

Aquatic chemistry and humic substances in bog lakes in Latvia

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Chemical composition of 19 bog lakes in Latvia has been studied. Peculiarities of chemical composition and their influence on community structure have been analysed as the main factors considering aquatic humic substances (AHS). AHS isolated from bog waters shows principally different elemental, functional and spectral characteristics, and also acidity, from humic substances isolated from other water bodies (eutrophic and mesotrophic) and reference humic samples. The observed differences are due to the dependence of humus properties on biological processes and pollution levels in the water bodies.

Introduction

Dystrophic lakes (commonly found in bog areas) in many of the lake classification systems are considered as the final stage of lake development (Wetzel 1983), they are common in northern countries, but many of their properties are not adequately studied. At the same time bog lakes are of interest considering existence of specific communities in conditions of comparatively high acidity, high concentrations of organic substances and low pollution levels (Gorniak *et al.* 1999). Bog lakes are traditionally viewed as unproductive and numbers of bacteria, mass of plankton and other living organisms in them are substantially lower than in other lake types (Druvietis 1992, Druvietis *et al.* 1998). Studies of factors controlling biological diversity and communities are of utmost importance in limnological investigations. At first, bog lakes are characterized by the presence of humic substances, that determine

the common dark brown color of waters of bog lakes, acidity, light and nutrient availability to biota (Gjessing 1976). It is considered that primary production is depressed due to formation of complexes between humic substances, nutrient ions (especially phosphates and iron) (Kullberg *et al.* 1993). Of substantial importance are bacterial communities able to use humic substances (commonly considered as refractory and highly resistant to degradation) as carbon source for their development (Jackson and Hecky 1980). Phytoplankton biomass and productivity in bog lakes are generally low and frequently below the levels observed in lakes with comparable phosphorus loading (Heyman 1983). The typical feature of bog lakes is a high zooplankton:phytoplankton biomass (Hessen *et al.* 1989). Even though biology of bog lakes has been comparatively widely studied, there are only a few studies dedicated to aquatic chemistry of bog lakes, especially considering natural organic matter (Keskitalo and

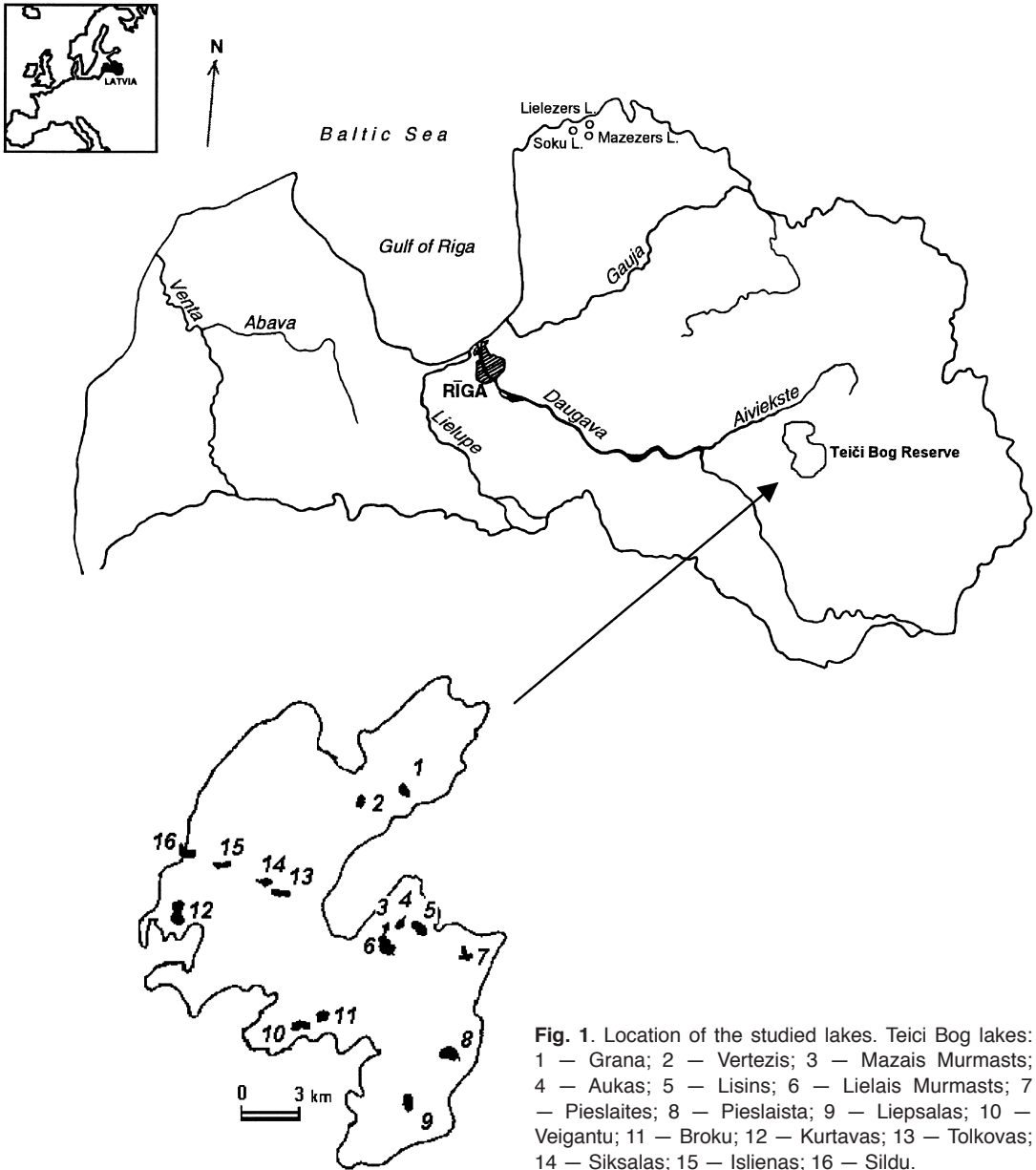


Fig. 1. Location of the studied lakes. Teiči Bog lakes: 1 – Grana; 2 – Vertezis; 3 – Mazais Murmasts; 4 – Aukas; 5 – Lisins; 6 – Lielais Murmasts; 7 – Pieslaites; 8 – Pieslaista; 9 – Liepsalas; 10 – Veigantu; 11 – Broku; 12 – Kurtavas; 13 – Tolkovas; 14 – Siksalas; 15 – Islienas; 16 – Sildu.

Eloranta 1999). The bulk of organic matter (60%–85%) in surface waters derived from natural sources consists of humic substances (Thurman 1985), and their concentrations in bog waters can be very high. Bog water humic substances were first isolated in 1836 by Berzelius, but major questions on bog water humus structure, properties, sources and sinks in environment, are still not answered.

The aim of the present study is to compare

aquatic chemistry of bog lakes in Latvia, and study the properties of humic substances isolated from their waters.

Materials and methods

Bog lakes in Latvia (Fig. 1) situated in protected areas were studied. Most of the studied lakes (16) are situated in the Teiči State Bog Reserve,

located on moraine hillocks in the East Latvian Lowland and covering an area of 19 587 ha. The remaining three are situated in North Vidzeme Biosphere reserve. Development of wetlands is attributed to the Sub Arctic period 9000–10 000 years B.C. when mineral depressions filled with melted water started to overgrow with vegetation. Genetically investigated lakes are formed from ancient lakes in which more intensive overgrowing occurred 7500–9000 years ago. The fact is proved by 0.5 m thick layer of sapropel or gyttja just on mineral soils — sand and sandy clay.

The bogs are feeding mainly by atmospheric precipitation and runoff is linked with waterproof features of active horizon. The thickness of the active water exchange horizon varies from 25 to 60 cm.

Twelve of the investigated lakes — Aukas, Broku, Grana, Lielais Murmasts, Liepsalas, Mazais Murmasts, Pieslaista, Pieslaistes, Tolkaja, Veigantu, Vertezis (Fig. 1) — are situated in the part of oligotrophic raised bog with typical feeding from atmospheric precipitation. The rest of the lakes — Islienas, Kurtavas, Lisins, Siksals, Sildu, Akacis, Soku — are located mainly at the marginal part of the bog which is characterized as mesotrophic transition fen. Shorelines of those lakes in some places are formed by mineral soils or wooded elevation. In most cases the size of lake does not exceed 50 ha. The largest of them — Kurtava, Pieslaista, Liepsalas, and Lisins — are situated in the marginal part of Teici Bog. The shape of those lakes are mostly round with an even and smooth shore line. The majority of investigated lakes are related to the group of water bodies with a small surface area. In general terms the bog lakes can be characterized as shallow, with average depth up to 2 m. Only a few of the lakes are characterized as relatively deep, with maximum depth of 6–7.8 m (Veigantu, Siksals, Tolkovas).

Water samples (3l) were taken in the central part of each lake from 0.5 m and 2 m depth. Chemical analyses of water composition were conducted using Standard methods (1992). The factors analysed included pH, conductivity color, basic inorganic ingredients, nutrients, COD. Concentration of humic substances were determined spectrophotometrically after enrichment

on sorbents (Standard methods 1992). Studies of communities in bog areas are conducted as indicated earlier (Druvietis *et al.* 1998). Phytoplankton samples were collected by Ruttner type water sampler in pelagial and littoral zones. Samples for quantitative analyses were immediately fixed with aqueous formaldehyde (40 ml l⁻¹). The biomasses of phytoplankton species were determined by counting their numbers and measuring their cell volumes. The algal volume was converted to biomass by assuming the specific density of the cells to be equal to that of water. Live samples used for qualitative species identification were taken with a 10 µm plankton net by vertical hauls through the water column. Cell numbers were determined by Zeiss microscope using magnifications of 100×, 200×, 400×, 600×. Zooplankton samples were collected with a conical net (mesh 64 µm). It was used to sample 100 l of lake water for zooplankton in each station. Samples were preserved with formaline to final concentration of formaldehyde 4%. The calculation for density of invertebrates was obtained by direct count method using a Zeiss microscope.

For isolation of humic substances a modified (Thurman and Malcolm 1981, Klavins 1991) XAD method was used. The obtained fulvic acid solutions were transferred to the H⁺ form and freeze dried, but the humic acid fraction was desalted by repeated washing with Milli-Q water. For comparison, Nordic Reference HA and FA samples were used.

The obtained humic substances were characterized as follows:

- elemental analysis: C, H, N and ash were determined by a Perkin Elmer 240/A CHN-Analyzer. Oxygen content was calculated by difference;
- content of functional groups (–COOH, –OH) was determined by methods suggested for analysis of soil humic substances (Methods of Soil Analysis 1989);
- molecular weight distribution was determined by gel filtration on a Sephadex G100 (bead diameter 40–120 µm) column (1 × 40 cm). The eluent, 0.001 mol l⁻¹ Na₂P₄O₇, was pumped through the column at 1 ml min⁻¹, gathering 0.5 ml fractions. A standard set of proteins was used for column

calibration. The void volume of the column was determined using Blue Dextran 20000. Detection was performed at 280 nm;

- UV-Vis spectra were obtained over a range of 200 to 700 nm using a Specord UV 40 UV-Vis spectrophotometer on 0.05 N NaHCO₃ solutions at a concentration of 50 mg l⁻¹ for FA and 33 mg l⁻¹ for HA and a pH between 8 and 9;
- IR spectra were recorded in the 4000 to 500 cm⁻¹ wavenumber range using Perkin Elmer 400 IR spectrophotometer on KBr pellets obtained by pressing mixtures of 1 mg samples and 400 mg KBr, with precaution taken to avoid moisture uptake.

Results and discussion

All studied bog lakes are situated in remote areas with minimal human impact.

The hydrochemical parameters in waters of the sampling stations are briefly described and summarized in Table 1. The waters of lakes have low TDS (< 100 mg l⁻¹) and they are soft with total hardness not exceeding 1 meq l⁻¹. Common ranges of inorganic ion concentrations are: Mg²⁺

1.2–10 mg l⁻¹; SO₄²⁻ 5–18 mg l⁻¹; Cl⁻ 2.2–6 mg l⁻¹; HCO₃⁻ ~1–100 mg l⁻¹. Studied bog lakes in North Vidzeme are slightly more mineralized. Bog lakes depending on their aquatic chemistry can be split into two groups, as indicated in previous studies of bog waters — dystrophic and dyseutrophic.

The dyseutrophic lakes can be characterised by a relatively high mean concentration and large variability of the main water parameters. (Fig. 2). The typical dystrophic lakes have relatively low mean values of pH, conductivity and a small variability of these parameters seasonally and annually. Common for studied lakes are high COD and color values, determined by presence of humic substances (Fig. 3). This determines high consumption of oxygen and common anoxic conditions in the bottom water layers. If the oxygen depletion in summertime commonly takes place below 2 m depth then under ice in wintertime there are anoxic conditions. At the same time high concentrations of humic substances determines speciation of nutrients, as can be seen from correlation between N_{tot} and concentration of humic substances (Fig. 3), indicating that major part of nitrogen compounds are

Table 1. Water composition in the studied bog lakes (summer).

Lake	pH	Conductivity ($\mu\text{S cm}^{-1}$)	APHA (Pt/Co color)	Humic subst. (mg l ⁻¹)	N _{tot} (mg l ⁻¹)	PO ₄ ³⁻ (mg l ⁻¹)
Aukas	3.8–3.9	36–49	140–172	–	0.5–2.3	0.02–0.1
Broku	4.1–4.4	36–49	69–75	43.3–53.6	0.8–2.6	0.008–0.012
Grana	3.8–4.1	27–47	170–205	–	1.1–1.9	0.011–0.016
Islienas	4.4–7.8	65–156	190–550	61.7–150.2	0.9–6.3	0.004–0.04
Kurtavas	4.7–6.3	27–38	117–259	58.2–92.7	0.8–3.4	0.004–0.119
L Murmasts	3.7–3.9	55–58	166–351	81.2–92.7	1.0–1.7	0.01–0.023
Lielezers	4.8–7.0	20–66	163–228	–	1.8–3.4	0.015–0.124
Liepsalas	4.1–4.3	31–44	20–23	23.7–26.6	0.7–1.3	0.011–0.021
Lisins	4.1–5.1	35–175	216–228	45.6–78.9	1.1–9.0	0.011–0.032
M. Murmasts*	3.7	56	140	–	1.4	0.01
Mazezers	4.9–6.8	20–253	225–259	–	2.8–3.6	0.01–0.026
Pieslaista	4.0–4.1	40–42	160–170	62.8–97.3	0.2–1.4	0.008–0.022
Pieslaistes	4.0–4.2	29–47	124–131	66.3–115.7	0.6–2.2	0.033–0.05
Siksalas	3.7–6.6	29–44	196–244	50.2–62.8	0.6–2.7	0.02–0.04
Sildu	5.8–8.1	120–196	300–475	76.6–142.1	1.0–5.7	0.04–0.109
Soku	4.5–5.5	20–195	124–147	–	1.4–2.5	0.014–0.055
Tolkovas	4.2–6.1	22–32	21–47	32.9–52.5	0.7–1.4	0.008–0.047
Veigantu	3.7–4.0	39–45	116–225	42.1–72.0	0.9–5.0	0.008–0.149
Vertezis*	5.2	32	30	–	0.5	0.009

* = mean.

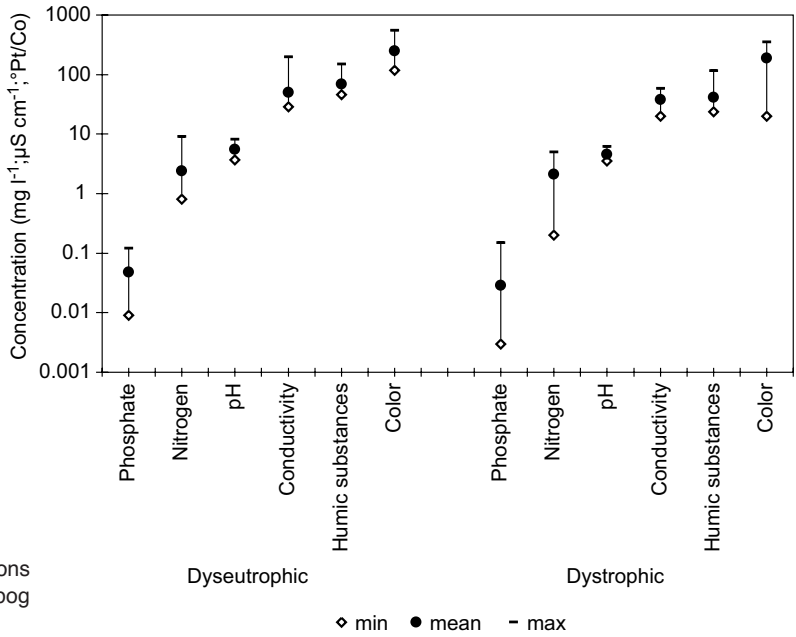


Fig. 2. Ranges of concentrations of dissolved substances in bog lakes in Latvia.

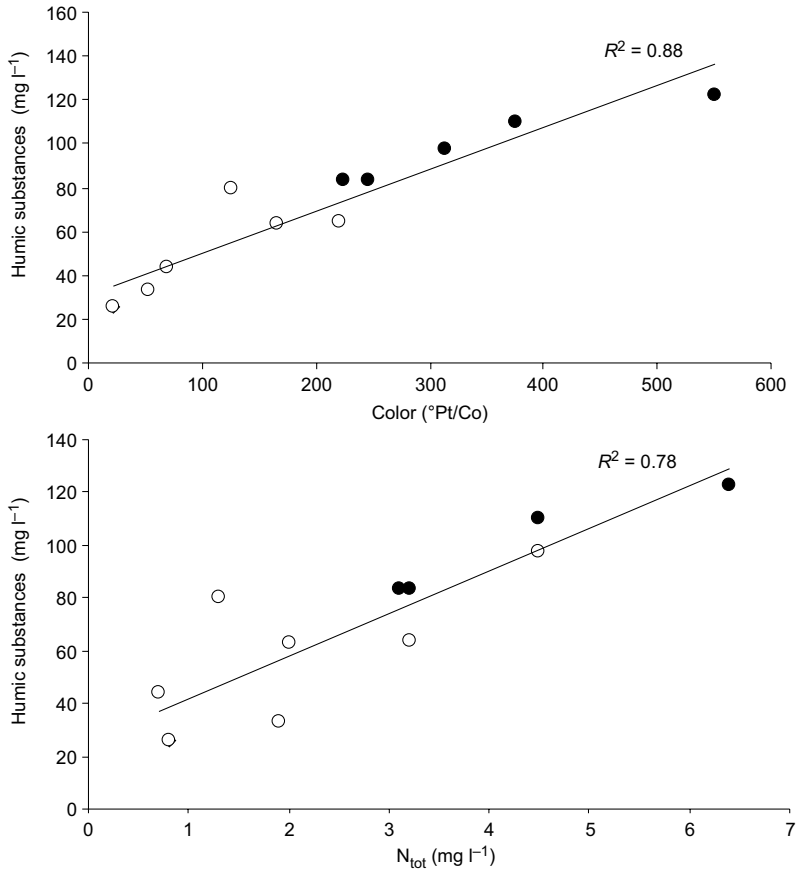


Fig. 3. Relationship between concentrations of humic substances and total nitrogen in waters of bog lakes in Latvia. ○ — dystrophic lake (Broku, Liepsalas, L. Murmasts, Pieslaista, Pieslaistes, Tolkovas, Veigantu); ● — dysetrophic lake (Islienas, Kurtavas, Lisins, Siksalsas, Sildu).

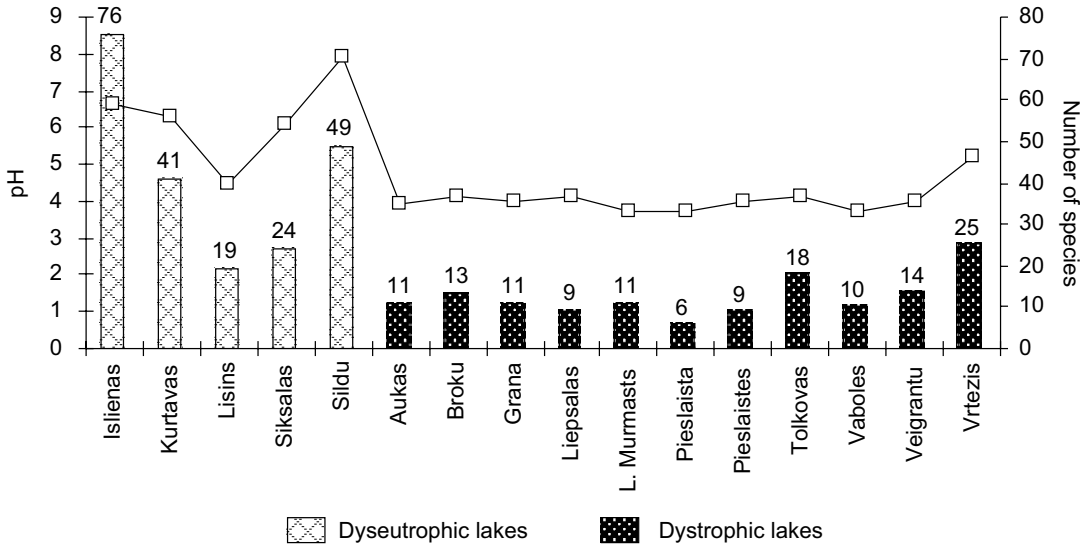


Fig. 4. Relationship between number of algae species and pH in the studied lakes.

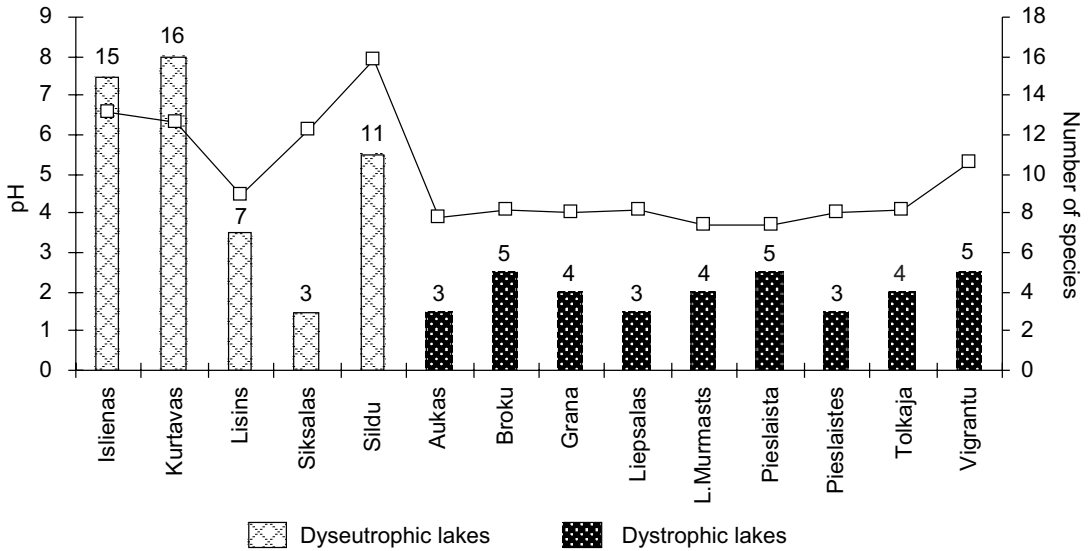


Fig. 5. Relationship between number of zooplankton species and pH in the studied lakes.

associated with humic substances.

Since humic substances in natural waters influence nutrient availability, pH and oxygen regime, and limit light availability, they have a major impact on community structure (Klavins 1998). The dominant phytoplankton species in brown-water lakes in Latvia are (in the vernal period) *Dinobryon* spp., (*Tabellaria* spp., Flagellates in the summer period), *Mallomonas acaroides*, *Botryococcus braunii* and *Oocystis*

spp. The dominant zooplankton communities in bog lakes can be briefly characterised by low numbers of true limnetic species (*Diaphanosoma brachyurum*, *Ceriodaphnia quadrangula*, *Bosmina coregoni obtusirostris*) and the occurrence of littoral or benthic and phytoplanktonic species in the limnetic samples (*Acroperus harpae*, *Alona gutatta*, *Alonopsis elongata*, *Polynemus pediculus* etc.). Only one species is dominant — *Diaphanosoma brachyurum* or *Bosmina core-*

goni obtusirostris (Urtane and Klavins 1995). The abundances of benthic species decrease in the following order: *Chironomidae* > *Trichoptera* > *Odonata* > *Coleoptera*. Brown water lakes have simplified community structure (benthic communities commonly have only 4–9 species) and reduced biodiversity. Humus content in water was found not to be correlated with community structure in Sweden (Bērziņš and Pejler 1987). This lack of effect of humus on community structure may be explained by compounding morphometric and chemical composition variability in the studied bogs.

At the same time the community structure of studied lakes, just as their chemical composition, can be split into 2 groups (dystrophic and dyseutrophic lakes), as can be seen from the dependence of found number of algae and zooplankton species on pH (Figs. 4 and 5). There is a clearly distinguished relationship in taxonomic composition of phytoplankton and state of lakes. Dystrophic lakes support very low number of species, low phytoplankton biomass (0.01–0.15 mg l⁻¹) and phytoplankton assemblage formed by *Dinobryon divergens*, *Mallomonas* spp., *Asterionella formosa*, *Botryococcus braunii*, *Euastrum* spp., *Staurastrum* spp., *Micrasterias* spp. Phytoplankton and periphyton are formed by *Mougeotia* sp., *Ulothrix* sp. and red algae *Batrachospermum* spp.

Dyseutrophic lakes are characterised by a comparatively higher number of species, higher phytoplankton biomass, higher numbers of chlorophyll a (2.3–6.25 mg m⁻³). Typical phytoplankton community is formed by *Aulacoseira italica*, *A. italica* var. *tenuissima*, *Dinobryon* spp., *Mallomonas* spp., *Asterionella formosa*, *Tabellaria flocculosa*, *T. fenestrata* var. *asterionelloides*.

Periphyton and phyto-benthos is dominated by *Spirogyra* sp., *Ulothrix* sp., *Mougeotia* sp. and *Oscillatoria* spp. Changes in phytoplankton quantity and quality showed definite seasonality in both types of lake (Table 2). The vernal growth was dominated by chrysophyceans, diatoms and flagellates. In early summer and late summer periods *Chrysophyceae* dominated, the most important being *Dinobryon* spp. and *Mallomonas* spp. Dystrophic lakes have a simple zooplankton species composition formed of Cladocera and Calanoida, but in dyseutrophic lakes zooplankton assemblage is formed by Cladocera, Rotatoria and Cyclopodia (Table 3).

So, the humic substances determine not only water color, low pH values but also influence aquatic biology in bog lakes.

Humic substances have been isolated from bog waters using known methods (Thurman and Malcolm 1981).

For comparison, humic substances from other typical water bodies (lakes Liepajas, Raznas, Rusonu) of Latvia were isolated using the same XAD method. The elemental composition, atomic ratios and ash content of HA and FA are presented in Table 4, together with the corresponding data from Nordic Reference samples. The fulvic and humic acids isolated from bog waters of Latvia had very similar elemental composition. Humic acids from bog waters typically have high carbon content (54%–58%) especially in comparison with carbon content in other lakes of Latvia (49%–51%). Carbon content in humic acids generally is higher than in fulvic acids. Humic acids are low in nitrogen and oxygen content, but in fulvic acids oxygen content typically is higher than in humic acids. The values of elemental composition obtained for humic acids

Table 2. Successional trends of phytoplankton of the dystrophic and dyseutrophic lakes.

Status of lakes	Vernal period	Early summer	Late summer
Dystrophic lakes	<i>Dinobryon divergens</i> , <i>Flagellates</i> <i>Tabellaria</i> spp. <i>Mallomonas</i> spp. <i>Euastrum</i> spp., <i>Staurastrum</i> spp., <i>Cosmarium</i> spp. <i>Micrasterias</i> spp. <i>Oocystis</i> spp., <i>Botryococcus</i> sp.		
Dyseutrophic lakes	<i>Asterionella formosa</i> , <i>Eudorina elegans</i> <i>Anabaena</i> spp. <i>Aulacoseira italica</i> , <i>Flagellates</i> <i>Peridinium bipes</i> <i>Fragillaria</i> spp., <i>Dinobryon</i> spp. <i>Gloeocapsa lacustris</i> <i>Asterionella formosa</i>		

from inland waters of Latvia are similar to those found for Nordic reference samples (obtained from marshwaters), but they differ from values found for other humic acids. For isolated humic

substances, the content of carboxyl groups and oxygen are significantly correlated. The unique elemental composition of humic substances from bog waters can be explained by high metabolic

Table 3. Biological characteristics of dystrophic and dyseutrophic lakes.

Biological features	Type of lake	
	Dystrophic	Dyseutrophic
Ground cover around the lakes	<i>Pinus silvestris</i> , <i>Betula pendula</i> , <i>B. nana</i> , <i>Ledum palustre</i> , <i>Vaccinium uliginosum</i> , <i>Calluna vulgaris</i> , <i>Rubus chamaemorus</i> , <i>Eriophorum</i> sp., <i>Drosera rotundifolia</i> , <i>Oxycoccus palustris</i> , <i>Chamaedaphne</i> sp., <i>Empetrum</i> sp., <i>Carex</i> spp., <i>Sphagnum</i> spp.	<i>Salix</i> sp., <i>Betula pendula</i> , <i>Pinus silvestris</i> , <i>Carex</i> spp., <i>Alnus incana</i>
Emergent macrophytes		<i>Menianthes trifoliata</i> , <i>Cicuta virosa</i> , <i>Calla palustris</i> , <i>Comarum palustre</i> , <i>Butomus umbellatus</i> , <i>Typha latifolia</i> , <i>Sparganium</i> sp., <i>Thelypteris palustris</i>
Floating macrophytes	<i>Nuphar lutea</i> , <i>Nymphaea candida</i>	<i>Nuphar lutea</i> , <i>Nymphaea candida</i> , <i>Stratiotes aloides</i> , <i>Hydrocharis morsus-ranae</i>
Submerged macrophytes		<i>Elodea canadensis</i> , <i>Potamogeton</i> sp.
Fish	<i>Perca fluviatilis</i>	<i>Perca fluviatilis</i> , <i>Esox lucius</i> , <i>Rutilus rutilus</i> , <i>Tinca tinca</i> , <i>Abramis brama</i>

Table 4. Elemental and functional composition and molecular weight distribution of humic substances from surface waters of Latvia. M_n = number average molecular weight, M_w = weight average molecular weight.

Humic substances	C (%)	H (%)	N (%)	O (%)	COOH (mmol g ⁻¹)	ArOH (mmol g ⁻¹)	M_n	M_w
FA-Akacis	54.13	3.68	1.15	39.61	3.75	1.24	1650	3450
HA-Akacis	57.38	3.94	1.61	36.34	3.18	1.46	2700	5450
FA-Sildu	55.48	3.75	1.07	38.12	3.88	1.22	1550	1800
HA-Sildu	57.21	3.94	1.03	37.11	3.65	1.42	2000	3800
FA-Aukas	54.60	3.70	0.93	39.16	3.78	1.15	1480	2150
FA-Grana	54.20	3.71	1.10	40.05	4.15	1.18	950	1340
HA-Tolkovas	54.33	4.23	0.96	37.63	3.85	1.25	3100	5750
FA-Tolkovas	51.02	4.72	0.87	41.90	5.32	1.21	1650	2800
FA-Vertezis	55.86	3.71	0.94	38.11	4.09	1.23	1150	1800
FA-Lielezers	56.14	3.93	1.18	36.32	3.72	1.22	1100	1650
FA-Lisins	58.34	4.72	2.64	32.67	3.46	1.38	860	920
HA-Islienias	57.18	4.12	0.63	36.83	3.75	1.64	1300	1920
FA-Islienias	56.41	3.85	0.87	37.12	4.17	1.43	1180	1350
FA-Pieslaistes	55.35	3.92	0.94	38.10	4.13	1.46	800	900
HA-Nordic Reference	55.20	4.12	1.04	39.02	4.12	1.21	4410	11190
FA-Nordic Reference	52.62	4.09	0.74	41.93	7.53	1.53	2180	3360
FA-Liepajas	49.38	4.22	0.61	45.34	5.78	1.36	1650	2700
FA-Rusonu	51.42	4.48	0.97	40.21	4.35	1.11	1800	3100
FA-Burtnieku	51.78	4.22	0.75	42.75	4.63	0.96	2200	3150
FA-Raznas	49.68	4.25	1.45	43.51	4.94	0.85	2040	2550

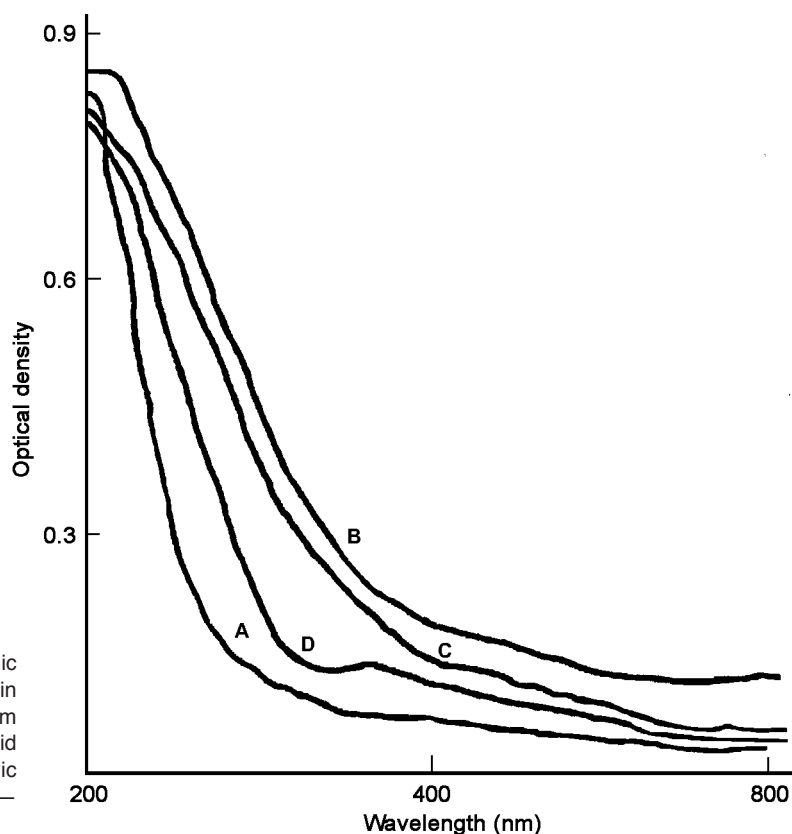


Fig. 6. UV-Vis spectra of humic substances from bog lakes in Latvia: 1 — humic acid from lake Islienas; 2 — humic acid from lake Akacis; 3 — fulvic acid from lake Islienas; 4 — humic acid from lake Raznas.

activity of groups of specialized microorganisms (Tranvik 1998).

The most important functional groups in the structure of aquatic humic substances are carboxyl and phenolic hydroxyl groups. If the content of carboxyl groups is higher in fulvic acids (3.5–6 mmol g⁻¹), then content of phenolic groups is higher in structure of humic substances (1.0–1.5 mmol g⁻¹). In humic substances from bog waters, the content of carboxyl groups is lower and of phenolic hydroxyl groups higher than in humic substances from other water bodies of Latvia and reference humic substances. The acidity of humic substances can be characterized not only by total content of acidic functional groups, but also their distribution and properties. The molecular weight distribution was characterized by number average molecular weight (M_n), weight average molecular weight (M_w) and their ratio (M_n/M_w) which characterizes the polydispersity of humic substances. According to the molecular masses, the humic substances can be arbitrarily divided depending on their type and

origin. The determined molecular masses of humic substances were always higher than those of fulvic acids and they are more heterogeneous. The degree of heterogeneity can be regarded as an estimate of humification degree (Klavins 1997). Also, humic substances from eutrophic and mesotrophic lakes are more heterogeneous and have higher molecular mass than humic substances from dystrophic waters.

The UV-Vis spectra of the FA and HA examined (Fig. 6) are featureless and monotonically decreasing with increasing wavelength with a more or less pronounced shoulder at about 270 nm exhibited by all HA. The slope of the adsorption curves, as measured by the ratios of absorbance at 465 and 665 nm, has been traditionally suggested to be inversely related to the amount of condensation of aromatic groups (aromaticity) and to particle size and molecular weight. This was found to be true also in our case. The differences in UV spectra of humic substances depend on their origin.

The IR spectra (Fig. 7) of the FA and HA

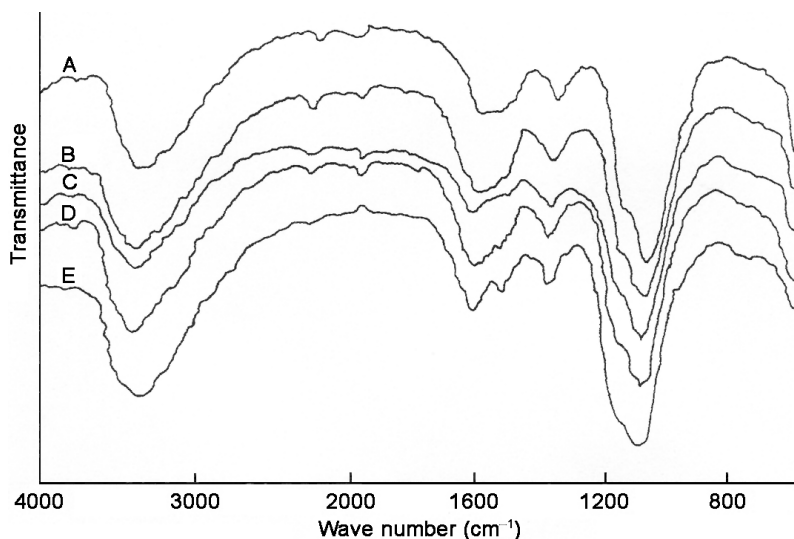


Fig. 7. Infrared spectra of humic substances from bog lakes in Latvia: 1 — humic acid from lake Islienas; 2 — humic acid from lake Akacis; 3 — fulvic acid from lake Islienas; 4 — humic acid from lake Kurtavas; 5 — humic acid from lake Raznas.

examined are similar in the principal positions of adsorption, but differences are apparent in the relative intensity of some bands, depending on the origin and nature of the sample. The IR spectra of the FA samples can be tentatively classified as type II spectra, while those of the HA samples resemble type I spectra.

All of the spectra are characterized by a strong band at 3390–3370 cm^{-1} (stretch of hydrogen bonded OH), which is relatively more intensive for HA than for FA of any origin, and broadens widely towards lower wavelengths (aromatic C–H stretching modes) for bog water FA. The presence of aliphatic groups is shown by a peak at 2920 cm^{-1} , with decreased intensity in humic substances from bogwaters in comparison with humic substances from other sources. The peak at 1618 cm^{-1} and broad band at 1400 cm^{-1} indicate the presence of C=C stretching of substituted aromatic rings. Intense sorption is observed at 1717 cm^{-1} and 1210 cm^{-1} indicating protonated carboxylic acids and ketonic C=O groups, C–O stretching vibration, and OH bending deformations of the COOH groups. Humic substances from bog waters do not show sorption common for carbohydrate residues.

Conclusion

The elemental, functional, mass-molecular and spectral characterization of bog humic sub-

stances indicates the major role their origin have on the properties. Humic substances from different water bodies principally differ in properties, indicating variable intensity of processes of biological transformation of organic matter.

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