions, HCO_3^-, Cl^-, and SO_4^{2-} with respective to total anionic charge. The discrepancy between anions and cations for clusters 1, 3, and 6 probably reflects the underestimation of HCO_3^- inasmuch as it was calculated based on atmospheric pCO_2, which probably was less than actual partial pressures. Organic anions were important in all clusters (6–46% of total anions), and greater than all other anions individually in clusters 1 and 5. Chloride and SO_4^{2-} were similar in all clusters except 6, which had the greatest concentration of these two anions. Base cations (C_B) provided most of the cationic charge, except in cluster 5 where H^+ and Al^{n+} were also significant. Driscoll et al. (1989) showed that in the Adirondack subregion, lakes with pH values less than 6.0 had SO_4^{2-} as the dominant anion, whereas in northeastern Minnesota lakes with pH values less than 6.5 were dominated by organic anions. In the lakes evaluated in this study, cluster 4 (primarily clearwater, acid lakes) and cluster 6 (East and West Bear Brook streams) had SO_4^{2-} as the dominant anion. This suggests that for cluster 4 lakes, which have relatively low pH's, SO_4^{2-} inputs were responsible for their acidification (Driscoll et al. 1989; Kahl et al. 1989).

Kahl et al. (1989) also found a range of organic to SO_4^{2-} dominated lakes in their extensive study of Maine lakes. They concluded that clearwater acidic lakes (about 60% of total acidic lakes) were acidic because of SO_4^{2-} deposition,



pH	6.46	6.66	4.93	4.41	5.87
ANC (µeq L^{-1})	81	55	-2	-36	22
DOC (µmol C L^{-1})	952	448	266	925	133

Fig. 12. Equivalence diagram for average lake or stream chemistry by cluster for lakes and streams sampled in central Maine (C_B = sum of Ca^{2+}, Mg^{2+}, K^+, and Na^+).

darkwater drainage lakes (10% of total) were acidic because of natural organic acids and SO_4^{2-}, and darkwater seepage lakes (30% of total) were acidic because of organic acids (Kahl et al. 1989). The lakes sampled in this study represented the clearwater acidic lakes (cluster 4) as well as the acid, organic seepage lakes (cluster 5). Kahl et al. (1989) suggested that the clear acidic seepage lakes (such as those in cluster 4) were essentially precipitation collectors with little watershed influence.

Formation and movement of organic acids

Organic acids in the seepage lakes and streams evaluated in this study may have both allochthonous (upland watersheds and wetlands) and autochthonous sources. For example, DOC in BBWM streams comprising cluster 6, was evidently controlled by upland soils which appeared to be the source of DOC and regulate the nature of the DOC reaching these surface waters. Stream levels of DOC are low, with the ratio of hydrophobic to hydrophilic acids similar to B horizon soil solutions (0.91), rather than the forest floor (David et al. 1989; Vance & David 1989; Vance et al. 1989). Adsorption by spodic B horizons appears to be the major controlling factor, similar to streams at Hubbard Brook (McDowell & Wood 1984). Short-term increases in DOC storm events or snowmelt may result from less hydrologic contact with deeper mineral soils by high DOC forest floor leachates (Cronan, 1990).

Cronan and Aikens (1985) found a depletion in hydrophobic acids in comparison with hydrophilic acids from forest floor DOC leaching through the B horizon and into surface waters. Vance and David (1989) found that hydrophobic acids are selectively adsorbed by B horizon soils in the BBWM watersheds. Jardine et al. (1989) found a similar preferential adsorption of hydrophobic acids relative to hydrophilic acids using an Ultisol.

Based on the above discussion, the hydrophobic to hydrophilic acid ratio would appear to be a good indicator of the contact of forest floor leachates with the B horizon. This is important to surface water quality because differences in the composition of hydrophobic and hydrophilic acids in soil leachates are significant to metal binding, acid-base chemistry, and the potential leachability of organic constituents. Acid additions (i.e., dilute H_2SO_4 and HNO_3 in laboratory studies) can also alter these fractions, decreasing the hydrophobic to hydrophilic acid ratio in forest floor leachates (David et al. 1989; Vance & David 1989). The influence of current acidic deposition levels on DOC fractions, however, have not yet been observed for leachates coming in contact with mineral soils.

The low DOC lakes of clusters 3 and 4 also had low hydrophobic to hydrophilic acid ratios (1.28 and 1.19, respectively), suggesting adsorption and leaching of DOC was controlled by the surrounding catchments for these seepage lakes. Although the average pH was quite different between the clusters (pH 6.66 and 4.93 for clusters 3 and 4, respectively), DOC character was similar. These lakes probably receive most of the water from seepage through surrounding soils. Apparently the hydrophobic acids are selectively removed during leaching through surrounding soils, resulting in low total DOC and low hydrophobic to hydrophilic acid ratios.

Seepage lakes (cluster 5) and streams (cluster 1) with high DOC had high hydrophobic to hydrophilic acid ratios (1.88 and 2.44, respectively), suggesting little contact of DOC with mineral soils. Many of the cluster 5 lakes had large areas of wetlands, some dominated by *Sphagnum*, which provided a source of DOC that did not contact the mineral soil or provide for generation of ANC by exchange of H^+ for Ca^{2+} or Mg^{2+}. Based on our results, DOC formed from either wetlands (including *Sphagnum*) or upland forest floors (David et al. 1989; Vance & David 1989; Vance et al. 1989) has similar characteristics such as hydrophobic to hydrophilic acid ratios, neutral and base fractions, and overall charge characteristics. The hydrophobic to hydrophilic acid ratio is decreased only after contact with mineral soil.

Cluster 1 streams are difficult to fit into our hypothesized control mechanism because the DOC concentrations and content reflects little contact with mineral soils, but the average ANC is 81 μeq L^{-1}. The base cations (primarily Ca^{2+}) responsible for this ANC are generally thought to originate in mineral soils. One possible explantion would be that wetlands contributed DOC to upper reaches of the streams and ANC was added from other sources (e.g., base flow or stream DOC would only be diluted, and not altered in character. This is clearly an area that requires further investigation.

McKnight et al. (1985) also found that DOC from a *Sphagnum* bog had a high hydrophobic to hydrophilic acid ratio (3.35) with high DOC, similar to our results for cluster 5. They concluded that the upper productive layer of peat and *Sphagnum* was the major source of DOC. Based on amino acid, carbohydrate, and carboxylic acid contents of hydrophobic and hydrophilic acids, they concluded that the latter acids are more labile intermediate compounds in the formation of hydrophobic acids. Our results are consistent with the McKnight et al. (1985) conclusions, with modification of DOC concentrations and constituents by mineral soil adsorption.

Conclusion

Dissolved organic carbon formation processes in upland soils, wetlands, and *Sphagnum* deposits were found to be important in determining the concentrations and character of DOC in seepage lakes and streams evaluated in central Maine. Quantity and quality of DOC fractions were strongly related to the hypothesized contact of soil leachates with B horizon soil material. Lakes with low DOC had low hydrophobic to hydrophilic acid ratios, regardless of pH and ANC, compared to lakes and streams with moderate to high DOC concentrations.

In all solutions, DOC was dominated by organic acids, which had a strong carboxylic character. Use of titration data from isolated DOC indicated that organic anions could be quantitatively predicted, and were strongly pH dependent. Caution must be used in comparing the "charge density" character of dissolved organic acids between various surface waters because of this pH dependence. We recommend using total charge density (i.e., exchange acidity) to represent the maximum dissociation that could occur at pH 7–8, and apparent charge density for the density at the solution pH.

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