Aquat. Sci. 66 (2004) 195–210
1015-1621/04/020195-16
DOI 10.1007/s00027-004-0710-0
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Aquatic Sciences

Research Article

Key site variables governing the functional characteristics of Dissolved Natural Organic Matter (DNOM) in Nordic forested catchments

Rolf D. Vogt [1,*], Jarkko Akkanen [2], Dag Olav Andersen [3], Rainer Brüggemann [4], Bimal Chatterjee [5], Egil Gjessing [1], Jussi V. K. Kukkonen [2], Håkon E. Larsen [1], Jörg Luster [6], Andrea Paul [4], Stephan Pflugmacher [4], Mike Starr [7], Christian E. W. Steinberg [4], Philippe Schmitt-Kopplin [5] and Ádám Zsolnay [5]

[1] Department of Chemistry, University of Oslo, N-0315 Oslo, Norway
[2] Department of Biology, University of Joensuu, FIN-80101 Joensuu, Finland
[3] Agder University College, N-4604 Kristiansand, Norway
[4] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, D-12561 Berlin, Germany
[5] GSF National Research Centre for Environment and Health, Neuherberg bei München, Germany
[6] Swiss Federal Research Institute, WSL, CH-8903 Birmensdorf, Switzerland
[7] Finnish Forest Research Institute, FIN-01301 Vantaa, Finland

Received: 23 June 2003; revised manuscript accepted: 18 November 2003

Abstract. The objective of this study was to extract and evaluate key catchment characteristics explaining the main variation in properties of dissolved natural organic matter (DNOM) in surface waters draining each catchment. A number of classical and sophisticated DNOM characterisation methodologies were used for this purpose. The explanatory capability of site characteristics on the variation in descriptors of DNOM, including elemental analysis (EA), DOC fractionation, SEC, CZE, CGE, UV/Vis- and FTIR spectra, FES, TLS, ESR, ESI/MS, ^{13}C-CPMAS-NMR, potentiometric pH titration as well as functional characteristics such as biodegradability, and the response of macrophyte and PAH partitioning coefficients were evaluated. The sampling sites were all natural, coniferous, heathery-forested catchments with bogs, forming a climatic, anthropogenic deposition and proportional biotope cover gradient.

Correlations to the main principal components, as well as a correlation matrix, singled out total S-deposition as the key explanatory site parameter, accounting for most of the variation in DNOM descriptors. Other significant factors were H^+ concentration, length of growing season, biotope coverage (i.e., peatland/ or lake/watershed ratio), site elevation and TOC concentration. Total sulphur (S) deposition was strongly negatively correlated to the molecular weight, aromaticity, carboxylic acidity and sorption capacity of PAH. A greater proportion of lake and Histosol biotopes of the sampling sites were reflected in a greater aliphatic character of the DNOM. PCA clustering of samples from the same site in spring and fall showed a basic site-specific DNOM quality and a weak season effect. The Hasse diagram technique (a method of partial order theory) was used to show, for instance, how the relative Histosol coverage may determine NOM quality and, in turn, how this coincides with high macrophyte response.

Key words. Explanatory parameters; DNOM characteristics; correlation; PCA; Hasse diagram technique; S-deposition.

* Corresponding author phone: +47 22 855696;
e-mail: rolf.vogt@kjemi.uio.no
Published on Web: June 16, 2004

Introduction

Dissolved natural organic matter (DNOM) is a term for a complex mixture of heterogeneous organic macromolecules that occur ubiquitously in surface waters. DNOM has an influential role on freshwater chemistry due to its various weak acid groups, strong metal complexation capacity and lipophilic sorption abilities, interactions with oxides and other mineral phases, and oxidation/reduction properties (Cronan and Aiken, 1985; Pohlman and McColl, 1988; Vance and David, 1991; Liechty et al., 1995). Furthermore, DNOM plays an important role as substrate for biological growth and determination of ecological niches (Steinberg, 2003). It also affects drinking water quality by providing precursor material for disinfection by-products (Reckhow and Singer, 1990; Reckhow et al., 1990). A widespread increase during the last 15 years in the amount of coloured DNOM in surface water has been reported throughout Europe and North America (Skjelkvåle, 2003). These changes have been linked to variation in climate, but possibly also to reduced acid deposition and land use changes. The effect of changes in these key factors on the amount and properties of DNOM cannot currently be predicted.

Characterisation of DNOM is necessary to explain its role in soil and fresh water as well as marine ecosystems. Numerous excellent ecological, physical and chemical analytical methods are available to characterize DNOM, enabling comparison of bio-physico-chemical properties. However, DNOM is generated from innumerable degradation- and by-products of living organisms. The characteristics of DNOM, therefore, rely both on the character of its source (e.g., edaphic and climatic factors as well as biota) and on the flow regime (i.e., water flow paths) prevalent during the sampling period. These spatial and temporal differences in the properties of DNOM are still not well described. Therefore, there is a need to link DNOM characterisation parameters and the ecological functions of DNOM with key descriptive qualities of the catchment. However, there has been a lack of multidimensional datasets characterizing the same set of DNOM; i.e., extensive characterisation of a set of DNOM material derived from a set of samples that have been isolated in the same manner. Such datasets must also comprise a wide range in values of the studied parameters. A shortcoming is also partly caused by the lack of descriptive data of the DNOM source; i.e., catchment characteristics. Furthermore, interpretation of the data characterizing the DNOM is often indecisive since we do not fully comprehend the denotation of the methodical defined analytical parameters.

The NOMiNiC project (Natural Organic Matter in the Nordic Countries) (Vogt et al., 2001, 2002a) produced a set of 10 Reverse Osmosis (RO) isolates of the spring and fall runoff from five coniferous-forested Nordic catchments differing in climate, anthropogenic S-loading and relative coverage of mineral soil, bogs and lakes. Sites with similar type of vegetation were selected, ensuring that the source of the DNOM was akin.

Here we report on the quantification of 3 biological properties and a number of chemical and physical DNOM properties. This paper focuses on the relationships and co-variations of DNOM characteristics with sampling site characteristics and water chemistry. The objective was to identify key site explanatory variables governing the functional characteristics of DNOM. Special attention was given the effects of spatial variation in S-deposition, climate and land coverage in dictating DNOM characteristics to predict their likely influence on future changes in the DNOM.

Site descriptions and material

Study sites

The location of the five catchments is shown in Figure 1. Selected characteristics of the raw water samples and sampling sites are presented in Table 1. The Hietajärvi catchment in Finland is a large (83 ha) pristine area (Bergström et al., 1995) comprisesing a large oligothrophic lake and several small ponds. The soils of Hietajärvi are deep (>10 m) with a high coverage of Histosols (Table 1). The chemistry of the surface water is characterised by relatively low TOC and high pH. Furthermore, the site has a dry and cold continental climate with a short growing season (102 d yr^{-1}). The Valkea-Kotinen catchment (Bergström et al., 1995) has relatively intermediate charac-

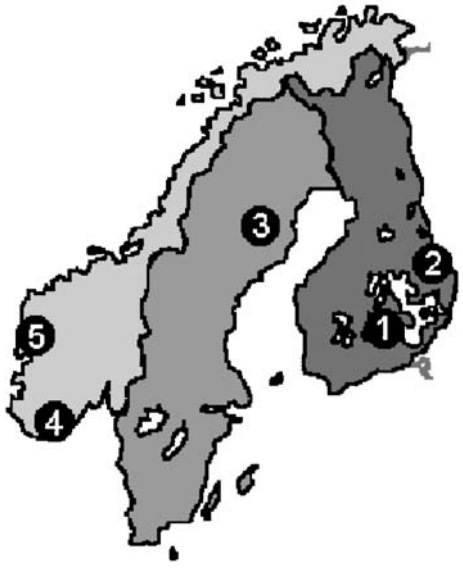


Figure 1. Map of the sampling sites: ❶ Hietajärvi (H), ❷ Valkea-Kotinen (V), ❸ Svartberget (Sv), ❹ Birkenes (B), and ❺ Skjervatjern (S).

Table 1. Selected characteristics of the raw water samples and sampling sites. Inorganic chemical parameters are the concentrations in the original water sample.

Site	Season	Abbr.	H^+	TOC	Al_a	SO_4^{2-}	Avg. temp.	Growing season	Precip.	Elevation	Tot S-dep.	Forest	Lake	Histosol
			µM	mg C L^{-1}	µM	µeq L^{-1}	ºC	d	mm yr^{-1}	m a. s. l.	g S yr^{-1}	%		%
Hietajärvi	Fall	H_f	0.63	6.4	0.5	33.5	2.0	102	592	165	0.5	39	24	35
	Spring	H_s	0.38	4.7	0.7	25.0								
Valkea-Kotinen	Fall	V_f	3.72	9.4	2.8	129	3.1	112	618	150	0.7	67	11	22
	Spring	V_s	9.33	11.1	3.5	130								
Svartberget	Fall	Sv_f	3.72	10.9	1.9	135	0.0	153	720	235	0.3	78	0	16
	Spring	Sv_s	37.1	18.6	8.3	70.9								
Birkenes	Fall	B_f	26.9	5.0	13	76.8	6.6	174	1500	190	0.9	83	0	13
	Spring	B_s	20.9	3.6	15	64.9								
Skjervatjern	Fall	S_f	27.5	10.2	4.3	17.6	5.4	180	2500	136	0.4	54	24	34
	Spring	S_s	31.6	5.9	2.5	<d.l.								

Al_a denote the monomeric Al fraction. Avg. temp. denote year averages. Growing season denote length of growing season (i.e., days with temp. > 5 °C). Tot S-dep. denote yearly averages of total deposition of sulfur during 1989–1999. For all sites the S-deposition has decreased substantially since 1980. Forest and lake coverage is relative to total catchment including also peatland. Histosol coverage is relative to other soil coverage (i.e., podzol, rock outcrop and thin humus soil).

teristics (Table 1), except for high evapotranspiration (432 mm yr^{-1}). The high evapotranspiration along with a moderate loading of acid rain produces the highest sulphate concentrations in runoff, despite lower total S-deposition than Birkenes. Inputs of mineral acidity appear to be neutralized by mobilization of calcium, leading to the highest calcium concentrations in runoff among the sites (114 µeq L^{-1}). This result is expected as the base saturation (BS) in the Dystric Cambisols found in Valkea-Kotinen are close to or greater than 20%, while at Birkenes the BS in the mineral soil horizons of its Podzols are all substantially below 20% (Larsen, 2003; Reuss and Johnson, 1986). The Svartberget site (Bishop et al., 1994; Köhler et al., 2002) in boreal Northern Sweden is relatively pristine with high levels of TOC (Table 1). The site consists of a large peat bog draining a catchment of well-developed Podzol profiles. The nemoral Birkenes site (Vogt, 1989) lies in the part of Scandinavia that receives the highest anthropogenic S-deposition (SFT, 2003). Surface water samples from this site, especially the base flow spring sample, had the lowest TOC concentrations and highest levels of aluminium (Al; Table 1) and nitrate as well as sodium chloride from sea salts (Larsen, 2003). Skjervatjern (Gjessing, 1994) is a small, pristine catchment with a deep dystrophic lake. The wet coastal boreal climate provides mild winters with a long growing season (180 d yr^{-1}) (Table 1). The soils at Skjervatjern are mainly organic (>70%; Hufthammar, 1994) and shallow (<0.5 m). The surface water is dilute ($I = 1.5 \cdot 10^{-4}$) with a pH that is the lowest among the sites, despite insignificant non-marine sulphate inputs. For more detailed data of the sites see Vogt et al. (2001, 2002a) and Larsen (2003).

Experimental design

DNOM was isolated twice from 500 to 1100 L of surface water at each site, each in spring and fall. Flow conditions during both sampling occasions were low (n.b. the spring sampling was after the snowmelt-related peak flow) and reflect base flow conditions. In aquatic ecosystems, DNOM undergoes significant variation governed mainly by fluctuations in flow conditions (Easthouse et al., 1992). Therefore, the two samples from each site do not represent the seasonal variability in DNOM. However, by sampling under base flow conditions, we minimised the effect of flow while optimising the effect of biological activity. The sample in fall represents the end of the growing season while that in spring represents the beginning of the growing season.

The isolation of DNOM was conducted using a mobile reverse osmosis (RO) unit (PROS2/2S) (Serkiz and Perdue, 1990). In the RO membranes (15 nm), the feed-water was separated into a permeate solution, containing virtually no solutes, and a retentive solution that contained nearly all the dissolved inorganic and organic material in the cation (Na^+) exchanged sample. The permeate solution was discharged and the retentive solution was recycled back to a sample reservoir. Additional water was fed into the sample reservoir to maintain a more or less constant volume. Each isolation resulted in a 25 L concentrated DNOM sample that was rotary-evaporated and freeze dried. This far, 154 bio-physico-chemical parameters characterizing the water quality and DNOM have been determined; i.e., water quality of raw water (20), water quality of re-dissolved RO isolates (26), DNOM composition (17) and -structure (63), and DNOM functionality: i.e., biological- (3), chemical- (11) and physical (14).

Catchment characteristics (i.e., anthropogenic S-loading, geography, edaphic factors, climate, topography, soil, vegetation, biotope, and lake coverage) were quantified by means of 30 different parameters. Correlation analysis and indirect principal component analysis (PCA) were conducted on the data.

Methods

Water quality

Acidity (pH) was measured in situ in accordance with the ISO method (1994). Major cation (Al, Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) concentrations were determined by Inductive Coupled Plasma (ICP) Atomic Emission Spectroscopy (AES) (ISO, 1996) while major anion (SO_4^{2-}, NO_3^{-} and Cl^{-}) concentrations were determined by Ion Chromatography (IC) (ISO, 1992). TOC was determined according to ISO (1999) using high temperature catalytic combustion and detection of CO_2 by IR. Total and organic monomeric Al fractions (Al_a and Al_o, respectively) in the water samples were determined in situ according to the Barnes/Driscoll procedure (Sullivan et al., 1986).

Elemental analysis (EA) of RO isolates

Carbon and nitrogen in the isolates were determined simultaneously after combustion at 940 °C (CHN-analyser). CO_2 was detected by IR absorption as described in ISO (1995). NO_2 was reduced to N_2 and detected by thermic conductivity as described in ISO (1998).

Fractionation characterisation

DNOM of raw water was fractionated into hydrophobic- (HPO-) and hydrophilic (HPI) -acids (-A), -bases (-B) -neutrals (-N), and phenolics (-Ph) according to Leenheer and Huffman (1979) with modifications from Qualls and Haines (1991). After removal of HPO-B (on XAD-8 at pH 7), HPO-A and -N were trapped on an XAD-8 resin at pH 2. The HPO-A fraction was determined as the amount of DOC that is eluted by 0.1 M NaOH, while the HPO-N fraction remains adsorbed. HPO-A, thought to be the main component of fulvic acids, is a mixture of complex aliphatic carboxylic acids, simple aromatic acids and phenols. HPO-N is a mixture of hydrocarbons and carbonyl compounds (Leenheer, 1981).

Size-exclusion chromatography (SEC) with UV- and organic carbon detection (Huber and Frimmel 1991, 1996) was used to fractionate the DOC into 4 groups; polysaccharides (PSac), humic substances (HS), low molecular weight carboxylic acids (LMWA) and low weight substances (LWR; after retention time of 45 minutes). The column was packed with Toyopearl HW-50S resin and a phosphate buffer was used as eluent. Molecular weights (Mw) were determined using a HRLC Biorad HPLC System with a TSK Gel Column SW 2000 (4.6 mm ID × 30 cm) with a 0.028 M phosphate buffer at pH 6.2 as eluent (flow rate 0.4 mL min^{-1}) as described in Kudryavtsev et al. (2000). Samples were dissolved in the buffer and 100 µL were injected. Detection was at 260 nm. Calibration was done using polystyrene sulfonates as internal standards representative of globular polyelectrolytes with a charge density in the range of humic materials as measured with capillary electrophoresis.

Capillary Electrophoresis (CE) was performed with a Beckman PA/CE 5510 in uncoated fused-silica CE columns (57 cm total length, 50 cm to the detector, 75 µm i.d.). The UV absorbency was detected by a diode-array detector set at 254 nm (Schmitt-Kopplin et al., 1999). The technique to determine hydrodynamic radii is based on combined electrophoretic measurements using Capillary Zone Electrophoresis (CZE) and Capillary Gel Electrophoresis (CGE). The conditions in CGE were the same as in CZE (0.025 M sodium carbonate buffer pH 9.3, 25 kV, 30 °C) except that the buffer was adjusted to 0.3% methylcellulose (sieving polymer). The variation in mobility from CZE to CGE is proportional to the molecular mass and the hydrodynamic radii can be calculated from the molecular mass distributions using polystyrene sulfonates as internal standards (Schmitt-Kopplin, unpublished).

The weighted average mass to charge (m/z) was measured with Flow Injection Electrospray Ionisation Mass Spectrometry (FI-ESI/MS) in negative and positive ionisation modus. The CE system described above was hyphenated to a Thermo Finnigan (San Jose, USA) LCQ Duo ion trap mass spectrometer (MS) using a self-modified Thermo Finnigan CE/MS (sheath liquid) interface which allowed accurate three-dimensional positioning of the CE outlet in respect to the MS orifice. The CE-system was used as a micropump in high pressure modus for FI-ESI/MS flushing 20 sec sample between buffers at 20 psi (Junkers et al., 2003). The 20 sec peaks were integrated (intensity), background subtracted and the weighted values (m/z_w) calculated with GelTreat software (Kudryavtsev et al., 2000).

The lack of congruence between the methods describing the size of the DNOM is because they are based on incompatible yet complementary principles. The difference between the SEC and the CGE is the pH (SEC at 6.95 and CGE at 9.3) and that there is no chromatographic effect in CGE. The ESI/MS is based on "selective" electrospray ionization and is very sensitive to solution conditions (conterions) and the ESI-source set-up (see Schmitt-Kopplin and Kettrup, 2003). This issue is not the topic of this paper but will be treated in more detail in a later publication. In fact, we find nice correlations between the m/z_w and the CGE estimated sizes when setting up optimal ESI-conditions.

Structural characterisation

Radiation absorbencies of surface water and re-dissolved RO isolates were measured at λ 254, 400 and 600 nm in 1 cm quartz cuvettes at sample pH. Optical densities of surface water were determined in situ. The absorbency at λ 254 nm was also measured at pH 2. The absorbencies were used to calculate the specific visible and UV absorbency (i.e., absorbtivity; sVISa = $A_{\lambda 400\,nm}$/mg C L^{-1}; sUVa = $A_{\lambda 254nm}$/mg C L^{-1}) and the specific absorption ratios (SAR_{UV} = $A_{\lambda 254nm}/A_{\lambda 400nm}$ and SAR_{VIS} = $A_{\lambda 400\,nm}/A_{\lambda 600\,nm}$ ratio). Only the SAR_{UV} values are reported as the SAR_{UV} and SAR_{VIS} values were strongly correlated (r = 0.92). SAR_{UV} values are hereafter referred to as SAR. The sUVa reflects the degree of aromaticity, while a low SAR is expected for material with a high molecular weight (Hautala et al., 2000) but not a high degree of condensed aromatic rings (Senesi and Steelink, 1989). In addition, SAR values were expected to increase with increasing oxygen content (Chen et al., 1977).

Fourier transformed infrared (FTIR) spectra were obtained from 400 to 4000 cm^{-1}; 1 mg of DNOM isolate was mixed with 200 mg KBr and pressed into pellets (Schmitt-Kopplin et al., 1998). Resolution enhancement was used systematically (deconvolution factor 50, smooth factor 30) as a quantitative tool to determine wave numbers of predominant bands. The following FTIR wavelength assignments were assumed: COO^- groups and aromatic C=C bonds = 1620–1565 cm^{-1}, O-H deformation and C-O stretching of phenolic O-H, aliphatic and carboxylate groups = 1400–1445 cm^{-1}. No sharp signals due to Si-O or SiO-H bonds were found between 3600–3700 cm^{-1}, in relation to 1030 and 1050 cm^{-1}, showing that in these RO isolates the FTIR bands in the lower range 1150 to 1100 cm^{-1} can be ascribed to vibration of C-O bonds of polysaccharidic structures only.

Fluorescence emission spectra (FES) were obtained as described in Zsolnay et al. (1999) and Cox et al. (2000) at sample pH and at pH 2 from 300 to 480 nm using a 254 nm excitation light. The fluorescence emission was corrected by multiplication with the factor e^A, where A is the absorbance at 254 nm. This correction is necessary since the exciting light is attenuated by its absorption within the solution being investigated. Based on these measurements the following parameters were calculated: RkL = relative fluorescence emission in bluer region (330–345 nm), presumably representing less condensed material; RkH = relative fluorescence emission in redder region (435–480 nm), presumably representing more condensed material; HIX = Humification Index (dimensionless, RkH/RkL) and SFE = fluorescence efficiency (Arbitrary Units; AU cm) at λ_{max} ~ 430 nm.

Total luminescence spectra (TLS) were recorded from 10 mg C L^{-1} solutions, which were adjusted to pH 4 with dilute HCl or NaOH, as a series of 10 synchronous scan spectra with differences between excitation and emission wavelength of 25, 50, 75, ..., 250 nm and excitation wavelengths between 250 and 550 nm. The spectra were corrected for inner filter effects by using the data from molecular absorption spectra between 250 and 700 nm that were recorded from the same solutions (Lakowicz, 1983). Finally, a blank spectrum of doubly deionised water was subtracted. For instrumental details, see Blaser et al. (1999). A peak is observed at $\lambda_{ex}/\lambda_{em}$ = 340/440. Among the many naturally occurring fluorophores in this wavelength region, simple phenolic compounds like hydroxyl-substituted benzoic and cinnamic acid derivatives are most likely the main contributors in aquatic environments. A weak shoulder at about $\lambda_{ex}/\lambda_{em}$ = 440/515 indicates the presence of small amounts of more highly conjugated aromatic compounds such as di-substituted coumarins and xanthones as well as quinones and Schiff-Base derivatives (Blaser et al., 1999). The chemical identity of the fluorophores responsible for the peak at $\lambda_{ex}/\lambda_{em}$ = 250/450 is unknown. However, such a peak seems to be typical for aquatic NOM as well as for all types of soil NOM (Blaser et al., 1999) and has been attributed to humified material (Zsolnay et al., 1999).

Cw X-band electron spin resonance (ESR) spectra of un-dissolved RO isolates exposed to 2 mW microwave radiation were recorded at room temperature. For the determination of g-values, MgO/Cr^{3+} (g = 1.9796) was used as reference. Spin contents of samples were determined by parallel recording of sample and reference and calculated using the integrated areas normalized to the reference. The obtained spin densities were then related to the Suwannee River Humic Acid Standard (SuRi-HA) at a value of $0.65 \cdot 10^{-17}$ g^{-1} (Paul et al., 2004a). High spin densities indicate high degrees of humification (Bayer et al., 2000; Maia et al., 2000) and aromaticity, and a low amount of carboxyl groups. Determination of singlet oxygen quantum yields (Φ_Δ) were performed by means of time-resolved luminescence measurements as described in Paul et al. (2004b). High values for Φ_Δ indicate more photoreactive DNOM.

The solid-state ^{13}C Cross Polarization Magic Angle Spin Nuclear Magnetic Resonance (CPMAS NMR) spectra were determined at 50.3 MHz. The rotor was the standard 4 mm diameter and 7 mm high. The acquisition conditions were as follows: acquisition time 0.1 sec; relaxation delay 2.0 sec; spectral window width 31000 Hz (30865.113 Hz); (contact time 1 ms, 90 dgr pulse 3.12 µsec); number of scans 131 000. The following NMR assignments were assumed; 0–45 ppm = Alkyl Carbon, 45–60 ppm = Methoxyl and N-alkyl Carbon, 190–220 ppm = Ketonic (or Carbonyl) Carbon (Malcolm, 1989). The ratio of integrated NMR signal between 110–220 and 0–110 nm is used as a measure for the aromaticity of the DNOM (i.e., Aromatic/Aliphatic). A correlation between sUVa and aromaticity is to be expected, but our were uncorrelated. This result is likely because

the samples included a large span in spatial and temporal DNOM variability. A separate manuscript addressing the expected and missing correlations between parameters should be published within a year.

Functionality characterisation

Carboxylic- and hydroxyl acidity was determined by potentiometric pH titrations. DNOM isolates (5 mg) were dissolved in distilled water (20 mL) and autotitrated with 0.02 N NaOH under an N_2 atmosphere at a rate of 40 µL every 60 sec. The pK spectra of Humic substances were calculated from the data of potentiometric titration (Garmash et al., 1998).

The partition coefficient of Benzo[a]pyrene and Pyrene ($K_{B[a]P}$ and K_P, respectively) between water and the DOC in samples was determined with the equilibrium dialysis technique (Carter and Suffet, 1982; McCarthy and Jimenez, 1985) using radioactive labelled (^3H or ^{14}C) compounds. The radioactive labelled compounds were quantified by liquid scintillation counting (for further details see Akkanen and Kukkonen, 2003 and Akkanen et al., 2004).

Biodegradable organic matter was determined as the net removal of DOC from the metabolisable pool under ideal conditions within 5 days. The DOC solutions (8 mL) in small Teflon vials were added 3 mL of a nutrient solution (0.1% NH_4NO_3 and 0.1% K_2HPO_4) and 50 µL of a microbial inoculum. The vials were then incubated in the dark at room temperature for 5 d. The DOC content was quantified before and at the end of the incubation after acidification and purging (i.e., NPOC) with a Shimadzu 5050 TOC Analyzer. To ensure that the assay was functioning, control samples containing 20 mg L^{-1} glucose were run alongside the six test solutions. In the control samples, approximately 88% of the DOC was removed after 7 d. All analyses were performed in triplicate. The inoculum consisted of agricultural soil slurry, which was allowed to settle until a relatively clear liquid could be pipetted off. The amount of DOC in the inoculum was negligible. An unpublished study indicated that, at least for agricultural soils, the source of the inoculum was of no importance.

The biological effect (i.e., macrophyte response) of the DNOM on Coontail (*Ceratophyllum demersum*), a free floating submersed plant, was measured by exposing the plant for 24 hours to 0.5 g C L^{-1} solutions of DNOM and determining the photosynthetic oxygen production (PS) as well as the amount of guajacol peroxidase (POD), an enzyme involved in oxygen stress detoxication of plants (Pflugmacher et al., 1999). PS was selected as a parameter for macrophyte response as photosynthesis is the main metabolic feature in plants and is also very sensitive to environmental stress. POD was chosen because it has proved to be a very good biomarker for environmental stress and, furthermore, due to a possible production of reactive oxygen species (ROS) in the plant during exposure to DNOM. ROS can cause serious damage to DNA. The peroxidase is one of the enzymes involved in the detoxification of ROS. A low PS value indicates that the plant is seriously stressed. A high POD value indicates that the plant was influenced by ROS, which might be a by-product of the detoxication metabolism.

Data treatment

Data on DNOM characteristics were generated by the authors, for whom the methods are very routine and established. Furthermore, their development is referenced in the list of literature.

The quality of DNOM material is described in respect to characteristics of each sampling site in the results chapter. Multivariate statistics using principal component analysis (PCA) on the correlation matrix (i.e., after transformations and scaling of the data) was conducted on the DNOM descriptors to understand the underlying data structure and to form a smaller number of uncorrelated variables (i.e., principal components (PC)). The key explanatory sampling site characteristics were then identified as the site descriptive parameters that were significantly correlated to the main PCs. A PCA on all DNOM descriptors proved to have moderate explanatory value. Since several DNOM descriptors are likely not dictated by the site characteristics, parameters with high loading in this PCA are not necessarily linked to any site explanatory variables. In order to refine the model relative to the site characteristics, a parameter screening was conducted to select 21 DNOM parameters that were conceptually or empirically linked to the site explanatory variables. The empirical parameter screening analysis was conducted by means of Pearson correlation. A new PCA was then conducted on the 21 parameters. In addition, the potential of using the Hasse diagram technique on such data was explored.

Results

General

Despite a similar biological origin (i.e., conifer forests) and overall base-flow conditions during sampling, there was a large range in values of the bio-physico-chemical characteristics of DNOM among the five sites (Table 2).

Differences in DNOM characteristics among sites were generally greater than those between the spring and fall samples. The main exceptions being the presence of generally more photo reactive DNOM, as indicated by singlet oxygen data (Φ_Δ; see Paul et al., 2004b), lower photosynthetic oxygen production of macrophyte exposed to the DNOM and lower mass to charge ratio in

Table 2. Selected characteristics of the DNOM. Site and season abbreviations are explained in Table 1.

Site_ Season	C:N	HPO-A	HS	Mw	Radii	m/z_w	sUVa	SAR	C=C; COO^-	O-H; C-O	Cond. mat.	HIX	SFE	Phenol	Phenol/ Arom	Spin density	Arom/ Aliph	Tot-COOH	BDOM	$K_{B[a]P}$	PS	POD
	Wt%	%	%	kD	Nm				%					AU		$10^{17} \cdot g^{-1}$		meq g^{-1}	%		O_2 L^{-1} h^{-1} mg FW^{-1}	Nkat mg^{-1} protein
H_f	47	67	78	4.4	2.10	1130	3.5	7.7	10	4.9	14.0	8.5	10.5	144	8.00	0.64	0.46	2.37	3	13	24	8.9
H_s	38	41	77	4.2	2.85	1189	3.7	7.8	8.8	4.9	18.9	8.1	14.0	162	7.75	1.03	0.68	2.16	–3	21	55	5.7
V_f	33	62	75	4.1	2.24	1127	3.6	7.9	7.8	4.4	17.5	9.5	12.8	169	8.67	0.04	0.45	2.19	11	23	23	8.3
V_s	35	64	75	4.0	2.73	1135	3.7	8.2	8.7	4.4	18.8	10.7	12.8	179	8.10	0.14	0.67	2.06	11	20	49	6.3
Sv_f	42	62	n. d.	4.7	1.88	1146	3.9	5.2	8.3	4.0	14.4	8.5	8.85	167	5.80	0.18	0.73	2.27	6	36	23	6.8
Sv_s	57	14	84	5.0	1.56	1080	5.1	7.8	8.5	4.7	19.4	9.5	8.38	174	5.63	0.06	0.68	2.59	8	21	45	4.3
B_f	33	48	80	4.3	2.75	1139	3.8	9.7	6.0	4.1	21.8	15.1	13.1	230	7.32	0.0	0.65	1.94	10	6.8	34	5.6
B_s	24	55	77	3.9	2.51	1179	2.5	10.7	5.3	4.3	19.0	9.1	19.0	227	7.57	0.0	0.90	1.77	17	3.8	58	4.8
S_f	55	63	80	4.6	2.22	872	4.9	7.2	9.3	5.5	19.8	12.9	9.20	160	6.43	0.01	0.59	2.30	1	27	62	2.7
S_s	58	59	76	4.6	2.38	1176	4.3	7.1	8.6	5.3	18.0	10.2	10.2	167	6.23	0.16	0.55	2.28	12	33	66	2.5

n. d. = not determined. C:N denotes ratio of carbon to nitrogen as determined by EA. HPO-A denotes the hydrophobic acid fraction of the DNOM as adsorbed by a XAD-8 resin and eluted by 1M NaOH. HS denote humic substances determined by SEC. Mw denotes molecular weight as determined using HPSEC. Radii denote molecular size based on combined CZE and CGE measurements. m/z_w denote weighted average mass to charge measured with ESI/MS in negative ionisation modus. sUVa denote the specific UV absorption ratio of λ_{254}/mg C L^{-1} measured at pH 2. SAR denote the specific absorption ratio of $\lambda_{254}/\lambda_{400}$ nm. C=C; COO^- denotes aromatic bonds and carboxylic groups as seen in FTIR spectra (1620–1565 cm^{-1}). O-H; C-O denote O-H deformation and C-O stretching of phenolic O-H, aliphatic and carboxylate groups as seen in FTIR spectra (1445–1400 cm^{-1}). Cond. mat. (Condenced material) denotes the relative fluorescence emission ($\cdot 10^4$) in redder region (435–480 nm) measured at pH 2. HIX denote Humification Index (fluorescence emission in redder region (435–480 nm) relative to emission in bluer region (330–345 nm). SFE denote fluorescence efficiency ($\cdot 10^6$) at λ_{max} ~ 430 nm. Phenol denotes phenolic content as expressed by TLS ($\lambda_{ex}/\lambda_{em}$ = 340/440). Phenol/Arom denote the intensity ratio of the TLS signal at $\lambda_{ex}/\lambda_{em}$ = 340/440 over $\lambda_{ex}/\lambda_{em}$ = 440/515, attributed to the ratio of phenolic over highly conjugated aromatic compounds. Spin density denote the spin content in ESR spectra relative to reference and related to IHSS-HA standard with a spin of 0.65×10^{17} g^{-1}. Arom/Aliph (Aromatic/Aliphatic) denotes the ratio of integrated NMR signal between 110–220 over 0–110 nm. Tot COOH denotes density of carboxylic acid functional groups as determined by potentiometric pH titration. BDOM denote Biodegradable organic matter determined as the amount of DOC which was metabolised under ideal conditions within 5 days. The $K_{B[A]P}$ denotes the partitioning coefficients for Benzo[a]Pyrene ($\cdot 10^4$). PS denotes the photosynthetic oxygen production of a macrophyte exposed to the DNOM. POD denotes the amount of guajacol peroxidase, an enzyme involved in oxygen stress detoxication of plants.

spring than in autumn (see PS and m/z_w, respectively, in Table 2). Large differences between the sampling occasions were also found for humification index (HIX) and the relative amount of hydrophobic acids (HPO-A), although without any consistent trend. The NMR spectra indicated that the main structural differences between the isolates lay in the relative amounts of aromatic compounds and carbohydrates (see Arom/Aliph in Table 2).

The Svartberget and Birkenes sites represent the end-members in a clear gradient within the dataset despite similarities in, e.g., proportional biotope cover. This trend is especially the case for the spring samples. Svartberget is a pristine and organic rich site, while Birkenes is an anthropogenic acidified site with low TOC concentrations (Table 1). The spring sample from the Svartberget was collected during a minor runoff episode and had elevated TOC concentrations, while low flow conditions prevailed during the sampling of the spring sample at Birkenes leading to an even lower TOC concentration than usual (Larsen, 2003).

Hietajärvi DNOM

DNOM samples from the Hietajärvi site (H) had a large FTIR spectra band at c. 1600 cm^{-1}, indicating the presence of especially aromatic parts and partially COOH groups (C=C; COO- in Table 2). On the other hand, the specific absorption in the UV range (sUVa) of the DNOM at Hietajärvi was low, suggesting low aromaticity. The FES indicates a very low humification index (HIX). Furthermore, the TLS showed a high ratio of the phenolic- to highly conjugated aromatic peak in this material (Phenol/Arom.; Table 2). Therefore, the spectra band at c. 1600 cm^{-1} is considered to be mainly due to carboxylic groups. This assumption is supported by a high content of carboxylic acids (Tot-COOH, Table 2). Considering the long residence time of the runoff water at this site (1.5 years; Bergström et al., 1995), these findings agree with a hypothesis that the more highly conjugated aromatic compounds are reduced with time, either by precipitation, by photodegradation (i.e., photobleaching) or by bio-degradation to simple phenolic compounds (Blaser et al., 1999). Photodegradation, producing reactive species, is mainly expected to play a significant role from spring to fall. Among the reactive species produced are reactive oxygen species like singlet oxygen (Φ_Δ) and radicals (Cooper, 1989; Hoigné et al., 1989). Major modifications of DNOM may then occur by hydrolysis, polymerisation and photomineralization (Salonen and Vähätalo, 1994) through complex interactions between light, DNOM and the reactive species. These processes result in the release of low molecular weight compounds that may serve as nutrients for heterotrophic bacteria (Bertilson and Tranvik, 2000). This modification of DNOM would explain why less reactive, more inert DNOM was found in autumn than in spring. Complying with the bio-degradation reasoning, we found on average no biodegradable DOC left in DNOM from the large Hietajärvi lake (BDOM, Table 2).

Both singlet oxygen and ESR data were especially unique for Hietajärvi, with exceptionally high singlet oxygen quantum yields (Avg. $\Phi_\Delta = 0.76$) and spin densities (Table 2). The latter is contradictory to the FTIR spectra and the theory of Senesi et al. (1987) and previous findings by Sposito et al. (1996). They state that the number of organic radicals correlates with the number of aromatic rings in the system, since high spin densities are believed to indicate a high degree of aromatisation and low amount of carboxyl groups (Sposito et al., 1996). The latter is also partly opposed by a high total acidity, as expressed by the high content of both carboxylic- and phenolic acids (2.15 and 2.98 µeq mgC^{-1} in the fall and spring samples, respectively). On the other hand, the amount of POD, an enzyme involved in the detoxification of the ROS, produced when exposing a macrophyte to the DNOM was highest for the material from Hietajärvi (Table 2). This concurs with the high singlet oxygen quantum yields (Φ_Δ) found for this material. The strong biological response to the DNOM from Hietajärvi may be related to the high coverage of Histosols at this site, as discussed by means of the Hasse diagram below.

Valkea-Kotinen DNOM

A relatively high N-deposition at Valkea-Kotinen (V) and Birkenes (B) (average (1989–97) bulk deposition of inorganic N deposition was 357 and 1028 mg m^{-2} yr^{-1}, respectively) may be the cause of the relatively low C:N-ratio (Table 2) of DNOM from these sites. The SEC analysis shows a relatively low humic substance content (HS, 75%) and, instead, a relatively high content of low weight substances (LWR; 24%) at Valkea-Kotinen. These findings comply with the FTIR spectra showing a predominantly aliphatic character (not presented).

Svartberget DNOM

The RO material from the spring sample from Svartberget (Sv) is found to be exceptional in terms of most parameters (Table 2) (Vogt et al., 2001, 2002a). While the hydrophobic acid fraction (HPO-A) is generally found to be the greatest DNOM fraction in surface waters (see e.g., Thurman, 1985; Malcolm, 1985), the Svartberget spring sample was dominated by the hydrophobic neutral (HPO-N; 62%) fraction. Complying with this, the FTIR spectra showed an exceptionally large difference between the two Svartberget samples, with a distinct carbonyl peak at 1750–1690 cm^{-1} in the spring sample. Size exclusion chromatography showed the highest percentage of humic substance (HS; 84%) and lowest percentage of

low molecular weight compounds (LWR; 16%) in the spring material. Furthermore, the NMR spectra revealed that the spring sample had, on average, the lowest alkyl content among the DNOM isolates.

The Svartberget samples (especially the spring sample) had the lowest content of simple phenolic compounds relative to highly conjugated aromatic compounds (see Phenol/Arom in Table 2). This finding was confirmed by the highest specific UV absorption value (sUVa; Table 2), indicating a high degree of aromaticity. The Svartberget samples possessed also the lowest fluorescence efficiency (SFE; Table 2), probably due to high self absorption. The latter was again supported by the HPSEC analysis, where samples from Svartberget were found to have the highest molecular weight (Mw) and lowest molecular size (radii) based on combined CZE and CGE (Table 2). Furthermore, the Svartberget samples had high total carboxylic acidity (tot-COOH; Table 2). The material from Svartberget therefore presumably represents large and more aromatic DNOM material with high functionality. These characteristics favour a high partitioning coefficient of PAH (e.g., $K_{B[a]P}$ in Table 2) (see also Akkanen et al., 2004).

Birkenes DNOM

The ash content of isolates from Birkenes (B) was high (55–66%; Vogt et al., 2002a) due to the high content of aluminium and chloride salts. Gjessing et al. (1999), comparing sites with similar sea salt loading, also found a higher ash content in RO material from Birkenes relative to RO material from less acidified sites. The Birkenes RO gave the weakest FTIR bands and peaks in the 1800 to 1200 cm^{-1} region; i.e., their structures appear to be simpler, less aromatic and composed essentially of polysaccharide residues. Furthermore, the aliphatic character was even more predominant in the spring sample than in the fall sample. Complying with these findings, the spring DNOM from Birkenes had the lowest sUVa and SFE values (Table 2). The latter is possibly caused by less self absorbance (or inner filter effect) due to fewer larger molecules being present. The spring isolate from Birkenes was also the material with lowest molecular weight (Mw). The very high SAR value for this material is also indicative of small molecules.

The TLS show apparently an especially high phenolic content (Table 2) in this DNOM material, but this signal is, instead, related to the formation of Al complexes with DNOM (Luster et al., 1996). The carboxylic acidity of especially the spring DNOM sample was the lowest among the studied sites. These rather small DNOM molecules had a very poor ability to sorb PAH (e.g., $K_{B[a]P}$, Table 2). Other studies have shown that the Al complexation ability of this relatively aliphatic DNOM from Birkenes is much lower than that of Svartberget (Østerhus, 2001) and Skjervatjern (Taugbøl and Seip, 1994).

Skjervatjern DNOM

Although clearly different in their site characteristics (e.g., soil coverage and climate), the quality of DNOM from Skjervatjern (S) resembled the large and more aromatic DNOM material from Svartberget (Sv), with a few exceptions (see Table 2). The FTIR spectra of the Skjervatjern material was more similar to the spectra of the DNOM material from Hietajärvi (not shown), having the same large band at c. 1600 cm^{-1} resulting from the presence of aromatic parts and partially of COOH groups. However, the aromatic content was higher and the polysaccharide content was lower in the DNOM from Skjervatjern. The FTIR spectra of the isolates from Skjervatjern also indicated a high content of phenolic, aliphatic and carboxylate groups (O-H; C-O, Table 2).

Exposing the macrophyte to DNOM from Skjervatjern caused only a minor biological response; i.e., slight inhibition of photosynthetic oxygen production and a low amount of guajacol peroxidase (PS and POD, respectively; Table 2). This result indicates that the plant was not seriously stressed by exposure to this DNOM material.

Discussion

A rudimentary principal component analysis (PCA) on all DNOM characteristics (i.e., using data on the water quality of re-dissolved RO isolates (26), DNOM composition (17) and -structure (63), and the DNOM functionality: i.e., biological- (3), chemical- (11) and physical (14)) gave principal components with moderate explanatory value (i.e., the first and second principal component (PC1 and PC2) explained only 27.3 and 19.8% of the variance in the dataset, respectively. The sample scores of PC1 was positively correlated to the total S-deposition (average deposition from 1989 to 1999; $r = 0.86$) and negatively correlated to the amount of DNOM as expressed by the absorbency at 600 nm, 400 nm, 254 nm and TOC ($r = -0.81$, -0.79, -0.76 and -0.74, respectively). PC2 was strongly correlated to inorganic water quality, as expressed by the K^+, H^+ and Ca^{2+} concentrations ($r = 0.84$, -0.77 and 0.74, respectively).

Many of the parameters with high loadings along the main principal components describing the variation in DNOM were not influenced by the site characteristics of their origin. Furthermore, many of the DNOM descriptors have low explanatory value and/or are inherently interrelated. It was therefore apparent that the PCA analysis could be improved by pre-screening parameters. Since the focus of this paper is to identify key site descriptors and water chemical parameters governing the characteristics of DNOM, we conducted first a correlation analysis to identify DNOM descriptors that appear to be most empirically explained by the site characteristics. Based on the correlation analysis, the explanatory value of the

parameter as described in the results chapter, as well as possible conceptual relation with the site characteristic, a selection of 21 DNOM descriptors was then made for more detailed study by PCA.

Correlation analysis and conceptual reasoning

The correlation (Pearson) coefficient matrix (MiniTab®, 1999) of all parameters gave several strong correlations between the 134 DNOM descriptors and 50 site characteristics (i.e., 30 catchment descriptors + 20 water chemical parameters). Generating close to $3.35 \cdot 10^3$ correlations, one is bound to produce several that are fortuitous. Furthermore, some of these correlations were uncertain due to a single data point relative to a cluster of values. Therefore, only those significant correlations (i.e., $r > 0.8$; $p < 0.005$) that can be considered to be due to real empirical relationships between DNOM parameters and explanatory site characteristics (Table 3) are further discussed. Some of the correlations may still have occurred by chance due to the limited number (N = 10) of samples. Some of the correlations may also be due to co-variation with a third parameter. The latter is especially the case regarding site characteristics, as many of these parameters are inherently inter-correlated (e.g., lake/watershed ratio vs. relative Histosols coverage). We therefore consider such correlations to only imply possible conceptual links between the empirically correlated parameters.

The correlation analysis also suggests that S-deposition (Table 1) has great explanatory value on the variation of several DNOM characteristics (Table 3). Bearing in mind the present regional decrease in S-deposition, it is interesting to note that, according to the correlations, the DNOM become better carriers of PAH (e.g., increased partitioning coefficient for pyrene; see also Akkanen et al., this volume), have larger molecular weight (Mw) and become more coloured (i.e., decreased SAR). The latter relationship may have relevance to the greater increase in colour than in TOC that has been observed over the last decades in many freshwater systems in Norway (see Liltved et al., 2000).

The increased ability to absorb organic micro pollutants with decreasing S-deposition may be explained by the observed negative correlation between Mw and S-deposition, as larger molecules are more lipophilic. The observed negative correlation between Mw and S-deposition may be conceptually warranted because of decreased ionic strength and mineral acidity. Less ionic strength allows for more open structure of the humic colloids (i.e., increased double layer) and less mineral acidity causes less protonization of the weak organic acids (e.g., R-COOH) and less complexation of mobilized aluminium, rendering a relative higher charge density and, thereby, less hydrophobic nature of DNOM. This inference is also supported by a positive (although not significant) correlation between amount of condensed material (Cond. mat.; Table 2), as found by FES at pH 2, and the acidity of the water (H^+, Table 1; $r = 0.62$). Larger molecules also allow for longer chains of conjugated double bonds and, thereby, explain the greater ability to absorb light with longer wavelengths, i.e., decreased SAR. It is noted that total carboxylic acid density (Tot-COOH; Table 2) increased with decreasing S-deposition. The conceptual reasoning for this result may be that in regions with high S-deposition and pH below 5 the carboxylate ion either becomes protonated ($pK_a \sim 4.1$) or is neutralized by complex binding to aluminium. DNOM material with relatively low contribution of carboxylic charge then becomes correspondingly less mobile than in regions with higher pH and less strong mineral acidity (i.e., less aluminium available for complexation). Gjessing (1990) also showed that the quality of DNOM from an acidified area differed from that of a non-acidified area by a higher content of organic sulphur. Likewise, the strong negative correlation between total S-deposition and the C:N ratio (Table 3) may be explained by co-variation between S and N anthropogenic deposition.

The amount of aluminium bound to DNOM (Al_o) was positively correlated to the forest coverage relative to peat land and lake surface (Table 3). The likely explanation for this is that conifers preferably grow on mineral soils, which are podzolic and leach DNOM rich in Al from the eluvial horizon. Sachse et al. (2001) studied the impact of land-use characteristics on DOC characteristics in temperate climates. They found that high-molecular weight and high aromaticity of the humic acid fraction are typical features of peat-influenced waters. They also found that humic substances in forest spring water are high in aromaticity, being attributed to the outwash of pedogenic DNOM from the upper soil horizons.

Table 3. Attention-grabbing strong ($r > 0.8$) real empirical relationships involving key site characteristics and DNOM descriptors. Parameter abbreviations are explained in Table 1 and 2.

Site characteristics	DNOM descriptor	r	p
	Tot – COOH	–0.877	0.001
Total	SAR	0.859	0.001
S-deposition	Mw	–0.840	0.002
	C:N	–0.840	0.002
	K_{Pyrene}	–0.834	0.003
Avg. temperature	Total acidity	–0.817	0.004
Biotope coverage			
% forest	Al_o	0.874	0.001
% lake	O-H; C-O	0.827	0.003
% histosol	O-H; C-O	0.839	0.002
Surface water chemistry			
TOC	Mw/radii	0.806	0.005
Al_a	Phenol	0.925	0.000

Using multivariate statistics on a similar data set of RO isolates from sites in southern Norway, Oug et al. (2003) found DOC concentration to be the key parameter explaining most of the variation in DNOM characteristics. Even variations in qualitative parameters like specific optical property (i.e., sUVa and SAR) were to a large extent empirically explained by the variation in DOC concentrations (see Oug et al., 2003). Despite a high correlation to PC1, as stated above, we found that, except for the expected correlations to the optical density at 254, 400 and 600 nm, TOC concentrations were significantly correlated only to the density (Mw/radii) of DNOM colloids (Table 3). Nevertheless, this result indicates that the physico-chemical properties of DNOM were influenced by the concentration of TOC and suggests a possible mechanistic explanation for the correlations between DNOM concentration and sUVa and charge density found by Gadmar et al. (unpublished).

The TLS was the most site-specific DNOM descriptor, as there was relatively little difference between the spring and fall samples (e.g., phenolic content and ratio of phenolic over highly conjugated aromatic compounds in Table 2). The phenolic peak was correlated with monomeric aluminium concentration in the original water sample (Table 3). Such an increase in the phenolic peak intensity with increased Al is characteristic for the formation of Al complexes with DNOM (Luster et al., 1996). Blaser et al. (1999) demonstrated a positive correlation between the phenolic/conjugated aromatic peak ratio in the TLS and the residence time of DNOM in the water. We also found such a correlation ($r = 0.98$) when omitting the samples from Birkenes, which had very high Al concentrations and, therefore, give a high intensity of the phenolic peak. Blaser et al. (1999) interpreted this correlation as a decrease in more highly conjugated aromatic compounds with increasing residence time in the water, either by precipitation or by degradation to simple phenolics. The latter is supported by strong positive correlations between the relative lake coverage (as well as Histosols) with the amount of phenolic and carboxylate groups (Table 3), as seen in the FTIR spectra.

The biological parameter POD was only significantly correlated to amount of precipitation and the H^+ concentration of the water ($r = -0.81$ for both). The negative correlation with acidity suggests that the macrophyte is less stressed by DNOM in acid waters. The PS was not significantly correlated to any of the catchment characteristics, but it did show a corresponding weak positive correlation with H^+ concentration and the precipitation amount ($r = 0.54$ and 0.66, respectively). The PS was also somewhat correlated to the day of year (i.e., season; $r = -0.68$), implying that the photosynthesis of macrophytes may be more strongly reduced when exposed to fall DNOM material than when exposed to spring material. No direct correlation was found between the biological response parameters and the relative Histosol coverage of the sites.

Principal component analysis

Based on the above correlation analysis, the information content of the 21 DNOM descriptors presented in Table 2 were selected for further investigation by PCA (Fig. 2). Data for humic substances (HS) were excluded due to missing data. The main goal of the PCA was to extract general groupings and trends present in the DNOM descriptive data and to quantify the significance of variables that explain the observed groupings and patterns in the inherent properties of the individual DNOMs. The parameter loadings and sample scores on the first two (most significant) principal components (PC1 and PC2) are plotted in Fig. 2.

There are several interesting features that may be recognized in Fig. 2. The Birkenes site (B), with a high sulphur deposition, had very strong sample scores along PC1. DNOM at the Birkenes site is characterized by small simple DNOM molecules with low degree of aromaticity. The pristine and TOC rich Svartberget and Skjervatjern sites (Sv and S), having negative sample scores, possess DNOM that are characterized by high molecular weight (Mw) with high content of carboxylic groups (Tot-COOH), low C/N ratio and a large capacity to sorb organic micro pollutants as PAH (i.e., large partitioning coefficient for e.g., Benzo-A-Pyrene; $K_{B[a]P}$). The Hietajärvi site (H), with a continental cold climate, high Histosol coverage and high pH, had, on average, a rather strong negative loading along PC2. Its DNOM was characterized by less condense material with low degree of humification and rich in HPO-A. Finally, the Valkea-Kotinen site (V) had mainly low sample scores along the first three principal components, suggesting a rather intermediate DNOM quality, which fits well with its rather intermediate site characteristics.

The first principal component (PC1), explaining 41.4% of the variation in the studied data sub-set, separates DNOM with high molecular weight, site density, colour and aromaticity from material with opposite characteristics and more phenolic character as well as high fluorescence efficiency (SFE). Correlations between the sample scores along the principal components and site characteristics show that PC1 is strongly related to S-deposition (Table 4). Other acid rain related parameters as inorganic monomeric aluminium ($Al_i = Al_a - Al_o$) and nitrate concentration in the water were also strongly correlated ($r = 0.832$ and 0.75, respectively) to PC1 scores. The biological parameters PS and POD had only minor loading along PC1, suggesting that the effect of S-deposition on DNOM does not influence its biological response.

The second principal component (PC2), explaining another 20.7% of the variation, separated mainly hy-

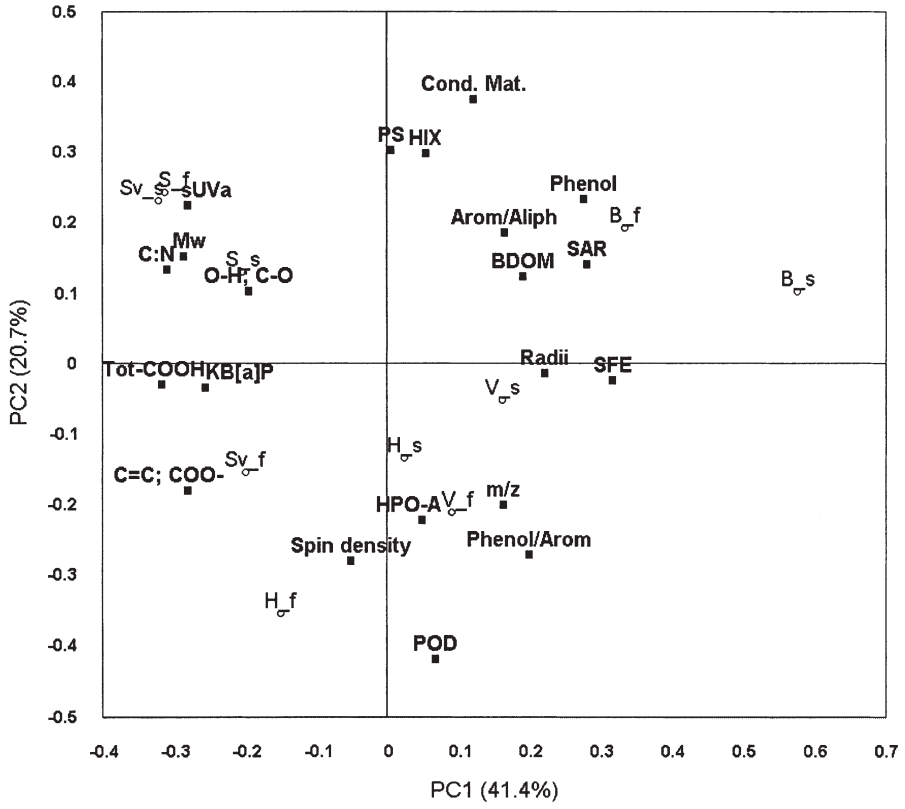


Figure 2. The DNOM parameter loading (black square ■) and sample scores (white circle **o**) along the two main principal components (PC1 and PC2). Sample and parameter abbreviations are explained in Table 1 and 2, respectively.

drophobic material with high spin densities from more condensed and humified material (Table 2). This gradient appears to have a strong influence by the biological response with a strong loading of PS and POD, possibly related to the high spin density of this material. This component is correlated to the acidity of the surface water as well as the length of growing season (Table 4), but not the relative Histosol coverage of the sites. Perpendicular to the PC1 and PC2 plane, PC3 (not shown) explained a further 12% of the variance and was strongly loaded by high biodegradability (BDOM) on the one side and material with large radii and with high content of O-H and C-O bonds on the other. PC3 scores were most strongly correlated to the elevation of the sites (Table 4). Oug et al. (2003) also found site elevation to be an important parameter correlated to the quality of DNOM. The fourth PCA component (PC4), which explained only an additional 9% of the variation, had a strong loading for spin density on the one side and loading of the humification index (HIX) on the other. The sampling day of year was strongly correlated to PC4 (Table 4). In accordance with this finding the spring and fall samples from each site received rather similar scores along PC1, PC2 (Fig. 2) and PC3, but not PC4. The main exception is the spring and fall samples from Svartberget, showing opposite loadings along PC2. This result is likely due to the minor discharge episode that occurred during the sampling of the spring sample.

Table 4. Key site characteristics explaining the main principal components (PC) of a 21-dimentinal data matrix on the bio-physicochemical characteristics of DNOM. Parameter abbreviations are explained in Table 1 and 2.

Principal components	Site characteristics	r	p
PC1	Total S-deposition	−0.946	0.000
PC2	H^+	0.933	0.000
	Growing season	0.825	0.003
PC3	Site elevation	−0.826	0.003
PC4	Day of year	−0.713	0.021

A PCA has previously been conducted on a combined dataset of 8 RO isolates from a study by Gjessing et al. (1999; i.e., NOM-typing) with these 10 RO isolates (Vogt et al., 2002b). The NOM-typing isolates also contained seasonal samples from sites as well as additional samples from Birkenes and Skjervatjern. This study showed the same clustering of sites in the PC1 vs. PC2 plane. Clustering of the sites suggests that the DNOM characteristics are site specific and that the effect of climatic variation between seasons is small. Our isolates were collected during relatively low base flow conditions. Likely large

fluctuations in DNOM characteristics during high flow episodes, as indicated by the effect of a minor episode captured in Svartberget, were therefore not found in this study.

Hasse diagram technique

The Hasse diagram technique is a specific method of partial order theory. Partial order theory is considered as a growing branch of discrete mathematics (Brüggemann et al., 2001). The main purpose is to identify order among a set of objects. There are many possibilities to define order relations. Here the simplest one is considered, namely the parameterwise order: two objects, x and y, are comparable (here we assume $x < y$) if all parameters of object x have lower values than those of object y. This simple method allows analyzing a set of objects with respect to main data structures and supplies a multivariate method based on variance or distance based considerations. The Hasse diagrams are read from bottom to top, as the value of a parameter (attribute) increases in this direction. Circles linked to each other arise from each other with the analyzed criteria; those on the same level next to each other have the same intensity, but are not comparable to each other in the marked attributes.

To exemplify the procedure, we combined selected classified environmental data (Al_a and % Histosol coverage; Table 1), NOM characteristic (HIX; Table 2), and effect data (PS; Table 2). The resulting Hasse diagram (Fig. 3) shows that the fall samples from Svartberget (Sv_f), Valkea-Kotinen (V_f) and Hietajärvi (H_f) lay below the corresponding spring samples. This means that all parameters of the fall samples have simultaneously lower values than those of spring samples. The Skjervatjern spring (S_s) sample, which is equivalent to Hietajärvi spring (H_s) sample, however, is lower than the fall sample from Skjervatjern (S_f). The fall and spring samples form Birkenes (B_f and B_s, respectively) are not comparable due to the antagonism in the first two parameters, i.e., HIX and macrophyte response. There are three branches stemming from the fall material from Svartberget. Each branch has a top-site. The left branch with fall material from Birkenes can be classified as: high HIX, low macrophyte response, high Al_a and low Histosol coverage. The top element of middle branch (Fall material from Skjervatjern) is characterized by: high HIX, high macrophyte response, low Al_a, and high Histosol coverage. All elements of this branch have one feature in common: they have very low Al concentrations. In addition, the elements of this branch are the only ones that have medium to high Histosol cover values. The right branch, with the top element being the spring material from Birkenes, is characterized by simultaneously low values of HIX and Histosol cover. High macrophyte response occurs only if the Histosol cover is above the medium range. This result was expected and we hypothesize that Histosols produce and release DNOM with high concentration of quinons, which have the potential to interfere with the photosynthesis of macrophytes.

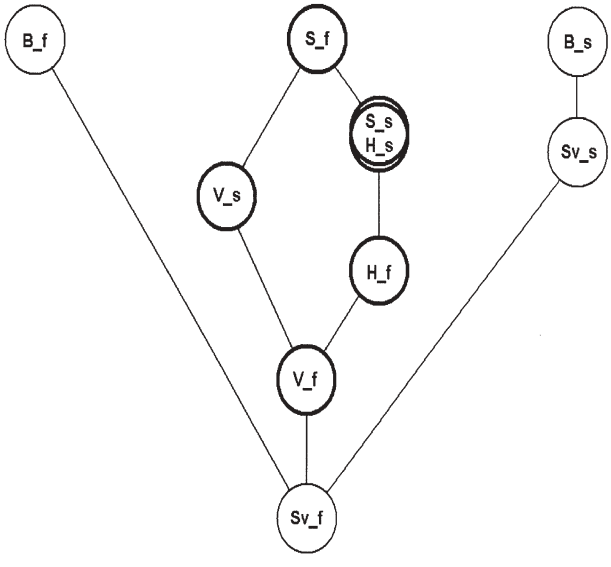


Figure 3. Hasse diagram, based on 5 sites, two sampling campaigns (spring and fall), and four classified parameters [humification index (HIX), inhibition of macrophyte photosynthetic oxygen production (PS; macrophyte response), aluminium concentration in surface waters (Al_a) and the percentage of Histosol in the soil (Histosol)]. Sample abbreviations are explained in Table 1.

A large number of parameters were available and the Hasse diagram presented only a small selection of all possible combinations. We intend in a future publication to show how Hasse diagrams and other more advanced methods can be used to derive hypotheses regarding which environmental features determine humic substance quality, and, in turn, how these determine biological effects.

Conclusions

Several clear environmental gradients existed among the 5 studied sites; e.g., from surface water dominated by organic to mineral acidity, from sites with continental climate with a short growing season to marine climate with a long growing season, and from sites consisting mainly of forest to sites with large lakes and extensive mires. Comparison of DNOM characteristics from a pristine and organic rich site (Svartberget) with DNOM characteristics from a similar site but exposed to acid rain (Birkenes) showed a number of distinct differences. The spectroscopic parameters (TLS, TFS, FTIR, sUVa, SAR) indicated more condensed and conjugated aromatic compounds at Svartberget relative to simple aliphatic compounds at Birkenes. A more condensed material at Svartberget was also supported by the results from capillary analytical methods (HPSEC, CZE, CGE), which showed that the highest

molecular weights and lowest molecular sizes were at Svartberget. Another distinct difference in DNOM was in the carboxylic and total acidity with high site density at Svartberget. These differences were promoted during elevated discharge at Svartberget and prolonged dry conditions at Birkenes.

The PCA on selected parameters gave high negative sample scores to the anthropogenic acidified Birkenes site along the main principal component (PC1). This component, which explained 41% of the variation in the data, was strongly related to S-deposition. Other important explanatory key variables were also H^+ activity in solution and absorbency at 600 nm, followed closely by TOC. The PCA showed that the bio-physico-chemical characteristics were strongly site specific and varied less between the beginning and end of the growing season. The Hasse diagram technique revealed that high Histosol coverage in the watershed coincided with a strong macrophyte response.

These empirical correlations, as well as conceptual reasoning, suggest that DNOM becomes more coloured and has greater capacity to sorb organic micro-pollutants due to an increased molecular weight as the load of S-deposition decreases.

Acknowledgments

Financial support from NordTest is greatly appreciated. The German Israeli Foundation is thanked as the development of the electrospray ionization interface was possible within *GIF Young Scientist grant* N°1084-307.2/2000. The TSK Gel Column SW 2000 used in HPSEC was a gift from TOSOH-Bioscience Stuttgart, Germany. Mrs H. Neumeier, B. Look and A. Wust from GSF are thanked for their helpful assistance. The authors are grateful to Drs. Per Olav Kvernberg and Olve Frederichsen (UiO), Arja Tervahauta and Veikko Kitunen (METLA) for laboratory assistance; Lars Evje and Helene Thorsen (UiO), as well as Noureddine Hajjar and Alois Zürcher (WSL) for field work and laboratory assistance; Kevin Bishop (SLU) for field work and Profs. Teodoro Miano, Hans Martin Seip, anonymous reviewers and subject editor Lars Tranvik for valuable comments.

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