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ISBN 951-46-9381-7
ISSN 0355-0982

Helsinki 1996. Valtion painatuskeskus
COMPARATIVE STUDY ON THE ESTIMATION OF HUMIC MATTER IN NATURAL WATERS

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Organic carbon (TOC, DOC, CODMn) and some optical measurements (colour, UV-VIS and fluorescence spectroscopy) were coupled with liquid chromatography in order to estimate aquatic humus in water from natural and artificial lakes. 36 forest lakes and 10 reservoirs without domestic or industrial effluents were studied at winter stratification and autumn overturn. Subfractions with different A254/A420 ratios representing particles, colloids and dissolved material were separated, without prior separation of humic material from non-humic organic material and metals. The interference caused by iron was considerable, especially in samples containing particles and colloids with high iron contents. Generally, the high molecular weight (HMW) and low molecular weight (LMW) fractions could be grouped as follows: CODMn, TOC, DOC, A254 and fluorescence were coupled with LMW fraction and colour (and A420) with HMW fraction. The colour thus indicated the variation of the HMW fraction, or as a matter of fact, the variation of iron present in colloids and particles. The monitoring of humic matter using non-specific measurements might be possible in homogenous materials but several inorganic and organic interferences should be investigated before further application.

Index words: Humus fractions, lakes, reservoirs, monitoring, optical measurements, interferences.

1. INTRODUCTION

The humic matter found in inland waters is mostly allochthonous, synthesized in the peatlands, forests and fields of the catchment area (Jackson 1975). The inputs of allochthonous organic carbon fluctuate seasonally (Wetzel and Otsuki 1973), especially in the regions of long winter. The natural organic matter suspended or dissolved in water bodies includes humic and non-humic material (Schnitzer and Khan 1972), but the accurate separation of these two fractions cannot be accomplished in most laboratories. The proportion of non-humic organic material (carbohydrates, amino acids, fatty acids etc.) is estimated to be rather low even in eutrophic waters (e.g. De Haan and De Boer 1978, Laane 1982).

The physico-chemical characteristics of humus fractions (humin, humic acid, fulvic acid; see Stevenson 1982) originating from terrestrial, aquatic and semi-aquatic systems are remarkably

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similar. New techniques have been developed for structural studies of humic substances, e.g. $^{13}$C-NMR (Thurman and Malcolm 1983). Separation and purification (c.f. Aiken et al. 1979) followed by degradation are obligatory steps before structural studies but in routine water analyses these are usually ignored. Simple methods based on optical properties of humus or on the high content of organic carbon are widely used in monitoring water quality (c.f. Buffle et al. 1982). Optical measurements as well as standard analyses for humic matter are all non-specific for humus and several causes of interference exist even in unpolluted humic waters (Table 1). The new material standards for aquatic humic and fulvic fractions prepared by Thurman and Malcolm (1983) might provide possibilities for comparing different methods and for identifying the actual interferences in different cases.

In this study some estimates of humic matter are compared using un-fractionated and fractionated samples. The lakes and reservoirs under investigation received no effluents from domestic or industrial sources or from intensive agriculture. Some effects of forest draining etc. might be evident and some of the forest lakes were slightly regulated.

2. MATERIAL AND METHODS

2.1 Material

The material for this study was collected from 36 forest lakes and 10 reservoirs without domestic or industrial loads. Eight of the reservoirs were located in western Finland and two in northern Finland (Verta et al. 1986b). The forest lakes were located in central Finland and they were chosen on the basis of colour and pH values of the lakes (Verta et al. 1986a). Water samples were collected at the end of winter stratification (2.3.–27.4.1983) and during the autumn overturn (22.9.–25.10.1983) from a depth of 1 m in polyethylene bottles. One duplicate of each water sample was analyzed in the water laboratories of the National Board of Waters and the other in the laboratory of the department of limnology of the university of Helsinki. The latter laboratory was responsible for the optical measurements and fractionation of the water samples.

2.2 Methods

The concentration of humic material was estimated approximately by standard methods (COD$_{Mn}$, colour and DOC) and selected optical measurements (A254, A420, F350/455). Gel chromatography was used in order to obtain qualitative information about humic matter and the coloured fractions of particulate, colloidal and dissolved matter were separated. Absorbance measurements in the UV (254 nm) and visible (420 nm) ranges were the first indicator of humic material both in the fractions and in the initial un-fractionated samples. Organic carbon analysis would be necessary for more accurate estimation of the concentration of organic matter in the fractions and initial samples, but measurements of organic carbon were available only for the un-fractionated samples in this material. It should be noted that none of the measurements used is strictly specific for humic matter and several interferences occur even in unpolluted humic waters (Table 1).

2.2.1 Organic carbon

Total organic carbon (TOC) and dissolved organic carbon (DOC) of winter samples were analyzed using an infrared gas analyzer developed by Salonen (1979). DOC-concentrations of the autumn samples were analyzed by the method of the National Board of Waters (National Board of Waters 1981).

2.2.2 Optical measurements

Absorbance measurements were performed with a spectrophotometer (Spectronic 200 UV, Shimadzu/Bausch and Lomb, Japan) against distilled water in 1 cm quartz cuvettes at the wavelengths 254 nm and 420 nm (A254, A420), representing ultra-violet and visible regions of the absorption spectra, respectively.

Fluorescence measurements were performed with an Amino-Bowman (USA) spectrofluorometer (model J4-8950, photomultiplier tube 1P21, microphotometer J10-222A). A xenon lamp was the high-energy source for the excitation. Fluorescence measurements were carried out at the emission wavelength of 455 nm when excitation wavelength was set at 350 nm (F350/455) according the observed maximum inten-
Table 1. The characteristics and disturbing factors of different estimates of aquatic humus.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Character on which analysis is based</th>
<th>Disturbing factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical oxygen demand (COD)</td>
<td>Chemically oxidized matter</td>
<td>Other oxidized organic or inorganic substances</td>
</tr>
<tr>
<td>Organic carbon (TOC, DOC)</td>
<td>The elemental composition of humus:</td>
<td>Other organics</td>
</tr>
<tr>
<td></td>
<td>40—50% organic carbon</td>
<td>Other dissolved organic or inorganic substances with the same colour: opacity caused by colloids or suspended particles</td>
</tr>
<tr>
<td>Visible colour</td>
<td>Yellow-brownish colour of natural humus</td>
<td>Other dissolved organic or inorganic substances</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with the same colour: opacity caused by colloids or suspended particles</td>
</tr>
<tr>
<td>UV-VIS spectroscopy</td>
<td>The wide absorption spectrum of humus</td>
<td>Other absorbing substances (organic or inorganic)</td>
</tr>
<tr>
<td>Fluorescence spectroscopy</td>
<td>The fluorescent units of humus (mainly in fulvic acids)</td>
<td>Other fluorescent materials, metal complexes, the pH of the solution</td>
</tr>
</tbody>
</table>

...sities in the (uncorrected) spectra. Both undiluted and diluted (10x, 14.3x) samples were studied routinely using a permanent setting as described above. The results (F350/455) presented in the study can be converted into approximative concentrations of quinine sulphate (mg QS 1⁻¹) with a factor of 0.08. Details of the calibration and dilution effect on fluorescence results will be presented by Pennanen and Mannio (1985).

2.23 Gel chromatography

The procedure for gel fractionation and manipulation of the elution results was a slight modification of the procedure developed by Pennanen (1975, 1982) on the basis of the earlier method first presented by Gjessing (1965).

2.23.1 Gel fractionation procedure

Unconserved, unfiltered water samples were warmed to 20°C and 250 ml of the sample was concentrated under reduced pressure (Rotavapor, Büchi, Switzerland) to 25 ml at 35±1°C. The possible precipitate in the concentrate was not removed by filtering or centrifugation before gel filtration.

A 10 ml portion of the concentrate was chromatographed immediately after concentration through a dextran gel bed column (ø 2.5 cm, height of the gel bed app. 35 cm). Distilled water was used as eluant and the water flow was adjusted to 0.5 ml min⁻¹ with a peristaltic pump (P-3, Pharmacia, Sweden). The effluent was collected automatically and subfractions of the eluate were adjusted to 15±1 ml (Frac-100, Pharmacia, Sweden and Hans Höslé, V 150, Switzerland).

2.23.2 The elution profile and nomenclature of the fractions

The elution profile of humic water consists of two peaks as observed before (Pennanen 1975, 1982). Because the coloured concentration peaks can be distinguished even visually, the absorbance measurements were not performed separately from every test tube but the combination to the fractions f₁, f₀ and f₂ was made as presented in Figure 1. The results of the elutions can be summarized as presented by Pennanen (1982). Thus for example the f₁-fraction in Figure 1 can be calculated as follows:

\[ f₁ = A_i V_i \]  

where

\[ A_i = \text{absorbance of a subfraction} \]
\[ V_i = \text{volume of a subfraction} \]

The observed recovery (R₀) of an elution is the sum of the fractions:

\[ R₀ = f₁ + f₀ + f₂ \]
The theoretical (100%) fractionation yield \( R_T \) of a portion can be calculated:

\[
R_T = \frac{V \cdot A_f}{V \cdot c \cdot v}
\]

where

- \( V = \) calculated volume of the fractionated portion
- \( A_f = \) measured absorbance of the unFractionated sample
- \( v = \) portion volume applied to the top of the column
- \( c = \) concentration coefficient

The fractionation yield \( R_0 \) was usually smaller than the theoretical (100%) fractionation yield \( R_T \) of a sample. Thus some of the coloured material was lost during the elution. This loss was mainly retained on the application cuvette and on the top of the gel bed. By calculating the loss of (coloured) material the fraction \( f_x \) is obtained, describing the particulate matter:

\[
f_x = R_T - R_0
\]  

Especially in autumn samples from the reservoirs, the amount of the colloidal fraction \( (f_1) \) did not increase with increasing amount of organic matter. By contrast, the amount of particulate fraction \( (f_0) \) increased clearly. This could be the true situation, but it could also be partly due to the fractionation procedure, in which some colloidal matter could have been retained on the top of the gel in the case of some reservoir samples. In lake samples the corresponding situation was not observed, probably because of their higher content of coloured Fe-containing colloidal matter, which would be eluated more easily through the gel bed than the coloured particulate matter of reservoirs. But, because it is still possible that the \( f_1 \)-fraction of some reservoirs was small because of the uncomplete fractionation between \( f_x \) and \( f_1 \) these fractions were summarized in this investigation. Further, the fraction between the main fractions in the elution \( (f_0) \) was not included in this study. Thus the discussion is restricted to two main fractions: the high molecular weight (HMW) organic matter, which includes particulate and colloidal matter \( (f_x + f_1) \), and the low molecular weight (LMW) organic matter \( (f_2) \), which includes most of the dissolved matter in a sample. The nomenclature and some properties of the fractions are presented in Table 2.

Table 2. Nomenclature of gel fractionated fractions with reference to average particle size and traditional humic fractions.

<table>
<thead>
<tr>
<th>Symbols of the gel fractionation</th>
<th>( f_x )</th>
<th>( f_1 )</th>
<th>( f_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symbols in the text</td>
<td>high-molecular weight fraction (HMW)</td>
<td>low-molecular weight fraction (LMW)</td>
<td></td>
</tr>
<tr>
<td>Approximate size class</td>
<td>particulate</td>
<td>colloidal</td>
<td>dissolved</td>
</tr>
<tr>
<td>Comparison with the traditional humus fractions</td>
<td>humin</td>
<td>humic acid</td>
<td>fulvic acid</td>
</tr>
</tbody>
</table>
3. RESULTS AND DISCUSSION

3.1 Correlations between estimates of organic matter

Different estimates of organic matter (including humus) based on the estimation of organic carbon (TOC, DOC, COD) or on optical properties (colour, A420, A254, fluorescence), were strictly correlated with each other, as was expected. The correlation coefficients varied in winter samples from 0.779 to 0.962 and in autumn samples from 0.780 to 0.974 (Table 3a and 3b), but as a rule the correlation coefficients were higher in the 1 m winter samples, indicating more homogenous water in the epilimnion at the end of winter stratification. For example, the correlations of COD with optical measurements were clearly higher during winter stratification than during the autumn overturn, probably due to fresh run off waters. In winter, COD correlated best with A254. The latter variable was also the best of the optical measurements for COD. The linear regressions between the variables were:

\[ \text{COD}_{\text{Mn}} = 27.4 \times A254 + 1.01 \quad r = 0.953; \quad n = 46 \quad \text{in winter} \quad (6) \]

\[ \text{COD}_{\text{Mn}} = 21.8 \times A254 + 4.01 \quad r = 0.857; \quad n = 45 \quad \text{in autumn} \quad (7) \]

Colour correlated best with the absorbance of visible light (A420), as was expected. The linear regressions between the variables did not differ significantly in the winter and autumn materials:

\[ \text{colour} = 2030 \times A420 + 5.28 \quad r = 0.923; \quad n = 46 \quad \text{in winter} \quad (8) \]

\[ \text{colour} = 1990 \times A420 + 19.5 \quad r = 0.923; \quad n = 45 \quad \text{in autumn} \quad (9) \]

All the optical measurements correlated well with organic carbon (TOC, DOC) and the highest correlation coefficients (0.94) were observed in the winter material for A254 (Table 3a). COD also showed very high correlations (0.91, 0.93) with organic carbon. Fluorescence correlated as well as A254 and COD with organic carbon.

3.2 Grouping of estimates of HMW and LMW fractions

Although all the correlations between different estimates of organic matter were very high, some differences were observed when considering the correlations between the fractions of HMW and LMW and the estimates of organic carbon ana-
Table 4. Correlation of the fractions with different estimates of humus. Material: 1 m samples (n=45-46) from 35-36 forest lakes and 10 reservoirs. All correlation coefficients were statistically significant (p < 0.001).

<table>
<thead>
<tr>
<th>Winter stratification</th>
<th></th>
<th>Full overturn</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HMW</td>
<td>LMW</td>
<td>HMW</td>
</tr>
<tr>
<td>colour</td>
<td>0.889&lt;sup&gt;1)&lt;/sup&gt; 0.840&lt;sup&gt;2)&lt;/sup&gt;</td>
<td>0.740 0.742</td>
<td>0.878 0.700</td>
</tr>
<tr>
<td>A420</td>
<td>0.922 0.900</td>
<td>0.795 0.808</td>
<td>0.905 0.833</td>
</tr>
<tr>
<td>A254</td>
<td>0.842 0.775</td>
<td>0.906 0.899</td>
<td>0.888 0.903</td>
</tr>
<tr>
<td>fluorescence</td>
<td>0.683 0.607</td>
<td>0.941 0.914</td>
<td>0.773 0.939</td>
</tr>
<tr>
<td>COD&lt;sub&gt;Mn&lt;/sub&gt;</td>
<td>0.765 0.705</td>
<td>0.900 0.892</td>
<td>0.737 0.769</td>
</tr>
<tr>
<td>DOC</td>
<td>0.748 0.682</td>
<td>0.892 0.868</td>
<td>0.698 0.836</td>
</tr>
<tr>
<td>TOC</td>
<td>0.758 0.691</td>
<td>0.885 0.866</td>
<td>0.785 0.759</td>
</tr>
</tbody>
</table>

1) Upper values: Absorbance measurements of fractions performed at 254 nm
2) Lower values: Absorbance measurements of fractions performed at 420 nm

Lyonized in unfraccionated samples. The high molecular weight fraction (HMW) was best correlated with colour (and A420), while the low molecular weight fraction (LMW) was best correlated with COD, TOC, DOC, A254, and fluorescence (Table 4). The same connections were revealed by factor analysis of the winter material (Table 5). The estimates of organic matter were divided into two factors, which explained 95% of the total variance. Fluorescence, COD, TOC, DOC and A254 were linked in the same factor with LMW, while A420 and colour remained in the same factor as HMW (Table 5). As presented and discussed before, the LMW fraction contained the major part of the organic carbon (Pennanen 1982), as well as the major part of the fluorescence of the humic water fractionated by the same techniques as in this study (Pennanen and Sederholm 1974, Pennanen 1982, 1984, Pennanen and Mannio 1985). The high correlation between colour (or A420) and HMW fraction might have been caused mainly by interference due to iron present in this fraction (Pennanen and Frisk 1984 and references in it). This correlation can be seen clearly in the UV/VIS ratios of the fractions (Figure 2) (cf. Pennanen 1975).

To summarize, organic matter can be fractionated into two main fractions of different size class (HMW and LMW fractions). These two classes have optical properties which are mainly explained by the interference of iron, which increases absorbances in the visible range and quenches fluorescence in iron-organic complexes of aquatic humus (cf. Buffle et al. 1982).

Table 5. Sorted rotated factor loadings of different estimates of organic matter. Material: 1 m samples (n=46) from 36 forest lakes and 10 reservoirs during winter stratification 1983.

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMW (A254)</td>
<td>.954</td>
<td>.261</td>
</tr>
<tr>
<td>LMW (A420)</td>
<td>.940</td>
<td>.267</td>
</tr>
<tr>
<td>fluorescence</td>
<td>.878</td>
<td>.399</td>
</tr>
<tr>
<td>DOC</td>
<td>.814</td>
<td>.503</td>
</tr>
<tr>
<td>COD&lt;sub&gt;Mn&lt;/sub&gt;</td>
<td>.813</td>
<td>.531</td>
</tr>
<tr>
<td>TOC</td>
<td>.806</td>
<td>.513</td>
</tr>
<tr>
<td>A254</td>
<td>.782</td>
<td>.613</td>
</tr>
<tr>
<td>HMW (A420)</td>
<td>.246</td>
<td>.958</td>
</tr>
<tr>
<td>HMW (A254)</td>
<td>.348</td>
<td>.926</td>
</tr>
<tr>
<td>A420</td>
<td>.621</td>
<td>.771</td>
</tr>
<tr>
<td>colour</td>
<td>.556</td>
<td>.765</td>
</tr>
<tr>
<td>VP</td>
<td>6.027</td>
<td>4.427</td>
</tr>
</tbody>
</table>
The humic material present in natural waters should be separated and purified from other organics present even in unpolluted waters and from inorganic species (metals especially) in order to obtain accurate estimates for aquatic humus. The usefulness of simple optical methods, however, can clearly be seen. Moreover, the two main fractions of coloured humic matter can quite easily be separated without drastic operations in unpolluted humic inland waters. It seems possible to separate these two main fractions on the basis of organic carbon and fluorescence measurements (Pennanen 1984).

4. CONCLUSIONS

1. Simple optical measurements (A254, fluorescence 350/455) were as good indicators of organic carbon (TOC, DOC) as was CODMn. The colour (or A420) of water was strongly correlated with the HMW fraction with high iron content.

2. Four subfractions with different optical properties and size classes were separated by gel fractionation without isolation from non-humic material and metals. The fractions thus obtained were comparable to the traditional humic and fulvic acid fractions.

3. The low molecular weight (LMW) fraction, representing the major part of (dissolved) organic carbon, was best characterized by fluorescence and A254 and by organic carbon (TOC, DOC, CODMn).

The preparative isolation of humic material was not included in this study. A more exhaustive investigation of inorganic and organic interferences affecting the monitoring of aquatic humus with non-specific methods is needed. Comparison of these results with aquatic humic and fulvic acid standards of the International Humic Substances Society will be carried out in a future work during 1985.

TIIVISTELMA


Preparatiivisesti voitiin erottaa liuennut fraktio kolloideista sekä laskennallisesti partikulaarinen fraktio. Kaikki fraktiot kvantifioitiin optisten määritteiden perusteella, mutta orgaanisen hiilen pitoisuus voitiin tässä selvityksessä määrittää ainostaan fraktioimatomoista näytteistä. Muiden selvitysten perusteella tiedetään, että suurin osa humuksesta on liuennettua ja kiipeä vaaleita, kun taas väriltään voimakkaan kolloidifraktion hiilissä on noin neljännes organisesta, mikäli se on perusteltu organisesta hiilen kokonaismäärästä. Mainittu epäsuhtailtaan halutessa suureltiin osan raudan aiheuttamasta interferenssistä. Kaiken kaikkiaan voitiin vääristetään humusainesta (jota ei kuitenkaan ollut eristetty muusta organisesta aineesta tai metalliehdosta) jaka kahteen selväpiirteiseen komponenttiin: suurimolekyyliseen (HMW) ja pienimolekyyliseen (LMW) fraktion. Mikäli humuk-
vat parametrit indikoivat jossakin määrin spesi­lien pitoisuus (TOC, DOC) valon absorptio ultra­violettialueella (A254) sekä fluoresenssi (F350/ 455) liittyvät parhaiten luonneeseen LMW-frak­tion. Väriluku ja A420, jotka molemmat ilmai­455) liittyvät parhaiten liuoen LMW-frak­tioiden eristämistä, humuksen määrää mittaa­sen käyttökelpoisuutta humuksen pitoisuuden mittana.

Kaikki humusanalyysit ovat epäspesifisiä lu­kuisten määritystä haittaavien interferenssien­vuoksi. Taulukkoon 1 on koottu muutamia näkö­kohtia, joita tulisi ottaa huomioon sovellettawa­eralaisia humuksen mittausmenetelmiä. Jotta hu­muksen määritysmenetelmät antaisivat entistä­tarkemmin estimaatin sekä humuksen määrälle­laadulle, tulisi menetelmää määrätietoisesti kehitteä, sekä selvittää perusteellisesti esim. opt­tisten mitausten soveltuuvuus. Viimeksi mainitut­taroavatkin erään etenemislinjan, jolla voidaan kehitellä jopa jatkuvasti rekisteröiviä mittausmenetelmiä. Toi­saalta humuksen ja muun orgaanisen aineen (sekä­luonnosta huuhtoutavat että teollisuudesta tai­organisidensiides) johdosta peräisin oleva) analyyttinen erotta­minen on välttämätöntä, kun halutaan tehdä laskelma­erilaisia kerrostajuudit. Parhaaseen menet­tävän voitaisiin päästä yhdistämällä vaativampi­määrityksiä yksinkertaisia, helposti toteu­tettavia optisia määrityksiä.


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