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692. SĒJUMS

Zemes un vides zinātnes

Humusvielas un to loma vidē

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Comparison of the chemical composition of sediments in lakes in catchments forested with beech and pine

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The aim of this work was to determine the influence of the forest catchments management on the properties of lake sediments and qualitative parameters of formed humic acids. The results showed that the forest type occupying the lake catchments area can significantly influence the properties of humic acids formed in sediments as indicated by their spectrometric properties. The presence of pine forests in the catchment area leads to acidification of the basin and this negatively influences the retention of pigments in bottom sediments. However, the conditions prevailing in lakes surrounded by beech forests aid the retention of pigments of plant origin in sediments and their bounding to humic acids by ways of the diagenetic transformations.

Introduction

The major source of organic matter in sediments of lakes is from the detritus of single-celled phytoplankton that have lived in their photic zone. Detritus from land plants can be an important additional contribution to the organic matter in sediments of many lakes. The relative contribution from these two general sources of organic matter to sediments is strongly influenced by algal productivity, land-plant productivity and transport processes (*Meyers* 1997). Unlike organic matter of the algal origins, which is characterised by high protein contents and undergoes degradation already in the free fall stage in water, detritus of land origins is characterised by high content cellulose and lignin type compounds resistant to decomposition (*Reeves* 1995, *Hu et al.* 1999, *Meyers* 2003). Relevant to the conditions prevailing in a lake the matter deposited at the bottom undergoes various diagenetic transformations. Humic substances and pigments originating from algae and higher plants constitute all important components of organic matter in sediments. Diagenetic transformations, as well as the sources of organic matter influence the contents and type of pigments present in sediments i.e. the diagenetic transformation of the chlorophyll into pheophytin is stimulated by the acidic conditions, the oxygen conditions and light can stimulate the decomposition of pigments present in water, as well as in sediments, whilst the retention is aided by a high primary production (*Meyers and Ishiwatari* 1995, *Bianchi et al.* 2000, *Kowalewska and Szymczak* 2001). Sometimes there are absorption bands noted in humic acid spectra – usually monotonic – and their presence is associated with fragments of the pigment origins (*Ishiwatari* 1973, *Povoledo et al.* 1975). Such properties are characteristic to humic acids extracted from sediments in lakes with the mid-field catchment, which contain considerable quantities of carbonates. The presence of HAs (humic acids) with such properties was not found in sediments of mid-forest lakes, which are characterised by the lack of carbonates (*Cieslewicz* 1999). The aim of this work was to determine the influence of the forest catchment management on the properties of the sediments and the qualitative parameters of humic acids.

Research location

The lakes under research are situated in the Drawa National Park on the Drawa Plain (South Pomeranian Lake District). The Drawa National Park was established in 1990 on 11018 ha of land including 368 ha under strict protection. Outwashed sands occupying 70% of the parks surface are the dominating geological feature. Also there are waters-glacial sands and gravel. Locally there are boulder-clay, loams, mud, hill-side sands and gravels, river sands and gravels. The types of soil occurring in the Drawa National Park most commonly are the reflection of its geological situation. The dominating type of soil in the Draw National Park is podzols and it is found in places of hydrogenic and semihydrogenic soils.

There were three mid-forest lakes selected for research: Arkonskie, Moczel and Piaseczno Male. Lake Arkonskie is a self-contained, eutrophic basin of 13.2 ha with maximum depth of 3.8 m, situated among pine forests. The total capacity of this lake is 250.8 thousand m^3 and it is not supplied by any visible water source. Lake Moczel is a small self-contained basin – 3.99 ha with fertile water and it is characterised by high quantities of sediments making it quite shallow. The maximum depth is 1.5 m and its capacity is 17.5 thousand m^3. It is surrounded by beech trees representing the complex of fertile Pomeranian beech trees. Further from the lake are swampy alder forests. Piaseczno Male Lake is situated in the eastern part of the Drawa National Park and its surface is 8.6 ha. It is also a self-contained basin with maximum depth of 6.8 m and capacity of 258.4 thousand m^3. It occupies a natural basin in the ground created as a result of melting of a massive lump of ice approximately 10 thousand years ago. Its situation among pine forests basin is evolving towards dystrophy (*Romanowicz and Szyper* 1998).

Materials and methods

Water samples for analyses were obtained during the spring circulation from the depth of 1 m. The following parameters were determined in the analysed samples: pH, specific conductivity (EC), the concentration of selected cations and anions by standard methods in water analysis.

The bottom sediment samples were obtained from the sediment layer 0–10 cm in the littoral and profundal with the Ekman sampler. The contents of total carbon (TC) and inorganic carbon (IC) were analysed in dried sediment samples with the Primacs^{SC} Analyser (Skalar, Breda, Netherlands), while total nitrogen (Nt) was determined with the Kjeldahl method. On the basis of these results, calculations were made of the contents of organic carbon (TOC) from difference TOC=TC-IC, as well as values of the TOC:Nt ratio. In the analysed sediment samples pH in water (in the ratio 1:10 w/v) and total phosphorus contents (TP) by the Bleck method (1965) were also determined. On the basis of the results calculation were made for the contribution percentages of the organic matter (TOCx1.74, *Mudroch et al.* 1997) and the mineral non-carbonate fraction.

Pigments were extracted from the freeze-drying sediment samples with acetone (ratio 1:100 w/v). Spectra in the range of 350-800 nm were made for the solutions with the Perkin-Elmer Lambda 20 UV-VIS Spectrometer. The contents of pigments were calculated, quoted in SPDU (sedimentary degradation pigment unit) per 1g of sediment (Equation 1) or 1g of organic matter in sediment (Equation 2) (*Vallentyne* 1955):

$$\mathrm{SPDU}\cdot g_{DW}^{-1} = (A_{662} - A_{750})\ (1/DW) \qquad (1)$$

$$\mathrm{SPDU}\cdot g_{OM}^{-1} = (A_{662} - A_{750})\ (1/OM) \qquad (2)$$

where: SPDU – sedimentary degradation pigment unit, A_{662} – maximum value of absorbance in the 657-666 nm range, A_{750} – absorbance value at 750 nm, DW – sample dry mass (in g), OM – organic matter contents in the analysed sample (in g).

Humic acid (HA) fractions from air-dried sediment samples were extracted with 0.5 M NaOH, precipitated at pH 2.0 with 5.0 M HCl, and then purified with the mixture HF-HCl, according to the method by Schnitzer and Skinner (1968). The extracted HAs were analysed for the elemental composition with the 2400 Perkin-Elmer Analyser (C, H, N). The oxygen content (O) was calculated by the difference: O=100%–(C+H+N). On the basis of the elemental composition analyses, calculations were made of atomic ratios and the degree of internal oxidation (ω) according to the equation (*Zdanov* 1965):

$$\omega = (2O+3N - H) : C$$

where: O, N, H, and C – are from the elemental composition, in atom percentage.

Spectral properties were measured with a Perkin-Elmer Lambda 20 UV-VIS Spectrometer (for 0.003% HA solutions in 0.05 M NaOH). Absorbance values were used for calculations of absorbance ratios $A_{2/4}$ (A_{280}:A_{465}), $A_{2/6}$ (A_{280}:A_{665}) and $A_{4/6}$ (A_{465}:A_{665}). The fourth derivative (D4) was calculated for normalised spectra at 404 nm and approximately 660 nm, at which the presence of local maximum in absorbance values were found (*Cieslewicz* 1999, *Manual of Lambda* 20 1996, *Cieslewicz and Gonet* 2004). Statistical analysis was conducted with the cluster analysis using the Ward method (*Ward* 1963).

Results and discussion

Waters

The water samples from Piaseczno Male Lake contained the lowest concentrations of the majority of analysed anions and cations (Table 1).

Water from this lake showed also the lowest EC amounting only to 47 $\mu S\cdot cm^{-1}$, while the concentrations of nitrate-nitrogen, ammonia-nitrogen and chlorides were relatively high. Water from Moczel Lake was characterised by clearly the highest concentrations of calcium, magnesium and hydrogencarbonates (Table 1).

Table 1

Physico-chemical properties of lake waters

Parameter	Unit	Lake		
		Arkonskie	Moczel	Piaseczno Male
pH		6.5	7.0	6.7
specific conductivity	$\mu S \cdot cm^{-1}$	108	185	47
chlorides	$mgCl^{-} \cdot L^{-1}$	9.7	9.9	12.4
hydrogencarbonates	$mgHCO_3^{-} \cdot L^{-1}$	35.1	114.4	19.8
phosphates	$mgPO_4^{3-} \cdot L^{-1}$	0.67	0.53	0.13
calcium	$mgCa^{2+} \cdot L^{-1}$	17.6	38.9	7.6
magnesium	$mgMg^{2+} \cdot L^{-1}$	3.6	4.4	1.0
potassium	$mgK^{+} \cdot L^{-1}$	2.1	2.4	1.3
sodium	$mgNa^{+} \cdot L^{-1}$	4.9	6.4	2.1
sulphates	$mgSO_4^{2-} \cdot L^{-1}$	26.5	18.5	2.0
nitrate-nitrogen	$mgN \cdot L^{-1}$	0.15	0.24	0.26
ammonia-nitrogen	$mgN \cdot L^{-1}$	0.07	0.78	0.53

Sediments

Table 2 shows the physico-chemical properties of the samples of bottom sediments from the lakes. The contents of total organic carbon ranged widely reaching the values as high as 457 $g \cdot kg^{-1}$ (littoral sediments in Piaseczno Male Lake).

Similarly, the concentrations of total nitrogen ranged widely and the lowest contents of this element were found in profundal sediments of Piaseczno Male Lake – 26.5 $g \cdot kg^{-1}$.

Table 2

Physico-chemical properties of lake bottom sediments. SD is the standard deviation

Name of lake and sample symbol		TOC	Nt	TP	TOC:Nt	pHH_2O	Pigments	
			$(g \cdot kg^{-1})$				$SPDU \cdot g_{DW}^{-1}$	$SPDU \cdot g_{OM}^{-1}$
Littoral								
Arkonskie	ArL	323	30.2	0.93	10.7	5.2	0.176	0.263
Moczel	MoL	385	38.5	1.21	10.0	5.2	0.268	0.344
Piaseczno Male	PiL	457	31.4	0.58	14.5	6.0	0.035	0.038
Profundal								
Arkonskie	ArP	386	34.8	1.19	11.1	5.2	0.192	0.245
Moczel	MoP	364	38.5	1.21	9.5	5.4	0.309	0.262
Piaseczno Male	PiP	315	26.5	0.74	11.9	5.5	0.090	0.138

High differentiation of organic carbon and total nitrogen values in the sediment samples found their reflection in the calculated values of the TOC:Nt ratio. The values of this ratio indicate a major share of algae in the organic matter pool in the sediments, because organic matter originating from algae, due to its chemical composition, is characterised by the values of the C/N ratio ranging from 5 to 10. Higher values of this ratio indicate an inflow of organic matter originating from land plants, which are characterised by much higher values of this ratio (C/N ≥ 20) (*Nakai and Koyama* 1987, *Meyers and Lallier-Vergès* 1999, *Meyers* 2003).

The lowest concentrations of total phosphorus were found in sediments of Piaseczno Male Lake. It is accepted that bottom sediments can contain on average from 0.5 to 6.0 $g \cdot kg^{-1}$ TP (*Kajak* 1995). However, higher values – 7.32 $g \cdot kg^{-1}$ (Hollingsworth Lake – Florida) (*Brenner et al.* 1999), and as high as 8.5 $g \cdot kg^{-1}$ (*Søndergaard et al.* 1996) have also been registered. The highest concentrations of total phosphorus were found in sediments from Moczel Lake. However, when comparing these values with those in the literature, it can be concluded that they are low.

Visible light spectra of acetone extracts of pigments contained in the sediments showed the presence of well marked absorption bands at the wavelengths of 410–414 nm and 660–665 nm (Figure 1). The highest contents of pigments were found in the sediments from Moczel Lake 0.268 and 0.309 $SPDU \cdot g_{DW}^{-1}$ (littoral and profundal respectively, Table 2), whilst the lowest concentrations were found in the sediments from Piaseczno Male Lake. Because of a wide differentiation of the chemical properties of the sediments the results are shown additionally in calculation per 1 gram of sediment organic matter. This confirmed dependencies obtained earlier (Table 2).

Humic acids

The elemental composition of humic acids did not differentiate the bottom sediments clearly (Table 3). The lowest values of the atomic ratios H:C and N:C and the highest values of the parameter ω were found for humic acid samples originating from the sediments of Piaseczno Male Lake, whilst the reverse dependencies were recorded for HA from Arkonskie Lake. The above dependencies were found in the samples obtained from both the littoral and profundal. The lower H:C ratio suggests a higher aromaticity of organic compounds (*Van Krevelen* 1950, *Klavins and Apsite* 1997). Recorded values of the H:C ratio were generally higher than the values quoted for HA originating from lake bottom sediments (1.30 ± 0.13) (*Ishiwatari* 1985). Low values of the H:C ratio 0.92 – were recorded for humic acids originating from sediments of dystrophic lakes, while these values were higher for eutrophic lakes – 1.34 (*Klavins and Apsite* 1997). The degree of internal oxidation (ω) describes the origin and the character of humic acids. The samples showed a negative degree of internal oxidation, confirming anaerobic conditions prevailing during diagenetic transformations. Positive values are characteristic for soil HAs with aerobic conditions (*Gonet* 1989, *Cieslewicz et al.* 1997) (Table 3).

UV-VIS spectra of soil humic acids are characterised by a monotonically diminishing course with a slight inflection at the wavelength of 280 nm, which is associated with the presence of the lignin type compounds (*Kononova and Aleksandrova* 1973, *Kumada* 1987, *Ramunni et al.* 1994). A similar course of spectra is usually characteristic for humic acids extracted from bottom sediments of lakes with a forest catchment (*Cieslewicz* 1999). Such course of spectra was observed for alkali solutions of humic acids originating from the lakes Arkonskie and Piaseczno Male (Figure 2 – exemplary spectrum for humic acids from Piaseczno Male Lake).

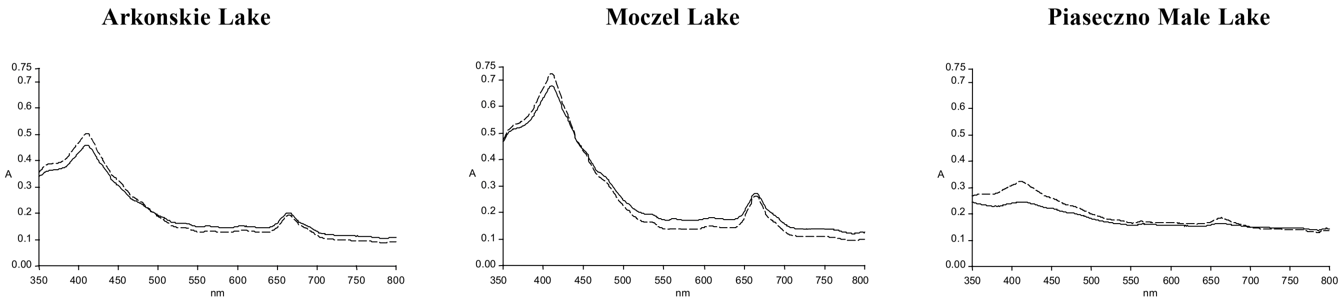

Fig. 1. Original spectra of pigments extracted from lake sediments. Littoral (____) and profundal (---).

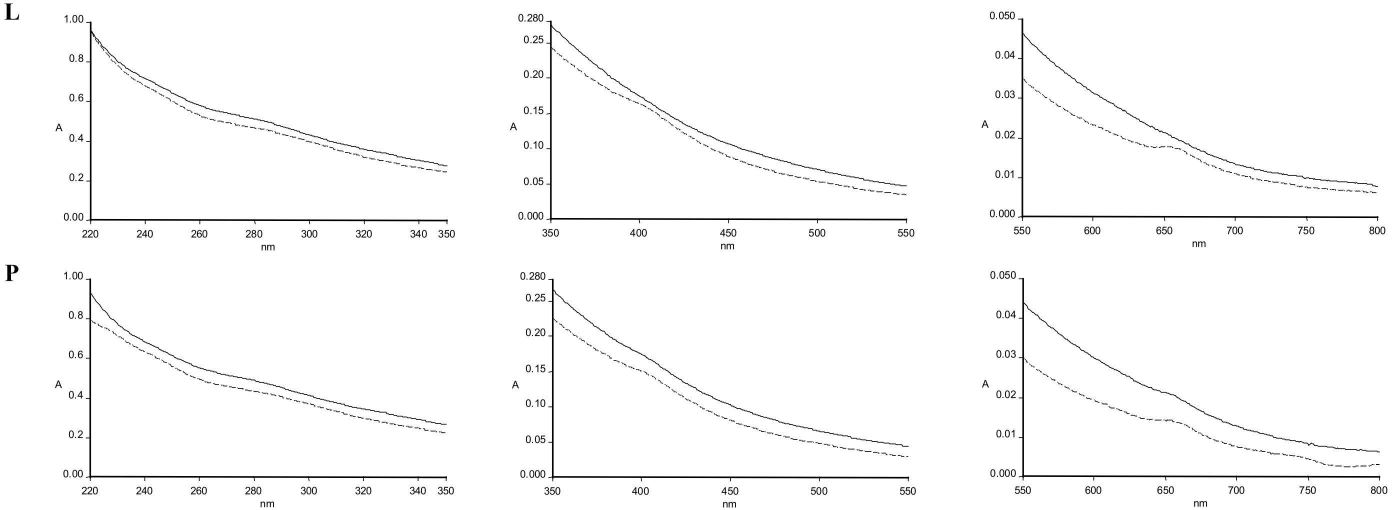

Fig. 2. Original UV-VIS spectra for alkali HA solutions. L – littoral, P – profundal, Piaseczno Male Lake (____) and Moczel Lake (----).

Table 3

Elemental composition (in atom percentage), atomic ratio values and internal oxidation degree (ω) of humic acids extracted from sediments. SD is the standard deviation

Name of lake and sample symbol		C	H	N	O	H:C	N:C	O:C	O:H	ω
Littoral										
Arkonskie	ArL	32.0	48.4	2.6	17.0	1.51	0.080	0.53	0.35	-0.206
Moczel	MoL	32.7	47.7	2.8	16.8	1.46	0.085	0.52	0.35	-0.173
Piaseczno Male	PiL	33.5	46.8	2.4	17.3	1.40	0.072	0.51	0.37	-0.153
Mean±SD		32.7±0.75	47.6±0.80	2.6±0.20	17.0±0.25	1.46±0.055	0.079±0.007	0.52±0.010	0.36±0.012	-0.177±0.027
Profundal										
Arkonskie	ArP	31.7	49.0	2.5	16.8	1.54	0.079	0.53	0.34	-0.249
Moczel	MoP	32.3	47.7	3.2	16.8	1.48	0.099	0.52	0.35	-0.139
Piaseczno Male	PiP	33.4	46.5	2.5	17.6	1.39	0.074	0.53	0.38	-0.116
Mean±SD		32.5±0.86	47.7±1.25	2.7±0.40	17.1±0.46	1.47±0.075	0.084±0.013	0.53±0.006	0.36±0.021	-0.168±0.071

Table 4

Absorbance and absorbance ratio values determined for alkali HA solutions. SD is the standard deviation

Name of lake and sample symbol		A_{280}	A_{465}	A_{665}	$A_{2/4}$	$A_{2/6}$	$A_{4/6}$
Littoral							
Arkonskie	ArL	0.427	0.067	0.013	6.40	31.66	4.95
Moczel	MoL	0.468	0.076	0.016	6.18	28.72	4.65
Piaseczno Male	PiL	0.511	0.094	0.018	5.45	27.64	5.08
Mean±SD		0.469±0.042	0.079±0.014	0.016±0.003	6.01±0.497	29.34±2.080	4.89±0.221
Profundal							
Arkonskie	ArP	0.428	0.072	0.014	5.97	29.92	5.01
Moczel	MoP	0.436	0.068	0.013	6.39	33.79	5.29
Piaseczno Male	PiP	0.488	0.089	0.019	5.51	26.23	4.76
Mean±SD		0.451±0.033	0.076±0.011	0.015±0.003	5.96±0.440	29.98±3.780	5.02±0.265

A different course of spectra of alkali solutions of humic acids was found in sediments of Moczel Lake. There were weakly marked additional absorption bands at the wavelengths of approximately 400 and approximately 660 nm (Figure 2). The presence of these bands is associated with the presence of structures originating from pigments (*Ishiwatari* 1973, *Povoledo et a*l. 1975). The presence of this band is sometimes used as a method for telling the difference between the matter of terrestrial and water origin (*Fooken and Liebezeit* 2000).

Absorbance values are usually recorded for alkali solutions of humic acids at three different wavelengths 280, 465 and 665 nm. Absorbance values at 280 nm are associated with the presence of structures of lignin origins, while those at 465 nm are connected with the contents of organic matter in early stages of decomposition, and at the wavelengths of 665 with the substances of a high humification degree (*Chen et al.* 1977). The calculated values of respective absorbance ratios – $A_{2/4}$, $A_{2/6}$ and $A_{4/6}$ are also analysed.

At the wavelengths of 280 and 465 nm the lowest absorbance values among littoral samples were found for the alkali solutions of humic acids originating from Arkonskie Lake. Absorbance values at the wavelength of 665 nm were ranging from 0.013 – 0.018. Absorbance values obtained from profundal were less variable (Table 4).

The highest values of the ratios $A_{2/4}$ and $A_{2/6}$ were recorded for littoral samples originating from Arkonskie Lake, whilst the values of the $A_{4/6}$ ratio were the highest for the alkali solutions of humic acids from sediments in Piaseczno Male Lake. Among the samples from profundal HA originating from the Moczel Lake sediments showed the highest values of all of the ratios (Table 4).

Higher values of the $A_{2/6}$ and $A_{4/6}$ ratios indicated that humic acids were characterised by a higher content of lignin-type compounds and a lower degree of humification, but so far this interpretation works well only for soil HA (*Chen et al.* 1977, *Cieslewicz* 1999). The presence of the additional absorption bands, which affect equally the recorded absorbance values, as well as the values of the absorbance ratios, makes the interpretation of data of the spectrometric analysis difficult.

Table 5

Fourth derivative (D4×1000) values of humic acids extracted from sediments. SD is the standard deviation

Name of lake and sample symbol		$D4_{404}$	$D4_{660}$
		Littoral	
Arkonskie	ArL	5.8	1.2
Moczel	MoL	8.1	1.7
Piaseczno Male	PiL	2.8	0.4
Mean±SD		5.57±2.658	1.10±0.656
		Profundal	
Arkonskie	ArP	5.5	0.8
Moczel	MoP	8.9	1.7
Piaseczno Male	PiP	5.9	1.0
Mean±SD		6.77±1.858	1.17±0.473

UV-VIS spectra (Figure 2) were transformed as the fourth derivative. Values of the fourth derivative were calculated at the wavelengths of approximately 404 and 660 nm ($D4_{404}$ and $D4_{660}$, Table 5). Values of the fourth derivative at wavelength 404 and 660 nm were significantly higher for HAs isolated from the sediments of Moczel Lake (Table 5) than for HAs from other sediments. The catchment of Moczel Lake is covered by the beech forest, but the catchments of lakes Arkonskie and Piaseczno Male are covered by the pine forests. These relations are clear for both $D4_{404}$ and $D4_{660}$ derivatives for HA samples extracted from the littoral and profundal.

Cluster analysis, also called segmentation analysis or taxonomy analysis, seeks to identify homogeneous subgroups of cases in a population. That is, cluster analysis seeks to identify a set of groups, which both minimize within-group variation and maximize between-group variation. Ward's method calculates the sum of squared distances from each case in a cluster to the mean of all variables. The cluster to be merged in the one which will increase the sum the last (*Ward* 1963).

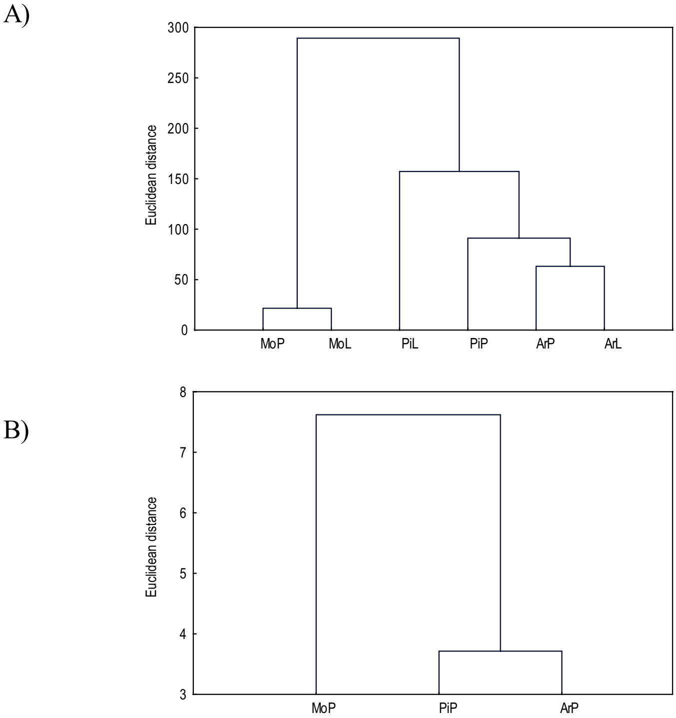

Fig. 3. Cluster analysis: A– for lake parameters (water, sediments, pigments, humic acids) and B – for profundal humic acid parameters, explanations in the text. L – littoral, P – profundal, Pi – Piaseczno Male Lake, Mo – Moczel Lake, Ar – Arkonskie Lake.

Cluster analysis divided the samples into two clusters, each reflecting the origin of humic acid samples (Figure 3A and 3B). Considering all parameters for water (pH electrical conductivity, contents of Cl^-, HCO_3^-, PO_4^{3-}, SO_4^{2-}, NO_3^-, NH_4^+, Ca^{2+}, Mg^{2+}, Na^+, K^+), for bottom sediments (TOC, Nt, TP, TOC:Nt, pH), for pigments

(SPDU – the contents of pigments calculated per 1 gram of sample dry mass, and per 1 gram of sediment organic matter) and for humic acids (C, H, O, N, H:C, N:C, O:C, O:H, ω, A_{280}, A_{465}, A_{665}, $A_{2/4}$, $A_{2/6}$, $A_{4/6}$, $D4_{404}$, $D4_{660}$), the cluster analysis clearly divided the three lakes (Figure 3A). For each of the lakes a division covering littoral and profundal were additionally obtained.

Figure 3B depicts the results of cluster analysis for humic acids from profundal sediments in all three lakes. For the analysis three HA parameters were considered; their values differed significantly: the fourth derivative for λ=404 nm, the absorbance ratio of $A_{2/6}$, ω – internal oxidation degree (from the elemental analysis). In this case, the cluster analysis showed differentiation of HAs being conditional upon their origins, where the properties of HAs in the sediments from lakes Arkonskie and Piaseczno (with the pine forest in the catchment) were more similar to each other than to HAs in sediments from Moczel Lake (with the beech forest in the catchment).

Conclusions

The results of water and sediment analyses in lakes showed diversification in the character of lake ecosystems relevant to the management of the catchment area. The primary feature characterising humic acids formed in the sediments of Lake Moczel is the presence of the additional absorption bands in their spectra obtained in the UV and visible light ranges, and their presence is associated with structures of the pigment origins. The conditions in this basin promote the retention of pigments of the algal origin in sediments and binding them with the humic acid molecules in the process of diagenetic transformations. The remaining lakes, due to the character of their catchment areas, were exposed to acidification, which in mid-forest basins, particularly in coniferous pine forest, is aided by the mor type humus containing soluble in water fulvic acids (*Adamczyk* 1980). Furthermore rainwater passing through the tree branches and flowing along the tree trunks undergo acidification down to pH=4 (*Grodzinska* 1976, *Wrobel and Bombowna* 1984). In contrast the catchment of Lake Moczel is covered by the beech forests and furthermore, its water contains high concentrations of calcium and magnesium, as well as the hydrogen-carbonate ions, which prevent pH fluctuations.

The qualitative parameters of humic acids and in particular the results of spectrometric analyses and the elemental composition can be used in the environmental evaluation of lake bottom sediments.

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Properties of organic matter and molecular size distribution of humic acids isolated from forest soil

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The aim of the present paper was to characterize the properties of humic substances of forest soils, especially the properties of humic acids. The research involved samples from the organic and humic horizon of forest soil taken from the monocultures of European silver fir (*Abies alba*) and European beech (*Fagus sylvatica*). The humic acids were characterized by their elemental composition, spectrometric properties for the UV-VIS and IR ranges as well as HPSEC. It was shown that the highest content of carbon, oxygen and the lowest of hydrogen as well as the lowest values of coefficients of $A_{2/6}$, $A_{4/6}$ and ΔlogK was obtained for humic acids isolated from deeper layers of the humic horizon, irrespective of the species of the forest stand. Based on the HPSEC analysis, it was noted that the process of humification of the materials of the duffs analyzed was connected with an increase in the polydispersity of humic acids molecules.

Key words: *forest soil, humic acids, elemental composition, spectral properties, HPSEC.*

Introduction

Soils in commercial forests undergo various transformations due to the forest economy (e.g. lumbering, planting), as well as a longer or a shorter period of agricultural management. The practices applied disturb the natural setting of the uppermost soil horizons stopping the continuity of development of soil as a whole. It also happens that the uppermost horizons (organic and humic horizons), while being exposed to growing treatments, can result in a complete degradation. Their reconstruction is slow and occurs together with the forest stand development. However quite frequently they do not reach the state of maturity as the forest stand reaches the felling age and the development of the uppermost soil horizons are disturbed (*Dziadowiec* 2003).

Undoubtedly one of the basic factors affecting the properties of the organic horizon is the forest stand species composition. The forest stand species composition also affects the properties of deeper horizons as a result of mobility of nutrients from the uppermost horizons (*Dziadowiec et al.* 1998, 2002, *Zech and Guggenberger* 1996). The intensity of mineralization processes (humification) considerably depends on the chemical composition and morphology of the plant fall. As it is seen from literature

(*Aleksandrova* 1980, *Gonet and Debska* 1998, *Zaujec* 1980), the chemical composition of plant materials remains one of the basic factors affecting the properties of humic substances produced and the course of the humification process. One of the parameters changing during the humification process is the degree of polydispersity of humic substances molecules.

High performance size exclusion chromatography (HPSEC) can be used to analyze the molecular size distribution (MSD) of organic macromolecules. In HPSEC molecules larger than the gel pores move rapidly through the column with the eluent but smaller molecules penetrating into the pores of gel in relation to both their size and shape as well as the pore size distribution of the gel (*Nissinen et al.* 2001, *Vartiainen et al.* 1987).

The aim of the present paper was to define the humic substances properties of forest soils, especially the properties of humic acids. The characteristics of humic acids were determined by their elemental composition, spectrometric properties for the UV-VIS and IR range as well as HPSEC.

Material and methods

The research involved the samples taken from the humic horizon of forest soil taken from the monocultures of European silver fir (*Abies alba)* (sample – RJ 2 and RJ 3) and European beech (*Fagus sylvatica)* (sample RB 2 and RB 3). Additionally in order to define the effect of the kind of plant fall on the properties of organic matter of soil, soil samples of the organic (litter) subhorizon (Ol) of fir – RJ1 and beech – RB1 were analyzed. The samples were obtained from the area of Forests of Experimental Stations at Rogowo (Poland), set up on Eutric Luvisols. The forest stand age varied and for fir – it was 30 years, while for beech – 90 years.

The following were determined in the soil samples and plant fall:

- total organic carbon (TOC) – using the TOC Primacs analyzer by Skalar,
- total nitrogen (Nt) – with the Kjeldahl's method.

Humic acids (HAs) were extracted and purified according to standard methods following the procedure of:

- decalcification with 0.05 M HCl at 1:10 (w/v) ratio, extraction time 24 h. After solution centrifugation, the residue was washed with distilled water until a neutral reaction was reached.
- extraction of humus acids: solid remaining after decalcification was subjected to 0.5 M NaOH at 1:10 (w/v) ratio for soil samples of humus horizons and 1:50 for samples of subhorizons OL, with 24 h extraction time, occasionally mixed, then centrifuged humus acids extract. In this solution values of absorbance were determined.
- precipitation of humic acids: the received alkaline extract was treated with 2 M HCl giving pH=2, after 24 hours the precipitate of humic acids was separated by centrifugation.
- purification of humic acids: the received residue of humic acids was treated with a mixture of HCl-HF (950 mL H_2O, 5 mL HCl and 5 mL HF) over

24-hour period and subsequently separated in a centrifuge. This action was repeated three times. After purification the residue of humic acids was treated with distilled water until a zero reaction to chlorides was achieved, and then freeze-dried.

During extraction the following were determined: the content of organic carbon and the content of nitrogen of humic acids fractions (C_{HA}, N_{HA}) and fulvic acids fractions (C_{FA}, N_{FA}) – using the TOCN Formacs analyzer by Skalar.

The extracted humic acids were analyzed for:

- elemental composition (Perkin Elmer 2400 CHN analyzer). On the basis of elemental composition the values of atomic ratios: H/C, O/C, O/H, N/C and ω-internal oxidation degree, were calculated according to the formula:

  $\omega = (2O+3N-H): C$, where: O,N,H,C – content in atomic percentage.
- absorption spectra in UV-VIS range (Perkin Elmer UV-VIS Spectrometer, Lambda 20). VIS spectra were performed for 0.02% humic acids solutions in 0.1M NaOH and UV-spectra were determined after fivefold dilution. Absorbance measured at the wavelengths of 280 nm (A_{280}), 400 nm (A_{400}), 465 nm (A_{465}), 600 nm (A_{600}) and 665 nm (A_{665}) were used to calculate the coefficient values:

  $A_{2/4}$ – 280 nm and 465 nm absorbance ratio;
  $A_{2/6}$ – 280 nm and 665 nm absorbance ratio;
  $A_{4/6}$ – 464 nm and 665 nm absorbance ratio;
  $\Delta \log K = \log A_{400} - \log A_{600}$.
- infrared spectra (Perkin-Elmer FT-IR Spectrometer, Spectrum BX). FT-IR spectra for the range 400 – 4400 cm^{-1} were done for HA samples of 3 mg in 800 mg KBr. To carry out IR spectra processing, the deconvolution method was applied, applying a filter making the bands of $\gamma = 4$ narrower, and the process of smoothing, for which the length parameter was $l = 80\%$ (*Debska* 2004, *Cocozza and Miano* 2002);
- high-performance size-exclusion chromatography (HPSEC) (Chromatograph HPLC Perkin Elmer Series 200, Diode Array UV detector operating at 254 nm). Humic acids were separated with the TSK G3000SW column (7.5 mm × 600 mm). Sodium acetate 0.01 M at pH 7 was used as eluent. The guard column was TSK G3000SW (7.5 × 75 mm). Solutions of humic acids were applied in 0.01M CH_3COONa of the concentration of 0.05 mg/cm^3. A sample of 500 μl was injected into the column. The peak area was determined: S_0 – total area, S1 – the first peak area, S_2 – the second peak area as well as the S_1/S_2 ratio.

Results and discussion

In forest soils humus accumulates in different horizons, however, mostly in organic and humic horizons. The organic horizon is produced from the accumulation of organic residue from the yearly tree and shrub fall as well as the ground cover on the surface of the mineral part of soils. The carbon to nitrogen ratio (C/N) remains on the same level as in the fall. The horizon constitutes an integral part of forest soils and it is closely connected with mineral horizons of soils, at the same time affecting their properties. The organic (litter) subhorizon soil sampled for analysis consisted of slightly changed plant fall residue at an early stage of transformation. The content of total organic carbon (Table 1) in poorly transformed fir needles was 471 g/kg, and the content of nitrogen – 16.0 g/kg. A higher content of TOC and a lower of nitrogen and, as a result, a higher value of the TOC/Nt ratio (37.3) was observed for the samples of Ol horizon taken from the beech stand. The content of TOC and Nt in the humic horizon was higher in samples taken from the fir stand than the beech stand.

Table 1

Content of carbon and nitrogen as well as fractional composition of organic matter

Sample	TOC	Nt	TOC/Nt	C_{HA}	C_{FA}	C_{HA}/C_{FA}	N_{HA}	N_{FA}	N_{HA}/N_{FA}
	g/kg			% of TOC			% of Nt		
RJ1	471	16.0	29.4	16.4	11.7	1.40	28.4	11.3	2.50
RJ2	36.3	3.0	12.2	28.7	23.5	1.22	23.4	14.8	1.58
RJ3	19.5	1.4	13.5	25.3	23.9	1.06	28.5	18.8	1.52
RB1	540	14.5	37.3	14.7	9.2	1.60	25.0	6.2	4.02
RB2	22.6	1.4	15.7	36.0	22.3	1.61	38.0	20.3	1.87
RB3	15.0	1.1	13.5	29.8	24.1	1.24	29.0	20.2	1.44

Fractional composition of organic matter

A higher share of humic acids and fulvic acids (C_{HA} and C_{FA}) was observed for organic material on the fir stand – 28.1% as compared with the beech stand – 23.9% (Table 1). Fractional composition of humic substances of duffs undergoing decomposition showed the highest share of humic acids fractions. A higher value of the C_{HA}/C_{FA} ratio was observed for the organic matter sampled from the beech stand, as compared with the duff sampled from the fir stand.

The humic horizon occurring right next to organic horizon recorded a higher share of humic acids fractions than the deeper layer of this horizon. The share of fractions of fulvic acids, irrespective of the depth of soil sampling for analysis, was similar. The humic horizon in the fir stand showed lower values of the C_{HA}/C_{FA} ratio, as compared with the beech stand.

According to numerous authors (*Arvisto* 1970, *Sotakova* 1983), the initial stages of humification always show the dominance of fulvic acids over humic acids. Sometimes fulvic acids prevail very considerably over humic acids. According to Aleksandrova (1980) at the initial humification stage humic acids prevail and along the course of the humification process the share of fulvic acids fractions increases. The research results obtained seem to confirm the thesis developed by the present author, as well as the observations made by, among others, Dziadowiec 1990 and Zaujec 1990, that the mutual quantitative relationships between those groups of humic compounds depend mostly on the kind of material exposed to humification and the environmental conditions (soil, moisture, temperature) under which the process takes place.

The organic matter of the duffs analyzed showed a high share of nitrogen of fractions of humic and fulvic acids – 39.7% in samples taken from the fir stand and 31.2% in samples taken from the beech stand (Table 1). The share of N_{HA} fractions was 2.5-fold higher in samples of the RJ1 variant and about 4-fold higher in samples of the RB1 variant, as compared with the nitrogen fraction of fulvic acids. In the humic horizon of soil samples taken from the fir stand, the value of the N_{HA}/N_{FA} ratio ranged from 1.58 to 1.52, while from the beech stand – from 1.87 to 1.44.

Elemental composition of humic acids

Molecules of humic acids isolated from the samples of organic (litter) subhorizon taken from the fir stand showed a lower content of carbon and a higher content of nitrogen, as compared with HAs variant RB1 (Table 2). In HAs isolated from the humic horizon layer right next to the organic horizon there were observed first of all a decrease in the content of hydrogen and an increase in the content of oxygen, while the lowest content of hydrogen and the highest content of carbon were observed for HAs variants RJ3 and RB3.

Table 2

Elemental composition of humic acids

Sample	C	H	N	O	H/C	O/C	O/H	N/C	ω
RJ1	33.92	44.84	2.06	19.18	1.32	0.566	0.428	0.061	-0.009
RJ2	33.65	43.29	2.41	20.65	1.29	0.614	0.477	0.072	0.155
RJ3	36.95	39.37	2.18	21.49	1.07	0.582	0.546	0.059	0.275
RB1	34.76	44.25	1.45	19.55	1.27	0.562	0.442	0.042	-0.023
RB2	34.12	42.53	2.03	21.32	1.25	0.625	0.501	0.059	0.182
RB3	36.70	40.43	2.40	20.47	1.10	0.558	0.506	0.065	0.210

As a result of changes in the content of respective elements in the composition of molecules of humic acids, changes occur in the value of atomic ratios of respective elements. The numerical values of atomic ratios allow for a rough determination of the structure of molecules of humic acids through the evaluation of the degree of condensation of aromatic rings (H/C ratio) as well as the degree of their maturity (O/C, O/H, ω). Some authors (*Dziadowiec* 1979, *Gonet and Debska* 1989, *Debska* 2004) consider it most justifiable to determine, based on the elemental composition, the values of the degree of internal oxidation (ω parameter). The parameter can be used both to evaluate the degree of the progress of humification process of 'fresh' organic matters as well as the characteristics of the humic substances produced. The values of the H/C ratio of samples of RJ1 and RJ2 as well as RB1 and RB2 variants were similar (Table 2).

Humic acids isolated from samples taken from the organic (litter) subhorizon showed lower values of the O/C, O/H ratios as well as the degree of internal oxidation, as compared with humic acids from humic horizon. High values of the O/H ratio and the degree of internal oxidation of humic acids isolated from deeper layers of the humic horizon show their greater 'maturity'. A varied elemental composition in the molecules of humic acids of respective layers of humic horizon suggests that the soil did not reach the dynamic balance disturbed by the agrotechnical practices applied. It also applies to soil in the stand of beech which was 90 year old as well as much younger fir – 30 years.

Spectrometric parameters of humic acids

The absorbance values at the wave lengths of 280, 400, 465, 600 and 665 nm were used to calculate the ratios of absorbance $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and ΔlogK coefficient (Table 3). Generally it is assumed that the value of absorbance for 280 nm defines the content of compounds of lignin type, A_{465} value – content of the substance at the initial stage of decomposition, while A_{665} reflects the content of substances showing a high degree of humification. Coefficients of $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and ΔlogK are the parameters which can be used to evaluate the progress of the humification process of organic matter and the characteristics of the humic substances produced. 'Young' humic acids, generally of a lower molecular weight and a lower degree of condensation of the aromatic structures, show higher values of the following coefficients: $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and ΔlogK, as compared with HA of a high degree of humification (*Gonet, Debska* 1998, *Debska* 2004).

The humic acids absorbance coefficient values obtained from the analyzed duffs show their 'young chemical age' (Table 3). At the same time it was observed that humic acids isolated from soil samples of the humic horizon taken from the beech stand show a higher degree of humification as compared with the soil sampled from the fir stand. It could have been a result of the chemical composition of organic material undergoing decomposition, but also of the forest stand age.

Table 3

Spectrometric parameters of humic acids

Sample	$A_{2/4}$	$A_{2/6}$	$A_{4/6}$	ΔlogK
RJ1	7.36	53.4	7.26	0.916
RJ2	5.42	29.1	5.38	0.605
RJ3	5.05	23.9	4.73	0.564
RB1	5.78	45.7	7.91	0.925
RB2	4.09	19.4	4.74	0.612
RB3	4.26	16.7	3.93	0.536

FTIR spectra of humic acids

Humic acids spectra were determined with the autodeconvolution method, applying a filter making the bands of $\gamma = 4$ narrower and applying the process of smoothing, for which the parameter of wavelength was $l = 78\%$ (Fig. 1 and 2). The application of such parameters of the process of autodeconvolution allowed obtaining clear absorption bands of the areas of interest and, at the same time, the occurrence of additional bands, which could lead to a wrong spectral interpretation, was avoided.

The spectra of the analyzed humic acids, depending on the soil sampling depth, differed in their pattern for the range of wave numbers 2920–2960 cm^{-1} and about 2850 cm^{-1}, conditioned by the presence of $-CH_3$ and $=CH_2$ groups and the range of 1000–1460 cm^{-1} (Figs. 1 and 2).

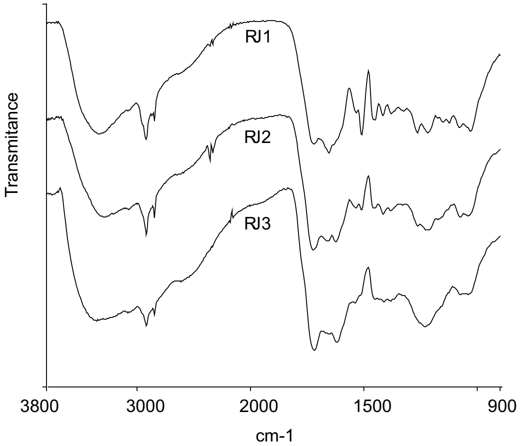

Fig. 1. FT-IR spectra of humic acids isolated from soil sampled from the fir stand.

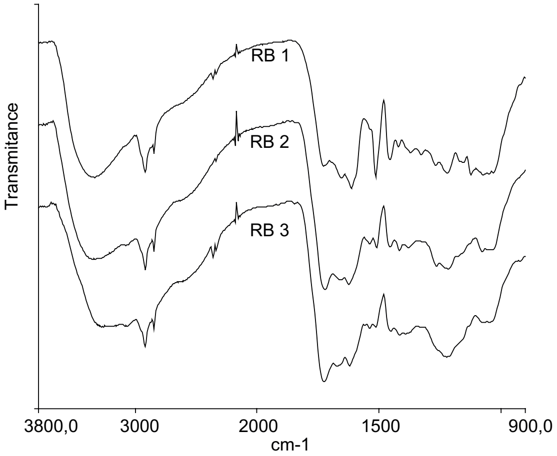

Fig. 2. FT-IR spectra of humic acids isolated from soil sampled from the beech stand.

The clear absorption bands in the range of wave numbers from 1460 to 1000 cm^{-1} in IR spectra of humic acids are due to the presence of lignin structures which have not undergone decomposition and show their 'young chemical age'. The intensity of bands in the range of wave numbers 2920–2960 cm^{-1} and 2850 cm^{-1} depends on the degree of aliphaticity of humic acids molecules. The higher the intensity of this band, the greater the share of aliphatic structures in HA molecules (*Gonet* 1989).

Based on the pattern of IR spectra, one may conclude that the greater the depth, the lower the share of aliphatic structures, methoxyl groups (1030–1080 cm^{-1} range) and the higher the share of non-lignin aromatic bonds and carboxyl groups (1720 – 1700 cm^{-1} range spectrum). Similar relationships were obtained by, e. g., Zech and Guggenberger (1996) based on the pattern of spectra ^{13}C NMR.

High-performance size-exclusion chromatography (HPSEC)

During the resolution of the analyzed humic acids with the HPSEC method, for samples taken from the organic (litter) subhorizon (Ol) of fir a single wide peak was obtained, while for the organic (litter) subhorizon (Ol) taken from the beech stand, although it was similar, at the retention time of 16 minutes one could observe a kink, which shows a tendency of the peak to resolve (Figs. 3, 3a). However a clear resolution into two peaks can be observed only for humic acids separated from humic horizons of both fir and beech.

Bearing that in mind, the total area of peaks for organic (litter) subhorizon is more than two-fold lower than the area of humic acids isolated from the humic horizon (Table 4).

Table 4

Absorbance values and parameters of chromatographic resolution

Sample	A_{280}	A_{465}	A_{665}	S_0 (cm^2)	S_1 (%)	S_2 (%)	S_1/S_2
RJ1	2.88	0.391	0.054	13.2	–	–	–
RJ2	4.49	0.830	0.154	36.9	27.1	72.9	0.37
RJ3	7.36	1.456	0.308	29.1	19.9	80.1	0.25
RB1	3.00	0.520	0.066	12.7	–	–	–
RB2	4.19	1.020	0.216	30.1	28.2	71.8	0.39
RB3	5.80	1.362	0.347	28.2	20.6	79.4	0.26

The share of the first peak area (S_1) ranged from about 20 to 28%, and so it was the second peak which showed dominant, which is also seen from the ratios of peak areas S_1/S_2. There were observed no significant differences in the chromatographic parameters analyzed between the molecules of humic acids isolated from soil sampled from the fir and beech stands. The measurement conditions applied as well as the column selection (TSK GEL 3000SW) allowed for the resolution of the isolated humic acids depending on the size of molecules (*Conte and Piccolo*, 1998). For that reason the first peaks, namely the ones of a shorter retention time, include molecules greater in size, while the peaks of a longer retention time are typical for molecules smaller in size.

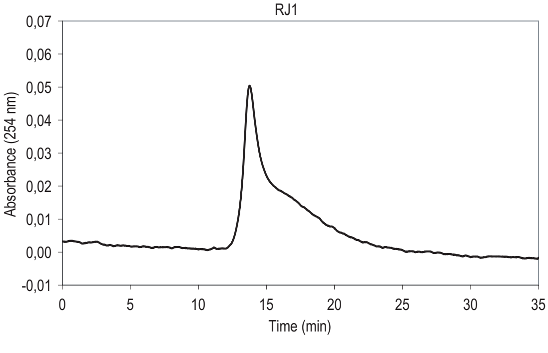

Fig. 3. HPSEC chromatograms spectra of humic acids isolated from soil sampled from the fir stand.

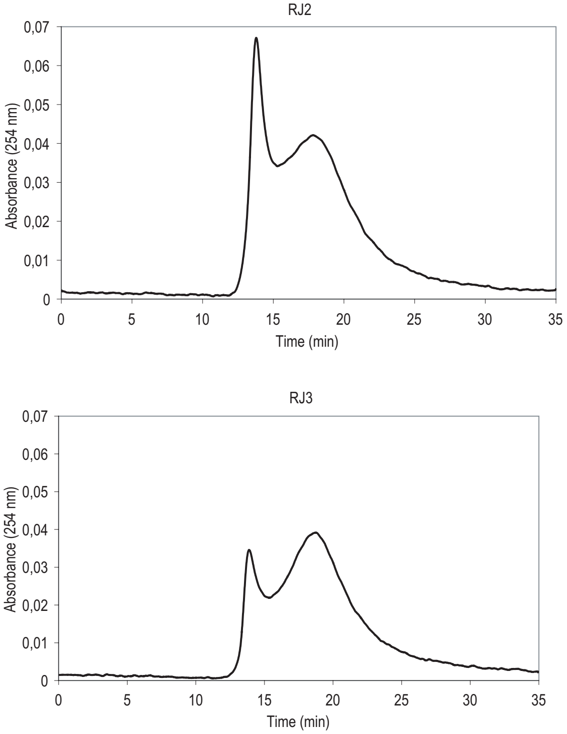

Fig. 3a. HPSEC chromatograms spectra of humic acids isolated from soil sampled from the fir stand.

The analysis of the chromatogram patterns shows that the newly-created humic acids, at a very early stage of organic matter transformations, show a lower polydispersity and a relatively high molecular weight, as compared with HAs of a greater degree of maturity (HAs of the humic horizon). In the course of the humification process the polydispersity of humic acids molecules increases and, as a result, the average molecular weight decreases. Also Aleksandrova (1980) stresses that humic acids at a very early stage of the humification process of plant material show a greater molecular weight, and in the course of the humification process the molecular weight of humic acids decreases. According to the author HAs are produced as a result of oxidation of high molecular products of plant residue decomposition.

Conclusions

1. Organic matter of duff sampled from the fir stand showed a lower content of carbon and a higher content of nitrogen and, as a result, a lower value of the TOC/Nt ratio, as compared with the organic matter of the duff sampled from the beech stand.
2. Organic matter of the organic (litter) subhorizon and humic horizon which occurred in the beech stand showed higher values of C_{HA}/C_{FA} and N_{HA}/N_{FA} ratios, as compared with the fir stand.

3. The highest content of carbon, oxygen and the lowest of hydrogen as well as the lowest values of coefficients $A_{2/6}$, $A_{4/6}$ and ΔlogK were observed for humic acids isolated from deeper humic horizon layers, irrespective of the species of the forest stand.
4. The process of humification of the duff materials analyzed was related to the increase in the polydispersity of humic acids molecules.

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Properties of humus in relation to soil particle size fraction

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A model experiment was carried out to evaluate the effects of mixing organic fertilizers (cattle manure, vermicompost and wheat straw) with sandy soil material. Soil samples were taken from the 0–20 cm layer (soil material mixed with organic fertilisers) and the 20–35 cm layer (soil material only), after one year as well as after three years of incubation. Total organic carbon (TOC), total nitrogen (TN) and fractional composition of humus (humic acids, fulvic acids) were analysed in two particle size fractions ($\phi > 0.1$ mm – coarse fraction and $\phi < 0.1$ mm – fine fraction).

The results showed that the $\phi < 0.1$ mm fraction accumulated three times more organic carbon than did the $\phi > 0.1$ mm fraction. Humus distribution between the two fractions depended on the kind of organic material added to the soil. Humus in the fine fractions was characterised by higher content of TN and insoluble fraction of organic matter, lower contribution of humic and fulvic acids if compared to the coarser fractions. Application of organic fertilisers to the soil caused an increase of the content of total nitrogen in both fractions. Three years after addition of organic fertilizers no migration of organic carbon and nitrogen in the soil profile was found.

Key words: *grain soil fractions, organic fertilizers, carbon, nitrogen, fractional composition.*

Introduction

Soil organic matter is one of the most basic and characteristic components of soil. It comprises numerous substances, more or less complex, depending on the kind of the starting material and the bioecological conditions during decomposition (*Gonet* 2003). The content of organic matter in arable soils is the result of the balance between decomposition processes and the input of fresh organic matter (*Aoyama and Kumakura* 2001, *Gonet* 1993, *Gonet and Debska* 1999). The amount and quality of humus is one of the factors crucial for soil productivity and fertility. It depends on the site conditions (climate, soil) as well as on management (soil tillage, plant cultivation and crop rotation). The processes of mineralization and humification of organic carbon compounds introduced into soil with organic fertilizers depend among others, on the C: N ratio as well as on the percentages of fast and slowly decomposing carbon fractions. Soil fractional composition has been considered one of the factors important for the intensity of organic matter transformations. (*Debska* 2003, *Debska et al.* 1999, *Gonet and Debska* 1999, *Jurcova et al.* 2001, *Sotakova* 1983).

Numerous authors (*Schmidt et al.* 1996, *Schmidt and Kogel-Knabner* 2002, *Schulten et al.* 1993, *Tarchitzky et al.* 2000) have indicated that the content of organic matter decreases with an increase of the diameter of particle grains. Therefore, sand fractions are often poor in carbon while most carbon is with larger fragments of decaying plants residues and attached to surfaces of fine mineral particles. According to a literature survey (*Debska et al.* 1999, *Jurcova et al.* 2001, *Chen and Chiu* 2003), humic substances linked with fine-grained soil fraction have a higher humification degree than those connected with coarse-grained ones and are more resistant against decomposition.

The objective of the study was to determine the relationship between the diameter of soil particles and the content of carbon and nitrogen. Another aim was to evaluate the effect of differentiated organic fertilization on accumulation and properties of humus contained in soil fractions under study.

Material and methods

In a model incubation experiment, cattle manure, vermicompost and wheat straw were mixed with sandy soil material (from the A horizon of Haplic Luvisol, TOC content – 5.8 g/kg, TN content – 0,53 g/kg). Vermicompost was produced from bovine manure by Californian worms (*Eisenia fedita Sav.*). The product was subsequently mixed with peat. The experiment was carried out over three years in plastic containers placed in soil in the field.

The containers were filled (in three replications) with the material as follows:

Variant	Incubation material
0	soil without additives (control)
1O	soil + manure (100 kg + 10 kg)
2S	soil + straw + NH_4NO_3 (100 kg + 2 kg + 0.014 kg N)
3E	soil + vermicompost (100 kg + 8.3 kg)
4OS	soil + manure + straw + NH_4NO_3 (100 kg + 5 kg + 1 kg + 0,007 kgN)
5ES	soil + vermicompost + straw + NH_4NO_3 (100 kg + 4.15 kg + 1 kg + 0.007 kgN)

In order to increase the organic carbon concentration by about 10g/kg, adequate quantities of organic fertilizers were added to the top 0–20 cm layer of soil. Organic carbon content of soil samples with fertilizers were calculated taking into consideration the quantities in the soil and in the fertilizer. Soil surface in the containers was maintained to represent a vegetation-free fallow, and then the containers were placed into soil and exposed to natural climatic conditions.

Soil samples were taken from the 0–20 cm and 20–35 cm layers after one year and after three years of incubation. Each sample was divided into two fractions with particle size >0.1 mm (coarse fraction) and particle size <0.1 mm (fine fraction), respectively.

The two particle size fractions were analysed for:

- total organic carbon (TOC) with the Skalar Primacs^{sc} Analyser,
- total nitrogen (TN) by the standard Kjeldahl method,
- fractional composition of humus (*Griffith and Schnitzer* 1975):
  - C_d – carbon in 0.1M HCl extract,
  - C_{HA} – carbon of humic acids fraction,
  - C_{FA} – carbon of fulvic acids fraction.

Variance analysis (Tukey test) for three-factor experiment was done:

- Factor I – fertilization variants (0, 1O, 2S, 3E, 4OS, 5ES),
- Factor II – particle size fraction (>0.1 mm – coarse fraction, <0.1 mm – fine fraction),
- Factor III – time of incubation of organic materials (after one year, after three years).

Results and discussion

Prior to experiment, the contents of organic carbon (TOC) and total nitrogen (TN) in the 0–20 cm and 20–35 cm soil layers was 5.8 g/kg and 0.53 g/kg, respectively. According to the experimental design, carbon concentration in soil samples with fertilizers in the 0–20 cm layer was by about 10 g/kg higher than in the non-fertilized soil. After the addition of organic and mineral fertilizers, total nitrogen content ranged between 0.90 – 1.08 g/kg, while the values of the TOC/TN ratio were between 14.3 and 16.0.

After one year samples of soil without additives and mixed with organic fertilizers were taken. After separation into fine and coarse-grained fractions it was found that organic carbon and nitrogen accumulated mainly in the fine-grained fraction (Table 1). In the 0–20 cm soil layer without additives, the carbon content in the fine-grain fraction was by 200% higher than in the fraction >0.1 mm. Similarly, the fine-grained fraction contained 184% more TN than the coarse-grained one. However, despite different concentrations of TOC and TN in both fractions, the value of the TOC/TN was almost the same and averaged to 10.9.

The samples of soil mixed with organic fertilizers collected after one year of incubation contained significantly (Table 2) more carbon in both fractions than in the soil without additives (by 46% and 116% for the fine and coarse fractions, respectively). Moreover, it also depended on fertilizer added. The highest TOC content in the coarse-grained fraction was found after addition of vermicompost (variant 3E), while the lowest one was noted for the introduction of manure (5.3 g/kg). TOC concentration in the fine fraction varied from 13.4 g/kg (variant 5ES) to 17.6 g/kg (variant 1O).

Similarly to TOC, the content of TN in the soil mixed with organic fertilizers was higher in the fine fraction than in the coarse fraction. Also an increase of the TN concentration after the introduction of organic fertilizers was observed (by 103%

and 29% for the coarse and fine fraction, respectively), but no statistically significant effect from the type of fertilizer applied.

After three years of decomposition of the fertilizers introduced to soil a decrease in TOC and TN were noted as compared with their content after one year (Table 1). After three years, concentrations of TOC in the coarse-grained fraction was on average by about 16% lower, while the nitrogen content was by 26% lower than before. The corresponding decrease in the fine-grained fraction was 12% (TOC) and 14.2% (TN).

Table 1

Organic carbon content in the 0–20 and 20–35 cm soil layer

Variant	Fraction (mm)	After 1 year			After 3 years		
		TOC	TN	TOC/TN	TOC	TN	TOC/TN
		g/kg			g/kg		
Soil layer 0–20 cm							
0	>0.1	3.5	0.33	10.6	3.5	0.32	11.0
	<0.1	10.5	0.94	11.2	10.4	0.84	12.4
1O	>0.1	5.3	0.49	10.9	5.1	0.38	13.3
	<0.1	17.6	1.51	11.7	14.3	1.23	11.6
2S	>0.1	6.9	0.64	10.8	4.9	0.35	14.1
	<0.1	13.4	0.85	15.7	12.2	0.74	16.4
3E	>0.1	10.9	1.06	10.3	8.4	0.81	10.4
	<0.1	15.2	0.97	15.7	13.9	1.12	12.4
4OS	>0.1	6.3	0.53	11.8	7.0	0.46	15.2
	<0.1	16.0	1.43	11.2	13.5	0.99	13.6
5ES	>0.1	8.4	0.71	11.8	6.4	0.46	14.0
	<0.1	14.3	1.20	11.9	13.5	1.11	12.2
Soil layer 20–35 cm							
0	>0.1	3.6	0.35	10.3	3.7	0.32	11.4
	<0.1	10.8	1.00	10.8	10.5	0.96	10.9
1O	>0.1	3.6	0.38	9.4	3.5	0.34	10.3
	<0.1	11.3	1.00	11.3	10.9	0.99	11.0
2S	>0.1	3.8	0.32	11.9	3.7	0.34	10.8
	<0.1	10.4	1.09	9.6	10.6	1.01	10.6
3E	>0.1	3.8	0.40	9.6	3.7	0.32	11.6
	<0.1	10.9	1.00	10.9	10.5	0.88	12.0
4OS	>0.1	3.7	0.32	11.6	3.8	0.30	12.7
	<0.1	10.0	0.97	10.4	11.0	1.05	10.5
5ES	>0.1	3.7	0.38	9.7	3.7	0.35	10.7
	<0.1	11.3	0.95	11.9	9.8	0.90	10.9

Table 2

Variance analysis of organic carbon (TOC) content and total nitrogen (TN) content in the soil samples (ns – not significant)

Variant	TOC	TN	TOC/TN	TOC	TN	TOC/TN
	g/kg			g/kg		
	Soil layer 0–20 cm			Soil layer 20–35 cm		
Factor I (variant)						
0	7.0	0.61	11.5	7.1	0.66	10.7
1O	10.6	0.90	11.8	7.3	0.68	10.7
2S	9.4	0.65	14.5	7.1	0.69	10.3
3E	12.1	0.99	12.2	7.2	0.65	11.1
4OS	10.7	0.85	12.6	7.1	0.66	10.7
5ES	10.6	0.87	12.2	7.1	0.64	11.1
LSD	2.96	ns	2.30	ns	ns	ns
Factor II (fraction)						
>0.1mm	6.4	0.55	11.6	3.7	0.34	10.9
<0.1mm	13.7	1.08	12.7	10.7	0.98	10.9
LSD	1.03	0.145	0.80	0.44	0.038	ns
Factor III (time)						
After 1 year	10.7	0.89	12.0	7.3	0.68	10.7
After 3 years	9.4	0.73	12.9	7.1	0.65	10.9
LSD	1.03	0.145	0.80	ns	ns	ns

As it has been shown earlier (*Mazur* 1996, *Mazur and Budzynska* 1994), the use of organic and mineral fertilizers can induce migration of labile carbon and nitrogen compounds down the soil profile. Considering this, our experiment was based on two soil layers: surface one (0–20 cm), corresponding to the arable layer, and the so-called subsoil (20–35 cm) similar to the sub-arable layer. It was shown that TOC and TN concentrations in the grain fractions in the 20–35 cm layer did not change according to the type of fertilizer, or the experimental time. The only factor affecting the TOC and TN was the grain-size. Both TOC and TN, were distributed unevenly between the fractions. The fine fraction ($\phi < 0.1$ mm) was almost three times richer in carbon and nitrogen than the coarse one ($\phi > 0.1$ mm). However, no significant differences were observed for the TOC/TN ratio values with both fractions (Table 2). The results clearly showed that under the experimental conditions no leading of carbon or nitrogen compounds to the deeper soil layers were observed.

It is known that the processes of mineralization of organic fertilizers are accompanied by humification. According to many authors (*Sotakova* 1983, *Dziadowiec* 1990) at initial steps of humification fulvic acids are more abundant than humic acids. Nevertheless, according to Dziadowiec (1990) the quantitative relations between both groups of humic compounds depend on the type of humified material. Fractional composition of organic matter, i.e. the content of fraction soluble in 0.1M HCl (C_d), humic acids (C_{HA}) and fulvic acids (C_{FA}) expressed as percent of TOC of the samples collected from the 0–20 cm layer is presented in Table 3, while the results of statistical analysis of the 0–20 cm and 20–35 cm layers is given in Tables 4 and 5.

Table 3

Humus fraction content as a percent of TOC in 0–20 cm soil layer

Variant	Fraction (mm)	After 1 year				After 3 years			
		C_d	C_{HA}	C_{FA}	C_{HA}/C_{FA}	C_d	C_{HA}	C_{FA}	C_{HA}/C_{FA}
0	>0.1	5.7	24.1	30.5	0.79	2.6	26.3	28.1	0.94
	<0.1	3.2	22.7	21.0	1.08	2.6	24.3	21.2	1.15
1O	>0.1	4.1	27.3	19.5	1.40	2.9	26.1	17.3	1.51
	<0.1	2.7	22.7	14.4	1.55	2.1	21.4	15.3	1.40
2S	>0.1	3.8	27.0	20.6	1.31	4.0	32.8	19.2	1.71
	<0.1	3.4	23.1	19.1	1.21	3.3	24.8	19.5	1.27
3E	>0.1	3.1	19.7	18.6	1.06	2.7	34.0	18.0	1.89
	<0.1	3.3	18.7	15.1	1.23	1.7	19.6	17.1	1.15
4OS	>0.1	4.3	34.9	29.2	1.20	2.4	20.5	19.1	1.07
	<0.1	3.2	21.3	17.4	1.24	2.5	14.8	19.8	0.75
5ES	>0.1	2.6	32.7	17.9	1.83	3.0	34.1	15.4	2.21
	<0.1	2.8	31.3	17.8	1.78	2.2	29.1	19.0	1.54

The fine-grained fractions of soil samples taken after one year of incubation had a similar percentage of humic and fulvic acids ($C_{HA}/C_{FA} \sim 1.08$), while in the coarse-grained fraction the share of fulvic acids was by 6.4% higher. After three years no significant changes in fractional composition was found for the soil without additives.

Introduction of organic fertilizers to the surface soil layer (0–20 cm) caused a significant decrease of the share of fulvic acids in both grain fractions (Table 3 and 4).

Statistical analysis showed also the significance of the interaction between the experimental factor II and III (grain fraction and experimental time, results not given in Table 4). The share of fulvic acids in the coarse fraction was higher in the samples taken after one year than those collected after three years. A reverse relationship was noted for fulvic acids concentrations in the fine fraction ($\phi<0.1$ mm, Table 3).

Changes in the share of humic acids in grain fractions depended also on the type of organic fertilizer introduced into soil. In general more humic acids and fulvic acids were found in the coarse fraction than in the fine fraction ($\phi<0.1$ mm).

Table 4

Results of variance analysis of humus fraction content as a percent of TOC in the 0–20 cm soil layer (ns – not significant)

Treatment	C_d	C_{HA}	C_{FA}	C_{HA}/C_{FA}
Factor I (variant)				
0	3.5	24.4	25.2	0.99
1O	3.0	24.4	16.6	1.47
2S	3.6	26.9	19.6	1.38
3E	2.7	23.0	17.2	1.33
4OS	3.1	22.9	21.4	1.07
5ES	2.7	31.8	17.5	1.84
LSD	ns	ns	6.18	0.407
Factor II (fraction)				
> 0.1 mm	3.4	28.3	21.1	1.41
< 0.1 mm	2.8	22.8	18.1	1.28
LSD	ns	3.64	2.15	ns
Factor III (time)				
After 1 year	3.5	25.5	20.1	1.31
After 3 years	2.7	25.7	19.1	1.38
LSD	0.74	ns	ns	ns

Table 5

Results of variance analysis of the humus fraction content as a percent of TOC in the 20–35 cm soil layer (ns – not significant)

Treatment	C_{deka}	C_{HA}	C_{FA}	C_{HA}/C_{FA}
Factor I (variant)				
0	4.1	23.3	25.3	0.95
1O	2.9	22.8	25.0	0.95
2S	3.1	22.3	26.6	0.87
3E	2.7	23.8	27.4	0.91
4OS	3.0	23.9	27.5	0.90
5ES	3.8	23.4	26.4	0.93
LSD	ns	ns	ns	ns
Factor II (fraction)				
> 0.1 mm	3.7	23.5	31.6	0.74
< 0.1 mm	2.8	22.9	21.1	1.09
LSD	0.57	ns	2.28	0.081
Factor III (time)				
After 1 year	3.2	23.6	27.2	0.89
After 3 years	3.3	22.9	25.5	0.94
LSD	ns	ns	ns	ns

A consequence of changes in the share of humic and fulvic acids after the introduction of organic fertilizers into soil were the changing C_{HA}/C_{FA} ratio values (Tables 3 and 4). The humic acids to fulvic acids ratio in the fertilized soil samples were on average higher than in the control soil. However, neither the influence of experimental time nor grain fraction on this ratio was noted.

The percentages of C_{HA}, C_{FA} and C_d also showed that organic matter of a higher share of the insoluble portion of organic matter was better accumulated in the fine-grained fraction ($\phi<0.1$ mm) than in the coarse-grained fraction.

No effects of the added fertilizers used nor experimental time on fractional composition of organic matter were found in the 20–35 cm soil layer (Table 5). This finding supported the lack of migration of carbon compounds down the soil profile. The only factor differentiating organic matter properties was the degree of grain size. Organic matter of the $\phi<0.1$ mm fraction had a lower share of the fulvic fraction than the $\phi>0.1$ mm, thus the C_{HA}/C_{FA} ratio was higher than in the coarse-grain fraction.

The results of the study showed that organic matter of the fine-grained fraction had a higher humification degree than that of the coarse-grained fraction (higher values of the C_{HA}/C_{FA} ratio). Moreover, it contained more substances resistant to decomposition (insoluble portion of organic matter). Therefore smaller losses of carbon and nitrogen noted in the case of fine-grained fraction were the consequence of a higher degree of humification of humus accumulated in the fine-grained fractions as compared with humic substances of coarse-grained fraction and/or the persistence of organic-mineral bonds occurring in them (*Schmidt and Kogel-Knabner* 2002, *Schulten et al.* 1993, *Tarchitzky et al.* 2000).

Conclusion

1. Fractions of the diameter smaller than 0.1mm accumulate almost three times more organic matter than the coarse fractions (>0.1 mm). Addition of organic fertilizers to soil causes an increase of organic carbon and nitrogen content in both fractions. TOC content depends on the type of the fertilizers.
2. Introduction of organic fertilizers changes the percentage of humic and fulvic acids. Organic matter accumulated in fine fraction has a higher humification degree (higher values of the C_{HA}/C_{FA} ratio) and contains more insoluble carbon fractions than the coarse fraction.
3. No migration of carbon and nitrogen compounds into deeper soil layers was noted under study conditions over three experimental years after the addition of organic fertilizers.

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Drinking water treatment from reservoirs under the impact of increasing DOC values of raw waters

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Long term investigation concerning Dissolved Organic Carbon (DOC) in central-European drinking water reservoirs indicates a significant increase of the total amount and the annual changes as well. In order to guarantee a high quality of drinking water the water companies have to consider this tendency. To gain insight into treatment process, the DOC of raw and treated waters was fractionated by gel chromatography. The highest removal efficiency could be obtained for polysaccharides (algae origin) and high-molecular size humic substances. With lower molecular size the removal efficiency was reduced. Optimizing conventional treatment trains is therefore urgently necessary. To give the water companies quick information about the efficiency of different measures a model for the calculation of the DOC-fractions and the biodegradability was developed. The model consists of two segments, «a» for raw and «b» for clear water. The results of the calculation are in good relation with data measured by gel-chromatography.

Key words: *DOC, humic substances, Gel Permeation Chromatography, drinking water reservoir.*

Introduction

The DOC (Dissolved Organic Carbon) concentration in the water of drinking water reservoirs in mountains of Middle Europe has been more than doubling during the last fifteen years. The reason of this undesirable effect is closely connected to the global climate change, which shows itself in increasing average temperatures during the winter period and more frequent heavy rain falls, e.g. during June till August (*Grunewald et al.*, 2003; *Hejzlar et al*, 2003). Nevertheless, the process is caused by much more complex factors. So, former examinations showed the influence of changing pH-values which is closely connected to acid rain (*Vogt et al.*, 2003). Another reason for this development, which is of real anthropogenic character, is brought about by the management of catchment areas. With the beginning of the 1990s, the general strategy was changed from consequent drainage systems to natural management with the result of re-wetting of peat bogs.

Water treatment facilities have to find strategies for upgrading their treatment processes, because a complete removal of the natural organic matter in form of the DOC is not possible in the case of the traditional conventional treatment trains – flocculation – fast filtration-disinfection – used in this region.

Systematic investigations have been carried out over a period of three years. The objective was the finding of general information concerning the character of the DOC. The interpretation of data set obtained during this time forms the base for a better understanding of the water treatment process applied. Additionally, a tool in the form of a model including a software package was developed, which gives the waterworks quick and detailed information concerning the removal of DOC in their treatment process under the impact of changing raw water quality.

Experimental

The data set was produced by an application of gel permeation chromatography (GPC) (*Huber and Frimmel*, 1996). The GPC-measurements were carried out using the LC-OCD (Liquid Chromatography – Organic Carbon Detection) system (DOC Labor Dr. Huber). As a result of this procedure, five extractable fractions of the DOC were obtained:

- Humic Substances (divisible into one high- and one middle-molecular fraction; = HS I, HS II)
- Building Blocks (humic substance hydrolyzates; = BB)
- Low-Molar Mass Organic Acids
  (oxidation products of organic substances; = lmA)
- Low-Molecular Weight Neutrals (not yet investigated in detail = lmN)
- Polysaccharides (cell components from algae and bacteria = P) (*Huber and Frimmel*, 1996)

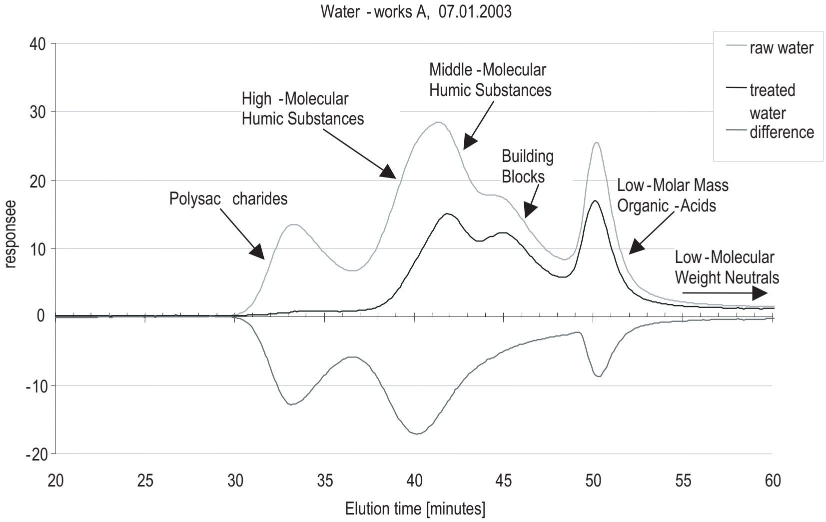

Fig. 1. Fractionation chromatograms of raw water, treated water, and difference chromatogram.

To quantify the fractions, the commercially available program FIFFIKUS (DOC Laboratory Dr. Huber, Germany) was used. Figure 1 exemplifies the OC-chromatogram of characteristic raw water in this region which is treated by conventional flocculation and filtration. The determination of the different DOC-fractions is used as the base for discussion of the DOC-character in this article.

In addition to DOC fractionation its biodegradable portion (BDOC) was determined. For BDOC measurements the water has been incubated for 28 days with a drinking water biofilm fixed to porous glass beads (*Korth et al.,* 2001). Further routine parameters produced by water authorities and waterworks such as the SAC-values at 254 and 436 nm were implemented in the interpretation of the data pool.

The statistical evaluation of the data bases on the calculation of the (annual) 10-, 50- and 90-percentiles. The minimum and maximum values are given additionally. The values are summarised by box and Whisker plots.

Results and discussion

Raw water

Figure 2 shows exemplarily the eight years tendency of the SAC_{254} value of the raw water in waterworks A.

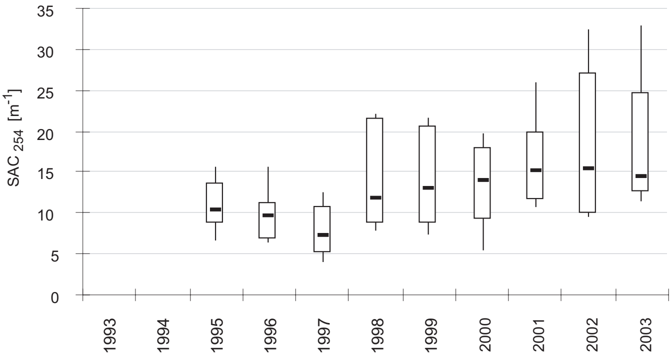

Fig. 2. Tendency of SAC_{254} of raw water in waterworks A, data set (24 measurements p.a.).

The median of the values clearly indicates the increasing level from SAC = 10 m^{-1} in 1995 up to SAC = 15 m^{-1} in 2003. The variance between the 10 and 90 percentile was becoming much more significant. So the water quality was not only changing in the average, moreover the water companies have to manage higher quality fluctuations in shorter periods.

Nevertheless, the possibilities for managing this undesirable situation are limited. One of the most important measures applied could be the optimisation of the raw water intake, because many reservoirs in Middle Europe are subjected by an annual periodic stratification. This process is shown by the temperature pattern of reservoir B in Figure 3. In comparison to that Figure 4 shows the distribution pattern of humic substances (fraction I).

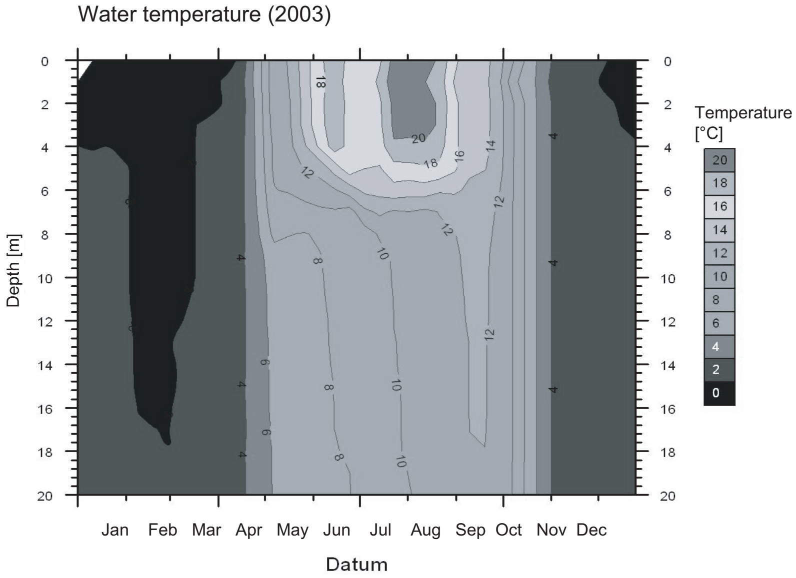

Fig. 3. Water temperature in reservoir B in 2003.

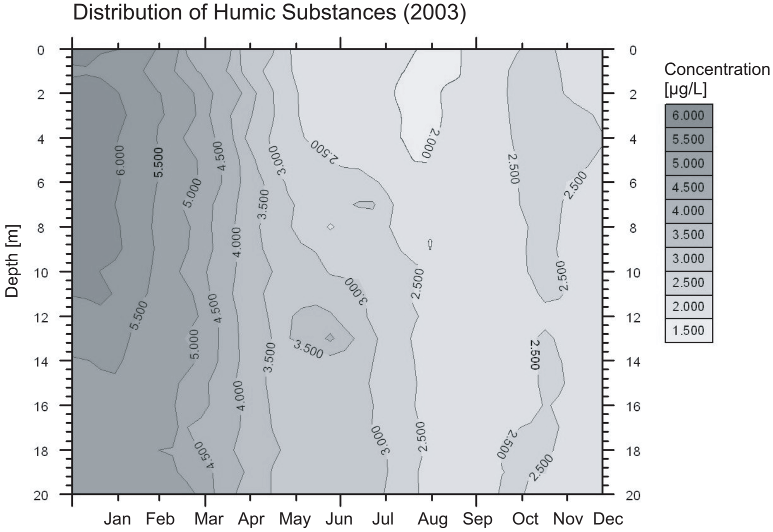

Fig. 4. Distribution of humic substances in reservoir B, 2003.

From these results a lower concentration of humic substances can be recognised in horizons of higher temperature during summer. This effect can be explained with microbial and photolytic degradation as well (*Brinkmann*, 2003; *Brinkmann et al.*, 2003). However, in general the water companies can profit only little from this effect, because the priority of water intake is given by the lowest level of turbidity and low temperatures. Very often the higher horizons during warmer periods are influenced by algal growth connected with the formation of taste and odorous compounds in the water.

Water treatment

In order to understand the complex relation between the character of the DOC and the removal efficiency during the treatment process, a systematic evaluation of the raw and clear water data sets in selected treatment trains has been carried out over several months. During the experimental period the August flood in Saxony occurred. Caused by this event the raw water quality was changing significantly and offered the possibility to check the treatment process under extreme conditions.

In Figure 5, the average removal efficiency of the different DOC-fractions is shown for two waterworks A and B. The data set consists of 26 and 22 measurements, respectively during the period from 2002 till 2004 (see Figure 5). The statistic evaluation of the data is expressed by the 10, 50, and 90-percentile values again.

The diagram shows the decreasing removal efficiency with lower molecular size. Additionally the variance, i.e. the difference between the 10- and 90-percentile values, was increasing. The best removal efficiency could be obtained for the high molecular fraction of polysaccharides, followed by the high molecular fraction of humic compounds and the other fractions Humic-II and the Building Blocks, both middle size fractions. The other fractions, the low molecular acids (lmA) and low molecular neutrals (lmN) are not under discussion in this article.

However, all the data discussed were obtained during different raw water qualities. Especially the flood (August 2002) had a significant impact on the removal efficiency of DOC in general. During that time the water companies tried to optimise their treatment trains by additional dosage of flocculation and several aid agents as well. Therefore, the discussion of the data has to consider these changing treatment conditions.

In Figures 6 and 7 the removal efficiency concerning the different DOC-fraction is exemplarily shown for waterworks A. The data-set was measured during the time from 2002 till 2004, and consists of 25 data per fraction.

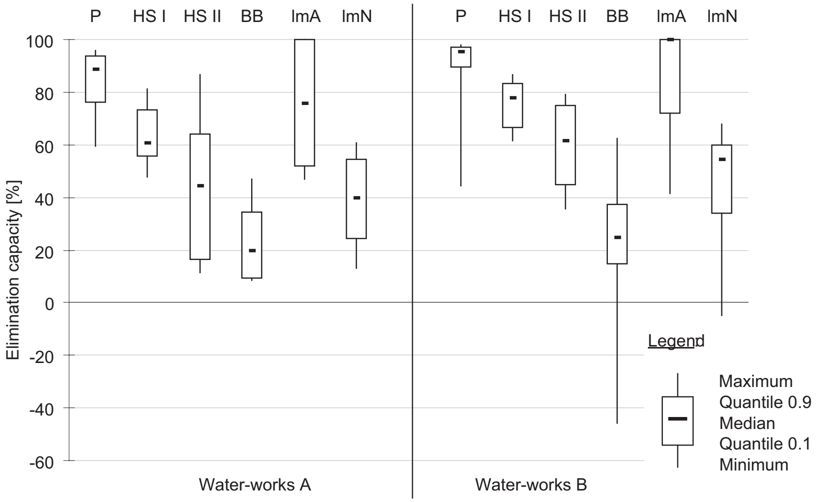

Fig. 5. Average removal efficiency of the DOC-fractions for the waterworks A and B.

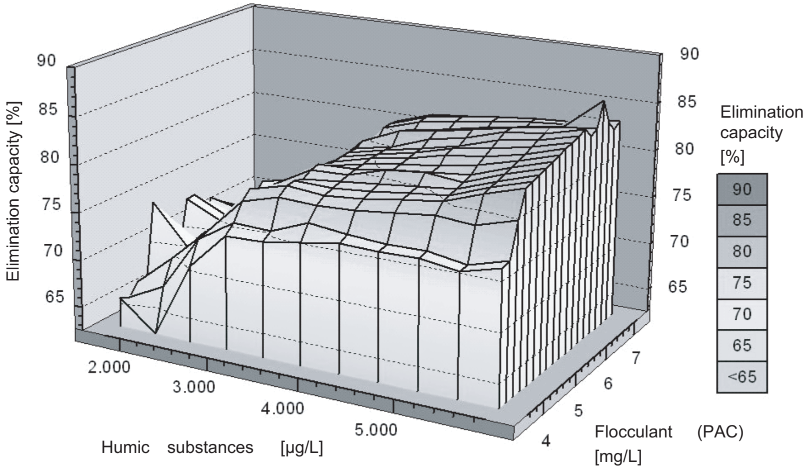

Fig. 6. Dependence of humic substances removal on the flocculant dosage and the starting concentration.

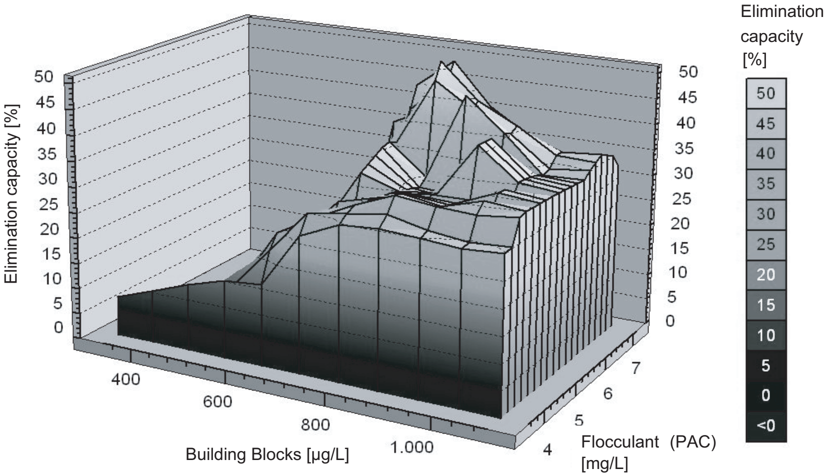

Fig. 7. Dependence of Building Blocks removal on the flocculant dosage and the starting concentration.

The removal efficiency of the high molecular humic fraction HS I in Figure 6 shows a relatively clear tendency. Higher percentage of removal can be expected with higher concentration of that fraction in the raw water and higher dose of flocculant (polyaluminium chloride, PAC).

In contrast to that, in the case of the lower molecular size fraction of Building Blocks the removal efficiency does not follow this rule. A maximum was obtained for medium levels in the raw water removed by high doses of flocculant. This effect means the better coagulation of high size molecules as it is well known. Nevertheless, treatment measures for improving the efficiency for reduction of the low molecular fraction are difficult and complex. The systematic optimisation of different treatment trains requires measures concerning the adjustment of parameters such as pH, type and dose of flocculant as well as type and dose of aid agents.

Therefore, the water companies need quick information concerning the removal efficiency of different DOC fractions. On the other hand, the method of gel permeation chromatography with OC-detection is complex and expensive. Many companies do not have the possibility to use this special kind of analysis. The possible solution could be found in the design of a model which is further developed to a software package as described in the following chapter.

Model concept

The intention of this development is based on the determination of online parameters such as SAC_{254} and DOC. Both parameters, being used as model input, are common and available for most of the waterworks. The output of the model is special information concerning the concentration of single DOC-fractions (HS I, HS II and BB) and their biodegradability. The connection between the online/onsite routine- and special data was found by correlation coefficients which where evaluated by statistical control (*Grunewald and Schmidt et al.*, 2005, in press.). The model consists of two different segments:

a) The calculation of the DOC-fractions and BDOC by SAC_{254} only and

b) The calculation of the DOC fractions and BDOC using the SAC_{254} and the DOC.

Exemplarily, in Figure 8 the correlation between the high-molecular humic fraction (HS I) and the SAC_{254} is shown in the case of raw water of waterworks A. The correlation coefficients were calculated for all fractions measured. The best correlation was always obtained in the case of the raw water. Therefore model segment «a» is more suitable for calculation of raw waters.

The estimation of total BDOC and their content to the single humic fractions in raw waters bases on empirically found portions on the single fractions and on the correlation coefficients with the SAC value. In Figure 9 the model structure of segment «a» is shown.

For clear water especially in the case of the fractions HS II and BB a low correlation with the corresponding SAC-values was measured. In order to improve the model the DOC was introduced in the concept. This step is carried out by segment «b». Figure 10 shows the structure of segment «b».

In segment «b» the concentration of remaining fractions such as polysaccharides, low molecular acids and amphiphilic substances are put to zero. This is possible, because in all treated waters these compounds were determined with a very low concentration. In segment «b» the fractions HS II and BB were summarised. They were calculated by the difference between DOC and HS I, which was determined in relation to segment «a». According to the quality of correlation coefficients segment «b» is more suitable for treated clear waters.

The estimation of the BDOC by segment «b» bases on the percentage amount of the BDOC on the DOC obtained from more than 30 measurements in clear water. In this segment the assessment was made that most of the easily biodegradable organic matter coming from algae was completely removed during treatment.

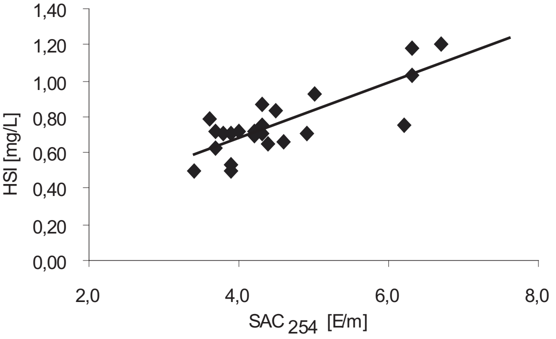

Fig. 8. Correlation between high-molecular humic substances and the SAC_{254} for treated water of water-works A.

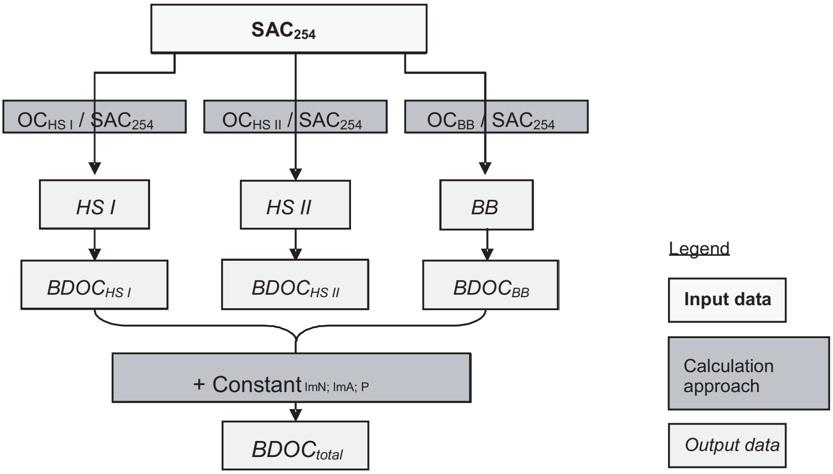

Fig. 9. Structure of model segment «a» for untreated raw waters.

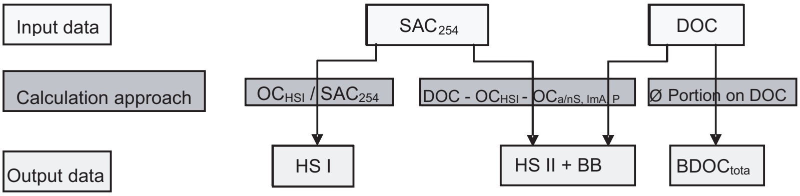

Fig. 10. Structure of model segment «b» for treated clear waters.

Assessment of calculated data

The Figure 10 shows the course of the HS I fraction in the raw water of waterworks A using segment «a» from January 2002 till April 2004. The comparison between measured and calculated data shows a suitable correlation.

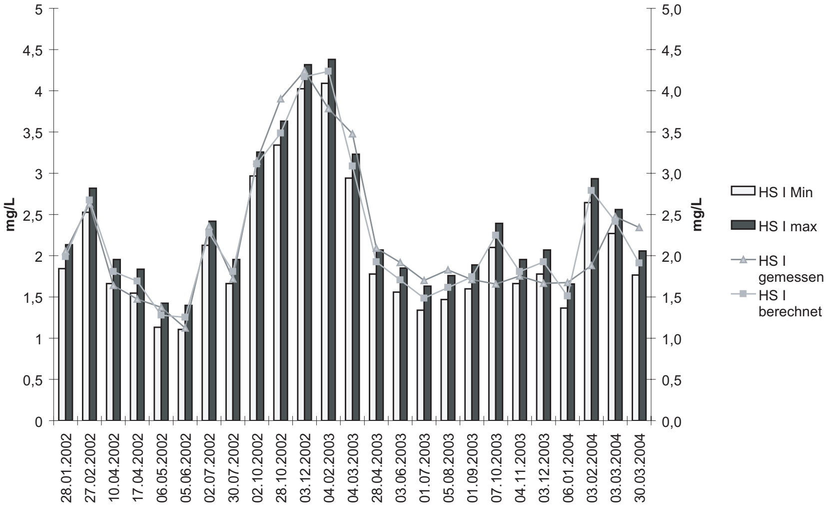

Fig. 11. Course of fraction HS I in raw water of waterworks A – Calculation with segment «a».

Corresponding to Figure 10 in Diagram 11 the HS I fraction of the treated water by segment «a» is shown for the same period. The calculation of lower molecular fractions HS II and BB was made by segment «b». The comparison of calculated and measured data in both figures is also suitable. Nevertheless, the range between the calculated minimum and maximum is wider in comparison to the raw water.

In general, all the results obtained by application of segment «a» in the case of raw water and segment «b» for treated waters have someting in common: that they show a good correlation with the measured values. The position and values of maxima and minima of measured and calculated data are very close. This fact indicates that especially significant changes in the water quality can be expressed by the model with sufficient accuracy. On the other hand, a lower quality of data was obtained for the biodegradable fraction.

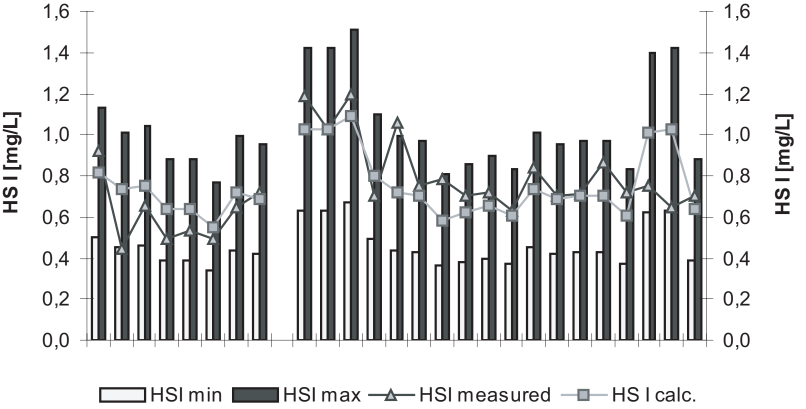

Fig. 12. Course of fraction HS I in treated water of waterworks A – Calculation with segment «b».

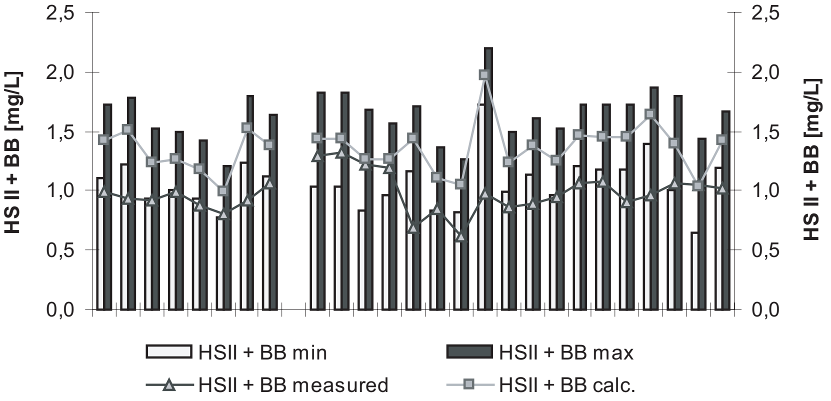

Fig. 13. Course of fraction HS II + BB in treated water of waterworks A – Calculation with segment «b».

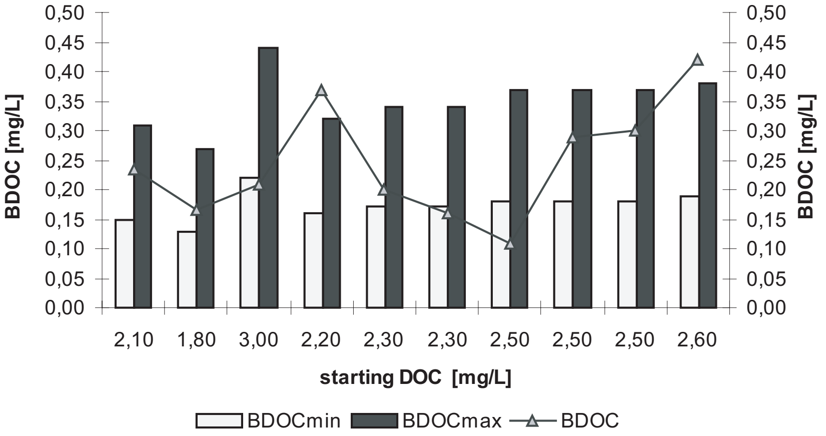

Fig. 14. Course of BDOC in treated water of waterworks A – Calculation with segment «b».

In Figure 13 the measured data set available is compared with calculated values. Although the general level of BDOC (calc.) is comparable with the measured data, the fraction's confidence level could not be reached. One of the reasons could be that the number of measured data was too low to estimate a sufficient correlation coefficient, which is needed for the model approach.

Conclusions

The long term data set produced for raw as well as clear waters by applying the method of gel permeation chromatography coupled with OC-detection forms a sufficient pool to estimate several correlation coefficients of these special data with online/onsite values such as SAC-254 and DOC.

The correlation coefficients are used for the design of a model including software for the calculation of the DOC-fractions according the molecular size and its biodegradability.

The correlation of calculated data with the measured values is sufficient to describe a changing annual tendency as well as the absolute values of the fraction. Improvements are necessary for the calculation of the BDOC. This could be reached by enlargement of the data set measured.

Anyway, this model approach provides water works with the opportunities to calculate the DOC fractions and the biodegradable portion on DOC by means of basic parameters. The author still works on the described approach, and the final version is going to be part of a doctorate.

Acknowledgements

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Paramagnetic properties of humic substances extracted from Albeluvisols of North-Eastern European Russia

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Free radicals content has been determined in humic acids (HAs). Samples of HAs were collected from soil horizons along a gradient of increasing hydromorphology. The soils sampled were: a Siltic Albeluvisol, a Silti-Stagnic Albeluvisol, and a Stagni-Histic Albeluvisol (Siltic). Free radicals' content in HA molecular structure increases with increasing soil moisture. High free radicals content in HAs from all horizons of the Stagni-Histic Albeluvisol (Siltic) is apparently caused by biohydrothermal conditions of humus formation in this soil. It has been found out that in all studied soils free radicals content in HAs decreases downwards the soil profile.

Key words: *humic acids, fulvic acids; free radicals; Albeluvisols.*

Introduction

Since electron paramagnetic resonance (EPR) was discovered by E.K. Zavoisky in 1944, very detailed data on the structure of many organic and inorganic compounds have been obtained with the help of this method. The first publication, dealing with the EPR method application to biological systems research appeared nine years later after the discovery of the EPR effect (*Commoner et al.* 1954).

Already in the first paper it was shown that many biological systems contain free radicals, and a certain correlation was shown to exist between radicals content and the material's metabolic activity. It turned out that to answer the question, what kind of free radicals precisely take part in particular processes, was much more difficult. It was established that many redox processes in biological systems pass through monoelectron stages. This fact suggests that free radicals are formed as intermediate products (*Michaelis* 1931).

In recent years the EPR method has been successfully used to study soil organic matter. It is highly sensitive and provides diverse structural information about chemical compounds containing free radicals. The latter play an important part in the majority of chemical reactions between organic, organomineral and mineral compounds in soils due to their large store of energy and high activity (*Chukov* 1998, 2001).

Numerous publications (*Flaig* 1964; *Shnitzer and Skinner* 1969) show that these radicals directly participate in the graded polymerization process of humus formation in soils. Studying the free radicals activity in humic substances and soils allows to make conclusions about the ability of these substances and soils to react, which is a top issue in the complex problem of formation and transformation of organic matter in soils.

The important role of non-paired (free) electrons in any chemical or biological system is caused by their large reserve of energy and, accordingly, by a high activity of these electrons. It is known that most of chemical reactions proceed through a stage at which free radicals with a non-paired electron are formed. A free radical may have a short lifetime (for example, in reactions of inorganic molecules) or it may be stable (usually in complex organic systems). In the literature devoted to the use of the EPR method studying natural high-molecular organic compounds, the term «paramagnetic center» is accepted to describe groups of atoms or parts of molecules being possessed by a non-paired electron (*Verts and Bolton* 1975, *Neto et al.* 1991, *Chukov* 1998, 2001).

All substances containing non-paired electrons can be divided into two big groups. In the first group of substances such electrons are connected either to the whole molecule, or to its biggest part. They move along strongly delocalized molecular orbits and cause activity of atomic groups included into the molecule. Studying these non-paired delocalized electrons is important for understanding specific features of the reactions proceeding through the free radicals stage and the mechanism of polymerization or formation of intermediate products in some biochemical reactions.

The second group includes such compounds in which a non-paired electron is connected to only one atom and does not move along the delocalized molecular orbit which incorporates many atoms. That is why a study of electron paramagnetic resonance in biological and biochemical systems containing paramagnetic metal atoms or ions often provides very useful information about the degree of oxidation, bonds and molecular surrounding of the atom under study. Analysis of publications shows that both various bioclimatic factors and human activity exert a strong influence on free radicals content in humic substances (*Babanin et al.* 1977, *Neto et al.* 1994).

Materials and methods

Samples of HAs and FAs were collected from soil horizons along a gradient of increasing hydromorphology. The soils sampled were: a Siltic Albeluvisol, a Silti-Stagnic Albeluvisol, and a Stagni-Histic Albeluvisol (Siltic) (*FAO, ISRIC and ISSS* 1998).

The soils were developed on mantle loams in the middle taiga zone of the Komi Republic. The sampling site was located on a hilltop, 6 km west from the city of Syktyvkar. The site was at the Maksimov field station of the Institute of Biology, Komi Science Center, Ural Division of Russian Academy of Sciences (61°39.6′ 50°41.0′).

Morphological descriptions of the studied soils are given in Table 1. Physico-chemical and biological properties are presented in Table 2.

Table 1

Morphological descriptions of soils

Soil	Horizon	Depth of sampling, cm	Soil horizon description	Site description
Siltic Albeluvisol	Oe	0–5	Weakly decomposed peaty, very dark brown litter covered by fallen leaves, needles, and branches; pierced by roots and fungal hypha, moist.	Altitude 170 m a.s.l., the top of a hill on the interfluve and the «ridge» of a microhigh 1.5 m height. Aspen-birch-spruce forest with bilberry, cowberry and green mosses. Many dry and rotten tree branches and trunks.
	AEh	5–7(10)	Light gray, light silty loam, friable, many roots.	
	E1	7(10)–18	Whitish with dark brown iron-organic soft segregations, strong platy, silty light loam, friable, roots are common.	
	E2	18–35	Whitish, platy, light silty loam, weakly compact, few roots, glossic boundary.	
Silti-Stagnic Albeluvisol	Oe	0–5	Dark brown peaty weakly decomposed litter with many inclusions of small branches and needles; many roots, at the bottom of the layer – carbonized plant residues.	Profile is located on the gentle slope of the same microheight. The composition of the tree stand is the same: spruce-birch-aspen-pine; the undergrowth is composed of wild sorrow and *Rosa sp.* In the ground cover bilberry and green mosses prevail.
	Ehg	5–7(12)	Dark brown and gray with reddish hue are alternating, apedal, silty light loam (loamy sand), moist, friable, concretions/nodules are common, many roots.	
	Eg1	7(12)–23	Whitish with dark brown humus streaks to the depth of 12 cm, loamy sand, many pores – there are vesicular voids, 3 mm large, vertical planes with humified walls, compact, concretions and nodules, to 1 mm in diameter, are frequent, not many roots.	
	Eg2	23–31(40)	Light gray, platy-crumby, silty light loam; with finer voids and lower abundance on concretions and nodules than in the previous horizon, moist, compact, few roots, glossic boundary.	
Stagni-Histic Albeluvisol (Siltic).	Oe	0–12	Sphagnum peat with inclusions: branches, needles, leaves; many roots, weakly moist.	Profile is located in a microhollow between small flat microelevations. Birch-spruce Sphagnum-Polytrichum forest. The cover of Sphagnum is prominent near the peat.
	Ehg	12–20	Brown, stratified, nodules (1–3 mm in size) are common, and some vertical planes with organic coatings. Roots up to 0.5 cm in diameter.	
	Eg1	20–28(30)	Light brown, apedal, thixotropic, coarse silty loam, compact, moist, pierced by vertical fissures with humified walls. Concretions and nodules up to 3 mm in diameter are frequent, they are more abundant in rusty mottles; few fine tree roots.	
	Eg2	28(30)–37	Rusty pale brown with bluish veins, silty light loam, compact, much reddish brown segregation, roots of horse tail, glossic boundary.	

Table 2

Physicochemical properties and quantity of fungi and bacteria in soils

Horizon	Hygroscopic moisture, %	Bulk density G/cm^3	pH		TOC (w/w), %	Quantity of fungi*, thousand cells/soil g	Quantity of bacteria*, thousand cells/soil g
			water	1 N KCl			
Siltic Albeluvisol							
Oe	13.3	0.10	5.3	4.5	33.5	208	374
AEh	10.2	n.d.	4.1	3.3	1.77	448	31
E1	9.0	0.90	5.1	4.0	0.45	1	27
E2	8.4	1.47	5.1	4.7	0.35	2	21
Silti-Stagnic Albeluvisol							
Oe	14.2	0.10	5.4	4.6	47.4	141	440
Ehg	12.1	n.d.	4.1	3.8	1.63	345	–
Eg1	12.3	1.53	4.4	4.1	0.57	1	11
Eg2	10.7	1.63	4.5	4.0	0.16	2.0	16.7
Stagni-Histic Albeluvisol (Siltic)							
Oe	20.0	0.15	4.5	3.6	37.6	56	41
Ehg	15.0	1.67	4.8	3.9	0.73	2	3
Eg1	15.0	1.69	5.1	3.8	0.34	1	6
Eg2	18.0	1.53	5.1	3.9	0.25	1	1

* – (*Khabibullina, 2001*) n.d. – not determined.

Preparations of humic and fulvic acids were extracted according to M. M. Kononova and N. P. Belchikova methods (*Kononova and Belchikova* 1961). Electron paramagnetic resonance (EPR) spectra of the samples under study were recorded with a SE/X2547 Radiopan spectrometer.

The experimental conditions were: modulation amplitude 1.6 Gauss peak to peak for free radicals (semiquinone), temperature 25 °C, microwave power 20–40 mW, microwave frequency 9.372 GHz. The relative area of EPR signals was obtained by approximation ($\Delta H^2 \times I$, ΔH – line width and I – signal swing) (*Neto et al.* 1991). Diphenylpicrylhydrazyl with a known content of radicals was used as a standard for the absolute concentration of free radicals. C, N and H contents of HAs and FAs were estimated with a Carlo Erba CHNS-O-EA1108 microanalyzer.

Results and discussion

The goal of this work was to determine the dependence of free radicals content in HAs and FAs on the degree of moistening of the studied soils.

Figure 1 shows an EPR spectrum typical for all investigated HAs and FAs.

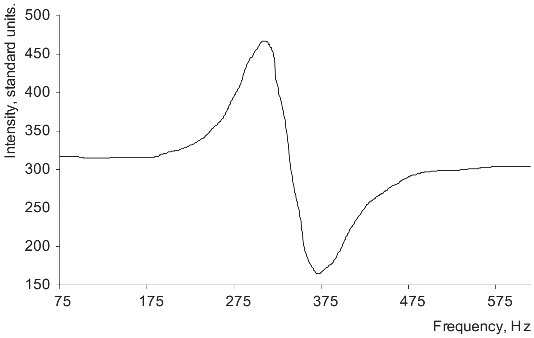

Fig. 1. The EPR spectrum typical for all investigated humic substances, according to the example of humic acids extracted from E hor. of Albeluvisols.

All the spectra have one intensive narrow line with g = 2.003 clearly suggesting presence of free radicals in structure of HAs and FAs. The obtained g – factor is close to that of a free electron (g = 2.0023), which points either to existence of strongly delocalized molecular orbits, or to such a state where the orbital component of the electron's magnetic moment is close to zero (*Verts and Bolton* 1975, *Chukov and Gurov* 1984). Calculation of the integral intensity of the absorption line allowed estimating the non-paired electrons content in the humic acid samples. It has been found out that the paramagnetic centers content in HA molecules from Stagni-Histic Albeluvisol (Siltic) is almost twice as higher than in HAs of Siltic Albeluvisol and Silti-Stagnic Albeluvisol.

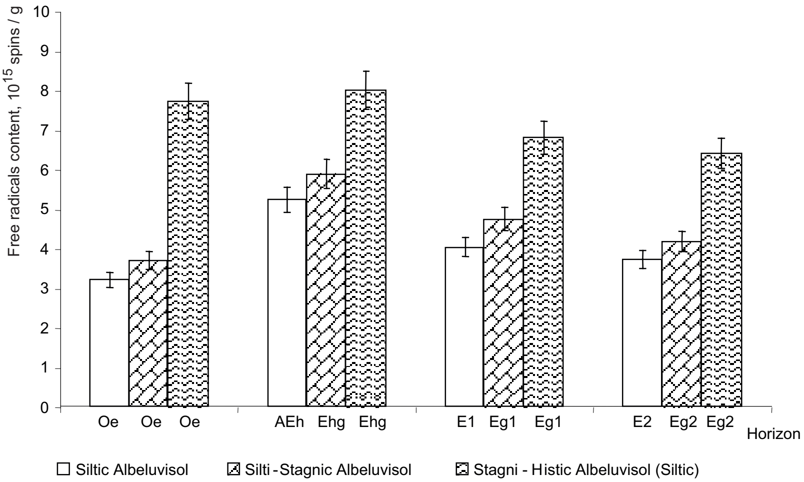

Fig. 2. Concentration of paramagnetic centers in humic acids extracted from soils of different degrees of moistening.

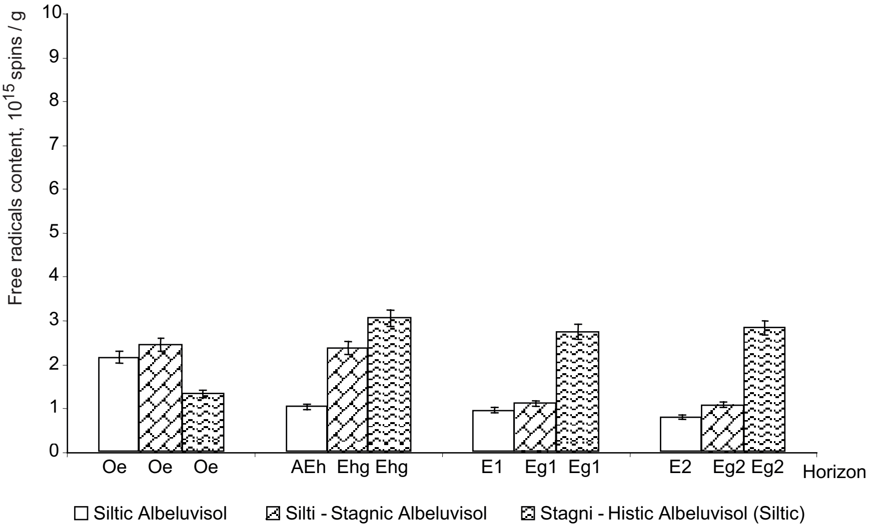

Fig. 3. Concentration of paramagnetic centers in fulvic acids extracted from soils of different degrees of moistening.

Figures 2 and 3 show how free radicals content varies in HA and FA preparations of soils of different degrees of moistening.

Figure 2 shows, free radicals content in HA molecular structure increases with increasing soil moisture. High free radicals content in all horizons of Stagni-Histic Albeluvisol (Siltic) is apparently caused by biohydrothermal conditions of humus formation in Stagni-Histic Albeluvisol (Siltic). Under these conditions all biochemical processes are hindered, which favors free radicals accumulation in HA molecules. It has been found out that in all studied soils free radicals content in HAs decreases downwards the soil profile; this proves a higher thermodynamic stability of the HA molecules from mineral horizons as compared to those from organic horizons. An exception is the HAs extracted from litters, in which free radicals content is much lower than that in the HAs from the underlying horizons. This must be due to the fact that the HAs extracted from the litters contain mainly weakly humified pro-humic acids, in which the free radicals content is strongly dependent on the biochemical composition of the incoming plant fall.

FA preparations show a slightly different picture with regard to the variation of free radicals content along the soil profiles (Figure 3). For example, FAs from Siltic Albeluvisol demonstrate more than a two time downward decrease in free radicals content within the set of mineral horizons. Thus, it can be concluded that for Siltic Albeluvisol and Silti-Stagnic Albeluvisol FA preparations from mineral horizons are biothermodynamically more stable than analogous FAs from organic horizons.

As for Stagni-Histic Albeluvisol (Siltic), its organic horizon clearly falls out of the pattern observed in the other two soils, which might be a result of long-term formation and low biochemical activity of peat in this horizon. In other respects, variations in free radicals content in FA and HA preparations along the profile of Stagni-Histic Albeluvisol (Siltic) are similar.

Conclusion

Thus, our research shows that in the studied soils the CPC is from 1.5 to 5 times higher in HA than in FA preparations. This causes higher ability of HAs for polymerization and complex formation based on the radical mechanism. The CPC in HA preparations decreases downwards the studied soil profiles. This indicates higher stability of HA molecules in lower mineral horizons as compared to the HAs in the organic horizons.

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Applications of capillary electrophoresis in aquatic dissolved organic matter analysis

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Capillary zone electrophoresis (CZE) has been effective for the separation of many organic biomacromolecules. In the present study the CZE with diode array UV detection at three different wavelengths (210, 254 and 280 nm) was applied to characterize dissolved organic matter (DOM) from aquatic environmental samples including lake- and seawater and sediment pore-water. The separation of DOM was carried out using polyvinyl alcohol (PVA) coated silica capillaries with phosphate background electrolyte at pH 6.8. The obtained results indicate the potential of this method for the fingerprint characterization of DOM from different pore-water sources. Thus the method could be useful in natural organic matter studies.

Key words: *CZE; Polyvinyl alcohol; Dissolved organic matter; Pore water.*

Introduction

The content and composition of organic matter are the important characteristics determining the quality of natural waters. In natural aquatic environments the dissolved organic matter is mostly in the form of humic substances (HS) where it constitutes between 10 and 20% and more than 80% of dissolved organic carbon, respectively, for open ocean, river and lake waters (*Harvey et al.* 1983, *Harvey and Boran* 1985). HS are considered as a general category of naturally occurring, biogenic, heterogeneous organic substances characterized by the yellow to black color, high molecular weight and refractory.

The porewater DOM is composed of defined organic compounds like fatty acids, free sugars and amino acids. The main fraction of porewater DOM consists largely of humic substances with soluble proteins, carbohydrates other non-humic macromolecules (*O'Loughlin and Chin* 2004). Lake-sediment pore-water DOM is thought to play an important role in both carbon remineralization and preservation in bottom sediments. During remineralization the organic matter in sediments generally passes through DOM intermediates of smaller molecular weight as it is oxidized to CO_2. The DOM is formed during the microbial decay of particulate organic matter in sediments. Therefore the concentrations and characteristics of DOM in sediment pore water may be as indicator of the susceptibility of sediment organic matter to mineralization or burial. It has been noted that low concentrations of DOM suggest rapid turnover and organic matter mineralization while high concentrations would indicate slow turnover, long residence times and the potential for organic matter accumulation (*Amon and Benner* 1996, *Burdige* 2001, *Burdige et al.* 2004, *Kappler et al.* 2001).

The ordinary water analysis does not cover the characterization or fractionation of highly complex, structurally heterogeneous mixture of dissolved and colloidal organic matter. Capillary zone electrophoresis (CZE) has been effective for the separation of many organic biomacromolecules due to the high separation efficiency. The results indicate the potential of this method for the fingerprint characterization of DOM from different sources (*Fetsch and Havel* 1998, *Lepane* 2001a, 2001b, *Schmitt-Kopplin et al.* 1998, *Schmitt-Kopplin and Junkers* 2003, *Parlanti et al.* 2002). Recently, the application of polyvinyl alcohol (PVA) coated capillaries for the separation of aquatic humic substances at low concentration ranges (up to 0.04 mg ml^{-1}) was found to yield moderately resolved electropherograms (*Peuravuori et al.* 2004).

The aim of this study was to analyze different aquatic samples in order to address the following questions: (1) The possibility to separate original, not concentrated aquatic DOM? (2) Separation of DOM in pore-water samples? (3) Differentiation of porewater DOM using electrophoretic separation data. According to the knowledge of the author the pore-water DOM has not been separated by CZE with coated capillaries before.

Materials and methods

Reagents

All solutions and buffers were prepared using analytical grade chemicals and MilliQ water. 0.02 M phosphate buffer with pH 6.8 was prepared from sodium dihydrogen phosphate and disodium hydrogenphosphate dissolving appropriate amounts in MilliQ water. All solutions were degassed and filtered through 0.45 μm Millipore filters.

Instrumentation and analysis

All CZE experiments were performed with $HP^{3D}CE$ capillary electrophoresis system (Hewlett Packard) equipped with diode array detector at wavelengths 210, 254 and 280 nm. Operation of the instrument, data collection and analyses were controlled by ChemStation system software. The polarity was negative. Samples were injected hydrodynamically at the temperature of 25 °C with 5 kPa pressure for 10 s.

Polyvinyl alcohol (PVA) coated capillary from Agilent (G1600–61419) was used for separation experiments. Capillary had a total length of 64.5 cm (56.0 cm to the detector). The capillary (100 μm i.d.) was rinsed first 15 min with the phosphate buffer and second conditioned 2 min with the analysis voltage of –10 kV. Between the runs 4 min rinse with the phosphate buffer was performed. At the end of the experiments the capillary was rinsed with the phosphate buffer for 5 min, with water for 15 min and dried with air for 10 min.

Samples

Seawater sample was taken from the Baltic Sea near Maasi and lake water sample from the Lake Koigi (island Saaremaa, Estonia). The isolation procedure as well the chromatographic and electrophoretic characterization of the samples have been reported previously (*Lepane* 1999, 2001).

The sediment samples were collected from two lakes in Estonia: Lake Harku (59° 25′ N, 24° 37′ E) and Lake Karujärv (58° 23′ N, 22° 13′ E). Detailed descriptions of the lakes and samples were presented earlier (*Lepane et al.* 2004, *Mäemets* 1977). Sampling was performed in 1994 with a modified Livingstone-Vallentyne piston corer. The coring sites were chosen in the accumulation part of the lakes at similar depths. In Lake Harku coring was performed at a depth of 2.5 m (northeastern part of the lake, the deepest place), in Lake Karujärv at 3.0 m (in the central basin). The length of the cores taken in Lake Harku was 46 cm and in Lake Karujärv 32 cm. The cores were immediately cut into subsamples and stored at –18 °C. For analysis the samples were let thaw slowly at 4 °C. The water emerged on the solid phase of sediment samples was separated and clarified by centrifugation (3500 rpm for 30 min). Before electrophoresis pore-water samples were filtered through a glass-fiber filter (type GF/F, Whatman).

Results and discussion

CZE of DOM

Characterization of the aquatic DOM ideally should be performed in the original matrix of the sample concerned. When the sample has been isolated, concentrated or modified the results may not be representative of the original sample or DOM structure and properties. For that reason the pore-water samples in present study have been analyzed in natural concentrations. The centrifugation of pore-water samples possibly caused some changes in the samples because of the colloidal nature of DOM. The obtained supernatant was analyzed by CZE and thus the water extraction of sediment was avoided. The water extraction has been reported to cause increased DOM concentrations (*Akkanen et al.* 2005). Another possible sample manipulation that is essential to perform before CZE analysis is the filtration of the sample through the 0.45 μm filter. The procedure might remove at least a part of macromolecules and thus change the original sample composition.

Results of DOM separation by CZE depend on buffer pH and concentration and in analyzing aquatic natural DOM samples they should remain close to original values. Parameters that could influence the efficiency of electrophoretic separation such as buffer pH, composition, concentration, injection time of the sample were optimized for the aquatic humic substances in an earlier study (*Peuravuori et al.* 2004). In the pH range of 5 to 9 the humic substances have negative charge thus making the electrophoretic separation possible. To avoid possible interaction with humic constituents and the silica capillary wall and to reduce EOF it is useful to perform CZE analysis with coated capillaries. The CZE with PVA coating has shown good resolution and reproducible results for the isolated aquatic humic and fulvic acids (*Peuravuori et al.* 2005).

As humic and fulvic acids are the major components of aquatic DOM, the electropherograms of DOM should be of similar shape. The usual separation pattern for humic substances is a broad peak with shoulders, like presented in Figure 1 for lakewater. An example of separation under optimized conditions for seawater is shown in Figure 2.

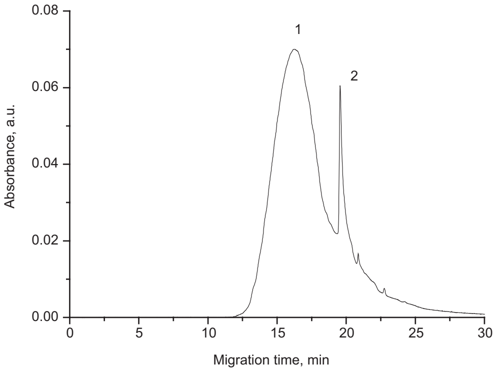

Fig. 1. Electropherogram of lakewater humic substances. For experimental conditions see text. The numbers denote migration times for peak maxima (1) 16.25 and (2) 19.61 min.

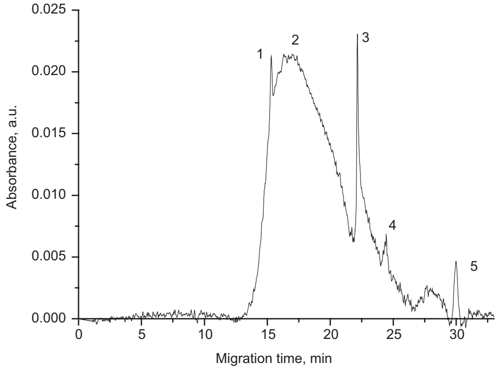

Fig. 2. Separation of seawater humic substances by CZE with background correction. Electrophoretic fractions indicated by numbers; corresponding migration times for peak maxima: (1) 15.21, (2) 17.00, (3) 22.25, (4) 24.66, (5) 29.82 min.

Interpretations of the electrophoretic fractions obtained can be done according to the migration times of different types of compounds. The main broad fraction is characteristic to humic-type compounds. The narrow fractions are characteristic to defined compounds (for example mono- and polyfunctional fatty acids (*Hofrichter and Steinbüchel* 2001)) but for exact identification CZE coupling with MS detector is essential.

CZE of pore-water DOM

UV detection is mostly used in humic substances separations but recently diode array and laser-induced fluorescence detections have been applied (*Havel and Fetsch* 2000). In the present study the CZE with diode array UV detection at three different wavelengths (210, 254 and 280 nm) was applied to characterize pore-water DOM.

Fig. 3 demonstrates the original electropherograms of surface layer pore water. The surface layer is considered to be the most active zone of the sediment where the biogeochemical reactions transform the organic matter from water and from sediment from deeper layers (*Wetzel* 1983, *Haglund et al.* 2003).

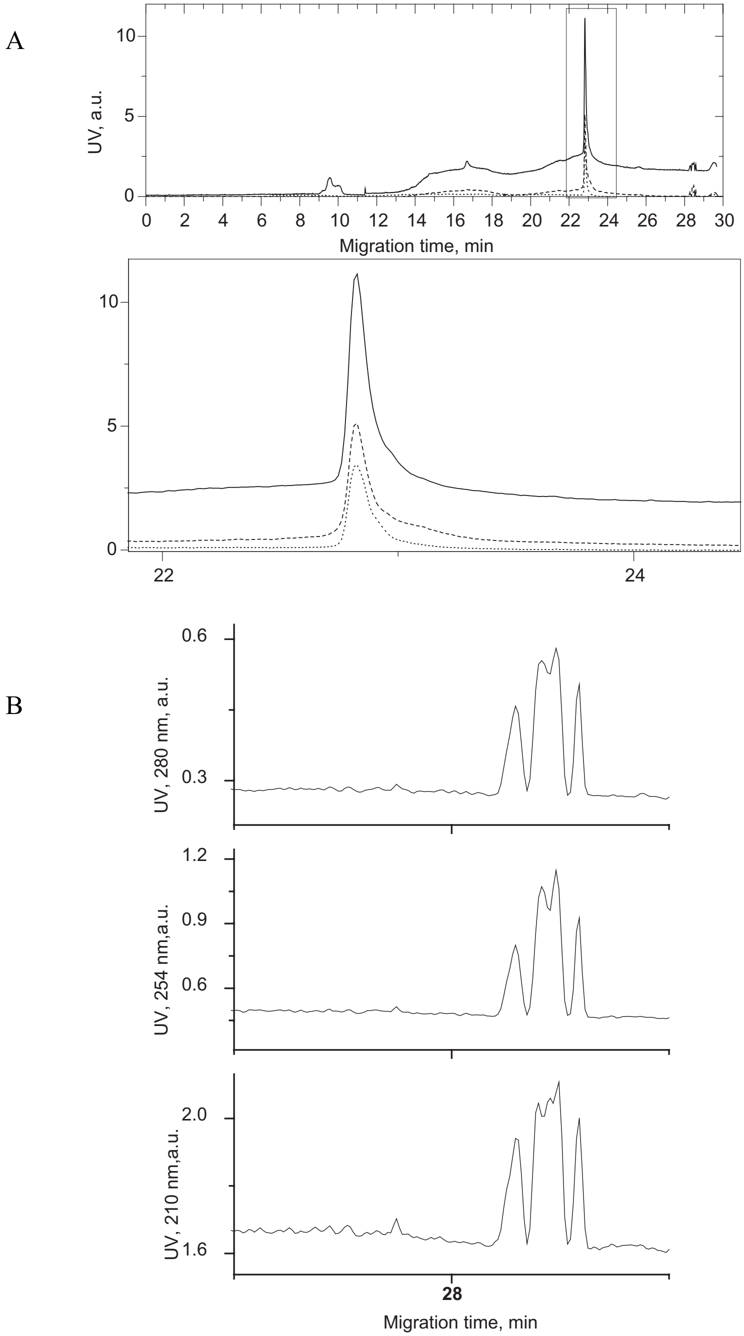

Fig. 3. Electropherograms of lake sediment surface layer pore-water obtained with diode array detection. In enlarged windows: (A) highest peak at 22.79 min. (B) separated group at migration times 28.30, 28.40, 28.46, 28.50 and 28.59 min for peak maxima from left to right at 210 nm; 28.30, 28.42, 28.48 and 28.60 min at 254 nm and 280 nm.

Electropherograms showing separation of pore-water DOM (Fig. 3) with different detection wavelengths used demonstrate the decrease of sensitivity as wavelength increases. The highest signal and more detailed electropherograms were obtained using 210 nm wavelengths for detection that was attributed to the greater impact of carboxylic groups. The 254 and 280 nm are commonly used to detect phenolic, benzene carboxylic and polycyclic aromatic components of aquatic DOM. The UV absorbance at 254 nm has been frequently used as a rough indicator of overall DOM concentration (*Leenheer and Croue* 2003).

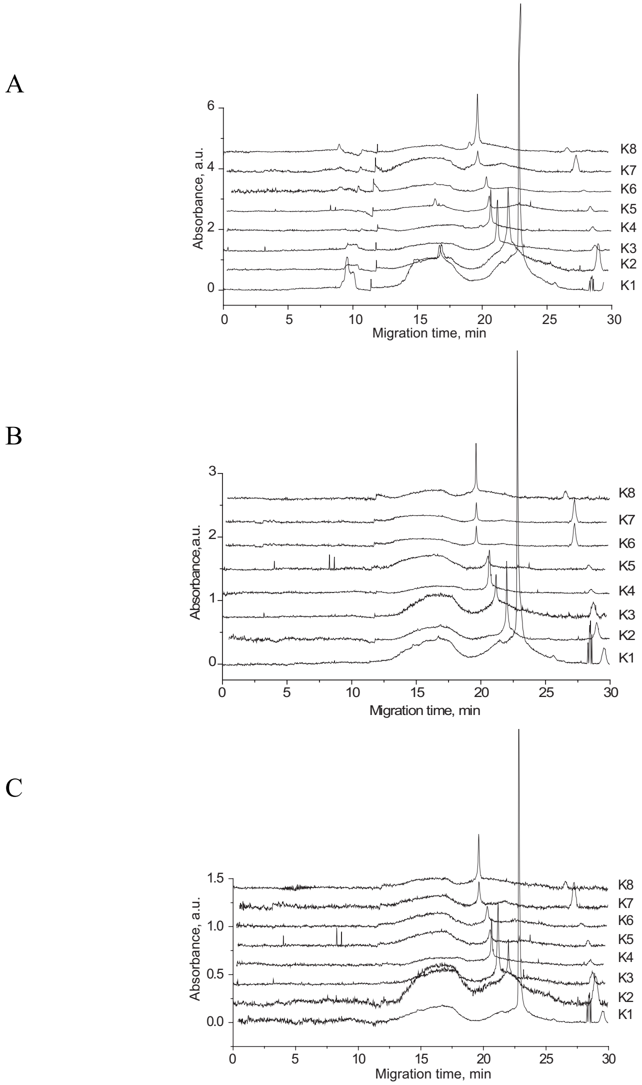

Fig. 4. Depth electropherogram profiles of sediment pore-water DOM from Lake Karujärv, Estonia. Detection wavelengths: (A) 210 nm, (B) 254 nm, (C) 280 nm. For conditions see text. Legend: K1 1–5 cm, K2 5–9 cm, K3 9–14 cm, K4 14–18 cm, K5 18–22 cm, K6 22–26 cm, K7 26–29 cm, K8 29–32 cm.

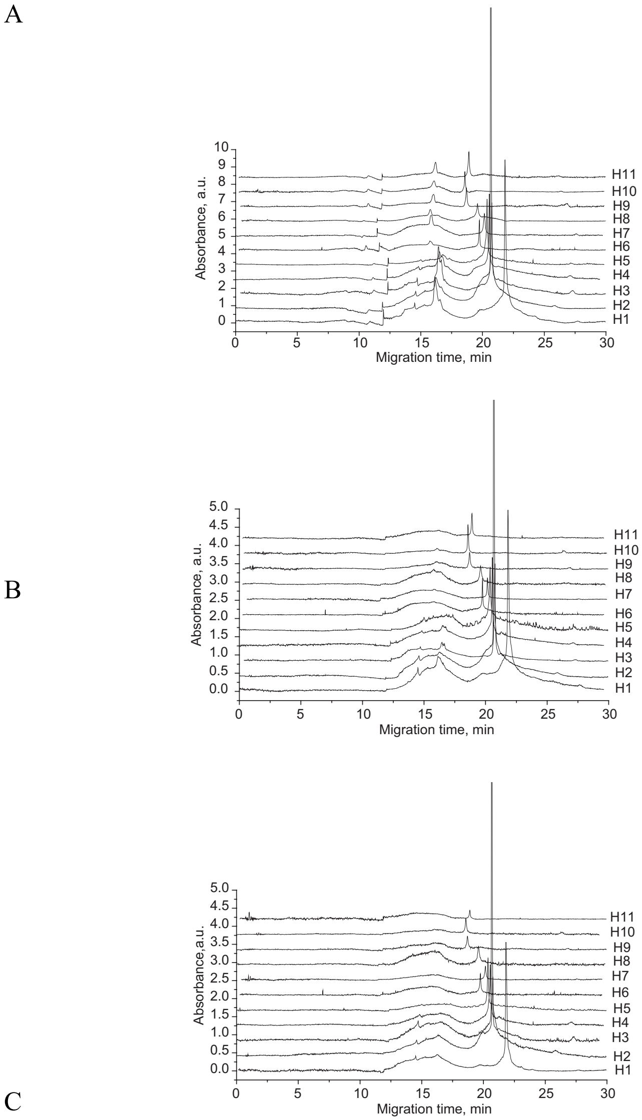

Fig. 5. Depth electropherogram profiles of sediment pore-water DOM from Lake Harku, Estonia. Detection wavelengths: (A) 210 nm, (B) 254 nm, (C) 280 nm. For conditions see text. Legend: H1 1–5 cm, H2 5–9 cm, H3 10–14 cm, H4 14–18 cm, H5 18–22 cm, H6 22–26 cm, H7 26–30 cm, H8 30–34 cm, H9 34–38 cm, H10 38–42 cm, H11 42–46 cm.

However the UV detection does not allow to detect all the DOM components as it has low response to proteins, sugars, aminosugars and aliphatic acids. The examples of electropherograms of sediment pore-water DOM of two lakes studied are shown in Figs. 4 and 5.

The electropherograms of pore waters differed from those obtained for aquatic humic substances. The characteristic feature was the separation of a broad peak at migration times 13–19 min followed by a sharp peak and small well-resolved peaks at longer migration times. The intensity and peak position somewhat changed depending on the sediment layer and on the lake studied.

From the electropherograms presented in Figs. 4 and 5 the differences in the substructure of the broad peak (13–19 min) are clearly observable for the both lake pore-waters. The broad peak could be associated with the fulvic acid fraction as the migration times were close (see *Peuravuori et al.* 2004). The nature of the sharp peak at longer migration time remains unknown. Obviously it represents quite well-defined or uniform electrophoretically separated fraction of pore-water DOM. Interesting feature was the shift of the sharp peak towards shorter migration times as the sediment depth increased (Figs. 4 and 5, Table 1).

Table 1

Electrophoretic data for highest sharp DOM fraction in pore-waters separated by CZE with PVA coated capillaries and detected at 254 nm

Lake	Sample	Depth, cm	Migration time, min	Area, mV × min	Height, AU	Width
Karu	K1	1_5	22.82	41.3	4.70	0.118
	K2	5_9	22.00	8.2	1.10	0.109
	K3	9_14	21.17	4.8	0.47	0.143
	K4	14_18	20.66	7.1	0.60	0.174
	K5	18_22	20.54	1.6	0.14	0.155
	K6	22_26	20.30	4.9	0.29	0.207
	K7	26_29	19.65	2.4	0.28	0.114
	K8	29_32	19.63	3.8	0.73	0.073
Harku	H1	1_5	21.83	44.9	4.20	0.154
	H2	5_9	20.67	68.7	16.37	0.061
	H3	10_14	20.75	19.7	2.49	0.104
	H4	14_18	20.55	13.8	1.97	0.102
	H5	18_22	20.36	16.1	1.40	0.150
	H6	22_26	19.75	4.8	0.81	0.082
	H7	26_30	20.15	3.9	0.53	0.111
	H8	30_34	19.60	6.2	0.44	0.192
	H9	34_38	18.70	4.3	0.43	0.132
	H10	38_42	18.57	5.5	0.75	0.109
	H11	42_46	18.89	4.4	0.59	0.106

The similar phenomenon was observed and attributed as oligomerization of humic acid with the increase of concentration (*Fetsch et al.* 1998). However in present study the concentration of DOM in sediment pore-water decreases with the increasing depth (*Lepane et al.* 2004). It is well known that the migration of molecules in CZE depends on their charge-to-size ratio. The shift towards shorter migration times means that biogeochemical processes in sediment and in pore-water have caused the change of

DOM constituent charge-to-size ratio. Because the shift was observed in both lakes pore-waters it suggests to the similarity of the biogeochemical processes in the aquatic environment.

Conclusions

The results of this work indicate that CZE separation of pore-water DOM using PVA coated capillary is useful for detailed sample characterization from different sources. Thus the method could find application in natural organic matter studies where the concentration procedure must be avoided. However, for more comprehensive identification of separated electrophoretic fractions the CZE should be coupled with MS detector.

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International comparison of analytical protocols for determining soil organic matter content on Lithuanian Albeluvisols

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Research data were obtained on sandy loam Eutric Albeluvisols at the Kaltinenai Research Station of the Lithuanian Institute of Agriculture on the undulating hilly topography of Western Lithuania. Results from 18-years of field investigations show significant increases in soil organic matter (SOM) content under grass-grain crop rotations compared with field and grain-grass crop rotations. SOM content was analyzed using the Tyurin titrimetric method, which is not widely used in Western Countries. Therefore, SOM data are also presented after split analysis using dry combustion, Walkley-Black (USDA) and loss-on-ignition (UK) methods. Transfer functions permit transfer between databases. International comparison of SOM databases will assist in many tasks, including evaluating the importance of SOM/SOC in international collaborative terrestrial ecosystem studies and the development of effective soil conservation policies.

Key words: *Albeluvisols; soil erosion; soil organic matter; SOM analyzing protocols; data conversion.*

Introduction

Soil organic matter (SOM) plays a central role in maintaining key soil functions, such as increasing soil porosity and infiltration rates. In turn, this increases the water holding capacity of soil and makes tillage operations easier. The resultant increased water availability for plants decreases runoff and the pollution of watercourses with agrochemicals. The soil organic fraction accounts for 50–90% of the cation exchange capacity (CEC) of mineral surface soils, which allows macronutrient cations (K, Ca, Mg) to be held in forms available to plants. Through CEC, organic matter also provides much of the soil pH buffering capacity (*Brady and Weil* 1999; *Lal, et al.* 1998).

Nitrogen, phosphorus, sulphur, and micronutrients are stored as constituents of SOM, which are slowly released by mineralization, thus aiding plant growth. Humic acids are constituents of SOM and these accelerate soil mineral decomposition, releasing macro- and micro-nutrients as exchangeable cations. Furthermore, organic matter decreases soil erodibility (*Jacinthe et al.* 2002, *Munshower* 1994).

Carbon (C) is a major component of SOM, which in turn plays a key role in the global C cycle. While ~8 gigatonnes (Gt) of anthropogenic C is emitted into the atmosphere annually, ~2 Gt of C is captured (sequestered) in SOM annually (*Lal* 2001, 2002). This underlines the importance of SOM in relation to climate change. There are, however, limits to the amount of organic matter and hence C that can be stored in soils.

SOM decline is of particular concern in many European areas. According to the European Soil Bureau, based on the limited data available, nearly 75% of the total area analysed in the Mediterranean region of Southern Europe has a low (3.4%) or very low (1.7%) SOM content. Typically, agronomists consider soils with <1.7% organic matter to be in a pre-desertification stage (*CEC*, 2002; *Gobin et al.*, 2002). The problem is widespread. For instance, SOM values for England and Wales show the percentage of soils with <3.6% organic matter rose from 35% to 42% in the period 1980–1995, which is chiefly due to changing management practices (*CEC* 2002, *Gobin et al.* 2002). For the same period, in the Beauce region, south of Paris, SOM decreased by half, which is attributed to the same reasons (*CEC* 2002). Because SOM decline is a crosscutting issue that also affects associated soil parameters, such as fertility, erosion, and conservation, it is extremely difficult to estimate its true environmental and financial cost.

Soils serve a crucial role in the global C cycle, providing an estimated 2300 Pg (2.3×10^{18} g) of the total C pool (*Batjes* 1996, *Lal* 2002, 2003). Consequently, C cycle models provide a valuable tool for understanding and predicting SOM turnover and thus assist national and international carbon sequestration estimates. Models require the input of characteristic soil and climate data, such as soil texture, SOM, rainfall, temperature and evapotranspiration (*King et al.* 1997, *Smith et al.* 1997). Therefore, transferable soil data, beyond those of institutional and national boundaries, has international importance for soil C model inclusion and quantification of the global C budget. Unfortunately, differences between international protocols employed to determine SOM content produce different estimates and interpretations.

Determination of organic matter content is a routine procedure carried out in soil analytical laboratories throughout the USA and other Western countries. However, there is no satisfactory universal method for determining SOM content. It can be determined indirectly by measuring soil organic carbon (SOC) content and multiplying the result by the ratio of organic matter to organic C normally found in soil. Direct determination of organic matter usually involves destruction of the organic fraction by oxidation or ignition of the soil at high temperature. Soil weight loss is taken as a measure of organic content. However, the oxidation method has serious limitations, mostly because the oxidation process is incomplete, and the extent of oxidation can vary between soils (*Page et al.* 1992).

Universal or harmonized quantification of SOM concentrations is essential and data comparability can be achieved by harmonization of analytical protocols. At present, due to methodological differences between regional and national laboratories, problems of SOM data comparison and acceptance exist, particularly where results are presented for international publication or inclusion in soil C models. Consequently, there was a need to develop transfer functions between analytical protocols used to determine SOM content by (1) dry combustion; (2) Walkley-Black; (3) loss-on-ignition; (4) Tyurin photometrical and (5) Tyurin titrimetrical methods (*Ball* 1964, *Александрова, Найденова* 1986, *Орлов, Гришина* 1981, *USDA* 1995, *Никитин* 1999). Traditionally, the latter two techniques have been and continue to be used in Central and East European countries.

Transfer equations were calculated using data from the pilot project 'Carbon sequestration in Lithuanian soils' (F/00630B), which was supported by the Leverhulme Trust, UK (*Jankauskas et al.* 2005 in press). Five SOM/SOC analytical protocols, used in laboratories of the LIA and the University of Wolverhampton (UK), were compared. Correlation coefficients between sets of results analysed by different methods varied between $r = 0.81–0.89$ (0-20 cm, $n = 46$, $P<0.001$). This promotes the possibility of data transfer, based on the strength and significance of correlation and regression relationships.

The objective of this paper is: (1) to present results of SOM content changes under various land use systems on Lithuanian Eutric Albeluvisols, based on 18-years of field investigations, and (2) explore the possibility to transfer SOM content results from the Tyurin titrimetric method to dry combustion (D_c), Walkley-Black (W–B) and loss-on-ignition (LoI_W) methods.

Material and methods

Study sites

Field investigations were conducted on the Zemaiciai Uplands of Western Lithuania. Data were obtained from the Kaltinenai Research Station (KRS) of the Lithuanian Institute of Agriculture (LIA) during 1983–2000, which is located on the southern-central Zemaiciai Uplands (55°34'N, 22°29'E). Study sites A, B and C were on slopes of 2–5°, 5–10° and 10–14°, respectively. The field experiments are part of the 'Core Research Programme of the Global Change and Terrestrial Ecosystem (GCTE) Project', a component of the 'International Geosphere Biosphere Programme (IGBP)' (*GCTE Focus 3* 1997).

Field experiments were performed on eroded Eutric Albeluvisol sandy loams (FAO-UNESCO, 1994). Soil was differentially eroded along the slopes, being slightly eroded on 2–5° slopes (study site A), moderately eroded on 5–10° slopes (study site B) and strongly eroded on 10–14° slopes (study site C), with colluvial deposits on basal slopes. Soil erosion was caused mainly by tillage and water erosion, under continuous intensive cropping. The average agro-chemical properties of Ap horizons (0–20 cm) before field experiments show the topsoil was slightly acid, P-deficient, moderately K-rich and contained varying SOM contents (Table 1). The highest SOM content was on the less eroded 2–5° slope and the lowest on the 10–14° slope. For historical reasons, soil analytical techniques follow mainly East European procedures.

In Lithuania, water erosion occurs mostly on arable slopes, as natural vegetation (woods, shrubs and grasslands) effectively protects soil from erosion (*Jankauskas* 1996). Mean annual precipitation in Lithuania is 626 mm, with ~858 mm on the central

Zemaiciai Uplands and 750–800 mm on the upland fringe. Annual precipitation during the study period was 635–1075 mm.

Table 1

Mean chemical soil properties of the arable layer (0–20 cm) before field experiments in 1981

Study sites	Slope Steepness (degrees)	pH_{KCl}	Hydrolytic acidity	Exchangeable bases	Available elements (mg kg^{-1})		Organic matter (g kg^{-1})
			cmol(+)kg^{-1}		P	K	
A	2-5	5.8	20.1	119	49.8	146.1	28.5
B	5-10	5.3	24.5	94	18.3	127.0	22.0
C	10-14	5.8	16.7	96	29.7	131.2	20.8

General methodological framework

Long-term field experiments were conducted since 1982. Four six-course crop rotations were compared. These were:

(a) The field crop rotation: 1: winter rye (*Secale cereale* L.), 2: potatoes (*Solanum tuberosum* L.), 3–4: spring barley, 5–6: mixture of clover-timothy (CT) (*Trifolium pratense* L.-*Phelum pratense* L.);

(b) The grain-grass crop rotation: 1: winter rye, 2–4: spring barley, 5–6: CT;

(c) The grass-grain I crop rotation: 1: winter rye, 2: spring barley, 3–6: CT;

(d) The grass-grain II crop rotation: 1 winter rye, 2: spring barley, 3–6: mixture of orchard grass-red fescue (OF) (*Dactylis glomerata* L.-*Festuca rubra* L.).

A multi-species mixture of perennial grasses for long-term use (sod-forming grasses: g) was sown on 10–14° slopes, instead of the field crop rotation (as tillage crops are not recommended on slopes >10° in Lithuania) (*Jankauskas* 1996). The grass mixture consisted of 20% each of common timothy, red fescue, white clover (*Trifolium repens* L.), Kentucky bluegrass (*Poa pratensis* L.) and birdsfoot trefoil (*Lotus corniculatus* L).

Winter rye and spring barley were the only crops included in all investigated rotations. A mixture of clover-timothy was grown in three crop rotations (field, grain-grass and grass-grain I). Potatoes were grown only in the field crop rotation, while a mixture of orchard grass-red fescue was grown only in the grass-grain II rotation.

Soil sample collection

A total of 88 Eutric Albeluvisol samples were collected from topsoil (0–20 cm) of three field experiments described in the 'General methodological framework', and analysed for SOM content by Tyurin titrimetric method, described below. A further 46 samples were collected from topsoil (0–20 cm) of 46 long-term experimental plots of the KRS for comparison of different SOM analytical methods. Soil samples were taken from three monitoring sites (each containing 10 plots), representative of six land management systems, on slopes of 7–9, 7–8 and 9–11° with a southerly aspect (duration 8 years). Samples were also taken from two monitoring sites (each containing 8 plots, duration 20 years), representative of four management systems, on slopes of

10–14° with a northerly aspect (*Jankauskas et al.* 2004) and slopes of 12–16° with a southerly aspect.

SOM determination methods

For SOM determination, each sample was sub-sampled and analysed by five separate techniques: (1) the traditional West European approach of loss-on-ignition (LoI) (*Ball* 1964), conducted in both the laboratories of the University of Wolverhampton (UoW), UK and the KRS of the LIA; (2) the East European Tyurin titrimetric ($T_{t.}$) method (*Александрова, Найденова* 1986), performed in the laboratory of KRS of the LIA; (3) the Tyurin photometric ($T_{ph.}$) method (*Орлов, Гришина* 1981; *Никитин* 1999); (4) the USDA Walkley-Black (W-B) method (*USDA*, 1995) and (5) the dry combustion (D_c) method using a automatic analyzer Vario EL III in the Chemical Research Laboratory of the LIA. A summary of analytical methods is presented in Table 2. Sample preparation for each method involved the removal of visible plant and animal residues from bulk soil samples and then sieving using a 0.2 mm sieve. Ponomariova and Plotnikova (1980) provided detailed descriptions of soil preparation for SOM (humus) analyses by the Tyurin method.

Table 2

Summary of SOC and SOM analytical methods

	Loss-on-ignition (LoI)		Tyurin titrimetric (T_t)	Tyurin photometric (T_{ph})	Walkley-Black (W-B)	Vario EL III (D_c)
Laboratory	UoW (LoI_W)	KRS (LoI_K)	KRS	LIA	LIA	LIA
SOC			+	+	+	+
SOM	+	+	Recalc. SOM=SOC×1.724	Recalc. SOM=SOC×1.724	Recalc. SOM=SOC×1.724×1.3	Recalc. SOM=SOC×1.724

NOTE: + indicates the type of measurement performed by the analytical technique.

Results and discussion

Changes in SOM content

SOM accumulation is a slow process and considerably slower than the decline (*Lal et al.* 1998). Fortunately, accumulation can be enhanced by positive farm management techniques, such as permanent grassland, cover crops, conservation tillage (including no-tillage cropping techniques), mulching, green manures and applications of farmyard manure and compost. Most of these techniques have also proved effective in preventing erosion, increasing fertility and enhancing soil biodiversity (*Lal* 2001, 2002). The natural fertility of Dystric Albeluvisols on the Zemaiciai Uplands has decreased by 21.7, 39.7 and 62.4% on slightly, moderately and severely eroded slopes, respectively, in turn causing the deterioration of soil physico-chemical properties (*Jankauskas and Fullen* 2002).

SOM content changes in long-term field experiments at the KRS illustrate multiple influences of land use systems on SOM dynamics (Table 3). Firstly, the variety of crops as constituents of the rotation can differentially affect C sequestration processes (*Jankauskas* 1996, *Lal et al.* 1998). Secondly, different land use systems require different intensities of soil tillage. Consequently, more intense soil tillage stimulates more SOM mineralization, which releases more C from the soil store to the atmosphere (*Lal* 1999). Thirdly, there were different soil losses due to water erosion under different land use systems: highest losses were under the field crop rotation and the lowest were under grass-grain rotations (*Jankauskas and Jankauskiene* 2003; *Jankauskas et al.* 2004). The higher soil losses lead to higher losses of SOM. Furthermore, different land uses influence C sequestration by changing soil physical properties, such as dry bulk density, total soil porosity and field capacity. At KRS, the erosion-preventive grass-grain crop rotations and long-term perennial grasses significantly increased total porosity and field capacity (*Jankauskas and Jankauskiene* 1999). There were small changes in % SOM after both the first and even the second crop rotation (Table 3). However, differences in % SOM become more evident after the third crop rotation in 2000. Significantly higher SOM values were found under the grass-grain crop rotations on the 2–5^{o} and 5–10^{o} slopes compared with the field crop rotation, and under the sod-forming perennial grasses on the 10–14^{o} slope compared with the grain-grass crop rotation.

Data presented in the last column of the 10–14^{o} slope (in brackets) represent results from plots sampled in 2002 for the project 'Carbon sequestration in Lithuanian soils'. The data in brackets represent two replications and are therefore unsuitable for analyses for significant differences. There is evidence of consistent SOM patterns from both data sets i.e. the lowest SOM values were under the grain-grass crop rotation and highest were under the sod-forming perennial grasses (Table 3).

Comparable results were found at the Hilton Experimental Site, Shropshire, UK Conversion of 10 erosion plots from bare arable to grass ley set-aside reversed the trend of declining SOM contents, which then significantly increased, especially in the first four years. Mean soil organic content (0–5 cm depth) significantly ($P < 0.001$) increased from 2.04% by weight (S.D. 0.45, n = 50 samples) in April 1991 to 3.11% (S.D. 0.68, n = 50 samples) in April 2001, compared with permanent grassland values of ~4.5%. Soil erodibility after six years of set-aside (sampling date 24/04/97) was determined using a drip-screen rainfall simulator. Soil aggregate stability was higher on the grassed soils, compared with set-aside and bare arable soils. Despite no significant ($P > 0.05$) differences between grassland and set-aside soils, both these treatments were significantly ($P < 0.001$) greater than bare soils (*Foster et al.* 2000, *Fullen et al.* 2002).

International comparison of analytical protocols

Traditionally, the main method for determination of soil humus (as equivalent of SOC content in mineral soils) in East European countries was the wet combustion method of Tyurin, or some modifications of this indirect method (*Пономарева, Плотникова* 1980). The Tyurin dichromate oxidation method does not require very expensive instrumentation and is not affected by the presence of lime. However, the disadvantage of this method is incomplete oxidation of organic matter (*Orlov et al.*

1993), which is especially obvious, for instance, when analyzing peaty soils, soils containing considerable decayed plant residues or upper horizons of forest soils (*Орлов, Гришина* 1981, *Rojkov et al.* 2002). Organic C values determined by the Tyurin method are ~90% of those determined by the dry combustion method of Gustavson (*Бельчикова* 1975). Moreover, similar data presented in the literature are diverse and even contradictory.

Table 3

Mean SOM contents under different land use systems (1983–2000)

Treatments (crop rotations)	SOM (%)		
	After 1st crop rotation, 1988	After 2nd crop rotation, 1994	After 3rd crop rotation, 2000
2–5° slope			
a) Field	3.47a*	2.73a,b	2.64a
b) Grain-grass	3.46a	2.54a	2.99b
c) Grass-grain I	3.08a	3.65b	3.39c
d) Grass-grain II	3.23a	3.47b	3.46c
LSD_{05}	0.412	0.301	0.284
5–10° slope			
a) Field	2.52a	2.37a	2.17a
b) Grain-grass	2.47a	2.35a	2.01a
c) Grass-grain I	2.48a	2.27a	2.75 b
d) Grass-grain II	2.41a	2.31a	2.67b
LSD_{05}	0.287	0.287	0.1.64
10–14° slope			
a) Grasses**	2.49a**	2.59b**	2.51b** (2.72**)
b) Grain-grass	2.42a	2.24a	1.99a (1.69)
c) Grass-grain I	2.71b	2.47b	2.45b (2.18)
d) Grass-grain II	2.50a	2.39a	2.43b (2.22)
LSD_{05}	0.232	0.221	0.328

* Values with the same letter subscript are not significantly ($P<0.05$) different.

** The sod-forming perennial grasses were grown instead of the field crop rotation on the 10–14° slope.

Large archive databases of SOM and SOC exist in Lithuania and in other Central and East European countries. Most of these data were generated using the Tyurin titrimetric protocol. Due to methodological differences between laboratory protocols, difficulties exist using these data for international eco-environmental assessments, for joint research projects and for data acceptance in international publications. Short descriptions of investigations at the KRS were presented above (see 'Introduction'). Consequently, the possibility exists to transfer data from methods widely used in Eastern Europe to other protocols, using simple linear regression equations, more complex paired regression equations or conversion coefficients (Table 4).

Table 4

Linear or paired regression equations and transfer coefficients for recalculation of SOM data received by Tyurin titrimetric method to other analytical protocols, 0–20 cm (n = 46)

Recalculation:		Linear regression	Paired modified power regression	Transfer coefficient
From – X	To – Y*			
Tyurin titrimetric	D_c	Y=0.504+0.833X	$Y=0.966\times1.469^X$	0.97
Tyurin titrimetric	W-B	Y=0.628+0.889X	$Y=1.130\times1.436^X$	1.07
Tyurin titrimetric	LoI_W	Y=0.172+1.364X	$Y=1.141\times0.742^X$	1.32

* SOM methods: D_c is dry combustion, W-B is Walkley-Black, LoI_W is loss-on-ignition method.

The presented equations and transfer coefficients were used to recalculate SOM content data received by the Tyurin titrimetric method to others using the last section of data (10–14° slope, 2000) presented in Table 3. This choice was made because there are available data received two years later, i.e. in 2002, from the soil erosion monitoring sites used for the above-mentioned project ‘Carbon sequestration in Lithuanian soils’. Recalculated data sets (Table 5) show that highest values of SOM were obtained using the loss-on-ignition method (2.63–3.60%), while much lower values were generated by the Walkley-Black (2.13–2.86%) and dry combustion (1.93–2.60%) methods. Noticeable variations in mean SOM data were achieved using different recalculation methods. The highest mean value (2.85%) was using linear regression; a lower mean value (2.76%) was achieved using paired modified power regression and the lowest value (2.63%) using transfer coefficients (Table 5).

Comparison with data sets from the ‘Carbon sequestration in Lithuanian soils’ Project in relative numbers enable the evaluation of the optimal recalculation procedures (Table 6). The closest association for this data set after recalculation using the various transfer approaches is using paired modified power regression. The association is less close after recalculation using transfer coefficients.

Several attempts have been made to establish transfer coefficients for different organic analytical methods. The following coefficients were suggested to recalculate the data of the dichromate method of Walkley-Black into dry combustion results: 1.24–1.32 (depending on soil parent material: 1.24 for soils developed on basalt, alluvium or sand, 1.32 for soils developed on granite or metamorphic rock) (*Gillman et al.* 1986), 1.33 (*Bornemisza et al.* 1979) and 1.41 (*USDA*, 1995). We used the correction factor of 1.3 recommended by Nilson and Somers (1982) to compensate for incomplete oxidation in the primary stage of calculations. Therefore, in this paper, presented mean values of W–B data are slightly higher than D_c equivalents. A generalized coefficient of 1.28±0.19 was suggested for the recalculation of organic carbon content determined by the Tyurin method into that determined by dry combustion by automatic analyzers. The following transfer coefficients have been suggested for different soils: typical chernozem (1.13); soddy-podzolic soil (1.30); light chestnut soil (1.26); leached chernozem (1.23); calcareous chernozem (1.29) and solonetzes (1.32) (*Когут, Фрид* 1993). A general coefficient of 1.34 was proposed for mineral soils (*Orlov et al.* 1993). However, calculation of transfer coefficients is simple, but not precise; while linear and paired power regression equations are more precise (Table 4).

Table 5

Comparison of SOM data sets recalculated by selected protocols to the Tyurin titrimetric method

Treatments on the 10–14° slope	SOM (%) data sets			
	Original, analyzed by T_t	Recalculated by equations or coefficients		
		Linear regression (lr)	MPR	Transfer coefficient (tc)
	Recalculated to Dry combustion method			
a) Grasses*	2.51b	2.60b	2.54b	2.43b
b) Grain-grass	1.99a	2.16a	2.08a	1.93a
c) Grass-grain I	2.45b	2.55b	2.48b	2.38b
d) Grass-grain II	2.43b	2.53b	2.46b	2.36b
LSD$_{05}$	0.328	0.344	0.334	0.318
Mean	2.35	2.46	2.39	2.28
	Recalculated to Walkley-Black method			
a) Grasses*	2.51b	2.86b	2.80b	2.69b
b) Grain-grass	1.99a	2.40a	2.32a	2.13a
c) Grass-grain I	2.45b	2.81b	2.74b	2.62b
d) Grass-grain II	2.43b	2.79b	2.72b	2.60b
LSD$_{05}$	0.328	0.370	0.370	0.351
Mean	2.35	2.72	2.65	2.51
	Recalculated to loss-on-ignition method			
a) Grasses*	2.51b	3.60b	3.46b	3.31b
b) Grain-grass	1.99a	2.89a	2.75a	2.63a
c) Grass-grain I	2.45b	3.51b	3.37b	3.23b
d) Grass-grain II	2.43b	3.49b	3.34b	3.21b
LSD$_{05}$	0.328	0.472	0.452	0.433
Mean	2.35	3.37	3.23	3.10

* The sod-forming perennial grasses; MPR** paired modified power regression.

Table 6

Comparison of recalculated SOM data (%) with the initial data set

Methods	Initial data set (n = 46)		Mean values from Table 5 recalculated by:					
	Values	Rel. n.**	Equation of lr*		Equation of MPR*		Transfer coefficient	
			Values	Rel. n.**	Values	Rel. n.**	Values	Rel. n.**
T_t	2.21±0.091	100	2.35	100	2.35	100	2.35	100
D_c	2.35±0.091	106.3	2.46	104.7	2.39	101.7	2.28	97.0
W-B	2.59±0.094	117.2	2.72	115.7	2.65	112.8	2.51	106.8
LoI_W	3.19±0.151	144.3	3.37	143.4	3.23	137.4	3.10	131.9

** relative numbers.

Development, refinement and employment of this calibration approach will assist the harmonization of international SOM/SOC data and appraisal of long-term global soil C storage trends. These include international collaborative studies on the role of SOM/SOC in terrestrial ecosystems and the development of effective soil conservation policies.

Conclusions

1. Generally, higher soil losses promote greater SOM loss. Furthermore, various land use systems influence erosion rates and changes in soil physical properties. Erosion-preventive grass-grain crop rotations and perennial grasses for long-term use significantly increased SOM on 2–5° and 5–10° slopes, compared to field crop rotations. Sod-forming perennial grasses significantly increased SOM on 10–14° slopes compared with the grain-grass crop rotation.
2. The feasibility of transferring data from the Tyurin titrimetric method to other protocols was investigated. The data sets appeared comparable and appropriate for recalculation. Successful recalculation was achieved using simple linear regression equations, more complex paired regression equations, or transfer coefficients. Employment of this approach will assist the harmonization of international SOM/SOC data and appraisal of long-term global trends in soil carbon storage.
3. Erosion-preventive cropping systems (grass-grain crop rotations and long-term perennial grasses) significantly increased SOM/SOC when maintained for ≥ 12 years.

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Long-term changes of dissolved organic carbon in surface waters of Latvia

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The concentrations and loading of organic carbon influence not only direct anthropogenic loading very much, but also character of landuse and climate. During the last decade, anthropogenic pressure to the environment has considerably reduced. Within this article, the impact of reduced human loading on the concentrations and flows of dissolved organic matter in Latvian rivers has been evaluated. Influence of land-use types and soil texture has been studied.

This study revealed an increasing trend of chemical oxygen demand and water color during the last decade. There is a positive correlation between estimates of organic matter content and water discharge. This study did not reveal a clear correlation between the concentrations of dissolved organic carbon (DOC) and land-use types within the river basin. A closer relationship was found between DOC concentrations and soil texture.

Key words: *DOC, trends, Latvia.*

Introduction

Dissolved organic substances (characterized as dissolved organic carbon – DOC) plays a significant role in the carbon global biogeochemical cycle, but at the same time influence mineral weathering, nutrient cycling, metal leaching as well as pollutant behavior and toxicity. The principal sources of DOC in this respect, flows of humic substances (major part of dissolved organic carbon – DOC) are very important. Humic substances constitute 70 to 85% of the amount of total organic matter and are indicators of water quality (*Thurman* 1985). Measurements of water color and chemical oxygen demand (COD) can be used as indirect estimates of humic substances since in many undisturbed rivers usually 40…65% of the COD is contributed by humic substances.

The proportion of area covered by wetlands in the catchment can explain much of the variability of DOC flows (*Gergel et al.* 1999). Flows of organic substances are influenced also by bedrock geology, intensity of agricultural use and other most significant features of the catchment area. Correll and co-workers (2001) have stressed the impact of precipitation and air temperature on flows of dissolved organic carbon. At the same time, the pattern and intensity of biological processes within the catchments of rivers and lakes can also affect the flows of dissolved organic matter (*Scott et al.* 1998). It is clear that there exist tight links between land-use patterns and flows of organic substances and these relations are regionally specific. On the other hand, natural processes interfere much with human-induced processes. Transport from adjacent areas, and also industrial effluents and non-point pollution sources, can be sources of increased fluxes of DOC. Leaching of humic substances from agricultural areas due to surface runoff, especially during flood periods, can be of key importance in intensively used agricultural areas. Flows of DOC have been analyzed worldwide (*Depetris and Kempe* 1993; *Pettine et al.* 1998; *Arvola* 1999; *Westerhoff and Anning* 2000), but there are few publications concerning water chemical composition and dissolved organic matter in waters of Latvia (*Klavins et al.* 1997, *Apsite and Klavins* 1998). In Latvia, the recent decade has witnessed a substantial reduction of human loads to the environment, due to transformation of the political, economic and social systems. For example, fertilizer and manure use has fallen by five times and the number of livestock has decreased by around three times (*Latvian Environmental Data Centre* 1999), so indicating main reduction of loading of organic matter to the surface water. The aim of this study is to describe long-term changes in concentration of organic matter in surface waters of Latvia, factors controlling its runoff, spatial variability of water chemical composition, possible impacts of pollution sources.

Materials and methods

The study site covers the entire territory of Latvia (Figure 1). Latvia is located on the north-western part of East European Plain on the coast of the Baltic Sea. The territory of Latvia occupies 64 000 km^2. Bedrock is covered by Quaternary deposits consisting of moraine material, limnoglacial or fluvioglacial deposits. The climatic conditions in Latvia can be characterized as humid (*Klavins et al.* 2002).

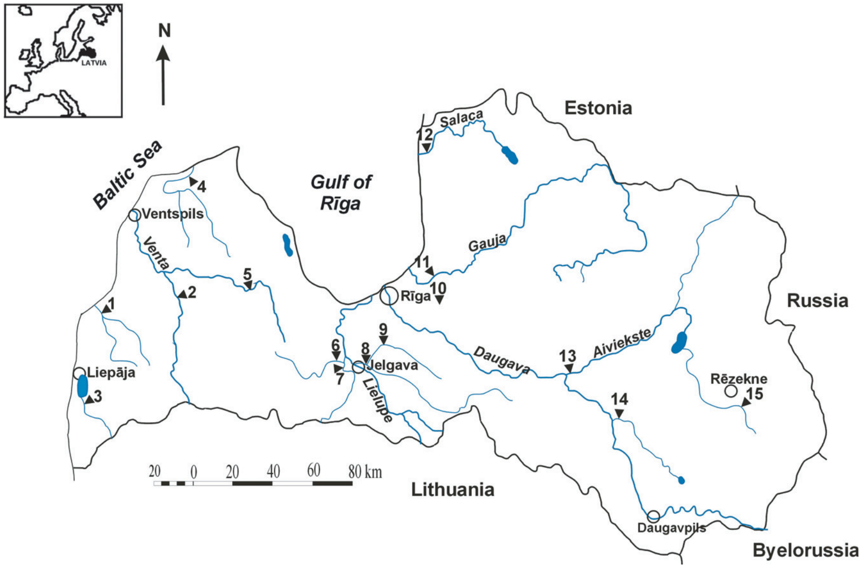

Fig. 1. Map of the study area (▼ – monitoring sites).

Data on COD, water color and river discharge used in this study were obtained from the Latvian Hydrometeorological Agency for time period 1977–2001. Water color was determined using Pt/Co scale spectrophotometrically. COD was determined by oxidation with $K_2Cr_2O_7$ and titration with ferrous ammonium sulfate (*Standard Methods*... 1973).

Data on land use within river subbasins were obtained from the GIS data base «Corine Land Cover Latvia», and information on soil texture was taken from a GIS-based soil map created by the Latvian Environment Agency (*Latvian Environmental Data Centre* 1999). Soils, depending on their texture, were divided into three groups: clay and loam, sandy soils and organic soils.

Long-term changes of river discharge, chemical oxygen demand and water color were studied by using the non-parametric Mann-Kendall test (*Hirsh et al.* 1982, *Hirsch and Slack* 1984). This test can be applied for data sets that have non-normal distribution, missing values or «outliers», and serial character (e.g., seasonal changes). The program *MULTIMK/CONDMK* was used to detect trends, as it allowed including covariates representing natural fluctuations (e.g., meteorological and hydrological data) (*Libiseller and Grimvall* 2002). If the Mann-Kendall test value is greater than 1.65, the trend is increasing (significance level $p<0.05$). If the test value is smaller than –1.65, the trend is decreasing at significance level $p<0.05$.

Results and discussion

Both the spatial and seasonal variability of concentrations of organic substances in the surface waters of Latvia can be considered to be comparatively high. The highest COD concentrations are usual for rivers in the Lielupe and Aivиekste basins (Figure 2), and also in bog lakes and eutrophic waters.

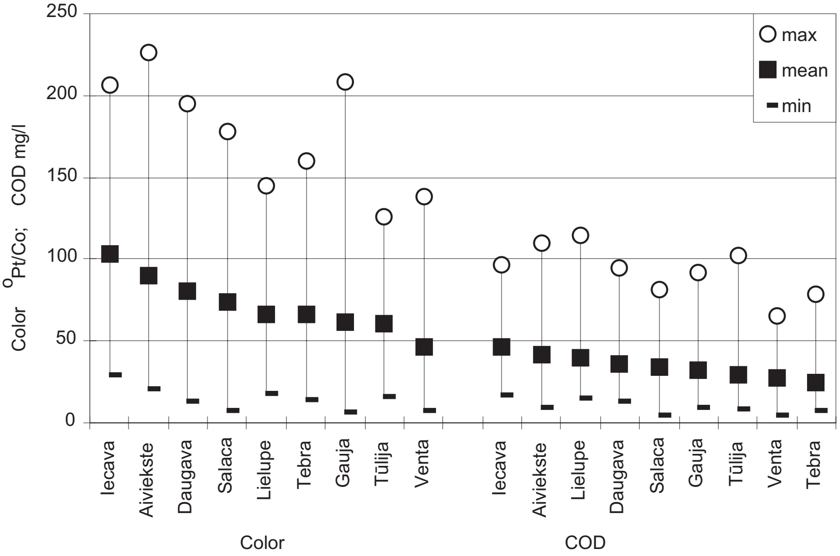

Fig. 2. Variation of COD and color in river waters of Latvia (1977–2001).

Export of COD calculated using monitoring data for the period 1977–1998 varies between river basins in Latvia by nearly twice, from 5226 kg/km^2/year for the Mūsa River up to 12731 kg/km^2/year for the Iecava River, both rivers are of the Lielupe basin. The highest values of COD export evidently are due to agricultural activities and a high percentage of wetlands in the basin. The lowest COD export values are more usual for highly forested river basins. High specific loading of organic substances is contributed by the Salaca River basin. The basin of this river has a relatively high coverage of bogs, and waters of the river originate from the eutrophic Lake Burtnieks. However, the runoff of substances from domestic and non-point sources for this river are among the lowest of the studied river basins in Latvia.

A conditional Mann-Kendall test was applied to detect changes of COD and water color during the last ten years. Despite the dramatically reduced anthropogenic pressure to the environment during this period, the Mann-Kendall test results show even increasing trends of estimates of organic matter content. Water color shows statistically significant linear trend at the 95 % level for all studied sites and COD for five sites of the eight sites (Table 1). River discharge does not show any statistically significant changes.

Table 1

Results of conditional Mann-Kendall test (*Libiseller and Grimvall* 2002) **for Latvian rivers (1991–2001)**

River	Discharge	COD	Color
Daugava	-0.333	1.557	2.049
Aiviekste	0.197	1.347	2.521
Dubna	-1.502	2.699	**1.916**
Gauja	-0.187	2.674	**2.786**
Salaca	-1.090	1.449	2.917
Lielupe	0.646	2.365	**2.857**
Venta	0.035	2.249	**2.668**
Tebra	-0.694	1.707	**2.018**

Bold – test is statistically significant at 95% level.

Increasing concentrations of COD and water color during the last 10 years coupled with a reduction of anthropogenic loading and oscillating patterns of long-term changes (Figure 3), allow suggesting that natural processes play a significant role in the actual flows of organic matter. For example, changes in the hydrological regime and climate can influence both the production and leaching of organic matter at a level exceeding the impact of human loading. Typically, a positive relationship exists between estimates of organic matter content and river discharge. However, this study did not reveal a close correlation between water discharge and COD and water color (Figure 4), likely because of the multitude of factors affecting the concentration of organic substances in waters.

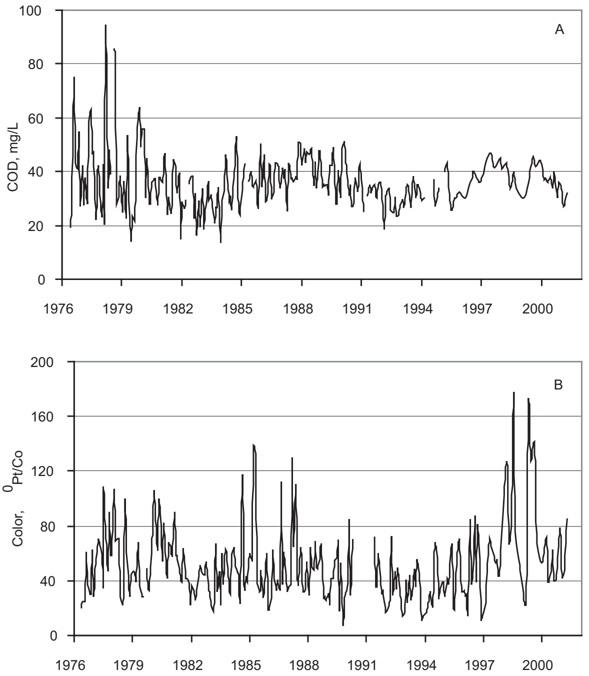

Fig. 3. Long-term changes of chemical oxygen demand and water color in the Daugava (A) and Venta (B) Rivers (1977–2001).

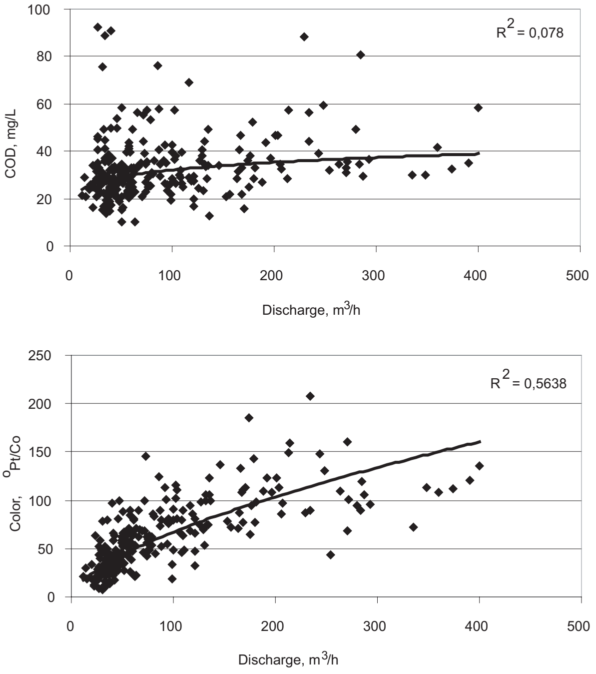

Fig. 4. Relationship between discharge and COD and water color in the Gauja River (1977–2001).

Soil composition can be mentioned among the factors influencing runoff of organic matter. Adsorption to mineral surfaces is an important process controlling natural organic matter leaching at relatively low pH, low ionic strength and in rapid flow systems (*Maurice et al.* 2002).

Our study revealed a positive correlation between dissolved organic carbon concentrations in river water and the relative coverage (% of area) of sandy and organic soils within the river catchment (r = 0.65 and r= 0.55, respectively). A negative relationship was observed between dissolved organic carbon concentrations and the proportion area covered by clay and loamy soil (r = -0.69) (Figure 5).

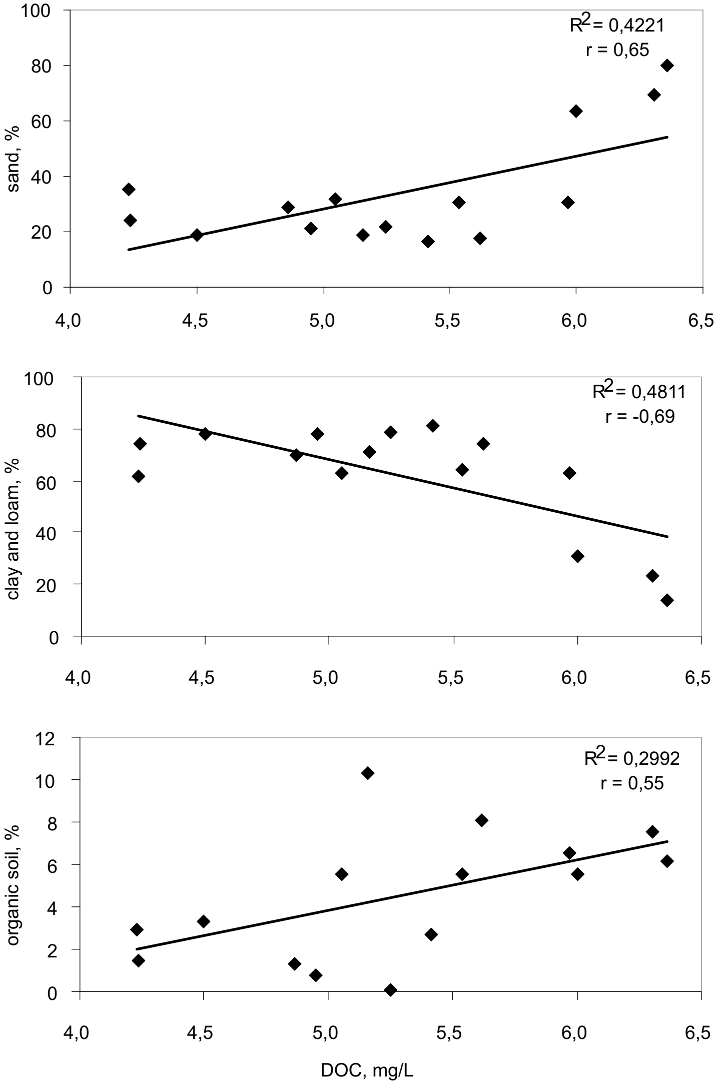

Fig. 5. Correlation between the proportions of catchment area covered by sandy soil, clay and loam, organic soil and DOC concentrations in river water.

A clear relationship between the concentrations of dissolved organic carbon in the river water and land-use classes was not observed. There was a positive relationship between DOC and the coverage (%) of forest area within the river basin (r = 0.33) and coverage (%) of mires area (r = 0.52), and a negative correlation existed between DOC and the coverage (%) of agricultural land (r = -0.38). The above relationships may be explained by the low anthropogenic pressure, as the DOC concentrations used for calculations were for the time period 1995–1999. Probably, soil texture and absorption of organic matter on clay particles have a more significant impact on flows and retention of organic substances.

Changing hydrological and climatic conditions also influence the structure (physiochemical characteristics, molecular weight) of dissolved organic matter, and changes in solar radiation can affect the photodegradation rates of organic matter (*Maurice et al.* 2002).

Conclusions

Long-term data on chemical oxygen demand were used within this study to estimate flows of dissolved organic carbon in Latvia. Long-term changes (1977–2001) of chemical oxygen demand and water color do not follow linear trends but rather show oscillating patterns. However, for the last decade (1991–2001), when loading to waters was substantially reduced, these parameters showed an increasing linear trend according to the Mann-Kendall test results. Typically, there is a positive relationship between estimates of organic matter content and water discharge. There was no clear relationship between the concentration of DOC and land-use pattern within the river basin. A closer relationship was found between the concentrations of DOC and soil properties: a positive correlation between DOC concentrations and the coverage (%) of sandy soils (r = 0.65) and of organic soils (r = 0.55) and a negative correlation with area percentage of clay and loam soils (r = -0.69) in the catchment area.

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Solubility enhancement of polycyclic aromatic hydrocarbons by humic substances and their derivatives

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Development of micellar structures in solutions of surfactants may increase the apparent aqueous solubility of polycyclic aromatic hydrocarbons (PAHs). In this study, we tested the effect of the structures of humic acids and their derivatives on the enhancement of solubility of six polycyclic aromatic hydrocarbons. Solubility of PAHs in presence of humic acids increased with increasing number of aromatic rings. Similar results demonstrated anionic surfactants with and without aromatic rings. The modified humic substances demonstrated more significant solubilizing effect of PAHs.

Key words: *humic substances; solubility; polyaromatic hydrocarbons.*

Introduction

Humic substances (HS) play a major role in the biogeochemical cycling of carbon and they are major organic substances in soils and waters, thus being of importance also in fossil organic deposits, especially peat and low rank coal (*MacCarthy* 2001). Due to multifunctional character (presence of numerous carboxylic and phenolic, but also keto-, and aminogroups) humic substances are able to complex heavy metals and persistent organic xenobiotics (*Leenheer et al.* 2003).

Many important processes in the environment are influenced through interaction with humic substances, for example with solid phases in soils and natural waters. In the interactions both organic (usually detritus particles) and inorganic (mineral matter) substances become covered with humic substances and thus contaminant movement largely can be modified by interaction with humic substances (*De Paolis and Kukkonen* 1997). As far as humic substances consist of a hydrophilic part and a hydrophobic part they can be considered as amphiphiles (*Warsaw* 1993).

In aqueous solutions, the sorption of hydrophobic organic compounds (HOCs) to humic substances can cause an increase in apparent water solubility, thus affecting the fate and transport of HOCs in both surface-water and groundwater systems (*Chiou et al.* 1986, *Johnson and John* 1999). Humic substances and surfactants as environmental amphiphiles are similar structurally and functionally and thus may affect the fate and transport of HOCs in the aquatic environment. Binding intensity of HOCs will be changed as the properties of the humic substances, such as aromaticity, molecular weight, dispersivity, and aliphatic composition, vary (*Jones and Tiller* 1999).

Interaction of humic substances with xenobiotics can modify the uptake and toxicity of these compounds and affect the fate of pollutants in the environment. The properties of HS, as well as their dominant structures depend on their origin (*Ritchie and Perdue* 2003). The aim of this study is to analyze dependence of solubility enhancement of polycyclic aromatic hydrocarbons (PAHs) by humic substances of different origin and their derivatives.

Materials and methods

Humic acids were extracted and purified using procedures recommended by the International Humic Substance Society (*Kļaviņš* 1998). Briefly, 1 kg of 2 mm (sieved) air dry soil or peat was reacted with 10 l of 0.1 M HCl for 1 h. The slurry was allowed to settle and the aqueous phase was decanted and discarded. Approximately 1 l of H_2O was added to the soil or peat mass and the resulting slurry was allowed to incubate for 30 min, after which the pH of the slurry was adjusted to 7 with the addition of 1 M NaOH. This was followed by the addition of a sufficient quantity of 0.1 M NaOH (done under N_2 gas); to bring the total volume of the solution phase to 10 l and the resulting slurry was stirred under N_2 gas. After 24 h the alkaline slurry was filtered through glasswool and the particle free filtrate was acidified to a pH value of 1, with the addition of 6 M HCl. This solution was then allowed to settle then it was centrifuged and the supernatant was discarded. The sediment (which contained humic acid) was washed with distilled water and repeatedly centrifuged discarding the supernatant. Solid residue after centrifugation was then suspended in a mixture of 0.1 M HCl and 0.3 M HF, to remove mineral particles. This treatment was repeated until the ash content was reduced less than 2%. Afterwards humic acid dispersion in distilled water was dialyzed against water to remove chlorides and resulting humic acids were lyophilized. Clear brown colored solution containing the fulvic acids (FA) were obtained after acidification with HCl. The obtained FA solutions were transferred to the H^+ form by passing through a strong cationite KY-23 column and finally freeze dried. To ensure representative samples for analysis two extractions were made for some soil samples. Humic and fulvic acids were isolated from waters in Latvia by Thurman and Malcolm method (1981). For comparison commercial HA's (Aldrich Chemical Company.) and FA after purification was used. The obtained humic substances were characterized as follows:

1. Elemental analysis: C, H, N and ash concentrations were determined by a Perkin Elmer 240/A CHN-Analyzer. Oxygen concentrations were calculated by difference.
2. Concentrations of functional groups were determined by standard methods (*Methods of Soil Analysis* 1989).
3. Molecular weight distribution was determined with gel filtration on Sephadex G-100 (bead diameter 40–120 μm) column (1 × 40 cm). The eluent: 0.01 M Tris-HCl buffer (pH 9.0) was pumped through the column at 1 ml/min, gathering 1 ml fractions. 25 mg of humic substances were applied on the top of the column as 1% solution in 0.1 M NaOH. A standard set of proteins was used for column calibration. The void volume of the column was determined using Blue Dextran 2000000. Detection was performed at 280 nm.

4. Poly (ethylene glycol) (PEG) – water distribution coefficient (*Zavarzina et al.* 2002) was determined mixing 1 ml of HA (2 mg/ml in 0.05 M NaOH), 1 ml 30% PEG 500 kDa Pharmacia Fine Chemicals, Sweden) and 1 ml 10% $(NH_4)_2SO_4$ (pH 5.6). After phase separation the distribution coefficient was determined as absorbance at 465 nm ratio.

Sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDDBS) were purchased from Sigma Chemical (USA) (Table 1).

Table 1

Properties of surfactants used in this study

Surfactant	Molecular weight	CMC*, mg/l
Sodium dodecyl sulfate	288.4	2100
Sodium dodecylbenzene sulfonate	348.0	1000

*CMC – critical micelle solution

The six PAHs were naphthalene, anthracene, phenanthrene, pyrene, coronene and perylene, of 99, 98, 98, and 99% purity, respectively. All compounds were purchased from Aldrich, used without any further purification, and their properties are given in Table 2.

Table 2

Polycyclic aromatic hydrocarbons used in this study

	Molecular weight	Water solubility, mg/l	Log K_{ow}
Napthalene	128.16	3.2 × 10	3.37^a
Anthracene	178.22	0.2	4.32
Phenantrene	178.22	1.6	4.57^b
Pyrene	202.26	1.6×10^{-1}	5.18^b
Perylene	252.32	4.0×10^{-4}	6.50^b
Coronene	300.22	3.2×10^{-6}	7.22

K_{ow} – octanol – water partition coefficient

^{a} – Chiou and Schmeddling 1982

^{b} – Schlautman and Morgan 1993

Humic acid was dissolved into 500 ml of distilled water with 1 ml of 0.1 N NaOH. Humic acid solution was added to a glass vial with a nominal volume of 40 ml and a Teflon lined septum cap. Then, excess amounts of PAHs were added to each vial. The vials were mixed in a rotary shaker at 250 rpm and 25 °C for 72 h, and the solution subsequently was filtered through a 0.1 μm inorganic membrane filter (Whatman, UK) mounted on a 10 ml syringe, to remove the undissolved PAHs. The concentration of PAHs in humic acid solution was measured with a high performance liquid chromatograph (HPLC; Waters model 515, St., USA).

The analysis was conducted at an ultraviolet wavelength of 254 nm, a flow rate of 1.8 ml/min, a mobile phase of 80% acetonitrile (Fisher Scientific, USA) and 20% water, and a 3.9 × 300 mm μ-bondapak C18 reverse phase column (Waters). The retention times of humic acid and naphthalene were about 0.6 and 2.6 min, respectively, so no hindrance for the analysis occurred from these. All experiments were at least duplicated.

The solubility enhancement of PAHs by humic substances can be expressed as:

$$\frac{S'_w}{S_w} = 1 + XK_{HA}$$

where: S_w is the aqueous solubility of the solute in pure water;

S'_w is the solubility of the solute in a humic substance solution at concentration X, in grams per milliliter of water;

K_{HA} is the solute partition coefficient between the humic substance and pure water, in milliliters per gram. The parameter KHA can be normalized with the organic carbon content of the sorbent (f_{oc}) as

$$K_{OC} = \frac{K_{HA}}{f}$$

where: K_{OC} is the solute partition coefficient normalized with the organic carbon content of the sorbent.

Results and discussion

Humic acids used in this study commonly have much higher carbon concentrations than fulvic acids, while oxygen and carboxyl group concentrations are higher in fulvic acids. In humic substances from peat and soil, concentrations of carboxyl groups and phenolic hydroxyl groups are lower (Table 3) than in humic substances from aquatic sources and peat.

The most dominant functional groups in the structure of humic substances are carboxyl and phenolic hydroxyl groups. The concentrations of carboxyl groups are higher in fulvic acids (3.5–6 mmol/g), while humic acids have more phenolic hydroxyl groups (1.0–1.5 mmol/g). The slope of the adsorption curves as measured by the ratios of UV absorbancy at 465 and 665 nm have been suggested to be inversely related to the condensation of aromatic groups (aromaticity), and also to particle size and molecular weight. The higher E_4/E_6 ratios measured for the aquatic FA, with respect to those of soil origin, are in general agreement with data in the literature and suggest a lower degree of condensed aromatic systems and smaller particle sizes or molecular weights than for aquatic HS. The E_4/E_6 ratio for fulvic acids is higher than for humic acids, thus this ratio is correlated with the changes in molecular mass of humic substances (Table 4). Humic substances from soils and peat have a high degree of aromaticity (as determined by ^{13}C-NMR) and molecular mass than humic substances from aquatic sources.

The sorption of PAHs to humic acids was studied using plots of ($\frac{S'_w}{S_w} - 1$) versus the concentrations of humic acid at 25°C (Figure 1), where the slopes of the fitted linear lines are K_{HA}. Even though a single value of K_{HA} was assumed for each compound by fitting a linear line, K_{HA} seems to increase somewhat as humic acid concentration increases, especially for perylene and coronene. It is likely that the aggregates of amphiphiles at lower concentrations are less efficient in sorbing PAHs than at higher concentrations.

Partitioning of PAHs into the organic phase was regarded as the main cause for the sorption of PAHs to humic acid by other researchers (*DePaolis and Kukkonen* 1997). However, the ability of the studied humic substances to solubilize PAHs differs quite much (Figure 2) and can be related to hydrophobicity of humic substances as evaluated by the K_{PEGW}.

Table 3

Elemental and functional composition of humic substances from different environments

Humic substances	C, %	H, %	N, %	O, %	COOH, mmol/g	ArOH, mmol/g
Humic acids						
HA-Podzol	52.34	4.83	3.08	38.92	2.85	1.94
HA-Rendzina	52.05	4.69	3.16	39.23	3.10	2.15
HA-Gleysoils	53.46	4.81	3.61	36.78	2.85	2.10
HA-Rāznas Lake	48.74	4.06	1.65	44.17	5.56	0.72
HA-IHSS	59.20	4.08	1.12	35.60	n.d.	n.d.
HA-Aldrich	60.00	4.47	0.96	34.50	n.d.	n.d.
HA-Nordic Reference	55.20	4.12	1.04	39.02	4.12	1.21
HA-Peat	48.11	5.60	1.95	43.00	n.d.	n.d.
Fulvic acids						
FA-Podzol	49.31	4.64	2.95	41.93	3.28	2.16
FA-Rendzina	48.65	4.54	2.87	42.98	3.80	2.20
FA-Gleysoils	43.68	4.81	3.65	46.18	3.68	1.75
FA-Rāznas Lake	44.36	4.31	3.48	46.51	3.75	1.60
FA-IHSS	48.75	3.85	2.63	43.59	3.30	2.20
FA-Aldrich	48.23	4.43	3.93	41.54	2.82	0.90
FA-Nordic Reference	49.35	4.56	2.76	42.19	2.85	1.15
FA-Peat	48.17	4.15	2.83	43.90	2.70	0.85

Table 4

Molecular properties of humic substances from different environments

Humic substances	Aromaticity, %	M_w, Da	K_{PEGW}
HA-Podzol	41.4	8200	12
HA-Rendzina	36.7	4250	10
HA-Gleysoils	32.4	3800	11
HA-Rāznas Lake	17.4	2200	5
HA-Peat	56.3	9300	18
FA-Podzol	32.5	4300	10
FA-Rāznas Lake	15.5	1250	3
FA-Peat	32.5	3500	14

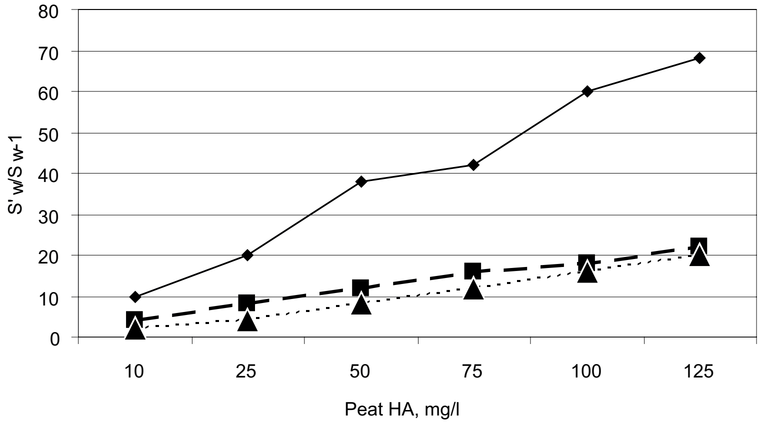

Fig. 1. Plot of $\frac{S_w^{'}}{S_w} - 1$ for coronene (–♦–), pyrene (-■-) and napthalene (···▲···) versus peat humic acid concentrations.

Table 5 contains the measured Koc values of six PAHs in humic acid solutions in this research.

Table 5

Solubilization of polycyclic aromatic hydrocarbons by peat humic acid

	Log K_{OC}
Napthalene	4.72
Anthracene	4.82
Phenantrene	4.96
Pyrene	5.62
Perylene	6.23
Coronene	6.71

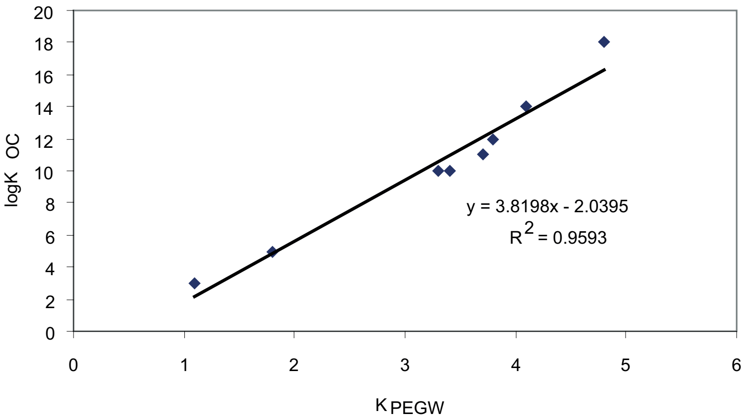

Fig. 2. Solubilization of anthracene by humic acids in this study.

In summary, enhancement of solubilities of PAHs by humic acid was studied in this research. Anionic surfactants, which, unlike humic acid, are amphiphilic and contain known molecular structures, were used for better understanding the sorption behavior to humic acid, the exact molecular structure of which is difficult to define. Surfactant and humic acid molecules may be structurally quite different, because the hydrophobic parts of surfactant micelles and humic acid are mainly composed of long alkyl chains and of aromatic rings, respectively. However, the approach in this study seems reasonable because surfactants and humic acid commonly are amphiphilic and act as sorbents for PAHs in water. The results from this study indicate that sorption of PAHs can be determined by their molecular structures as well as their hydrophobicity.

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The effect of cropping and fertilizer management on soil humus content and composition

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Experiments were carried out on clay loam *Endocalcari – Endohypogleyic Cambisol* during 1995–2002 with a view to identifying the effects of legume crops and their biomass introducing for green manure and different fertilization systems – mineral, organic – mineral and organic on humus accumulation in the soil and its quality. It was revealed due to a longer period on soil dormancy and systematic introduction of necrotic fine rootlets that the highest content of humus and mobile humic acids were found in the soil after lucerne cultivation. Positive changes in humus were identified in all organic – mineral with the application of 40; 60 and 80 t ha^{-1} farmyard manure and organic – 80 t ha^{-1} fertilization systems compared with mineral fertilization systems.

Key words: *clay loam, legume crops, fertilization systems, humus and its composition.*

Introduction

Humus is composed of complex organic compounds that have formed during the various – level organic matter decomposition processes. Humus is an important factor that affects the whole agrosystem since humus particles bound to clay minerals make up complex formations that remain stable for a long time. Humus formation, its fractional composition depends on the amount of organic matter that gets into the soil and its composition, specific characteristics of soil and phytocenoses (*Heinrich, Hergt* 2000; *Weniger, Engels* 1996). Humus content is largely dependent on the intensity of crop cultivation technologies. With increasing intensity of agricultural production humus reserves are depleted through continual soil tillage, narrow specialization of crop rotations, intensive and heavy mineral fertilization, application of plant protection measures and other factors (*Kuliukin* 1997, *Freyer* 2003).

Humus breakdown and formation balance is achieved when the constantly replenished organic matter content in the soil compensates for mineralized and removed in the form of plant nutrients organic matter (*Lejins, Lejina* 2000). It is common to maintain organic matter content in the soil by heavy application of farmyard manure and by cultivating in the crop rotation perennial grasses that leave large amounts of dead plant residues in the soil (*Arvidsson* 1998, *Hofman* 1986, *Barber* 1988, *Teit* 1991, *Freyer* 2003). It was manure (10 t ha^{-1} per year) stored for a longer period with which part of already humified organic matter is introduced into the soil (*Arvidsson* 1998, *Hofman* 1986).

Experimental evidence suggests that in the case of manure fertilization, about 75 % of this fertilizer mineralises and about 25 % increases humus reserves in the soil (*Lacko-Bartasova et al.* 1999). However the recent developments in agriculture resulting in the emergence of solely crop production farms creates shortage of manure, furthermore, a reduction in the number of livestock declines the demand for the biomass of perennial grasses. Searching for alternative sources of organic matter replenishment in the soil, green manure becomes an increasingly attractive research subject. Some researchers assert that green manure is an important source for organic matter maintenance in the soil, other researchers indicate that nitrogen – rich mass of green manure is prone to rapid mineralization and stimulates humus mineralization and supply the plants with nutrients only for a short period of time (*Nadezkin, Korjagin et al.* 1998, *Kudiejarov* 1999, *Ziezjukov et al.* 1999).

The effect of green manure on humus content depends on the chemical composition of organic matter, whose breakdown process – mineralization, micro-organism immobilisation and incorporation into stable organic compounds, bound with soil particles and humification direction are determined by many factors. The ratio of carbon (C) to nitrogen (N) and content of lignin in organic matter, genetic properties of soil, granulometric composition and aeration have a significant effect on stable accumulation of humus (*deNeergard* 2002, *Wivstad et. al* 2003, *Buciene* 2003).

Some researchers suggest that an effective means for the maintenance of soil potential is cultivation of perennial grasses and using their aboveground mass as a green manure. Organic-carbon rich root mass left in the soil by legumes supplemented with nitrogen – rich aboveground biomass plays an important role in the maintenance of balance between mineralization and humification processes (*Barber* 1998, *Merbach et. al.* 2000).

The objective of the present study was to ascertain the effects of incorporation of perennial legume crops aboveground biomass as green manure and different fertilisation systems mineral, organic – mineral and organic on soil humus content and its fractional composition.

Materials and methods

The data from two experiments were generalised in order to estimate the effects of perennial legumes and their biomass used as green manure and fertilising systems differing in intensity on the variation of soil humus and its fractional composition.

Site, soil characteristics and sampling

Experiments were conducted at the Joniskelis Research Station of the Lithuanian Institute of Agriculture located in the northern part of Central Lithuania Lowland. According to the FAO soil classification system, the soil of the experimental site is characterised as *Endocalcari-Endohypogleyic Cambisol (CMg-n-w-can)*, according to the texture – clay loam on silty clay. The soil agrochemical properties in the 0–20 cm layer were as follows: pH_{KCl} – 6.1–6.4; humus – 1.98–2.18%; total nitrogen – 0.131–0.138%; plant available P_2O_5 and K_2O – 108–130 and 241–279 mg kg^{-1} of soil respectively. The legume crops and cereals were grown using conventional technology.

Soil samples for the determination of chemical composition were taken from the backgrounds of legume crops before applied organic manure and after growing cereals at a depth of 0–20 cm. Soil samples for $N_{min.}$ were taken in spring, after the renewal of winter wheat vegetative growth from the 0–40 cm depth.

The hydrothermal regime (HTC) of the crops vegetative growth period (April–August) was characterised as optimally wet in 1996–1999, 2002 and weak drought in 2000, and excessively wet in 2001 (*Kelcevskaja* 1971).

Experimental design

Experiment 1. «Effects of legume crops and their biomass incorporated as green manure on the variation of humus and its composition» was conducted during 1996–2002 according to the following experimental design: *Factor A.* Preceding crops for cereal sequence (winter wheat-winter wheat-spring barley): 1. Red clover *(Trifolium pratense L.),* 2. sown lucerne (*Medicago sativa* L.), 3. vetch and oat mixture (*Vicia sativa* L., *Avena sativa* L.);

Factor B. Organic manure: 1. Without manures, 2. Ist crop without manure, 3. Ist crop – green manure, 4. Ist crop – 40 t ha^{-1} of farmyard manure (FYM).

Experiment 2. Research in the long – term fixed trial «The effects of various fertilisation systems mineral, organic – mineral and organic on soil humus content» was carried out during 1995–2000 according to the following experimental design: 1. Without fertilisers; 2. Mineral fertilisation system $N_{56}P_{48}K_{60}$; 3. Organic – mineral fertilisation system I – 40 t ha^{-1} farmyard manure+$N_{56}P_{48}K_{60}$; 4. Organic – mineral fertilisation system II – 60 t ha^{-1} farmyard manure + $N_{56}P_{48}K_{60}$; 5. Organic – mineral fertilisation system III – 80 t ha^{-1} farmyard manure + $N_{56}P_{48}K_{60}$; 6. Organic fertilisation system – 80 t ha^{-1} farmyard manure. The trial was conducted in a five – course crop rotation: sugar beet, spring barley, perennial grasses of the 1st year of use, perennial grasses of the 2nd year of use, and winter wheat. In the organic – mineral fertilisation systems mineral fertilisers were distributed in the following way: for sugar beet $N_{120}P_{90}K_{120}$, spring barley $N_{30}P_{60}K_{60}$, perennial grasses of the 1st year of use $P_{50}K_{60}$, perennial grasses of the second year of use N_{60}, winter wheat $N_{70}P_{40}K_{60}$, of which the average annual rate was $N_{56}P_{48}K_{60}$. Farmyard manure (containing dry matter 19.81 %, N 0.38 %, P_2O_5 0,21 %, K_2O 0,67 %) in the organic – mineral and organic systems was given to sugar beet.

Data analysis

Total nitrogen in the soil samples was determined by the Kjeldahl method. Humus content was determined after picking out visible rootlets from the soil samples by the Tyurin method, and humus fractional composition was identified according to the Tyurin method modified by Ponomariova and Plotnikova (1980) at the Lithuanian Institute of Agriculture (LIA) analytical laboratory. Mineral nitrogen (NO_3 + NH_4) was determined by distilling and colorimetry (in KCl extraction) method. Nitrogen content in the green material of legume crops, their plant residues, farmyard manure were determined by Kjeldahl method (*Official methods of analysis* 1984), carbon by the analyser «Heareus».

Humification coefficient of farmyard manure was calculated according to the formula:

$$y = (a : b) \times 100, (1)$$

where y – humification coefficient, a – humus content t ha^{-1}, that formed per rotation from farmyard manure, b – DM content t ha^{-1} (without ash) incorporated with farmyard manure per rotation. Humus content t ha^{-1} that had formed from farmyard manure per rotation was calculated according to the formula:

$$a = m - n, (2)$$

where m-increase of humus per rotation t ha^{-1}, n-humus content from plant residues t ha^{-1}. Humus content that had formed from plant residues was calculated by multiplying crop yield by the coefficient for individual crop rotation plants (*Lukosiuniene* 1992). The amount of manure necessary per rotation in order to cover the losses of mineralised humus was calculated as follows:

$$x = c : d, (3)$$

where c – humus losses per rotation t ha^{-1}, d – humus received from 1 t of farmyard manure.

The experimental data were processed by methods of dispersion and correlation analysis, applying the programmes ANOVA (for bi-factorial experiment) and STATENG for statistical data treatment (*Tarakanovas* 1999). Credibility of equations was determined according to Fisher criterion (r or η), at 95% probability level (marked as*), 99% (marked as**).

Results and discussion

Experiment 1. **Effects of legume crops and their biomass incorporated as green manure on the variation of humus and its composition.**

Quality of legume plant residues and organic manure. Averaged data from three experimental years indicate that together with the roots of legume crops and their residues the dry matter incorporated was 1.5–3.5 times higher than that incorporated together with the aboveground biomass used as green manure. The highest contents of all nutrients were left in the soil after lucerne and clover, while the lowest contents after vetch and oats mixture, this was determined by the mass of plant residues and their chemical composition. After bastard lucerne the soil received with plant residues 234.8 kg N ha^{-1}, the largest part of which 156.5 kg ha^{-1} was made up from the atmospherically fixed nitrogen. Here the nitrogen content was 2.4 times higher than after clover and 6.1 times higher than after vetch and oats mixture. The ratio of carbon to nitrogen, determining the processes of the organic matter transformation in the soil, differed in the aboveground and belowground parts of plants between separate plant species and within individual plants. This ratio for lucerne residues was the narrowest 18, and that for vetch and oats mixture was the widest 35. The data obtained by Janusiene suggest that 68–79% of plant residue mass breaks down per year and C:N ratio is 16–24. Furthermore, humification coefficient of legumes is higher than that of spiked cereals (*Janusiene* 2002).

Less nutrients were introduced into the soil with the aboveground mass of legumes than with roots. Averaged data show that the highest content of nitrogen 114.2 kg N ha^{-1} were introduced with lucerne green material, which is 1.4 and 3.2 times more than with clover or vetch and oats mixture. The content of atmospherically fixed symbiotic nitrogen in the aboveground mass of plants incorporated into the soil as green manure was lower than in residues, however it was significant: of clover 50.5 kg N ha^{-1}, lucerne 72.1 kg N ha^{-1}. The narrower carbon to nitrogen ratio (C:N) in lucerne green material determined a slightly more rapid organic matter mineralization than for clover.

The content of nitrogen incorporated with farmyard manure varied from 120.0 to 188.0 kg ha^{-1} and in separate years was equal to that incorporated with lucerne aboveground biomass. However, with farmyard manure the soil received much more phosphorus and potassium 4.0–5.8 and 2.2–3.0 times more than with green manure.

The content of mineral nitrogen in the soil is one of the major parameters that define decomposition of organic matter incorporated into the soil. Statistical analysis showed that having incorporated organic fertilisers in the first year $N_{min.}$ depended not only on the nitrogen content incorporated with organic fertiliser DM, their C : N ratio but also on the soil total nitrogen, labile humic acids and on the conditions of organic matter mineralization in heavy soils – bulk density and aeration porosity (Table 1).

Table 1

Soil mineral nitrogen (y) in relation to the incorporated organic matter, their quality and soil properties (x)

Factors (x)	Member of sequence	
	first	second
	w. wheat	w. wheat
Incorporated of dry matter t ha^{-1}	0,85**	0,85**
Incorporated of nitrogen kg ha^{-1}	0,88**	0,84**
C:N of organic matter	-0,95**	0,33*
Humus before experiment%	0,77*	0,45 ns
Total nitrogen%	0,78*	0,44 ns
Mobile acids, (HR)%		0,71**
Humic acids, (HR)%		0,57 ns
Soil bulk density Mg m^{-3}	-0,86*	-0,46 ns
Aeration porosity%	0,85**	0,43 ns

** p<0,01; *p<0,05; n.s. – statistically is not significant.

In the second year, when growing winter wheat $N_{min.}$ variation was less dependent on soil properties. A stronger relationship remained with the nitrogen incorporated with organic matter and soil labile humic acids.

Humus composition. In clay loam soil fulvic acids accounted for the largest share in soil humus (39.3–44.8%), while humic acids and humin accounted for a smaller share (26.6–31.4%). The highest content of humic acids was determined after vetch and oat mixture (on average by 30.2%) and in the plots fertilised with farmyard manure (30.1%) (Table 2). Analyses of humic acids composition revealed that the fraction of mobile humic acids (HA1), which is attributed to active forms of humus and is characterised by an increased content of hydrogen and nitrogen, accounted for a small share from the sum total of humic acids (HA total). After all the legume crops studied, the highest content of these acids (HA1) (11.0% from the total content of HA) accumulated after lucerne and the lowest (8.5%) after vetch and oat mixture. Green manure increased their content by 4.4%, however less than farmyard manure. The rest of the larger part of humic acids comprised acids bound with Ca and strongly bound with clay particles (HA2 and HA3).

Table 2

Effect of preceding crops and organic fertilizers on humus composition in soil when growing winter wheat

Treatment	% of C from the total amount of carbon					
		% of HA fraction from HA total				
	HA total	mobile (HA1)	bound with Ca (HA2)	tightly bound (HA3)	FA total	HA/FA
Preceding crop-red clover						
Without fertilizers	26.7	8.4	44.0	47.6	41.2	0.65
I st crop without fertilization,	26.6	8.4	44.0	47.6	41.0	0.65
I st crop – green manure,	27.5	9.6	42.4	48.0	41.9	0.66
I st crop – FYM 40 t ha^{-1}	28.1	9.5	43.1	47.4	39.3	0.71
Preceding crop-sown lucerne						
Without fertilizers	28.9	10.9	40.6	48.5	40.2	0.72
I st crop without fertilization,	29.1	10.9	40.6	48.5	40.4	0.72
I st crop – green manure,	29.2	10.4	37.6	51.9	41.1	0.71
I st crop – FYM 40 t ha^{-1}	30.7	11.6	36.8	51.6	44.4	0.68
Preceding crop-vetch and oats mixture						
Without fertilizers	29.4	8.1	35.8	54.2	43.2	0.68
I st crop without fertilization,	29.3	8.1	35.8	54.2	43.1	0.68
I st crop – green manure,	31.0	8.4	38.0	51.7	44.8	0.69
I st crop – FYM 40 t ha^{-1}	31.4	9.5	35.7	52.8	42.3	0.71
Average after preceding crops						
Clover	27.2	9.0	43.3	47.7	40.8	0.67
Lucerne	29.5	11.0	48.9	50.1	41.6	0.71
Vetch and oats mixture	30.2	8.5	36.3	53.2	43.4	0.69
Average in fertilization treatments						
Without fertilizers	28.4	9.1	40.1	50.1	41.6	0.68
I st crop without fertilization,	28.3	9.1	40.1	50.1	41.5	0.68
I st crop-green manure,	29.3	9.5	39.3	50.5	42.6	0.68
I st crop – FYM 40 t ha^{-1}	30.1	10.2	38.5	50.6	42.0	0.70
LSD_{05} fact. A	1.78	1.30	3.19	2.48	2.03	0.043
LSD_{05} fact. B	2.05	1,50	3.69	2.86	2.35	0.050
LSD_{05} fact. AB	3.56	2.59	6.38	4.95	4.07	0.087
Sx	4.2	9.3	5.5	3.4	3.3	4.3

They are attributed to stable or inert humus forms, which are more resistant to decomposition and show a higher level of soil cultivation. The highest content of humic acids bound with Ca was found after clover (43.3%). The content of fulvic acids (FA total), which have a negative effect on plant growth and soil properties, under the effect of individual preceding crops and organic fertilisers differed insignificantly when wheat was grown.

Humus quality is defined by the ratio of humic and fulvic acids ($C_{HA} : C_{FA}$). In our experiments it did not differ much after various legume crops, but it did after lucerne – 0.71. When fertilising by farmyard manure, this ratio tended to increase. Due to earlier described humus composition indicators, the humus of clay loam soil can be characterized as low mobile and tightly bound.

Soil total nitrogen. Averaged data suggest that before trial establishment the highest total nitrogen content in the soil was accumulated on the background of lucerne (0.140%), however, these data for clover and vetch mixture differed inappreciably (0.135 and 0.134%, respectively) (Table 3).

Average data suggest that when cereals had been grown for three years in succession (winter wheat – winter wheat – spring barley) after legume preceding crops and their biomass and farmyard manure had been incorporated the content of total nitrogen consistently declined by on average 10.3% in all treatments. When estimating only the preceding crops, it was identified that the greatest reduction in the total nitrogen occurred after clover and lucerne, 14.8 and 9.3%, respectively, while a slightly less reduction was identified after vetch and oats mixture 7.5%, compared with the data of the respective preceding crop before the trial establishment.

When estimating fertilisation treatments, the greatest reduction in nitrogen content (11.0%) occurred in the plots applied only with mineral nitrogen fertiliser. Significant reductions in nitrogen were also recorded for the other treatments: in unfertilised treatment 10.3%, in the treatment applied with manure 9.6%, and the treatments fertilised with green manure 8.8%. The most dramatic reduction in the total nitrogen content was identified when cereals had been grown after clover and had been fertilised with mineral nitrogen fertilisers and without mineral fertilisation by 17.8 and 14.8%, respectively, compared with the previous data. When cereals were grown after annual mixture as preceding crop, the total nitrogen content in the soil in the analogous treatments declined by 6.7 and 8.2% compared with the data before the trial establishment. Such results were determined by a markedly lower yield of cereals after this preceding crop and lower nitrogen removal than that after perennial legumes.

After cereals growing a significantly higher total nitrogen content remained after lucerne preceding crop 0.127%, which was by 10.4 and 2.4% more than after clover or mixture. Furthermore, after various preceding crops the total nitrogen content in the treatments fertilised with green manure and especially with farmyard manure, tended to increase compared with unfertilised treatments. The highest content of total nitrogen after cereals growing was identified after lucerne preceding crop when fertilising with lucerne biomass and farmyard manure 0.128% or 11.3% more than in the control treatment. Statistical analysis suggests that the relationship between the total nitrogen content after cereals growing and the dry matter content of organic matter incorporated was moderate (r=0.42*).

Table 3

The influence of preceding crops and organic manure on the variation of total nitrogen and organic carbon in the soil (0–20 cm), after growing cereals, Joniskelis, 1996–2002

Treatments	After growing of cereals			
	total nitrogen	organic carbon	C:N	humus
Preceding crop-red clover				
Without fertilizers	0.115	1.11	9.7	1.92
I st crop without fertilization	0.111	1.14	10.3	1.96
I st crop – green manure	0.119	1.12	9.4	1.94
I st crop – FYM 40 t ha^{-1}	0.117	1.18	10.1	2.04
Preceding crop-sown lucerne				
Without fertilizers	0.127	1.23	9.7	2.11
I st crop without fertilization	0.127	1.21	9.6	2.08
I st crop – green manure	0.128	1.21	9.5	2.09
I st crop – FYM 40 t ha^{-1}	0.128	1.28	10.0	2.21
Preceding crop-vetch and oats mixture				
Without fertilizers	0.123	1.17	9.5	2.02
I st crop without fertilization	0.125	1.18	9.4	2.03
I st crop – green manure	0.124	1.20	9.7	2.07
I st crop – FYM 40 t ha^{-1}	0.124	1.25	10.1	2.15
Average after preceding crops				
Clover	0.115	1.14	9.9	1.97
Lucerne	0.127	1.23	9.7	2.12
Vetch and oats mixture	0.124	1.20	9.7	2.07
Average in fertilization treatments				
Without fertilizers	0.122	1.17	9.6	2.02
I st crop without fertilization	0.121	1.18	9.8	2.02
I st crop – green manure,	0.124	1.18	9.5	2.03
I st crop – FYM 40 t ha^{-1}	0.123	1.24	10.1	2.13
Average of experiment	0.122	1.19	9.8	2.05
LSD$_{05}$ fact. A	0.005	0.024		0.040
LSD$_{05}$ fact. B	0.005	0.028		0.047
LSD$_{05}$ fact. AB	0.009	0.048		0.081

Soil carbon and humus. The highest organic carbon content in the 0–20 cm soil layer was identified after lucerne 1.25%, after clover and annual mixture less by 1.16 and 1.18%, respectively or by 7.2 and 5.6% less than after lucerne.

After a tree-year cereal link growing the content of organic carbon markedly declined after lucerne (1.6%) and clover (2.6%), and after vetch and oats mixture it slightly increased (2.1%), compared with the previous values. This resulted from the fact that cereals left less plant residues and at the same time less organic carbon in the soil compared with perennial grasses. More over, an increase in soil aeration during the soil preparation for cereals activated soil microbiological processes and increased mineralization of readily decomposed humic substances that accumulated after perennial grasses. Annual mineral nitrogen fertilisation of cereals negligibly declined (1.7%) carbon content in the soil. This resulted from the stimulating effect of nitrogen fertiliser on organic matter mineralization which causes deficiency of carbon assimilated by soil micro-organisms, this fact has been also reported by other authors (*Kudiejarov* 1999). The content of organic carbon increased only for the treatment fertilised with farmyard manure, which made up on average 3.3% through all preceding crops, compared with the previous level.

After the cereal crop rotation link on the backgrounds of legume preceding crops the highest carbon content 1.23%, like at the sequence of the experiment, remained after lucerne preceding crop, its content there was 7.9% higher than after clover and 1.7% higher than after vetch and oats mixture (Table 3).

On average, after all preceding crops, incorporation of different amounts of organic matter had a diverse effect on the variation of organic carbon content. The highest increase in the organic carbon content occurred in the plots fertilised with farmyard manure, significant difference compared with the unfertilised treatment made up 6.0%. When estimating individual treatments, the lowest organic carbon content was identified for the plots after unfertilised clover and vetch and oats mixture. A trend of organic carbon increase was identified after clover and annual mixture preceding crops in the treatments fertilised with green manure.

Results of the correlation – regression analysis suggest that the variation of organic carbon after a three – year cereal crop rotation link was dependent on the dry matter incorporated with the aboveground and underground biomass of preceding crops r=0.56** and nitrogen content present in them r=0.60**. The relationship of organic carbon content in the soil with the C:N ratio of organic matter incorporated was weak and insignificant, however significantly correlated with soil carbon content (r= 0.59*) before the trial establishment and total nitrogen (r=0.77**).

The relationship between organic carbon variation and soil physical properties was nonessential, though a slight carbon increasing trend was identified with a reduction in soil bulk density and an increase in aeration porosity and in the content of valuable structural soil aggregates 0.25–10.0 mm in size.

The ratio of carbon to nitrogen (C:N) in the soil at trial establishment differed inconsiderably (8.7–8.9) in different backgrounds, however, it was inappreciably higher after lucerne. Growing of cereals which are demanding in terms of nitrogen for three years in succession resulted in a substantial increase in the carbon to nitrogen ratio, by on average 10.2%, compared with this ratio before the trial establishment. This was determined by disproportional variation of soil total nitrogen and carbon. Having compared C:N changes after different legume preceding crops it was found that the greatest increase in this ratio occurred after clover (15.1%) due to the reduction in total nitrogen, compared with the previous data.

On average in all fertilisation treatments owing to the same reason C:N was the greatest in unfertilised soil and fertilised with only nitrogen fertiliser treatments. The lowest soil C:N was found when fertilising with only nitrogen fertilisers or fertilising with nitrogen under the effect of green manure, whereas in the treatment fertilised with manure the soil carbon to nitrogen ratio increased by 12.5%, compared with the data before the trial establishment. This increase resulted from the increase in carbon.

Experiment 2. **Effects of various fertilisation systems – mineral, organic – mineral and organic on soil humus content.**

Variation of soil humus. After the five – course rotation with two fields of perennial grasses in the unfertilised treatment (control), the content of humus in the 0–20 cm depth did not change, compared with the initial level (Figure 1).

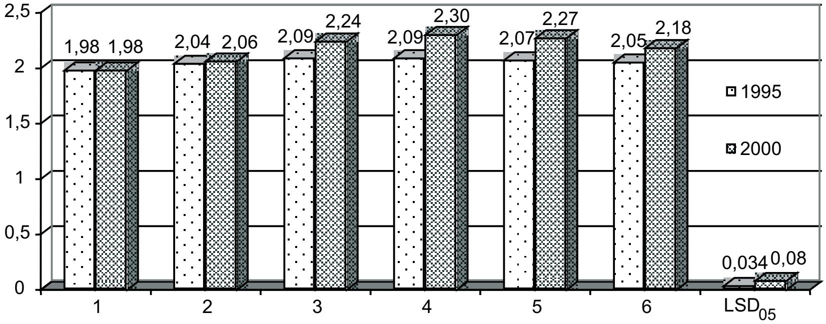

Fig. 1. Effect of fertilization systems on soil humus content – 20 cm layer of soil. Fertilization systems: 1. Without fertilizers; 2. Mineral NPK; 3. I – organic mineral 40 t ha^{-1} of manure + NPK; 4. II – organic mineral 60 t ha^{-1} of manure + NPK; 5. III – organic mineral 80 t ha^{-1} of manure + NPK; 6.80 t ha^{-1} of manure.

In the mineral fertilisation system which received on average $N_{56}P_{48}K_{60}$ annually (treatment 2) the content of humus remained close to that before the trial establishment. Positive changes in humus in the 0–20 cm layer were identified in all organic – mineral fertilisation systems (with the application of 40; 60 and 80 t ha^{-1} farmyard manure), i. e. significantly 13.1; 16.2 and 14.6% more than in the control treatment and 7.2; 10.0 and 9,7%, compared with its initial level.

In the organic fertilising system (80 t ha^{-1} farmyard manure) humus content was significantly 10.1% higher compared with the control treatment, 6.3% compared with its initial level and 5.8%, compared with mineral fertilisation system. In the organic – mineral fertilisation systems compared with mineral fertilisation system humus content also significantly increased through the application of 40 t ha^{-1} farmyard manure 8.7%, 60 t ha^{-1} – 11,7% and through 80 t ha^{-1} – 10.2%. In the organic – mineral fertilising systems when increasing manure rate between different manure fertilisation levels an increase in humus content was not significant.

These data suggest that in the organic – mineral fertilisation systems more favourable conditions are created for humification and humus accumulation in the soil compared with organic or mineral fertilisation systems. When applying in the crop rotation organic – mineral fertilisation systems with 40; 60 and 80 t ha^{-1} farmyard manure its humification coefficient was 36; 38 and 26%, respectively, and 94; 98 and 67 kg ha^{-1} of humus per ton of manure was produced. In the organic fertilisation system humification coefficient was the lowest 19% and 48 kg ha^{-1} of humus was produced per 1 ton of manure. Experimental evidence suggests that in order to maintain non-deficient balance of humus and to secure its increase in the crop rotation one should apply 12–16 t ha^{-1} of farmyard manure together with mineral fertilisers annually.

Correlation-regression analysis shows that soil humus content at the 0–20 cm depth was strongly dependent on DM (r=0.79**) incorporated with organic fertiliser and on nutrients incorporated with organic and mineral fertilisers – nitrogen (r=0.87*).

Conclusions

1. Bastard lucerne as preceding crop increased soil humus content most markedly. The content of mobile humic acids (HA1) and the ratio of humid to fulvic acids ($C_{HA} : C_{FA}$) was significantly higher after lucerne preceding crop, compared with clover or annual mixture.
2. More intensive soil aeration during soil preparation for cereals promoted organic matter mineralization and reduction in humus content, especially in the plots applied with mineral nitrogen fertilizer.
3. On clay loam cambisol various fertilisation systems – mineral, organic – mineral and organic had a marked effect on the variation of soil humus content. In organic – mineral fertilisation system application of 40; 60 and 80 t ha^{-1} per crop rotation of farmyard manure and $N_{56}P_{48}K_{60}$ average per year resulted in a significant soil humus content increase by 8.7%; 11.7%, and 10.2 %, and in the organic system by 5.8%, compared with mineral fertilisation system.
4. The most rational fertilisation system in terms of humus accumulation was organic – mineral with the application of 40 and 60 t ha^{-1} of farmyard manure and NPK as much as in the mineral system since the highest humification coefficient was achieved, which was 36 and 38 percentage units or 38.5 and 46.1% higher compared with the application of 80 t ha^{-1}.

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Quantification of fluxes of humic substances in forested drinking water catchments in the low mountain range Ore Mountains (Erzgebirge), Germany

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The humic matter loads of a drinking water catchment area in the Ore Mountains in Saxony were quantified for the years 1993–2003. Dissolved organic carbon was separated into its fractions by the molecular size with an LC-OCD system. Depending on their retention times in the gel column of the LC-OCD we identify the concentrations of 6 fractions. The high molecular mass fraction (HS I) and the middle molecular mass fraction (HS II) represent humics in a natural water. The values for the sum of the humic fractions I and II were between 5.7 and 21.6 kg*ha^{-1}*a^{-1}, whereby the individual brooks of the subcatchment areas, depending on their catchment area features, played a very different role in the total load of the receiving stream. Here, the peatlands and the histic gley soils represent the main source of the humic matter in the brooks. A subcatchment area with a proportion of peatlands and histic gley soils of 27% had a 10 times higher load than from a catchment area with only mineral soil formations twice its size. Extreme weather conditions influence the input of humic substances into the brooks and rivers.

Introduction

Since the beginning of the 90s an increase in NOM (natural organic matter) concentrations has been observed in the rivers in several parts of Northern and Central Europe (*Freeman et al.* 2001, *Mc Cartney et al.* 2003, *NORDTEST* 2003, *Grunewald et al.* 2003, *Worrall & Burt* 2004, *Hongve et al.* 2004). This phenomenon also occurs in the low mountain ranges of Germany. The reasons which are responsible for the NOM increase in rivers and lakes are complex and not entirely known. Key factors are climate change (*Freeman et al.* 2001, *Hejzlar et al.* 2003), decreasing sulphur depositions (*McCartney et al.* 2003), increasing microbiological soil activities (*Bragg* 2002, *Evans and Monteith* 2001) and increasing carbondioxide concentrations in the atmosphere (*Freeman et al.* 2004, *Kang et al.* 2001). In the Ore Mountains (Erzgebirge), Saxony the case of dissolved organic substances has increased in a number of drinking water reservoirs. These must be removed by the waterworks at great expense because it has become a sizeable cost factor in drinking water treatment. Adapting to the changed situation, the question about the development of NOM inputs in drinking water reservoirs has arisen.

The catchment areas of the drinking water reservoirs in the Ore Mountains are forested and, for the most part, consist of a considerable proportion of peatlands, which constitute the main source of humic substances in surface waters. Past and current fluxes must be estimated in order to be able to assess the further development. In the brooks of the investigation area, continuous measurements of the parameters DOC (dissolved organic carbon) and the SAC (spectral adsorption coefficient) at 254 nm and 436 nm wavelength have been taken place since the 90s.

The aim of this work is the calculation of the recent and past humic matter loads for a drinking water reservoir, in order to investigate the influence of different catchment area conditions on the humic matter inputs in surface waters under changing environmental conditions.

Investigation area

Our investigation areas are situated in the east and the west of the Ore Mountains to the south of the Free State of Saxony in the south-east of Germany. This involves the catchment areas of the drinking water reservoirs Rauschenbach and Flàje in the eastern Ore Mountains, whereby the reservoir Flàje is located on the territory of the Czech Republic. In the western Ore Mountains the catchment areas of the drinking water reservoirs Muldenberg and Carlsfeld are being investigated. In this paper the discussion is restricted to the main channel (brook «Rauschenbach») to the drinking water reservoir Rauschenbach in the eastern Ore Mountains (Figure 1). The characteristic of the catchment area is shown in Table 1.

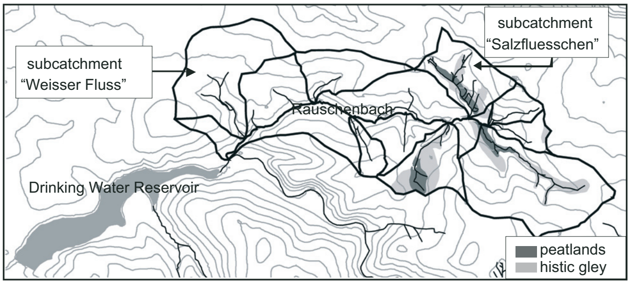

Fig. 1. Investigation area catchment Rauschenbach.

Table 1

Catchment area characteristics

Area		931 ha
Altitude		600–870 mNN
Landuse	Forests	75%
	Grassland	24%
	Settlement	1%
Soils	Peatlands and histic gley soils	10%
	Terrestrial soils	90%
Climate	Precipitation	950 mm/yr
	Temperature	6,3 °C

The catchment area of the Rauschenbach is shaped by continental climatic conditions and is considered as part of the medium to upper wet to very wet mountain ranges. Whereas on the German side the land use is predominantly characterized by forests of Norway spruce (picea abies), the landscape on Czech territory is determined by pioneer foliage forests and clearings covered with grass.

Different soil formation conditions are mirrored in the heterogenous spatial pattern of terrestrial, semi-terrestrial or rather fully hydromorphous soils and are marked by a different potential for humic substances release.

The catchment area of the brook Rauschenbach can be divided into several subcatchments which have a different influence on the humic matter load of the stream.

Materials and methods

Since 1993, the brook Rauschenbach has been monitored on a monthly base by the Landestalsperrenverwaltung (state dam administration) of Saxony for the parameters DOC (dissolved organic carbon) and SAC (spectral adsorption coefficient) at 254 and 436 nm wavelengths.

In the year 2001 a detailed measuring network for chronological and spatially higher distributed monitoring of the brooks was installed in the investigation area. The DOC from selected locations was gelchromatographically fractionated with an LC-OCD system. This method allows the classification of DOC into 6 fractions, according to Huber & Frimmel (1992) (Table 2).

Table 2

Fractions of the DOC

Fraction	Term
HS I	High-Molar Mass Humic Substances (I),
HS II	Middle-Molar Mass Humic Substances (II)
BB	Building Blocks
nS	Low-Molar Mass Organic-Acids
anS	Low-Molar Mass Neutrals and Amphiphilics
Psac	Polysaccharides

The separation criterion is the molecular size. The fractions HS I and HS II represent the humics. Building Blocks (BB) are Hydrolysates of the humics and highly substituted aromatic or conjugated acids. Low-Molar Mass Organic-Acids (nS) are the final degradation product of organics. Low-Molar Mass Neutrals and Amphiphilics (anS) make up about 10%–30% of TOC (total organic carbon) and are rare in aquatic systems. The last fraction, the polysaccharides (Psac), is very high in molar mass (> 50.000 – 2 Mio. $g*mol^{-1}$). Psac includes amino sugars, polypeptides and proteins.

For the years 1993–2001 the DOC fractions were calculated with a regression model based on the measured values of DOC, SAC_{254} and the colour (SAC_{436}) (Table 3). This way it was possible to set up and evaluate a timeframe of 11 years (Figure 2).

Table 3

Regression parameters of the form: a * SAC_{254} + b * DOC + c * SAC_{436} + d

Fraction	a	b	c	d	R^2	p
HS I	0.073	0.364	-0.089	-0.265	0.94	0.05
HS II	0.059	0.024	0.091	0.026	0.89	0.05
BB	0.010	0.052	-0.102	0.112	0.82	0.05
nS	0.002	0.047	-0.126	0.013	0.19	0.05
anS	0.014	0.042	-0.151	0.103	0.31	0.05
Psac	0.011	-0.012	0.008	0.001	0.83	0.05

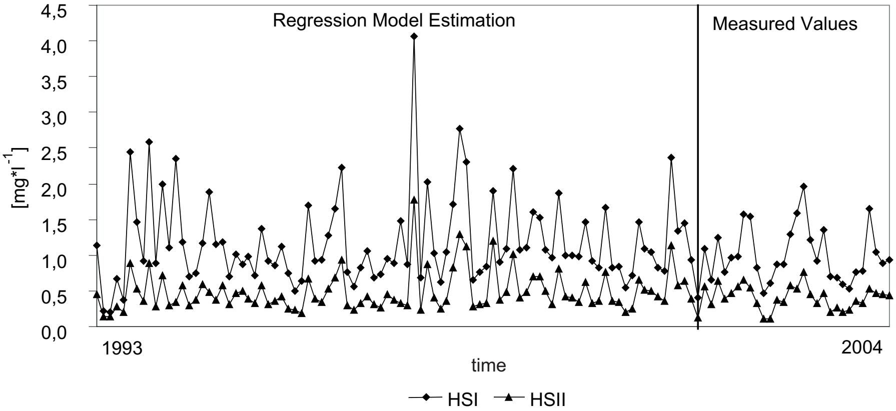

Fig. 2. Time series for the fractions I and II, estimated and measured values (monthly medians)

For the calculation of the water balance, the physical catchment area model SWAT 2000 (*Arnold et al.* 1998) was used. SWAT 2000 was developed for the prediction of the influence of land usage on sediment loads and chemical substances from agriculture in rivers. With this model, the water balance for a number of catchments and subcatchments can be modelled in one simulation run.

Advantages of this model are the free availability of the usage and the source code, a user-friendly Arc View GIS interface, as well as extensive documentation. SWAT has been employed successfully for several years, especially in the USA and Europe (*Boorman* 2003, *Eckhardt & Ulbrich* 2003, *Fontaine et al.* 2002, *Grizzetti et al.* 2003). Up to now the model SWAT has been used in the Ore Mountains for testing by simulation calculations. In its standardized form, the routine available in the model for the calculation of «wetlands» has shown itself to be unsuitable. For this reason, the freely available source code by SWAT 2000 (Fortran 95) was adapted to the conditions in the testing areas. With the modified version it is possible to calculate the evaporation from forests and the runoff from the peatlands more adequately than with the original model.

Results

The humic substances (HS I and HS II) represent the greatest part of the fractions of DOC followed by the Building Blocks, the low molar mass substances and the polysaccharides. (Figure 3).

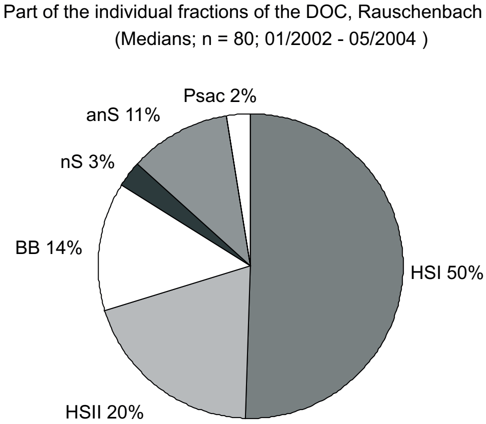

Fig. 3. Average composition of DOC – Rauschenbach.

Since 1993, a remarkable increase of the loads of humic substances (sum of HS I and HS II) has been recorded, with a maximum of 21.6 kg*ha^{-1}*a^{-1} in the year 2002 (during the flood in Saxony) and a minimum of 5.7 kg*ha^{-1}*a^{-1} in the year 2003 (extreme drought) (Figure 4).

Only a few changes were detected in the composition of DOC in the median values between 1993 and 2003. The humic substances have slightly increased for the entire period (Figure 5). The DOC-concentration and the fraction of humic substances are strongly dependent on short-term fluctuations in hydrology. So, depending on drainage and weather conditions, differences of up to 30% can occur for the part of the humic matter of the DOC within a month.

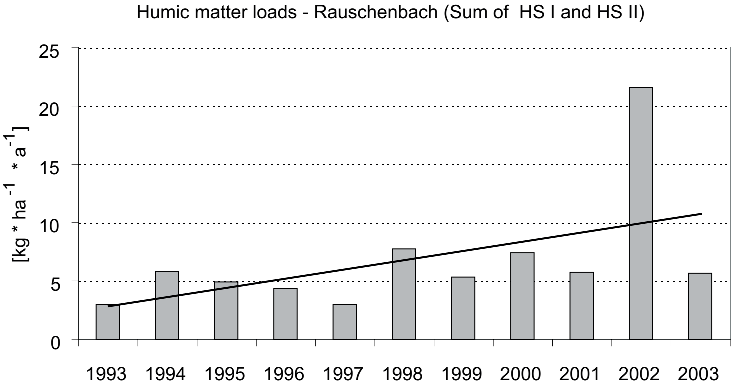

Fig. 4. Annual average humic matter loads – Rauschenbach.

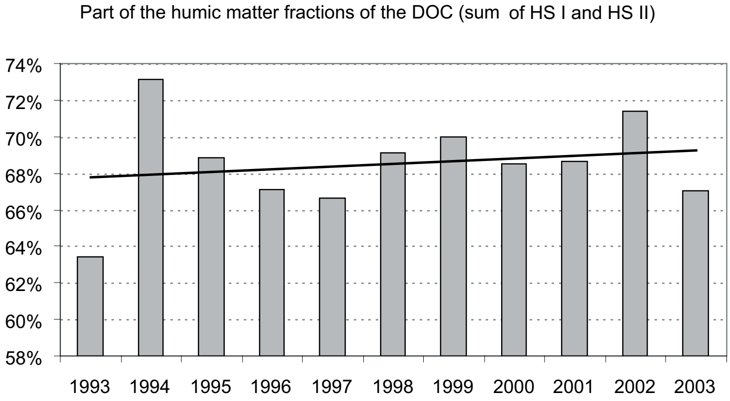

Fig. 5. Development of the humic matter fractions of the DOC.

The subcatchments participate in the humic matter load of the Rauschenbach in different ways. I.e., the median concentrations of DOC and the size of the humic matter fractions I and II strongly correlate with the proportion of peatlands in the catchments (*Grunewald et al.* 2004). Therefore they represent the major source for humic matter in the Ore Mountains.

For comparison, two differently equipped subcatchments are being juxtaposed here (see Figure 1). The subcatchment «Weisser Fluss» (area = 148.2 ha) possesses only mineral soil formations (cambisol). In contrast, the subcatchment «Salzfluesschen» (area = 76.7 ha) has 27% peatlands and histic gley soils. The median DOC concentration of the «Weisser Fluss» is 1.4 mg*l^{-1} and the median spectral adsorption coefficient at 2.5 m^{-1}. In contrast, the «Salzfluesschen» shows a median DOC of 5.7 mg*l^{-1} and a median SAC$_{254}$ of 23.4 m^{-1}. The average proportions of the fractions in the DOC also differ noticeably.

I.e., the DOC of the «Weisser Fluss» shows lower proportions of the humic matter fractions I and II in favour of the building blocks and the neutrals and amphiphilics (Table 4).

Table 4

Average proportions of the fractions in DOC for the subcatchments «Weisser Fluss» and «Salzfluesschen»

Fraction	«Weisser Fluss»	«Salzfluesschen»
HS I	38%	61%
HS II	15%	19%
BB	21%	7%
nS	1%	1%
anS	22%	8%
Psac	2%	2%

The relative loads calculated for the years 2002 and 2003 are shown in Figure 6. It becomes clear that the individual subcatchments have a different influence on the load of the receiving brook. In 2002 (flood) the loads are approximately 4 times higher than during the extreme drought in 2003.

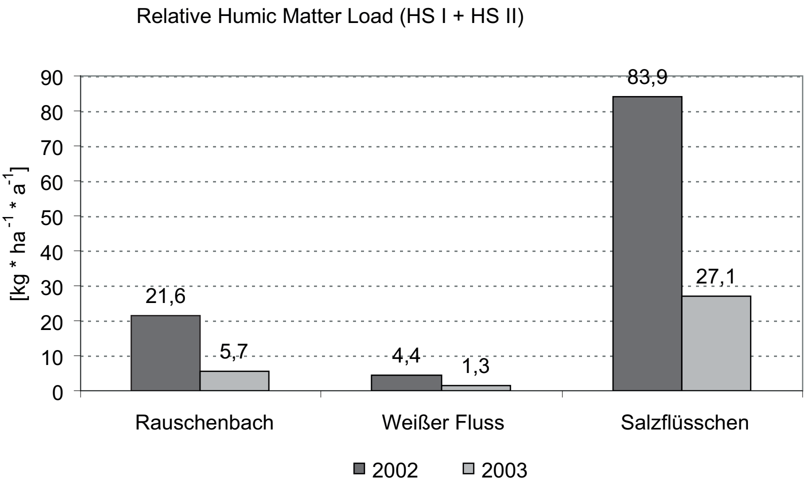

Fig. 6. Average humic matter loads (sum of fraction I and II) in comparison.

The absolute drainage amounts of high molecular weight humic matter (Figure 7) show what a large role the subcatchment area «Salzfluesschen» plays for the total organic load. In both years, the loads of the subcatchment area «Salzfluesschen» are approximately 10 times higher – although only half its size – than those of the subcatchment area «Weisser Fluss». This clearly shows the great influence of the peatlands in the subcatchment «Salzfluesschen».

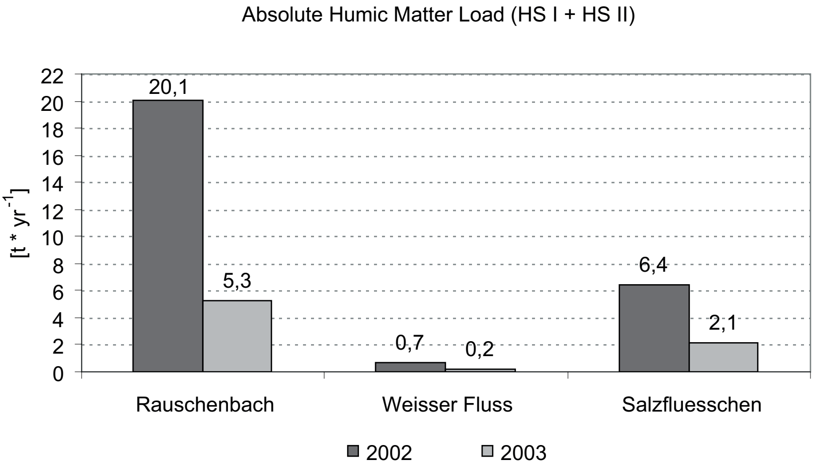

Fig. 7. Absolute average humic matter loads (sum of fraction I and II) in comparison.

Outlook

The results represented here are showing the relationship between catchment area characteristics and humic matter loads. Estimating future humic matter inputs into surface waters necessitates a model approach which allows the calculation of past inputs and the assessment of future developments. We have been in process of developing such a base for the catchment areas.

Above all it is essential to clear up the processes which lead to the activation and the transfer of dissolved humic matter. This includes the quantification of the production and decomposition rates as well as the identification of the factors causing the release of humic matter. In particular, the water-and material-balancing processes in the peatlands have not yet been completely determined.

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The effect of the treatment process on nitrogen content in humic acids extracted from sewage sludge

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Humic acids were extracted from sludge from three sewage treatment plants with different treatment technologies. Investigations of the extracted humic acids were carried out by means of elementary analysis and spectroscopic methods.

Results of elementary analysis show that percentage content of nitrogen is different for humic acids extracted from sludge derived from the subsequent treatment stages. The influence of the treatment process on nitrogen content was also observed. The percentage content of nitrogen was of the range from 5.05% to 8.99%. The highest value of nitrogen for humic acids extracted from sludge was obtained from the nitrification and denitrification chamber.

Key words: *humification process; humic acids; nitrogen; sludge; sewage treatment plants.*

Introduction

Elemental analysis is one of the most commonly used tool for the characterization of humic acids and provides information on the distribution of major elements, mainly: C, H, N, O and S (*Sparks* 1999).

The percentage content of nitrogen bounded by humic substances in sewage sludge and in compost is one of the main criterions of usefulness of sewage sludge compost for agricultural purposes (*Jerzykiewicz et al.* 1999).

The aim of this work was to observe the influence of the individual processes of the sewage treatment process on nitrogen content in humic acids extracted from sewage sludge collected from three treatment plants with different technological processes.

Materials and methods

Sludge samples for studies were collected according to Polish standards (in broad range according to ISO/5667/3 standard), from three biologic-mechanical sewage treatment plants in Sosnowiec-Zagórze (P-I), Jastrzębie Zdrój (P-II) and Dąbrowa Górnicza (P-III) (Polish standard, 1988). From the sewage sludge treatment in Sosnowiec-Zagórze samples were taken from: the primary settling tank (1a), the recirculation chamber (2a), the digestion chamber (3a) and the drying bed (4a) (Fig.1a); from the sewage sludge treatment in Jastrzębie Zdrój samples were taken from: the primary settling tank (1b), the nitrification chamber (2b), the denitrification chamber (3b), the digestion chamber (4b) and the drying beds (5b) (Figure 1b); from the sewage sludge treatment in Dąbrowa Górnicza samples were taken from the dephosphatation chamber (1c), the recirculation chamber (2c), the nitrification and denitrification chamber (3c), after sludge the densifier (4c) and after the press (5c) (Figure 1c).

Humic acids were also extracted from the sampled sludge. For this purpose 40 g of air-dried sludge was first shaken for 24 h with 200 cm^3 of 0.5 M NaOH and then centrifuged. Residual sludge after centrifugation was again flooded with base solution (200 cm^3) and again shaken. Solutions from these two extractions were then combined. Raw humic acid was precipitated from the solution of humic and fulvic acids by acidifying with a (10%) solution of hydrochloric acid to pH=1. Residual solution after centrifugation contained fulvic acids. To obtain pure humic acid, the raw humic acid was dissolved in 200 cm^3 of 0.5 M NaOH solution, centrifuged, and next filtered to remove impurities. Humic acid was then reprecipitated with hydrochloric acid and washed several times with water. Pure humic acid in the form of powder was obtained by lyophilization (*Stevenson* 1982).

To determine the elementary composition changes in the extracted humic acids at each stage of purification, the percentage content of carbon and nitrogen was determined by means of Series II CHNO/S 2400 PERKIN ELMER elementary analyzer.

Results and discussion

The obtained results of the percentage content of nitrogen in humic acids extracted from sewage sludge sampled from subsequent treatment stages from three plants are high and they range from 5.01% to 8.99% (Table 1) while the percentage content of nitrogen in humic acids extracted from soil or peat usually do not exceed 4% (*Filip et al.* 2000, *Stevenson* 1982, *Struyk et al.* 2001). One can conclude that the humic acids extracted from sewage sludge are rich in nitrogen. However, the percentage content of nitrogen in humic acids extracted from compost and sewage sludge were higher and ranged from 5.10% to 7.90% (*Amir et al.* 2004, *Jerzykiewicz et al.* 1999, *Reveille et al.* 2003).

The obtained results of the percentage content of nitrogen in humic acids extracted from sewage sludge after the nitrification and denitrification chamber from P-III (3b-8.99%) is significantly higher than the percentage content of nitrogen in humic acids extracted from soil, peat, compost and sewage sludge determined previously (*Amir et al.* 2004, *Filip et al.* 2000, *Jerzykiewicz et al.* 1999, 2001, *Reveille et al.* 2003, *Stevenson* 1982, *Struyk et al.* 2001).

The character of changes of the percentage nitrogen content in humic acids extracted from sewage sludge collected from subsequent stages of treatment from P-I, P-II and P-III varies for each plant. It is probably caused by different unit processes and differences in the technological treatment process used in these plants. However, the influence of the nitrification and denitrification processes seems to be crucial for the nitrogen content (Table 1).

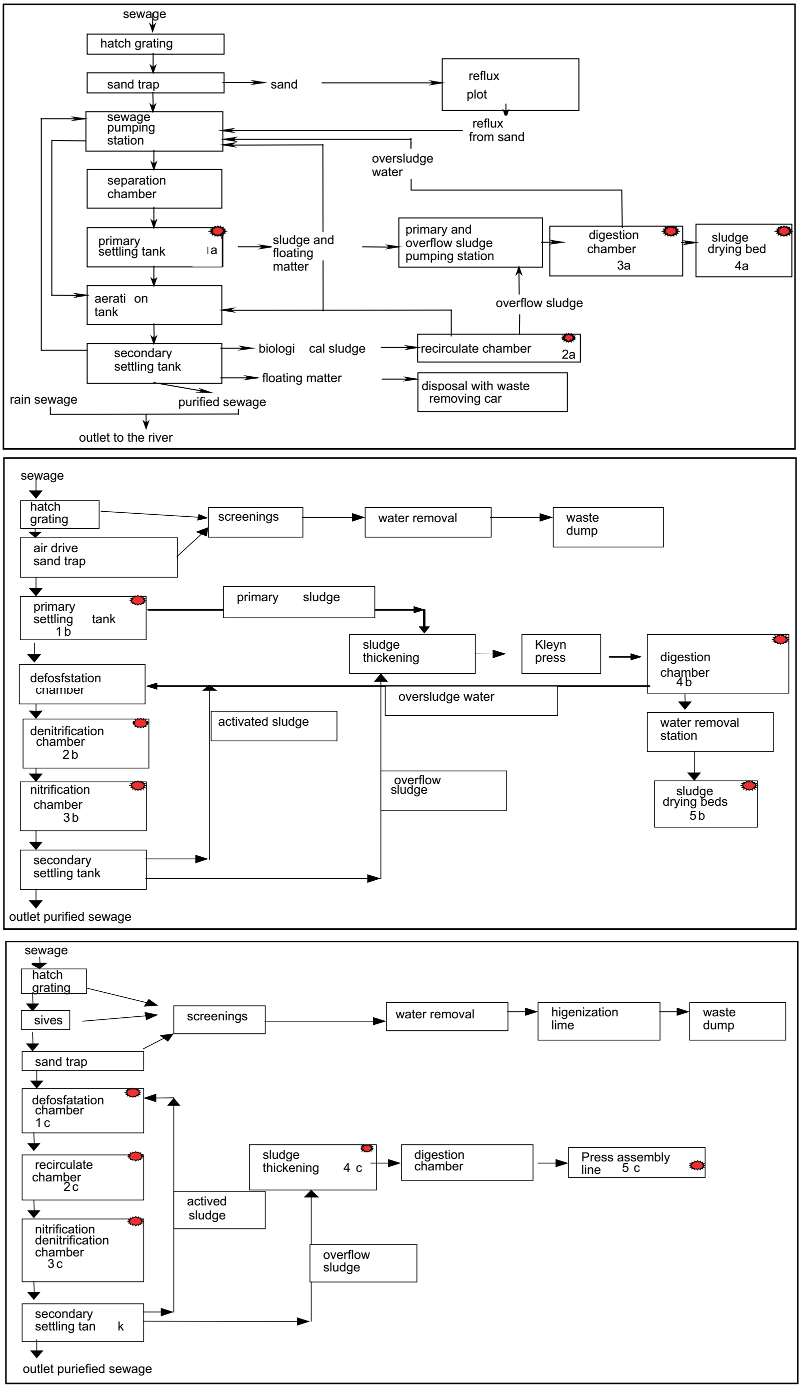

Fig. 1. Block diagrams of the treatment plants in Sosnowiec-Zagórze P-I (Fig 1a) Jastrzębie Zdrój P-II (Fig 1b) Dąbrowa Górnicza P-III (Fig 1c) with marked points of sampling.

Table 1

Elementary composition and H/C, O/C and C/N atomic ratio values of humic acids extracted from sludge collected from treatment plants in Sosnowiec Zagórze (PI), Jastrzębie Zdrój (PII) and Dąbrowa Górnicza (PIII)

The samples are numbered according to diagram on Figure 1.

Sample	%C	%N	C/N	Sample	%C	%N	C/N	Sample	%C	%N	C/N
1a	57.40± 0.07	5.26 ± 0.16	12.7± 0.64	1b	50.45 ± 0.72	5.21 ± 0.12	11.35 ± 0.57	1c	40.80± 0.96	7.72 ± 0.54	6.18 ± 0.31
2a	54.56± 0.72	5.05 ± 0.02	7.47 ± 0.37	2b	42.81± 0.38	6.39 ± 0.02	7.76 ± 0.39	2c	41.27 ± 0.71	6.54 ± 0.15	7.30± 0.37
3a	49.30± 0.33	6.57 ± 0.32	8.69 ± 0.43	3b	41.10± 0.05	6.21± 0.025	7.80 ± 0.39	3c	39.09 ± 0.9	8.99 ± 0.14	4.30 ± 0.21
4a	47.65± 0.15	5. 32 ± 0.02	10.2± 0.51	4b	40.10 ± 0.87	5.69 ± 0.33	8.14± 0.41	4c	41.08 ±0.89	7.08 ± 0.32	6.60± 0.33
				5b	39.54± 0.16	5.01 ± 0.02	9.14 ± 0.46	5c	41.01 ± 0.65	6.62 ± 0.37	7.20 ± 0.36

± SD

The percentage content of nitrogen in humic acids extracted from sewage sludge after leaving the primary settling tank (1a and 1b) from P-I and P-II are comparable. However, the percentage content of nitrogen is much higher in humic acids extracted from the sewage sludge after the dephosphatation chamber (1c) in the treatment plant P-III. Hence it can be assumed that the dephosphatation process is responsible for the increase of percentage content of nitrogen. Yet, the essential changes of nitrogen content in humic acid extracted from sewage sludge take place after the nitrification and denitrification processes. Both these processes are carried out in P-II and P-III. However, they are realized in a different way. The nitrification and denitrification processes in P-II are carried out in two separate chambers, while in P-III these processes are realized in one chamber whereas the sewage sludge is treated alternately i.e. nitrification is followed by denitrification. Probably this technological difference results in the varied percentage content of nitrogen in humic acid extracted from sewage sludge from plants P-II and P-III after these processes (2a, 3a, 3b).

The humic acids extracted from sewage sludge after the nitrification and denitrification processes are enriched in nitrogen both in treatment plant P-II and in P-III. However, in plant P-III, where nitrification and denitrification processes are carried out separately, nitrogen concentration is lower.

In the humic acids extracted from sewage sludge from P-II the nitrogen content increases after the denitrification process but after nitrification a slight decrease of nitrogen content is observed. The nitrification and denitrification processes carried out in one chamber (P-III) enrich the humic acids in nitrogen much more effectively.

It can be assumed, that if these processes are carried out separately nitrogen escapes into the atmosphere, what makes nitrogen enrichment of humic acids in sewage sludge difficult.

After the subsequent stages of the treatment process the nitrogen content in humic acids extracted from sewage sludge decreases to 27% and 22% in P-III and P-II, respectively. However, humic acids extracted from sewage sludge from P-III after treatment process still contain the highest nitrogen concentration.

Due to the absence of the nitrification and denitrification process in the sewage sludge treatment in P-I the change of the percentage of nitrogen content differs significantly in comparison with plants P-II and P-III. In humic acids extracted from sewage sludge from treatment plant P-I the increase of nitrogen content is observed after leaving the digestion chamber where in the case of P-II and P-III after the fermentation processes a decrease of nitrogen content takes place.

This may be due to the fact that in the case of P-I the fermentation process is also accompanied by the process of nitrification and denitrification of the sludge not conducted before. Since the fermentation is carried out in anaerobic conditions denitrification may dominate. Whereas the decrease of the nitrogen content after the fermentation process in the case of treatment plants P-II and P-III may be associated with the ripening of the sludge and particularly with its aromatisation.

Conclusions

The obtained results show that percentage content of nitrogen in humic acid extracted from sewage sludge is relatively high and depends on the treatment technology applied in the studied plants. It was observed that unit processes e.g. nitrification, denitrification, fermentation and dephosphatation influence the nitrogen concentration. It was found that nitrification and denitrification processes carried out in one chamber enrich the humic acids in nitrogen more effectively than in the case of conducting these processes in two separate chambers.

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The effect of heavy metals on the reduction and stabilisation of the g-factor value of humic acids extracted from sewage sludge

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The presence of heavy metals complexes with humic substances can influence the value of g-factor value. This effect was investigated for humic acids, extracted from sludge at the biologic-mechanical sewage treatment plant in Jastrzębie Zdrój by means of EPR spectroscopy. The presence of metals was confirmed by ICP. Moreover, IR spectroscopy shows that dissociated carboxylic groups occur in the humic acids.

Sludge samples for studies were taken from the primary settling tank, the sludge nitrification and denitrification chamber, the sludge digestion chamber and the sludge drying bed.

The value of g-factor at each process stage was determined. The mean g-factor of the studied humic acids from sludge was low in comparison with the values obtained for humic acids originating from the other sources. The low value of g-factor results from the formation of metal complexes.

Key words: *EPR; IR; ICP; g-factor; sludge; humification process; humic acids.*

Introduction

Electron paramagnetic resonance (EPR) is frequently applied to study the humification processes of soil, peat and sludge (*Deiana et al.* 1990; *Jerzykiewicz et al.* 1999; *Stevenson* 1983). Major spectra of humic acids originating from various materials (soil, peat, brown coal, compost) exhibit broad lines from transition metals and narrow lines from free radicals (*Jezierski et al.* 2000a, *Jezierski et al.* 2000b, *Sparks*1999). On the basis of EPR spectra free radical concentration and the g-factor value were determined. The g-factor value commonly measured for humic acids does not differ significantly from the one obtained for another organic compounds and ranged from 2.0023 to 2.0050 (*Senesi* 1990a, *Senesi* 1990b).

The changes of g-factor for humic acids of free radicals may be used as an indicator of the oxidation, aromatisation and maturation processes during humification in soils, composts and sludge (*Jerzykiewicz et al.* 1999, *Jezierski et al.* 2000a, *Jezierski et al.* 2000b, *Sparks* 1999). The oxidation processes taking place in the course of the humification processes can enrich humic acids in oxygen functional groups (OH, COOH, CO), interacting with free radicals. This in consequence can result in the increase of the g-factor. Metals bounded by the humic acid can be an additional reason for the change of the value of the g-factor (*Czechowski et al.* 2004).

During sewage treatment both oxidation and aromatisation processes take place. Moreover, the presence of metal ions in sewage sludge is found. All these factors can influence the g-factor values and thus provide important information on the progress of humification process. Therefore, studies of the changes of g-factor for humic acids extracted from sewage sludge collected from the subsequent treatment stages seem to be of interest.

Materials and methods

Sewage sludge samples were taken from the primary settling tank (1), the nitrification chamber (2), the denitrification chamber (3), the sludge digestion chamber (4) and the sludge drying beds (5) at the sewage treatment plant in Jastrzębie Zdrój according to Polish standards and ISO/5667/3 (Figure 1) (*Polish standard* 1988).

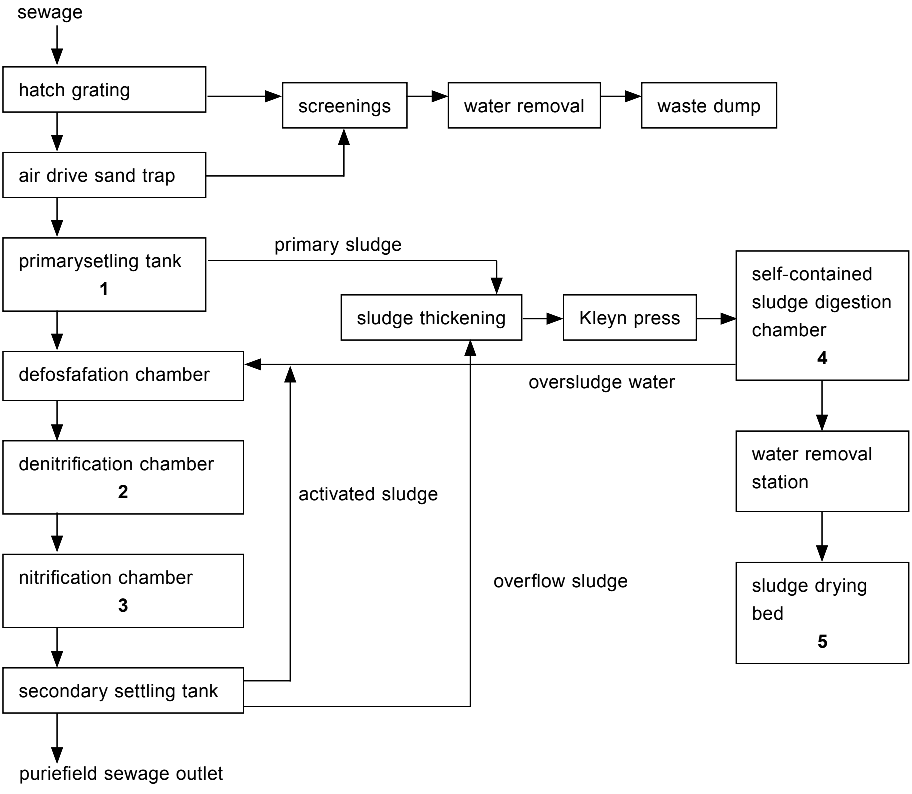

Fig. 1. Block diagram of the treatment plant in Jastrzębie with marked points of sampling.

Humic acids were extracted from sludge samples according to the method described before (*Pajaczkowska et al.* 2003)

Electron paramagnetic resonance (EPR) spectra were obtained with Bruker EMX EPR spectrometer operating at X-band frequency at room temperature. The EPR was applied for qualitative (g-factor) analysis. At each stage of sewage treatment, the extraction was performed three times. The obtained g-factor is the mean value of three measurements.

The identification of functional groups was done by means of infrared spectroscopy (IR). IR spectra of humic acids samples were recorded with a Perkin Elmer FTIR Spectrum One spectrometer, from KBr pellets (1 mg sample and 100 g KBr).

In order to determine the total metal concentration for humic acids, the analysis by means of ICP – AES method was done. The measurements were performed for mineralized humic acids extracted from sludge from the treatment plant in Jastrzębie Zdrój. Mineralized sample of humic acid was obtained by using the following procedures: 0.5 g of humic acid was oven-roasted at the temperature 500 ºC for 24 h, then solubilized in 5 ml of concentrated nitric acid and warmed in order to eliminate the oxide.

Results and discussion

On the basis of EPR spectra of humic acids extracted from sludge taken at different stages of sewage treatment, g-factor values were determined (Figure 2, Table 1). G-factor values obtained for humic acids extracted from sludge from the treatment plant in Jastrzębie Zdrój do not change during the treatment process and exhibit relatively low values (Table 1). The typical g-factor values for semiquinone radicals in the structure of humic acids extracted from soils, peats and composts amount to 2.0034–2.0040 (*Jerzykiewicz et al.* 1999, *Jezierski et al.* 2000a) and 2.0030–2.0034 for humic acids extracted from sewage sludge (*Jezierski et al.* 2000a, *Pajaczkowska et al.* 2003).

Table 1

The values of g-factor obtained for humic acids extracted from sewage sludge sampled from treatment plant in Jastrzębie Zdrój (Poland)

Sample	g-factor
1	2.0028 ± 0.0002
2	2.0027 ± 0.0001
3	2.0027 ± 0.0002
4	2.0027 ± 0.0003
5	2.0027 ± 0.0006

± sd

It was observed that the g-value of humic acids extracted from sludge at each stage of sewage treatment in Sosnowiec Zagórze varies during sewage treatment from 2.0031 at the beginning of the treatment process to 2.0034 at the end of this process. The increase of g-factor value indicates the formation of oxygen-rich groups (*Pajaczkowska et al.* 2003, *Polak et al.* 2005).

Table 2

Metal concentration for humic acids extracted from sewage sludge sampled from treatment plant in Jastrzębie Zdrój (Poland)

Sample	Fe [μg/g]	Mn [μg/g]	Ti [μg/g]	Cr [μg/g]	Cu [μg/g]	Pb [μg/g]	Zn [μg/g]
1	12929.65	44.01	68.69	11.09	86.93	81.93	499.6
5	18143.37	67.71	115.64	11642.8	85.08	68.96	358.43

It is difficult to explain why such low g-factor values were obtained for humic acids extracted from sludge from treatment plant in Jastrzębie Zdrój. The low value of g-factor could be caused by the presence of heavy metal in the humic acids structure. Particularly Pb and Hg can cause the decrease of the value g-factor from the value typical for semiquinone radicals i.e. 2.0034 down to 1.9999. Simultaneously metals can cause characteristic change of the shape of the EPR line (*Czechowski et al.* 2004).

The results of IR and ICP measurements show that metals appear in the studied samples of humic acids extracted from the sewage sludge from the treatment plant in Jastrzębie Zdrój.

On IR spectra (Figure 3) the absence of the band characteristic for carboxylic groups at 1710 to 1720 cm^{-1} and concurrent enhancement of the absorption band 1659 cm^{-1} characteristic for the dissociated carboxylic group may indicate the presence of metal complexes with carboxylic groups in the humic acids.

The metal content has been determined by means of ICP method (Table 2). The content of Pb in humic acids extracted from sludge oscillates from 55.96 μg/g to 81.93 μg/g sample-dependent. However, the obtained results of metal content cannot be used as quantitative ones since complete mineralization of the humic acids samples was not achieved. Therefore the presence of metals (especially Pb) could explain the low value of the g-factor. It should be noticed that the change of the EPR signal shape for the studied humic acids was not observed (Figure 2).

Conclusions

The g-factor values for humic acids extracted from sewage sludge collected from the treatment plant in Jastrzębie Zdrój are unexpectedly low. It could be a result of heavy metals presence in the humic acids. However, the characteristic change of the shape of EPR lines caused by metals was not observed. Hence, the explanation of the g-factor value is questionable and requires further studies.

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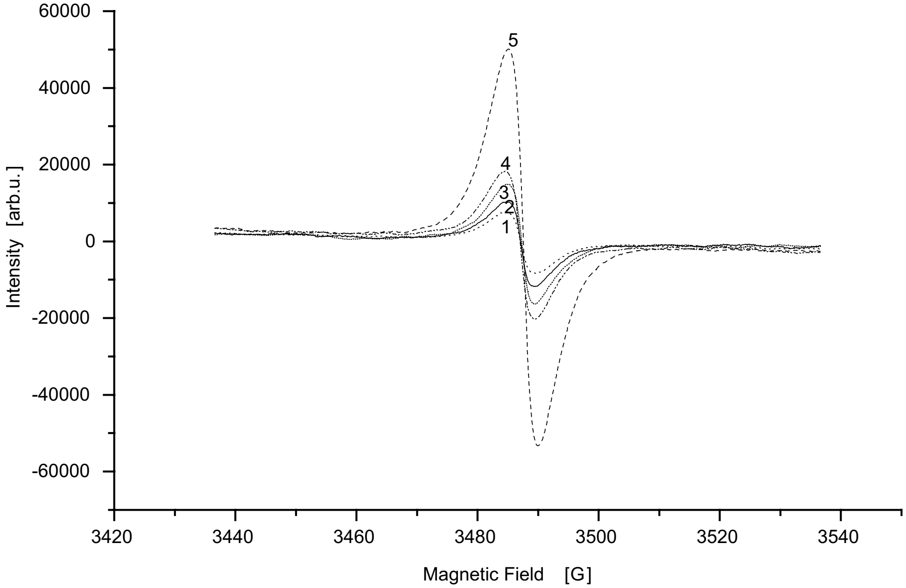

Fig. 2. EPR spectra of humic acids extracted from sludge sampled from respective places of the treatment plant.

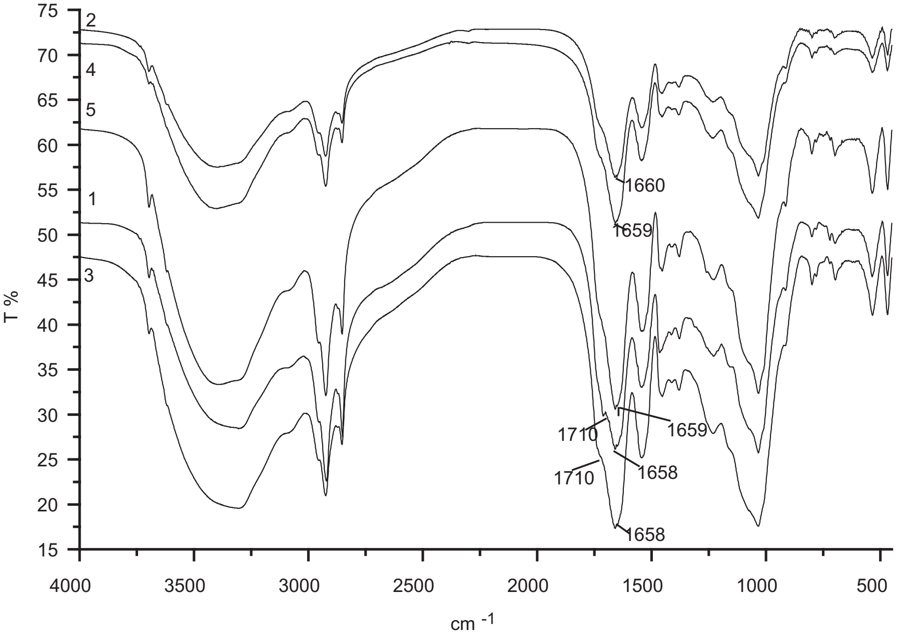

Fig. 3. IR spectra of humic acids extracted from sludge sampled from respective places of the treatment plant.

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NOM quality and transfer from peat bogs due to environmental changes in the mountains «Erzgebirge» (Germany)

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Drastic environmental changes have occurred in the mountains «Erzgebirge» during the last decades. Before 1989 large amounts of sulphur were transferred into the mountains. Increased pH values in precipitation have been observed after the political change in 1989. Extreme climatic events such as drought and heavy rainfalls occur more often. At the same time the NOM inputs in water reservoirs have raised to a large extent. Peat bogs are the main source for NOM. Laboratory experiments on peat bogs were conducted in order to investigate the causes. It was found that where sulphur is present, lower pH values reduce the NOM transfer. More NOM is discharged from peat bogs if the pH value increases. Dry periods followed by wet periods intensify the peat bogs'matter output as well.

Key words: *natural organic matter, peat bogs, environmental changes, mountains, black triangle.*

Introduction

The mountains «Erzgebirge» are low mountain ranges in Central Europe. Its summit altitudes reach up to more than 1000 metre a.s.l. High precipitation amounts characterize the climate. A dense forest, the so-called «Miriquidi primeval forest» used to cover the mountains. Numerous peat bogs appeared due to the climatic conditions and the features of the substratum that occurred during the holocene.

Since centuries there has been mining in the mountains «Erzgebirge». Settlement and mining entailed the use of forests and peat bogs. The forests were cleared due to the high demand for wood for mining purposes. Many peat bogs were drained and then mined. About 200 years ago, after the mountains were almost clear-cutted, forests were systematically afforested. Spruces (Picea abies) were planted and therefore monocultures of the same age appeared. The afforestation took place at several locations, even at peat bogs. All peat bogs found were damaged and degraded because of regular drainage.

The mountains «Erzgebirge» are situated in that German-Czech border region, where drastic environmental changes had occurred in the last few decades. There was a high air pollution from the centres of industry and mining in Saxony, Northern Bohemia, and Silesia, which gave that region the name «Black Triangle». Extreme climatic and natural geological acid conditions in the upper mountain range, as well as sulphur emissions and acid rain led to an additional increase of soil and surface waters acidification. Spruces were not able to cope with those influences anymore and perished to a large extent. Since the political change in 1989 the quality of the air has been improving. The silvicultural rehabilitation of the destroyed forests commenced (afforestation with alternative tree species, liming).

From the beginning of the 20th century many dams were built in the mountains «Erzgebirge». On the one hand dams served for flood protection. Events like the flood in 2002 had always happened. On the other hand, the inhabitants of the mountains and the adjacent cities such as Dresden and Chemnitz are supplied with the drinking water from the reservoirs.

Since the beginning of the 1990's, the phenomenon of a changing NOM input was observed in numerous brooks and reservoirs of the mountains «Erzgebirge» (fig. 1). In particular between 1998 and 2001 a very strong increase of humic matter concentration took place. At the same time changes in climate and emissions were measured. At present there is no acid rain like it used to be in the 70th and 80th but pH value of the precipitation and the frequency of heavy rainfall events are increasing. Moreover, the composition of natural organic matters (NOM) was subject to changes (*Grunewald et al.* 2003). Furthermore, cultivation strategies in the catchments have changed. This applies in particular to spruce forests and peat bogs as well as other sites with peat layers and histic layers respectively.

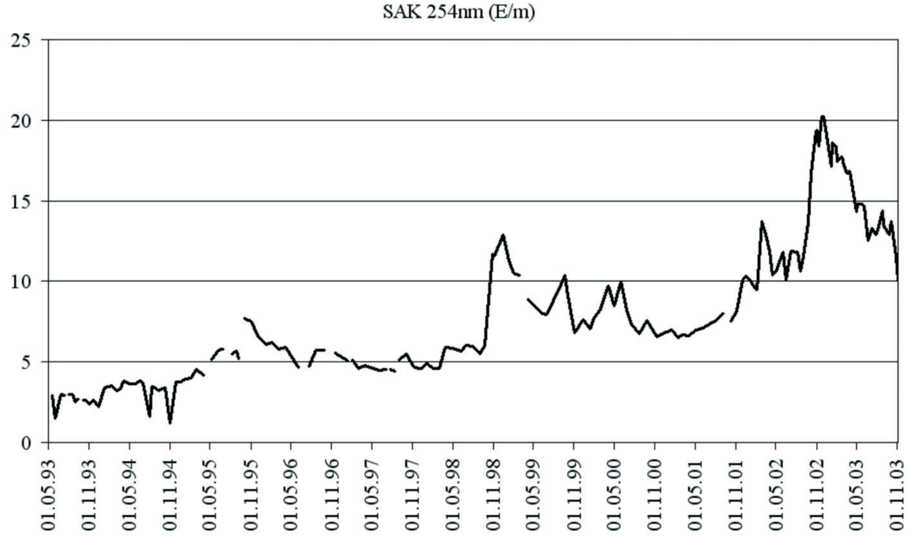

Fig. 1. SAC 254nm development between 1993 and 2003 of one drinking water reservoir in the mountains «Erzgebirge» (data source: Landestalsperrenverwaltung Sachsen).

Due to the increasing NOM tendency the water suppliers face many problems. Research projects were established in order to investigate the causes and to develop strategies to solve the problem. In the course of the past few years it was found that the above-mentioned phenomenon does not occur only in the mountains «Erzgebirge». In the last decade a NOM increase was also observed in Western and Northern Europe

(*Bragg* 2002, *Evans & Monteith, Harriman et al.* 2003, *Hongve et al.* 2004, *Soulsby* 2002, *Vogt et al.* 2003). However, the relevant factors are estimated differently or can be merely presumed respectively (*Nordtest* 2003). It is a fact that a large number of influencing and controlling factors had caused a change of the matter output. Many studies consider peat bogs as the most important source areas for NOM. Consequently it was necessary to find out to what extent the changed environmental conditions had influenced the transformation in the peat bogs and led to their balance as well as to an increased NOM output.

Several laboratory experiments were carried out on soil samples because the remote changes in the peat bogs and environment cannot be analyzed posterior at the actual place. This is the focus of the paper.

The following theses were investigated in the experiments:

1. Peat bogs and certain peaty layers are the main NOM suppliers.
2. A changed matter input situation through atmospheric deposition has an impact on the chemistry of soil solution and bog water, which results in changed processes and NOM output.
3. Weather events such as drought periods and frequent heavy rainfalls (as well as a longer growing period), have an impact on the water and matter balance of peat bogs. In the case of an increase of those events the matter output then also changes (NOM increase). Similar to the change of drought and wet periods or years respectively, drainage and rewetting activities cause a changed situation with regard to the natural organic matter transfer.
4. Changes of deposition and climate entail changes of microbiological conditions and activities in the peat bog which also have an impact on the NOM fluxes.

Methods

Area and site description

The studies were conducted in the catchment areas of drinking water reservoirs. The reservoir «Rauschenbach» is located in the eastern part of the mountains. Silicate rocks (gneisses, granite, ryolithe) determine the geology and the hilly surface. Cambisols, podzols and peat bogs are common due to the geological, relief and climatic conditions. Approximately 900 mm annual precipitation and annual temperature means less than 5 ºC characterize the mountainous region with average altitudes from 700 to 800 m a.s.l. Spruce forests are the main land use.

Besides the establishment of water networks and periodical sampling of the streams several investigations of the soils were done in the catchments between 2001 and 2005. The sites were analyzed along slope sequences according to the German soil investigation standard «KA4» (*AG Boden* 1994) and sampled in their depth profile. Field studies always dealt with parameters such as colour, soil moisture, and degree of decomposition (peat layers).

First, three soil types of the subcatchment «Salzflüßchen» of the reservoir «Rauschenbach» were analyzed and compared with each other for purposes of laboratory experiments. Samples were taken from the humic layer and all horizons of a shallow peat bog with grassy cover (site 1), typical cambisol under spruce (site 2) as well as podzolic cambisol under beech (site 3). The following studies emphazised on peat bogs and peat layers. A geogen influenced (site P3) and an exclusively ombrogen supplied (site P1) peat bog, merely 100 metres away, were examined in order to show the different response of a peat bog to slope water and rain water chemistry respectively.

Laboratory experiments and analyses

All taken soil samples were air-dried, sieved (2 mm) and stored under lab conditions. By way of exception two peat layers were kept in a freezer to compare «freshness» and «drought». 5 gram of each sample was shaken for 24 hours at a medium intensity by using different solutions in order to investigate the potential mobilizable matters. Yet the results may not be overestimated. The applied procedure «shaking extraction» is an unnatural intervention with soils. Nevertheless, the lab experiment shall give an insight into some basic mechanisms of NOM sorption and desorption intensity. Distilled water (MQ) and for simulation of changes in rain and soil water chemistry water which was enriched with lime (MQk) and acid (MQs) were used as extraction solutions. The increase of the acid capacity by adding lime was based on the assumption that usually 4.5 tons lime per hectare (= 0.45 kg/m^2) were distributed by forestry every four or five years. However, lime has a low solubility. The monthly average rain water amount of approximately 90 l / m^2 dissolves the lime gradually within that period. Consequently 0.05 g Ca/MgCO$_3$ had to be mixed with 0.5 litre of distilled water. The impact of atmospheric acid input was simulated by reducing the acid capacity by adding acid. In 1994 in the upper altitudes of the mountains «Erzgebirge» the deposition rate amounted to 80 kg sulphur per hectare and 40 kg nitrogen per hectare with an effective acid load of 5.2 keq (SMUL 2003). Therefore sulphur acid and saltpetre acid were added to the distilled water to reduce the pH value down to between 3.0 and 3.5.

Moreover the soil respiration was measured in one series of experiments (method according to Isermeyr in Schlichting & Blume 1995) and then compared with the peat bogs'chemical-physical features as well as with the matter mobilization behaviour.

All water extracts were filtered (0,45 μm). Organic parameters such as spectral absorbance (SAC in Ext/m, by photometer, wavelength 254 nm), dissolved organic carbon (DOC in mg/l, by Analyzer TOC 5000 / 5050 Shimadzu), fractions of natural organic matter (NOM in %), and pH value were determined in the water of the extracted solutions (methods according to Hütter 1990). The fractionation of NOM was realized by means of liquid-chromatography and detection of organic carbon (LC-OCD-system, Huber and Frimmel 1996). Dissolved NOM is subdivided in humic matters (fraction 1 and 2 in %; humine, humic acids, fulvic acids), building blocks, low-molecular acids, amphiphil and neutral matters and polysaccharides.

The soil samples were analyzed in order to determine their chemical and physical attributes. Organic carbon (Corg), nitrogen (N), phosphorus (P) and pH value as well as degree of decomposition are all relevant parameters (methods according to Schlichting & Blume 1995). The grade of peat decomposition was divided in stages from «less decomposed» (1) to «strong decomposed» (5). Ratios of C/N and C/P were calculated to estimate the nutrition state of the peat soils. Furthermore the left water content of the samples was analyzed by weighing and drying (105 °C).

Results and discussion

Simulation 1: Peat bogs as NOM source

The comparative study of the three different soil types brought the expected results. Highest amounts of extractable DOC in relation to the dried soil mass show the peat layers and the humic layers. Peat layers and humic layers showed the highest amounts of extractable DOC in relation to the dried soil mass (tab. 1). That is not surprising as these layers have a high content of organic carbon. The SAC (254 nm) distribution was similar to DOC (even in relation to dried soil mass). Furthermore the specific absorbance (SAC/DOC ratio) as a parameter for the aromaticy of the organic compounds indicated that peat bog is the main source of humic matter. Low concentrations of DOC and low values of SAC values characterize the NOM solubility from the mineral subsoil layers of the two cambisols. By calculating the site-specific DOC mobilization (all layers in consideration of bulk density and depth) it was found that the shallow peat bog delivers five to eight times more soluble organic carbon than terrestrial soils.

Table 1

Selected NOM parameters from the three different soil types

Site	Soil type	Layer	DOC (mg/g)	SAC (E/g)	SAC/DOC
1	peat bog	L/H	5,9	49,4	8,4
		HI	5,8	45,0	7,8
		HII	6,9	45,7	6,6
2	typical cambisol	Of	2,6	10,1	3,9
		Oh	5,4	27,4	5,1
		Ah	2,1	9,6	4,6
		Bv	1,1	3,4	3,0
3	podzolic cambisol	Of	4,9	31,5	6,4
		Oh	4,6	34,7	7,6
		Ahe	2,2	11,9	5,4
		Bhs	0,7	2,6	3,5

The relationship between the high amount of soil organic carbon in peat bogs and the high level of DOC export and the increasing NOM flux from these sites was observed in several European mountains and wetland regions, in particular in Western and Northern Europe (*Bragg* 2002, *Evans & Monteith, Harriman et al.* 2003, *Hongve et al.* 2004, *Soulsby* 2002, *Vogt et al.* 2003). The reasons and responsible factors are seen differently in changes of climatic conditions, atmospheric deposition, soil microbiology, peat bog hydrology or land use.

Simulation 2: Changes in atmospheric deposition, NOM transfer and quality

Compared with other peat bogs investigated in the mountains «Erzgebirge» the two sites have typical depth profiles in their low pH values, high contents of organic carbon, wide C/N ratios and changing degrees of decomposition (tab. 2). The upper 1.0 metre was sampled. The total depth was 3.8 and 1.9 but samples were only taken from the first 1.0 metre. All the upper peat layers are strongly decomposed due to drainage that took place in the last decades. Deeper layers showed less decomposition and macrofossil rests especially from Sphagnum mosses.

Table 2

Soil characteristic of site P1 and P2

Site	Layer	pH value	Organic carbon (%)	C/N ratio	degree of decomposition
P1	P1/1	3,3	65,1	58	5,0
	P1/2	2,7	54,1	37	4,5
	P1/3	2,6	65,3	42	4,0
	P1/4	2,6	52,9	33	4,5
	P1/5	2,9	66,5	46	3,0
	P1/7	2,9	64,9	36	3,0
	P1/8	3,1	62,6	52	2,0
	P1/9	3,3	61,3	44	2,5
P3	P3/1	4,0	45,2	23	no data
	P3/2	2,9	53,5	35	5,0
	P3/3	2,7	54,7	46	5,0
	P3/4	2,7	54,1	37	3,5
	P3/5	3,4	47,1	30	3,0

The extraction with distilled water (MQ), limed water (MQk) and acidic water (MQs).

Showed interesting differences depending on the peat properties. Parameter SAC 254 nm showed in the extracts at both sites a clear decrease from the top to the bottom of the profiles (fig. 2a). Between the strong decomposed, upper (P1/1 – P1/4, P3/1 – P3/3) and the less decomposed deeper (P1/5 – P1/9, P3/4 – P3/5) peat layers a breakdown of the curve could be established. The DOC solubility is similarly characterized except for P1/1 and P1/5 (fig. 2b). The amount of both parameters has a comparable level to analyzed water from the peat bog leaving ditches.

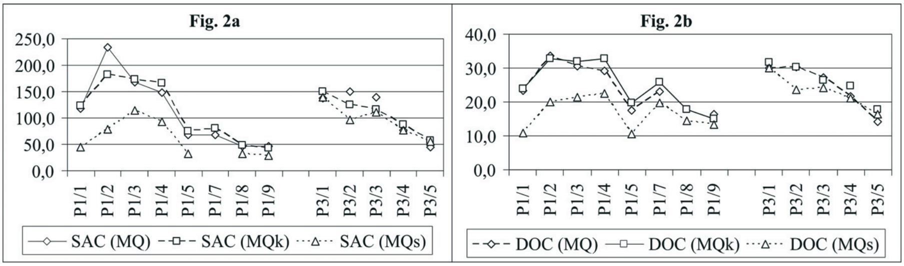

Fig. 2a and 2b. SAC and DOC from P1 and P3 (MQ, MQk, MQs).

The chemism of the solution if limed or acidic also determines the amount of extractable organic matter. From most samples the highest SAC and DOC were measured under use of MQk with an initial pH value of 85. After extraction the pH level of the solution however dropped down to half. In this case the difference between MQk and MQ was less than to MQs. In the acidic environment SAC and DOC are reduced. The pH value does not show any change after extraction (3.4).

The NOM composition in all extracts is dominated by the humic matter fraction (Figure. 3a-d). Where the solution has a higher pH value (MQk) the part of dissolved humic matter on NOM is at the highest level (except for P3/3). Both sites are characterized by a change / a decrease on depth. Ombrogen influenced site show much bigger differences between MQ, MQk and MQs than P3.

In general the lowest content was found similar to SAC and DOC in the less decomposed peat layers. Adding an acidic solution reduces in particular fraction 2 with lower molecular weights (Figure. 3c). Otherwise after adding lime the fraction shows the highest share. The curves of fraction 1 and 2 usually run opposite to each other, as clearly seen in the layer P1/5 (Figure. 3d). Increased share of higher molecular humic matter (H1) induces decrease of lower molecular weighted compounds (H2) or the other way round.

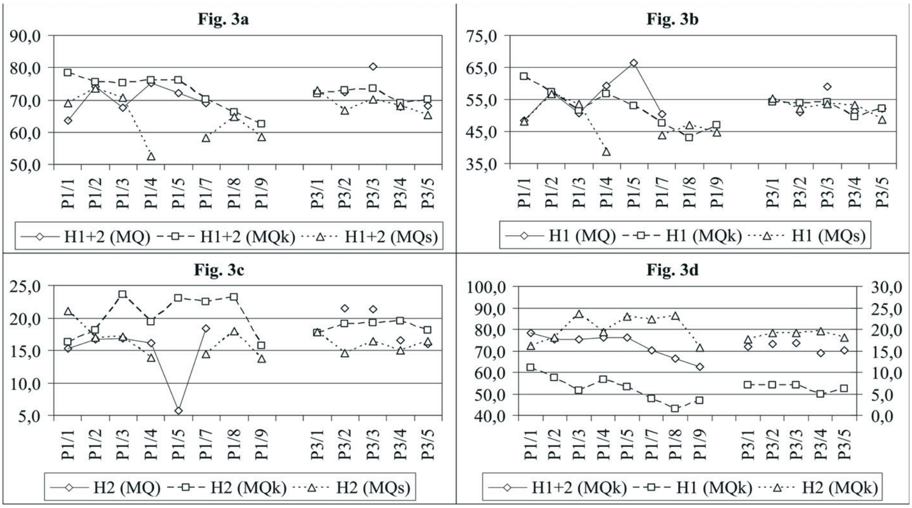

Fig. 3a-d. Humic matter fractions from P1 and P3.

The comparison of the two peat bog sites with different peat properties along depth gradients and the use of distilled; limed and acidic water allows a careful estimation with regard to the responsible mechanism of NOM mobilization and transfer. Due to enormous sulphur input in the ecosystems over decades from the early 1990's the matter balance has probably changed such a reduction of microbiological activity, decrease of primary production or changed peat decomposition. On its way through the Sphagnum peat bog, water chemistry and pH value change due to cation exchange (*Bragg* 2002). Most likely the ionic flux controls hydraulic the peat decomposition, caused by sulphur (*Edom in Succow & Joosten* 2001). On the other hand the role of nitrogen (ammonium) deposition can be seen as a steering factor of the eutrophic conditions and therefore enhance microbiological activities. The significant increase of pH value and the decrease of sulphur loads while relatively enhancing the nitrogen compounds in the precipitation since the mid 1990's should be important exogen driving forces on the endogen peat properties in connection with changes in the NOM transfer and in NOM quality.

Simulation 3: Changes in climatic conditions, NOM transfer and quality

Several studies reported about the influence of extreme dry seasons with drought over many weeks on water table connected to increase of DOC export from peat bogs in the following «climatic usual» years (*Hongve et al.* 2004, *Blodau et al.* 2004, *Burt* 2003). Therefore parts of the samples from the upper, stronger decomposed peat layer P1/1 and the deeper, less decomposed P1/5 were stored under cool conditions in order to keep them «fresh». The conducted experiments and used solutions MQ, MQk and MQs are similar to Simulation 2. The shares on dry soil mass were calculated on the extracted matter concentrations to guarantee the comparability between «fresh» and «dried» samples with different water contents. Independent of the chemism of the solution all extracts showed higher contents of DOC, higher SAC 254nm and higher shares of humic matter fractions after the samples were dried for 3 days (Table 3). The upper P1/1 shows an increase of approximately ten times and the less decomposed P1/5 shows an increase of about five times in the SAC levels. After acidic water was added the mobilization was again hampered (as opposed to) limed water as well as the relative enhance of humic matter fractions on NOM.

Table 3

Comparison of NOM quality in the extracts MQ, MQk and MQs from «fresh» (F) and «dried» (T) peat samples

Sample	SAC (E/g)	DOC (mg/g)	SAC/DOC	HS1 (%)	HS2 (%)	HS1+2 (%)
P1/1-F-MQ	22,2	4,1	5,4	no data	no data	no data
P1/1-T-MQ	26,4	5,2	5,1	48,4	15,4	63,7
P1/5-F-MQ	9,7	2,4	4,0	50,0	19,6	69,5
P1/5-T-MQ	15,4	3,9	3,9	66,4	5,7	72,1
P1/1-F-MQk	27,9	4,8	5,8	51,4	16,2	67,5
P1/1-T-MQk	27,6	5,3	5,2	62,1	16,3	78,4
P1/5-F-MQk	10,8	2,0	5,4	54,6	16,4	71,0
P1/5-T-MQk	16,9	4,5	3,8	53,1	23,0	76,2
P1/1-F-MQs	7,8	1,6	4,8	55,1	12,1	67,1
P1/1-T-MQs	10,1	2,4	4,2	48,0	21,1	69,1
P1/5-F-MQs	4,0	1,2	3,4	45,8	13,6	59,5
P1/5-T-MQs	7,3	2,4	3,0	no data	no data	no data

Simulation 4: Microbiological activity, NOM transfer and quality

From site P3 further experiments on the relationship between ... NOM transfer were done (tab. 4). At present the aggregated results give a first insight into possible connections between the matter and water balances and microbiological processes. The upper, more composed layers supply the most NOM (here characterized by SAC 254 nm) similar to the other simulations. The highest activity of microorganisms was measured in the humic layer. Under wet conditions the respiration rates in the deeper layers were reduced in comparison to the upper layers.

Table. 4

Overview of peat properties, microbiology and SAC at site P3

Parameter	Humic layer	Upper peat layers	Deeper peat layers
depth	0–5 cm	5–55 cm	55–105 cm
peat pH value	2,9	2,8	3,7
organic carbon	42,5%	55%	45%
degree of decomposition	strong (5)	strong (5)	medium (3)
relative moisture	dry–medium	medium–wet	wet
soil respiration	90–110 mg CO2/g	60–77 mg CO2/g	31–76 mg CO2/g
SAC 254 nm	200 Ext/g	220–250 Ext/g	55–130 Ext/g

The shown phenomena between microbiology, peat properties and NOM transfer can be explained by the seasonal variation of the water table (*Blodau et al.* 2004, *Kalbitz et al.* 2002). Changes in atmospheric deposition with less sulphur input into the peat bogs appear to regulate the encymic activity as one controlling factor for DOC export (*Freeman et al.* 1998). Nevertheless the increased atmospheric CO_2 concentration causes the microbiological activity followed by the NOM transfer from peat bogs (*Kang et al.* 2001).

Conclusions

The laboratory experiments gave a prime insight into the behaviour of peat bogs in the face of simulated environmental changes. A statistic verification of the data is not possible. Yet it is a good indicator as far as NOM mobilization is concerned (with regard to NOM mobilization). The largest amounts of NOM and humic matters are transferred from the peat bog into surface water. Peat bogs respond sensitive to a changed matter input. Low pH values and large amounts of sulphur in precipitation and soil water reduce the NOM output. More organic matters dissolve when the pH value increases. A better quality of the air and perhaps lime from forestic measures cause the increase of the pH value. The portion of humic matter fraction with regard to organic carbon also ascends. However, further studies are necessary regarding the relevance of currently high nitrogen inputs in correlation to microbiological processes. For the future it is expected that both pH value and nitrogen input will rise. Both the changed matter input and the climatic conditions play an important role. Periods of drought and heavy rainfall are the relevant factors for changed matter outputs. If the upper layers of a peat bog dry up like for instance during a long dry period which is then followed by wet conditions, a large amount of NOM will be transferred into soil solution and surface water. According to the climate prediction for Saxony more extreme incidents are expected for the «Erzgebirge» as well (*SMUL* 2005). The weather as well as drainage measures and rewetting measures can cause the above-mentioned processes.

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Acid-base properties of water soluble organic matter of podzols in the North-East European Russian taiga

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Acid-base properties of water-soluble organic matter from podzol soils have been studied. Five acidic groups composed of different substances with pK values around 3.6; 4.8; 6.7; 8.7 and 9.7 have been identified. Testing the properties of soluble soil fraction, it is to be taken into account that when it is isolated from non-soluble soil matter, some water-soluble substances remain in soil and do not pass into the solution. Most firmly adsorbed in soil are water-soluble components with pK 9.6–9.8.

Key words: *water-soluble organic matter, acid-base properties, pK-spectrum.*

Introduction

Systemic research of soil organic matter is needed considering a variety of its composition, forms, and ecological functions. An important component of soil humus is organic matter water-soluble fraction. Being of a non-homogeneous character and also differing by molecular-weight composition, its various functional groups are responsible for a unique role of these compounds for formation of genetic horizons of podzols.

The computer treatment of potentiometric titration curves for aqueous solutions of the mixture of weak acids has found a wide application in recent years that allows one to obtain dependences of molar fraction or concentration of the relevant acid or acid-base group on the *pK* value characterizing this species. This dependence constructed for the entire range of physically reasonable *pK* values was named the pK-spectrum (*Danchenko N. N. et all* 1998, *Ryazanov M. A. et all* 2001, *Ryazanov M. A. et all* 2003).

Materials and methods

The study was conducted on the soil samples, collected from organic horizons of differently moistened sod-podzolic soils that are situated in south taiga north part of Komi Republic (Russia): a Non-Gleyed Sod-Podzol (Sample 1), a Poorly Gleified Sod-Podzol (Sample 2), and a Strongly Gleified Sod-Podzol (Sample 3). The forest plot is located 59°38′25″ N, 49°22′40″ E.

The aliquots of water extracts from the soil samples and 'scour waters' were titrated with HCl and NaOH solutions (molar concentrations (0.02000 ± 0.0026) mol dm^{-3}); the pH was measured 2 min after having added the next portion of titrant. The titration procedure was in the pH ranging from the titration starting point to 3, when acid-titrated, and to 10, when base-titrated (Figure 1). In order to elucidate the nature of water-soluble components that do not enter the water extract after one-time eluant treatment, the filter with substrate was scoured with distilled water until the volume of 'scour waters' reached 20.0 cm^3.

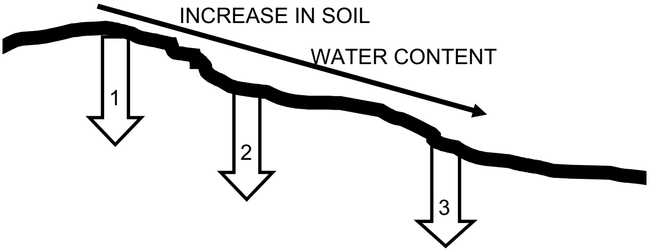

Fig. 1. Soil distribution across the topography.

The potentiometric titration experimental curves of the water extracts and 'scour waters' were interpreted by a special computer program that enables drawing the pK spectra.

Results and discussion

In any point of titration of solution, the number of H$^+$ (OH$^-$) is equal to

$$n/m(\mathrm{pH})=\frac{c_{\mathrm{HA}}\cdot V_{\mathrm{HA}}-\left([\mathrm{H^+}]-{K_{\mathrm{W}}}/{[\mathrm{H^+}]}\right)\cdot(V_0+V_{\mathrm{HA}})}{m}\text{, mmol/g} \quad (1)$$

$$n/m(\mathrm{pH})=\frac{-c_{\mathrm{BOH}}\cdot V_{\mathrm{BOH}}-\left([\mathrm{H^+}]-{K_{\mathrm{W}}}/{[\mathrm{H^+}]}\right)\cdot(V_0+V_{\mathrm{BOH}})}{m}\text{, mmol/g} \quad (2)$$

Here c_{HA}, c_{BOH} – molar concentration of a strong monoatomic acid or base in the initial substrate solution, V_{HA}, V_{BOH} is titrant volume (cm^3), V_0 is the aliquot volume (cm^3), *m* is the substrat in the aliquot (g).

The *n/m*(pH) value is related to the concentrations of acid-base groups of substrate by an evident relationship

$$n/m(\mathrm{pH})=\sum_i[\mathrm{HA}_i]=\sum_i\frac{[\mathrm{HA}_i]}{[\mathrm{HA}_i]+[\mathrm{A}_i]}\cdot([\mathrm{HA}_i]+[\mathrm{A}_i]) \quad (3)$$

The value $n/m(\mathrm{pH})_i = [\mathrm{HA}_i] + [\mathrm{A}_i]$ represents the number of acid-base groups of particular type and supposedly remains constant throughout the titration, the ratio of HA_i and A_i species being only changed. If we consider a substrate particle as an original supramolecule with the studied surface acid-base groups, the apparent dissociation constant of the *i*-th acid-base group can be described as

$$K_i = \frac{[\mathrm{H}^+]\cdot[\mathrm{A}_i]}{[\mathrm{HA}_i]} \tag{4}$$

Using Eq. (4), we transform Eq. (3) into the following form:

$$n/m(\mathrm{pH}) = \sum_i n/m(pH)_i \frac{[\mathrm{HA}_i]}{[\mathrm{HA}_i]+K_i} \tag{5}$$

or by passing to pH and p*K* units

$$n/m(\mathrm{pH}) = \sum_{pK_i=pK_{\min}}^{pK_i=pK_{\max}} n/m_i\left(\frac{10^{-pH}}{10^{-pH}+10^{-pK}}\right) \tag{6}$$

The result obtained allows one to find the total amount of acid-base groups of substrate:

$$n/m(\mathrm{pH}) = \sum_i n/m(pH)_i \tag{7}$$

and molar fractions ($q_i = {n/m(pH)_i}\big/{n/m(pH)}$).

In order to diminish the effect of errors on the calculation results, we used the spline program, which smoothes the experimental dependence $n/m(\mathrm{pH}) = f$ (pH) and decreases the ΔpH value to 0.1 by interpolation (table 1, 2, figure 2) (*Forsythe G. et all*, 1980).

The mean square error total of titration curve was calculated according to the formula:

$$s = \sqrt{\frac{\sum_k^K s_k^2}{K}} \tag{8}$$

where *K* is the number of points on a balanced dependence chart of the arithmetic mean nb_{mean} on the pH in solution. Just these results were used for drawing the pK spectra.

Table 1

Experimental data for three titration replications of soil water extracts from organic horizon of Strongly Gleified Sod-Podzol

n/m_1, mmol/g	pH_1	n/m_2, mmol/g	pH_2	n/m_3, mmol/g	pH_3
0.029405	2.88	0.027795	3.03	0.026611	3.02
0.025902	3.05	0.02535	3.25	0.023331	3.25
0.023812	3.31	0.021986	3.47	0.020415	3.49
0.020631	3.55	0.019022	3.71	0.017586	3.71
0.01751	3.77	0.016337	3.88	0.014668	3.93
0.014717	3.95	0.013679	4.18	0.011895	4.25

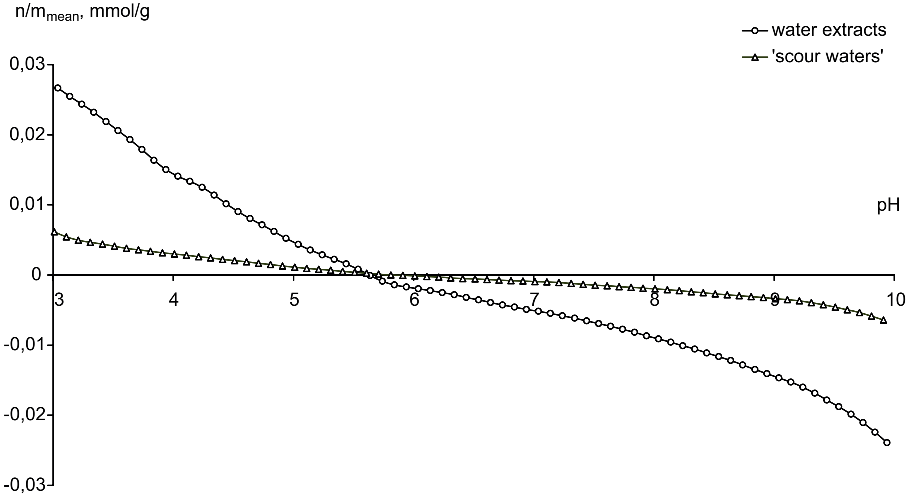

Fig. 2. Relation of the amount of the –H and –OH groups of organic compounds to the pH in soil sample from organic horizon of Strongly Gleified Sod-Podzol.

Table 2

Balanced *(n/m$_i$)* values for three titration replications of soil water extracts from organic horizon of Strongly Gleified Sod-Podzol with the known pH values, arithmetic means (*n/m$_{mean}$*), selected dispersions (s^2_k)

k	pH	*n/m$_1$*, mmol/g	*n/m$_2$*, mmol/g	*n/m$_3$*, mmol/g	*n/m $_{mean}$*, mmol/g	$s_k^2 \times 10^7$
1	3.04	0.026053	0.027682	0.026314	0.026683	2.55
2	3.14	0.24945	0.026534	0.02485	0.025443	2.98
3	3.24	0.024317	0.025272	0.023463	0.02435	2.47
4	3.34	0.023518	0.023833	0.022201	0.023184	2.5
5	3.44	0.022244	0.022386	0.02101	0.02188	1.91
6	3.54	0.020776	0.021139	0.019807	0.020574	1.58
7	3.64	0.019382	0.019968	0.018533	0.019294	1.73
8	3.74	0.017969	0.018556	0.017164	0.017897	1.63
9	3.84	0.016367	0.016923	0.015766	0.016352	1.11
10	3.94	0.014845	0.015627	0.014563	0.015011	1.01
11	4.04	0.013823	0.014761	0.013682	0.014089	1.15

After one-time treatment of soil sample with DL water only (70 ÷ 80)% groups of water-soluble organic compounds entered the water extract (Table 3). Besides, after one-time treatment of soil sample with DL the groups with the pK 9.6 ÷ 9.8 were extracted only by 60% in case of Non-Gleyed Sod-Podzol and Poorly Gleified Sod-Podzol, and by 70% for Strongly Gleified Sod-Podzol. The fourth pK range groups were extracted most by (85 ÷ 90)% (Table 4).

The obtained results on water extracts and 'scour waters' from organic horizons of three soils by the method of pK spectroscopy have indicated that one-time DL treatment is not enough to extract all water-soluble organic compounds. As the composition of compounds that entered the extract and remained in wet remnant is different, we cannot determine the composition and properties of water-soluble compounds based only on the water extracts.

Table 3

The total of acid-base groups of soil water-soluble compounds that entered the water extract after one-time DL treatment and repeated treatment of wet remnant

Number of soil sample	Amount of groups, *n/m(pH)*, mmol/g			Molar portion of the groups that entered the water extract, *x*, %
	water extract	'scour waters'	total	
1	0.078	0.028	0.105	74
2	0.110	0.045	0.155	71
3	0.051	0.015	0.065	78
Error (± Δ*n*, mmol/g)	±0.006	±0.004	±0.005	

Table 4

Molar portions of acid-base groups that did not enter the water extract after one-time soil sample treatment with DL water from the total molar concentrations in extracts and 'scour waters'

Band number in the pK-spectrum	pK range	Number of soil sample		
		1	2	3
		Molar portions of groups in 'scour waters', *x*		
1	3.5 ÷ 3.8	0.13	0.19	0.28
2	4.6 ÷ 4.9	0.19	0.24	0.15
3	6.4 ÷ 7.1	0.18	0.27	0.21
4	8.6 ÷ 8.9	0.15	0.09	0.15
5	9.6 ÷ 9.8	0.43	0.38	0.28

Conclusions

The pK spectroscopy method revealed the presence of five acid-base groups with the pK values 3.5 ÷ 3.8, 4.6 ÷ 4.9, 6.4 ÷ 7.1, 8.6 ÷ 8.9, and 9.6 ÷ 9.8. It is to be taken into account in studies of composition and properties of water-soluble soil components that some part of them remain in wet soil and do not enter the solution, their composition in wet soil and extracted solution is different.

Acknowledgments

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Plankton communities of the humic lakes in Teici Bog Reserve, Latvia

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Teici State Reserve is a unique bog ecosystem with a diversity of vegetation and animals. The presence of humic substances causes dark brown water colour, high COD and low pH. In these waters plankton was formed by species tolerant to low values of pH and high humic substance concentrations. The composition of plankton species is simplified. The significant correlation between the number of planktonic species and pH is detected. Dyseutrophic lakes are characterized by comparatively higher algae biomasses than dystrophic ones. The relationship between the concentration of humic substances and total bacterial numbers are observed. Accordingly to the placement of lakes in the territory that is relatively isolated from human impact the development of planktonic organisms is determined mainly by the presence of humic substances.

Key words: *humic substances, brown water, biodiversity, trophic state, Latvia.*

Introduction

Teici State Reserve was established in Latvia in 1982 for the purpose to conserve the largest unimpacted rised bog ecosystem in Baltic countries characterised by diverse flora and fauna. Since 1995 the environmental significance of Teici Bog Reserve has been recognized internationally by its inclusion in Ramsar convention. There are 18 bog lakes in the territory of the reserve with the area from 1.5 to 74.0 ha and mean depth from 1.0 to 5.2 m. From hydrobiological point of view water bodies of Teici Bog Reserve are characterized by different trophic states based on their genesis. Planktonic communities of the lakes are closely related to different content of humic substances in water.

Materials and methods

Location and geomorphology of the study place

Teici Bog Reserve is located on moraine hillocks in East-Latvian lowland on the watershed between basins of the River Aiviekste and the River Nereta and covers an area of 19 587 ha (Figure 1). Teici Bog has started to form in Preboreal, about 10 300 BP. It has formed by several bogs joining together which had developed in relief depressions around lakes where silty gyttja had sedimented.

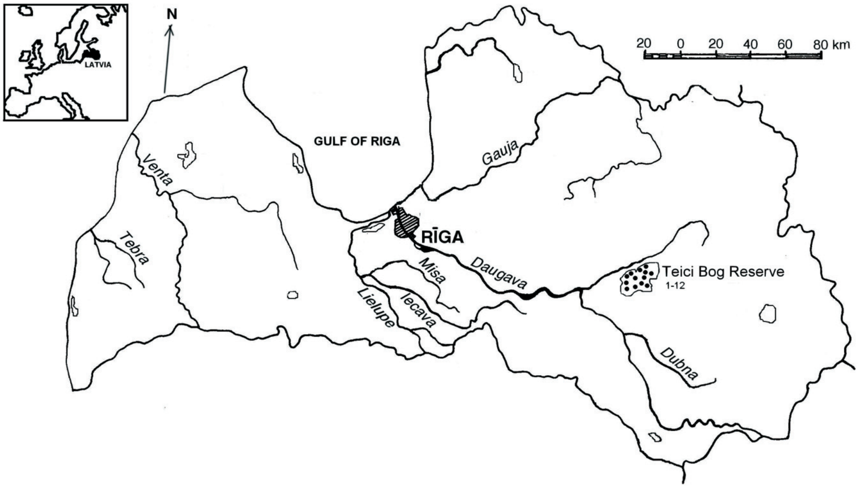

Fig. 1. Location of study area.

The maximum thickness of peat layer in the bogs of the reserve is 9.3 m, and an average one about 4.5 m (Lacis and Kalnina, unpubl. data). The average amount of precipitation is 611 mm yearly, and the average amount of drained water is 211 mm per year. On the average 330 mm evaporate yearly from the water surface. The bog is 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. There is a lack of transit runoff due to existence of several globes. The ditches nets made at the beginning of the century around the bog have lost their importance.

Methods of investigations

Samples from 12 lakes in Teici bog were collected in summer stagnation period 1992, 1994, 1996 and 1997 at the depth of 0.5m (Figure 1).

The chemical composition of water was determined by standard methods (*APHA* 1988). Concentration of humic substances was determined spectrophotometrically after diazotization and conjugation with p-nitroaniline (*Methoden der Wasseruntersuchung* 1988). Bacteriological analyses (total bacterial numbers, saprophyte numbers) were done according to methods accepted in freshwater microbiology (*Romanenko and Kuznetsov* 1974).

Phytoplankton samples were collected by the Ruttner type water sampler in pelagial and littoral zones. Samples for quantitative analyses were immediately fixed with aqueous formaldehyde. The biomass of phytoplankton species were determined by counting their number 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 10 μm plankton net by vertical hauls through the water column. Cell numbers were determined by a Zeus's microscope using magnifications of 100, 200, 400, and 600.

Results and discussion

Hydrochemistry

The results of analyses of main water parameters for several years and seasonal fluctuation as well allow to figure out two groups of lakes.

Relatively low concentration of pH, conductivity, colour, nutrients and comparatively little variability of these parameters characterize the lakes situated in the central part of raised bog (Table 1). COD of water is 18-90 mg L^{-1}. These lakes belong to dystrophic lake systems.

Regarding the lakes bordering with mineral soils in comparison with lakes of the first group higher concentrations and larger variability of main water parameters are typical (Table 1). COD values are stated in the limits 33–120 mg L^{-1}.

Table 1

The variances and mean values of the water composition of the Teici Reserve's Lakes (summer)

Lake	pH	Conductivity (μS cm^{-1})	APHA Pt/Co Colour	Humic substances (mg L^{-1})		N_{tot} (mgL^{-1})	PO_4^{3-} (mg L^{-1})
				min-max	Mean		
Islienas	6.6	65–156	550	61.7–150.2	122.6	0.9–6.3	0.004–0.04
Kurtavas	6.3	27–38	260	58.2–92.7	83.5	0.8–3.4	0.004–0.119
Lisins	4.5	35–175	225	45.6–78.9	83.0	1.1–9.0	0.011–0.032
Siksalas	6.1	29–44	183	50.2–62.8	50.2	0.6–2.7	0.02–0.04
Sildu	8.0	120–196	144	76.6–142.1	110.0	1.0–5.7	0.04–0.109
Dyseutrophic	**6.3**	**27–196**	**272**	**45.6–150.2**	**89.9**	**0.6–9.0**	**0.01–0.119**
Broku	4.1	36–49	69	43.3–53.6	44.0	0.8–2.6	0.008–0.012
Liepsalas	4.1	31–44	22	23.7–26.6	26.0	0.7–1.3	0.011–0.021
L Murmasts	3.7	55–58	166	81.2–92.7	63.0	1.0–1.7	0.01–0.023
Pieslaista	4.0	40–42	170	62.8–97.3	67.5	0.2–1.4	0.008–0.022
Pieslaistes	4.1	29–47	130	66.3–115.7	85.8	0.6–2.2	0.033–0.05
Tolkovas	5.3	22–32	44	32.9–52.5	32.9	0.7–1.4	0.008–0.047
Veigantu	4.0	39–45	196	42.1–72.0	64.0	0.9–5.0	0.008–0.149
Dystrophic	**4.2**	**22–58**	**114**	**23.7–115.7**	**54.7**	**0.2–5.0**	**0.008–0.149**

The water mineralization (sum of inorganic ions) of most lakes is low (< 100 mg L^{-1}) and waters can be regarded as very soft (total hardness < 1 mg-eq L^{-1}). Low mineralization is determined by low concentrations of cations (Ca^{2+}, Mg^{2+}, Na^+) and anions (HCO_3^-, Cl^- SO_4^{2-}), and less expressed interannual and seasonal variability of water chemical composition than it is common for freshwaters in Latvia. However, there is an exception

(Lake Islienas) where mineralization of the lake water is low and concentration of Ca^{+2}, Mg^{+2} and HCO_3^- reaches values common for surface waters of Latvia. In this lake chemical composition of the water is not determined by the presence of peatlands in its basin but due to receiving waters rich in substances from surrounding dry hillocks, water chemistry is similar to that in Latvia in general (*Klavins and Apsite* 1997, *Springe et al.* 1999).

All studied lake waters contain high concentration of humic substances: 23.7 to 150.2 mg L^{-1} (Table 1). On average amount of humic substances reaches 54.7 mg L^{-1} in dystrophic lakes and 89.9 mg L^{-1} in dyseutrophic ones. Presence of humic substances causes dark brown water colour for all of dyseutrophic (on average 272 Pt/Co colour) and most of dystrophic (on average 114 Pt/Co colour) lakes. The obvious correlation between concentrations of humic substances and water colour is found in all of investigated lakes as well as correlation between humic substances and dissolved inorganic nitrogen (Figure 2).

To characterize humic substances in lake waters under investigation, they have been preoperatively isolated and characterized by elemental composition and spectral properties. Sediments of the studied lakes have been characterized as well. The organic matter – humus (85.0–99.5%), dominated the sediment composition. It is found that there exists the similarity of isolated humic substances from the water of the lakes in Teici Reserve with peat humus. (*Klavins et al.* 1998).

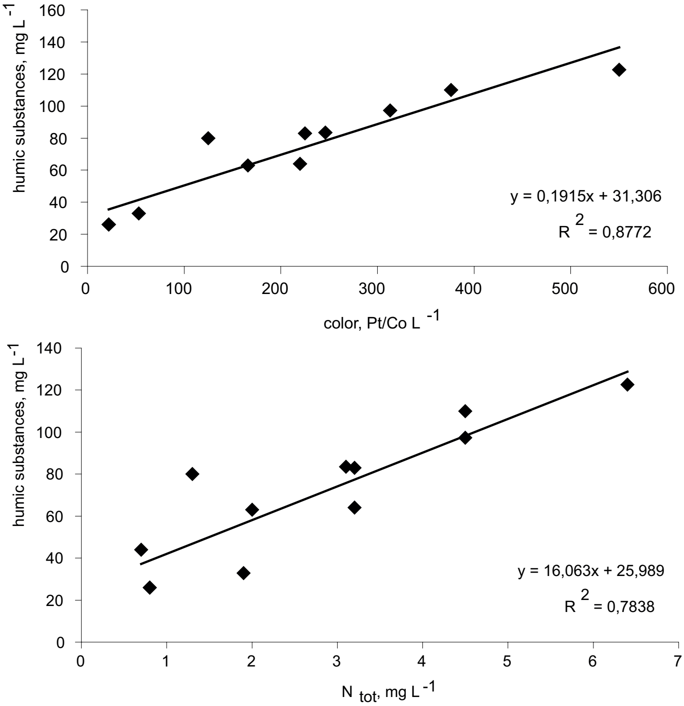

Fig. 2. Correlation between concentration of humic substances, dissolved inorganic nitrogen and water colour.

Presence of high concentrations of humic substances determines substantial changes in concentrations of minor components of waters studied and also reduced intensity of nitrification processes as well as formation of ammonium fulvates. Total concentration of nitrogen in waters of lakes reaches 0.2–9.0 mg L^{-1} N although concentration of nitrates in summer time may reach even zero.

The found tight correlation between concentrations of humic substances and dissolved inorganic nitrogen in studied waters clearly indicates that most of nitrogen is bound to humic substances. It can be supposed that presence of humic substances also reduces the intensity of nitrification processes. Thus unless nitrogen concentrations can reach extremely high values for uncontaminated waters (up to 9.0 mg L^{-1}) it can be supposed that biota suffer from available nutrients resulting in reduced primary production. Also binding of iron to humic substances may reduce its availability to plankton organisms. Thus presence of humic substances influence primary production level and determines trophic state of the lakes.

Humic substances can reduce the adverse impact of water acidification especially in carbonate deficient waters and this process is typical for the most of lakes of Teici Bog. There has also been stated close relationship between content of humic substances and such parameters of plankton as total number of bacteria ($r = 0.79$, $\alpha = 0.05$) and zooplankton organisms ($r = 0.65$, $\alpha = 0.05$) (*Druvietis et al.* 1998).

Bacterioplankton

Bacterioplankton of investigated dystrophic lakes generally is characterised by low total bacterial number – in average it varies from 1.2 to 3.3 × 10^6 cells mL^{-1}. In dyseutrophic lakes lying under some impact of anthrophogenic load a tendency of eutrophication is observed as essential increase of total bacterial number which can reach 7.0×10^6 cells mL^{-1} (Figure 3).

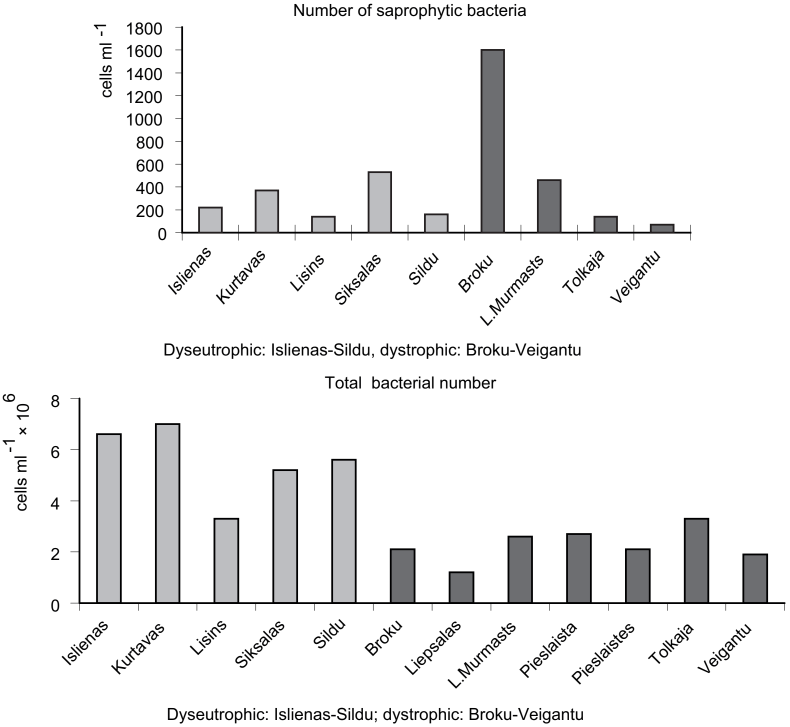

Fig. 3. The mean summer values for total bacterial number and saprophytic bacteria in plankton in surface (0.5 m) waters (1992, 1994, 1996).

There is no so clearly distinguished difference between dystrophic and dyseutrophic lakes regarding to numbers of saprophytic bacteria which are recorded on average from 70 to 530 cells mL^{-1} in all lakes investigated (Fig. 3).

In most cases saprophyte's ratios of total bacterial numbers are thousandths of per cent. The total bacterial number in 0.5 m horizon in pelagial zone of the dyseutrophic lakes exceeds that in dystrophic lakes about 3 times and there is a good correlation between the total bacterial number and pH level in Teici bog lakes: $r = 0.83$ ($\alpha = 0.01$).

For the time being the content of humic matter impacts the development of planktonic bacterial community – the total bacterial numbers are higher in the lakes with higher concentrations of humic matter. A correlation between total bacterial number and concentration of humic substances in all lakes investigated is stated: correlation coefficient $r = 0.79$ ($\alpha = 0.05$). Definite links between saprophytic bacteria and humic substances in Teici bog lakes have not been found but the numbers of saprophytes correlate to Secchi disk transparency: $r = 0.73$ ($\alpha = 0.05$) which reaches up to 0.70m in dyseutrophic lakes, and up to 1.30 m in dystrophic ones.

Humic lakes are traditionally viewed as unproductive and numbers of bacteria are markedly lower in acidic dystrophic lakes which contain high concentrations of humic matter (*Wetzel* 1983). In our case the content of humic matter positively impacts the development of planktonic bacterial community – the total bacterial numbers are higher in the lakes with higher concentrations of humic matter. It could be explained by the fact that bacterial yield is stimulated by the presence of the colloid concentrate and it is stated that in lake water much of dissolved organic matter may be of molecular weights that would render it colloidal than truly dissolved (*Tranvik* 1990, 1994).

As the total bacterial number differs clearly between dystrophic and dyseutrophic lakes – in the latter it is on average 3 times higher – it can be used as a characteristic of trophic state (*Aizaki* 1985).

Phytoplankton

According to the phytoplankton biomasses and number of algae species it is possible to group the investigated lakes in two groups: phytoplankton of the first group of the lakes is characterized by very low number of species (36 species) and support very low phytoplankton biomass (0.03–0.10 mg L^{-1}).

The phytoplankton communities of the second group is formed by algae divisions Chrysophyta, Bacillariophyta, Chlorophyta and Dinophyta which includes specific algae assemblages dominated by *Dinobryon divergens, D. sertularia, Mallomonas* sp.*, Asterionella formosa, Botryococcus braunii, Aulacoseira italica, Glenodinium gymnodinium*. The second group of the lakes is characterized by higher diversity of planktonic algae. Phytoplankton communities formed by algae divisions Bacillariophyta, Euglenophyta, Chrysophyta, Dinophyta, Chlorophyta, Cyanophyta have got higher biomasses (0.18–0.60 mg L-1). Phytoplankton assemblage is dominated by *Asterionella formosa, Tabellaria* spp., *Aulacoseira italica, Trachelomonas* spp., *Euglena* sp., *Phacus* spp., *Dinobryon divergens, Glenodinium gymnodinium, Oocystis* spp., *Microcystis pulverea*.

Positive correlation between pH and number of species in the lakes investigated is obtained (Figure 4).

In total 112 species of algae are found in the observed lakes (Cyanophyta – 11; Chrysophyta – 7; Baccillariophyta – 36; Xantophyta – 2; Dinophyta – 4; Euglenophyta – 9; Chlorophyta – 43). Rare periphytic species of Rhodophyta *Batrachospermum boryanum, B. gelatinosum* and *B. vogesiacum* are found on submerged tree stumps, branches and rushes. Bloom of cyanobacteria is not observed.

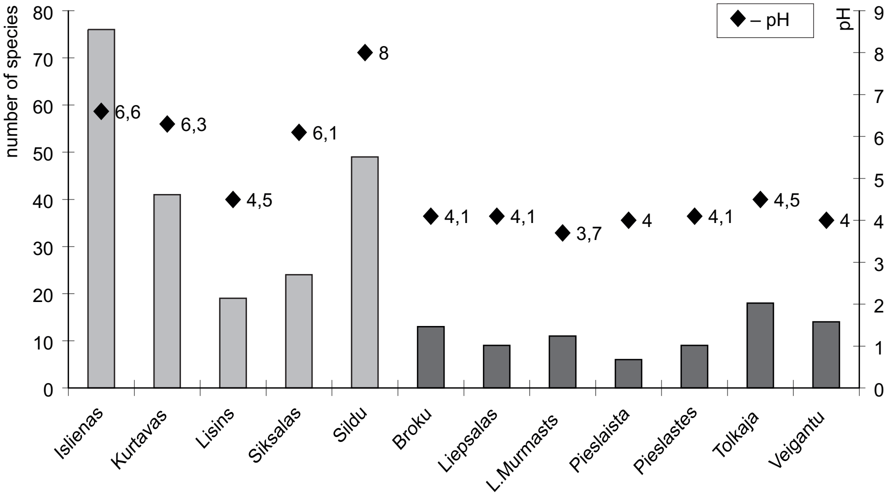

Fig. 4. Relationships between number of phytoplankton species and pH in the dyseutrophic (Islienas – Sildu) and dystrophic (Broku – Veigantu) lakes.

Phytoplankton communities in brown water lakes in Reserve Teici are formed by species tolerant to a low pH and high humic substance concentration and there is positive correlation between pH and number of species obtained in the lakes investigated. In total the simple community structure for phytoplankton determined by low pH is characterized.

The clear relationship between taxonomic composition of phytoplankton and trophic state of the lakes is found (Table 2).

Table 2

Comparison between phytoplankton communities of the dystrophic and dyseutrophic lakes

Status of the lakes dominated	Species number	Mean biomass, mg/l	Species
Dystrophic (n=7)	36	0.12	*Dinobryon divergens* *D. sertularia* *Mallomonas sp.* *Botryococcus sp.*
Dyseutrophic (n=5)	79	0.39	*Asterionella formosa Tabellaria spp.* *Aulacoseira italica* *Euglena spp.*

Conclusions

In total, humic substances in waters of Teici Bog Reserve are regarded as the main factor affecting water pH, colour and transparency. According to the biological features and hydrochemical parameters communities, the studied lakes can be divided into the following groups: dystrophic lakes and dyseutrophic lakes.

Dystrophic lakes are characterized by: low total bacterial number, low number of plankton taxa. *Dyseutrophic lakes* are characterized by: essential increase of the total bacterial number, comparatively higher numbers of plankton taxa.

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Occurrence and removal of natural organic matter (NOM) in drinking water treatment

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The amount and characteristics of NOM in Finnish raw water sources differ considerably particularly in rivers. The removal of the high molecular mass NOM fraction is relatively easy in the conventional water treatment processes. The intermediate and small molecular mass fractions of NOM require either optimisation of the existing processes or investment in the additional advanced treatment units. Enhanced coagulation, ozonation, activated carbon filtration and membrane techniques can significantly improve NOM removal efficiency. Small and medium size water work doesn't usually have resources to invest in the best available drinking water treatment techniques. The enhancement of NOM removal and stable drinking water quality should be obtained by selecting the best possible water source and by protecting it. More data is needed of the characteristics of NOM for the risk assessment and selection of proper treatment technologies.

Key words: *drinking water, water treatment, natural organic matter (NOM), high-performance size-exclusion chromatography (HPSEC).*

Introduction

Water sources used for drinking water purposes generally contain dissolved natural organic matter (NOM). The character and properties of NOM in water of different origin differ considerably (*Gjessing et al.* 1999). Particularly in boreal areas the humic substances are ubiquitous in all surface waters. NOM can cause several problems in drinking water production and distribution. It has been demonstrated that NOM is the basis for the production of potentially hazardous disinfection by-products. NOM also contributes to annoying colour, taste and odour problems and acts as a carrier of metals and hydrophobic organic chemicals, as well as causes biological regrowth and promotes corrosion in distribution system (*Jacangelo et al.* 1995).

NOM can be removed from water by a number of different treatment processes (*Jacangelo et al.* 1995). The most common and economically feasible process to remove NOM from drinking water is coagulation and flocculation followed by sedimentation/flotation and sand filtration. Organic matter with high molar mass (HMM) is removed efficiently from water in coagulation process. However, low molar mass (LMM) and intermediate molar mass (IMM) organic compounds are removed only partially (*Jacangelo et al.* 1995, *Nissinen et al* 2001, *Matilainen et al.* 2002, *Matilainen et al.* 2005a). Coagulation is a process where chemicals are used in forming a precipitate with NOM and subsequently removing them by settling or flotation. Historically coagulation has been employed in water treatment practise to decrease turbidity. However, optimum conditions for turbidity removal are not always the same as those for NOM removal (*Budd et al.* 2004, *Semmens and Field* 1980). The effectiveness of coagulation to remove NOM and particles depends on several factors, including coagulant type and dosage, pH, particle and NOM properties, such as size and hydrophobicity, temperature and presence of divalent cations (*Vrijenhoek et al.* 1998). In order to enhance the removal of NOM, the best way is to optimise coagulation conditions in regard to pH and coagulant dose (*Bell-Ajy et al.* 2000, *Semmens and Field* 1980). Moreover, the removal can be enhanced by a choice of a proper coagulant and by employing possible coagulation aids (polymers) (*Matilainen et al.* 2005a, *Lindqvist et al.* 2004).

A part of the organic matter is passing through the conventional drinking water treatment process (*Matilainen et al.* 2005 a and 2005 b). This part consists predominantly of LMM organic compounds. The residual NOM after coagulation/flotation can be further removed by granular activated carbon (GAC) and biologically activated carbon filtration, and membrane processes (*Jacangelo et al.* 1995). Coagulation prior to GAC filtration removes particles that might clog GAC beds and coagulation also removes NOM, which reduces the loading on GAC filters (*Jacangelo et al.* 1995). GAC is an effective adsorbent used widely for drinking water treatment. Its major use is the removal of both man-made and natural micro-pollutants such as pesticides, industrial chemicals, tastes and odors and algal toxins. GAC also adsorbs NOM to some degree (*Jacangelo et al.* 1995, *Matilainen et al.* 2005b). The IMM organic compounds and a part of the LMM organic matter are removed quite efficiently in the GAC filtration process (*McCreary and Snoeyink* 1980). The adsorption behaviour of NOM is particularly difficult to understand due to the heterogeneous nature of both AC and NOM. According to study by Newcombe et al. (2002) the adsorption of the NOM is controlled predominantly by the relationship between the molecular size distribution (MSD) of the NOM and the pore size distribution of the carbon.

The NOM removal results by ozonation can vary significantly depending on the characteristics of the water and the type of ozonation, ozone dose applied and conditions. NOM consists of diverse range of compounds with very different chemical properties. It can affect on ozonation by various ways: NOM can act whether as an initiator, promoter or inhibitor of ozone decomposition. The ozonation of NOM practically never leads to a complete mineralization of the organic compounds, but rather to the formation of organic products with different physical and chemical properties. Ozonation increases the concentration of assimilable organic carbon (AOC) which can enhance the biological regrowth in water distribution system (*Miettinen et al.* 1998) if not properly removed by AC filtration.

Membrane processes are a relatively new innovation for NOM removal from waters (*Jagelangelo et al.* 1997). Nanofiltration (NF) has proven to be reliable for the removal of organic and inorganic compounds (*Amy et al.* 1990, *Thorsen* 1999, *Siddiqui et al.* 2000). Foulants with molar masses bigger than the membrane cut off are removed mainly by a sieving mechanism, and molecules with molar masses smaller than the membrane cut off by diffusion and charge repulsion based mechanisms. Molecule polarity, hydrophobicity and configuration also affect the retention (*Cho* *et al.* 1999, *Van der Bryggen et al.* 1999). However, most studies have concentrated on the NOM removal from natural surface waters, ground waters or model waters, and little is known about the NOM removal from rather pure chemically pre-treated surface waters.

Due to these water quality problems and stricter regulations for drinking water quality, there is a need for more efficient and still economical methods for the removal of the residual organic matter. The need for more efficient removal of NOM necessitates more knowledge of the matter. The nature of organic matter is very complex and there is a need for a simple and rapid method to characterize NOM. The traditional analytical methods are measurements of $KMnO_4$, total organic carbon (TOC) content, UV_{254nm}, SUVA and colour. One of the most common innovative method to characterize NOM is the determination of the molecular size and molar mass distribution (MSD) by high-performance size-exclusion chromatography (HPSEC) (*Myllykangas et al.* 2002, *Nissinen et al.* 2001, *Matilainen et al.* 2001, *Peuravuori and Pihlaja* 1997, *Vartiainen et al.* 1987).

1. Materials and methods

Sampling of different surface waters

Waters used in these experiments were gathered from eight different locations in Finland and from one location in Estonia. The coagulation jar-tests were made with eleven different raw water samples (lake, river, bank-filtrated) (*Ketonen* 2003). Water form Lake Roine was used in the laboratory scale coagulation and ozonation experiments.

Water Treatment Plants

The main partner in our studies has been Tampere Waterworks, Rusko Water Treatment Plant, which has been studied since 1999 (*Matilainen et al.* 2001, *Matilainen et al.* 2005a and 2005 b, *Lindqvist et al.* 2001). The Rusko Water Treatment Plant pumps its raw water from Lake Roine, seven kilometres southeast of the plant and produces 68% of the distributed drinking water for the city of Tampere, Finland. The plant has a maximum treatment capacity of 55 000 m^3/d and an average water flow of about 1 400 m^3/h (Fig. 1.). Raw water is typical Finnish lake water having low alkalinity (about 0.25 mmol/l) and quite a low turbidity (about 2 FTU). Organic carbon content is fairly low (about 5 mg/l) compared to typical Finnish surface waters. The treated water has a TOC value of about 2.5 mg/l. This is a quite typical amount in the conventional water treatment facilities with GAC filtration (*Nissinen* 2001).

In the beginning of the study (1999), all 14 filters were ordinary one-layer GAC filters, operating as parallel filters. Six of the GAC filters were changed to sand filters during the study, in May 2002. After that in-line two-fold filtration was taken in use, where water from coagulation/flotation were first filtered through sand filters (6 parallel filters) followed by GAC filtration (8 parallel filters) (Figure 1). At the same time, the coagulant agent was changed from aluminium sulphate to ferric sulphate.

Water samples from five different surface water treatment plants and one artificial ground water treatment plant were collected in the NF study (*Matilainen et al.* 2004).

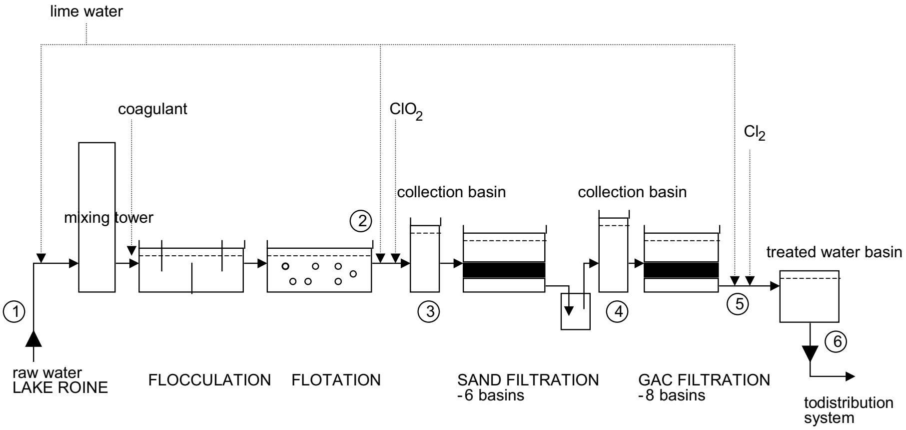


Fig. 1. Schematic picture of Rusko Water Treatment Plant.

2. Analysis

Sampling procedure in Rusko

Water samples were taken about once a week. Samples were taken from the following stages of the treatment sequence:

1) raw water,
2) water after the coagulation/flotation, prior to ClO_2 addition,
3) water after the coagulation/flotation, after ClO_2 addition,
4) water after the filters S1 and S2,
5) water after two parallel activated carbon filters (AC1 and AC2),
6) combined purified water from all AC filters (See Fig. 1).

Basic water analyses were done at the day of sampling. The 10 ml samples were collected and frozen until analysed with HPSEC.

Basic water analysis

UV_{254} absorbance was measured with a spectrophotometer. The raw water sample and the samples after flotation and sedimentation were centrifuged before UV measurement. TOC samples were filtered through 0.45 μm membrane and they were measured as dissolved organic carbon (DOC) according to the SFS-EN 1484 standard and by using a Shimadzu TOC-5000A with a high sensitivity catalyst.

HPSEC measurements

The HPSEC samples were first filtered through a Gelman 0.45 μm membrane and then size-exclusion fractionated with a Hewlett-Packard HPLC 1100-series system, equipped with a diode array UV-detector (λ=254) and a TSKgel G3000SW 7.5 mm (ID) × 30 cm column. Pre-column was not used. Sodium acetate (0.01 M) was used as eluent at a flow rate of 1 ml/min. Injection volume was 30 μl. The method was selected and operated on the basis of results from earlier investigations (*Vartiainen et* *al.* 1987, *Peuravuori and Pihlaja* 1997, *Nissinen et al.* 2001). It must be emphasized that HPSEC columns gels might have charge repulsion effects (*Peuravuori and Pihlaja* 1997), as well as adsorption interactions with the humic compounds (*Myllykangas et al.* 2002), which can affect the measurement of NOM with HPSEC.

When using UV adsorption as detecting method, one should also remember that small aliphatic compounds do not adsorb UV-light due to the lack of conjugated double bonds, and thus are not indicated by the UV_{254} measurements. Still, the similar TSK-columns as ours has been used for NOM measurements more than 17 years and the method has proved to be highly informative in studying changes in MSD of NOM (*Myllykangas et al.* 2002, *Nissinen et al.* 2001, *Peuravuori and Pihlaja* 1997, *Vartiainen et al.* 1987).

Heights of the peaks in the HPSEC chromatogram were used in the data analysis (*Vartiainen et al.* 1987, *Peuravuori and Pihlaja* 1997). The height of the peak refers to the amount of NOM in a specific molecular size fraction with a peak having the lowest retention time referring to the highest MM fraction of NOM and peak having the highest retention time to the lowest MM fraction of NOM. The sum of all peak heights (SOPH) represents the total amount of NOM in the sample.

Jar-test conditions

The jar-tests were done with the Kemira mini-flocculator. It consists of six parallel agitators, in which 1.0 dm^3 glass beakers were placed. The pH adjustment chemical (H_2SO_4 or NaOH) was added and the sample was stirred intensively (400 rpm) for 30 seconds. The coagulant was immediately added after previous stage and the sample was again stirred intensively (400 rpm) for 30 seconds. The flocculation stage lasted 30 min and the speed of the agitator was 30 rpm. After the flocculation the agitators were carefully removed from the beaker and the flocks were allowed to settle for 30 min. The optimal coagulation pH was defined first. In the optimal coagulant dose test different amounts of coagulant was added to the beakers and the pH was adjusted to the optimal in all the beakers.

Ozonation experiments

Ozonation experiments were performed with continuous flow pilot plant and semi-batch laboratory scale systems by Ristimäki (2003). Pre-purified surface water, from Lake Roine, and Milli-Q were spiked with different pharmaceuticals and ozonated with different ozone doses and with addition of hydrogen peroxide.

3. The occurrence of NOM in Finnish surface waters

There are usually 5–7 different peaks in Finnish surface water samples. (*Nissinen et al.* 2001, *Matilainen et al.* 2001, *Myllykangas et. al.* 2002, *Ketonen* 2003). Concentration of NOM is highest in rivers (Table 1, Figures 2 and 3) and the SEC profile is dominated by highest molar mass fractions (Figure 2).

Table 1

Quality of different raw waters examined

Water supply	pH	Turbidity (NTU)	UV-254 (cm^{-1})	DOC mg/l	SUVA (l/mgm)	HPSEC (mAU)
Lake Näsijärvi	6,9	1,6	0,19	7,0	2,7	18,81
Lake Roine	6,3	1,3	0,12	5,8	2,0	11,85
Lake Ülemine	8,5	10,0	0,25	13,2	1,9	21,70
Hietasalo	6,2	10,0	0,18	6,0	2,9	17,27
Hietasalo&Lake Kallavesi	6,6	8,9	0,19	6,4	3,0	20,06
River Aurajoki 1	7,0	82,0	0,64	18,2	3,5	44,80
River Aurajoki 2	6,9	10,0	0,55	17,1	3,2	37,55
Basin Maarian allas	7,1	10,0	0,35	12,6	2,7	24,43
River Kyrönjoki	6,5	5,0	1,12	26,3	4,3	81,44
Basin Uusikaupunki	6,5	0,9	0,14	6,5	2,2	16,21

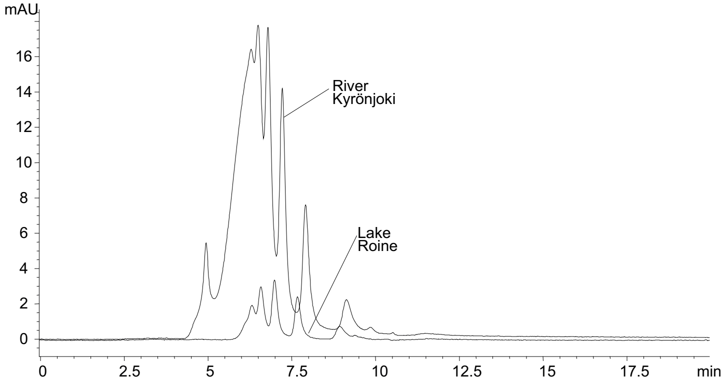


Fig. 2. HPSEC chromatograms of River Kyrönjoki and Lake Roine. The components are eluted in order of decreasing molecular sizes; the small molecules are the last to elute from the column.

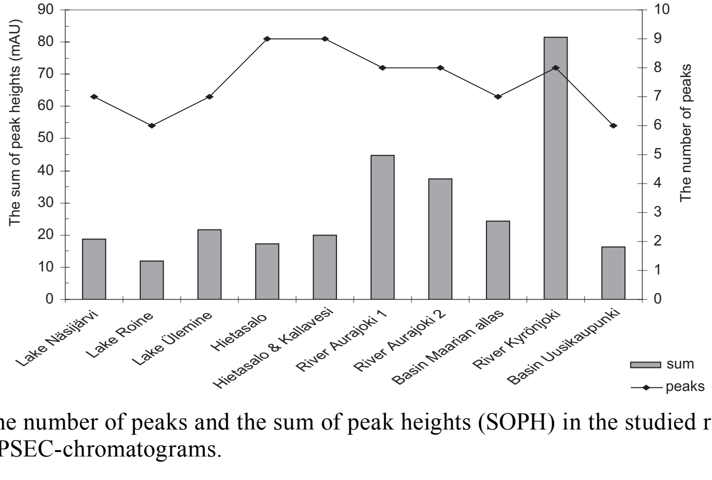
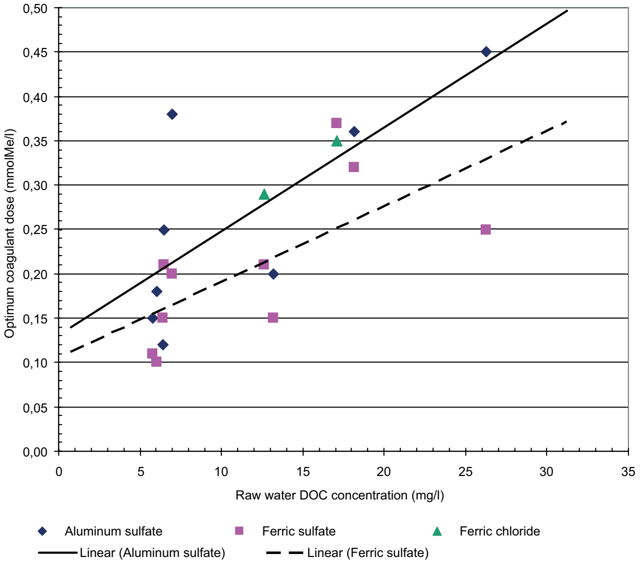


Fig. 3. The number of peaks and the sum of peak heights (SOPH) in the studied raw waters HPSEC-chromatograms.

4. Removal of NOM in water treatment

Coagulation and flocculation

The different raw waters gathered where used in jar-test coagulation investigation, where the waters were coagulated with three different coagulation agents (*Ketonen* 2003). The optimum coagulant dose versus initial DOC concentration in raw water can be seen in the Fig. 4. There is no linear correlation between DOC content of raw water and optimum coagulant dose. For example when raw waters DOC content varies from ca. 6.0 to 7.0 mg/l, the optimum coagulant dose of aluminum sulfate varies greatly depending on the raw water.



Fi.g. 4. The correlation between DOC content of the raw water and the optimum coagulant dose of different coagulant agents studied.

High molar mass (HMM, peaks I and II) NOM is generally removed very easily by coagulation – also in our studies both in full and in laboratory scale (Figs. 5 and 6). The use of iron instead of aluminium sulphate at optimal conditions improved particularly the removal of intermediate molar mass (IMM, peaks III and IV) fractions of NOM from 20% to 50%. The increase of total removal of NOM was 15%. In full-scale treatment process at Rusko (Figs. 5 and 6), the removal of the low molar mass (LMM, peaks V–VII) matter was very poor with both aluminium and ferric sulphate. Nearly 90% of the LMM matter was still present in water after the coagulation process with both of the coagulants (Fig. 6). LMM fraction is very hard to remove in the coagulation. According to the jar-test studies (*Lindqvist et al.* 2004, *Tuhkanen et al.* 2004) the LMM fraction was not removed any better even when the process was optimised in regard to NOM removal. However, in the study made by Lindqvist et al. (2004), almost 40% removal of the smallest MM fraction was observed if polyelectrolytes were used as coagulation aids in the process.

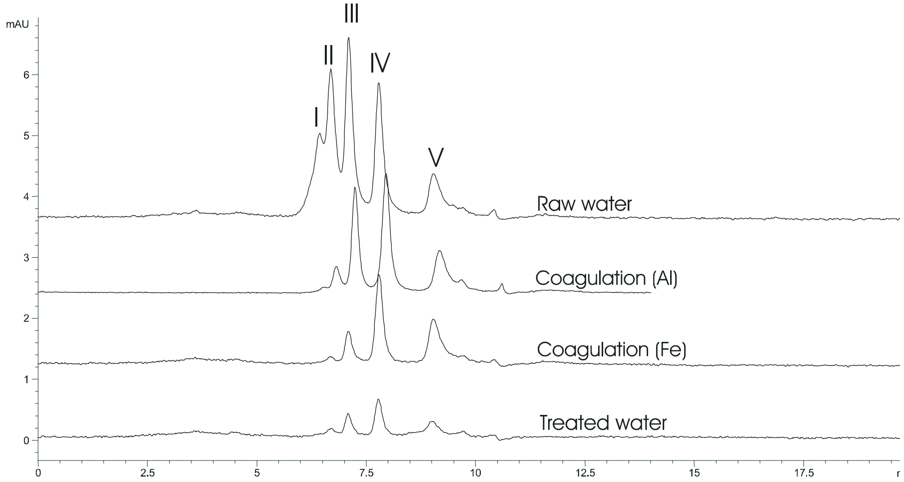


Fig. 5. HPSEC chromatograms of raw (above), aluminium sulphate coagulated, ferric sulphate coagulated and treated (below) water samples from Rusko Water Treatment Plant.

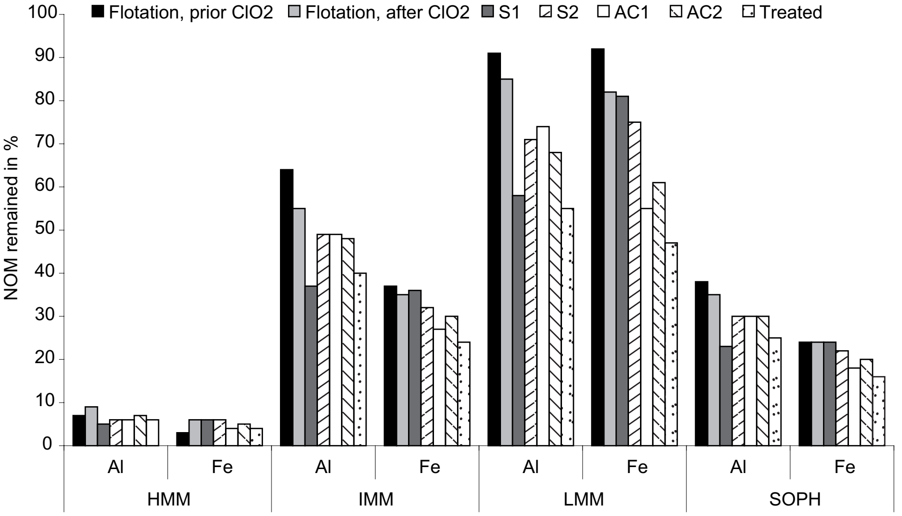


Fig. 6. Different MM fractions of NOM remained (according to the HPSEC results) after the each purification step in Rusko (in % compared to the raw water results), calculated as an average (Al-coagulation: May 2001–May 2002 and Fe-coagulation: June 2002-April 2003). S1 and S2 were GAC filters during the Al-period and changed to sand filters in the beginning of the Fe-period.

GAC filtration

Two of the studied GAC filters (S1 and S2) were changed to sand filters in the middle of the study, at the same time as the coagulant agent was changed to ferric sulphate. By this way the age and efficiency of the rest of the GAC filters were increased and filtration costs were decreased. The GAC filter S1 was regenerated in the beginning of the study (May 2001). NOM removal rate in the GAC filter S1 was high during the period May 2001–May 2002. Especially the removal of the IMM and the LMM NOM was generally much higher with this filter than with other GAC filters studied during the Al-coagulation period (Fig. 6).

The efficiency decreased gradually after the regeneration. During the period of ferric sulphate coagulation (June 2002–April 2003), the carbons in the GAC filters AC1 and AC2 were changed to new ones in June 2002 and in September 2002, respectively. The removal capacities were noticed to increase immediately after the change quite dramatically. However, within few months the capacities returned to the levels before the change. It was also noticed that the GAC filtration enhanced the removal of the IMM matter of NOM most effectively. On the contrary, GAC filtration did not enhance the removal of HMM or LMM matter fractions any further (*Matilainen et al.* 2005a and 2005b).

Ozonation and ozone/hydrogen peroxide

Ozonation of NOM does not affect the total amount of organic carbon so much (*Tuhkanen et al.* 1994), but it is, however, slightly reduced. The extent and rate of DOC removal typically increases as ozone dose increase. The removal rate depends also on NOM characteristics. The addition of hydrogen peroxide and UV radiation during ozonation enhances the removal of NOM, as DOC and as sum of peak heights (SOPH) measured by HPSEC, compared to ozonation alone (*Kainulainen et al.* 1994, *Kainulainen et al.* 1995). MSD of NOM changes considerably during ozonation. IMM fraction of NOM is reduced the most. With ozone dose of 2 mg/l the SOPH reduced 30–40%, but the amount of DOC did not decrease. The combination of ozone and hydrogen peroxide enhanced the NOM removal. The DOC was reduced 5% with O_3/H_2O_2 molar ratio of 2:1, 10% with ratio of 1:1 and 18% with ratio of 1:2 (Fig. 7) (*Ristimäki* 2003).

Membrane techniques

The standard treatment operations are not able to remove the LMM fraction of NOM and the IMM matter is only partly removed. The removal of the residual NOM from drinking water by nanofiltration (NF) was evaluated in our study (*Matilainen et al.* 2004).

Three different NF membranes were compared in filtering six pre-treated surface waters. The DOC content of the feed waters varied from 2.0 to 4.2 mg/l. The NOM removal efficiencies of the membranes were good and varied between 100% and 49%, and between 85% and 47% according to HPSEC and DOC measurements, respectively. Removal of different molecular size fractions varied from 100% to 56%, 100% to 54% and 88% to 19%, regarding HMM, IMM and LMM organic matter, respectively. The Desal-5 DL membrane produced the highest NOM removals rates (*Matilainen et al.* 2004).

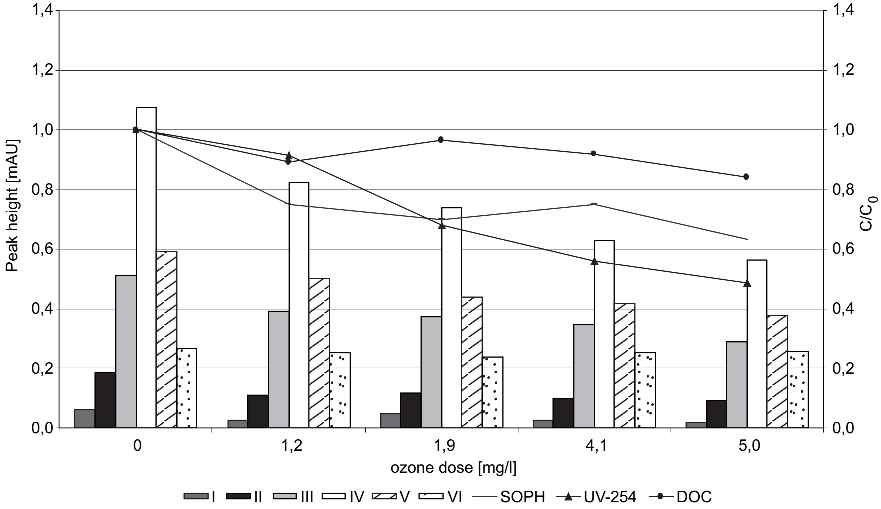


Fig. 7. Changes in NOM measured as MSD, SOPH, UV_{254} and DOC during pilot plant ozonation. (Co = 3200 ng/l, UV-254 $_0$ = 0.029 cm^{-1}, DOC_0 = 3.0 mg/l, t_R = 20 min, T_w = 21 °C and pH 7.0).

When considering the performance of a NF membrane one should bear in mind that the aim is to achieve the required drinking water quality at the lowest possible costs. Accordingly, the membrane with the highest NOM removal capacity may not always be the best choice, as some other membrane may produce a high enough water quality at a lower cost due to higher productivity. Also, in Finland the quality of drinking water produced from surface waters is generally high already without NF and the need for NF is restricted to special cases. Still, especially the LMM fraction of NOM, which is hard to remove by other means, can be further removed by NF. Microbes easily use this fraction, and thus its enhanced removal is desirable for improved quality of drinking water.

5. Further research and development needs and the consequences of increased colour and NOM in raw waters

The ground water has always been preferred as the raw water source in Finland. The ground water resources are in the Northern and Eastern Finland but the majority of the population is in the coastal area in Southern and Western Finland. Rivers and lakes with high amount of NOM have been used for the production of drinking water. Significant increase of colour and NOM has been reported by literature (*Nordtest Position paper* 2003). The development of the trend should be analysed from existing follow-up data of waterworks. The quantity of NOM is not the only parameter affecting to the treatment efficiency. In principle, the HMM molecules are easier to remove than intermediate or small NOM molecules.

During the last two decades there have been huge investments for the improvement of drinking water quality. Still there is a pressure to further improve the quality and safety of drinking water. The most obvious solution would be the enhancement of the performance of existing processes; coagulation, flocculation and filtration. The possible increase of NOM in the raw water will lower the efficiency of water treatment processes and increase the demand of water purification chemicals such as inorganic coagulants, flocculation aids and disinfections chemicals, which leads to the increase of the amount of sludge formation. The treatment costs are increased directly due to the price of chemicals and indirectly by the disposal of sludge. The increase of NOM in the raw water leads also to the need of backwashing of filters. When AC filtration is used to polish the drinking water, the carbon is going to be saturated sooner, which increase the need of regeneration or exchange of activated carbon.

If the existing water treatment process is considered insufficient, there are still unit operations for the removal of the residual organic matter and possible micro-pollutants from water: membrane techniques, oxidisation of organic matter followed by assimilation of organic matter by micro-organisms, and artificial groundwater recharge. If the increase of NOM in the raw water cannot be removed by traditional unit operations the use of additional advanced and/or innovative processes is unavoidable. However, particularly the small waterworks don't have resources to invest in additional unit operations for NOM removal. The enhancement of NOM removal and stable drinking water quality should be obtained by process optimisation and raw water source selection and protection. More data is needed of the characteristics of NOM for the risk assessment and selection of proper treatment technologies.

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Effect of sustainable soil and crop management on humus changes

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Organic matter is one of the most important factors for saving clay soils from their degradation. In order to increase the amount of organic matter in the soil and to improve its quality applying different practices of soil and crop management, complex researches on a glacial lacustrine clay loam on silty clay (*Gleyic Cambisol)* were made in Joniskelis Research Station of the Lithuanian Institute of Agriculture.

It was assessed, that increasing the area of longer vegetation wintering crops in the heavy soil had a positive influence to the amount of organic carbon and humic acids and degree of humification. Application of sustainable soil tillage systems especially in blends with organic manure determined the reduction of organic substances mineralization and increased the amount of humus and fulvic acids.

Key words: c*ambisol, soil and crop management, organic matter, humic and fulvic acids.*

Introduction

One of the main soil quality indexes, which shows its resistance to physical and biological degradation, is the amount of organic matter in the soil (*Loveland and Web* 2003). According to the amount of organic matter and especially humus in the soil it is possible to judge about the tame level of soil (*Franzluebbers* 2002, *Kay and Van den Bygaart* 2002, *Kristensen et al.* 2003).

Clayey soil becomes resistant to compaction and suitable for reduced tillage, when the amount of humus exceeds 5% in the topsoil (*Balesdent et al.* 2000). The amount of humus in the most of heavy soils in Lithuania doesn't reach 3%; consequently there is a tendency of degradation (*Lithuanian soils* 2001).

Selection of the best crops in the most effective using of their alternation advantages has a diverse importance reaching to enrich heavy soils. Soil tillage and other complex agricultural practices have to promote organic carbon accumulation in the soil and to increase its biological activity, to fit biological requirements of plants and soil protection requirements (*Arlauskas and Slepetiene* 1997, *Balesdent* 2000).

Winter crops growing longer period of time and having a stronger system of roots, use potential fertility of heavy soils much better and suffer from extreme climate less than spring crops (*Golub* 1996, *Rasmussen* 1999). After their growing in the soil there are left more vegetative remains, than after the spring ones. Admittedly, winter crops are improving biological, chemical and physical soil properties when they are cultivated like catch crops. Climate factors do less damage for the soil properties when the field is taken longer (*Dabney et al.* 2001).

The indexes of total humus, its humus forming matters accumulation, their composition and changes are very important factors in investigating the systems of rational agriculture, which could let to warrant the big productivity of cultivated crops. The soils are becoming more resistant for degradation, declining intensity of soil tillage and strengthening positive influence of plants and other living organisms. Supposedly, that such influence is effective in the heavy soils (*Franzluebbers* 2002, *Kay et al.* 2002).

There are lots of records about positive influence of sustainable soil tillage to accumulation of organic carbon, especially in the upper layer of the soil. It is affirmed that it depends on the insertion depth of organic remains. Many authors state that the amount of humus is increasing in the upper layer when working without overturn of the soil, but it becomes worse in the bottom layer (*Gamzikov et al.* 1992, *Sdobnikov and Mielcajev* 1998, *Rasmussen* 1999, *Christensen* 2001, *Loveland and Webb* 2003).

In Lithuanian Institute of Agriculture the soil differentiates into the richer upper layer of humus and into lower with less amount of organic matters layer because of shallow soil tillage (*Arlauskas and Slepetiene* 1997). In the Joniskelis Research Station of the Lithuanian Institute of Agriculture it was set that using processed amount of organic matters in the conventional soil tillage system in the clay loam soil, marked improvement of humus that could better physical properties of the soil wasn't obtained. (*Maiksteniene* 1996).

One of the most important aims in the agriculture is stable supplement of organic matters in the soil and balanced transformation of them to humus. Between different agricultural practices where organic matters stimulating segmentation dominate less attention is paid to keeping these matters.

The aim of this research was to evaluate the effect of extension of winter crop area and application of sustainable soil tillage systems on organic matter changes in clayey soil.

Materials and methods

Site and soil description

The study was conducted at the Lithuanian Institute of Agriculture's Joniskelis Research Station situated on the soils of the northern part of Central Lithuania's lowland (56°21′ N, 24°10′ E) during the period 1998–2002. The experiments were carried out on drained, clay loam on silty clay with deeper lying sandy loam *Endocalcari-Endohypogleyic Cambisol* (FAO classification), whose parental rock is glacial lacustrine clay. Clay particles < 0.002 mm in A_a horizon (0–30 cm) made up 27.0%, in B_1 horizon (52–76 cm) – 51.6%, in C_1 horizon (77–105 cm) -10.7%, in C_2 horizon (106–135 cm) – 11.0%.

Before the start of the experiment soil humus content (0-25 cm layer) was 2.20%, pH (KCl 1M, w/v 1:2.5), determined using pH-meter, was 6.6–6.8; available phosphorus (P_2O_5)– 154 mg kg^{-1} and available potassium (K_2O) determined by A–L method -304 mg kg^{-1} (*Egner et al.* 1960).

Experimental designs

The experiment on feasibility of extension of winter crop area in the crop rotation and soil tillage systems was done according to the scheme:

Factor A. Crop rotations with different area of winter and spring crops: 1. Without winter crops (1. Annual grasses; 2. Spring wheat; 3. Spring triticale; 4. Spring barley). 2. 25% winter crops (1. Perennial grasses; 2. Spring wheat; 3. Spring triticale; 4. Spring barley, undersown crop). 3. 50% winter crops (1. Perennial grasses; 2. Winter wheat; 3. Spring triticale; 4. Spring barley, undersown crop). 4. 75% winter crops (1. Perennial grasses; 2. Winter wheat; 3. Winter triticale; 4. Spring barley, undersown crop). 5. 100% winter crops (1. Perennial grasses; 2. Winter wheat; 3. Winter triticale; 4. Winter barley, undersown crop).

Factor B. Soil tillage systems: 1. Conventional (primary soil tillage is performed by ploughing by a mouldboard plough). 2. Sustainable (after grasses the soil for wheat is ploughed by a mouldboard plough; after cereals ploughless soil tillage is applied for all crops). The experiment was established using the fully expanded crop rotation method.

The experiment on improvement of heavy soil by incorporation of amendments and applying reduced sustainable tillage was made according to the scheme:

Factor A. Amendments for soil improvement:

1. Without amendments;
2. Farmyard manure – 60 t ha^{-1};
3. Green manure – 27 t ha^{-1};
4. Lime mud – 10 t ha^{-1}.

Factor B. Incorporation methods of amendments:

1. With a moldboard plough at 25 cm depth;
2. With a segment plough at 40 cm depth.

Factor C. Primary soil tillage technologies after the incorporation of amendments:

1. Moldboard ploughing at 25 cm depth;

2–3. Reduced non-ploughing tillage at 25 and 15 cm depths.

Measurements and assessments

For preparation of samples for chemical analyses all visible plant and animal residues were removed from soil sample and then sieved using a 0.25 mm sieve. Humus content was determined by the Tyurin method modified by Nikitin and calculated by multiplying C_{org} content by 1.724 (*Nikitin* 1999). Humus fractional composition was identified according to the Tyurin method modified by Ponomareva and Plotnikova (1980). For humus fractional composition, the solutions of different NaOH concentrations were used for extraction: 0.1 M NaOH (room temperature);

0.02 M NaOH (hot extraction) also 0.05 M H_2SO_4 (for decalcitation, room temperature) at a soil solution ratio at 1:20. The extracted humic substances were then separated into humic and fulvic acid fractions by acidifying the extract to pH 1.3–1.5 using 0.5 M H_2SO_4 at 68–70 °C and humic acids were separated by filtering. Separated humic acids were re-dissolved in 0.1 M NaOH solution. Some humic and fulvic acid solutions of each fraction were evaporated, oxidized and organic carbon content determined, using the same procedures as soil samples. Carbon content in the fractions of humic and a fulvic acid was determined by the dichromate oxidation procedure (*Ponomareva and Plotnikova* 1980, *Nikitin* 1999).

The following humic acids fractions were identified: HA1 – «free and weakly bound with clay minerals» or otherwise called «mobile» humic acids fraction; HA2 – bound with calcium; HA3 – strongly bound with soil clay minerals. The content of humic acids bound with calcium (HA2) was calculated as follows: (HA2+HA1)–HA1. Respectively fulvic acids fractions were determined. Degree of humification was calculated: (carbon of humic acid / total organic carbon × 100).

According to Orlov-Grishina, humification degree (HD) of OM, determined by Ponomareva–Plotnikova method, 40–30% is high, 30–20% – middle, 20–10% – low (*Orlov and Grishina* 1981). A more detailed description of the fractionation methodology can be found in Ponomareva and Plotnikova (1980), Slepetiene and Butkute (2003).

Statistics. The experimental data were processed by ANOVA and STATENG. Significant differences are presented at 95% probability level.

Results and discussion

The influence of the winter crops and sustainable soil tillage for the humus amount in the soil. The influence of the different structure of winter and spring crops and system of sustainable soil tillage applied in the crop rotations to the amount of humus and humus fractional composition evaluated in the topsoil (0–25) layer (Table 1).

Averaged data, having increased the area of winter crop in the crop rotation till 75 and 100 %, amount of humus in the soil basically increased in comparison with the version without winter crop accordingly 2.14 % and 2.15 % (compare with 2.09 %).

The amount of mobile humic acids (HA1), between versions the sum of HA and FA had no essential difference. The amount of humic acids (HA2) bound with calcium, while increasing the plot of winter crop in the crop rotation till 100%, it was quite big in comparison with crop rotation without winter crop, or under winter crop area 25%.

The amount of humic acids (HA3) is strongly bound with soil minerals, their total amount and humification degree in the soil was bigger with 100% of winter crops in the crop rotation, than without winter crops.

As preceding crop, winter crops also had a positive influence to the soil humification index in comparison with spring crops.

Applying the system of sustainable soil tillage, basically these indexes increased: HA1, HA2, HA3, the sum of HA, FA, humification degree, but HA/FA index had a tendency to increase, in comparison with the common soil tillage system (Figure 1).

Table 1

Effect of area of winter crop in rotations and soil tillage systems on humus indicators 1999–2002 averaged data

Area of winter crops % (Factor A)	Humus indicators						
	humus content %	HA1[a]	HA2[b]	HA3[c]	∑HA[d]	∑FA[e]	degree of humification[f]
Soil tillage systems (Factor B)							
1. Conventional							
0	2.03	0.043	0.127	0.175	0.359	0.474	30.1
25	2.05	0.044	0.125	0.177	0.359	0.474	30.2
50	2.06	0.045	0.127	0.179	0.363	0.479	30.4
75	2.09	0.048	0.130	0.182	0.373	0.482	30.7
100	2.09	0.047	0.138	0.187	0.385	0.485	31.7
Average of factor B1	2.06	0.045	0.129	0.180	0.368	0.476	30.6
2. Sustainable							
0	2.15	0.050	0.129	0.193	0.388	0.504	30.8
25	2.15	0.050	0.132	0.193	0.388	0.504	31.0
50	2.16	0.050	0.136	0.190	0.389	0.500	31.1
75	2.18	0.051	0.136	0.193	0.396	0.508	31.2
100	2.21	0.051	0.139	0.200	0.407	0.514	31.8
Average of factor B2	2.17	0.050	0.134	0.194	0.394	0.506	31.2
Averages of factor A							
0	2.09	0.047	0.128	0.184	0.374	0.489	30.5
25	2.10	0.047	0.129	0.185	0.374	0.489	30.6
50	2.11	0.048	0.132	0.185	0.376	0.490	30.8
75	2.14	0.050	0.133	0.188	0.385	0.495	31.0
100	2.15	0.049	0.139	0.194	0.396	0.500	31.8
$LSD_{05}A$	0.055	0.005	0.010	0.010	0.018	0.014	1.1
$LSD_{05}A$	0.021	0.002	0.004	0.004	0.007	0.053	0.4
$LSD_{05}AB$	0.080	0.007	0.015	0.015	0.026	0.020	1.6

[a] Mobile humic acids extracted with 0.1M NaOH and separated by acidifying solution at pH 1.3–1.5.

[b] Humic acids, bound with calcium, extracted with 0.1M NaOH after removing calcium and separated from fulvic acids by acidifying solution at pH 1.3–1.5.

[c] Humic acids, strongly bound with soil clay minerals, extracted with 0.02M NaOH (hot extraction) and separated by acidifying solution at pH 1.3–1.5.

[d] Total content of humic acids in all fractions.

[e] Total content of fulvic acids in all fractions.

[f] Degree of humification (carbon of humic acid/total organic carbon × 100).

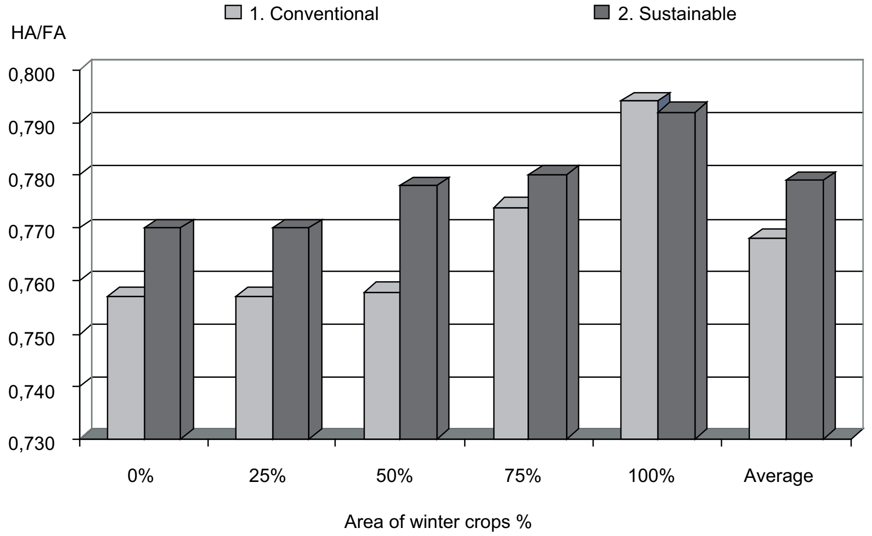

Fig. 1. Effect of area of winter crops in rotations and soil tillage systems on ratio among humic (HA) and fulvic (FA) acids, 1999–2002.

The results showed that increasing the area of winter crops in the crop structure is beneficial factor for improvement of the heavy, humus poor soil. Systematically growing winter crops in the crop rotation, combining with the sustainable soil tillage, it is possible to increase more effective accumulation of organic matter in the heavy soil, to enrich humus quality index and to save sensitive soil from degradation.

The influence of organic and mineral amendments, and sustainable soil tillage for soil humus. Improving used amendments of heavy soils, their incorporation means and sustainable soil tillage, and the influence of these combination practices were set in the upper 0–15 cm and lower 15–25 cm of the soil layers. Farmyard manure had a tendency to increase the amount of humic acids in the 0–15 cm layer used for improving soil from the research. The same tendency was set in the 15–25 cm layer. After ploughing down of farmyard manure with a moldboard plough, and shallow soil tillage with a moldboardless loosener in depth of 15 cm – mostly investigated combination of agricultural practices, stimulating accumulation of humus, humic acids and influencing increase of humification level in the upper layer of the soil (Tables 2, 3).

Concerning segment ploughing, humus had a tendency to decrease, because there was less humic soil raised from the subsoil to the topsoil. In the upper soil layer (0–15 cm) the amount of humus was less if ploughed in amendments with segment plough in depth of 40 cm, than with a moldboard plough in the depth of 25 cm. Ploughing amendments with a moldboard plough, in both layers of soil resulted in about 9% humus more, than if ploughing with a segment plough. By averaged data, the humification degree in the 0–15 cm soil layer was smaller ploughing in amendments with a segment plough, than a moldboard plough, but the difference in the soil depth of 15–25 cm wasn't very important.

Table 2

Effect of soil amendments and sustainable tillage on humus content and degree of humification 2001–2003 averaged data

Treatment	Layer cm	Humus %	Degree of humification %[a]
Amendments (Factor A)			
Without amendments	0–15 15–25	2.25 2.11	30.5 30.6
Farmyard manure	0–15 15–25	2.32 2.24	30.3 29.7
Green manure	0–15 15–25	2.22 2.22	29.4 29.8
Lime-mud	0–15 15–25	2.21 2.14	28.9 29.2
Incorporation methods of amendments (Factor B)			
With a moldboard plough at 25 cm depth	0–15 15–25	2.36 2.26	30.3 30.0
With a segment plough at 40 cm depth	0–15 15–25	2.14 2.10	29.2 29.7
Primary soil tillage (Factor C)			
Ploughed with a moldboard plough at 25 cm depth	0–15 15–25	2.22 2.18	30.0 30.0
Loosened with a moldboardless loosener at 25 cm depth	0–15 15–25	2.23 2.13	29.4 29.4
Loosened with a moldboardless loosener at 15 cm depth	0–15 15–25	2.30 2.22	29.9 30.1
$LSD_{05}A$	0–15 15–25	0.028 0.079	0.94 1.10
$LSD_{05}B$	0–15 15–25	0.016 0.046	0.54 0.64
$LSD_{05}C$	0–15 15–25	0.023 0.065	0.77 0.90

[a] Degree of humification was calculated: (carbon of humic acid / total organic carbon × 100).

Shallow soil tillage in the depth of 15 cm after incorporation of amendments basically increased the amount of humic acids in the depth of 0–15 cm in the soil layer, in comparison with a moldboard plough in the depth of 25 cm. The ratio among humic acids and fulvic acids showed that investigated practices settled the humus type of humous-fulvius. This index was most influenced by the way of ploughing in amendments, but the difference was most noticeable in the layer of 0–15 cm (ploughing down with moldboard plough HA/FA was 0.75, but segment plough – 0.68).

Table 3

Effect of amendments and sustainable tillage on soil humus quality 2001–2003 averaged data

Treatment	Layer cm	$\sum HA^a$	$\sum FA^b$
Amendments (Factor A)			
Without amendments	0–15 15-25	0.398 0.375	0.74 0.75
Farmyard manure	0–15 15–25	0.408 0.389	0.70 0.68
Green manure	0–15 15–25	0.378 0.385	0.72 0.74
Lime-mud	0–15 15–25	0.370 0.363	0.70 0.71
Incorporation methods of amendments (Factor B)			
With a moldboard plough at 25 cm depth	0–15 15–25	0.414 0.393	0.75 0.73
With a segment plough at 40 cm depth	0–15 15–25	0.363 0.362	0.68 0.70
Primary soil tillage (Factor C)			
Ploughed with a moldboard plough at 25 cm depth	0–15 15–25	0.386 0.381	0.74 0.74
Loosened with a moldboardless loosener at 25 cm depth	0–15 15–25	0.380 0.364	0.71 0.69
Loosened with a moldboardless loosener at 15 cm depth	0–15 15–25	0.399 0.389	0.70 0.72
$LSD_{05}A$	0–15 15–25	0.013 0.023	0.039 0.045
$LSD_{05}B$	0–15 15–25	0.007 0.013	0.022 0.026
$LSD_{05}C$	0–15 15–25	0.010 0.019	0.032 0.049

[a] Total content of humic acids in all fractions.
[b] Total content of fulvic acids in all fractions.

With reference to the results of research it is possible to affirm, that reclamation of farmyard manure was the most effective for improving heavy soils according to the amount of humus. On the purpose to improve the subsoil layer, by incorporating amendments into it, the topsoil suffers, because it is mixed with less humic subsoil. Therefore farmlands have to be of mixed specializations of crop production and stockbreeding, in order not to allow their degradation in Lithuanian heavy soils. Accumulative farmyard manure in the farm should be used systemically for humic enriching in the soil. It is purposeful having incorporated organic amendments to use sustainable shallow soil tillage in order to slow down mineralization processes of organic matter and to increase the amount of the humus in the soil. Raising the amount of humic matters in the upper layer of the soil makes it more resistant to degradation caused by technogenic and climatic factors.

Conclusions

1. While increasing the area of winter crops in the crop rotation, the amount of humus, mobile humic acids, common amount of humic acids and humification level had increased in the soil. Applying sustainable system of soil tillage, especially combining it with systematic growing of winter crops in comparison with conventional tillage had increased the amount of humus, mobile and common humic acids in the soil.
2. Application of farmyard manure was the most effective amendment for improving heavy soil according to humus quantitative and qualitative indexes. On the purpose to improve the subsoil layer by incorporating amendments into it, the topsoil suffers due to mixing it with less humic subsoil.

   Applied sustainable system of soil tillage in amendment using for the improvement of soil forced accumulation of organic matter in the soil.

   After ploughing down farmyard manure with moldboard plough in depth of 25 cm shallow soil tillage (15 cm) had improved most of all investigated agricultural practices combinations accumulating of humus, humic acids and increasing of humification level in the upper layer of the soil.

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