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VIDES ZINĀTNES NODAĻA

Linda Eglīte

Promocijas darbs

Humusvielas, to mijiedarbība ar augsni veidojošiem komponentiem un humusvielu imobilizācija

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Ievads

Darba aktualitāte

Viens no ap mums esošās dabas vides izpētes uzdevumiem ir izprast, kas vidē notiek ar dzīvos organismus veidojošajām vielām. Dabiskas izcelsmes organiskās vielas ir ne tikai dzīvos organismus veidojošās vielas, to metabolīti, bet arī dzīvo organismu sadalīšanās procesu noturīgie produkti. Tie līdz ar to uzskatāmi par nozīmīgu dabas vides sastāvdaļu, kas ietekmē dabā noritošos procesus, kā arī piesārņojošo vielu pārvērtības, tām nonākot apkārtējā vidē.

Pēc masas nozīmīgāko daļu no dabiskas izcelsmes organiskajām vielām veido humusvielas. Tās var uzskatīt par visvairāk izplatīto organisko vielu grupu uz Zemes (Stevenson, 1994). Humusvielas (HV) raksturo augsts noturīgums vidē, kas tās atšķir no individuālām identificējama sastāva organiskajām vielām. Humusvielas ir bioloģiski noturīgi, ķīmiski heterogēni augstmolekulāri savienojumi, kuru sastāvā ir ar dažādām skābekli saturošām funkcionālām grupām aizvietotas aromātiskas struktūras, ko savstarpēji saista alifātiskas virknes. Humusvielas veidojas, sadaloties dzīvajiem organismiem, kā arī biosintēzes rezultātā no zemmolekulāriem savienojumiem. Humusvielu vecums vidē var sasniegt pat vairākus desmitus tūkstošus gadu. HV multifunkcionālais sastāvs un sarežģītā uzbūve nosaka to spēju mijiedarboties ar dažādām vides sastāvdaļām.

Augsnes un ūdeņu humusvielu veidošanās gaitā dzīvo organismu organiskās vielas transformējas un mijiedarbojas ar vidē jau esošām vielām. Līdz ar to, lai izprastu augsnes un ūdeņu organisko vielu veidošanos mehānismu, ir būtiski izpētīt HV un vides sastāvā esošo neorganisko vielu, mikroorganismu un dzīvo organismu palieku savstarpējo iedarbību. Šo iedarbību izpēte ir svarīga ne vien lai izprastu augsnes veidošanās procesu raksturu, bet arī lai izvērtētu ūdens vidē suspendēto vielu veidošanos. HV un augsnes komponentu mijiedarbības izpēte ir nozīmīga, lai novērtētu vidi piesārņojošo vielu migrācijas raksturu – gan hidrofobās vidi piesārņojošās organiskās vielas, gan metālu joni migrē kompleksu veidā ar HV.

HV un augsnes ingredientu mijiedarbības izpēte ir nozīmīga arī, lai pētītu un adekvāti aprakstītu piesārņojošo vielu izturēšanos un plūsmas vidē, vispirms – piesārņojošo vielu degradācijas gaitu. Šīs mijiedarbības izpēte ir būtiska arī dzeramā ūdens sagatavošanas tehnoloģiju pilnveidošanai. Sagatavojot dzeramo ūdeni, ir svarīgi, lai humusvielas, kas atrodas ūdenī, tiktu izdalītas, pirms ūdens tiek dezinficēts, jo hlorēšanas gaitā var veidoties hlororganiskie savienojumi, kas negatīvi ietekmē ūdens patērētāju veselību.

HV un augsni veidojošo minerālu daļiņu mijiedarbības rezultātā minerālu daļiņu virsma dabas vidē tiek pārklāta ar humusvielām, respektīvi, humusvielas tiek imobilizētas. Par nozīmīgu problēmu uzskatāma imobilizēto HV un vidi piesārņojošo vielu mijiedarbības rakstura izpēte, jo šī mijiedarbība savukārt var ietekmēt piesārņojošo vielu kustīgumu un pārvērtību raksturu.

Darba mērķis ir izpētīt dažādas izcelsmes humusvielu un Latvijas apstākļiem nozīmīgāko augsni veidojošo minerālo sastāvdaļu mijiedarbības raksturu, kā arī iespējas iegūt ar nesējiem kovalenti saistītas (imobilizētas) HV un pētīt vidi piesārņojošo vielu sorbciju uz tām.

Darba uzdevumi:

1) izdalīt humusvielas no Latvijas apstākļiem tipiskajām vidēm un tās raksturot;

2) pētīt humusvielu sorbcijas raksturu, tās kinētiku, atkarību no jonu spēka uz augsni veidojošām minerālām un organiskām sastāvdaļām;

3) pētīt humusvielu sorbcijas raksturu uz augsnē ietilpstošiem mikroorganismiem;

4) izstrādāt humusvielu imobilizācijas metodes un raksturot imobilizētās HV;

5) izpētīt vidi piesārņojošo vielu sorbciju uz imobilizētām humusvielām.

Darba novitāte:

1) preparatīvos daudzumos no Latvijas dabas vides izdalītas un izpētītas humusvielas;

2) izpētīts humusvielu sorbcijas raksturs uz neorganiskajiem augsnes vidi veidojošajiem minerāliem;

3) izpētīts humusvielu sorbcijas raksturs uz augsnes vidi veidojošiem mikroorganismiem;

4) izstrādātas metodes humusvielu imobilizēšanai;

5) pierādīts, ka imobilizētās humusvielas ir perspektīvas kā sorbenti vidi piesārņojošo vielu sorbcijai.

Darba praktiskā nozīmība:

1) izpētītais humusvielu un augsni veidojošo vielu mijiedarbības modelis ir nozīmīgs videi draudzīgu humusvielas saturošu lauksaimniecībā izmantojamu mēslojumu izstrādei;

2) humusvielu sorbcijas rakstura izpēte uz pazemes ūdeņu mākslīgās papildināšanas infiltrācijas laukus veidojošajām minerālajām fāzēm ir nozīmīga, lai pilnveidotu gruntsūdeņu mākslīgās papildināšanas tehnoloģiju;

3) imobilizētās humusvielas uzskatāmas par perspektīviem sorbentiem.

Pētījumu rezultātu aprobācija

Par pētījumu rezultātiem ziņots starptautiskās konferencēs:

1. Eglite, L., Juhna, T., Klavins, M. (2000) Sorption of humic substances onto solid sorbents during natural and artificial water purification process. In: Abstracts of Symposium on Refractory organic substances in the environment. Karlsruhe, Germany, 198–200.

2. Eglite, L., Odham, G., Rozenbaha, I., Klavins, M. (2001) Reductive degradation of humic substances for elucidation of their core structures. In: Proceedings of 8th Nordic IHSS Symposium, Copenhagen, 44–45.

3. Eglīte, L., Kļaviņš, M. (2001) Humusvielu imobilizācijas metodes. II Pasaules latviešu zinātnieku kongress, Rīga, 351.

4. Kļaviņš, M., Eglīte, L., Juhna, T. (2001) Sorption of humic substances onto solid phases during natural and artificial groundwater recharge process. In: Abstracts of ISEB 15 Biogeochemical processes and cycling of elements in the environment, Wroclaw, 33.

5. Eglite, L., Klavins, M. (2002) Sorption of humic substances on soil inorganic and organic components. In: Use of humates to remediate polluted environments: from theory to practice, Zvenigorod, Russia, 15–16.

6. Eglite, L., Peuravuori, J., Lehtonen, T. (2005) Characterization of humic substances of different origins of Latvia using pyrolysis-gas chromatography/mass spectrometry. In: Abstracts of 10th Nordic IHSS Symposium on Character of natural organic matter and its role in the environment, Riga, Latvia, 22.

Disertācijas nozīmīgākie rezultāti publicēti:

1. Kļaviņš, M., Seržāne, J., Eglīte, L. (1999) Methods for analysis of aquatic humic substances. *Crit. Rev. Analyt, Chem.*, 29, 187–203.

2. Kļaviņš, M., Juhna, T., Eglīte, L. (2000) Removal of humic substances during treatment of drinking water using sorbents. *Vatten,* 56, 79–83.

3. Klavins, M., Eglite, L. (2002) Immobilisation of humic substances. *Colloids and Surfaces A: Physicochem. Eng. Aspects,* 203, 47–54 .

4. Eglite, L., Klavins, M. (2002) Sorption of humic substances on aquifer material and soil components. In: Proceedings of 20th Anniversary Conference of IHSS "Humic substances: Nature's most versatile materials", Boston, USA, 146–149.

5. Rozenbaha, I., Odham, G., Jarnberg, U., Eglite, L., Klavins, M. (2002) Characterization of humic substances using reduction and acid catalysed transesterification. In: Proceedings of 20th Anniversary Conference of IHSS "Humic substances: Nature's most versatile materials", Boston, USA, 74–77.

6. Juhna, T., Klavins, M., Eglite, L. (2003) Sorption of humic substances on aquifer material at artificial recharge of groundwater. *Chemosphere,* 51(9), 861–868.

7. Kļaviņš, M., Šīre, J., Eglīte, L. (2004) Humic substances and the potential of their use in agriculture. *Proc. Latv. Acad. Sci.,* Ser B, 58(2), 39–49.

8. Eglīte, L., Rozenbaha, I., Odham, G., Järnberg, U., Kļaviņš, M. (2003) Reductive degradation of humic substances. *Latv. Ķīm. Ž.,* 2, 313–320.

9. Peuravuori, J., Monteiro, A., Eglite, L., Pihlaja, K. (2005) Comparative study for separation of aquatic humic-type organic constituents by DAX-8, PVP and DEAE sorbing solids and tangential ultrafiltration: elemental composition, size-exclusion chromatography, UV-vis and FT-IR. *Talanta,* 65, 408–422.

10. Klavins, M., Eglite, L., Zicmanis, A. (2006) Immobilized humic substances as sorbents. *Chemosphere,* 62, 861–868.

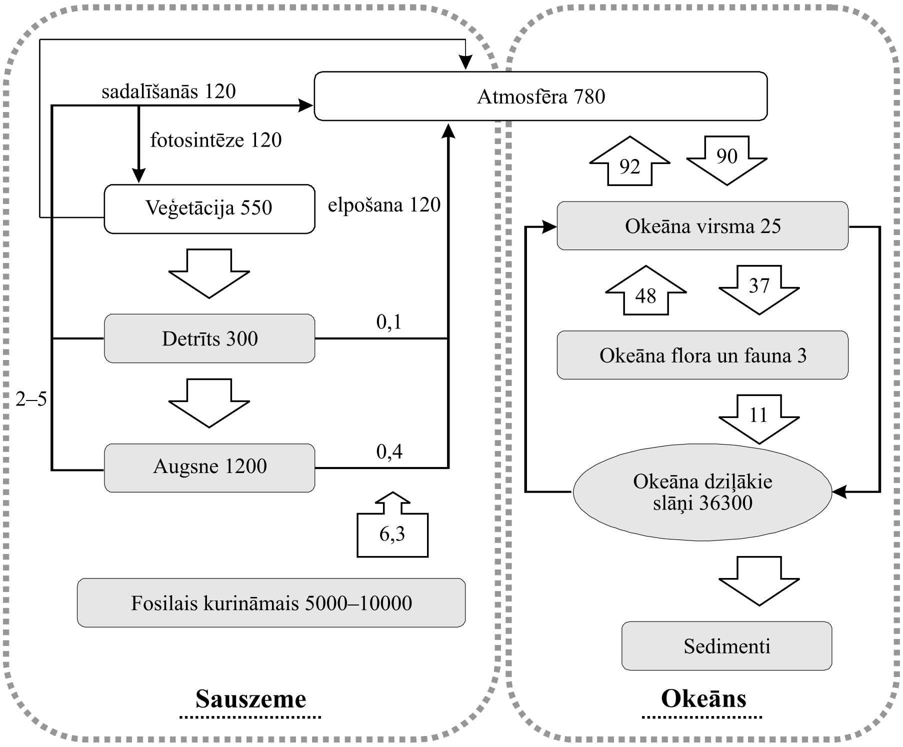
Darbā izmantotie saīsinājumi

HV – humusvielas
FS – fulvoskābes
HS – humīnskābes
DEAE – dietilaminoetil-
GH/MS – gāzu hromatogrāfija/masspektrometrija
ŠH/MS – šķidruma hromatogrāfija/masspektrometrija
UV/Vis – ultravioletā/redzamā gaisma
KMR – kodolmagnētiskā rezonanse
IS – infrasarkanā gaisma
EPR – elektronu paramagnētiskā rezonanse

1. Literatūras apskats

1.1. Humusvielu veidošanās avoti un to loma vidē noritošajos procesos

Humusvielas (HV) ir dabas vidē bioloģiski noturīgi augstmolekulāri heterogēnas uzbūves daudzfunkcionāli polikatjonīti, kas veido augsnes, ūdeņu un daudzu fosilo oglekli saturošo minerālu (kūdras, sapropeļa, brūnogļu) galveno masu, uz Zemes (1. attēls) tā sastāda 2–3×10^{10} t (Jones and Bryan, 1998). Lielākie humusvielu daudzumi atrodas izšķīdušā veidā Pasaules okeānā, kā arī ietilpst augsnes organiskās vielas sastāvā (Ziechman, 1994). Līdz ar to humusvielas ir nozīmīgs oglekļa bioģeoķīmiskās aprites cikla elements.



1. attēls. **Oglekļa bioģeoķīmiskās aprites cikls (tilpes un plūsmas Gt vai Gt/gadā) (Hedges and Oades, 1997)**
Figure 1. **Carbon biogeochemical cycle (reservoirs and flows Gt or Gt/year) (Hedges and Oades, 1997)**

Humusvielas atkarībā no to šķīdības tradicionāli iedala trīs grupās: a) ***humīnskābes*** (HS) – humusvielu daļa, kas šķīst ūdenī, ja vides pH>2; b) ***fulvoskābes*** (FS) – humusvielu daļa, kas šķīst ūdenī neatkarīgi no pH; c) ***humīns*** – ūdenī nešķīstošā humusvielu daļa (Thurman, 1985). Humusvielas veidojas, sadaloties dzīvajai organiskajai vielai, kā arī tās degradācijas produktiem un dzīvo organismu metabolītiem reaģējot savā starpā un mijiedarbojoties ar vidē un dzīvajos organismos esošām neorganiskajām vielām. Dzīvās organiskās vielas degradācija mikroorganismu un ekstracelulāro un intracelulāro fermentu darbības rezultātā noved pie nestabilāko savienojumu sabrukšanas. Tā rezultātā HV veido struktūras, kas vidē ir bioloģiski noturīgas. Paralēli norit arī humusvielu struktūrelementu sintēze no zemmolekulāriem savienojumiem, kuru avots var būt gan dzīvo organismu metabolisms, gan arī biomolekulu sabrukšana (Aiken et al., 1985).

Nozīmīgākās vielu grupas, kas būtiskas HV izveidei, ir lignīns un tā transformācijas produkti, poliaromātiskas struktūras saturoši savienojumi, polisaharīdi, nepiesātinātās taukskābes, polifenoli (Bollag, 1983). Humusvielu veidošanās notiek gan hidrosfērā, gan litosfērā (Steinberg, 2003). Lai gan pastāv dzīvos organismus veidojošo vielu daudzveidība (1. tabula), humifikācijas procesu rezultātā veidojas vielu grupa ar izteikti līdzīgām īpašībām – humusvielas (Orlov et al., 2005).

1. tabula / Table 1

Dzīvos organismus veidojošo organisko vielu sastāvs (%) (Orlov et al., 2005)
Composition of organic substances in living organisms (%) (Orlov et al., 2005)

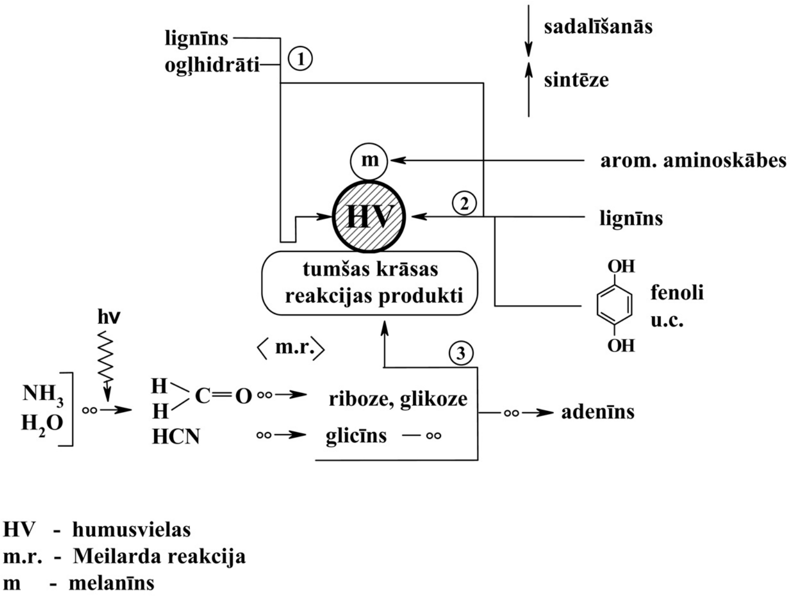
Organisms	Neorganiskas vielas	Olbaltumvielas	Hemiceluloze, pektīns	Celuloze	Lignīns	Lipīdi, tanīni
Baktērijas	2–10	40–70	Nenozīmīgs	0	0	1–40
Aļģes	20–30	10–15	50–60	5–10	0	1–3
Ķērpji	2–6	3–5	60–80	5–10	8–10	1–3
Sūnas	3–10	5–10	30–60	15–25	0	5–10
Papardes	6–7	4–5	20–30	20–30	20–30	2–10
Skujkoki:						
stumbrs	0,1–1	0,5–1	20–30	40–50	20–25	5–15
skujas	2–5	4–10	10–20	15–25	20–30	5–15
Lapu koki:						
koksne	0,1–1	0,5–1	20–30	40–50	20–25	5–15
lapas	3–8	4–10	10–20	15–25	20–30	5–15
Graudaugi	5–10	5–12	25–35	25–40	15–20	2–10
Pākšaugi	5–10	10–20	15–25	25–30	15–20	2–10

Humifikācija sākas pēc biosintēzes un metabolisma procesu izbeigšanās organismā (pēc organisma nāves), vispirms ekstracelulārajiem fermentiem citoplazmā sagraujot viegli degradējamās vielas, bet pakāpeniski degradācijā iesaistās arī uz šūnas membrānām saistītie fermenti. Pēc šūnas membrānas sadalīšanas humifikācijā iesaistās mikroorganismi, iedarbojoties uz augu un dzīvo organismu atliekām. Organiskajās atliekās esošie savienojumi, kas sintezēti augu un dzīvo organismu dzīves laikā, ir barības vielas un enerģijas avots dažādām baktērijām un sēnēm, kas ir iesaistītas destrukcijas procesā. Tādējādi mikroorganismi, nodrošinot savus dzīvības procesus, piedalās oglekļa apritē un noturīgu organisko savienojumu – humusvielu sintēzē (MacCarthy, 2001).

Humifikācijas procesā mikroorganismi noārda un metabolismam patērē lielāko daļu no organisko atlieku sastāvā esošajiem ogļhidrātiem, lipīdiem, nukleīnskābēm un olbaltumvielām. Šī mikroorganismu veiktā barības vielu asimilācija ir uzskatāma par pirmo soli humusvielu veidošanās procesā. Dažas no minētajām vielām, piemēram, cukuri un ciete, ir vieglāk sadalāmas, turpretī celuloze un hemiceluloze mikroorganismu darbības ietekmē noārdās grūtāk, bet tauki, vaski un lignīns ir visnoturīgākie pret bioloģisko degradāciju (Ziechmann, 1994).

Fulvoskābes ir mazāk aromātiskas (Thomsen et al., 2002) un ar augstāku karboksilgrupu koncentrāciju. Tiek uzskatīts, ka humusvielas var savstarpēji pārvērsties humifikācijas gaitā (Kögel-Knabner, 2002). Dzīvo organismu atliekām sadaloties veidojas mazākas molekulas (fulvoskābes), bet tālākā humifikācijas procesa gaitā tās var savstarpēji reaģēt un veidot lielākas molekulas (humīnskābes). Savukārt humīnskābes, tām sadaloties, var atkal veidot fulvoskābes, kurām ir maza molekulmasa, bet augsta aromātisko grupu koncentrācija (Kögel-Knabner, 2002).

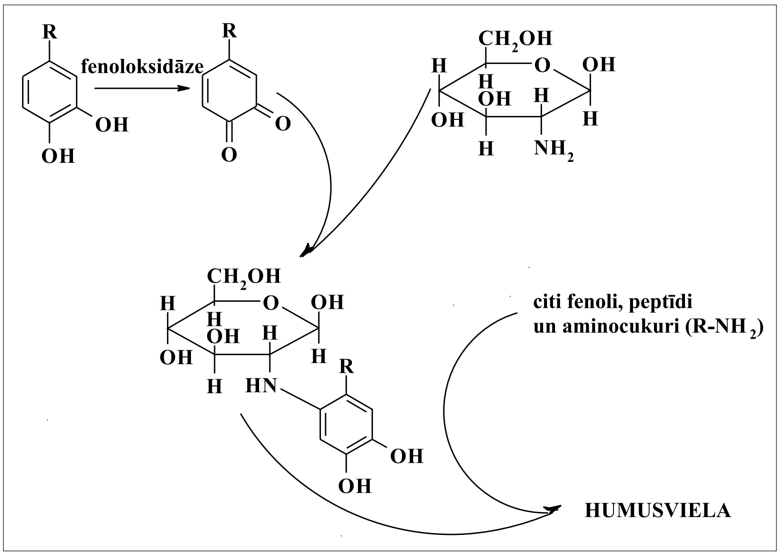
Humifikācijas gaitu ietekmē organismu, kas sadalās, sastāvs; dominējošo mikroorganismu grupu, kas atbildīgas par organisko vielu sagraušanu, vides reakcija un reducēšanas–oksidēšanas apstākļi; skābekļa klātbūtne un arī tādu vielu klātbūtne, kas var paātrināt vai kavēt humifikācijas gaitu un kas var arī ietekmēt to vielu spektru, kuras tiek sagrautas vai izmantotas humusvielu struktūras izveidei. Piemēram, ja vide ir anoksiska un tās reakcija ir skāba, organisko vielu degradācija norit ievērojami lēnāk nekā bāziskas vai neitrālas vides apstākļos. Līdz ar to šādā vidē veidotajās humusvielās ir ievērojami augstāks ogļhidrātu palieku daudzums. Vidē jau izveidojušās humusvielas var kavēt organismu atlieku degradāciju un humifikācijas procesu (Piccolo et al., 1992; Ziechmann, 1994). Bioloģisko procesu intensitāte vidē var ietekmēt humusvielu vecumu. Ja humusvielu vecumu, tās izdalot no fosilajiem nogulumiem (lignīta, leonardīta, kūdras utt.), var vērtēt pat ar kārtu miljoni gadu, tad humusvielas ūdeņos var būt dažus simtus gadu vecas, bet produktīvos ūdeņos vai atkritumos – gadu vai pat mēnešu vecas (Steinberg, 2003).



2. attēls. **Principiālā humifikācijas shēma (Ziechman, 1994)**
Figure 2. **Humification principle of organic matter (Ziechman, 1994)**

Vērā ņemami humusvielu veidošanos ietekmējoši faktori ir arī temperatūra un ūdens daudzums vidē, kā arī oglekļa/skābekļa attiecība organiskajās atliekās, kas tiek sadalītas. Paaugstinoties vides temperatūrai, palielinās arī mikroorganismu aktivitāte, kas darbojas humusvielu sadalīšanas procesos, tādēļ, analizējot humusvielu veidošanās apstākļus un izveidoto humusvielu struktūru, ir būtiski ņemt vērā visus humifikācijas reakcijas ietekmējošos faktorus (Nardi et al., 1996; Kļaviņš, 1998; Hayes and Clapp, 2001).

HV raksturīga gan īpašību dažādība, gan arī neapšaubāma līdzība. To nosaka atšķirīgās vidēs notiekošu humifikācijas procesu rakstura līdzība – humifikācijas apstākļi vidē, fizikāli ģeogrāfiskie apstākļi un bioloģisko procesu intensitāte. Tas pierāda, ka no atšķirīgām izejvielām (dzīvajām organiskajām vielām) var rasties humusvielas – savienojumi ar izteiktu īpašību un uzbūves līdzību –, un to nosaka humifikācijas procesa gaita.



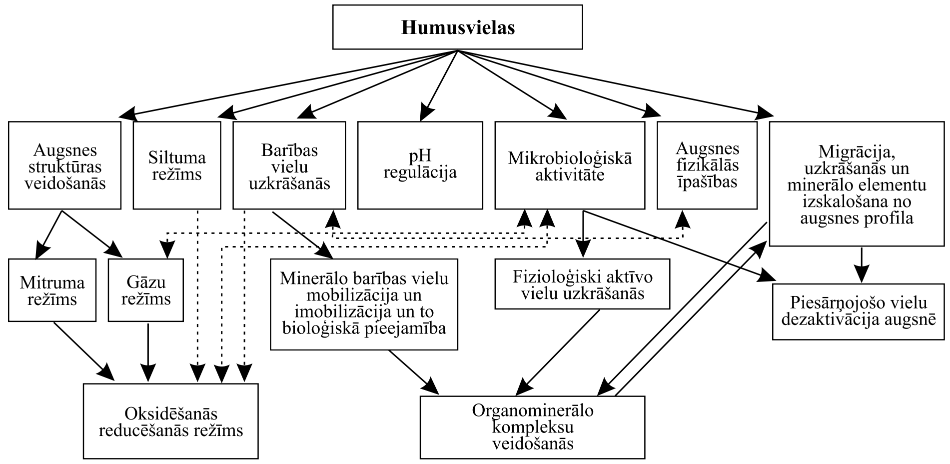
3. attēls. **Iespējamā ogļhidrātu un lignīna atlikumu iesaistīšanās shēma humusvielu sastāvā (pēc Hessen and Tranvik, 1998)**

Figure 3. **Incorporation of remains of carbohydrate and lignin in structure of humic substances (after Hessen and Tranvik, 1998)**

Augsnēs nozīmīga ir humusvielu veidošanās no lignīna (2., 3. attēls), mikroorganismu degradācijas ceļā nepilnīgi sadalītā lignīna atlikumiem iekļaujoties augsnes humusa sastāvā. Izmaiņas lignīna molekulā ietver metoksigrupu (-OCH$_3$) atšķelšanu, veidojot fenolus un hinonus, un alifātisko sānu ķēžu oksidēšanu līdz karboksilgrupām (Stevenson, 1982). Tālāk tās, mijiedarbojoties ar ogļhidrātiem un aminosavienojumiem, veido humīnskābes, un pēc tam, notiekot oksidācijai un fragmentācijai, rodas fulvoskābes (Qualls, 2003).

Humusvielas var veidoties vidē, kurā tās atrodas (autohtonā izcelsme), vai arī var tikt ienestas no saistītām vidēm (alohtonā izcelsme), piemēram, augsnes humusvielām ieskalojoties virszemes vai pazemes ūdeņos (Aiken et al., 1985).

Humusvielu uzbūve ļauj tām mijiedarboties ar dažādu vielu grupām, piemēram, ar neorganiskajiem joniem, veidojot sāļus un kompleksus savienojumus, kā arī ar hidrofobām organiskajām vielām (Wershaw et al., 1969; Carter and Suffet, 1982; Senesi et al., 1986). Humusvielu spēja aktīvi mijiedarboties ar dažādām vidē esošām vielām nosaka to lielo nozīmi dabā noritošajos procesos (4. attēls), turklāt vienlaikus, ņemot vērā to lielo masu dažādās vidēs (augsnē, kūdrā), tās spēj būtiski ietekmēt arī pašas vides īpašības, ieskaitot bioloģisko procesu raksturu tajā (Chen and Aviad, 1990).



4. attēls. **Humusvielu nozīme augsnē (pēc Orlov et al., 2002)**
Figure 4. **The role of humic substances in soil (after Orlov et al., 2002)**

Humusvielu nozīmīgākās īpašības vidē noritošajos procesos ir:

1. Noturība pret ķīmisko un bioloģisko degradāciju. HV ir noturīgas pret mikrobiālo degradāciju, tādēļ HV vecums var sasniegt pat 15 000 un vairāk gadus (Stevenson, 1982; Malcolm, 1985), taču tajā pat laikā humusvielas var tikt izmantotas kā oglekļa avots anaerobā vidē (Tranvik and Hofle, 1987; Lindell et al., 1995). Humusvielas ir arī stabilas attiecībā pret vidē sastopamo skābju un bāzu iedarbību un oksidētāju iedarbību. Intensīva humusvielu degradācija notiek fotoķīmiski, īpaši katalizatoru klātbūtnē (Brinkman et al., 2003).

2. Spēja saistīties ar augsnes un ūdens minerālajiem komponentiem un organiskajiem savienojumiem (Stevenson, 1982; Lassen 1994). Tas ietekmē piesārņojošo vielu migrācijas raksturu (Kļaviņš and Apsīte, 1998).

3. Spēja saistīties ar metālu joniem. Humusvielās ir augsts tādu funkcionālo grupu saturs, kuras nosaka humusvielu spējas veidot savienojumus ar metālu joniem. Tādēļ ar HV bagātos ūdeņos metāli galvenokārt atrodas nevis brīvu jonu, bet gan humīnskābju un fulvoskābju sāļu un kompleksu savienojumu veidā (Buffle, 1988; Baker and Khalili, 2003). Līdz ar to HV ietekmē metālu (īpaši smago metālu) transportu un likteni vidē (Town and Filella, 2000; Nierop et al., 2002).

HV spējai veidot stabilus kompleksus ar augsnes vai ūdens neorganiskajiem un organiskajiem komponentiem ir būtiska loma vides piesārņojuma līmeņa veidošanā un piesārņojošo vielu migrācijā, kā arī to transformācijas procesā.

Nozīmīgākās humusvielu funkcijas vidē ir šādas (Орлов, 1990):

1. Akumulatīvā funkcija. Nozīmīgākie biogēnie elementi (slāpekļa, fosfora savienojumi) kā arī K, Ca, Mn, Fe un gandrīz visi mikroelementi vidē atrodas saistīti ar humusvielām.

2. Transporta funkcija. Humusvielas nosaka minerālo un organisko vielu ģeoķīmisko plūsmu veidošanos, īpaši ūdens vidē, veidojot noturīgus, bet salīdzinoši viegli šķīstošus HV savienojumus ar metālu joniem, hidroksīdiem, dažādām organiskajām vielām.

3. Regulējošā funkcija. Humusvielas tieši ietekmē daudzus vidē noritošus procesus:

- augsnes, kūdras struktūras un fizisko īpašību veidošanos;
- reakciju līdzsvara uzturēšanu jonu apmaiņas reakcijās, skābju–bāzu apmaiņu;
- dzīvo organismu minerālo komponentu barošanos (regulē minerālo komponentu šķīšanu ūdenī un pieejamību dzīvajiem organismiem);
- siltuma režīmu vidē un fotosintēzes intensitāti (iespaido spektrālās atstarošanās spējas, augsnes masas siltumietilpību un siltumvadāmību).

4. Protektorā funkcija. Humusvielas var funkcionēt kā barjera elementu ģeoķīmiskajās plūsmās:

- aizsargā augsni no sausuma un pārmērīga mitruma;
- pasargā augsni no erozijas un deflācijas;
- saglabā augsnes fizikālās īpašības antropogēno slodžu gadījumos;
- mazina toksisko vielu negatīvo iedarbību un aiztur to migrāciju vidē.

5. Fizioloģiskā funkcija. Izpaužas galvenokārt kā humusvielu tiešā fizioloģiskā iedarbība uz augiem un citiem dzīvajiem organismiem.

Tātad humusvielas kā viens no nozīmīgiem vides ingredientiem būtiski ietekmē tajā noritošos procesus.

1.2. Humusvielu izdalīšanas un attīrīšanas metodes

Humusvielas dabas vidē atrodas saistītas gan ar detrītu un citām organiskām vielām (zemmolekulārām un arī augstmolekulārām), gan ar neorganiskām vielām un minerāliem. Līdz ar to viena no nozīmīgākajām problēmām un būtisks uzdevums humusvielu izpētē joprojām ir augsti attīrītu humusvielu paraugu izdalīšana, lai šo paraugu iegūšanas laikā būtu līdz minimumam novērsta humusvielu destrukcija, bet iegūtais paraugs būtu ar izejvielā esošajām vielām identisku uzbūvi. Lai nodrošinātu izdalīto humusvielu preparātu salīdzināmību, humusvielu paraugu iegūšanai no dažādām vidēm tiek ieteikts lietot standartmetodes (Stevenson, 1982), kuru izmantošana nodrošina minimālās pieļaujamās dabiskā materiāla īpašību izmaiņas, kā arī iegūto humusvielu attīrīšanu no neorganiskajiem piemaisījumiem un minimālu humusvielu makromolekulu degradāciju.

Humusvielu izdalīšanas metodes visai ievērojami atšķiras atkarībā no tā, no kādas vides HV tiek izdalītas. Humusvielu izdalīšanai no cietas fāzes vides materiāliem (augsnes, kūdras, nogulumiem, lignīta) vispirms nepieciešams atdalīt kalcija un citu divvērtīgo jonu pamatmasu, kas ar humusvielām var veidot nešķīstošus sāļus. Šim mērķim tradicionāli izmanto paraugu apstrādi ar skābēm vai kompleksus veidojošiem reaģentiem (Aiken et al., 1985). Humusvielu ekstrakcijai no cietas fāzes paraugiem izmantojami sārmu metālu hidroksīdi, karbonāti, hidrogēnkarbonāti, pirofosfāti, kā arī citi savienojumi (Aiken et al., 1985). Visplašāk kūdras HV ekstrahēšanai izmanto nātrija hidroksīda šķīdumus (Kononova, 1966; Schnitzer, 1978), jo tie salīdzinājumā ar citiem ekstrahentiem ļauj izdalīt 30–60% augsnes vai kūdras humusvielu. Tomēr daudzi pētnieki nātrija hidroksīda šķīduma izmantošanu neatzīst, jo sārma klātbūtnē hidrolīzes un oksidēšanās rezultātā izmainās iegūto humusvielu īpašības un struktūra (Stevenson, 1982).

Nātrija hidroksīda un nātrija pirofosfāta izmantošana tiek rekomendēta arī Starptautiskās Humusvielu pētnieku savienības (IHSS) ieteiktajā standartizētajā humusvielu izdalīšanas procedūrā (Schnitzer, 1978; Thurman and Malcolm, 1981). Tiek rekomendēts HV ekstrahēšanu ar nātrija hidroksīda šķīdumu veikt N_2 atmosfērā, tas samazina izdalīšanas procesā radušās izmaiņas to struktūrā (Kononova, 1966).

Ievērojamas priekšrocības piemīt minerālskābju sāļu, īpaši nātrija pirofosfāta ($Na_2P_4O_7$), izmantošanai. Šo šķīdumu lietošanas efektivitāti nosaka humusvielu sāļu labā šķīdība, lai gan pastāv uzskats, ka HV cieši saistītas ar augsnes, nogulumu un kūdras minerālo daļu un, lietojot $Na_2P_4O_7$ vai citus šķīdumus, ekstrahēšanas efektivitāte ir zemāka, nekā HV ekstrahējot ar sārmiem. Vienlaikus atzīmējams tas, ka daudzos pētījumos pierādīts, ka humusvielu izdalīšanas gaitā atkarībā no ekstrakcijas apstākļiem notiek ievērojamas izmaiņas to struktūrā, bet tas var novest pie artefaktiem. Piemēram, apstrāde sārmainā vidē skābekļa klātbūtnē var izsaukt fenolu grupu saturošo HV struktūrelementu oksidēšanos, bāziskā vide var izsaukt labilāko sturktūrelementu hidrolīzi utt. Līdzīgi ekstrakcija ar etanolu skābā vidē noved pie humusvielu esteru veidošanās, tie ilgu laiku tika uzskatīti par humusvielu frakciju – himatomelānskābēm. Ir piedāvāti arī daudzi citi neorganiskie ekstrahenti, kā arī organiski šķīdinātāji, taču to izmantošana nodrošina vai nu ievērojami zemākus humusvielu iznākumus, vai arī rada citas iespējamās nevēlamās ietekmes uz humusvielu struktūru.

Ir vairāki faktori, kas ietekmē humusvielu izdalīšanas efektivitāti: reaģenta veids un koncentrācija, ekstrahenta un cietās fāzes attiecība, temperatūra un pH, pie kuras notiek izdalīšana, procedūras ilgums, skābekļa klātbūtne un citi. Pēc humusvielu izdalīšanas no cietās fāzes sastāva tās nepieciešams papildus attīrīt, lai pazeminātu neorganisko jonu un individuālo organisko vielu saturu tajās. Parasti humusvielu attīrīšanai izmanto sorbcijas metodi (uz jonu apmaiņas polimēriem vai hidrofobiem sorbentiem), atkārtotu izgulsnēšanu vai ultrafiltrāciju, reverso osmozi. Humīnskābes izdala, vidi paskābinot līdz pH 2, bet fulvoskābes iesaka izdalīt, izmantojot to sorbciju uz hidrofobiem sorbentiem vai vājiem anjonītiem un turpmāku liofilizāciju (Thurman and Malcolm, 1981).

Humusvielu nešķīstošo frakciju jeb humīnu galvenokārt veido organiskās vielas, kuras ir cieši saistītas ar augsnes (nogulumu) minerālo daļu un kuras raksturo liels daudzums hidrofobu struktūru. Humīna izdalīšanai rekomendē augsni, kūdru vai nogulumus apstrādāt ar HCl, HF un HCl–HF šķīdumiem. Tomēr šāda veida apstrāde izmaina organisko vielu ķīmisko struktūru, tādēļ tiek piedāvāta metilizobutilketona metode (Rice and MacCarthy, 1989). Saskaņā ar šo metodi pēc ekstrakcijas ar metilizobutilketonu HV tiek izolētas suspensijas veidā. Tā tiek samazināta ekstrakcijas procesa ietekme uz izdalītā materiāla īpašībām.

Humīnskābes iegūst, sārma ekstraktu paskābinot ar HCl šķīdumu līdz pH 1,5. Humīnskābe izkrīt kā tumši brūnas vai melnas krāsas nogulsnes, bet ekstraktā paliek fulvoskābes (FS), kas parasti ir dzeltenā krāsā.

Humusvielas no ūdens vides parasti koncentrē, izmantojot sorbcijas metodi, vai izdala lietojot membrāntehnoloģijas. Humusvielu izdalīšanai var izmantot to sorbciju uz anjonītiem (DEAE celuloze, DEAE Sephadex) vai arī stipri skābā vidē uz hidrofobiem sorbentiem (XAD 2, XAD 8). Sākotnēji izmantotie stipri bāziskie anjonīti nodrošināja visai zemus humusvielu iznākumus, mūsdienās iesaka izmantot vāji bāziskus sorbentus uz hidrofilu matricu pamatnes (dekstrāns, celuloze, polialkilmetakrilāta geli) ar makroporainu uzbūvi un zemu funkcionālo grupu saturu. Humusvielu izolēšanai no ūdens iesaka lietot polimērus: Dowex A-7, Lewatit MP-500, dažādus Sephadex jonītus (DEAE Sephadex) un DEAE celulozi. XAD un DEAE sorbenti tiek izmantoti HV izolēšanā, apstrādājot lielus ūdens daudzumus, pat ja humusvielu koncentrācijas ir niecīgas (Aiken et al., 1985; Kļaviņš, 1998). Jāatzīmē, ka, izmantojot DEAE sorbentus, mazāk tiek pārveidots ūdeņu HV dabiskais sastāvs (Hejzlar et al., 1994).

Hidrofobo sorbentu izmantošana ir ieguvusi ievērojamu popularitāti, kaut gan izdalīšanas process notiek stipri skābā vidē (pH <2), kas ietekmē humusvielu paraugu degradāciju, un faktiski šajā izdalīšanas procesā var notikt humusvielu paraugu frakcionēšana. Visos gadījumos pēc humusvielu izdalīšanas no ūdens vides tiek veikta to sadalīšana humīnskābēs un fulvoskābēs un tālāka attīrīšana, parasti izmantojot kolonnu hromatogrāfiju.

Humusvielu izdalīšanas un attīrīšanas procesa sarežģītība ietekmē arī humusvielu preparātu ražošanu. Maz attīrītas humusvielas jau tiek ražotas lieltonnāžas apjomos, un ar to ražošanu un izplatīšanu nodarbojas vairāki simti uzņēmumu, bet augsti attīrītu references paraugu cenas salīdzināmas ar augsti attīrītu fermentu cenām.

Humusvielas no kūdras pirmo reizi tika izdalītas 1786. gadā (Achard, 1786), no ūdens tās izdalīja Berceliuss (Berzelius, 1839). Pašlaik izmantotās humusvielu metodes ir standartizētas un to lietošana ļauj iegūt salīdzināmus preparātus, tomēr joprojām turpinās tādu metožu izstrāde, kas ļautu iegūt humusvielas, kurām izdalīšanas laikā būtu iespējami maz ietekmētas īpašības (McDonald et al., 2004; Moredo-Pineiro et al., 2004; Sierra et al., 2005).

1.3. Humusvielu īpašības

Ņemot vērā humusvielu heterogēno uzbūvi, to raksturošanai nepieciešams izmantot veselu rindu metožu, ieskaitot visai tradicionālas, jo bieži vien tikai kompleksa īpašību izpēte (2. tabula) ļauj tās raksturot (Abbt-Braun et al., 2004).

Oglekļa saturs humusvielās svārstās robežās no 40% līdz 60%, skābekļa saturs 30–35%, slāpekļa saturs 0,8–3%, ūdeņraža saturs 4–6%. Vienlaikus pastāv būtiskas atšķirības starp dažādas izcelsmes humīnskābju, fulvoskābju un humīna elementsastāvu (3. tabula).

Humusvielu elementsastāvs analizējams, izmantojot ģeoķīmijā plaši lietotos van Krevelena grafikus, kuros tiek attēlotas elementsastāva attiecības (C/H, O/C, N/C, M, funkcionālo grupu koncentrācijas), kas atsedz kopsakarības starp aromātisko struktūru veidošanos un skābekļa un slāpekļa savienojumu daudzuma izmaiņām. Van Kravelena grafiki ir izmantojami, lai pētītu humusvielu ģenēzes jautājumus (Killops and Killops, 1994; Barancikova et al., 1997). Ūdeņu humusvielas satur ievērojami vairāk oglekļa, bet mazāk skābekļa, salīdzinot ar augsnes HV. Fulvoskābes ir ar mazāku molekulmasu un augstāku O/C attiecību nekā humīnskābes, turklāt C/N attiecība ūdens fulvoskābēm (45–55:1) ir ievērojami augstāka augšņu fulvoskābēm (20:1), tas norāda, ka ūdeņu humusvielās ir daudz mazāk slāpekļa nekā augsnes HV sastāvā (Ishiwatari, 1971).

2. tabula / Table 2

Nozīmīgākās humusvielu raksturošanai izmantotās metodes
Methods used for characterization of humic substances

Metodes		Informācija	Literatūra
Fizikāli ķīmiskās	elementanalīze	elementu sastāvs (C, H, N, O, S)	Huffman and Stuber, 1985; Abbt-Braun and Frimmel, 2002
	derivatizācija (metilēšāna, sililēšana)	funkcionālās grupas (-OR, -OOR)	Schnitzer, 1978; Stevenson, 1982
	skābes/bāzes titrēšana	protonu kapacitāte	Barak and Chen, 1992; De Wit et al., 1993; Perdue, 1998; Abbt-Braun and Frimmel, 2002
	termālā degradācija (pirolīze) kombinācijā ar GH/MS vai ŠH/MS	degradācijas produkti (monomēri)	Bracewell et al., 1989; Abbt-Braun et al., 1989; Schulten et al., 2002
	oksidatīvā un reducējošā degradācija kombinācijā ar GH/MS vai ŠH/MS	degradācijas produkti (monomēri)	Schnitzer and Khan, 1972, 1972; Christman et al.,1989; Parson, 1989
	mikroskopija	izmērs, forma	Chen and Schnitzer, 1989
	viskozimetrija	izmērs, forma	Clapp et al., 1989
Spektroskopija	ultravioletajā/ redzamajā gaismā (UV/ Vis)	UV/Vis absorbcijas grupu noteikšana, E4/E6	Stevenson, 1982; Hayes et al., 1989; Abbt-Braun and Frimmel, 2002
	infrasarkanajā gaismā (IS, FT-IS, DRIFT)	funkcionālo grupu noteikšana	Hayes et al., 1989; Abbt-Braun, 1992; Niemeyer et. al., 1992
	fluorescences spektri	fluorescento grupu noteikšana	Senesi, 1990; Senesi et al., 1991; Kumke et al., 1998
	kodolu magnētiskā rezonanse (KMR ^{1}H un ^{13}C)	funkcionālo grupu kvalitatīvā noteikšana: aromātiskas, alifātiskas uzbūves, ogļhidrātu sastāvā ietilpstošo oglekļa atomu daudzuma noteikšana	Wilson, 1987; Frimmel et al., 2002
	KMR (^{1}H, ^{13}C)	kvalitatīvā/kvantitatīvā monomēro vienību un aizvietojuma veida noteikšana	Lambert et al., 1992; Hertkorn et al., 2001
	KMR (^{15}N)	struktūras elementu kvalitatīvā noteikšana	Knicker et al., 1997
	Ramana spektri	funkcionālo grupu kvalitatīvā noteikšana	Bloom and Leenheer, 1989
	EPR	organisko radikāļu un paramagnētisko metālu pāreju kvantitatīvā noteikšana	Senesi and Steelink, 1989
	Masspektrometrija (MS)	organisko molekulu noteikšana kombinācijā ar citām metodēm (fizikāli ķīmiskām degradācijas metodēm, pirolīzes-GH/MS, hidrolīzes-GH/MS, oksidācijas- GH/ MS)	Schulten et al., 2002; Parson, 1989; Christman et al., 1989
Hromatogrāfija	gēlhromatogrāfija	molekulu izmērs, masa	Perminova et al., 1998; Schmitt-Kopplin et al., 1998; DeNobili and Chen, 1999; Abbt-Braun and Frimmel, 2002
	apgrieztās fāzes hromatogrāfija (C18)	hidrofobums	Gremm et al., 1991

3. tabula / Table 3

Humusvielu elementsastāvs (pēc Kļaviņa, 1998)
Elemental composition of humic substances (after Kļaviņš, 1998)

Humusvielas	C, %	H, %	N, %
Ziemeļvalstu references ūdeņu fulvoskābe	53,13	4,60	0,8
Ziemeļvalstu references ūdeņu humīnskābe	54,62	4,53	1,02
References augsnes humīnskābe	58,13	3,68	4,08
References kūdras humīnskābe	56,37	3,82	3,69
Leonardīta humīnskābe	63,81	3,70	1,23
Rūpnieciski ražota humīnskābe (Aldrich)	49,89	4,76	2,30

4. tabula / Table 4

Humīnskābju un fulvoskābju funkcionālo grupu tipiskas vērtības (meq/g) (Schnitzer and Khan, 1972)
Functional group concentration in humic and fulvic acids (meq/g) (Schnitzer and Khan, 1972)

Funkcionālās grupas	Humīnskābe	Fulvoskābe
Kopējais skābums	6,7	10,3
Karboksilgrupas	3,6	8,2
Fenolu hidroksilgrupas	3,9	3,0
Spirtu hidroksilgrupas	2,6	6,1
Karbonilgrupas (C=O)	2,9	2,7
Metoksigrupas (OCH_3)	0,6	0,8

Nozīmīgs humusvielu raksturošanai ir arī funkcionālo grupu saturs molekulā, tas ir atkarīgs no humusvielu tipa (4. tabula). Fulvoskābes satur ievērojami vairāk karboksilgrupu un hidroksilgrupu nekā humīnskābes. Humusvielu skābes–bāzes īpašības nosaka atsevišķu funkcionālo grupu klātbūtne humusvielu molekulā, šīs īpašības ietekmē, piemēram, karboksilgrupas, fenolu hidroksilgrupas, spirti, sulfonskābes un aktīvās metilēngrupas. Faktisko skābju grupu koncentrāciju un skābju disociācijas konstantes (pK_a) vērtības nosaka HV struktūra, to izolēšanas procedūra un analīzes apstākļi. Pat nelielas atšķirības šajos procesos būtiski ietekmē iegūto datu vērtības. Galvenais parametrs, kuru izmanto, lai aprakstītu HV skābes–bāzes īpašības, ir skābju saturošo funkcionālo grupu kopējais saturs. Ilgajā humusvielu izpētes vēsturē ir izmēģinātas daudzas analītiskās metodes skābju saturošo funkcionālo grupu satura noteikšanai – tiešā titrēšana, attitrēšana kombinācijā ar destilēšanu vai ultrafiltrēšanu, reakcijas ar dažādiem organiskiem un neorganiskiem reaģentiem, kompleksometriskā titrēšana u. c. Galvenā problēma, nosakot skābes saturošās funkcionālās grupas, ir hidrolīzes procesa ietekmes samazināšana uz humusvielu struktūru (HV oksidēšanās gaisa skābekļa klātbūtnē). Plašāk izmantotās metodes ir tiešā titrēšana – bārija hidroksīda metode, kalcija acetāta jonu apmaiņas metode, potenciometriskā titrēšana ūdens vidē un bezūdens vidē (Kļaviņš, 1998).

Funkcionālajām grupām humusvielās ir atšķirīga protonu saistīšanas spēja, kā arī saites ar humusvielu struktūrelementiem. Līdz ar to ir svarīgi noteikt gan HV kopējo skābju funkcionālo grupu koncentrāciju, gan to sadalījumu. Piedāvāti vairāki matemātiskie modeļi (Perdue, 1985; Dempsey and O'Melia, 1983), kas apraksta humusvielu skābes–bāzes īpašības.

Vienkāršākie modeļi parāda HV kā vairāku vienkāršu skābju maisījumu, bet plašāk tiek izmantoti modeļi, kas pamatojas uz nepārtrauktu saistīšanās vietu sadalījumu. Saskaņā ar šo modeli humusvielu makromolekulu potenciometriskās titrēšanas līknes apraksta pēc modificēta Hendersona–Haselbaha vienādojuma (Ephraim et al., 1989). Titrēšanas līknes, protams, ir atkarīgas no karboksilgrupu tipa, koncentrācijas un polimēru kodola hidrofobajām īpašībām, kā arī no HV izcelsmes.

Lai pētītu HV skābes–bāzes īpašības, tiek izmantoti arī statistiskie modeļi (Gamble, 1972), kuros summē atsevišķas funkcionālās grupas.

Funkcionālo grupu nepārtrauktā sadalījuma modeļi parāda karboksilgrupu (tāpat kā hidroksilgrupu un citu funkcionālo grupu) sadalījumu, kas atbilst Gausa sadalījumam. Šo līdzību nosaka nenoteiktais molekulu skaits un atšķirīgais saistīšanās tips katrā humusvielu molekulā.

Atšķirības funkcionālo grupu koncentrācijā ir novērojamas arī atkarībā no humusvielas izcelsmes. Līdz ar to šo funkcionālo grupu raksturošanai ir izmantojamas dažādas titrimetriskās analīzes metodes (skābes–bāzes titrēšana), lai galvenokārt pētītu to spēju mijiedarboties ar bāzēm (Aleixo et al., 1992; Avena et al., 1999; Ritchie and Perdue, 2003).

Humusvielas raksturo gan to hidrofobās, gan hidrofilās īpašības. Humusvielu hidrofobuma raksturošanai var izmantot gan funkcionālo grupu koncentrācijas to molekulās gan arī ūdens–oktanola vai ūdens–polietilēnglikola sistēmu sadalījuma koeficientus (De Paolis and Kukkonen, 1997; Zavarzina et al., 2002). Saskaņā ar esošajiem priekšstatiem ūdeņu humusvielas ir hidrofilākas un ar mazāku molekulmasu nekā augsnes HV, kuras savukārt satur vairāk aromātisko struktūrelementu un līdz ar to ir hidrofobākas (Zavarzina et al., 2002).

5. tabula / Table 5

Humusvielu molekulmasa (dal) (Kļaviņš, 1998)
Molecular weights of humic substances (Dal) (Kļaviņš, 1998)

Humusvielas	Humīnskābes	Fulvoskābes
Augsnes	10 000–100 000	1 000–30 000
Kūdras	50 000–300 000	5 000–50 000
Ūdeņu	2 000–5 000	500–2 000

Viens no nozīmīgākajiem humusvielu makromolekulu raksturojumiem ir to molekulmasa. Tās noteikšanai sākotnēji (no 20. gs. sešdesmito gadu vidus) izmantoja gelfiltrāciju (Gjessing, 1965), bet mūsdienās humusvielu molekulmasu raksturo, izmantojot gan augstefektīvo šķidruma hromatogrāfiju (Conte and Piccolo, 1999; Perminova et al., 2003; Abbt-Braun et al., 2004), gan arī citas metodes: ultrafiltrāciju (Aiken, 1984), plūsmas frakcionēšanu (*field-flow fractionation*) (Becket et al., 1987), ultracentrifugēšanu (Reid et al., 1990), kapilāro elektroforēzi (Schmitt-Kopplin et al., 1998). Tajā pat laikā visas izmantotās humusvielu molekulmasas analīzes metodes parāda, ka tās raksturo augsta to molekulmasu dažādība, respektīvi, humusvielas ir daudzu līdzīgu molekulu maisījums. Humusvielu molekulmasa svārstās no 500 līdz 100 000 daltonu, tomēr bieži tās veido koloīdas un suspendētas daļiņas. Augsnes FS ir ar zemāku molekulmasu (1000–30 000 dal), salīdzinot ar HS molekulmasu (10 000–100 000 dal) (Paul and Clark, 1989). Ūdens FS, līdzīgi augsnes fulvoskābēm, ir ar zemāku molekulmasu (500–2000 dal), bet ūdens HS molekulmasa svārstās no 2000 līdz 5000 dal.

Tā kā humusvielas veidojas, sadaloties dzīvajai organiskajai vielai, tad tās sastāvā saglabājas arī individuālas zemmolekulāras vielas, kuras (ja tās ir saistītas labili) var tikt atbrīvotas hidrolīzes rezultātā. Humusvielu elementsastāvā ietilpst zināms daudzums slāpekļa (parasti mazāk par 5%), tas norāda uz slāpekli saturošu savienojumu klātbūtni humusvielu sastāvā. Slāpekļa saturs ir atkarīgs no humusvielu vecuma un humifikācijas pakāpes, un, pieaugot humusvielu vecumam, slāpekļa daudzums to sastāvā samazinās. Tiek uzskatīts, ka no kopējā slāpekļa savienojuma daudzuma augsnes humīnskābēs 25–55% veido aminoskābes, 1–2% aminocukuri, bet atlikumu veido heterociklisku savienojumu sastāvā ietilpstoši slāpekļa savienojumi (Turski and Chmielewska, 1986). Savukārt ūdeņu humusvielu slāpeklis galvenokārt atrodas amonija sāļu veidā (Hejzlar et al., 1994). Veicot humusvielu hidrolīzi skābā vidē un hidrolizāta aminoskābju analīzi, ir pierādīta daudzu aminoskābju klātbūtne humusvielās (Kļaviņš, 1993). Izmantojot ^{13}C KMR (kodolmagnētiskās rezonanses spektroskopiju) un ^{15}N KMR, pierādīta piridīna, nitrozofenolu, oksīmu, izocianīdu, amīdu, laktāmu, izoksazola un formazāna, kā arī citu slāpekļa savienojumu klātbūtne humusvielās (Thorn et al., 1992; Knicker et al., 1993). Slāpekļa savienojumi nosaka humusvielu lielo nozīmi bioloģiskajos procesos augsnē un ūdens vidē, jo humusvielas var kļūt par biogēno elementu avotu, saistītajiem slāpekļa savienojumiem atbrīvojoties bioloģiski pieejamās formās (Tranvik, 1990).

Ir pierādīts, ka līdz 6% no humusvielu masas veido dažādi ogļhidrāti, kas var tikt atbrīvoti hidrolīzes gaitā skābā vidē (Barriuso et al., 1985). Humusvielu sastāvā atrastas pentozes, heksozes, glikuronskābes un aminocukuri: glikoze, galaktoze, mannoze, ksiloze, arabinoze, ramnoze, fukoze, glikuronskābe, kā arī galaktozamīns. Arī ogļhidrātu daudzums humusvielās ir atkarīgs no to izcelsmes un vecuma, un augstākais tas ir humusvielās, kas izdalītas no eitrofiem ūdeņiem (Kļaviņš, 1993). Humusvielu sastāvā ietilpstošo ogļhidrātu analīze izmantojama, lai izsekotu to ģenēzes procesam, jo mikroorganismu darbības rezultātā veidoto ogļhidrātu spektrs ievērojami atšķiras no augu sastāvā esošajiem ogļhidrātiem (Moers et al., 1990).

Humusvielu raksturošanai plaši izmanto dažādas spektroskopiskās analīzes metodes, sākot no vienkāršākajām līdz pat visjaunākajām.

UV redzamās gaismas spektri visai plaši tiek izmantoti humusvielu identificēšanai, lai arī to sniegtā informācija ir visai ierobežota, ņemot vērā spektru monotono raksturu (Traina et al., 1990; Filip and Alberts, 1992; Chin et al., 1994). UV redzamās gaismas spektri ļauj konstatēt aromātisko grupu, hromoforu grupējumu klātbūtni humusvielu molekulās, bet absorbcijas attiecību izmantošana ieteikta arī aromātisko grupu (aromātiskuma) un humusvielu molekulmasas novērtēšanai (Kononova, 1966; Chin et al., 1994).

Humusvielu infrasarkanie (IS) spektri ļauj novērtēt un pierādīt -OH, $-CH_3$, $-CH_2-$, C=O, N-H, C=C un citu funkcionālo grupu klātbūtni humusvielu struktūrā (Senesi et al., 1986; Senesi and Sakellariadou, 1984). Šīs metodes priekšrocība ir tā, ka humusvielu raksturošanai nav nepieciešams izmantot augsti attīrītus paraugus. Humusvielu IR spektrus izmanto, lai salīdzinātu dažādas izcelsmes humusvielas, novērtētu funkcionālo grupu daudzumu to molekulās (Niemeyer et al., 1992).

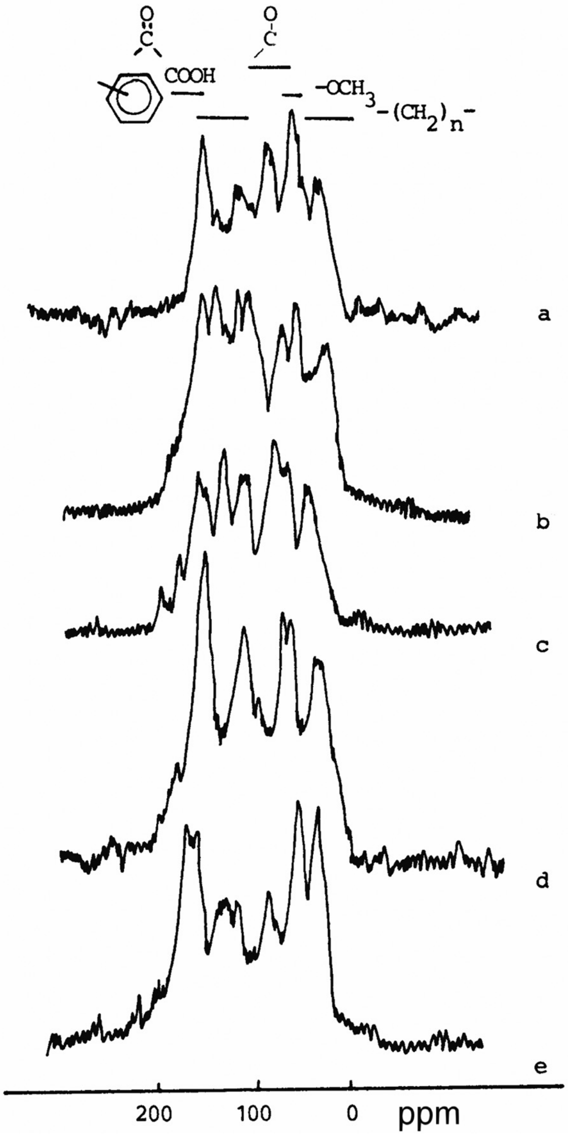
Humusvielu fluorescences spektrus parasti raksturo viens izteikts maksimums, kura novirze vai papildu „plecu" parādīšanās saistāma ar fluoroforu struktūru klātbūtni un koncentrāciju humusvielu molekulās (Senesi et al., 1989; Belin et al., 1993). Humusvielu fluorescences spektru analīze izmantota, lai pētītu lignīna fragmentu klātbūtni humusvielu molekulās. Visai plaši humusvielu fluorescences spektru analīzi izmanto, lai pētītu humusvielu mijiedarbību ar organiskajām vielām (piemēram, policikliskajiem aromātiskajiem ogļūdeņražiem) vai metālu joniem, jo šo mijiedarbību rezultātā notiek fluorescences signāla intensitātes samazināšanās (dzēšana), tā ir proporcionāla kompleksu veidošanās intensitātei (Kumke et al., 1998).

Elektronu paramagnētiskās rezonanses (EPR) spektrus raksturo izteikta simetriska rezonanses līnija. Šī signāla un pavadošo rezonanses līniju intensitāte humusvielu spektros mainās atkarībā no humusvielu izcelsmes. Pierādīts, ka signālu EPR spektros nosaka semihinona struktūras, kas saistītas ar kondensētām aromātiskām struktūrām, kurās ir alifātiskie vai heteroatomu aizvietotāji (Senesi and Steelink, 1989; Peuravuori, 1992). EPR spektrus plaši izmanto, lai pētītu humusvielu un paramagnētisko metālu jonu mijiedarbību.

Kodolmagnētiskās rezonanses spektroskopija (KMR) tiek izmantota kā viena no visefektīvākajām metodēm humusvielu raksturošanai. Protonu magnētiskā spektroskopija (KMR ^{1}H) tiek izmantota protonu saistības veida un ar tiem saistīto funkcionālu grupu daudzuma novērtēšanai humusvielu molekulās. KMR ^{1}H spektri ļauj novērtēt metilgrupu un metilēngrupu protonu daudzumu, metilēngrupās esošos protonus, kas atrodas α stāvoklī attiecībā pret aromātiskiem gredzeniem, karboksilgrupām vai karbonilgrupām. KMR ^{1}H spektri ļauj arī novērtēt protonus, kas saistīti ar metoksigrupām, kā arī ar olefīnos un aromātiskajās struktūrās esošajiem oglekļa atomiem (Peuravuori, 1992; Frimmel et al., 2002). Ievērojami plašāk izmanto ^{13}C KMR, tas ļauj raksturot humusvielas veidojošās pamatstruktūras un novērtēt oglekļa atomu daudzumu, kas saistīti ar nozīmīgākajiem humusvielu uzbūves elementiem (Leinweber et al., 1993; Frund et al., 1994; Hertkorn et al., 2001; Gelinas et al., 2001; Peuravuori et al., 2003; Keeler and Maciel, 2003). Plaši attīstās divdimensiju un trīsdimensiju KMR izmantošana, un tas ļauj pilnīgāk raksturot humusvielu uzbūvi (Chien and Bleam, 1998; Kingery et al., 2000; Hertkorn et al., 2002; Simpson et al., 2003).

Arī citu humusvielu sastāvā ietilpstošo elementu (N, O, F, Cl) KMR spektri ir izmantoti šo elementu nozīmes raksturošanai humusvielu molekulās (Almendros et al., 1991; Thorn et al., 1992; Knicker et al., 1997; Kögel-Knabner, 2002).

Humusvielu īpašību izpētē nozīmīgu vietu ieņem destruktīvās metodes, kuru gaitā humusvielu molekulas tiek sagrautas līdz zemmolekulāriem struktūrelementiem, kurus tālāk iespējams sadalīt un identificēt, izmantojot hromatogrāfijas metodes. Humusvielu degradācija ir veikta, tās hidrolizējot un atšķeļot to uzbūves elementus gan skābā, gan sārmainā vidē. Plaši tika izmantota humusvielu oksidēšana (ar $KMnO_4$, O_3, HNO_3 un citiem oksidētājiem), kā arī reducēšana (ar $NaBH_4$, Zn amalgamu, CuO un citiem). Lai nodrošinātu pietiekamu iegūto humusvielu degradācijas produktu gaistamību, tie tiek derivatizēti un tālāk analizēti ar gāzu hromatogrāfiju–masspektrometriju (GH-MS) (Hayes and Swift, 1978; Schnitzer and Khan, 1972; Liao et. al., 1982; Morinaga and Ishiwatari, 1987; Langvik et al., 1994). Mūsdienās visai plaši izmanto humusvielu pirolīzi un tās produktu GH-MS analīzi (Abbt-Braun et al., 1989; Schulten et al., 2002).



5. attēls. **Kūdras (a) un augsnes (b) humīnskābju, kūdras (c) un augsnes (d) fulvoskābju, kā arī kūdras humīna (e) ^{13}C KMR spektri (Kļaviņš, 1998)**
Figure 5. **^{13}C NMR spectra of peat (a) and soil (b) humic acid, peat (c) and soil (d) fulvic acids and peat humin (e) (Kļaviņš, 1998)**

Kaut arī humusvielu īpašību izpētē ir lietotas gandrīz visas mūsdienās zināmās organisko vielu un makromolekulu analīzes metodes, ir acīmredzams, ka neviena no tām, atsevišķi lietota, neļauj pilnībā aptvert šo vielu komplekso un sarežģīto uzbūvi un ka humusvielu raksturošanai nepieciešams izmantot vairāku metožu kombināciju (Abbt-Braun et al., 2004; Thomsen et al., 2002).

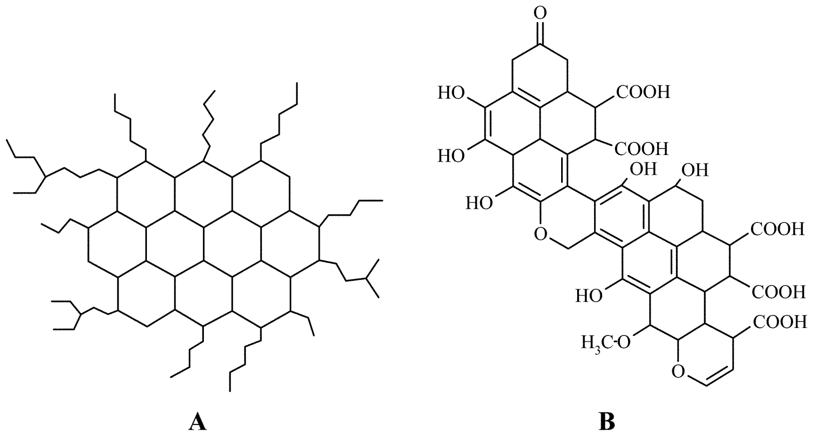
1.4. Humusvielu struktūra

Pilnveidojoties zināšanām par humusvielu veidošanos un attīstoties jaunām pētniecības metodēm, mainās arī priekšstati par HV molekulas uzbūvi. Analizējot HV struktūrmodeļus, parasti neizdala ūdens, augsnes un kūdras humusvielas, lai gan ir acīmredzami, ka pastāv ievērojamas atšķirības starp humusvielām atkarībā no to izcelsmes.

Ir vairākas pieejas humusvielu struktūras noteikšanai, un tās var iedalīt šādās grupās (Kļaviņš, 1998):

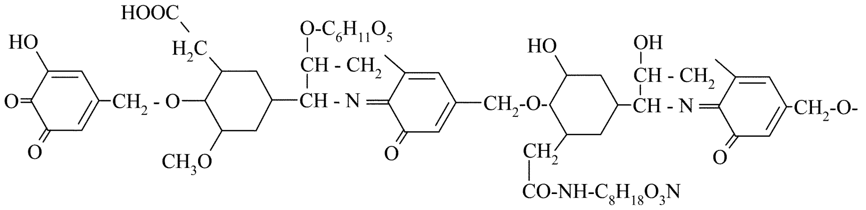
- teorētiskā pieeja – izmanto atsevišķus HV raksturlielumus: elementsastāvu, spektrālās īpašības, skābes–bāzes īpašības, esošos HV molekulu struktūras modeļus;
- degradācijas pieeja – pamatojas uz tādu zemmolekulāro savienojumu analīzi, kurus iegūst, humusvielas sadalot; iegūtie dati tiek izmantoti HV struktūras modeļu izveidošanai;
- nedestruktīvā pieeja – pamatojas uz tādām analīzes metodēm, kas minimāli iespaido humusvielu oriģinālo struktūru;
- modelēšanas pieeja – attīsta humusvielu struktūras modeļus, pamatojoties uz pētījumiem par HV lomu apkārtējā vidē.

Ņemot vērā to, ka ilgu laiku nebija vienotas koncepcijas par standartizētām HV paraugu iegūšanas metodēm, aprakstītie HV struktūras modeļi bija principiāli atšķirīgi. Pirmos HV struktūru modeļus izstrādāja, pamatojoties uz tā laika priekšstatiem par augsnes humusa struktūru un izmantojot samērā trūcīgo informāciju par pētāmo materiālu. Humusvielu uzbūves modeļi, ko piedāvāja Fukss (Fuchs, 1930) un Kasatočkins (Kasatockhin, 1951), tika attēloti kā telpiskas struktūras, kuras veidoja kondensēti aromātiskie gredzeni ar alifātiskām sānu virknēm, kā arī ar karboksilgrupām un hidroksilgrupām (6. att.). Šie modeļi parādīja HV nozīmi organiskā materiāla transformēšanā un to iespējamo lomu ogļu, naftas un citu fosilo nogulumu veidošanā.



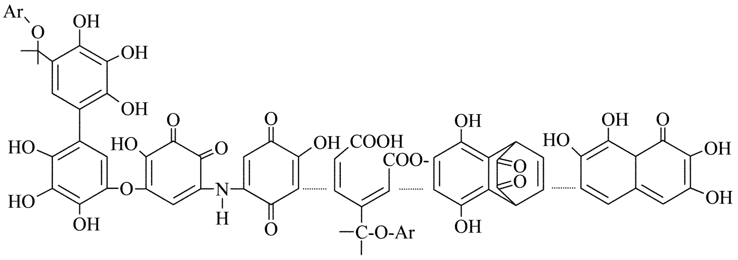
6. attēls. **Humīnskābes hipotētiskā struktūra: (A) Kasatockhin, 1951, (B) Fuchs, 1930**
Figure 6. **Hypothetical structures of humic acid molecule: (A) Kasatockhin, 1951, (B) Fuchs, 1930**

Otra pieeja balstījās uz dažādu struktūrelementu (kondensētu hinoidālu struktūru, lignīna atlikumu) grupēšanu, iegūstot hipotētiskas struktūras, kas izmantojamas, lai izskaidrotu dažādas HV īpašības (Dragunov, 1948). Šis modelis (7. att.) bija izveidots, apkopojot pieejamo informāciju par humusvielu pamatīpašībām un to veidošanās gaitu.



7. attēls. **Humīnskābes hipotētiskā struktūra (Dragunov, 1948)**
Figure 7. **Hypothetical structure of humic acid molecule (Dragunov, 1948)**

Lai gan Dragunova izstrādātais modelis balstījās uz teorētiskiem pieņēmumiem, vairākas viņa atziņas eksperimentāli apstiprinājās 20. gs. 90. gados. Dragunova modelis sastāv no kondensētām hinoīdgrupas saturošām struktūrām, lignīnu struktūrelementiem un ogļhidrātu atlikumiem. Šo modeli iespējams izmantot HV raksturošanai, jo tas atspoguļo humusvielu pamatīpašības, ļauj paredzēt HV lomu un īpašības apkārtējā vidē.



8. attēls. **Hipotētiskā humīnskābes struktūra (Flaig, 1960)**
Figure 8. **Hypothetical structure of humic acid molecule (Flaig, 1960)**

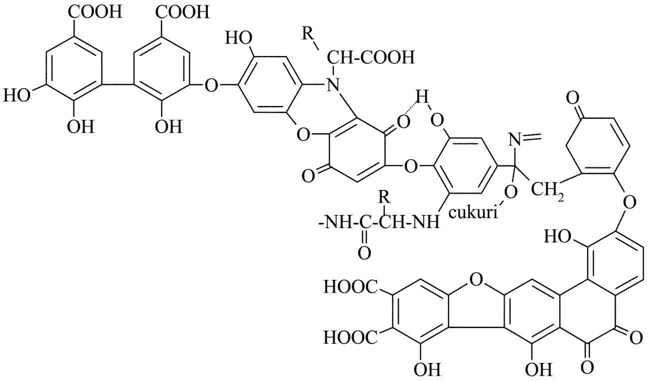
Kļūdaina datu interpretācija un būtiskas informācijas trūkums par humusvielu struktūru noteica tīri teorētisku HV modeļu izveidošanu, kas galvenokārt izpaudās kā zināmu ideju brīva kombinācija. Piemēram, Kleinhempela (Kleinhempel, 1970), Flaiga (Flaig, 1960) piedāvātā HV molekulas struktūra (8. att.) tika balstīta uz zināšanām par lignīna struktūru 20. gs. 60. gados.

Pavērsienu humusvielu īpašību izpētē izraisīja HV makromolekulu degradēšanas metodes izmantošana. Šo metodoloģiju plaši izmanto arī šodien. Humusvielu sadalīšanai izmanto tādus procesus kā oksidēšanu, reducēšanu, hidrolīzi un termisko degradēšanu (pirolīzi). Galvenie etapi HV degradācijas analīzē ir :

- no dabas vides izdalītā humusvielas parauga attīrīšana;
- priekšapstrāde, lai aizsargātu pret oksidēšanu labilās HV struktūras;
- humusvielu degradācija;
- degradācijas produktu izdalīšana;
- degradācijas produktu frakcionēšana, identificēšana un kvantitatīvā analīze.

Tomēr, ņemot vērā HV izteikti heterogēno dabu, iespējams noteikt tikai daļu humusvielu struktūrvienību degradācijas produktu maisījumā, un šī metode nedod tik labus rezultātus, kā analizējot citas biomolekulas (proteīnus, nukleīnskābes, lignīnus).

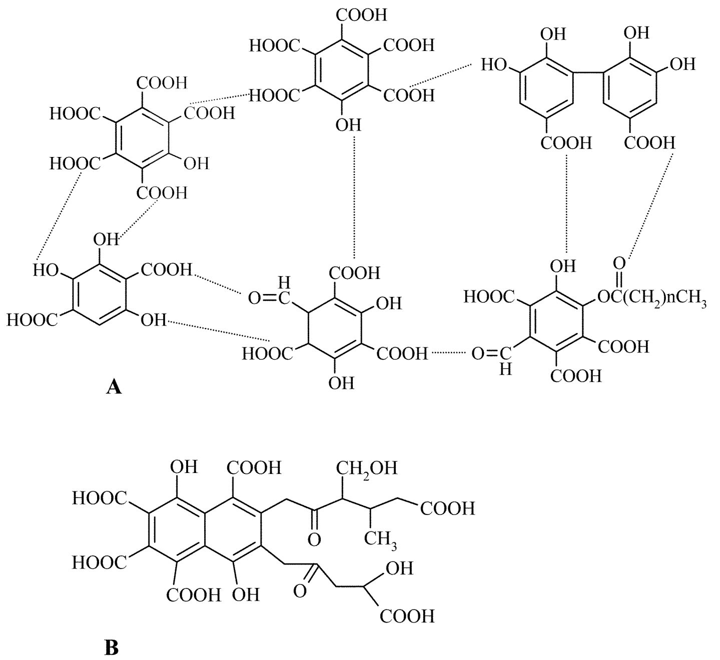
Humusvielu struktūras analīzei izmantotas arī nedestruktīvās metodes: ultravioletā, infrasarkanā spektrometrija, elektronu paramagnētiskā rezonanse (EPR), kodolmagnētiskā rezonanse, rentgenstaru analīze, elektronmikroskopija, viskozitātes un virsmas spraiguma noteikšana, dažādas metodes molekulmasas noteikšanai (piemēram, gelfiltrācija), titrimetrijas metodes un citas. Pēdējā laikā šīm metodēm ir bijusi noteicošā loma humusvielu struktūras noteikšanā, taču bieži tās kombinētas ar informāciju, kas iegūta, izmantojot humusvielu pirolīzi/masspektrometriju, šķidruma hromatogrāfiju/masspektrometriju, gāzes hromatogrāfiju/masspektrometriju un citas metodes. Tomēr neviena no šīm metodēm nedod visu informāciju, kas nepieciešama humusvielu struktūras noteikšanai. Tādēļ, lai izveidotu humusvielu molekulu uzbūves modeļus, izmantotas vairākas metodes, ieskaitot arī kvantu ķīmijas pieejas. Līdz ar to jaunākās humusvielu struktūras koncepcijas pamatojas uz detalizētāku izpratni par HV lomu vidē un HV netiek uzskatītas par viendabīgām ķīmiskām vielām (Stevenson, 1982) (9. att.).



9. attēls. **Humīnskābes hipotētiskā struktūra (Stevenson, 1982)**
Figure 9. **Hypothetical structure of humic acid molecule (Stevenson, 1982)**

Analizējot iespējamos humusvielu uzbūves modeļus, tiek ņemti vērā to iespējamie prekursori. Aplūkojot humusvielu struktūras elementus, tajā var saskatīt dažādu vielu sadalīšanās produktu sastāvdaļas. Tā, piemēram, slāpekļa esamību humusvielu molekulā nodrošina amonjaka vai aminogrupu (veidojušās no aminoskābēm) savstarpējās reakcijas ar ogļhidrātiem. Nākamā svarīgākā savienojumu grupa ir vielas ar aromātiskām serdēm, no kurām rodas dažādi fenoli un hinoni, kuri ir tiešie humusvielu prekursori un kuru avots galvenokārt ir augu atliekas. Humusvielu struktūras veidošanā nozīmīgs ir lignīns, ne tikai ņemot vērā tā lielo saturu augu atliekās un augsto noturību, bet galvenokārt tā neregulārās sazarotās polimērās uzbūves dēļ, jo to veido aizvietotie fenoli. Lignīnam pārveidojoties, augsnē samazinās hidroksilgrupu saturs un palielinās karboksilgrupu koncentrācija molekulā – tā var sasniegt 140–160 mgekv/100 g, un tas jau ir pielīdzināms karboksilgrupu saturam humusvielu molekulās. Tādējādi lignīna sadalīšanās fragmentus var uzskatīt par humusvielu veidošanās izejmateriāliem (Орлов, 1990). Par humusvielu aromātiskās struktūras vēl vienu izejvielu grupu var minēt flavonoīdus, lai gan tie ir mazāk izplatīti un tiem piemīt augstāka reaģētspēja. Visbeidzot par humusvielu aromātisko fragmentu avotu ir jāmin daudzie pigmenti (gan augu, gan mikroorganismu producētie), kas var ieslēgties humusvielā bez izmaiņām vai pēc daļējas sadalīšanās. Humusvielu struktūrā var tikt iekļauti dažādu vidē nonākušo piesārņojošo vielu transformācijas produkti, kā izplatītākos var minēt lauksaimniecībā izmantojamos pesticīdus (Kļaviņš, 1998).

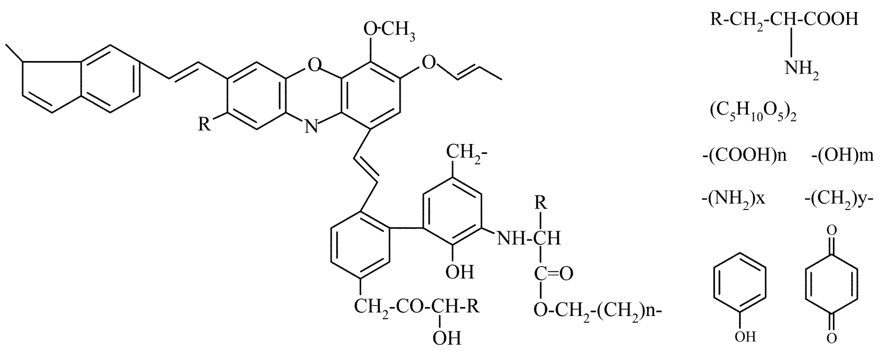
Saskaņā ar mūsdienās dominējošajiem priekšstatiem, humusvielu molekulas veido polimēras dabas struktūrelementi, kuru galvenajā struktūrā ietilpst divas vai trīs hidroksilgrupas saturoši fenolu aromātiskie gredzeni ar -O-, -CH-, -NH-, -N=, -S- u. c. grupu saitēm, kā arī slāpekļa savienojumi cikliskās formās un peptīdu ķēdēs. Šajā modelī N parādās visās struktūrās (Stevenson, 1982).



10. attēls. **Fulvoskābes hipotētiskā struktūra: (A) Schnitzer & Kahn, 1972; (B) Buffle, 1977**
Figure 10. **Hypothetical structures of fulvic acid molecule: (A) Schnitzer & Kahn, 1972, (B) Buffle, 1977**

Pastāv uzskats (Schnitzer and Khan, 1972), ka FS molekulu veido fenolkarbonskābes un benzolkarbonskābes jeb beznzoskābes (10. att. A), kuras kopā satur ūdeņraža saites, veidojot stabilas polimēra struktūras. Savukārt citi pētnieki (J. Buffle, 1977) FS molekulas struktūrā saskata aromātiskos un alifātiskos komponentus (10. att. B), kas aizvietoti ar skābekli saturošām funkcionālām grupām.

Fulvoskābes molekulai ir mazāk izteiktas aromātiskās struktūras nekā humīnskābes molekulai, jo tā satur mazāk C un vairāk H. Tādēļ FS ir vāji izteikts centrālais kodols (aromātiskos ogļūdeņražus saturošs kodols) un tās vairāk satur alifātiskās sānu virknes (Орлов, 1990) (11. att.).



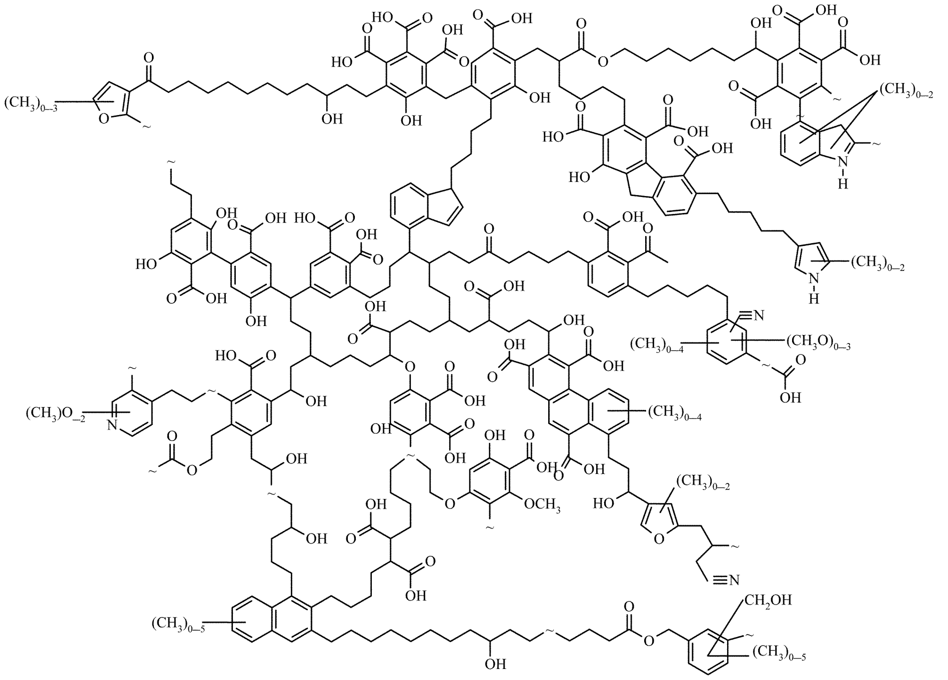
centrālā kodola daļa *hidrolizējamā daļa*

11. attēls. **Humīnskābes hipotētiskā struktūra (Орлов, 1990)**
Figure 11. **Hypothetical structure of humic acid molecule (Орлов, 1990)**

Mūsdienās par visbiežāk citētajiem var uzskatīt augsnes humīnskābes (Schulten and Schnitzer, 1993) un ūdeņu fulvoskābes struktūrmodeļus (Leenheer et al., 1995). No iepriekšminētajiem būtiski atšķirīgs ir H. Šultena un M. Šnitcera izstrādātais augsnes humusvielu struktūras modelis. Saskaņā ar to humusvielas molekulas veido lignīna degradācijas rezultātā radies aromātisku kodolu tīkls, kuru savstarpēji sasaista alifātiskas struktūras un ūdeņraža saites. Nesen izstrādātais humusvielu struktūrmodelis (12. attēls) tās aplūko kā alkilaromātisku struktūru tīklu, ko veido augsti aizvietotas fenolkarbonskābes. Divu un trīs locekļu ar karboksilgrupām un fenolu hidroksilgrupām aizvietotas aromātiskas vai heterocikliskas (skābekli un slāpekli saturošas) struktūras (saskaņā ar šo struktūras modeli – aromātiskas struktūras elementus) saista alkilvirknes, kuru garums var būt līdz 20 oglekļa atomiem un kuras var būt aizvietotas ar hidroksilgrupām, karboksilgrupām un keto grupām. Šis struktūrmodelis izskaidro HV un metālu jonu, un hidrofobo organisko vielu mijiedarbības raksturu, bet jautājumi par šādas HV struktūras ģenēzi humifikācijas procesā ir diskutabli.

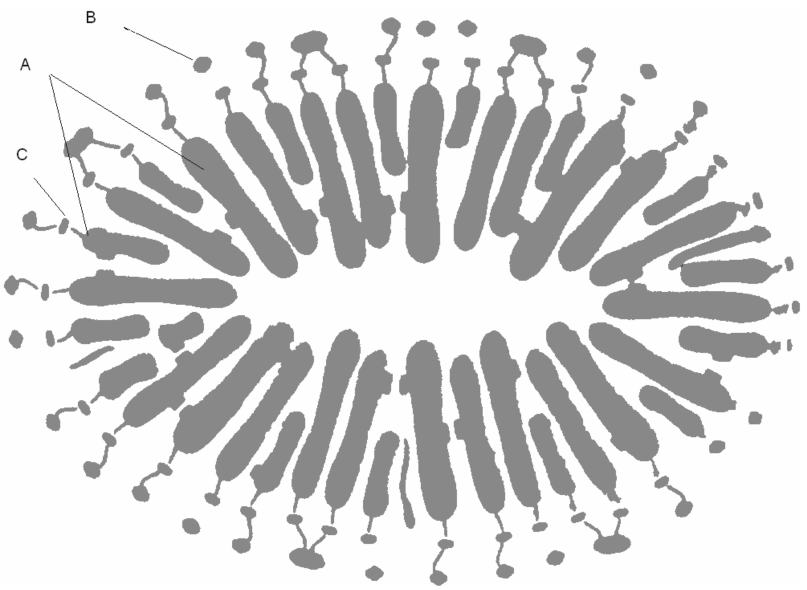
Izmantojot ievērojamu skaitu dažādu pētījumu metožu, ir izstrādāti Suvanī upes (ASV) ūdeņu fulvoskābju iespējamie struktūras modeļi. Saskaņā ar tiem ūdeņu fulvoskābes ir relatīvi zemas masas struktūras, kurās liela nozīme ir alifātiskiem uzbūves elementiem, kas izkārtoti ap aromātisku policiklisku "molekulas centru". Šo modeļu izmantošanas iespējas humusvielu īpašību izpētē un aprakstīšanā ir plaši dokumentētas. Ūdeņu fulvoskābes modelis apraksta struktūras ar ievērojami mazāku molekulmasu nekā augsnes humīnskābes, un to kodolu veido augsti aizvietotas poliaromātiskas struktūras, kas aizvietotas ar fenolu un monokarbonskābju un dikarbonskābju atlikumiem.

Piedāvātie humusvielu struktūras modeļi ļauj aprakstīt hipotētiskas struktūras, kādas varētu būt raksturīgas kādai (vai dominējošai) humusvielu veidojošo molekulu grupai, bet nekādā gadījumā tie nav identificējami ar to, ar ko struktūras jēdzienu saprot, piemēram, organiskajā ķīmijā. Vienlaikus nav apšaubāma mēģinājumu nozīmība izstrādāt humusvielu struktūrmodeļus, jo tieši pieeja izdalīt vielu no objekta, kurā tā atrodas, to attīrīt, noteikt tās īpašības, noteikt struktūru ir pamatā mūsdienu sasniegumiem biomolekulu un biopolimēru izpētē. Pēdējā laikā attīstās datormodelēšanas izmantošana humusvielu struktūras izpētē (Diallo, 2003).



12. attēls. **Augsnes humīnskābes molekulas hipotētiskā struktūra (Schulten and Shnitzer, 1993)**
Figure 12. **Hypothetical structure of soil humic acid molecule (Schulten and Shnitzer, 1993)**

Papildus tam pieaug izpratne par humusvielu nozīmi dabas vidē noritošajos procesos, kā arī par to, ka humusvielu izdalīšanas gaitā to īpašības tiek izmainītas un līdz ar to izolētu un augsti attīrītu humusvielu preparātu īpašības atšķiras no tām, kādas tās ir dabas vidē. Attīstoties priekšstatiem par humusvielu uzbūvi, tos papildina jaunas koncepcijas, saskaņā ar kurām humusvielas tiek uzskatītas par micellu agregātiem, respektīvi, dabas vidē humusvielas veido supramolekulārus agregātus. Šāda tipa humusvielu agregātiem var būt no attīrītiem preparātiem atšķirīgas ķīmiskās un fizikālās īpašības. Tā kā humusvielu makromolekulā ir gan polārie, gan nepolārie reģioni, tad, humusvielu saturam ūdens šķīdumā pārsniedzot kritisko micellu koncentrāciju, veidojas agregāti, kuru iekšpusē atrodas hidrofobākas molekulu daļas (alifātiskās ķēdes, aromātiskās un poliaromātiskās struktūras), bet micellu virsmu veido humusvielu sastāvā esošās polārās grupas (karboksilgrupas un fenolu hidroksilgrupas) (13. attēls). Šādas micellārās struktūras ir viegli sagraujamas sārmu klātbūtnē, un līdz ar to izdalītie humusvielu preparāti var visai maz atgādināt tās struktūras, kuras atrodas dabas vidē. Šajā humusvielu agregātu veidošanās procesā liela nozīme var būt gan citām organiskajām vielām (gan zemmolekulārām, gan augstmolekulārām), gan arī metālu joniem un minerālu daļiņām (Wershaw, 1986; Wershaw, 1990). Humusvielu micellāro dabu apliecinājuši daudzi pētījumi (Terashima et al., 2004; Yates and Wandruszka, 1999; Guetzloff and Rice, 1994; Conte and Piccolo, 1999).



13. attēls. **Humusvielu micellārās uzbūves shematisks attēlojums: A – amfifilā humusvielu molekula; B, C – ar humusvielām saistītie joni (Wershaw, 1993)**
Figure 13. **Schematic representation of micellar structure of humic molecules, A- amphiphilic molecule; B,C- binded cations (Wershaw, 1993)**

Kā jau iepriekš minēts, pašreizējās humusvielu struktūras noteikšanas koncepcijas balstās uz izpratni par HV lomu apkārtējā vidē un neuzskata HV par viendabīgām ķīmiskām vielām. Tomēr joprojām nav izstrādāta teorija, kas atbildētu uz visiem jautājumiem, līdz ar to pilnīga HV struktūras modeļa izveidošana ir viena no vissvarīgākajām humusvielu ķīmijas problēmām.

Lai gan humusvielas ir intensīvi pētītas, joprojām to struktūras definēšanas un izziņas jautājums nav uzskatāms par atrisinātu, jo, no vienas puses, neviens esošajiem modeļiem pilnībā neatspoguļo humusvielu īpašības (Burdon, 2001), bet, no otras puses, esošie priekšstati par organiskās vielas humifikācijas gaitu un bioloģiskās degradācijas procesiem nespēj izskaidrot piedāvāto struktūru izveidošanos.

1.5. Humusvielu mijiedarbība ar augsnes komponentiem un to sorbcijas loma pazemes ūdeņu mākslīgās papildināšanās procesā

Humusvielas dabas vidē veidojas un atrodas ciešā mijiedarbībā ar daudzām organiskām un neorganiskām vielām, kā arī ar dzīvo organismu sastāvā esošām vielām. Dažādo funkcionālo grupu klātbūtne humusvielu molekulās nosaka to spēju mijiedarboties ar dažādiem gan augsni, gan ūdens vidi veidojošiem minerālajiem komponentiem: katjoniem, oksīdiem, hidroksīdiem, silikātiem, alumosilikātiem. Katrs atsevišķs mijiedarbības process pakļauts termodinamikas likumiem, taču dažādo faktoru mijiedarbība un vienlaicīgi vidē noritošās ķīmiskās reakcijas un bioloģiskie procesi nosaka to, ka ne augsnē, ne ūdens vidē noritošos ķīmiskos procesus nevar aprakstīt, izmantojot klasiskās likumsakarības. Augsnēs un ūdeņos disperģētajās minerālu daļiņās notiek mijiedarbība starp organiskajām un minerālu fāzēm, fizikālā sorbcija un hemosorbcija, organisko vielu iespiešanās starp minerālu struktūrām un koagulācija, vienlaikus notiek arī organisko vielu bioloģiskā degradācija un jaunu struktūru veidošanās. Acīmredzot tāpēc humusvielu, augsnes un ūdeņu neorganisko vielu (minerālu daļiņas veidojošās vielas) mijiedarbība līdz šim ir visai maz pētīta, lai gan šāda mijiedarbība būtiski ietekmē vidē noritošos procesus (Schulten and Leinweber, 2002). Tomēr, lai šādu izpratni attīstītu, katru no šiem mijiedarbības procesiem ir nepieciešams pētīt atsevišķi, lai pēc tam izzināto apvienotu, veidojot izpratni par procesu komplekso raksturu, kas ietekmē humusvielu veidošanos, pastāvēšanas formas un procesu norisi dabas vidē. Izpratne par humusvielu un minerālu mijiedarbības raksturu ir būtiska arī, lai novērtētu humusvielu mijiedarbību ar piesārņojošajām vielām un, piemēram, to kustīgumu ūdens un pazemes vidēs. Tas ir nozīmīgi, jo humusvielu sorbcija augsnē var samazināt ar tām saistīto piesārņojošo vielu kustīgumu, bet absorbētās humusvielas var būtiski izmainīt augsnes daļiņu īpašības (kustīgumu elektriskajā laukā, koloīdu stabilitāti) un līdz ar to paaugstināt vielu pārneses procesu ātrumu (McCarthy et al., 1989). Par vienu no nozīmīgākajiem procesiem, no šī viedokļa, var uzskatīt mijiedarbību starp humusvielu un augsnes un ūdeņu minerālajiem ingredientiem.

Humusvielu sorbciju uz minerālu virsmām (Kretzschmar et al., 1997) nosaka šādi procesi: a) elektrostatiskā mijiedarbība; b) jonu apmaiņa; c) ligandu apmaiņa; d) hidrofobā mijiedarbība; e) ūdeņraža saišu un jonu saišu veidošanās.

Humusvielu īpašības, kas var ietekmēt to spēju mijiedarboties ar augsnes un ūdeņu minerālajām sastāvdaļām, kā arī adsorbēt citas organiskas vielas, ir:

- liels lādiņu blīvums, ko nosaka daudzo funkcionālo grupu klātbūtne;
- liels virsmas laukums, kas rodas lielo un sazaroto humusvielu molekulu dēļ, kā arī to dabas vidē pastāvošās micellārās formas dēļ.

Vairāki no faktoriem, kas ietekmē humusvielas un augsnes un ūdeņu neorganiskās fāzes veidojošos minerālus, ir vairāk vai mazāk pētīti, lai gan pētījumi pamatā veikti, izmantojot visai strikti definētas modeļsistēmas. Detalizēti pētīts humusvielu sorbcijas process uz dzelzs un alumīnija oksīdu virsmas, to galvenokārt ietekmē ligandu apmaiņas mehānisms (Gu et al., 1993). Pierādīts, ka ezeru nogulumu humīnskābes visai labi sorbējas uz gibsīta un ka sorbcijas intensitāte ir atkarīga no pH. Pierādīts, ka sorbcijas process norit divās stadijās un ka pēc sākotnējās sorbcijas notiek humusvielu molekulas konformācijas izmaiņas uz sorbenta virsmas (Aven and Koopal, 1999). Sorbcijas norisei savukārt nozīmīgs ir humusvielu transporta process šķīdumā un to mijiedarbība ar sorbenta virsmu. Sorbcijas atkarību no vides pH nosaka tas, ka, pieaugot pH, palielinās aktīvo funkcionālo grupu molekulas kopējais negatīvais lādiņš. Šādos apstākļos šīs funkcionālās grupas var piesaistīt katjonus no apkārtējās vides vai minerālu virsmas, tā radot katjonu apmaiņu. Pretējs process norit, samazinot pH, – palielinot ūdeņraža jonu koncentrāciju šķīdumā, humusvielu molekula iegūst pozitīvu lādiņu un spēj iesaistīties anjonu apmaiņas reakcijās. Tas, kurš no šiem procesiem kļūst par dominējošo, ir atkarīgs no tās minerālās fāzes īpašībām, uz kuras sorbcija tiek pētīta.

Šķīduma pH nebūt nav vienīgais apstāklis, kas nozīmīgi ietekmē sorbciju. Ja ir augsts pH un liels jonu spēks, negatīvais lādiņš uz humusvielu molekulas un minerālu virsmas var būt ekranēts, un līdz ar to elektriskā atgrūšanās starp minerālu virsmu un humusvielu molekulu var kļūt vājāka. Humusvielu sorbcijas pieaugumu, palielinoties jonu spēkam, var ietekmēt to makromolekulas konfigurācijas izmaiņas, jo liela jonu spēka ietekmē humusvielu molekulas var saritināties, tādējādi kļūstot kompaktākās un hidrofobākas. Pēc tam, samazinoties jonu spēkam šķīdumā, negatīvie lādiņi sāk atgrūsties un sorbētā humusvielu molekula uz minerāla daļiņas virsmas var atritināties kā kustīga lineāra polimēra molekula. Šādas konfigurācijas molekulas aizņem vairāk vietas uz virsmas, un tā rezultātā samazinās sorbcijas kapacitāte (Davis, 1982). Līdzīgi secinājumi iegūti, pētot humusvielu sorbciju uz alumīnija oksīda (Tombacz et al., 2000). Tajā pat laikā nepieciešams atzīmēt, ka praktiski visos šajos pētījumos izmantotas sorbcijas sistēmas, kas atšķiras augsnes iežu sastāva ziņā. Turklāt līdz šim veiktajos sorbcijas pētījumos ignorēta iespēja, ka humusvielu molekulas var atrasties koloīdu daļiņu vai micellu formā.

Adsorbcija uz alumosilikātiem notiek tikai specifiskās lokalizētās vietās uz sorbenta virsmas (sorbcijas centriem), un piesātinājums liecina par to, ka sorbcijas centri var tikt pilnībā aizņemti. Līdz ar to tipiski humusvielu sorbcija uz augsnes ir atkarīga no alumosilikātu daudzuma tajā (Shen, 1999). Alumosilikātu un dzelzs oksīdu sorbcijas spēja ir ievērojami augstāka nekā citiem augsnes vidi veidojošajiem minerāliem. Savukārt, kā tas pierādīts pētījumā, kurā izmantoti montmorilonīta māli (Koyama, 1995), adsorbcija faktiski notiek tikai uz kristāliskā režģa defekta vietām, bet, tās piesātinot, notiek mazāk efektīva humusvielu saistīšanās ar jau sorbētām humusvielu molekulām (daudzslāņu struktūru veidošanās). Līdzīgs ir sorbcijas raksturs uz getīta un kaolinīta (Meier et al., 1999).

Nozīmīga augsnes vides sastāvdaļa ir dzelzs oksīdi un hidroksīdi. Pierādīts, ka humusvielu sorbcija uz ar dzelzs oksīdiem bagātas tropu augsnes galvenokārt notiek kā fizikālā sorbcija (Sibanda and Young, 1986; Jardine et al., 1989). Hidratēti dzelzs oksīdi var nodrošināt humusvielu sorbciju skābā vidē, jo to virsma kļūst pozitīvi lādēta un iegūst spēju saistīt arī fulvoskābes, īpaši – ja tās satur daudz karboksilgrupu. Kvarca virsma ir negatīvi lādēta neitrālā vides reakcijā, līdz ar to elektrostatiskā mijiedarbība nevar nodrošināt humusvielu sorbciju un humusvielu sorbciju uz kvarca vispirms nosaka hidrofobā mijiedarbība (Aven and Koopal, 1999).

Nozīmīgi augsnes vides ingredienti ir arī tādi minerāli kā karbonātu ieži, tomēr humusvielu sorbcijas raksturs uz tiem praktiski nav pētīts. Augsnes vidi veido arī organiskā viela (detrīts) un mikroorganismi (sēnes, raugi, baktērijas). Humusvielu sorbcijas raksturs uz šiem dzīvajiem organismiem pētīts, pēdējā laikā izmantojot rūpnieciski kultivētu baktēriju biomasu.

Vairākos pētījumos pierādīts, ka humusvielu sorbcijas procesā uz minerālu virsmas notiek to frakcionēšana, jo vispirms tiek sorbētas hidrofobākās un lielākās (ar augstāku molekulmasu) humusvielu frakcijas (Dunnivant et al., 1992; Meier et al., 1999). Šāds pat secinājums izdarīts, pētot daudzu dažādas izcelsmes humusvielu sorbciju uz getīta virsmas (Kaiser, 2003). Jāatzīmē tas, ka galvenais humusvielu sorbcijas efektivitātes determinants ir to hidrofobums, respektīvi, aromātisko struktūru daudzums to molekulās.

Tajā pat laikā nepieciešams atzīmēt, ka veiktie pētījumi nav līdzīgi sorbcijas procesiem dabas vidē, galvenokārt ņemot vērā praktiski jebkuras augsnes sarežģīto uzbūvi, kā arī to, ka augsnes dabiski elementi ir dažādas organiskās vielas (to skaitā arī humusvielas) un ka augsnes vidē vienlaikus ar sorbcijas procesiem notiek augsnes sastāvā esošo vielu desorbcija.

Humusvielu un augsni veidojošo minerālu mijiedarbības rakstura izpēte ir īpaši būtiska, pētot pazemes ūdeņu mākslīgās papildināšanas procesus (Bower, 2002). Pazemes ūdeņu mākslīgā papildināšana nodrošina virszemes ūdeņos esošo piesārņojošo vielu saistīšanu, tiem filtrējoties cauri pietiekami biezam augsnes/nogulumu/iežu slānim, lai tiktu saistīti ūdeņu nevēlamie ingredienti un piesārņojošās vielas. Šī tehnoloģija tika izstrādāta vairāk nekā pirms 150 gadiem, lai attīrītu ūdeni no tajā esošajiem mikroorganismiem un suspendētajām vielām. Mūsdienās izvirzās prasība, lai pazemes ūdeņu mākslīgās papildināšanas gaitā tiktu saistītas arī ūdeņu humusvielas, jo tās ir trihalometānu prekursori (Rook, 1974), kā arī lai tiktu saistītas vielas, kas var nodrošināt daudzu piesārņojošo vielu transportu (Tan, 2003). Izmantojot modeļkolonnas, ir pierādīts, ka humusvielu sorbcija uz infiltrācijas baseinus veidojošajiem iežiem ir visai lēna, bet filtrācijas procesā notiek humusvielu molekulmasas un īpašību izmaiņas (Dunnivant et al., 1992). To nosaka to hidrofobākās frakcijas intensīvāka sorbcija uz minerālu virsmas. Pētījumi par humusvielu aizturēšanas efektivitāti pazemes ūdeņu mākslīgās papildināšanas gaitā ir visai pretrunīgi: ir atzīmēta visai efektīva humusvielu aizturēšana infiltrācijas baseina augšējos slāņos (Alborzfar et al., 2001) un arī baseinu veidojošo iežu masā (Miettinen et al., 1994), savukārt citos pētījumos atzīmēta mazāka (~15%) humusvielu koncentrācijas samazināšanās infiltrācijas procesa gaitā (Hanson, 2000) vai pat to koncentrācijas pieaugums. Lai gan humusvielu aizturēšanas mehānisms infiltrācijas gaitā nav ticis īpaši pētīts, tiek uzskatīts, ka tas ir līdzīgs humusvielu sorbcijai uz augsni veidojošajiem iežiem (Fryckland and Jack, 1997).

2. Pētījuma materiāli un metodes

Detalizēti izmantoto humusvielu izpētes un imobilizācijas metožu apraksti sniegti disertācijas rezultātu publikācijās (Kļaviņš et al., 2000; Kļaviņš and Eglite, 2002); Eglite and Kļaviņš, 2002; Rozenbaha et al., 2002; Juhna et al., 2003; Eglīte et al., 2003; Klavins et al., 2006; Peuravuori et al., 2005).

Humusvielas tika izdalītas no Latvijas purvu kūdras, kas pārstāv dažādus kūdras tipus un to veidošanās mehānismus. Augsnes paraugi tika ievākti dažādās Latvijas vietās no augsnes augšējā horizonta. Izvēlētie augšņu paraugi (dažām augsnēm tika ievākti vairāki paraugi) no visas Latvijas teritorijas pamatā ataino visus Latvijā sastopamos augšņu tipus. Augsnes tika klasificētas, pamatojoties uz Latvijas augšņu klasifikācijas sistēmu (Kārkliņš, 1995). Organiskā oglekļa saturs tika noteikts, izmantojot Volklija-Bleka (Walkley-Black) metodi (Tan, 2005). Augšņu un kūdras pH noteikts 0,1 M KCl šķīduma ekstraktā.

Humusvielas tika izdalītas un attīrītas pēc Starptautiskās Humusvielu biedrības ieteiktajām metodēm (Stevenson, 1994). Kilograms gaisa sausas augsnes vai kūdras tiek izsijāts caur 2 mm sietu, apliets ar 10 litriem 0,1 M HCl šķīduma un izturēts 1 stundu. Pēc masas nostāvēšanās un atdzišanas ūdens fāze tiek dekantēta. Atlikušajai masai pielej apmēram 1 l ūdens un atstāj uz 30 minūtēm. Pēc tam, pievienojot 1 M NaOH, panāk, ka vides pH ir 7. Turpmāk vides pH uzturēšana notiek N_2 atmosfērā, pievienojot 0,1 M NaOH, līdz kopējais tilpums sasniedz 10 litrus, un maisa 24 stundas. Pēc tam sārmaino masu filtrē caur stiklšķiedras vati vai audumu un filtrātu paskābina ar 6 M HCl, līdz pH ir 1. Iegūto šķīdumu nostādina, tad filtrē vai centrifugē. Atdalīto fāzi, kas satur humīnskābes, skalo ar destilētu ūdeni, atkārtoti centrifugējot un nolejot šķidro fāzi. Cietais atlikums pēc centrifugēšanas tiek suspendēts 0,1 M HCl un 0,3 M HF maisījumā, lai atbrīvotos no minerālajām daļiņām. Šis process tiek atkārtots, līdz pelnu saturs nepārsniedz 2%. Pēc tam humīnskābju dispersija destilētā ūdenī tiek dializēta, lai attīrītu no neorganisko sāļu piemaisījumiem, un pēc filtrēšanas – izžāvēta.

Fulvoskābju iegūšanai tika izmantota dzidrā brūni krāsotā šķidrā fāze, kas iegūta pēc humusvielu izgulsnēšanas ar sālsskābi. Iegūtais fulvoskābju šķīdums tika pārvērsts skābes formā (H^+), laižot cauri stipra katjonīta KY-23 kolonnai, un sausā veidā izdalīts, liofili žāvējot Lai nodrošinātu reprezentatīvus paraugus tālākai analīzei, tika veiktas divas ekstrakcijas no viena un tā paša augsnes parauga. Humīnskābes un fulvoskābes no ūdens tika izdalītas pēc Turmana un Malkolma (Thurman and Malcolm, 1981) metodes. Salīdzinājumam tika izmantotas četras rūpnieciski ražotas humīnskābes (Aldrich Chemical Company, Arctech Inc, IHSS, Tehum), un tad humīnskābes un fulvoskābes tika šādi analizētas:

1. Elementanalīze: C, H, N un pelnu saturs tika noteikts ar Carlo Erba Elemental Analyser EA-1108. Skābekļa koncentrācija tika aprēķināta kā starpība.
2. Funkcionālo grupu saturs tika noteikts, izmantojot standartmetodes (Tan, 2005).
3. Molekulmasu sadalījums tika noteikts, izmantojot gelfiltrāciju uz Sephadex G-100 (frakcija 40–120 μm) kolonnā (1×40 cm). Eluents 0,01 M Tris-HCl buferšķīdums (pH 9,0) tika sūknēts cauri kolonnai ar ātrumu 1 ml·min^{-1}, sakrājot 1 ml frakcijas. 25 mg humusvielas tika uznesti uz kolonnas nesēja kā 1%

šķīdums 0,1 M NaOH. Kolonnas kalibrēšanai tika izmantots proteīnu standartu komplekts. Kolonnas brīvais tilpums tika noteikts, izmantojot zilo dekstrānu 2000000 (Blue Dextran). Noteikšana tika veikta pie 280 nm.

4. UV-Vis spektri tika uzņemti 200–700 nm apgabalā, izmantojot 1 cm kvarca kivetes ar ThermoSpectronic Heλios γ 50 $mg \cdot l^{-1}$FA šķīdumam un 33 $mg \cdot l^{-1}$HA 0,05 N $NaHCO_3$ (pH 8–9).
5. Attiecība E_4/E_6 tika mērīta, kā norādīts literatūrā (Chin *et al.* 1994). Humusvielu paraugi tika izšķīdināti 0,05 M $NaHCO_3$ un gaismas absorbcija mērīta pie 465 un 665 nm pret šķīdinātāju 0,05 M $NaHCO_3$ ar spektrofotometru HACH 2000.
6. Fluorescences spektri tika uzņemti, izmantojot Hitachi 850 fluorescences spektrometru humusvielu ūdens šķīdumiem ar koncentrāciju 100 $mg \cdot l^{-1}$, panākot pH 8 ar 0,05 M NaOH. Emisijas spektri tika reģistrēti viļņu garumu apgabalā 360–640 nm (skanēšanas ātrums 60 nm/min, atbildes laiks 2 s) ar konstantu ierosinošo viļņu garumu 335 nm.
7. Humusvielu Furjē transformācijas infrasarkanie spektri (FT-IS) tika uzņemti ar Galaxy 6030 FT-IS spektrofotometru (Mattson Instruments), paraugus sagatavojot KBr tabletē. Apmēram 1,5 mg eksikatorā izžāvētu humusvielu un 200 mg KBr pulvera tika kopā samalti un hidrauliski saspiesti tabletē (13×0,5 mm). Sapresētās KBr tabletes tika žāvētas eksikatorā līdz analīžu veikšanai, lai samazinātu absorbētā ūdens ietekmi. FT-IS spektri tika uzņemti no 4000 līdz 400 cm^{-1}.
8. Elektronu paramagnētiskās rezonanses spektri (EPR) tika uzņemti, izmantojot RE-1306 spektrometru ar 100 kHz magnētiskā lauka modulāciju.
9. Humusvielu struktūras pētījumi tika veikti ar pirolīzes–gāzu hromatogrāfijas/masspektrometrijas (Py-GH-MS) metodi. Paraugs, ievietots kvarca turētājā, tika metilēts 10 min ar tetrametilamonija hidroksīda (TMAH) 25% ūdens šķīdumu 100 ºC temperatūrā un pēc tam pirolizēts 10 s 600 ºC CDS Pyroprobe 1000 pirolīzes iekārtā, kas tieši savienota ar gāzu hromatogrāfijas injektoru (karsēšanas ātrums 15,00 ºC m/s) He plūsmā. Pirolīzes produkti tika 5 min uzkrāti tērauda uztvērējā, kas tika dzesēts šķidrajā slāpeklī. Pēc tam uztvērējs tika pievienots GH/MS kapilārajai kolonnai (NB-1701, 50 m, iekšējais diametrs 0,32 mm) un tika uzņemta hromatogramma, bet pirolīzes produkti tika detektēti un identificēti, uzņemot to masspektrus.
10. Protonu kodolmagnētiskās rezonanses (^{1}H-KMR) spektri tika uzņemti ar Jeol GX 400 KMR spektrometru. 10 mg analizējamās humusvielas tika izšķīdinātas 0,5 ml D_2O, pievienojot dažus pilienus NaOD, tad paraugs tika filtrēts un tika uzņemti ^{1}H-KMR spektri, pie 400 MHz, uzstādot šādus spektra reģistrācijas apstākļus: detektēšanas laiks 4,68 s, skanēšanas reižu skaits – 200.
11. Oglekļa 13 krospolarizācijas kodolmagnētiskās rezonanses spektri (^{13}C-KPML-KMR), kas uzņemti zem maģiskā leņķa, tika iegūti, izmantojot Bruker DSX300 NMR spektrometru ar 7 mm Zr rotoru un 4 kHz rotācijas ātrumu. Spektra uzņemšana tika veikta 200 mg paraugam.

3. Rezultāti un to apspriešana

3.1. Pētījumā izmantoto humusvielu īpašības un raksturojums

Disertācijas izstrādes gaitā no Latvijas dabas vides (augsnes, kūdras, ūdeņiem, (6. tabula), izmantojot standartizētas Starptautiskās Humusvielu pētnieku savienības rekomendētas metodes, laikā no 1998. līdz 2003. gadam preparatīvos daudzumos (5–250 g) tika izdalīti humusvielu paraugi – humīnskābes. Tā kā humusvielu uzbūvi raksturo lielmolekulāra un heterogēna struktūra, to raksturošanai izmantotas dažādas fizikāli ķīmiskās analīzes metodes. Visas humusvielas, kas izdalītas no Latvijas vides, un rūpnieciski ražotās humusvielas tika analizētas, izmantojot gan spektroskopiskās analīzes metodes, gan arī molekulu struktūru sagraujošas analīzes metodes. Pie izmantotajām spektrometriskajām analīzes metodēm var minēt infrasarkanā un ultravioletā starojuma spektrometriju, kā arī protonu un oglekļa kodolmagnētiskās rezonanses spektrometriju. Humusvielu elementu (C, H, N) saturs tika noteikts, izmantojot CHN analizatoru. Savukārt ieskatu humusvielu struktūras uzbūvē deva to analīze, kas veikta, izmantojot pirolīzes–gāzu hromatogrāfu/masspektrometru. Šīs analīzes metodes gaitā humusvielas tika sadalītas atsevišķos to struktūras fragmentos pirolīzes kamerā un radušies mazmolekulārie savienojumi tika atdalīti gāzes hromatogrāfā, produktu struktūras tika noteiktas ar masspektrometrijas detektoru.

6. tabula / Table 6

Pētīto humīnskābju izcelsme
Origin of investigated humic acids

Vides, no kuras humīnskābes izdalītas	Humīnskābju saīsināti apzīmējumi
Daugava (lejpus Rīgas HES)	Daugava
Islienas ezers	Isliena
Siksalas ezers	Siksala
Salaca (Mērnieki)	Salaca
Baltezera pazemes ūdeņi, kvartārnogulumi no 18 m dziļuma	Baltezers kv
Baltezers pazemes ūdeņi, devona nogulumi no 64 m dziļuma	Baltezers de
Inčupe, pie ietekas jūrā	Inčupe
Irkutskas (Krievija) purvu kūdra	Irkutska
Kačoru purva kūdra	Kačoru
Olaines purva kūdra	Olaine
Bohēmijas (Čehija) oksihumolīts	Tehum
Augsnes standartpreparāts	Aldrich

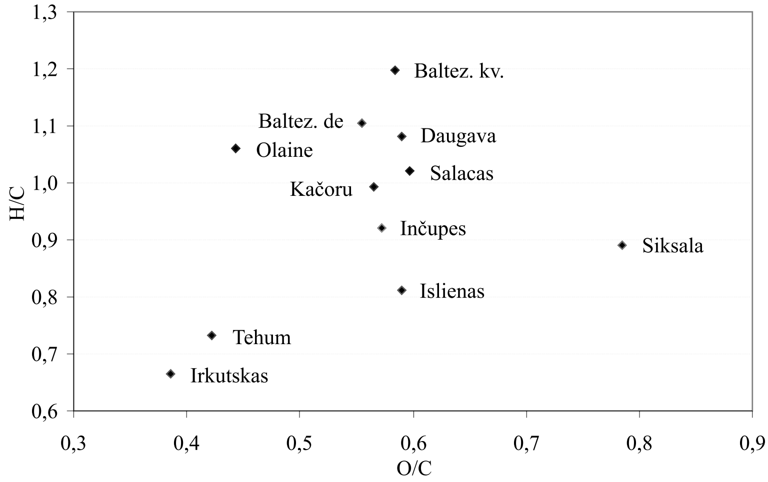
Analizējot no Latvijas vides izdalīto humīnskābju elementsastāvu (7. tabula), kā arī komerciāli ražotās humusvielas, iegūtie rezultāti labi saskan ar literatūrā norādītajiem (Stevenson, 1994; Kļaviņš, 1998). Oglekļa saturs mainās no 50 līdz 60%, un mazāks tas ir humīnskābēs, kas izdalītas no ūdens vides. Nedaudz lielāks oglekļa saturs ir no kūdras izdalītajās un komerciāli ražotajās humusvielās. Ūdeņraža saturs humusvielu uzbūvē sastāda 4–6%. Slāpekļa saturs humusvielās ir atkarīgs no konkrētās humusvielas izcelsmes avota. Upju ūdeņu humīnskābēs slāpekļa saturs ir nedaudz augstāks, salīdzinot ar purvu ezeru ūdeņu humīnskābēm. Tas varētu būt izskaidrojams ar noteci no lauksaimniecības zemēm un atšķirībām ūdeņu organisko vielu veidošanās procesos. Abi ezeri (Islienas un Siksalas), no kuriem tika izdalītas un analizētas humīnskābes, atrodas mežu ieskautā apvidū Teiču rezervāta teritorijā, kur ūdens sastāvu maz ietekmē lauksaimnieciskā darbība. Skābekļa saturs analizētajos paraugos tika aprēķināts kā starpība starp parauga masu (ņemot vērā mitrumu un pelnu saturu) un elementanalīzes gaitā noteikto oglekļa, slāpekļa un ūdeņraža saturu.

7. tabula / Table 7

Pētīto humīnskābju elementsastāvs
Elemental composition of investigated humic acids

Humīnskābes	C, %	H, %	N, %	O, %
Daugava	52,1	4,7	2,2	41,0
Isliena	53,4	3,6	1,0	42,0
Siksala	46,8	3,5	0,8	48,9
Salaca	51,9	4,5	2,3	41,3
Baltezers kv	51,6	5,2	3,0	40,2
Baltezers de	53,3	5,0	2,4	39,3
Inčupe	53,6	4,2	1,3	40,9
Irkutska	62,7	3,5	1,7	32,1
Kačoru	53,0	4,4	2,6	40,0
Olaine	57,6	5,1	3,2	34,1
Tehum	60,7	3,7	1,5	34,1
Aldrich	49,9	4,7	2,3	43,1

Humusvielu elementsastāva attiecību grafisku attēlojumu (Van Krevelena grafikus) plaši izmanto ne tikai fosilo nogulumu raksturošanā, bet arī humusvielu izbūves izpētē (Barancikova et al., 1997; Ziechman, 1994).



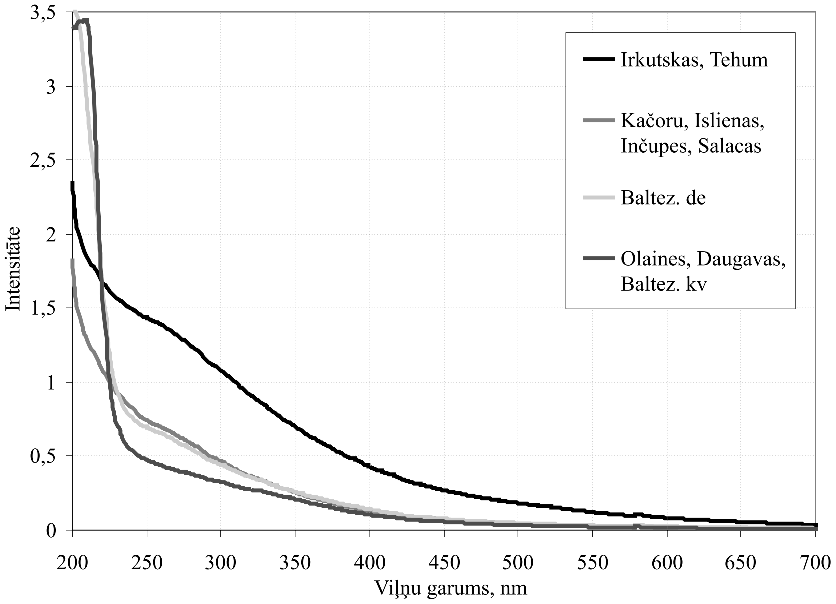
14. attēls. **Humīnskābju van Krevelena diagramma**
Figure 14. **Van Krevelen diagram of humic acids**

Humusvielu transformācijas pakāpi var raksturot, izmantojot elementsastāva attiecības:

- **H/C** attiecību. Ar šīs attiecības palīdzību var noteikt organiskā materiāla avotu. Jūras organismi un aļģes, piemēram, pamatā ir veidoti no taukiem un proteīniem bagāta organiska materiāla, kur attiecība H pret C ir augstāka nekā ar ogļhidrātiem bagātajos sauszemes augus veidojošajos savienojumos.
- **O/C** attiecība. Šī attiecība ir augsta ar polisaharīdiem bagātajās sauszemes augu atliekās.

Šīs attiecības ievietojot grafikā, var iegūt van Krevelena diagrammas (14. attēls). Pētīto humusvielu izvietojums van Krevelena diagrammās uzrāda rūpnieciski ražoto humīnskābju paraugu un ūdeņu humīnskābju ievērojamu līdzību, savukārt purvu ūdeņu un pazemes ūdeņu humīnskābes atrodas starpstāvoklī.

Humusvielu raksturošanai plaši izmanto spektrālās analīzes metodes, lai gan katrs atsevišķi ņemts paraugs sniedz ierobežotu informāciju par humusvielu uzbūvi un īpašībām.



15. attēls. **Humusvielu paraugu spektri ultravioletajā un redzamās gaismas apgabalā**
Figure 15. **UV/Vis spectra of humic substances**

Humusvielu šķīdumu sorbcija samazinās, palielinoties viļņu garumam (15. attēls). Pēc UV/Vis spektra formas tos var nosacīti iedalīt trīs grupās:

- ***I*** grupas humusvielu šķīdumiem ir augsta gaismas absorbcijas intensitāte īso viļņu apgabalā no 200 līdz 215 nm, bet tā strauji samazinās līdz 230 nm, tālāk absorbcija samazinās pakāpeniski, spektra daļā no 240 līdz 300 nm novērojams plecs, kas, iespējams, liecina par hinoīdu struktūru klātbūtni. Šajā grupā ietilpst humusvielas, kas izdalītas no Daugavas ūdens, no Baltezera gruntsūdeņu mākslīgās papildināšanas akām devona un kvartāra iežu slānī, kā arī no Olaines kūdras. Vairāk atšķiras humīnskābes, kas izdalītas no Baltezera devona slānī, jo šīm humusvielām ir izteiktāks absorbcijas plecs 240–300 nm apgabalā un, palielinoties viļņu garumam, absorbcija samazinās mazāk nekā pārējām minētajām humusvielām.

- ***II*** grupas humusvielām spektrā īso viļņu apgabalā absorbcijas intensitātes samazināšanās nav tik ļoti izteikta kā pirmās grupas humusvielām, savukārt spektra apgabalā no 240 līdz 325 nm ir novērojams izteikts maksimums un, palielinoties viļņu garumam, absorbcijas intensitāte samazinās pakāpeniski. Šai grupai pieder rūpnieciski ražotās humīnskābes (Irkutskas un Tehum).
- ***III*** grupas humusvielām spektra īso viļņu apgabalā absorbcija strauji samazinās, 225–325 nm apgabalā sorbcijas intensitātes kritums kļūst lēzenāks, bet nav izteikta absorbcijas maksimuma. Tālāk spektra daļa ir ļoti līdzīga pirmās grupas humusvielām. Pie trešās grupas var pieskaitīt humīnskābes, kas izdalītas no Islienas ezera, Inčupes un Salacas upes ūdeņiem, kā arī no Kačoru purva kūdras.

Humusvielu raksturošanai plaši izmanto absorbcijas attiecību 465/665 nm (E4/E6). Šī attiecība nav atkarīga no humusvielu koncentrācijas šķīdumā, bet tā samazinās, pieaugot humusvielu molekulu izmēram, līdz ar to liecinot par polikondensācijas pakāpes palielināšanos (Chin et al., 1994). Tas uzskatāmi parādās 8. tabulā. No ūdens izdalītajām humīnskābēm šī attiecība ir ievērojami lielāka nekā kūdras humīnskābēm.

8. tabula / Table 8

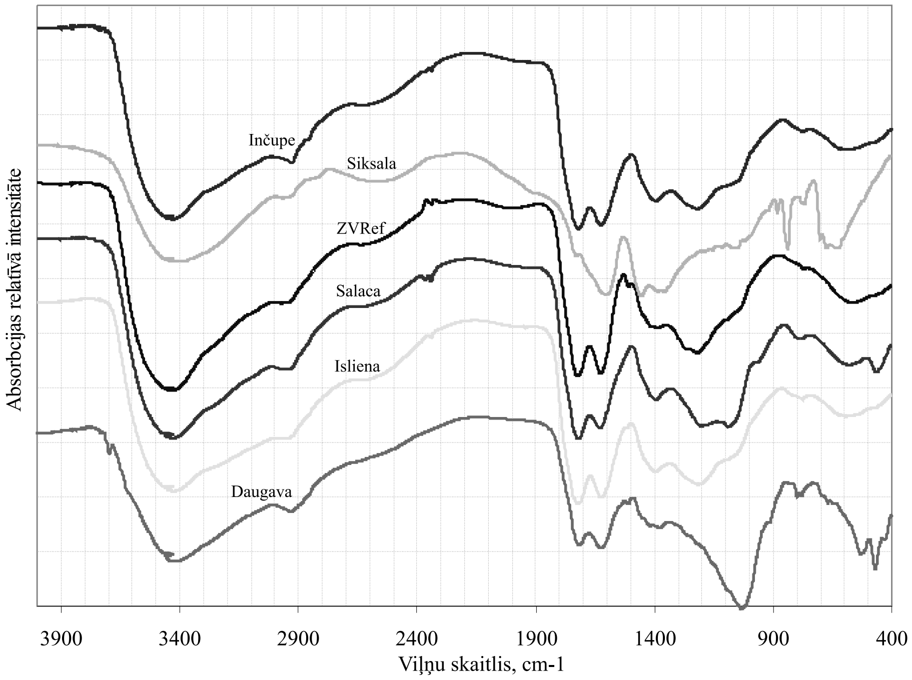
Humīnskābju šķīdumu absorbciju attiecība E4/E6
Adsorption ratio E4/E6 of solution of humic acids

Humīnskābes	E4/E6
Daugava	8,97
Isliena	6,22
Salaca	7,79
Baltezers kv.	10,38
Baltezers dev.	7,27
Inčupe	6,09
Irkutska	4,17
Kačoru	4,24
Olaine	5,95
Tehum	4,29

Gan no ūdens, gan arī no kūdras izdalīto un pētīto humīnskābju FT-IS spektri savstarpēji ir līdzīgi (16., 17. attēls), jo daudzi humusvielu struktūrelementi un to funkcionālās grupas ir vienādas un humusvielu izcelsme tos ietekmē relatīvi maz.

Visu humīnskābju FT-IS spektros (9. tabula) nozīmīgākie maksimumi ir spektra daļā no 3700 līdz 3300 cm^{-1}, kas raksturīga hidroksilgrupām, kas atrodas vai nu brīvā formā, vai saistītas ar ūdeņraža saitēm. Atšķirības starp humusvielām izpaužas tikai kā šī maksimuma intensitāte attiecībā pret citiem FT-IS spektra maksimumiem. Slāpekļa saturs humīnskābēs parasti ir zems, tādēļ arī izteikti amīnu un amīdu absorbcijas maksimumi FT-IS spektros nav novērojami. Absorbciju 2950–2850 cm^{-1} rada alifātiskās metilgrupu un metilēngrupu C-H valences svārstības, un šī josla vairāk izteikta ir humusvielām, kas izdalītas no kūdras. Relatīvi intensīva absorbcija šajā spektra daļā ir īpaši izteikta

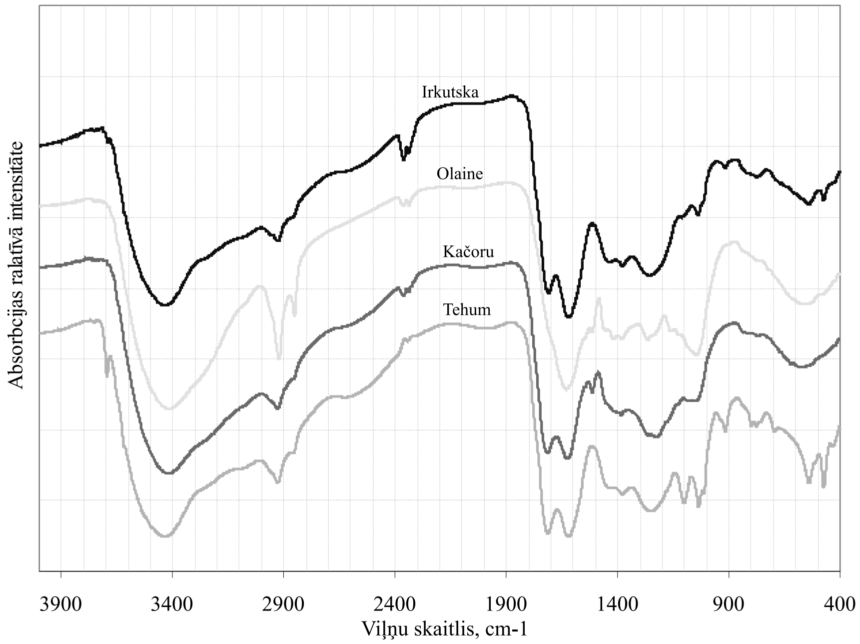
Olaines kūdras HS, kas saskaņā ar humīnskābju struktūras pētījumiem apstiprina to, ka šajās no kūdras izdalītajās humusvielās ir lielāks aromātisku struktūru saturs nekā humusvielās, kas izdalītas no ūdens (Kļaviņš, 1998). Spektra maksimums, kas raksturīgs karboksilātjonu klātbūtnei (2850–2500 cm^{-1}), skaidrāk ir redzams visu no ūdens izdalīto humīnskābju spektros. Visizteiktākie šie maksimumi ir no Siksalas ezera izdalīto humīnskābju spektrā, kā arī Islienas ezera humīnskābju parauga spektrā. Karbonskābju C=O valences svārstības (1725–1640 cm^{-1}) ūdens humusvielu spektros ir ar lielāku intensitāti, savukārt kūdras humīnskābju paraugiem izteiktāks absorbcijas maksimums ir tieši ketonu un aldehīdu C=O dubultsaišu svārstību dēļ gan cikliskos savienojumos, gan arī ketonos un hinonos (1640–1585 cm^{-1}).



16. attēls. **No Latvijas upēm un ezeriem izdalīto humīnskābju un Ziemeļvalstu references humīnskābju paraugu FT-IS spektri**
Figure 16. **FT-IR spectra of aquatic humic acids from lakes and rivers of Latvia and Nordic lake reference sample**

Gandrīz visos ūdeņu humusvielu spektros ir neizteikts absorbcijas maksimums, kas raksturīgs benzolu un piridīnu oglekļa dubultsaišu deformācijas svārstībām (1515 cm^{-1}), bet šis nelielais maksimums ir novērojams arī divos no kūdras izdalīto Kačoru un Olaines purva kūdras humīnskābju paraugu FT-IS spektros. Reģionā zem 1500 cm^{-1} humīnskābju IS spektru ir grūti interpretēt, jo šajā reģionā raksturīgās spektra līnijas var piederēt ļoti dažādām struktūrelementu grupām. Neliels absorbcijas maksimums pie 1400 cm^{-1} ir novērojams visos analizētājos humīnskābju paraugu spektros, tas varētu būt raksturīgs hidroksilgrupu deformācijām un C-O valences svārstībām fenolu OH grupās.

Gan ūdeņu, gan kūdras humīnskābju spektros ir novērojama absorbcijas josla 1200 cm^{-1} apgabalā, kas raksturīga C-O valences svārstībām un O-H deformācijas svārstībām COOH grupās, kā arī 1050 cm^{-1} apgabalā redzama spirtos vai ogļhidrātos esošo C-O grupu valences svārstību josla.



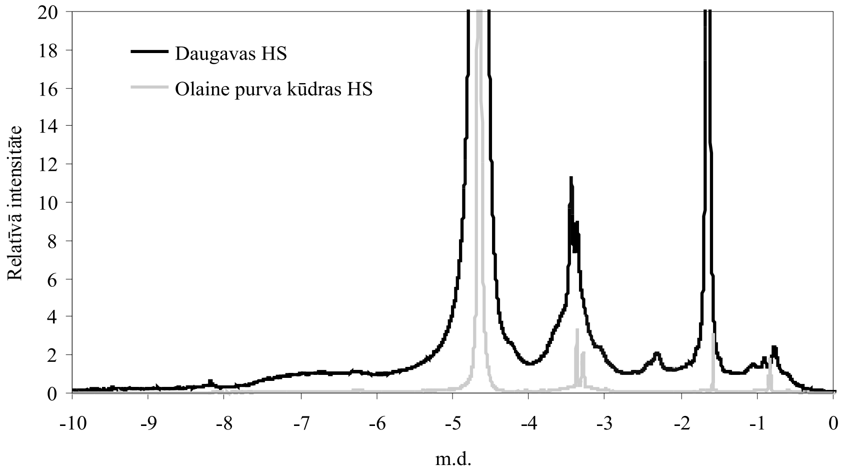
17. attēls. **No purva kūdras izdalīto un rūpnieciski ražoto humīnskābju FT-IS spektri**
Figure 17. **FT-IR spectra of humic substances isolated from peat and commercial humic acid**

Humusvielu šķīdumu protonu kodolmagnētiskās rezonanses spektros (18. attēls), ņemot vērā HV komplicēto uzbūvi, neparādās izteikti maksimumi, kas būtu izmantojami to struktūras atšifrēšanai. Tomēr ir iespējams relatīvi novērtēt, kādās struktūrās humusvielu molekulā esošie ūdeņraža atomi ir iesaistīti (10., 11. tabula). Pirmais spektra apgabals no 0–3,0 m.d. atbilst alifātiskajiem protoniem, tai skaitā apgabalā no 2,0–2,8 m.d. protoni ir saistīti ar elektronegatīviem atomiem (O vai N). Rezonanses maksimumu no 0,9 līdz 1,3 m.d. var attiecināt uz protoniem metilgrupās sazarotās alifātiskās struktūrās un metilgrupās alifātiskās virknēs. Pētītajās humusvielās intensīvākā sorbcija šajos spektra apgabalos ir raksturīga ūdeņu humusvielām. Spektra apgabalā 3,0–4,5 m.d. rezonē protoni, kas saistīti ar oglekļa atomiem, kas savukārt saistīti ar skābekli. Šādi rezonanses signāli raksturīgi ogļhidrātiem, poliēteriem vai metoksigrupu protoniem. Intensīvāki signāli no pētītajām humusvielām raksturīgi kūdras un ūdeņu humusvielām, bet rūpnieciski ražoto humusvielu paraugos tieši šo signālu intensitāte ir ļoti zema. Visbeidzot trešo protonu kodolmagnētiskās rezonanses spektra maksimumu 6,0–8,5 m.d. var attiecināt uz ūdeņradi, kas atrodas heterocikliskos un augsti aizvietotos aromātiskos gredzenos. Signāla intensitāte šajā reģionā ir maz izteikta pazemes un Inčupes ūdeņu humusvielām.

9. tabula / Table 9

Galvenās humusvielu absorbciju līnijas to FT-IS spektros
(pēc Aiken et al., 1985; Sposito, 1989)
Typical FT-IR spectra sorption lines for humic substances
(after Aiken et al., 1985; Sposito, 1989)

Viļņu skaitlis (cm^{-1})	Raksturojums
3670–3300	Ar ūdeņraža saitēm saistītas OH grupas, brīvās OH
3077–3030	Aromātiskās C–H valences svārstības
2950–2850	Alifātiskās C–H, CH$_2$, CH$_3$ valences svārstības
2850–2500	Karboksilātjoni
1725–1640	karbonskābju C=O valences svārstības
1640–1585	C=O dubultsaišu deformācijas svārstības cikliskos un acikliskos savienojumos, ketonos un hinonos
1515	Benzola un piridīna gredzenu C=C deformācijas svārstības
1470–1420	Alifātiskās C–H deformācijas svārstības
1400–1390	OH deformācijas un C–O valences svārstības fenolu OH, C–H deformācijas CH$_3$ grupās
1390–1332	Karbonskābju sāļu C=O
1280–1137	C–O esteru, ēteru un fenolu valences svārstības
1090–1040	C–O spirtu un polisaharīdu valences svārstības
880–750	Ar ūdeņraža saitēm saistītās OH grupu deformācijas svārstības karboksilgrupās
650–510	COOH deformācijas svārstības



18. attēls. **Protonu kodolmagnētiskās rezonanses (^{1}H-KMR) spektri humīnskābēm, kas izdalītas no Daugavas ūdens un Olaines purva kūdras**
Figure 18. **^{1}H-NMR spectra of humic acid isolated from water of River Daugava and peat from bog Olaine**

10. tabula / Table 10

^{1}H-KMR spektru raksturīgie reģioni
Typical regions in ^{1}H-NMR spectra

Ķīmiskā nobīde, m.d.	Protona pozīcija
0-2	H-C-C
~2	H-C-C=O
~3	H-C-N
~4	H-C-O
~5	H-C=C
6-7	H-aromātiskais
~9	H-C=O
~12	H-O-C=O

11. tabula / Table 11

Signālu intensitātes relatīvais sadalījums (%) humusvielu protonu kodolmagnētiskās rezonanses (^{1}H-KMR) spektros
Relative distributions of signal intensity (%) in ^{1}H-NMR spectra of humic substances

Humīnskābes	5,6 – 8 m.d.	2,8 – 4,3 m.d.	1,4 – 2,8 m.d.	0 – 1,4 m.d.
Daugava	14,2	34,4	42,3	9,2
Isliena	24,8	32,6	29,2	13,4
Siksala	21,9	31,6	31,2	15,3
Salaca	17,7	32,5	31,4	18,4
Baltezers kv	9,9	34,7	43,1	12,3
Baltezers de	15,0	39,0	24,4	21,6
Inčupe	6,6	41,9	37,9	13,6
Irkutska	33,9	18,4	24,0	23,7
Kačoru	15,2	42,8	24,3	17,6
Olaine	22,9	42,9	16,8	17,4
Tehum	31,8	16,3	27,3	24,6

Ievērojami vairāk informācijas par humusvielu veidoj ošo struktūru sadalījumu sniedz ^{13}C kodolmagnētiskās rezonanses spektri (19., 20. attēls), kas uzņemti zem “maģiskā” leņķa.

12. tabula / Table 12

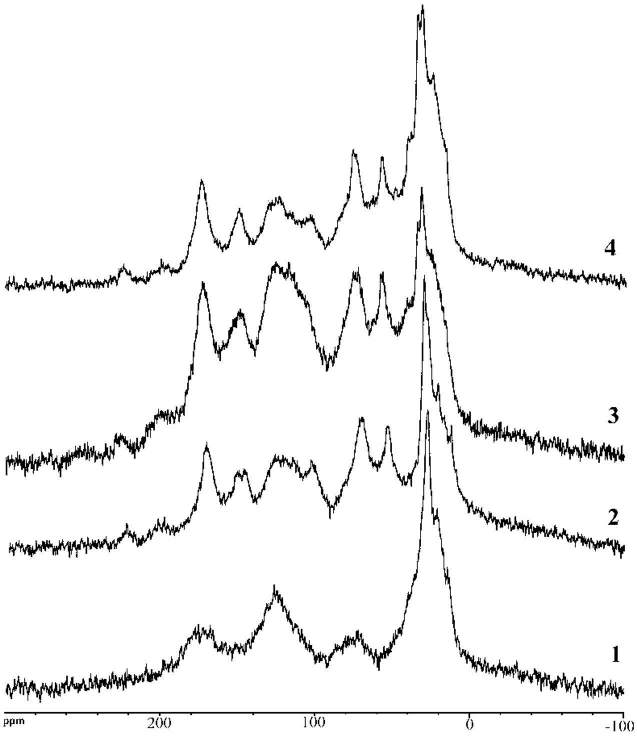
^{13}C-KMR spektru raksturīgie reģioni (Shin et al., 1999)
Typical regions in ^{13}C -NMR spectra

Ķīmiskā nobīde, m.d.	Oglekļa savienojuma veids
0–50	Alifātiskais ogleklis vai ogleklis, kas saistīts tikai ar citiem oglekļa atomiem
50–110	C-O, C-N saites ogļhidrātos, spirtos, esteros un amīnos
110–160	Oglekļa atomi, kas atrodas aromātiskās un fenolu struktūrās
160–190	Oglekļa atomi, kas atrodas karboksilstruktūrās, esteru un amīdu struktūrās
190–215	Oglekļa atomi, kas atrodas karbonilgrupu, ketonu, aldehīdu struktūrās

13. tabula / Table 13

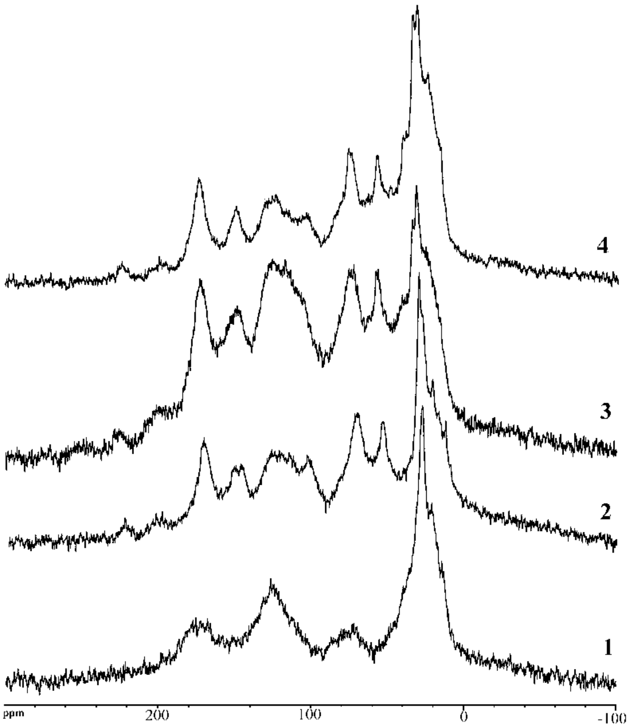
Signālu intensitātes relatīvais sadalījums (%) humusvielu oglekļa kodolmagnētiskās rezonanses (^{13}C-KMR) spektros
Relative distributions of signal intensity (%) in ^{13}C -NMR spectra of humic substances

Humīnskābes	Ķīmiskā nobīde, m. d.				
	0–50	50–100	100–160	160–190	190–215
Daugava	15	44	29	7	5
Baltezers kv.	10	53	25	8	4
Baltezers dev.	14	43	28	11	4
Irkutska	30	15	31	21	2
Kačoru	24	22	39	11	4
Olaine	32	25	30	11	3
Tehum	45	12	26	13	4
Podzolētās augsnes	39	24	24	10	2
Aldrich	52	10	23	11	4



19. attēls. **Oglekļa kodolmagnētiskās rezonanses (^{13}C-KMR) spektri Tehum (1), Olaines purva kūdras (2), Kačoru purva kūdras (3), velēnu podzolētās augsnes (4) humīnskābēm**
Figure 19. **^{13}C-NMR spectra of Tehum (1), Olaine peat (2), Kačoru peat (3), podsolic soil (4) humic acids**

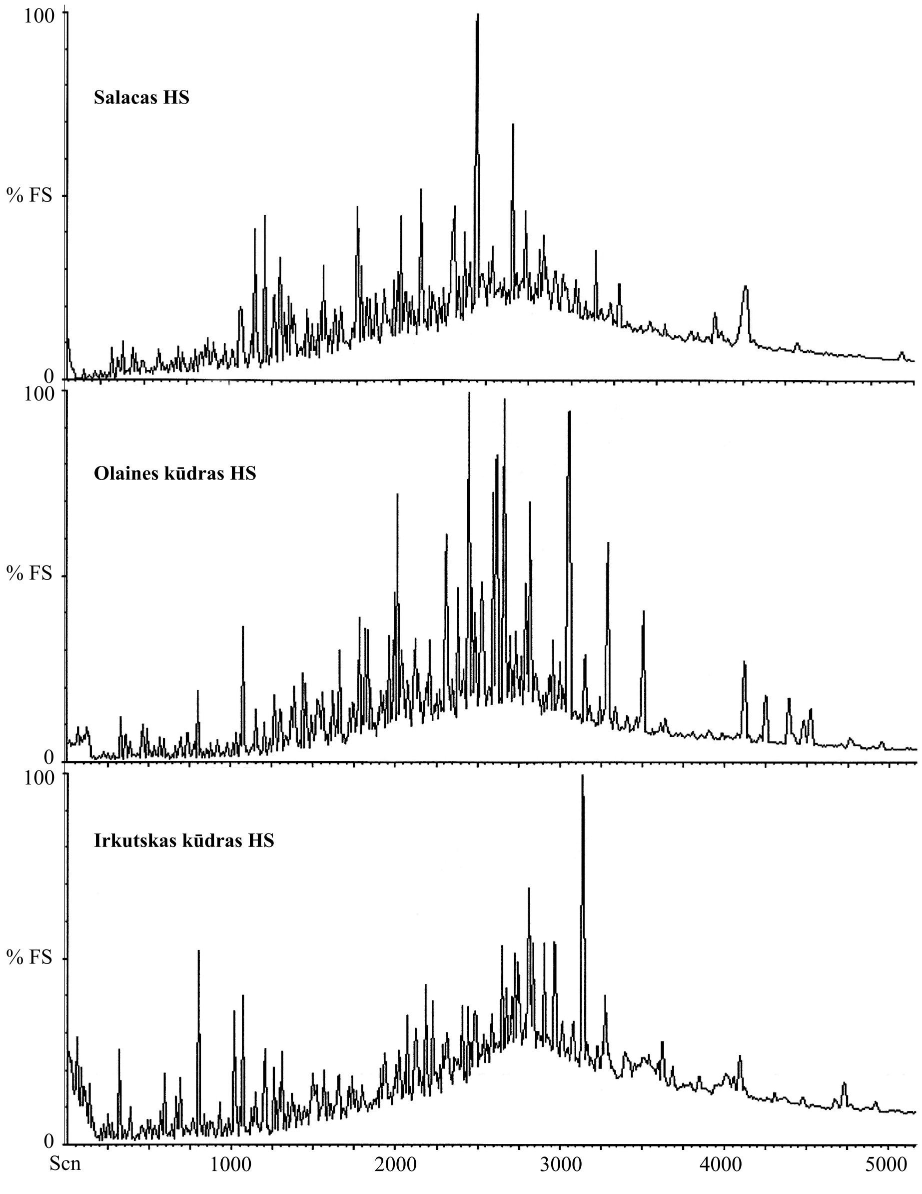
Salīdzinot humusvielu ^{13}C kodolmagnētiskās rezonanses spektru integrēšanas rezultātus (12., 13. tabula), var secināt, ka alifātiski saistīts ogleklis būtiski vairāk ir humusvielās, kas izdalītas no kūdras un no podzolētās augsnes. Savukārt ogleklis, kas saistīts ar skābekļa vai slāpekļa atomiem, relatīvi vairāk ir humīnskābēs, kas izdalītas no ūdens vides. Aromātiski saistītā oglekļa attiecības būtiski nemainās atkarībā no humusvielu izdalīšanas avota. Salīdzinot oglekļa kodolmagnētiskās rezonanses spektra maksimumus 160–190 m. d. apgabalā, var novērot, ka oglekļa atomi, kas veido karboksilgrupas, esterus un amīdus, nedaudz mazāk ir atrodami humusvielās, kas izdalītas no Daugavas ūdens un Baltezera mākslīgās papildināšanas infiltrācijas akām. Visām analizētajām humīnskābēm ir līdzīga relatīvā intensitāte spektra maksimumiem, kuros rezonē oglekļa atomi, kas iesaistīti karbonilgrupu, ketonu un aldehīdu struktūrās.



20. attēls. **Oglekļa kodolmagnētiskās rezonanses (^{13}C-KMR) spektri humīnskābēm, kas izdalītas no Daugavas (1), pazemes ūdens Baltezers kv. (2), Baltezers dev. (3), un komerciāli ražotajām Aldrich (4) humīnskābēm**

Figure 20. **^{13}C-NMR spectra of humic acids isolated from River Daugava (1), Baltezers (Quaternary) groundwater (2), Baltezers (Devonian) groundwater (3) and Aldrich commercially produced humic acid (4)**

Humusvielu struktūras izpētē būtiska loma ir ne tikai destruktīvajām metodēm, bet arī HV uzbūves izpētei, kas veikta, izmantojot destruktīvās pieejas. Pētījuma ietvaros tika izmantota humusvielu pirolīze, pirms tās veicot labilo funkcionālo grupu aizsardzību metilējot (ar tetrametilamonija hidroksīdu – TMHA) un pēc tam iegūto maisījumu analizējot, izmantojot gāzu hromatogrāfiju/masspektrometriju (Peuravuori et al., 2005). Piedāvātā pieeja ļauj samazināt artefaktu veidošanās iespēju un pasargā vispirms fenolu struktūras no to sašķelšanās pirolīzes procesa gaitā, līdz ar to ievērojami uzlabojas iegūto rezultātu ticamība. Pētīto humusvielu pirolīzes gāzu hromatogrāfiju/masspektrometrijas analīze (21. attēls, 14. tabula) uzrāda ievērojamas atšķirības starp humusvielām atkarībā no to izcelsmes. Tā kā izdevās identificēt vairāk nekā 150 dažādus degradācijas produktus, bija iespējams iegūt padziļinātu priekšstatu par humusvielu uzbūvi.



21. attēls. **Salacas ūdens, Olaines un Irkutskas purvu kūdras humusvielu pirolīzes produktu, kas apstrādāti ar TMHA, kopējo jonu hromatogrammas**

Figure 21. **Total ion chromatogram of humic acids isolated from river water Salaca, Olaine and Irkutska peat after TMAH thermo hemolysis**

14. tabula / Table 14

Humusvielu pirolīzes degradācijas produktu relatīvais iznākums, %
Relative yield (%) of pyrolysis degradation products of humic substances

Produkti	Virszemes ūdeņu HS				Pazemes ūdens HS		Kūdras HS		Rūpnieciski ražotās HS	
	Inčupe	Salaca	Isliena	Daugava	Baltez. kv.	Baltez. dev.	Kačoru	Olaine	Irkutska	Tehum
Alkilbenzoli	0,8	2,5	2,3	1,6	0,5	1,3	2,2	1,3	6,9	4,7
Fenoli	5,8	6,0	9,0	8,3	7,2	10,2	6,9	4,7	6,9	1,7
Alkilfenoli	5,6	12,4	10,5	10,0	4,2	6,0	12,5	13,4	10,7	4,6
Aromātiskie aldehīdi un ketoni	–	1,2	1,4	0,8	0,3	0,4	1,5	0,9	–	–
Fenolu aldehīdi un ketoni	5,0	1,7	3,5	5,9	3,7	4,1	8,5	4,7	–	0,2
Benzokarbonskābes	5,0	4,2	4,0	2,9	5,4	2,5	1,4	0,3	9,8	10,6
Alkilbenzokarbonskābes	0,7	2,0	1,0	0,7	–	0,6	1,0	0,3	2,4	2,1
Fenolkarbonskābes	27,7	18,9	24,8	22,0	11,2	14,5	22,0	13,6	6,0	9,4
Alkilfenolkarbonskābes	6,7	1,7	2,5	1,9	1,2	2,7	1,1	–	4,7	5,9
Citas fenolkarbonskābes	3,5	1,7	3,0	4,2	3,0	2,7	14,1	11,2	–	0,2
Poliaromātiskie sav.	–	1,1	0,3	–	–	0,2	1,0	0,1	2,9	5,6
Citi neklasificēti aromātiskie sav.	5,8	3,4	3,7	3,2	3,4	2,7	4,6	4,6	8,5	9,6
Arom. (bifenili)	1,2	0,8	1,1	0,6	0,4	0,4	1,5	0,6	–	0,6
Alkāni	–	–	–	–	–	–	–	0,2	2,9	5,0
Alkēni	–	–	–	–	–	0,3	–	–	2,0	0,5
Spirti	–	–	–	0,2	0,2	0,2	0,1	4,5	1,3	3,8
Alifātiskie aldehīdi un ketoni	0,3	0,3	0,3	0,1	0,2	0,3	0,1	0,7	–	0,5
Alifātiskās monokarbonskābes	4,5	5,9	4,0	6,8	11,3	7,0	7,1	20,9	23,1	21,5
Alifātiskās dikarbonskābes	10,8	14,1	10,1	7,4	3,7	4,6	3,4	6,2	2,4	3,2
Alifātiskās trikarbonskābes	2,7	3,0	2,7	1,8	–	1,9	–	–	–	–
Neklasificēti alifātiskie sav.	0,4	0,4	–	0,4	2,5	0,8	0,7	1,8	1,6	1,9
Furāni	1,8	3,5	3,8	1,9	1,4	2,6	1,7	1,9	1,5	2,8
Alifātiskie cikliskie sav.	0,2	0,4	0,4	0,4	0,4	0,0	0,3	0,1	0,9	1,3
Citi ogļūdeņražu atvasinājumi	–	0,0	–	6,1	8,5	5,4	0,0	1,4	–	0,3
Pirolu atvasinājumi	0,1	0,9	0,5	1,2	1,4	2,9	0,6	0,6	0,4	0,4
Piridīna atvasinājumi	–	–	–	–	0,3	0,6	0,2	–	–	–
Aromātiski N savienojumi	–	0,1	0,1	0,1	0,6	1,1	0,4	0,0	–	–
Citi N savienojumi	2,8	2,1	2,1	4,6	11,2	9,2	3,3	1,5	2,4	–
P savienojumi	–	0,9	0,1	0,7	1,2	0,7	–	–	–	–
S savienojumi	0,3	0,1	0,3	0,3	–	0,1	0,0	0,1	0,4	0,7
Neidentificētie savienojumi	8,3	10,8	8,6	6,2	16,8	13,8	3,9	4,5	2,2	2,9
Pirolīzes prod. skaits	158	158	155	150	140	160	170	52	72	37

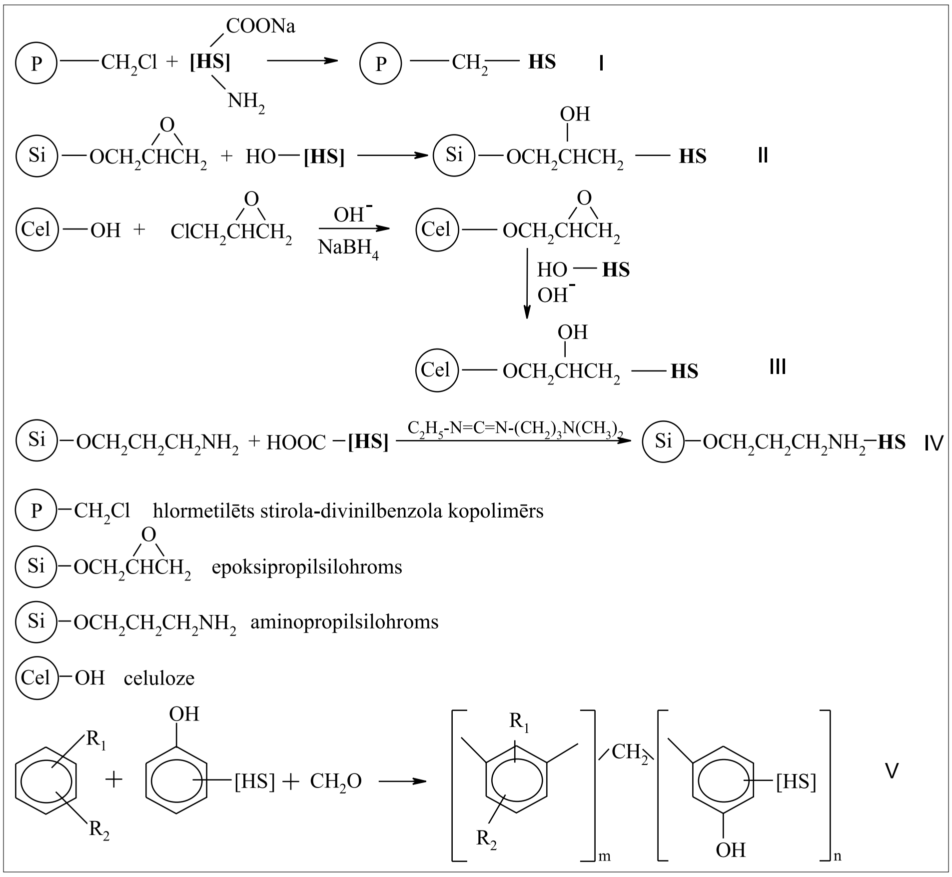
Analizējot humusvielas ar pirolīzes gāzu hromatogrāfiju/masspektrometrijas metodi, tika iegūta informāciju par humusvielu struktūru veidojošiem elementiem. Vidēji humusvielu pirolīzes gaitā veidojas 150 hromatogrāfiski izdalāmi un identificējami pirolīzes produkti. No virszemes un pazemes ūdens ekstrahētajās humīnskābēs pirolīzes produktu skaits atšķiras relatīvi maz, tas variē no 140 līdz 160 individuāliem sadalīšanās produktiem. Līdzīgais pirolīzes produktu skaits varētu liecināt, ka arī no virszemes un pazemes ūdeņiem izdalītās humusvielas ir ar līdzīgu struktūras dažādību. Situācija ir atšķirīga humīnskābēm, kas izdalītas no Latvijas purvu kūdras, un rūpnieciski ražotajām humusvielām, kurās pirolīzes produktu skaits ir ļoti atšķirīgs. Visvairāk to šajā grupā ir humīnskābēm, kas izdalītas no Kačoru purva kūdras (170 produkti). Olaines purva kūdras humīnskābes pirolīzes gaitā tika sadalītas līdz 52 individuāliem produktiem. Savukārt Irkutskas rūpnieciski iegūtajām humīnskābēm ir 72 dažādi pirolīzes produkti. Vismazākais pirolītiskās degradācijas produktu skaits – 37 ir no oksihumolīta komerciāli iegūtajām humusvielām. To varētu izskaidrot gan ar agresīvajiem humusvielu ekstrahēšanas apstākļiem rūpnieciskās ražošanas gaitā, kuru laikā netiek saglabāta informācija par izejas materiālu struktūru, gan arī ar būtisko atšķirību izejvielas uzbūvē (oksihumolīta organiskās vielas transformācijas – humifikācijas pakāpe ir ievērojami augstāka nekā organiskajai vielai kūdrā un ūdeņos).

Pirolīzes produkti tika iedalīti 31 apakšgrupā, pamatojoties uz to ķīmiskajām struktūrām.

3.2. Humusvielu imobilizācijas metožu izstrāde un imobilizēto humusvielu izmantošanas iespēju izpēte

Viena no pieejām, kas ir plaši izmantota biomolekulu pētījumos, ir to imobilizācija, jo pētāmajā vidē saistītas biomolekulas iespējams ievērojami plašāk izmantot (vispirms rūpnieciskiem mērķiem), ir vieglāk izpētīt to īpašības, pieaug stabilitāte un atkarībā no izmantotās imobilizācijas metodes ir iespējams saglabāt to īpašības. Viss iepriekš teiktais pilnā mērā attiecas uz humusvielu imobilizācijas iespēju izpēti. Dažādu biomolekulu (olbaltumvielu, nukleīnskābju, polisaharīdu un citu) imobilizācijas metodes ir plaši pētītas, un mūsdienās tās tiek izmantotas liela apjoma ražošanas procesos un pašu biomolekulu īpašību izpētē. Tajā pat laikā ir tikai daži pētījumi, kas veltīti humusvielu imobilizācijas metožu izstrādei un to praktiskas izmantošanas iespēju izpētei. Ir pētītas principiālās iespējas iegūt pie cietiem nesējiem saistītas humusvielas (Kļaviņš, 1993), piemēram, jonu saistības veidā vai izmantojot glutāraldehīdu (Yang and Koopal, 1999) imobilizētas humusvielas uz aminogrupas saturošu silīcija nesēju virsmas, lai noskaidrotu radioaktīvo izotopu migrācijas raksturu ūdeņos (Szabo and Bulman, 1994).

Disertācijas darba ietvaros pētītas iespējas imobilizēt humusvielas, izmantojot dažādas to funkcionālās grupas (vispirms hidroksilgrupas un karboksilgrupas, kas pieejamas humusvielu makromolekulās lielākās koncentrācijās), kā arī humusvielu struktūrelementus (fenolu gredzenus), kuru izmantošana ļauj humusvielu molekulas iesaistīt polimēru struktūru sastāvā. Piedāvātās pieejas mērķis līdz ar to ir nodrošināt humusvielu molekulas stabilas saistības ar polimēro nesēju izveidošanos (22. attēls).



R_1, R_2 – H; OH; CH_3

V_A – humīnskābju polikondensācija

V_B – R_1=H, R_2=OH

V_C – R_1=H, R_2=CH_3

V_D – R_1, R_2=OH

22. attēls. **Humusvielu imobilizācijai izmantotās metodes**
Figure 22. **Methods used for immobilisation of humic substances**

Kā izejas polimēri (nesēji) izmantoti biomolekulu imobilizācijā plaši lietotie hlormetilētie stirola divinilbenzola kopolimēri (gelveida Merifilda polimērs un makroporaini kopolimēri), celuloze (modificējot to ar epoksigrupām), epoksipropilgrupas vai aminopropilgrupas saturoši silīcija nesēji (humusvielas piesaistei izmantojot ūdenī šķīstošus karbodiimīdus). Humusvielas imobilizētas, tās iesaistot polikondensācijas reakcijās ar formaldehīdu un vielām, kas aktīvi spēj veidot fenola-formaldehīda sveķus.

Iegūtās imobilizētās humusvielas raksturotas, veicot iegūto polimēru elementanalīzi un titrimetriski nosakot nozīmīgākās funkcionālās grupas (karboksilgrupas), analizējot IS spektrus iegūtajām imobilizētajām humusvielām, kā arī spektrofotometriski nosakot to degradācijas produktu koncentrāciju pēc hidrolīzes sārma vidē. Tā, piemēram, imobilizēto humusvielu IS spektrus raksturo intensīva absorbcija spektra reģionā 3600–2800 cm^{-1}, kas raksturīga hidroksilgrupām, un absorbcija reģionā ~1700 cm^{-1}, kas raksturīga karboksilgrupām. Izmantoto metožu kopums ļauj kontrolēt imobilizācijas gaitu un raksturot iegūtos polimērus. Izmantotās imobilizācijas metodes līdz ar to ļauj iegūt imobilizētas humusvielas uz dažādu polimēru bāzes ar mainīgu humusvielu koncentrāciju un dažādām polimēra īpašībām (15. tabula).

15. tabula / Table 15

Imobilizēto humusvielu īpašības
Properties of immobilised humic substances

Imobilizētās HS	C, %	H, %	N, %	HS saturs, mg/g	Uzbriešana ūdenī, ml/g	Uzbriešana benzolā, ml/g
I-HS_{Olaine}	86,17	7,29	–	20	0,87	1,85
I-$HS_{Daugava}$	76,06	8,95	1,95	223	0,54	1,03
I-$HS_{augsnes}$	76,12	9,16	0,97	228	0,59	1,06
I-$HS_{Kačuru}$	77,03	8,21	0,43	208	0,48	1,01
II-HS_{Olaine}	12,32	3,74	0,12	57	0,11	0,35
II-$HS_{augsnes}$	12,31	4,76	0,32	85	0,12	0,45
III-$HS_{augsnes}$	43,86	7,30	1,50	83	0,18	0,56
III-HS_{Olaine}	42,20	6,13	0,30	87	0,22	0,64
IV-HS_{Olaine}	46,70	0,59	0,31	7	0,12	0,12
V_A-HS_{Olaine}	53,93	3,59	1,33	373	1,15	2,35
V_B-HS_{Olaine}	66,24	6,39	0,05	340	0,73	1,87
V_C-HS_{Olaine}	58,60	6,51	0,13	185	0,82	1,84
V_D-HS_{Olaine}	60,98	6,30	0,78	430	1,14	2,40

Par galvenajiem kritērijiem sekmīgas humusvielu imobilizācijas nodrošināšanai var uzskatīt reaģētspējīgu grupu klātbūtni izejas polimērā, pietiekamu reakcijas laiku un temperatūru, kā arī augstu humusvielu koncentrācijas nodrošināšanu reakcijas vidē. Līdz ar to, lai humusvielas imobilizētu uz stirola divinilbenzola kopolimēriem, par būtiskāko uzskatāma to pietiekami augstas humusvielu šķīdības nodrošināšana, vienlaikus novēršot hlormetilgrupu hidrolīzes iespējas. Lai iegūtu uz stirola divinilbenzola kopolimēriem imobilizētas humusvielas ar pietiekami augstu aizvietotības pakāpi, reakciju nepieciešams veikt bāziskā vidē, ūdens/dimetilformamīda šķīdumā 15–30 stundas 100 ºC temperatūrā. Reakcija ar nesēja hlormetilgrupām norit visai lēni, un to, iespējams, ietekmē humusvielu difūzijas ātrums gelveida polimēra struktūrā un reakcijā iesaistīto grupu visai zemā reaģētspēja. Jāatzīmē, ka šajos visai bargajos apstākļos iespējams imobilizēt dažādas izcelsmes humusvielas, turklāt to reaģētspēja pētītajās reakcijās atšķiras nebūtiski.

Ievērojami augstāku imobilizācijas efektivitāti iespējams sasniegt, izmantojot ar reaģētspējīgām funkcionālām grupām aktivētus hidrofilus polimērus – epoksigrupas saturošos silikagela un celulozes atvasinājumus. Imobilizēto humusvielu sintēzei līdz ar to izmantojami šādi polimēri, kas pieejami komerciāli vai arī vienkārši iegūstami, apstrādājot izejas polimēru matricu ar epoksigrupas saturošiem reaģentiem (piemēram, epihlorhidrīnu vai epoksipropilsilānu). Izejas polimēru matricu iespējams aktivēt ar epoksigrupām, mainot to ievadīšanas pakāpi. Savukārt epoksigrupu augstā reaģētspēja ļauj imobilizēt humusvielas relatīvi maigos apstākļos (t = 20–60 ºC, τ = 0,5–3 st), imobilizācijas reakciju veicot ūdens vidē.

Humusvielas tiek imobilizētas, arī izmantojot to karboksilgrupu aktivēšanu ar ūdenī šķīstošiem karbodiimīdiem un saistot ar aminogrupas saturošu silikagela nesēju.

Šo reakciju iespējams veikt maigos apstākļos, kas novērš humusvielu degradāciju, bet, ņemot vērā izmantoto reaģentu augsto reaģētspēju, var sasniegt augstu imobilizēto humusvielu koncentrāciju gala polimērā. Šīs metodes izmantošana ļauj saglabāt izejas polimēra struktūru nemainīgu, un līdz ar to var iegūt imobilizētas humusvielas, kuras potenciāli iespējams izmantot kā nesējus augstefektīvajā šķidruma hromatogrāfijā, piemēram, farmācijas rūpniecībā un citur.

Visas iepriekšminētās metodes ļauj saistīt humusvielas pie polimēra matricas, atdalot tās ar distancētāja starpniecību, līdz ar to vienlaikus tiek nodrošināta vairākcentru saistības iespēja un tiek saglabāts augsts humusvielu struktūrelementu kustīgums. Humusvielas iespējams imobilizēt arī, izmantojot to struktūrā ietilpstošo fenolu spēju stāties polikondensācijas reakcijās ar formaldehīdu. Tā kā fenolu struktūrelementi humusvielu molekulās tipiski ir poliaizvietoti vai atrodas stēriski grūti pieejamās pozīcijās, tad to reaģētspēja polikondensācijas reakcijās ir zema un iegūto polimēru mehāniskā stabilitāte arī ir zema. Taču, reakcijas maisījumam pievienojot vielas ar augstāku reaģētspēju polikondensācijas reakcijās (piemēram, fenolus, toluolu un citas), iespējams vienlaikus nodrošināt kvantitatīvu humusvielu iesaistīšanos polimēra sastāvā, ievērojami uzlabojot iegūto imobilizēto humusvielu mehānisko stabilitāti. Izmantojot polikondensācijas reakciju, iespējams iegūt gelveida blokpolimērus.

16. tabula / Table 16

Imobilizēto humusvielu hidrolītiskā stabilitāte
Hydrolytic stability of immobilised humic substances

Imobilizētās HS	**Hidrolītiskā stabilitāte (desorbētās HS pēc 24 stundām, %)**		
	0,05 N HCl	0,05 N NaCl	0,05 N NaOH
$\text{I-HS}_{\text{Olaine}}$	1	0	3
$\text{I-HS}_{\text{Daugava}}$	2	0	4
$\text{I-HS}_{\text{augsnes}}$	2	0	5
$\text{II-HS}_{\text{Olaine}}$	6	0	3
$\text{III-HS}_{\text{Olaine}}$	4	0	2
$\text{V}_{\text{B}}\text{-HS}_{\text{Olaine}}$	0	0	5
$\text{V}_{\text{B}}\text{-HS}_{\text{Olaine}}$	5	0	8

Izmantojot minētās humusvielu reakcijas, ir iespējams iegūt imobilizētas humusvielas, kas noturīgas pret hidrolīzi (16. tabula), bet kuru mehāniskā stabilitāte ir atkarīga no izejas polimēra matricas īpašībām. Imobilizētajām humusvielām noteikta to īpatnējā virsma un sorbenta poru izmēra sadalījums. Imobilizēto humusvielu īpatnējā virsma galvenokārt atkarīga no izejas polimēra virsmas. Tā tipiski ir zema polikondensācijas reakcijās iegūtajiem polimēriem (0,326–0,580 m^2/g), bet, piemēram, humusvielām, kas iegūtas uz makroporainā stirola-divinilbenzola bāzes, tās sastāda 51,6 m^2/g un attiecīgi uz aminopropilsilikagela bāzes –106 m^2/g. Tipiski, imobilizācijas reakcijas gaitā modificējot nesēju ar humusvielām, izejas sorbenta īpatnējā virsma nesamazinās. Līdz ar to var uzskatīt, ka izstrādātās humusvielu imobilizācijas metodes var nodrošināt humusvielu imobilizāciju ar augstu iznākumu uz atšķirīgas struktūras un īpašību polimēriem un ka ir iespējams iegūt potenciālus sorbentus, kuru īpašības nosaka humusvielu polifunkcionālā daba.

Imobilizēto humusvielu praktiskās izmantošanas iespējas saistās ar to lietojumu pašu humusvielu izpētē. Par nozīmīgu uzskatāmas to izmantošanas iespējas ne tikai lai modelētu humusvielu izturēšanos šķīdumā, bet arī lai pētītu uz cietas fāzes sorbētu humusvielu īpašības (šādā veidā tās atrodas augsnē un nogulumos, sorbējoties gan uz augsni un nogulumus veidojošo minerālu daļiņām, gan arī uz detrīta). Tieši pēdējo procesu izpētei imobilizēto humusvielu izmantošana uzskatāma par īpaši perspektīvu, jo dabiskā vidē sorbēto humusvielu mijiedarbības raksturu ar šķidrumā esošām vielām kavē sorbēto humusvielu līdzsvara desorbcijas procesi, kā tas redzams, pētot humusvielu sorbciju uz infiltrācijas baseinu nogulumiem. Tajā pat laikā par perspektīvāko imobilizēto humusvielu izmantošanas jomu var uzskatīt to izmantošanu par sorbentiem, kas izmantojami vielu attīrīšanai un izdalīšanai, notekūdeņu attīrīšanai un citiem mērķiem.

Disertācijas darba ietvaros pētīta metālu jonu un dažādu organisko vielu grupu modeļsavienojumu sorbcija uz imobilizētām humusvielām.

Pētījumā izmantoto polimēru matricas (silīciju saturošas matricas, stiroladivinilbenzola kopolimērs, celuloze) raksturo zema to spēja sorbēt metālu jonus, un līdz ar to var uzskatīt, ka to sorbcija notiek imobilizēto humusvielu fenolu hidroksilgrupu un karboksilgrupu mijiedarbības dēļ. Metālu jonu (Cu^{+2}, Co^{+2}, Mn^{+2}, Cd^{+2}) sorbcija norit relatīvi ātri, un tipiski sorbcijas līdzsvars tiek sasniegts vismaz 30 minūšu laikā. Minēto divvērtīgo jonu starpā nepastāv ievērojamas atšķirības to spējā mijiedarboties ar imobilizētajām humusvielām (17. tabula). Katra konkrētā sorbenta sorbcijas spēja ir atkarīga no polimēra matricas īpašībām un imobilizēto humusvielu koncentrācijas. Augstāka sorbcijas spēja ir raksturīga sorbentiem, kas iegūti, izmantojot polikondensācijas reakcijas.

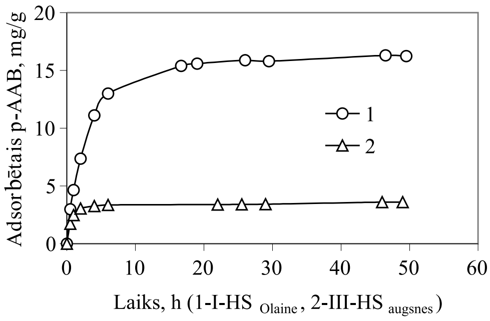
17. tabula / Table 17

Metālu jonu sorbcija uz imobilizētajām humusvielām
Sorption of metal ions on immobilised humic substances

Polimērs	**Cu^{2+}, μmol/g**	**Co^{2+}, μmol/g**	**Mn^{2+}, μmol/g**	**Cd^{2+}, μmol/g**
I-HS_{Olaine}	39	23	44	80
I-$HS_{Daugava}$	39	27	34	80
I-$HS_{augsnes}$	34	33	33	80
II-HS_{Olaine}	–	–	104	120
III-HS_{Olaine}	72	38	50	85
V_B-HS_{Olaine}	108	61	60	99
V_B-HS_{Olaine}	187	166	183	177

Tika pētīts dažādu organisko vielu sorbcijas raksturs uz imobilizētajām humusvielām, izmantojot gan hidrofobas organiskas vielas (p-aminoazobenzols), gan arī hidrofilas vielas (kristālvioletais, metilēnzaļais, flavīna mononukleotīds). Pētīto organisko vielu sorbciju var nodrošināt to mijiedarbība ar humusvielu sastāvā ietilpstošajām funkcionālajām grupām (vispirms karboksilgrupām vai fenolu hidroksilgrupām) vai arī hidrofobā mijiedarbība ar imobilizēto humusvielu hidrofobajiem uzbūves sturktūrelementiem.

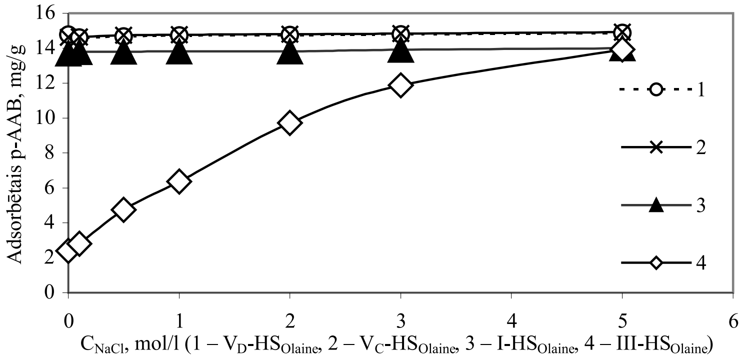
Sorbcijas ātrums, to pētot statiskos apstākļos, visos pētītajos gadījumos ir bijis atkarīgs no sorbenta matricas tipa, kā tas redzams aminozobenzola sorbcijas piemērā (23. attēls). Ja sorbcija notiek uz makroporainā polimēra vai celulozes, sorbcijas maksimums parasti tiek sasniegts dažu minūšu laikā, bet, ja sorbcija ir uz gelveida polimēriem (polikondensācijas polimēriem un gelveida stirola-divinilbenzola kopolimēriem), sorbcijas maksimums var iestāties pat pēc 10 un vairāk stundām. Sorbcijas gaita ir atkarīga no sorbcijas vidē izšķīdušo sāļu koncentrācijas (24. attēls), un jonu spēka ietekme ir liela, ja sorbcijā ir iesaistītas hidrofobas vielas, bet, ja polimēra matrica ir blīva vai arī vielas un polimēra matrica ir pietiekami hidrofila, jonu spēka ietekme ir niecīga.



23. attēls. **p-Aminoazobenzola sorbcijas kinētika uz humusvielām, kas imobilizētas uz aminopropilsilohroma un hlormetilētā stirola-divinilbenzola kopolimēra**

Figure 23. **Sorption kinetics of p-aminoazobenzene (p-AAB) on the immobilised humic acids onto aminopropilsilica and chloromethylated styrene-divinylbenzene copolymer**

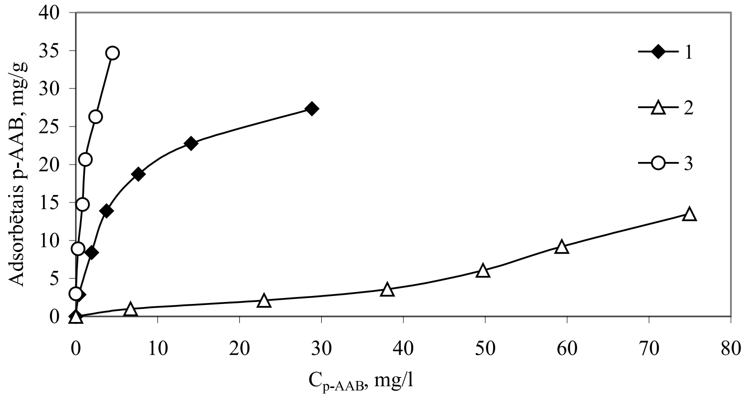
Pētīto vielu sorbcijas efektivitāti vispirms ietekmē humusvielu koncentrācija pētītajās imobilizētajās humusvielās un to matricas īpašības. Šo faktoru ietekme labi redzama (25. attēls), analizējot p-aminoazobenzola sorbcijas izotermas uz imobilizētām humusvielām: uz stirola divinilbenzola kopolimēra bāzes, izmantojot polikondensācijas pieeju, un uz aminopropilsilohroma, humusvielu piesaistei izmantojot ūdenī šķīstošos karbodiimīdus. Jāatzīmē, ka sorbcijas efektivitāti šajā konkrētajā sorbenta-sorbāta sistēmā ietekmē ne tikai humusvielu, bet arī stirola divilnilbenzola un polikondensācijas polimēru veidojošo struktūru spēja mijiedarboties ar aminoazobenzolu.



24. attēls. **p-Aminoazobenzola sorbcijas uz imobilizētajām humusvielām atkarība no NaCl koncentrācijas sorbcijas vidē**

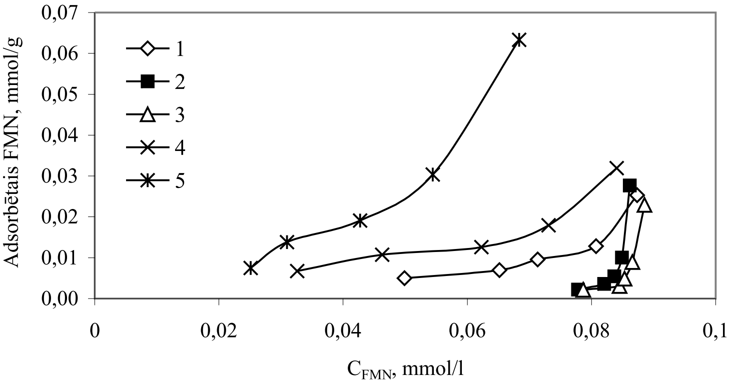
Figure 24. **Sorption of p-aminoazobenzene on immobilised humic acids depending on the ionic strength of the solution**

Ievērojami citādāka ir kristālvioletā, metilēnzaļā un flavīna mononukleotīda sorbcijas gaita. To ilustrē flavīna mononukleotīda sorbcijas uz 5 imobilizētām humusvielām izotermas (26. attēls).



(1 – I-HS_{Olaine}, 2 – III-HS_{Olaine}, 3 – V_D-HS_{Olaine})

25. attēls. **p-Aminoazobenzola sorbcijas izoterma uz imobilizētajām humusvielām**
Figure 25. **Sorption of p-aminoazobenzene onto immobilised humic acids**



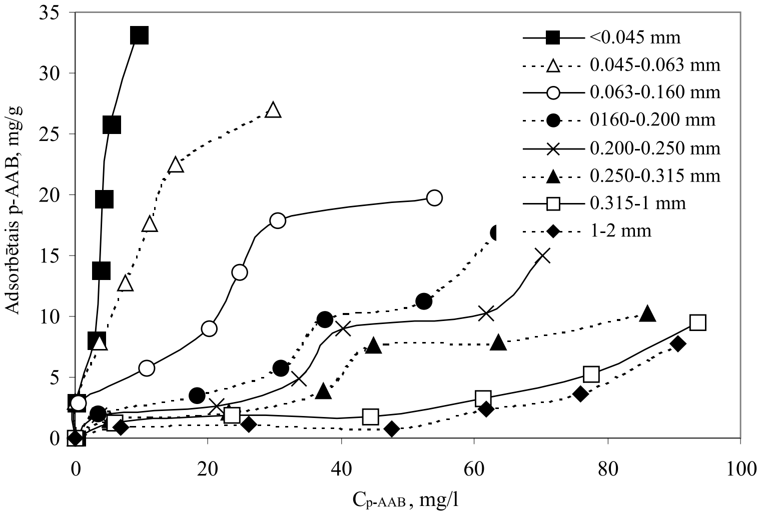
(1 – V_D-HS_{Olaine}, 2 – V_C-HS_{Olaine}, 3 – I-HS_{Olaine}, 4 – III-HS_{Olaine})

26. attēls. **Flavīna mononukleotīda sorbcijas izoterma uz imobilizētajām humusvielām**
Figure 26. **Sorption of flavine mononucleotide (FMN) onto immobilised humic acids**

Hidrofila rakstura sorbātu sorbcijas intensitāte nav tik ļoti atkarīga no sorbenta matricas īpašībām kā hidrofobo sorbātu gadījumā (26. attēls). Turklāt redzams, ka sorbcijas izotermas nevar aprakstīt ar klasiskajiem sorbcijas modeļiem un ka sorbcijas gaitu nosaka sorbcija noteiktos sorbcijas centros, kas acīmredzami ir imobilizētās humusvielu molekulas.

Par nozīmīgu faktoru, kas ietekmē sorbcijas gaitu, var uzskatīt sorbāta molekulu difūziju polimēra daļiņu iekšienē. Polimēra daļiņu izmērs līdz ar to izšķirīgi ietekmē sorbcijas spēju un sorbcijas izotermu raksturu (27. attēls).

Sorbcijas izotermu raksturs ir atkarīgs no procesiem, kas notiek uz cietās fāzes virsmas (difūzija/sorbcija). Jāatzīmē, ka daļiņu izmērs korelē ar to īpatnējo virsmu un ka 1–2 mm daļiņām īpatnējā virsma ir 0,326 m^2/g, 0,2–0,25 mm daļiņām – 0,481 m^2/g, bet 0,063–0,160 mm daļinām – 0,580 m^2/g. Vielu difūzija makroporaino polimēru sorbcijas raksturu daļiņas iekšienē neietekmē, un par galveno sorbcijas raksturu ietekmējošo procesu kļūst polimēra matricas un imobilizēto humusvielu spēja saistīt sorbātus.



27. attēls. **p-Aminoazobenzola sorbcijas uz polimēra V_D-HS_{Olaine} atkarības raksturs no sorbenta daļiņu izmēra**

Figure 27. **Sorption of p-aminoazobenzene onto immobilized humic substances V_D-HS_{olaine} depending on the size of polymer particles**

Veiktais pētījums kopumā pārliecinoši pierāda, ka imobilizētās humusvielas uzskatāmas par perspektīvu sorbentu grupu, tās var izmantot ne tikai pašu humusvielu izpētei, bet arī analītisku un preparatīvu problēmu risināšanai, kā arī tās var izmantot piesārņotu notekūdeņu attīrīšanai.

3.3. Humusvielu mijiedarbība ar pazemes ūdeņu mākslīgās papildināšanas baseinus veidojošajiem iežiem un minerāliem

Pazemes ūdeņu mākslīgās papildināšanas process tiek īstenots, virszemes ūdeņiem filtrējoties caur iežu slānim, līdz tie sasniedz ūdensnesošos horizontus. Ir pierādīts, ka šādā veidā iespējams nodrošināt mikroorganismu un ūdenī suspendēto vielu aizturi, tādējādi ūdeni attīrot (Huisman and Olsthoorn, 1983). Mūsdienās par aizvien aktuālāku kļūst jautājums par humusvielu atdalīšanu dzeramā ūdens sagatavošanas procesā. Virszemes ūdeņu eitrofikācijas un piesārņošanas rezultātā humusvielu koncentrācijas virszemes ūdeņos ir ievērojami pieaugušas, un to klātbūtne pasliktina ūdens estētisko kvalitāti, kā arī var izraisīt nepatīkamu ūdens garšu vai smaku. HV var palielināt toksisko organisko vielu, metālu un radionuklīdu transportu. Dezinficējot dzeramo ūdeni, no kura nav pietiekami attīrītas humusvielas, hlorēšanas ietekmē var veidoties hlororganiskie savienojumi, kas ir kancerogēnas vielas – trihalometāni (Rook, 1974).

Disertācijas izstrādes ietvaros tika pētīta humusvielu sorbcija uz Baltezera pazemes ūdeņu mākslīgās papildināšanas baseinus veidojošajām iežiem, minerāliem un modeļvielām. Baltezera pazemes ūdens mākslīgās papildināšanas baseini ir izveidoti kvartāra nogulumos, un tos veido 17 baseini. Baseinu materiālu pamatā veido kvarca smiltis ar alumosilikātu piejaukumu, turklāt tie var būt viendabīgi piejaukumi visai infiltrācijas baseina iežu masai (māla daļiņu piejaukumiem nepārsniedzot 1–3%) un arī koncentrēti nogulumi, kuru izplatība nav viendabīga visā baseina teritorijā (mālu lēcas), kā arī tie var būt dzelzi saturoši minerāli (getīts, celadonīts un glaukonīts). Līdz ar to kopumā infiltrācijas baseina iežu sastāvs ir līdzīgs augsnes sastāvam, tomēr ar ievērojami zemāku organisko vielu, dzelzs, mangāna oksīdu saturu. Baseinu iežu granulometriskais sastāvs ir raksturīgs kvarca smilšu nogulumiem, bet organisko vielu saturs tajos parasti ir mazāk par 0,5% (18. tabula). Lai gan humusvielu sorbcijai ir liela nozīme augsnes sastāva veidošanā, nepieciešams atzīmēt, ka šim jautājumam veltīto pētījumu skaits ir niecīgs (Sposito, 1989; McBride, 1994) un to rezultātā izteiktie viedokļi ir vērtējami visai pretrunīgi (Shen, 1999; Jardine et al., 1989; Gu et al., 1993). Esošie pētījumi par humusvielu sorbciju uz augsni veidojošajiem iežiem uzsver jonogēnās sorbcijas lielo nozīmi, un galvenokārt uz dzelzs oksīdu un alumosilikātu virsmas.

18. tabula / Table 18

Infiltrācijas materiālu fizikāli ķīmiskais raksturojums paraugiem, kas ievākti no Baltezera mākslīgā papildināšanas dīķu virsējiem slāņiem (0–4 cm) un salīdzināti ar Jardine et al. (1989) analizēto grunts sastāvu
Physical and chemical characteristics of aquifer material (filtersand) recovered from surfacial layers (0–4 cm) of infiltration ponds used for artificial groundwater recharged from Lake Baltezers compared to composition of soil analysed by Jardine et al. (1989)

Paraugs	Virsmas laukums, m^2/g	C, %	Parauga sastāvs, %			Fe, mg/g
			Smiltis	Smilšmāls	Māli	
8B[a]	–	0,5	77,0	22,0	1,0	0,64
3A[a]	0,49	0,6	65,7	31,0	3,3	0,71
4A[a]	0,85	0,5	40,8	37,2	22,0	0,69
References grunts[b]	–	0,2	32,2	44,5	23,6	-

[a] infiltrācijas dīķa numurs un parauga ievākšanas vieta dīķī
[b] Jardine et al., (1989)

Izvērtējot humusvielu sorbcijas spējas uz iespējamiem Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošajiem materiāliem, nepieciešams atzīmēt, ka sorbcija uz māliem acīmredzami ir izteiktāka (19. tabula), turklāt tā ir atkarīga no pētīto humusvielu īpašībām.

19. tabula / Table 19

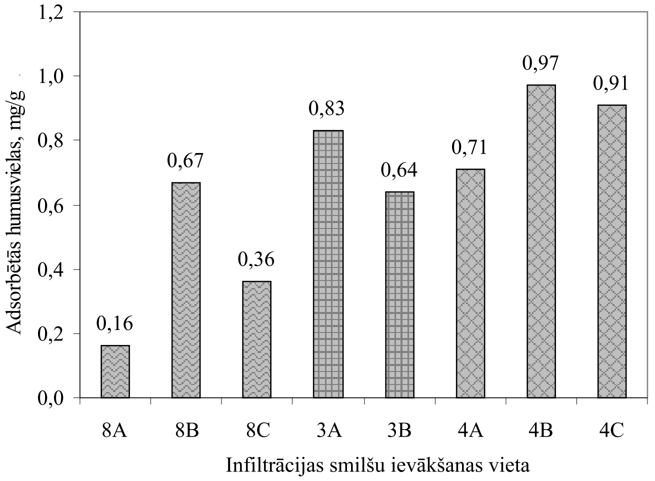
Dažādu humīnskābju (HS) un fulvoskābju (FS) sorbcija (mg/g) uz dažādiem mālu paraugiem
Sorption (mg/g) of different humic and fulvic acids on different clay samples

Humusvielas	Mālu paraugs no. 1[a]	Mālu paraugs no. 2[b]	Mālu paraugs no. 3[c]
Aldrich HS	27,1	29,2	16,0
Augsnes HS	26,3	28,4	13,5
Daugavas FS	12,1	14,2	6,4

[a] dominējoši – smektīts; [b] dominējoši – smektīts; [c] dominējoši – kaolinīts

Humusvielu sorbcija uz Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošajiem iežiem, kā tas redzams 28. attēlā, ir atkarīga no to mineraloģiskā sastāva un mainās visai plašā diapazonā pat viena infiltrācijas baseina robežās.

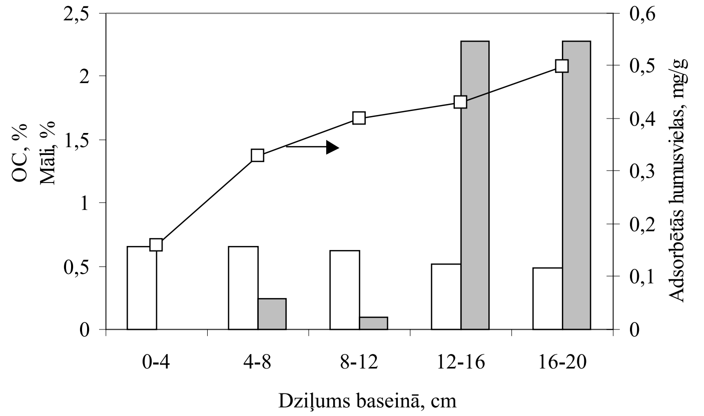
Faktoru vidū, kas ietekmē humusvielu sorbciju uz Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošajiem iežiem, var minēt alumosilikātu daudzumu tajos un organisko vielu saturu. Nepieciešams atzīmēt, ka sorbcijas kapacitāte palielinās, pieaugot attālumam no baseinu virsmas. To, iespējams, nosaka augšējo slāņu piesātināšanās ar ūdenī esošām organiskajām vielām, kā arī to kolonizācija ar mikroorganismiem, par ko liecina organisko vielu satura relatīvi paaugstinātas vērtības baseinu veidojošo iežu augšējos horizontos. Tas pierāda, ka liela daļa no potenciāli pieejamiem sorbcijas centriem var būt aizņemti, līdz ar to izmainot arī ar organiskajām vielām pārklāto daļiņu virsmas lādiņu (Day et al., 1994). Organisko vielu klātbūtnes lielo nozīmi humusvielu augstas sorbcijas spējas veidošanā apliecina arī tas, ka humusvielu sorbcijas spēja uz Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošajiem iežiem kritiski samazinās, ja tajās esošās organiskās vielas tiek aizvāktas, tās oksidējot. Humusvielu sorbcija pieaug, palielinoties alumosilikātu (mālu) daudzumam iežu masā, ko nosaka ne tikai alumosilikātu daļiņu lielā virsma, bet arī virsmas lādiņš.



28. attēls. **Aldrich humīnskābju sorbcija uz smilšu paraugiem, kas ievākti no dažādiem infiltrācijas dīķiem**

Figure 28. **Sorption of Aldrich humic acid on filtersand samples from different infiltration basins**

Humusvielu sorbcija uz Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošajiem iežiem ir atkarīga no vides pH reakcijas, un skābākā vidē tā ievērojami pieaug (29. attēls). To var saistīt gan ar karboksilgrupu dominējošo lomu sorbcijas procesā, gan arī ar to, ka skābā vidē humusvielu molekulas to savstarpējās mijiedarbības rezultātā veido lielākus agregātus. Savukārt vides pH reakcijas izmaiņas, protams, ietekmē Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošo iežu virsmas lādiņu.

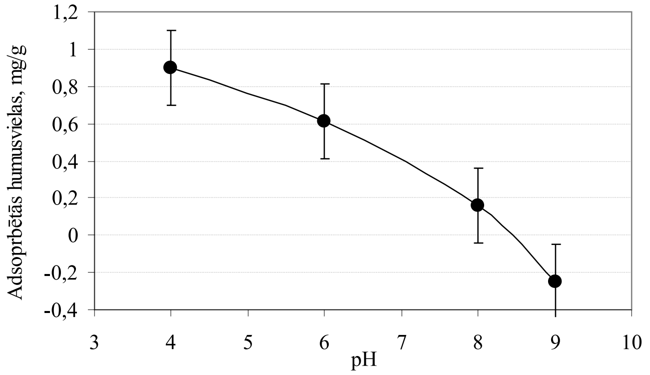


Leģenda: kreisā y-ass: □ – OC (organiskā oglekļa saturs masas procentos); ■ – mālu saturs masas procentos; labā y-ass: adsorbētās humusvielas (mg/g)

Legend: left y-axis □ – OC, organic carbon; ■ – clay; right y-axis, adsorbed humic substances (mg/g)

29. attēls. **Aldrich humīnskābju sorbcija (-□-) uz 8A dīķa infiltrācijas smiltīm atkarība no to dziļuma zem baseina**

Figure 29. **Relationship between sorption (-□-) of Aldrich humic acid to filtersand and the different depths below infiltration basin no. 8A.**

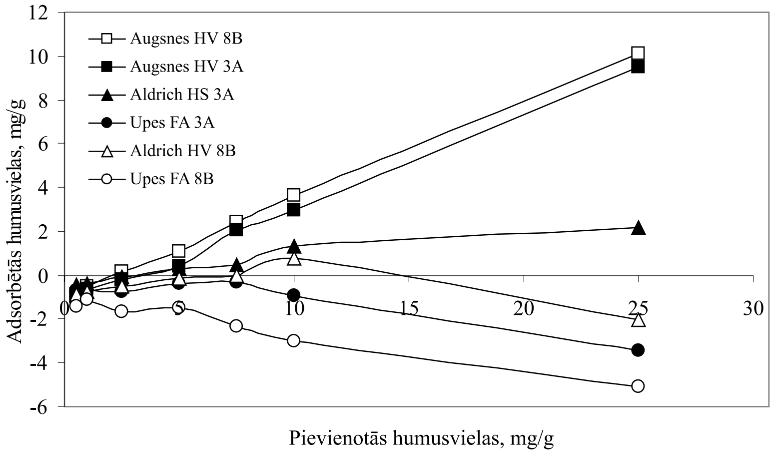


30. attēls. **Humusvielu sorbcijas atkarība no pH infiltrācijas smilšu paraugos, kas ievākti no visiem pētītajiem dīķiem dažādos dziļumos un vietās**

Figure 30. **Relationship between the sorption of humic substances and pH of filtersand samples from the infiltration basins. Each data point represents mean values of filtersand collected from all basins at different depths and positions**

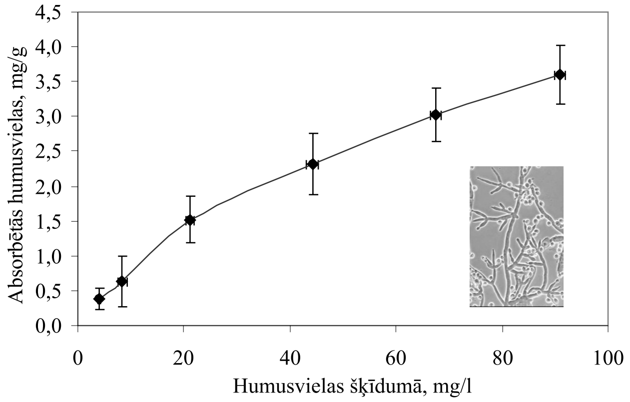
Salīdzinot atšķirīgas izcelsmes humusvielu sorbciju uz Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošajiem iežiem (30. attēls), ir jāatzīmē ievērojamas atšķirības gan humusvielu sorbcijas kopējā spējā, gan arī sorbcijas raksturā. Augsnes un rūpnieciski ražoto humusvielu sorbcijas spēja pieaug, palielinoties humusvielu koncentrācijai šķīdumā, bet, piemēram, ūdeņu humusvielu sorbcija pat samazinās, pieaugot to koncentrācijai vidē. Tas, iespējams, ir saistīts ar organisko vielu desorbciju, ko nosaka Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošie ieži. Iespējams, ievērojama daļa no organiskās vielas, kas ietilpst Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošajos iežos, ir vāji saistīta (adsorbēta) un var tikt atbrīvota vai aizvietota mijiedarbības rezultātā ar humusvielām, kuras tiek piesaistītas ķīmiskas sorbcijas ceļā. Kā pierāda veiktie eksperimenti, tas raksturīgs ūdeņu fulvoskābēm, kuras raksturo augsts karboksilgrupu saturs. Šāds humusvielu sorbcijas procesu kopums ir būtisks, izvērtējot pazemes ūdeņu mākslīgās papildināšanas metodes attīstību, jo tas ir tieši saistāms ar pazemes ūdens mākslīgās papildināšanas baseinus veidojošo iežu ekspluatācijas laiku, kā arī ar to, ka šī tehnoloģija var nenodrošināt humusvielu pilnīgu atdalīšanu papildināšanās procesa gaitā, ja iežu masā ir noteikts organisko savienojumu koncentrācijas līmenis.

Salīdzinoši ir pētīta arī humusvielu sorbcija uz Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošo iežu modeļsavienojumiem (31. attēls). Kā redzams, humīnskābes sorbējas labāk nekā fulvoskābes, izņemot dzelzs oksīdu gadījumā, kad fulvoksābju sorbcija ir augstāka. Humīnskābas ir ar augstāku molekulmasu un hidrofobākas nekā fulvoskābes, un līdz ar to sorbciju lielā mērā nosaka fizikāli procesi, nevis ķīmiska mijiedarbība (hemosorbcija). Savukārt fulvoskābju molekulas ir ar augstāku karbosilgrupu koncentrāciju, un tas var paaugstināt to sorbcijas efektivitāti uz dzelzs oksīdus saturošiem iežiem. Nepieciešams atzīmēt, ka pēc humusvielu molekulu sorbcijas uz iežu virsmas izveidojas pārklājuma slānis, kas ievērojami izmaina citu humusvielu molekulu sorbciju. Kopumā sorbcijas mehānismu atšķirības ļauj izskaidrot visai ievērojamās atšķirības starp humīnskābju un fulvoskābju sorbciju. Lai gan kvarcs ir galvenais Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošo iežu ingredients, tomēr sorbcija uz tā ir ar relatīvi mazāku nozīmi, ko apliecina arī humusvielu sorbcijas izpēte uz augsnes (Spark et. al., 1997).



31. attēls. **Dažādas izcelsmes humusvielu sorbcija uz augšējo slāņu infiltrācijas smiltīm no dīķiem 8B un 3A**
Figure 31. **Sorption of different humic substances on the upper layer of filtersand from basin 8A and 3A**

Nozīmīgs procesu kopums, kas ietekmē humusvielu sorbciju pazemes ūdeņu mākslīgās papildināšanas gaitā, ir to mijiedarbība ar iežu masu veidojošajiem mikroorganismiem. Tieši humusvielu mijiedarbības procesiem ar mikroorganismu sēnīšu biomasu – biosorbcijai pēdējā laikā tiek pievērsta īpaša vērība, uzskatot to par nozīmīgu procesu, par kopumu, kas ietekmē augsnes sastāva veidošanos. Disertācijas ietvaros tika veikti pētījumi, izmantojot imobilizētu mikroorganismu biomasu, un tika noteikta humusvielu sorbcija uz tās (33. attēls).



32. attēls. **Aldrich humusvielu sorbcija uz Trichodermas spp. atkarībā no humusvielu koncentrācijas**
Figure 32. **Sorption of Aldrich humic substances on** *Trichoderma spp.* **depending on the concentration of humic acid solution**

Veiktais pētījums pierāda visai sarežģīto humusvielu aizturēšanas procesu pazemes ūdens mākslīgās papildināšanas norisi un humusvielu saistīšanos to gaitā. Tā kā centru daudzums Baltezera pazemes ūdens mākslīgās papildināšanas baseinus veidojošajos iežos ir zems un acīmredzami daļa no tiem jau ir aizņemti, tad ūdeņos esošās humusvielas (galvenokārt fulvoskābes) var aizvietot sorbētās vielas. Savukārt humīnskābes, kas ir hidrofobas un ar augstāku molekulmasu, tiek ievērojami intensīvāk sorbētas nekā fulvoskābes. Līdz ar to tiek nodrošināta humusvielu kopuma frakcionēšana mākslīgās papildināšanas procesa gaitā, turklāt vispirms tiek saistītas humīnskābes, bet ūdeņi papildinās ar mikrobiālas izcelsmes un zemākas molekulmasas fulvoskābām.

Tomēr tieši augstmolekulārā humusvielu daļa (humīnskābes) ir galvenais trihalometānu un ūdeņu krāsainības avots, un līdz ar to humīnskābju sorbcija var būtiski sekmēt ūdeņu kvalitātes paaugstināšanos dzeramā ūdens sagatavošanas procesā.

4. Secinājumi

1. No Latvijas dabas vides izdalītie, references un rūpnieciski ražotie humusvielu paraugi uzrāda ievērojamas atšķirības to īpašībās (funkcionālo grupu daudzums, kas raksturots izmantojot funkcionālo grupu analīzi, IS, UV spektroskopiju; humusvielu pamatstruktūru uzbūve, kas raksturota izmantojot elementanalīzi, ^{1}H KMR, ^{13}C KMR spektroskopiju). Humusvielu īpašības ir atkarīgas no to veidošanās apstākļiem, kas ietekmē aromātisko/alifātisko struktūrelementu relatīvo daudzumu (noteikti izmantojot pirolīzi GH-MS analīzi) humusvielas molekulā, kā arī to struktūru veidojošo elementu saturu.
2. Galvenie faktori, kas ietekmē humusvielu mijiedarbības raksturu (sorbcijas kapacitāti un tās kinētiku, kā arī atkarības raksturu no jonu spēka un vides pH reakcijas) ar Latvijas apstākļiem nozīmīgākajām augsni veidojošajām minerālajām sastāvdaļām, pazemes ūdeņu mākslīgās papildināšanās gaitā, ir humusvielu struktūras specifika un molekulmasa, kā arī minerālā sorbenta īpašības.
3. Humusvielas iespējams imobilizēt, tās kovalenti saistot ar dažādu noturību gan uz neorganiskas izcelsmes, gan uz organiskiem nesējiem iegūstot praktiski izmantojamus sorbentus. Imobilizētās humusvielas ir hidrolītiski stabilas un to mehāniskā stabilitāte ir atkarīga no izejas polimēra matricas īpašībām, vienlaikus humusvielas saglabā tām raksturīgās spējas mijiedarboties ar citām vielām.
4. Imobilizējot humusvielas iespējams iegūt sorbentus ar atšķirīgām īpašībām un dažādu funkcionālo grupu aizvietotības pakāpi, kas uzskatāmi par perspektīviem materiāliem vidi piesārņojošo vielu sorbcijai un to mijiedarbības rakstura izpētei ar humusvielām.

Pateicība

Šī darba tapšanas process ir bijis ilgs, un tā gaitā ir saņemta nenovērtējama palīdzība un atbalsts no kolēģiem gan tepat, gan arī laboratorijās ārpus Latvijas, kurās man bija iespēja pastrādāt – Leipcigā, Stokholmā un Turku. Es gribētu pateikties tiem cilvēkiem, bez kuru līdzdalības šis darbs būtu grūti iedomājams.

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Linda Eglīte

Literatūras saraksts

Abbt-Braun, G., Laukes, U., Frimmel, F. H. (2004) Structural characterization of aquatic humic substances – the need for a multiple method approach. *Aquat. Sci*., 66, 154–176.

Abbt-Braun, G. (1992) Spectroscopic characterization of humic substances in the ultraviolet and visible region and by infrared spectroscopy. In: Matthess, G., Frimmel, F. H., Hirsch, P., Schulz, H. D., Usdowski, E. (eds). *Progress on hydrogeochemistry: organics-carbonate system-silicate system-microbiology-models*. Berlin: Springer, 29–36.

Abbt-Braun, G., Frimmel, F. H. (2002) The relevance of reference materials: isolation and general characterization. In: Frimmel, F. H., Abbt-Braun, G., Heumann, K. G., Hock, B., Ludemann, H.-D., Spiteller, M. (eds). R*efractory organic substances (ROS) in the environment.* Weinheim: Wiley- WCH, 1–38.

Abbt-Braun, G., Frimmel, F.H., Schulten, H.-R. (1989) Structural investigation of aquatic humic substances by pyrolysis- field ionization mass spectrometry and pyrolysis- gas chromatography/ mass spectrometry. *Water Res.,* 23, 1579–1591.

Achard, F.K. (1786) Chemische Untersuchung des Torfs. *Crell's Chem. Ann*., 2, 391–403.

Aiken, G. and Cotsaris, E. (1995) Soil and hydrology: their effect on NOM. *J. Am. Water Works Ass.,* 87(1), 36–45.

Aiken, G. R. (1984) Evaluation of ultrafiltration for determining the molecular weight of fulvic acid. *Environ. Sci. Technol.*, 18, 978–981.

Aiken, G. R., McKnight, D. M., Wershaw, R. L. (1985) *Humic substances in soil, sediment, and water. Geochemistry, isolation and characterization*. N.Y.: Wiley

Alborzfar, M., Villumsen, A., Gron, C. (2001) Artificial recharge of humic ground water. *J. Environ. Qual.,* 30(1), 200–209.

Aleiho, L. M., Godinho, O. E. S., De Costa, W. F. (1992) Potentiometric study of acid-base properties of humic acid using functions for treatment data. *Anal. Chim. Acta,* 257, 35–39.

Aleiho, L. M., Godinho, O. E. S., De Costa, W. F. (1992) Potentiometric study of acid-base properties of humic acid using functions for treatment data. *Anal. Chim. Acta,* 257, 35–39.

Almendros, G., Fründ, R., Gonzalez-Vila, F. J., Haider, K. M., Knicker, H., Lüdemann, H. D. (1991) Analysis of ^{13}C and ^{15}N CPMAS NMR-spectra of soil organic matter and composts. *FEBS Lett*., 282(1), 119–121.

Aven, M. J., Koopal, L. K., van Reimsdijk, W. H. (1999) Proton binding to humic acids: Electrostatic and intrinsic interactions. *J. Colloid Interface Sci.,* 217(1), 37-48.

Baker, H., Khalili, F. (2003) Comparative study of binding strengths and thermodynamic aspects of Cu (II) and Ni (II) with humic acid by Schubert's ion-exchange method. *Anal. Chim. Acta,* 497, 235–248.

Bano, N., Moran, M. A., Hodson, R. E. (1997) Bacterial utilization of dissolved humic substances from a freshwater swamp. *Aquat. Microb. Ecol.,* 12, 233-238.

Barak, R., Chen, Y. (1992) Equivalent radii of humic macromolecules from acid-base titration. *Soil Sci.,* 154, 184–195.

Barancikova, G., Senesi, N., Bruneti, G. (1997) Chemical and spectroscopic characterization of humic acids isolated from different Slovak soil types. *Geoderma*, 78, 251–266.

Barriuso, E., Andreux, F., Portal, J. M. (1985) Etude de la repartion des glucides associes aux constituants humiques dans un sol humifere de montagne. *C.R. Acad. Sci. Paris*, 300(11), 827–832.

Beckett, R., Jue, Z., Giddings, J. C. (1987) Determination of molecular weight distributions of fulvic and humic acids using flow field flow fractioanation. *Environ. Sci. Technol.,* 21, 289–294.

Belin, C., Quellec, C., Lamotte, M., Ewald, M., Simon, Ph. (1993) Characterisation by fluorescence of the dissolved organic matter in natural water. Application to fractions obtained by tangential ultrafiltration and XAD resin isolation. *Environ. Technol*., 14, 1131–1144.

Berzelius, J. J. (1839) *Lehrbuch der Chemie*, Dresden.

Bloom, P. R., Leenheer, J. A. (1989) Vibrational, electronic, and high-energy spectroscopic methods for characterizing humic substances. In: Hayes, M. H. B., MacCarthy, P., Malcolm, R. L., Swift, R. S. (eds). *Humic substances II: In search of structure.* N.Y.: Wiley, 409–446.

Bollag, J. M. (1983) Cross-coupling of humus constituents and xenobiotic substances. In: Christman, R. F., Gjessing, E. T. (eds.) *Aquatic and Terrestrial Humic Substances*, Ann Arbor, MI: Ann Arbor Science Publishers, 127–141.

Bower, H. (2002) Artificial recharge of groundwater: hydrology and engineering. *Hydrol. Sci. J.,* 10, 121–142.

Braccwell, J. M., Haider, K., Larter, S. R., Schulten, H.-R. (1989) Thermal degradation relevant to structural studies of humic substances. In: Hayes, M. H. B., MacCarthy, P., Malcolm, R.L., Swift, R.S. (eds). *Humic substances II: in search of structure.* New York: John Wiley & Sons, 181–222.

Brinkmann, T., Horsch, P., Sartorius, D., Frimmel, F. (2003) Photoformation of low-molecular-weight organic acids from brown water dissolved organic matter. *Environ. Sci. Technol*., 37, 4190–4196.

Buffle, J. (1977) Les substances humiques et leurs interaction avec les ions mineraux. In: *Conference Proceedings de la Commission d'Hydrologie Applique de l'A.G.H.T.M.* L'Universite d'Orsay. 3–10.

Buffle, J., (1988) *Complexation reactions in aquatic systems*. Chichester: Ellis Horwood.

Burdon, J. (2001) Are the traditional concepts of the structures of humic substances realistic? *Soil Sci*., 166(11), 752–769.

Carter, C. W., Suffet, I. H. (1982) Binding of DDT to dissolved humic materials. *Environ. Sci. Technol*., 16, 735–740.

Chen, Y., Aviad, T. (1990) Effects of humic substances on plant growth. In: MacCarthy, P., Clapp, C. E., Malcolm, R. L., Bloom, P. R. (eds.), *Humic Substances in Soil and Crop Sciences: Selected Readings.* Madison, WI: ASA and SS SA, 161–186.

Chen, Y., Schnitzer, M. (1989) Sizes and shapes of humic substances by electronic microscopy. In: Hayes, M. H. B., MacCarthy, P., Malcolm, R. L., Swift, R. S. (eds). *Humic substances II: In search of structure.* N.Y.: Wiley, 621–638.

Chien, Y. Y., Bleam, W.F. (1998) Two-dimensional NOESY nuclear magnetic resonance study of pH-dependent changes in humic acid conformation in aqueous solutions. *Environ. Sci. Technol.,* 32, 3653–3658.

Chin, Y. P, Aiken, G., O'Loughner, E. (1994) Molecular weight polydispersity and spectroscopic properties of aquatic humic substance. *Environ. Sci. Technol.,* 28, 1853–1858.

Chiou, C. T., Porter, P. E., Schmeddling, D. W. (1983) Partition equilibria of nonionic organic compounds between soil organic matter and water. *Environ. Sci. Technol*., 17, 227–231.

Christman, R. F., Norwood, D. L., Seo, Y., Frimmel, F. H. (1989) Oxidative degradation of humic substances from freshwater environment. In: Hayes, M. H. B., MacCarthy, P., Malcolm, R. L., Swift, R. S. (eds). *Humic substances II: In search of structure*. N.Y.: Wiley, 33–67.

Clapp, C.E., Emerson, W.W., Olness, A.E. (1989) Sizes and shapes of humic substances by viscosity measurements. In: Hayes, M. H. B., MacCarthy, P., Malcolm, R. L., Swift, R. S. (eds). *Humic substances II: In search of structure.* N.Y.: Wiley, 497–514.

Conte, P., Piccolo, A. (1999) Conformational arrangement of dissolved humic substances. Influence of solution composition on association of humic molecules. *Environ. Sci. Technol.,* 33, 1682–1690.

Conte, P., Piccolo, A. (1999) High pressure size exclusion chromatography (HPSEC) of humic substances: molecular sizes, analytical parameters and column performance. *Chemosphere*, 38(3), 517–528.

Davis, J. A. (1982) Adsorption of natural dissolved organic matter at the oxide/water interface. *Geochim. Cosmochim. Acta*, 46, 2381–2393.

Day, G. M., Hart, B. T., McKelvie, I. D., Beckett, R. (1994) Adsorption of natural organic matter onto goethite. *Colloids Surf., A*, *Physicochem., Eng. Aspects,* 89, 1–13.

De Nobili, M., Chen, Y. (1999) Size exclusion chromatography and humic substances: limits, perspectives and prospectives. *Soil Sci.,* 164, 825–833.

De Paolis, F., Kukkonen, J. (1997) Binding of organic pollutants to humic and fulvic acids - influence of pH and the structure of humic material. *Chemosphere,* 34 (8), 1693–1704.

De Wit, J. C. M., van Riemsdijk, W. H., Koopal, L. K. (1993) Proton binding to humic substances. 1. Electrostatic effects. *Environ. Sci. Technol.,* 27, 2005–2022.

Dempsey, B. A., O'Melia, C. R. (1983) Proton and calcium complexation of four fulvic acid fractions. In: *Aquatic and terrestrial humic materials* (Eds. Christman, R. F., Gjessing, E. T.) Ann Arbor:AnnArbor Science, 239–273.

Diallo, M. S. (2003) 3-D structural modeling of humic acids through experimental characterization, computer assisted structure elucidation and atomistic simulations. 1. Chelsea soil humic acid. *Environ. Sci. Technol.,* 37, 1783–1793.

Dragunov, C. C., Zhelokhotseva, H. H., Strelkova, E. J. (1948) A comparative study of soil and peat humic acids. *Pochvovedenije*, 7, 409–420 (in Russian)

Dunnivant, F. M., Jardine, P. M., Taylor, D. L., McCarthy, J. F. (1992) Transport of naturally occuring dissolved organic carbon in laboratory columns containing aquifer material. *Soil Sci. Soc. Amer. J*., 56(2), 437–444.

Edwards, M., Benjamin, M. M., Ryan, J. N. (1996) Role of organic acidity in sorbtion of natural organic matter (NOM) to oxide surfaces. *Colloids Surf., A*, *Physicochem., Eng. Aspects,* 107, 297–307.

Eglite, L., Klavins, M. (2002) Sorption of humic substances on aquifer material and soil components. In: Proceedings of 20th Anniversary Conference of IHSS "*Humic substances: Nature's most versatile materials*", Boston, USA, 146–149.

Eglīte, L., Rozenbaha, I., Odham, G., Järnberg, U., Kļaviņš, M. (2003) Reductive degradation of humic substances. *Latv. Ķīm. Ž.,* 2, 313–320.

Ephraim, J., Alegret, S., Mathuthu, A., Bicking, M., Malcolm, R. L., Marinsky, J. A. (1986) A unified physicochemical description of the protonation and metal ion complexation equilibria of natural organic acids (humic and fulvic acids). 2. Influence of polyelectrolyte properties and functional group heterogeneity on the protonation equilibria of fulvic acid. *Environ. Sci. Technol*., 20, 354–366.

Ephraim, J. H., Boren, H., Pettersson, C., Arsenie, I., Allard, B. (1989) A novel description of the acid-base properties of an aquatic fulvic acid. *Environ. Sci. Technol*., 23(3), 356–362.

Farrah, S. R., Goyal, S. M., Gebra, C. P., Manajan, V. K., Wallis, C., Melnick, J. L. (1978) Concentration of humic acid from tapwater. *Water Res.,* 12, 303–306.

Filip, Z., Alberts, J. J. (1992) Humic substances and some microbial analogs from two thermal sites in Iceland. *Sci. Total Environ*., 117/118, 227–239.

Flaig, W. (1960) Chemie der Humusstoffe. *Suomen Kem*., 33, 229–251.

Frimmel, F. H., Abbt-Braun, G., Heumann, K. G., Hock, B., Ludemann, H.-D., Spiteller, M. (eds), (2002) *Refractory organic substances (ROS) in the environment.* Weinheim: Wiley-VCH.

Fründ, R., Haider, H., Lüdemann, H. D. (1994) Impacts of soil management practices on the organic matter structure investigations by CPMAS ^{13}CNMR-spectroscopy. *Z.Pflanzenernähr. Bodenkunde*, 157, 29–35.

Frycklund, C. (1995) Total organic carbon retention by filtersand in an infiltration ponds for artificial groundwater recharge. *Aqua Fennica,* 25, 5–14.

Frycklund, C., Jack, G. (1997) Iron and artificial recherge of groundwater. *Boreal Environ. Res.*, 2, 171–181.

Fuchs, W. (1930) *Die Chemie der Kohle*. Berlin

Gamble, D. S. (1972) Potentiometric titration of fulvic acid: equivalence point calculations and acidic functional groups. *Can. J.Chem*., 50, 2680–2690.

Gelinas, Y., Baldock, J. A., Hedges, J. I. (2001) Demineralization of marine and freshwater sediments for CP/MAS ^{13}C NMR analyses. *Org. Geochem.,* 32, 677–693.

Gerlach, M., Gimbel, R. (1999) Influence of humic substance alteration during soil passage on their behaviour. *Water Sci. Technol*., 40(9), 231–241.

Ghosh, K., Chattopadhyay, A., Varadachari, C. (1983) Electron exchange behaviours of humic substances with iron, copper and manganese. *Soil. Sci*., 135, 193–196.

Gjessing, E. T. (1965) Use of Sephadex gels for the estimation of molecular weight of humic substances in natural waters. *Nature*, 208, 1091–1092.

Gremm, T., Abbt-Braun G., Frimmel, F. H. (1991) Influence of different parameters on the HPLC-characterization of organic acids. *Vom. Wasser,* 77, 231–241.

Grøn, C., Tørsløv, J., Albrechtsen, H.-J., Jensen, H. M. (1992) Biodegradability of dissolved organic carbon in groundwater from an unconfined aquifer. *Sci. Total. Environ.*, 117/118, 241–251.

Gu, B., Schmitt, J., Chen, Z., Lian, L., McCarthy, J.F. (1993) Adsorption and desorption of natural organic matter on iron oxide: mechanisms and models. *Environ. Sci. Technol.,* 28(1), 38–46.

Guetzloff T. F., Rice J. A. (1994) Does humic acid form a micelle. Sci. Total Environ., 152, 31–35.

Gustafsson, J. P. (2001) Modeling the acid-base properties and metal complexation of humic substances with the Stockholm Humic Model. *J. Colloid Interface Sci*., 244, 102–112.

Hautala, K., Peuravuori, J., Pihlaja, K. (2000) Measurement of aquatic humus content by spectroscopic analyses. *Water Res.,* 34(1), 246–258.

Hayes, M. H. B., Clapp, C. E. (2001) Humic substances: considerations of compositions, aspects of structure and environmental influences. *Soil Sci*., 166(11), 723–737.

Hayes, M. H. B., MacCarthy, P., Malcolm, R. L., Swift, R. S. (eds) (1989) *Humic substances II: In search of structure* N.Y.: Wiley.

Hayes, M. H. B., Swift, R. S. (1978) The chemistry of soil organic colloids In: *The Chemistry of Soil Constituents* (Eds. Greenland, D. J., Hayes, M. H. B.) N.Y.:Wiley, 179–320.

Hedges, I. J., Oades, J. M. (1997) Comparative organic geochemistries of soils and marine sediments. *Org. Geochem.,* 27, 319–361.

Hejzlar, J., Szpakowska, B., Wershaw, R. L. (1994) Comparison of humic substances isolated from peatbog water by sorption on DEAE-cellulose and Amberlite XAD-2. *Water Research*, 28, 1961–1970.

Hertkorn, N., Permin, A., Perminova, I., Kovalevskii, D., Yudov, M., Petrosyan, V., Kettrup, A. (2002) Comparative analysis of partial structures of a peat humic and fulvic acids using one- and two- dimensional nuclear magnetic resonance spectroscopy. *J. Environ. Qual.,* 31, 375–387.

Hertkorn, N., Schimtt-Kopplin, P. Perminova, I. V., Kovalevski, D., Kettrup, A. (2001) Two dimensional NMR spectroscopy of humic substances In: Swift, R., Spark, K. M. (eds). *Understanding and managing organic matter in soils, sediments and waters.* Proceedings of the 9th International Conference of the International Conference of the International Humic Substances Society, Adelaide, 149–158.

Hessen, D. O., Tranvik, L. J. (eds.) (1998) Aquatic Humic Substances. Ecology and Biogeochemistry. Berlin: Springer.

Hu, X., Kitano, M., Takenaka, N., Badow, H., Meada, Y., Zhang, D. (1994) Sensitive determination of humic acid in natural water by chemiluminiscence-flow-injection method. *Bunseki Kagaku*, 43(12), 1077–1082.

Huffman, E. W. D., Stuber, H. A. (1985) Analytical methodology for elemental analysis of humic substances. In: Aiken, G. R., McKnight, D. M., Wershaw, R. L., MacCarthy, P. (eds). *Humic substances in soil, sediment and water.* N.Y.: Wiley, 433–455.

Huisman, L., Olsthoorn, T. N. (1983) *Artificial Groundwater Recharge*. Boston: Pitman Books Ltd.

Hunt, A. P., Parry, J., Hamilton-Taylor, D. (1999) Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria. *Limnol. Oceanogr.*, 45(1), 237–241.

Ishiwatari, R. (1971) Molecular weight distribution of humic acids from lake and marine sediments. *Geochem. J.,* **5**, 121–132.

Jardine, P. M., Weber, N. L., McCarthy, J. F. (1989) Mechanisms of dissolved organic carbon adsorption on soil. *Soil. Sci. Soc. Am. J.,* 53, 1378–1385.

Jones, M. N., Bryan, N. D. (1998) Colloidal properties of humic substances. *Adv. Colloid. Interface Sci.*, 78, 1–48.

Juhna, T., Klavins, M., Eglite, L. (2003) Sorption of humic substances on aquifer material at artificial recharge of groundwater. *Chemosphere*, 51(9), 861–868.

Juhna, T., Klavins, M., Sprogis, J. (1998) Retention of humic substances on filtersand from artificial groundwater recharge infiltration basins. *Latv. Ķīm. Ž.,* 4, 87–93.

Kaiser, K., Zech, W. (1997) Competitive sorption of dissolved organic matter fraction to soil and related mineral phase. *Soil. Sci. Soc. Am. J.*, 61, 64–69.

Kārkliņš, A. (1995) Starptautiskā augšņu klasifikācijas sistēma. Jelgava: LLU

Kasatochkhin, V.I. (1951) The structure of carbonized substances. *Izv. AN SSSR. Otd.Tehn. Nauk*, 9, 145–186.

Keeler, C., Maciel, G. E. (2003) Quantitation in the solid-state C-13 NMR analysis of soil and organic soil fractions. *Anal. Chem.,* 75, 2421–2432.

Killops, S. D., Killops, V. J. (1994) *An introduction to organic geochemistry.* N.Y.: Wiley.

Kingery, W. L., Simpson, A. J., Hayes, M. H. B., Locke, M. A., Hicks, R. P. (2000) The application of multidimensional NMR to the study of soil humic substances. *Soil Sci.,* 165, 483–494.

Kļaviņš, M. (1993): Immobilization of humic substances. *Latv. Ķīm. Ž.,* (1), 96–102.

Kļaviņš, M. (1998) *Aquatic humic substances: characterisation, structure and genesis*. Rīga: LU.

Kļaviņš, M., Apsīte, E. (1998) Sorption of humic substances on clays from Latvia. *Latv. Ķīm. Ž,* (1), 67–72.

Klavins, M., Eglite, L. (2002) Immobilisation of humic substances. *Colloids Surf. A: Physicochem., Eng. Aspects,* 203, 47–54.

Klavins, M., Eglite, L., Zicmanis, A. (2006) Immobilized humic substances as sorbents. *Chemosphere,* 62, 861–868.

Kļaviņš, M., Juhna, T., Eglīte, L. (2000) Removal of humic substances during treatment of drinking water using sorbents. *Vatten*, 56, 79–83.

Kļaviņš, M., Seržāne, J., Eglīte, L. (1999) Methods for analysis of aquatic humic substances. *Crit. Rev. Anal. Chem*., 29, 187–203.

Kļaviņš, M., Šīre, J., Eglīte, L. (2004) Humic substances and the potential of their use in agriculture. *Proc. Latv. Acad. Sci., Ser B*, 58(2), 39–49.

Kleinhempel, D. (1970) Ein Beitrag zur Theorie des Huminstoffzustandes. *Albrecht Thear Archives*, 14, 3–14.

Knicker, H., Fründ, R., Lüdemann, H. D. (1993) The chemical nature of nitrogen in native soil organic matter. *Naturwissenschaften*, 80, 219–221.

Knicker, H., Frund, R., Ludemann, H. D. (1997) Characterization of nitrogen in plant compost and native humic material by natural- abundance ^{15}N CPMAS and solution spectra. In: Nanny, M. A., Minear, R. A., Leenheer, J. A., (eds). *Nuclear magnetic resonance spectroscopy in environmental chemistry.* Oxford: Oxford Univ. Press, 272–294.

Koyama, M. (1995) Adsorption of Humic Acid on Ca-Montmorillonite. *Soil Sci. Plant Nutr.,* 41, 215–223.

Kögel-Knabner, I. (2002) The macromolecular organic composition of plant and microbial reisdues as inputs to soil organic matter. *Soil Biol. Biochem.,* 34, 139–162.

Kononova, M.M. (1966) *Soil organic matter*, Oxford: Pergamon Press.

Kretzschmar, R., Barmettler, K., Grolimund, D., Yan, Y., Borkovec, M., Sticher, H. (1997) Experimental determination of colloid deposition rates and collision efficiencies in natural porous media, *Water Resour. Res*., 33, 1129–1137.

Kullber, A. (1994) Mobilisation and transport of dissolved organic matter and its biological effect in acidified humic freshwaters. PhD thesis, Department of Ecology, Lund University, Sweden.

Kumke, M. U., Abbt-Braun G., Frimmel, F. H. (1998) Time-resolved fluorescence measurements of aquatic natural organic matter (NOM). *Acta hydrochim. hydrobiol.,* 26, 73–81.

Lambert, J., Burba, P., Buddrus, J. (1992) Quantification of partial structures in aquatic humic substances by volume integration of two-dimensional ^{13}C nuclear magnetic spectra. Comparison of one- and two dimensional techniques. *Magn. Res. Chem.*, 30, 221–227.

Langvik, V. A., Akerback, N., Holmbom, B. (1994) Characterization of aromatic structures in humic and fulvic acids. *Environ. Internat*., 20(1), 61–65.

Lassen, P., Randall, A., Jorgenson, O., Warwick, P., Carlsen, L. (1994) Enzymaticaly mediated incorporation of 2-chlorophenol and 4 chlorophenol into humic acids. *Chemosphere*, 28(4), 703–710.

Leenheer, J. A., Wershaw, R. L., Reddy, M. M. (1995) Strong acid, carboxyl-group structures in fulvic acid from the Suwannee River, Georgia. 2. Major structures. *Environ. Sci. Technol*., 29(2), 399–405

Leinweber, P., Fründ, R., Reuter, G. (1993) A ^{13}C NMR study on the formation of soil organic matter from grass residues. *Z.Pflanzenernähr. Bodenk*., 156, 415–420.

Liao, W., Christman, R. F., Johnosn, J. D., Millington, D. S. (1982) Structural characterization of aquatic humic material. *Environ. Sci. Technol*., 16, 403–410.

Lindell, M. J., Granéli, W., Tranvik, L. J. (1995) Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. *Limnol. Oceanogr*. 40, 195–199.

Lovley, D., Coates, J. D., Blunt-Harris, E. L., Philips, E. J. P., Woodward, J. C. (1996) Humic substances as electron acceptors for microbial respiration. *Nature,* 383(1), 445-448.

MacCarthy, P. (2001) The principles of humic substances. *Soil Sci*., 166 (13), 738–751.

McCarthy, J. F., Roberson, L. E., Burrus, L. W. (1989) Association of benzo(α)pyrene with dissolved organic matter: prediction of K_{dom} from structural and chemical properties of the organic matter. Chemosphere 19: 1911–1920.

Malcolm, R. L. (1985) Geochemistry of stream fulvic and humic substances. In: *Humic subsbtances in soil, sediments and water* (Eds. Aiken, G. R., McKnight, D. M., Wershaw, R. L., MacCarthy, P.) N.Y.: Wiley, 181–210.

Maurice, P. A., Pullin, M. J., Cabiniss, S. E., Zhou, Q., Namjesnik-Dejanovic, K., Aiken, G. R. (2002) A comparison of surface water natural organic matter in raw filtered water samples, XAD, and reverse osmosis isolates. *Water Res.,* 36(9), 2357–2371.

McBride, M. (1994) *Environmental chemistry of soils.* Oxford: Oxford University Press.

McDonald, S., Bishop, A. G., Prenzler, P. D., Robards, K. (2004) Analytical chemistry of freshwater humic substances. *Anal. Chim. Acta,* 527, 105–124.

Meier, M., Namjesnik-Dejanovic, K., Maurice, P. A., Chin, Y.-P., Aiken, G. R. (1999) Fraction of aquatic natural organic matter upon sorption to goethite ans kaolinite. *Chem. Geol.,* (157), 275–284.

Miettinen, I. T., Martikainen, P. J., Vartiainen, T. (1994) Humus transformation at the bank filtration water plant. *Water Sci. Technol*., 30(10), 179–187.

Miettinen, I. T., Martikainen, P. J., Vartiainen, T. (1998) Mutagenicity and amount of chloroform after chlorination of bank filtered water. *Sci. Total Environ.*, 215, 9–17.

Miettinen, I. T., Vartiainen T., Martikainen, P. J. (1997) Changes in water microbial quality during bank filtration of lake water. *Can. J. Microbiol.,* 43, 1126–1132.

Moers, M. E. C., Baas, M., de Leeuw, J. W., Boon, J. J., Schenk, P. A. (1990) Occurence and origin of carbohydrates in peat samples from a red mangrove environment as reflected by abundances of neutral monosaccharides. *Geochim. Cosmochim. Acta*, 54, 2463–2472.

Moreda-Piñeiro, A., Bermejo-Barrera, A., Bermejo-Barrera, P. (2004) New trends involving the use of ultrasound energy for the extraction of humic substances from marine sediments. *Anal. Chim. Acta,* 524, 97–107.

Morinaga, S., Ishiwatari, R., (1987) Gas chromatographic determination of C_1-C_5 low-moleculart-weight organic acids in alkaline permanganate oxidation products of humic substances. *J.Chromatogr*., 403, 225–231.

Nardi, S., Concheri, G., Dell'Agnola, G. (1996) Biological activity of humic substances. In: Piccolo, A. (Ed.), *Humic substances in terrestrial ecosystems*, Amsterdam: Elsevier, 361–406.

Niemeyer, J., Chen, Y., Bollag, J. M. (1992) Characterization of humic acids, composts, and peat by diffuse reflectance Fourier transform infrared spectroscopy. *Soil. Sci. Soc. Am. J.*, 56, 135–140.

Nierop, K. G. J., Jansen, B., Vrugt, J. A., Verstraten, J. M. (2002) Copper complexation by dissolved organic matter and uncertainty assessment of their stability constants. *Chemosphere,* 49, 1191–1200.

Orlov, D. S. (1990) *Soil humic acids and general humification theory*. Moscow: MGU (in Russian).

Orlov, D. S., Sadovnikova, L. K. (2005) Soil organic matter and protective functions of humic substances in the biosphere 37-52, in Use of Humic Substances to remediate Polluted Environments: From Theory to Practice. Edited by Irina V. Perminova, Kirk Hattfield and Norbert Hertkorn, NATO Sciences Series IV Earth and Environmental Sciences- Vol. 52

Parson, J. W. (1989) Hydrolytic degradation of humic substances. In: Hayes, M.H.B., MacCarthy, P., Malcolm, R.L., and Swift, R.S. (eds). *Humic substances II: In search of structure*. N.Y.: Wiley, 99–120.

Paul, E. A., and Clark, F. E. (1989) Soil microbiology and biochemistry.Acadamic Press, Inc., San Diego, CA.

Perdue, E. M. (1983) Association of organic pollutants with humic substances: Partitioning equilibria and hydrolysis kinetics. In: *Aquatic and terrestrial humic materials* (Eds. Christman, R. R., Gjessing, E. T.). Ann Arbor: Ann Arbor Science, 441–460.

Perdue, E.M. (1985) Acidic functional groups of humic substanes. In: *Humic substances in soil, sediments and water* (Eds. Aiken, G. R., McKnight, D. M., Wershaw, R. L., MacCarthy, P.) N.Y.: Wiley, 86–98.

Perdue, E.T. (1998) Chemical composition, structure and metal binding properties. In: Hessen, D. O., Tranvik, L. J. (eds). *Aquatic humic substances.* Berlin: Springer, 41–61.

Perminova, I. V., Frimmel, F. H., Kovalevskii, D. V., Abbt-Braun, G., Kudryavtsev, A. V., Hesse, S. (1998) Development of predictive model for calculation of molecular weight of humic substances. *Water Res.,* 32, 872–881.

Perminova, I. V., Frimmel, F. H., Kudryavtsev, A. V., Kulikova, N. A., Abbt-Braun, G., Hesse, S., Petrosyan, V. S. (2003) Molecular weight characteristics of humic substances from different environments as determined by size exclusion chromatography and their statistical evaluation. *Environ. Sci. Technol.,* 37, 2477–2485.

Peuravouri, J., Pihlaja, K. (1998) Multi-method characterization of lake aquatic matter isolated with two different sorbing solids. *Anal. Chim. Acta,* 363, 235–247.

Peuravuori, J. (1992) Isolation, fractionation and chracterization of aquatic humic substances. Does a distinct humic molecule exist. *Finnish Humus News*, 4(1), 1–334

Peuravuori, J., Ingman, P., Pihlaja, K. (2003) Critical comments on accuracy of quantitative determination of natural humic matter by soil state ^{13}C NMR spectroscopy. *Talanta,* 59, 177–189.

Peuravuori, J., Monteiro, A., Eglite, L., Pihlaja, K. (2005) Comparative study for separation of aquatic humic-type organic constituents by DAX-8, PVP and DEAE sorbing solids and tangential ultrafiltration: elemental composition, size-exclusion chromatography, UV–vis and FT-IR. *Talanta*, 65, 408–422.

Peuravuori, J., Pihlaja, K. (1997) Molecular size distribution and spectroscopic properties of aquatic humic substances. *Anal. Chim. Acta,* 337, 133–149.

Piccolo, A., Nardi, S., Concheri, G. (1992) Structural characteristics of humus and biological activity. *Soil Biol. Biochem*., 24, 273–380.

Qualls, R. G., Takiyama, A., Wershaw, R. L. 2003. Formation and loss of humic substances in the floor of a pine forest. *Soil Sci. Soc. Am. J.* 67, 899-909.

Ray, C., Grischek, T., Schubert, J., Wang, J. Z., Speth, T. F. (2002) A perspective of riverbank filtration. *J. Am. Water Works Asoc.,* 94(4), 149–160.

Reid, P. M., Wilkinson, A. E., Tipping, E., Jones, N. M. (1990) Determination of molecular weights of humic substances by analytical UV scanning ultracentrifugation. *Geochim. Cosmochim. Acta,* 54, 131–138.

Rice, J. A., MacCarthy, P. (1989) Isolation of humin by liquid-liquid partitioning. *Sci. Total Environ*., 81/82, 61–69.

Ritchie, J. D., Perdue, E. M., (2003) Proton-binding study of standard and reference fulvic acids, humic acids, and natural organic matter. *Geochim. Cosmochim. Acta*, 67, 85–96.

Rook, J. J. (1974). Formation of haloforms during chlorination of natural waters. *J. Water Treat. Exam.*, 23, 234–243.

Rozenbaha, I., Odham, G., Järnberg, U., Alsberg, T., Klavins, M., (2002) Characterisation of humic substances by acid catalysed transesterification. *Anal. Chim. Acta,* 452, 105–114.

Rozenbaha, I., Odham, G., Jarnberg, U., Eglite, L., Klavins, M. (2002) Characterization of humic substances using reduction and acid catalysed transesterification. In: Proceedings of 20th Anniversary Conference of IHSS "*Humic substances: Nature's most versatile materials*", Boston, USA, 74–77.

Schmitt-Kopplin, P. A. W., Garrison, W., Perdue, E. M., Freitag, D., Kettrup, A. (1998) Capillary electrophoresis of humic substances. Facts and artifacts. *J. Chrom., A*. 807, 101–109.

Schnitzer, M. (1978) Humic chemistry: chemistry and reactions. In: Schnitzer, M., Khan, S. U. (eds). *Humic substances in the environment*. N.Y,: Marcel Dekker, 1–64.

Schnitzer, M. (1991) Soil organic matter – the next 75 years. *Soil Sci.,* 151(1)41–58.

Schnitzer, M., Khan, S.U. (1972) *Humic substances in the environment*. N.Y.: Marcel Dekker.

Schulten, H.-R., Leinweber, P., Jandl, G. (2002) Analytical pyrolysis of humic substances and dissolved organic matter in water. In: Frimmel F. H., Abbt-Braun, G., Heumann, K. G., Hock, B., Ludemann, H.-D., Spiteller, M. (eds). Refractory organic substances (ROS) in environment. Weinheim: Wiley- WCH, 163–187.

Schulten, H.-R., Leinweber, P., Jandl, G. (2002) Analytical pyrolysis of humic substances and dissolved organic matter in water. In: Frimmel F. H., Abbt-Braun, G., Heumann, K. G., Hock, B., Ludemann, H.-D., Spiteller, M. (eds). *Refractory organic substances (ROS) in environment.* Weinheim: Wiley- WCH, 163–187.

Schulten, H.-R., Schnitzer, M. (1993) A state of the art structural concept for humic substances. *Naturwissenschaften*, 80, 29–30.

Senesi, N. (1990) Molecular and quantitative aspects of the chemistry of fulvic acids and its interaction with metal ions and organic chemicals. II The fluorescence spectroscopy approach. *Anal. Chim. Acta,* 232, 77–100.

Senesi, N., Miano, T. M., Provenzano, M. R. (1991) Fluorescence spectroscopy as a means of distinguishing fulvic and humic acids from dissolved sedimentary aquatic sources and terrestrial sources. In: Allard, B., Boren, H., Grimvall, A. (eds). *Humic substances in the aquatic and terrestrial environment.* Berlin: Springer, 63–73.

Senesi, N., Sakellariadou, F. (1984) Structural and functional chemical properties of marine and coastal sedimentary humic acids. *Environ. Internat*., 20(1), 3–9.

Senesi, N., Sposito, G., Martin, J. P. (1986) Copper(II) and iron(III) complexation by soil humic acids: and IR and ESR study. *Sci. Total Environ*., 55, 851–362.

Senesi, N., Steelink, C. (1989) Application of ERS spectroscopy to study of humic sunstances. In: Hayes, M. H. B., MacCarthy, P., Malcolm, R. L., Swift, R. S., (eds). *Humic substances II: In search of structure.* N.Y.: Wiley, 373–408.

Shen, Y. H. (1999) Sorption of natural dissolved organic matter on soil. *Chemosphere,* 38 (7), 1505–1515.

Sibanda, H. M., Young, S. D. (1986) Competitive adsorption of humic acids and phosphate on goethite, gibbsite and two tropical soils. *J. Soil Sci.* 37, 197–204.

Sierra, M. M. D., Giovanela, M., Parlanti, E., Soriano-Sierra, E. J. (2005) Fluorescence fingerprint of fulvic and humic acids from varied origins as viewed by single-scan and excitation/emission matrix techniques. *Chemosphere,* 58, 715–733.

Simpson, A. J., Kingery, W. L., Hayes, M. H. B., Spraul, M., Humpfer, E., Dvortsak, P., Kerssebaum, R., Godejohann, M., Hofmann, M. (2002) Molecular structures and associations of humic substances in the terrestrial environment. *Naturwissenschaften,* 89, 84–88.

Simpson, A. J., Salloum, M. J., Kingery, W. L., Hatcher, P. G. (2003) The identification of plant derived structures in humic materials using three-dimensional NMR spectroscopy. *Environ. Sci. Technol.,* 37, 337–342.

Spark, M. K., Wells, J. D., Johnson, B. B. (1997) Characteristics of the sorption of humic acids by soil minerals. *Aust. J. Soil. Res.*, 35, 103–112.

Sposito, G. (1989) *The chemistry of soils*. Oxford: Oxford University Press

Steinberg, C. E. W., 2003, Ecology of Humic Substances in Freshwaters, Springer-verlag Berlin Heidelberg, 440.

Stepan, S. F., Smith, J. F., Fego, U., Reukers, J. (1978) Apparatus for on-site extraction of organic compounds from water. *Water Res*., 12, 447–449.

Stevenson, F. J. (1982) *Humus chemistry*. N.Y.: Wiley.

Stevenson, F. J. (1994) *Humus Chemistry: Genesis, Composition, Reactions*, N.Y.: Wiley.

Szabo, G., Bulman R. A. (1994) Comparison of adsorption coefficient (K_{oc}) for soils and HPLC retention factors of aromatic-hydrocarbons using a chemically immobilized humic-acid column in RP-HPLC. *J. Liquid Chromat.* 17, 2593–2604.

Tan, K. H. (2003) Humic Matter in Soil and the Environment Principles and Controversies. Marcel Dekker Inc., New York, 386.

Tan, K. H. (2005) Soil sampling, preparation, and analysis. Taylor & Francis Group, Boca Raton, 623.

Terashima M., Fukushima M., Tanaka S. (2004) Evaluation of solubilizing ability of humic aggregate basing on the phase separation model. *Chemosphere,* 57, 439–445.

Thomsen, M., Lassen, P., Dobel, S., Hansen, P. E., Carlsen, L., Mogensen, B. B. (2002) Characterisation of humic materials of different origin: a multivariate approach for quantifying the latent properties of dissolved organic matter. *Chemosphere,* 49, 1327–1337.

Thorn, K. A., Arterburn, J. B., Mikita, M. A. (1992) ^{15}N and ^{13}C NMR investigation of hydrohylamine derivatized humic substances. *Environ. Sci. Technol*., 26, 107–116.

Thurman, E. M. (1985) *Organic geochemistry of natural waters*. Wageningen: Martinus Nijhoff/ Dr. W. Junk Publishers

Thurman, E. M., Malcolm, R. L. (1981) Preparative isolation of aquatic humic substances. *Environ. Sci. Technol.*, 15 (4), 463–466.

Tipping, E. (1981) The adsorption of aquatic humic substances by iron oxides. *Geochim. Cosmochim. Acta,* 45, 191–199.

Tombácz, E., Csanaki, Cs., Márk, Cs., Szekeres, M. (2000) Particle interactions in complex aquatic systems. In: Proceedings of 19th Anniversary Conference of IHSS "*Entering the Third Millenium with a common approach to humic substances and organic matter in water, soil and sediments*", Tolouse, France, 146–149.

Tombácz, E., Dobos, Á., Szekeres, M., Narres, H. D., Klumpp, E., Dékány, I. (2000) Effect of pH and ionic strength on the interaction of humic acid with aluminium oxide. *Colloid Polym. Sci.*, 278, 337–345.

Town, R. M., Filella, M. (2000) A comprehensive systematic compilation of complexation parameters reported for trace metals in natural waters. *Aquat. Sci*., 62, 252–295.

Traina, S. J., Novak, J., Cmeck, N. E. (1990) An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids. *J.Environ. Qual*., 19, 151–153.

Tranvik, L. J. (1990) Bacterioplankton growth on fractions of dissolved organic carbon of different molecular weights from humic and clear waters. *Appl. Environ. Microbiol*., 56(6), 1672-1677.

Tranvik, L. J., Höfle, M. G. (1987) Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. *Appl. Environ. Microbiol.,* 53, 482–488.

Turski, R., Chmielewska, B. (1986) Soil humic acids. *Rocz. Gleb*., 37 (2/3), 107–115.

Vermeer, A. W. P., van Riemsdijk, W. H., Koopal, L. K. (1998) Adsorbtion of humic acid to mineral particles. 1. Specific and electrostatic interactions. *Langmuir*, 14, 2810–2819.

Wershaw, R. L. (1986) A new model for humic materials and their interactions with hydrophobic organic chemicals in soil-water and sediment-water systems. J.*Contam.Hydrol*., 1, 29–45.

Wershaw, R. L. (1989) Application of a membrane model to the sorptive interactions of humic substances. *Environ. Health Perspect*., 83, 191–203.

Wershaw, R. L. (1990) The importance of humic substance-mineral particle complexes in the modelling of contaminant transport in sediment-water systems. In: *Organic substances and sediments in water.* Vol. 1*, Humics and soil* (Ed. R.A.Baker), Boca Raton: Lewis Publ., 23–35.

Wershaw, R. L. (1993) Model for humus. *Environ. Sci. Technol*., 27(5), 814–817.

Wershaw, R. L., Bulcar, P. J., Goldberg, M. C. (1969) Interaction of pesticides with natural organic material. *Environ. Sci. Technol*., 3, 271–273.

Wilkinson, K. J., Nègre, J. C., Buffle, J. (1997) Coagulation of colloidal material in surface water: the role of natural organic matter. *J. Contamin. Hydrol.*, 26, 229–243.

Williamson, C. E., Morris, D. P., Pace, M., Olson, O. G. (1999) Dissolved organic carbon and nutrients as regulators of lake ecosystem: Resurrection of a more integrated paradigm. *Limnol. Oceanogr*. 44(3/2), 795–803.

Wilson, M. A. (1987) *NMR techniques and applications in geochemistry and soil chemistry.* Oxford: Pergamon.

Winner, R. W. (1985) Bioaccumulation and toxicity of copper as affected by interactions between humic acid and water hardness . *Water Res*. 19 (4), 449–455.

Yang, Y. H., Koopal L. K. (1999) Immobilisation of humic acids and binding of nitrophenol to immobilized humics. *Colloids Surf. A: Physicochem., Eng. Aspects,* 151, 201–212.

Yates L., Wandruszka R. (1999) Efects of pH and metals on the surface tension of aqueous humic materials. Soil. Sci. Am., 63, 1645–1649.

Zavarzina, A. G., Demin, V. V., Nifanteva, T. I., Skhinev, T. V., Danilova, T. V., Spivakov, B. Y. (2002) Extraction of humic acids and their fractions in poly(ethylene glycol) based aqueous systems. *Anal. Chim. Acta,* 452, 95–103.

Zhou, J. L., Rowland, S., Mantoura, R.F.C., Braven, J. (1994) The formation of humic coatings on mineral particles under simulated esturine condition – a mechanistic study. *Water Res*., 28(3), 571–579.

Ziechmann, W. (1994) *Humic substances*. Mannheim: BI Wissenschafts Verlag.

Орлов, Д. С., Садовникова, Л. К., Лозановская, И. Н. (2002) Экология и охрана биосферы при химическом загрязнении, Высшая школа, Москва: Высшая школа, 334 [Orlov, D. S., Sadovnikova, L. K., Lozanovskaja, I. N., (2002) Ecology and protection of biosphere on chemical pollution, Moscow, (in Russian)].

Орлов, Д. С. (1990) Гумусовые кислоты почв и общая теория гумификации. Москва: МГУ. 275.

UNIVERSITY OF LATVIA
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Humic substances, their interaction with soil components and immobilisation of humic substances

Summary of thesis for applying for the Doctoral degree

Riga, 2007

The research was carried out between 1999 and 2003 at University of Latvia, Faculty of Geography and Earth sciences in Laboratory of Environmental Chemistry. Some parts of experimental work were performed at Stockholm University, Department of Applied Environmental Science (Sweden), Centre for Environmental Research (Leipzig-Halle, Germany), and University of Turku, Department of Chemistry (Finland). Work was supported by European Social Fund.

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The defence of doctoral thesis will be held at 16th of March, 2007 at 1 p.m. in a public session of the Doctoral Committee at the Faculty of Geography and Earth Sciences, 10 Alberta Street, Riga.

The dissertation is available at Scientific Library of University of Latvia, 4 Kalpaka blvd. and Latvian Academic Library, 4 Lielvardes Street.

Aim of the study

Aim of the research was to investigate interaction process between humic substances isolated from water, soil and peat with inorganic soil components typical for Latvia, as well as to obtain immobilised humic substances by covalent binding with different carriers and to study their properties and sorption process of pollutants on immobilised humic substances.

Main tasks:

1) to isolate humic substances from different natural environments of Latvia, and characterise them;

2) to investigate the sorption characteristic of humic substances on soil mineral and organic components,

3) to investigate sorption characteristics of humic substances on soil microorganisms;

4) to develop methods for immobilisation of humic substances and characterise obtained immobilised humic substances;

5) to investigate the sorption of organic pollutants on immobilised humic substances.

Scientific novelty:

1) isolation of humic substances from different natural environments of Latvia, and characterisation of isolated samples were done;

2) investigation of humic substances sorption characteristic on inorganic soil minerals were done;

3) investigation of humic substances sorption characteristic on soil microorganisms were done;

4) method for immobilisation of humic substances were developed;

5) immobilised humic substances were tested as sorbents for removal of organic pollutants.

Practical importance of the study:

1) investigation of humic substances interaction with soil mineral components is important contribution for development of environmentally friendly humic containing fertilizers for use in agriculture;

2) investigation of humic substances sorption characteristic on inorganic materials forming groundwater artificial recharge infiltration layers, is important for optimization of groundwater artificial recharge technology;

3) immobilised humic substances can be considered as a prospective sorbents.

Approbation of Results

Main results of this study were presented in the following international conferences:

1. Eglite, L., Juhna, T., Klavins, M. (2000) Sorption of humic substances onto solid sorbents during natural and artificial water purification process. In: Abstracts of Symposium on Refractory organic substances in the environment. Karlsruhe, Germany, 198-200.
2. Eglite, L., Odham, G., Rozenbaha, I., Klavins, M. (2001) Reductive degradation of humic substances for elucidation of their core structures. In: Proceedings of 8th Nordic IHSS Symposium, Copenhagen, 44-45.
3. Eglīte, L., Kļaviņš, M. (2001) Methods of immobilisation of humic substances. II World Latvians Scientists congress, Riga, 351.
4. Kļaviņš, M., Eglīte, L., Juhna, T. (2001) Sorption of humic substances onto solid phases during natural and artificial groundwater recharge process. In: Abstracts of ISEB 15. Biogeochemical processes and cycling of elements in the environment, Wroclaw, 33.
5. Eglite, L., Klavins, M. (2002) Sorption of humic substances on soil inorganic and organic components. In: Use of humates to remediate polluted environments: from theory to practice, Zvenigorod, Russia, 15-16.
6. Eglite, L., Peuravuori, J., Lehtonen, T. (2005) Characterization of humic substances of different origins of Latvia using pyrolysis-gas chromatography/mass spectrometry. In: Abstracts of 10th Nordic IHSS Symposium on Character of natural organic matter and its role in the environment, Riga, Latvia, 22.

The main results of the study are described in publication:

1. Kļaviņš, M., Seržāne, J., Eglīte, L. (1999) Methods for analysis of aquatic humic substances. *Crit. Rev. Analyt, Chem*., 29, 187-203.
2. Kļaviņš, M., Juhna, T., Eglīte, L. (2000) Removal of humic substances during treatment of drinking water using sorbents. *Vatten,* 56, 79-83.
3. Klavins, M., Eglite, L. (2002) Immobilisation of humic substances. *Colloids and Surfaces A: Physicochem. Eng. Aspects,* 203, 47 - 54.
4. Eglite, L., Klavins, M. (2002) Sorption of humic substances on aquifer material and soil components. In: Proceedings of 20th Anniversary Conference of IHSS "Humic substances: Nature's most versatile materials", Boston, USA, 146-149.
5. Rozenbaha, I., Odham, G., Jarnberg, U., Eglite, L., Klavins, M. (2002) Characterization of humic substances using reduction and acid catalysed transesterification. In: Proceedings of 20th Anniversary Conference of IHSS "Humic substances: Nature's most versatile materials", Boston, USA, 74-77.
6. Juhna, T., Klavins, M., Eglite, L. (2003) Sorption of humic substances on aquifer material at artificial recharge of groundwater. *Chemosphere,* 51(9), 861-868.
7. Kļaviņš, M., Šīre, J., Eglīte, L. (2004) Humic substances and the potential of their use in agriculture. *Proc. Latv. Acad. Sci.,* Ser B, 58(2), 39-49.

8. Eglīte, L., Rozenbaha, I., Odham, G., Järnberg, U., Kļaviņš, M. (2003) Reductive degradation of humic substances. *Latv. Ķīm. Ž.,* 2, 313-320.
9. Peuravuori, J., Monteiro, A., Eglite, L., Pihlaja, K. (2005) Comparative study for separation of aquatic humic-type organic constituents by DAX-8, PVP and DEAE sorbing solids and tangential ultrafiltration: elemental composition, size-exclusion chromatography, UV–Vis and FT-IR. *Talanta,* 65, 408-422.
10. Klavins, M., Eglite, L., Zicmanis, A. (2006) Immobilized humic substances as sorbents. *Chemosphere,* 62, 861-868.

1. Introduction

Humic substances (HS) are the main component of soil organic matter (SOM), composing up to 60 – 70 % of them, but at the same time humic substances can be considered as one of the key elements in the biogeochemical turnover of carbon, possibly being the most abundant of naturally occurring organic macromolecules on the Earth (2–3 $\times 10^{10}$ t) (Jones and Bryan, 1998). As far as HS are able to interact with inorganic and organic substances, they act as carriers of them much influencing fluxes of elements in the environment. They also play an important role also in the formation of fossil fuels and mineral deposits (MacCarthy, 2001).

Humic substances are a general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight, and refractory (Aiken et al., 1985).

They consist of several groups of substances that depending on their solubility can be grouped as follows: humin is the fraction of humic substances that is not soluble in water at any pH; humic acid (HA) is the fraction of humic substances that is not soluble in water under acidic conditions (below pH 2), but becomes soluble at greater pH; fulvic acid (FA) is the fraction of humic substances that is soluble under all pH conditions (Aiken et al., 1985).

Humic substances have many functions in the environment (Orlov et al., 2002). In soils they participate in the formation of soil structure and thermal regime, but interacting with dissolved substances, they influence the accumulation and release processes of nutrients and trace elements. Humic substances much influence soil biota and especially microbial activity.

The interaction of humic substances with organic compounds can change their properties and fate in the environment. The most important impacts are:

1. increased apparent solubility of nonionic hydrophobic substances;
2. reduced solubility of ionic organic substances;
3. reduced volatility of organic substances;
4. modified chemical reactivity of organic substances in the environment;
5. change in rate of organic bioaccumulation in the environments;
6. increased association of organic substances with sedimentary phases and particulate matter (Kļaviņš, 1998).

In general terms, humic substances can be considered as a matrix onto which environmental processes are imprinted, but on the other hand they can be considered as an reactive ingredient, actively participating in reactions and processes going on in the soil and aquatic environment. As far as the major reservoir of humic substances are soils, they are an important factor in various areas of agriculture, such as soil chemistry, fertility, plant physiology and others. At the same time humic substances can be considered as an important resource, as far as they can be extracted in industrial amounts from sources (soil, peat, coal and others) in which they are abundant and consecutively applied to achieve or increase their positive impacts.

Humic substances are generally extracted from soil and peat by extracting with a basic solution (Stevenson, 1994), while humic and fulvic acids are solubilised, but the residue contains the humin. After the acidification of the alkaline extract by addition of a strong acid, humic acid precipitates, and the remaining organic material in solution is referred to as the fulvic acid (Stevenson, 1994). Often further purification is needed to clean up the HA and to separate the FA from other materials in the fulvic acid fraction, as well as to reduce the ash contents of the humic and fulvic extracts, and to fully convert the acid salts to their hydrogen forms. Evidently quite a lot of chemical degradation occurs during the extraction of HS. Thus, base-extracted HS generally is a combination of native and altered materials. The extracted HS are frequently dried by conventional evaporation or by lyophilization. It is also likely that some chemical changes, such as the formation of anhydrides and lactones and/or loss of carbon dioxide, occur when HS are dried, particularly when dried at elevated temperatures. There are numerous variations of the extraction procedure including the variation of the nature and concentration of the extractant; the temperature at which the extraction is performed; period of contact with base; steps taken to minimize ash content of extracted products; and the choice of aerobic versus anaerobic conditions during the extraction. Many other extraction procedures and variations have been used, some involving various organic solvents such as dimethylsulfoxide, dimethylformamide, and formic acid (Hayes and Clapp, 2001). It is not surprising that materials extracted from soils or sediments according to procedures based on the above definitions actually consist of mixtures and their properties depend on the specific conditions of extraction. Nevertheless, there is a remarkable uniformity in the average properties of all HA, FA, and humins (Schnitzer and Khan, 1972). Elemental contents of HA, FA from very different sources are remarkably consistent (Kļaviņš, 1998). Humic acids have been reported to have average MW varying from about 800 Da for aquatic materials to greater than 1×10^6 Da for soil- and peat derived materials (Kļaviņš, 1998). Humic substances have an abundance of oxygen-containing functional groups (carboxyl-, phenolic-, alcoholic-) which dominate their chemical properties. Humic substances occur in close association with other organic and inorganic materials in soil and sediments. Aquatic HS also occur in association with nonhumic materials and may exist in colloidal or larger aggregate forms.

The concept of structure of aquatic humic substances is closely linked with the understanding of their genesis. Generally, the approaches used for identification of humus structure can be divided into the following groups:

- speculative approaches, e.g., based on some integrative characteristics, such as elemental composition, spectral properties, acidity, and developed structural models;
- degradative approaches. Degradation of the original humus structure and analysis of the formed low molecular compounds, with their later combination in structural models;
- nondegradative approaches. Analysis of humus structure by methods which minimally affect the original structure;
- environmental approach. Development of structural models based on the understanding of the environmental roles of humic substances.

The use of a wide variety of analytical methods, including degradative and nondegradative approaches, to characterise Suwanee River fulvic acids, led to a model proposed by Leenheer et al., (1995). This model shows the most possible average structural formulas and considers also the possible precursors in the humification process. The aromatic and aliphatic structures in the proposed formulas indicate hypothetical residues of parent compounds, such as lignins, tannins, carbohydrates and lipids, which are incorporated into the structure of humic substances. The multitude of possible links of these structural units and their degree of biodegradation can explain the large diversity of structural formulas for the possible humus structure.

Schulten and Schnitzer (1993) proposed a carbon skeleton model for humic acid (Figure 1) in which alkylbenzene structures played a dominant role. This model is based on extensive mass spectrometric (pyrolysis GC/MS and pyrolysis-FIMS), degradative studies (chemical, oxidative and reductive degradation), colloid-chemical and electron microscope investigations of humus samples.

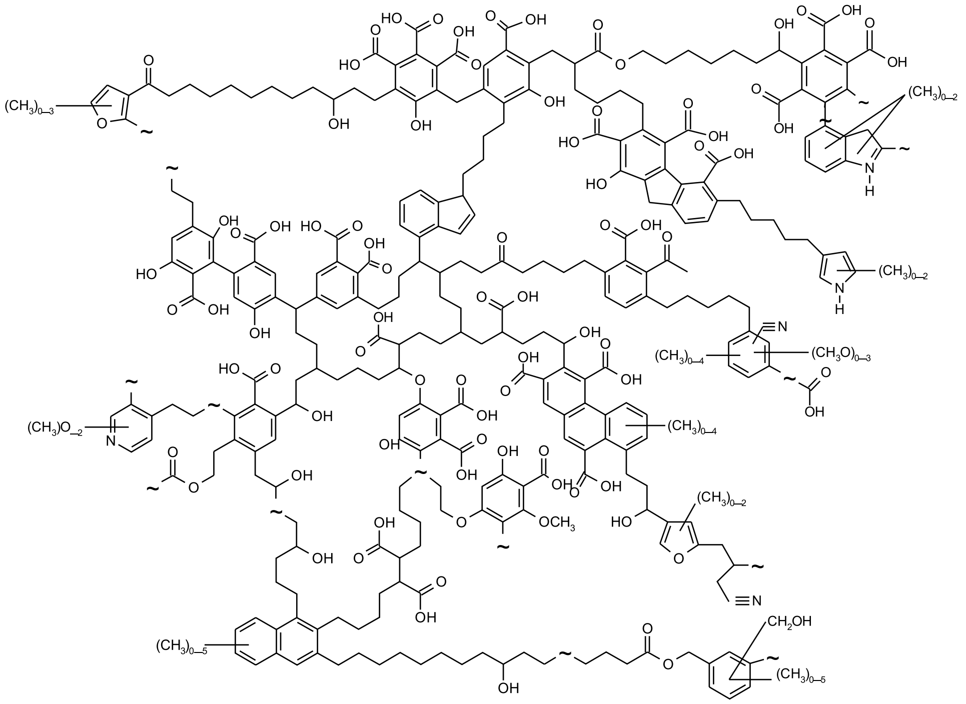

Figure 1. **Biomacromolecular humic acid structure after Schulten and Schnitzer (1993)**

Oxygen according to this model is present in humus structure in the form of carboxyls, phenolic and alcoholic hydroxyls, carboxylic esters and ethers. Nitrogen occurs in heterocyclic structures and as nitriles. The elemental composition of the proposed HA is about $C_{308}H_{328}O_{90}N_5$ with an approximate molecular weight of 5540 Da. Carbohydrates and proteinaceous materials are regarded to be present as loosely bound materials, but possessing the integral characteristics of humic substances. The comparison of the elemental and functional composition of the proposed structure with existing data reveals good agreement. The network structure allows to explain binding not only with metals and hydrophobic organic molecules, but also binding and incorporation into the molecular structure of molecules such as carbohydrates and proteins, as well as inorganics such as clay minerals and hydrous oxides. It can be expected that this chemical network structure is flexible in accordance with previous observations from surface pressure and viscosity measurements and from electron microscopy of humic substances.

Ziechman (1994) stressed the role of humus genesis in the development of the understanding of its structure.

Wershaw introduced the phase concept in the theory of humus structure. He regarded humic substances as mixtures that cannot be represented by conventional structural diagrams of functional groups held together by covalent bonds. Rather, they exist as membranelike or micellelike aggregates (Wershaw, 1989; 1993).

Furthermore, the physical and chemical properties of these humic aggregates are more a function of the structure of the aggregates, rather than the properties of individual components. In terms of this model, the division of humic substances into humic acid, fulvic acid and humin is a totally artificial division that tends to obscure the close interactions between the organic constituents of natural water systems. According to Wershaw (1993), the humic aggregates are composed of partially degraded molecular components of plants. This partial degradation consists, at least, of the oxidation and formation of carboxylic acid groups. These carboxylic groups are attached to hydrophobic or less polar functional groups. Therefore, the constituent molecules of the humic aggregates are amphiphiles.

The recent structural concepts of humic substances focus on the understanding of their environmental role, and no longer regard them as chemical molecules in the environment. A final understanding and unanimously regarded theories have not been developed, and the final definition of humus structure is one of the most important problems in humus research (Burdon, 2001).

The complexation of metals by humic substances is of particular interest because this complexation alters the toxicity and bioavailability of metal ions. The complexation of metals ions much reduce their toxicity in comparison with that of free, hydrated metal ions; however, substantial differences exist between different metal ions, and the modification of metal ion toxicity depends also on properties of humic matter (Winner, 1985). The interaction of humic substances with metals is important in plant nutrition, and availability of metal ions as well as nutrients to plants in soils is a function of the speciation of the metals in the soil solutions surrounding the plant roots. These forms have seen classified these forms into the following categories: a) particulate (metals incorporated into sediment particles); b) simple hydrated metal ion; c) simple inorganic complex; d) simple organic complex; e) stable inorganic complex; f) adsorbed on inorganic complexes; g) adsorbed on organic complexes. Considering the much differing toxicity and biological availability of different speciation forms, thus it is important to consider not so much the total amount of metals, but rather their extractable forms. Humic substances exist both in the bound and in the dissolved phase. In the solid phase the humic substances are present as coatings on mineral grains and possibly, in some instances, as separate particles. The fact that humic substances are present as thin coatings on the soil mineral grains causes the humic substances to be one of the most important components in soils, even when the organic carbon content of them may be only a few percent. Thus, the distribution of metals between the dissolved and solid phase will involve interactions with humic substances in both phases. The most common type of interaction of metal ions with humic substances is ion exchange, in which protons on carboxylic acid groups are replaced by metal ions. The analysis of fulvic acid titration data (Ephraim et al., 1986) indicated that fulvic acid molecules, in general, behave as rigid polyions that are impermeable to salt ions; however, of importance can be the presence of micellelike structures with charged groups on the exterior surfaces of the micelles. The simple picture of metal ions interacting with humic and fulvic acid molecules or micelles in solution is complicated by the fact that polyvalent cations cause aggregation of humic and fulvic acids (Ghosh et al., 1983). Humic and fulvic acids also form charge-transfer complexes with metal ions. Senesi et al. (1986) have found that soil humic acids have several different classes of binding sites for metal ions: stronger sites in which the metal-ligand complexes are covalent in character and which are stable to proton exchange, and sites that form weaker complexes that are readily disrupted by protons.

Organic compounds interact with humic substances in a number of different ways. Nonionic organic compounds partition into insoluble humic substances in soils, whereas soluble humic substances solubilize nonionic organics (Wershaw et al., 1969; Chiou et al., 1983). Ionic organic compounds can undergo ionic exchange reactions and charge-transfer complexation. In addition, some evidence exists for oxidative coupling reactions between xenobiotic organic compounds and humic substances. The sorption of nonionic organic compounds by wet soils involves partitioning of the organic compounds between the soil-water phase and the soil-organic phase. This partitioning is analogous to partitioning between water and an immiscible organic solvent phase (Chiou et al., 1983) and can be described by the same equations that are used for solutions of high molecular weight polymers in low molecular weight solvents, suggesting that the polymer molecule is a long chain composed of segments that are about the same size as the solvent molecules. An analogous process to partitioning into an insoluble organic phase is solubilization of hydrophobic organic compounds by dissolved humic substances. It has been found that humic acid enhances the solubility of DDT, but also of other hydrophobic pesticides in water. This increase in solubility apparently is brought about by the partitioning of the DDT molecules in the hydrophobic interiors of humic acid micelles. In a number of studies a variety of other hydrophobic organic compounds has been included as well as other humic substances, and generally similar solubilization behavior has been found (Carter and Suffet, 1982).

It has been found (Perdue, 1983) that partition of an organic substrate into a humic micellelike phase can modify the rate of hydrolysis of the substrate. Acid-catalyzed hydrolysis reactions will be accelerated, and base-catalyzed reactions will be inhibited. In addition to the effects on acid-base hydrolysis, partition into a humic micelle phase can also alter the general reactivity of a substrate. Recent studies demonstrated that the catalytic activity of humic substances can be considered as a key factor influencing the fate of contaminants in soil environments (Kļaviņš et al., 1999).

In dry soil hydrophobic organic compounds adsorb to the surface of soil particles (Chiou et al., 1983). This adsorption is characterized by different equations than the partition reactions that take place in wet soils and the adsorption that takes place on mineral surfaces. However, a number of studies have shown that metal oxide surfaces in natural waters bind humic substances and that this alters the surface properties of the metal oxides (Tipping, 1981). Clay minerals also form complexes with humic substances (Kļaviņš and Apsīte, 1998). These complexes consist of humic substances coating the surfaces of the clay particles and, in some instances, entering into interlayer positions in the clay particles. These results suggest that a substantial part of the mineral surfaces in soils and sediments are coated with humic substances; however, uncoated surfaces also may be present.

A number of different types of ionic interactions have been reported between humic substances and organic compounds. In the simplest case, the organic compound exists in solution as cations that can be bound by carboxylate groups of humic substances. Amino acids and triazine herbicides would bind to humic substances by this mechanism at low pH values where the nitrogen containing groups would be protonated, but hydrogen bonding can also take place between humic substances and basic herbicides such as substituted urea herbicides. The groups most likely to enter into hydrogen-bonding interactions would be hydroxyl and carboxylic acid groups on the surfaces of the humic substance membranelike aggregates (Kļaviņš, 1998).

Humic substances contain significant quantities of stable free radicals. The presence of these radicals has led some researchers to conclude that oxidative coupling reactions are important both in the formation of humic substances and in their interactions with other organic compounds (Bollag, 1983). Most of the work on the oxidative coupling in humic substances has been concerned with coupling of phenolate free radicals. Evidence for the presence of radicals of this type in humic substances is obtained from electron spin resonance (ESR) studies.

Therefore the aims of the thesis includes the isolation of humic substances from common environments in Latvia, the study of their properties as well as possibilities to isolate them from water, soil, peat, studying their basic properties indicating their structural composition, as well as the development of immobilization metods of humic substances and both sorption of organic and inorganic substances onto immobilized humic substances, both the sorption of humic substances onto inorganic materials of importance at the development of soil structure.

2. Properties of humic substances from different sources

Properties of humic substances are influenced by geographical, geological, geochemical and many other processes. The selected aquatic, soil and peat samples can be considered as representative for Latvia, and so are the studied humic substances. The selected humic substances have been compared with humic substances studied previously and references, and commercial humic substances.

The elemental composition of humic substances from soils and peat of Latvia is generally similar to that of soil humic substances presented previously, however, differing from composition of commercially available preparations, possibly isolated from coal and other sources.

Humic acids commonly have much higher carbon concentrations than fulvic acids, but at the same time oxygen, carboxyl group concentrations are higher in fulvic acids. To characterise elemental composition, atomic ratios (Van Krevelen graphs) are often used. H/C versus O/C ratios reflect the relative percentage of aromaticity in structures of humic substances. Chemical processes influencing the formation of HA's can be evaluated by the Van Krevelen diagram. As far as most important processes influencing the structure of HS can be described as demethylation (removal of $^{-}CH_3$ group), dehydration and decarboxylation (removal of –COOH group), the same diagram can be also used to study humification process. Demethylation, dehydration and decarboxylation processes result in increased aromaticity and an increased degree of humification. Demethylation and dehydration processes dominate in the generation of humic acids. Highest is the aromaticity of peat HA and HA from soils and peat as well as in commercial preparations. Lowest among humic acids is the humification degree for HA's from aquatic sources.

The UV-Vis spectra of FA and HA examined are featureless, and they monotonically decrease with increasing wavelength. Only in case of humic substances from peat shoulders at 360 nm can be observed. The ultraviolet spectra of both humic and fulvic acids are similar, differing only slightly in optical density.

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 (Chin *et al.,* 1994). 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 correlates with the changes in molecular mass of humic substances. Within the UV spectra, the molar absorptivities of the humic substances were measured at 280 nm. This wavelength was chosen, since π-π^* electron transition occurs in this region for phenolic substances, aniline derivatives, benzoic acids, polyenes, and polycylic aromatic hydrocarbons (Chin *et al.*, 1994). Since many of these substances are precursors of components of certain types of humic substances, molar absorptivity (э) may serve as a basis for the calculation of aromaticity of humic matter.

The IR spectra of the FA and HA examined are in general similar to one another in the main position of adsorption, but differences of various entities are apparent in the relative intensity of some bands, depending on the origin and nature of the sample. IR spectra of analysed humic substances can be divided by regions depending on the information given by them and the presence of important functional groups. Absorption bands in the spectral region 3600–2800 cm^{-1} are very broad and are typical not only for humic substances, but also for mineral constituents. Absorbance in this spectral region is determined by the presence of -OH groups. Sorption at wavelengths 2920 and 2860–2850 cm^{-1} identifies the presence of $–CH_3$ and $–CH_2$- groups. IR spectra data of soil humic substances show that methylene groups $–(CH_2)_n–$ exist in the form of comparatively short alkane chains (n< 4). Typical intensive sorption lines are common for the region around 1700 cm^{-1} (1725–1700 cm^{-1}), which is characteristic for carbonyl groups in aldehydes, ketones and carbonic acids. The actual sorption maximum greatly depends on the conjugation degree, presence of substituents and hydrogen bonding. In the spectral region 1690–1500 cm^{-1} it is possible to identify the sorption maximum of amide bonds (1650–1640 cm^{-1} and 1550–1540 cm^{-1}). In region 1625–1610 cm^{-1}, the sorption indicates the presence of aromatic C=C and carbonyl groups, quinones. At wavelengths 1470–1370 cm^{-1}, there are bands typical for C–H and O–H bonding and sorption maximums typical for C–O. For wavelengths below 1000 cm^{-1} fingerprint patterns are evident. Sorption in this spectral region provides information about possible role of carbohydrate percentage in the structure in humic molecules. Sorption at 1080 cm^{-1} shows OH deformation or C–O stretch of phenolic and alcohol OH groups, and 1040 cm^{-1} indicates C–O stretch of polysaccharide components.

The distinctive distribution of functional groups and the major building blocks of humic substances may reflect the way of their production and structure alteration due to microbial degradation and geochemical alteration. Fulvic acid formation may occur through condensation reactions involving compounds derived from precursor organic materials or through the production of residual recalcitrant compounds in the microbial degradation of precursor organic material. For peat humic acids, lignin is not included in the set of precursor materials and carbohydrates and aliphatic structures are relatively abundant. Carbohydrate structures, however, are the most labile and are rapidly degraded by microorganisms. Similarity between humic substances from peat and soil indicate either the dominance of allochthonous peat humus or the deep transformation of autochthonous humic material.

The molecular weight distribution of HS can be characterized by number average molecular weight (M_n), weight average molecular weight (M_W) and their ratio, which indicates the polydispersity. According to the molecular masses, the humic substances can be arbitrarily divided, depending on their type and origin. In all cases, the found molecular masses of humic substances are higher than those of fulvic acids and they are more heterogeneous. Also, AHS from eutrophic waters are more heterogeneous, but with lower molecular mass than humic substances from oligo- or dystrophic waters. Fractionation of isolated humic substances in Tris-HCl buffer confirmed that AHS in eutrophic waters are more heterogeneous, since more individual fractions were evident.

The studies of the structure of humic matter are confirmed using ^{13}C NMR spectroscopy (crosspolarization/magic angle spinning (CP/MAS) technique) which allows direct evaluation of the dominant structural units of HS. Comparison of ^{13}C CP/MAS NMR spectra makes it possible to directly determine major structural units of humic substances. The signals in these NMR spectra's represent different types of carbon atoms and makes it possible to determine their concentrations. The ^{13}C NMR spectra can be divided into eight chemical shift ranges. Region A (0-50 ppm) consists primarily of aliphatic carbon resonance's (methyl, methylene and methine carbons). Region B (50 to 60 ppm) consists of methoxylcarbon (-OCH_3) resonance signals. Region C (60 to 90 ppm) is believed to be primarily due to C-O, since the nitrogen content of humic substances is commonly low. In this region carbohydrate-type compounds and ether type bonds can resonate. Region D (90 to 110 ppm) is determined by resonance signals of dioxygenated carbons (present in polysaccharides). Signals ranging from 50 to 110 ppm correspond to carbons bonded to electronegative atoms (O, N, Cl), but this range can be dominated by the resonance signals originating from carbohydrate structures. Region E (110 to 140 ppm) is assigned to the resonances of unsubstituted and alkysubstituted aromatic carbons (aromatic carbon *ortho* to the oxygen- substituted aromatic carbon). Region F (140 to 160 ppm) consists of the resonance of the aromatic carbons substituted by oxygen and nitrogen (e.g. phenols, aromatic ethers or amines). Region G (160 to 190 ppm) represents resonances mostly due to carboxyl carbons, and region H (190 to 220 ppm) is characteristic of the carbonyl carbons (aldehyde and ketone carbons). Analyzing ^{13}C NMR spectra of analyzed HS there are evident differences in their structure: namely higher aromaticity of humic acids, in comparison with fulvic acids, but higher content of aliphatic structures in the molecules of the latter. In general, the pattern of ^{13}C NMR spectra confirms the information provided by elemental, functional and other spectroscopic studies of humic substances and the proportions of aliphatic structures calculated based on the spectral properties of HS.

3. Immobilisation of humic substances

The immobilisation of humic substances (HS) on solid carriers is an interesting approach for investigation of their interaction with various substances, as shown by Klavins (1993), Szabo and Bulman (1994). Although immobilisation has shown its usefulness for investigation of the structure, catalytic activity and other properties of proteins, nucleic acids and other macromolecules, in the case of HS there are few publications. Also, immobilised HS can serve as sorbents for organic and inorganic substances, enabling study of their interactions. Since humic substances mostly are present in immobilised form (bound to mineral or organic matrixes) in natural environments, they are likely to be important in the fate of different xenobiotics.

The aim of this part of the study was to compare different methods for immobilisation of humic substances and their subsequent use as sorbents. The most probable structures in humic substances that can be used for their immobilisation, according to hypothetical structural models, are residues of phenolic structures in their core, and functional groups such as phenolic hydroxylgroups, carboxylgroups, and aminogroups (Figure 1) in the peripheral part of the humic macromolecules. Alkylation, acylation and condensation are potential reactions for use in immobilisation by linking of humic substances via these structures to the solid phase of the polymer. For effective sorption, the responsible functional groups should be present in sufficient quantities.

Five different methods were used for the immobilisation of HS:

1. Immobilisation by grafting on chloromethylated styrene-divinylbenzene copolymer;
2. Immobilisation on epoxypropylsilica;
3. Immobilisation on epoxypropylcellulose;
4. Immobilisation on aminopropylsilica using coupling by means of water soluble carbodiimide;
5. Polycondensation of humic substances with formaldehyde and molecules able to enter in condensation reactions (phenols and alkylaromatic substances).

Immobilisation of HS on chloromethylated styrene-divinylbenzene copolymers (Merrifield resin) was conducted in a mixture of water with organic solvents (under conditions similar to those used in solid phase organic synthesis) in the presence of alkaline reagents, yielding stable polymers with high concentrations of immobilised humic substances.

Hydroxylgroups dominate among the functional groups in humic substances, and they are sufficiently reactive to react in the presence of catalysts in alkaline media with epoxygroups. This reaction was used to immobilise HS on cellulose or silica matrix *via* carriers containing epoxygroups. The conditions used for immobilisation of humic substances, selected as those commonly used for immobilisation of similar biomolecules, made it possible to obtain immobilised HS with high yield.

Carboxylgroups can be used for immobilisation using approaches widely adopted in peptide synthesis and solid phase organic synthesis – coupling by means of carbodiimides. Also, immobilised humic substances can be obtained using as a matrix silica carrier containing amino groups removed from the surface of the polymer *via* a spacer and water-soluble carbodiimide.

The basic properties of the humus core can be utilised for immobilisation, by virtue of the presence of phenolic units in the structure of humic substances. As phenols and ethers can enter in polycondensation reactions with formaldehyde, humic substances can form polycondensation polymers. Phenolic groups in the humic substances are in constrained positions and thus the corresponding sites in the aromatic core of HS are of low reactivity, yields of polycondensation polymers using pure humic substances are low, and the mechanical stability of the obtained polymers is poor. If other low-molecular weight substances (phenols, alkylaromatic substances) capable of entering in condensation reactions are added to the reaction mixture, the yields of the obtained polymers increase greatly and it is possible to obtain polymers with high mechanical stability.

The proposed immobilisation methods make it possible to immobilise not only commercial humic substances, but also humic substances from water, peat and soil with different properties. The immobilisation efficiency of different humic substances tested was found to differ only slightly.

The obtained polymers were characterised by weight gain during reaction, elemental analysis and IR spectra of polymers after the reaction. The IR spectra of immobilised humic substances show general features common for humic substances, sometimes with overlapping of lines from the polymer matrix used for immobilisation of HS. Absorption bands in the spectral region 3600-2800 cm^{-1} are very broad and due to the presence of -OH groups. Sorption at wavelengths 2920 and 2860-2850 cm^{-1} indicates the presence of CH_3- and CH_2- groups. Typical intensive sorption lines are common for the region around 1700 cm^{-1} (1725-1700 cm^{-1}), characteristic for carbonylgroups.

The total content of immobilised HS was determined by alkaline hydrolysis followed by spectrophotometric analysis. All immobilised humic substances were coloured polymers, and the porosity and particle size of the polymer matrix during the immobilisation process decreased minimally. The content of immobilised humic substances varied between 7 mg/g and 430 mg HS/g sorbent. The immobilised HS were hydrolytically stable.

The observed uptake of metal ions (Cu^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}) by obtained sorbents is efficient. The uptake of metals was relatively rapid, and commonly the equilibrium conditions were reached within less than 30 minutes. The initial polymer matrix did not absorb appreciable quantities of metals. The uptake capacity for different metals depended on the polymer matrix and type of humic matter bound to the polymer. Among the tested humic substances, the highest sorption capacity was found for immobilised humic substances obtained using polycondensation reaction.

The sorption kinetics on the immobilized humic acids depends on porosity of the polymeric matrix and the hydrophilicity/hydrophobicity balance of the sorbates, being fast for sorption on macroreticular polymers, but comparatively slow on gel-type polymers (immobilized humic substances on styrene-divinylbenzene copolymers or polycondensation polymers).

The character of the sorption process depends on the salt concentration in the solution. Also in this case the sorption capacity depends on the porosity of the polymer structure, which is insignificant for gel-type polymers and substantial for sorbents on aminopropylsilica.

The adsorption of p-aminoazobenzene on immobilized humic substances differs substantially depending on the properties of the original polymeric carrier. On silica polymers the sorption capacity is low (Yang and Koopal, 1999), while on humic substances immobilized on Merrifield resin it can be quite high. However, for polymers obtained using polycondensation with formaldehyde, the specific polymeric matrix as such does not exist. To compare the obtained immobilized humic substances, the sorption was expressed as mg of p-AAB/g of sorbent. The experimental data were fitted to empirical Freundlich and Langmuir adsorption equations. We found that the Langmuir isotherm gave the best fit for the description of the p-aminoazobenzene sorption. Comparison of adsorption isotherms shows substantial differences between the obtained polymers and possibly also the major impact of the original polymeric matrix: for polymer obtained by grafting humic acid onto Merrifield resin the sorption capacity is higher and can be described by the Langmuir model, while the sorption capacity on humic substances immobilized onto silica is lower. The Langmuir constants K and b, indicating bonding energy and the maximum sorption capacity, respectively, differed from sample to sample, suggesting that various mechanisms of sorption may occur.

The sorption on HS immobilized on a carrier, especially on a polymer carrier, obtained using polycondensation much depends on the size of the polymeric particles. The sorption takes place faster for small polymer particles and follows the Langmuir model. However, on large polymer particles obtained using polycondensation, the sorption follows the so-called S-type form, indicating a low surface affinity that increases with surface saturation with sorbate. The polycondensation polymers are gel-type, their swelling in aquatic media much depends on the polymer composition, the sorption in this case is evidently lower than on macroreticular polymers, it takes place on the surface layers of sorbent, and it is diffusion controlled.

The obtained immobilized humic acids demonstrated good sorption properties for column processes. For column proceses, the most effective sorbents are obtained using more rigid matrices (silica, crosslinked styrene-divinylbensene copolymer with a high degree of crosslinking) and the retention processes much depend on the concentration of sorbate.

In comparison to the sorption process of p-aminoazobenzene the character of sorption differs for higher molecular-weight polar sorbates, such as the dyes Crystal Violet, Methylene Green, and flavine mononucleotide. All of these isotherms clearly exhibited evidence for a sequence of adsorption steps and a comparatively low affinity of immobilized humic substances with respect to hydrophilic cationic sorbates.

The first stage of the adsorption is characterized by comparatively low sorption efficiency, but is followed by a significant increase of the sorbed amount. The affinity of selected sorbates towards the sorbent significantly increases after surface saturation of sorbents is reached. However, immobilized humic substances cannot be considered a good sorbent for sorption of hydrophilic cationic sorbates.

As it is well known, humic substances in solution can form stable complexes with metal ions as well as with organic molecules. The same is also true for immobilized humic substances, as shown by the observed sorption of some metal ions and some organic substances. Immobilized HS have a rather good sorption capacity for a wide range of substances. They are prospective for the investigation of humus properties and can serve as a potential sorbent with an inexpensive ligand for purification procedures.

4. Interaction of humic substances with aquifer material during artificial groundwater recharge

Artificial recharge of groundwater (ARG) is achieved by putting surface water in basins or other facilities where it infiltrates into the ground and moves through porous media downwards to recharge aquifers. In this process, without adding any reagents, suspended particles and human pathogens are effectively removed from the water (Huisman and Olsthoorn, 1983). Therefore, in areas where permeable aquifers are available, ARG has been used for drinking water production for more than 150 years. Nowadays, it is recognized that removing humic substances (HS) (humic acids (HA) and fulvic acids (FA)) from drinking water is important, because they impart a yellow colour to water and may lead to the formation of carcinogenic by-products during disinfections (Rook, 1974) as well as undesirable bacterial biofilm growth in distribution systems. The removal efficiency of HS during ARG varies from place to place; the causes of these variations are often not understood. So, to advance knowledge about interaction between humic substances and mineral materials of importance at the ARG basin, but also during development of the soil composition, the retention of humic substances onto different model compounds has been studied.

The composition of the filtersand used for ARG systems was similar to that of soils but with lower amounts of clay and iron oxide, the surfaces bearing major binding sites for sorption of HS. A relatively high amount of OC indicated that a large proportion of these sites had already been occupied with organic matter, making their surfaces negatively charged, and thus unavailable for ligand exchange. Quartz was a major constituent of the filtersand. Ligand exchange of HS with quartz surfaces is minor, as proven in experiments by Spark et al., (1997), in which no proton consumption was observed in the interaction between quartz and HS. Thus, under conditions where filtersand has a limited number of binding sites, ligand exchange is probably not the dominant process for removing HS during ARG.

Sorption of Aldrich HS increased with increased amounts of clay and decreased with increased amount of OC in the filtersand. Sorption was heterogeneous among samples, but generally increased with the depth below the basins. Sorption experiments of different HS on clays revealed that HS sorption exceeded the FA sorption, while it was better on smectite (the expandable clays) compared to koalinite. Sorption to the filtersand from which organic matter was chemically removed was low or negative. At the same pH, Aldrich and soil HA were sorbed better than river FA to typical filtersand. Regardless of the filtersand composition, the lowering of pH significantly increased the sorption efficiency of HS. To understand the influence of different phases composing the filtersand, the sorption of HA and FA to iron, aluminium oxides, and organic rich sediments were tested. HA were always better or similarly sorbed to all filtersand components except iron oxide, which better sorbed FA instead.

It can be suggested that chemisorption (ligand exchange) is the dominant mechanism for sorption of HS to soil; however, physical sorption driven by entropy changes (hydrophobic attraction) can also be important as far as most positively charged sites (e.g iron oxides, broken edges of clay surfaces) are occupied with organic matter, the ligand exchange is unlikely to be a dominant interaction in the soil system.

The results showed that sorption of HS increases with an increase in the fraction of clay in the filtersand. Due to their small size, clay particles have a large specific surface area that, depending on chemical and physical properties of clay (not identified herein), can be conductive for either physical or chemical sorption to occur. The results from this study showed that HA, more aromatic and with higher molecular mass fraction of HS were better removed than FA, more acidic fraction with higher amounts of functional groups, Fig. 3. This might indicate that physical interaction rather than ligand exchange between HS and the filtersand are important. However, this should be carefully considered since lower sorption efficiency of HS with a higher amount of functional groups (e.g. FA) is not always indicative of a lack of coordinative interaction (ligand exchange). HS are macromolecules that behave like polyelectrolytes; thus their sorption is determined not only by the availability of sites, but also by lateral interaction between themselves and the steric arrangements of the macromolecule. Due to a high amount of functional groups, HS stretches (due to lateral repelling between the functional groups) and when it becomes sorbed on the surfaces occupies a large area that makes overall sorption efficiency of FA lower than that of HA. This is also demonstrated by the finding that HS, with high amounts of aromatic groups and lower amounts of carboxyl groups, can be preferably sorbed to the surface with high amounts of binding sites such as iron or aluminium oxides. This occurs even though ligand exchange is the dominant mechanism of interactions of HS with those surfaces. In the sorption process HS replaced nearly all previously retained HS before any sorption could occur. This indicates that HS were weakly sorbed on the bare filtersand, perhaps as a result of physical interaction. Elemental analyses showed an insignificant increase of oxygen and carboxyl groups in the filtersand HS compared to HS of the water used for recharge. Thus, chemisorption, which generally contributes to a higher level of theses moieties in the sorbed HS, was not significant.

A change of pH affected both HS and filtersand properties. A decrease of pH should increase the positive charge of oxides, thereby making an electrostatic attraction with negatively charged HS molecules possible. However, at acidic pH HS become more hydrophobic, thus increasing the possibility for hydrophobic attraction. Since the filtersand contained low amounts of iron and clay, the strong positive effect of sorption that was due to a lowering of pH was perhaps also due to a hydrophobic effect rather than an increase of electrostatic attraction. Therefore, the sorption may be dominated not so much by the forces between the filtersand surface and HS, but rather by the intermolecular association forces between HS and its incompatibility with water.

However, we observed that background organic matter also plays an important role. HS are better attracted to organic rich sediments than to filters and had a relatively lower amount of OC. Elemental analyses revealed that filtersand showed significantly lower H/O ratio compared to sediments. The H/O ratio indicates the humification degree of humic substances. Thus, the higher ratio indicated that HS in sediments were more humified than the filtersand HS, which apparently also increases the ability to remove HS from water.

From this discussion, we conclude that due to low amounts of binding sites in filtersand, a hydrophobic fraction such as HA is preferably removed over a more acidic fraction such as FA. This may lead to a fractionation of HS during ARG where large hydrophobic and aromatic molecules are removed earlier in the process, though FA are conserved unless the aquifer is rich with iron oxides or clay. The aromatic fractions of HS are a major source of by-products and colour, thus ARG decreases the risk of carcinogenicity and improves the aesthetic quality of surface water. If filtersand, through which ARG is accomplished, is poor in iron oxide minerals or clay, the removal of FA would be low even after long infiltration distances.

Sorption experiments showed that clay content increased whereas organic matter decreased the sorption of HS to filtersand at ARG. Organic matter was released because it was not chemically bound and completely stabilized. More aromatic, hydrophobic fractions of HS (HA) were to a higher extent sorbed than acidic fractions of HS (FA). Chemisorption appears to be less important than physical sorption (hydrophobic sorption enhanced by polyelectrolytic effect) for HS removal during ARG.

Conclusions

1. The humic substances isolated from different natural environments of Latvia, commercially produced and reference samples of humic substances shows significant dissimilarities in their properties. The properties of humic substances depend on conditions of their formation, which affect relative amount of aromatic/aliphatic structural elements in molecule of humic substances as well as content of other functional group in molecule structure.
2. The main factor influencing interaction of humic substances with major soil mineral components typical for Latvia, during artificial groundwater recharge process, is specific character of structure and molecular weight of humic substances, as well as properties of soil mineral components.
3. The immobilisation of humic substances on inorganic or organic carriers can be performed by covalent binding. Immobilised humic substances are hydrolytically stable and their mechanical properties depend from primary polymer.
4. By immobilisation of humic substances sorbents with different properties of matrices and various substitution degrees of functional groups can be obtained, that can be used as a sorbents for removal of pollutants.

References

Aiken, G. R., McKnight, D. M., Wershaw, R. L. (1985) *Humic substances in soil, sediment, and water. Geochemistry, isolation and characterization*. N.Y.: Wiley

Bollag, J. M. (1983) Cross-coupling of humus constituents and xenobiotic substances. In: Christman, R. F., Gjessing, E. T. (eds.) *Aquatic and Terrestrial Humic Substances*, Ann Arbor, MI: Ann Arbor Science Publishers, 127–141.

Burdon, J. (2001) Are the traditional concepts of the structures of humic substances realistic? *Soil Sci*., 166(11), 752–769.

Carter, C. W., Suffet, I. H. (1982) Binding of DDT to dissolved humic materials. *Environ. Sci. Technol*., 16, 735–740.

Chin, Y. P, Aiken, G., O'Loughner, E. (1994) Molecular weight polydispersity and spectroscopic properties of aquatic humic substance. *Environ. Sci. Technol.,* 28, 1853–1858.

Chiou, C. T., Porter, P. E., Schmeddling, D. W. (1983) Partition equilibria of nonionic organic compounds between soil organic matter and water. *Environ. Sci. Technol*., 17, 227–231.

Ephraim, J., Alegret, S., Mathuthu, A., Bicking, M., Malcolm, R. L., Marinsky, J. A. (1986) A unified physicochemical description of the protonation and metal ion complexation equilibria of natural organic acids (humic and fulvic acids). 2. Influence of polyelectrolyte properties and functional group heterogeneity on the protonation equilibria of fulvic acid. *Environ. Sci. Technol*., 20, 354–366.

Ghosh, K., Chattopadhyay, A., Varadachari, C. (1983) Electron exchange behaviours of humic substances with iron, copper and manganese. *Soil. Sci*., 135, 193–196.

Hayes, M. H. B., Clapp, C. E. (2001) Humic substances: considerations of compositions, aspects of structure and environmental influences. *Soil Sci*., 166(11), 723–737.

Huisman, L., Olsthoorn, T. N. (1983) *Artificial Groundwater Recharge*. Boston: Pitman Books Ltd.

Jones, M. N., Bryan, N. D. (1998) Colloidal properties of humic substances. *Adv. Colloid. Interface Sci.*, 78, 1–48.

Kļaviņš, M. (1993): Immobilization of humic substances. *Latv. Ķīm. Ž.,* (1), 96–102.

Kļaviņš, M. (1998) *Aquatic humic substances: characterisation, structure and genesis*. Rīga: LU.

Kļaviņš, M., Apsīte, E. (1998) Sorption of humic substances on clays from Latvia. *Latv. Ķīm. Ž,* (1), 67–72.

Kļaviņš, M., Seržāne, J., Eglīte, L. (1999) Methods for analysis of aquatic humic substances. *Crit. Rev. Anal. Chem*., 29, 187–203.

Leenheer, J. A., Wershaw, R. L., Reddy, M. M. (1995) Strong acid, carboxyl-group structures in fulvic acid from the Suwannee River, Georgia. 2. Major structures. *Environ. Sci. Technol*., 29(2), 399–405

MacCarthy, P. (2001) The principles of humic substances. *Soil Sci*., 166 (13), 738–751.

Perdue, E. M. (1983) Association of organic pollutants with humic substances: Partitioning equilibria and hydrolysis kinetics. In: *Aquatic and terrestrial humic materials* (Eds. Christman, R. R., Gjessing, E. T.). Ann Arbor: Ann Arbor Science, 441–460.

Rook, J. J. (1974). Formation of haloforms during chlorination of natural waters. *J. Water Treat. Exam.*, 23, 234–243.

Schnitzer, M., Khan, S.U. (1972) *Humic substances in the environment*. N.Y.: Marcel Dekker.

Schulten, H.-R., Schnitzer, M. (1993) A state of the art structural concept for humic substances. *Naturwissenschaften*, 80, 29–30.

Senesi, N., Sposito, G., Martin, J. P. (1986) Copper(II) and iron(III) complexation by soil humic acids: and IR and ESR study. *Sci. Total Environ*., 55, 851–362.

Spark, M. K., Wells, J. D., Johnson, B. B. (1997) Characteristics of the sorption of humic acids by soil minerals. *Aust. J. Soil. Res.*, 35, 103–112.

Stevenson, F. J. (1994) *Humus Chemistry: Genesis, Composition, Reactions*, N.Y.: Wiley.

Szabo, G., Bulman R. A. (1994) Comparison of adsorption coefficient (Koc) for soils and HPLC retention factors of aromatic-hydrocarbons using a chemically immobilized humic-acid column in RP-HPLC. *J. Liquid Chromat.* 17, 2593–2604.

Tipping, E. (1981) The adsorption of aquatic humic substances by iron oxides. *Geochim. Cosmochim. Acta,* 45, 191–199.

Wershaw, R. L. (1989) Application of a membrane model to the sorptive interactions of humic substances. *Environ. Health Perspect.*, 83, 191–203.

Wershaw, R. L. (1993) Model for humus. *Environ. Sci. Technol.*, 27(5), 814–817.

Wershaw, R. L., Bulcar, P. J., Goldberg, M. C. (1969) Interaction of pesticides with natural organic material. *Environ. Sci. Technol.*, 3, 271–273.

Winner, R. W. (1985) Bioaccumulation and toxicity of copper as affected by interactions between humic acid and water hardness . *Water Res.* 19 (4), 449–455.

Yang, Y. H., Koopal L. K. (1999) Immobilisation of humic acids and binding of nitrophenol to immobilized humics. *Colloids Surf. A: Physicochem., Eng. Aspects,* 151, 201–212.

Ziechmann, W. (1994) *Humic substances*. Mannheim: BI Wissenschafts Verlag.

Орлов, Д. С., Садовникова, Л. К., Лозановская, И. Н. (2002) Экология и охрана биосферы при химическом загрязнении, Высшая школа, Москва: Высшая школа, 334 [Orlov, D. S., Sadovnikova, L. K., Lozanovskaja, I. N., (2002) Ecology and protection of biosphere on chemical pollution, Moscow, (in Russian)].

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Methods for Analysis of Aquatic Humic Substances

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Methods for Analysis of Aquatic Humic Substances

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ABSTRACT: Methods designed for analysis of aquatic humic substances are summarized and compared. A need to evaluate just the concentration of true humic substances, rather than summary parameters, characterizing dissolved organic substances such as COD, DOC, TOC, and others is stressed. Possible future trends in the studies of dissolved humic substances are touched on.

KEY WORDS: humic substances, analysis, natural waters.

I. INTRODUCTION

Among the various naturally occurring organic substances, humic substances (HS) are the most widespread. Humic substances form most of the organic component of soil, peat, lignite, natural waters, and their sediments. Up to 70% of the soil organic carbon and up to 90% of dissolved organic carbon may occur in the form of humic substances. Humic substances influence groundwater properties and the process of formation of fossil fuels; however, in concert they play a major role in the global carbon geochemical cycle. The global pool of humic matter is an important component in the formation of atmospheric carbon dioxide. The estimated amount of carbon in the atmosphere is 638 to 702 × 10^{15} g C, which is in the same range as carbon accumulated as soil humus (700 to 3000 × 10^{15} g C), peat humus (250 × 10^{15} g C), and aquatic humic substances (850 to 1700 × 10^{15} g C) (Klavins, 1998). Recently, it has been estimated that the global transport of organic carbon to oceans is about 0.4 to 0.9 × 10^{15} g C $yr.^{-1}$ (Hope et al., 1994).

The roles of humic substances is determined by their formation during the humification (decay) process of living matter. Humic substances form an intermediate phase in the transformation process of living matter (organic carbon reservoir) that continue in the organic carbon cycle or be deposited (as fossil materials). Humic substances are known to complex heavy metals and persistent organic xenobiotics. The interaction of humic substances with xenobiotics may modify the uptake and toxicity of these compounds by living organisms and affect the fate of pollutants in the environment. Considering these aspects the roles of humic substances in the environments may be regarded as important. Humus properties depend on its sources. Depending on the character of humification process, the greatest reservoirs of humic matter are distinguished as soil, peat, and aquatic humic substances (AHS).

The present level of knowledge indicates that humic substances form one of the most important groups of biomolecules. However, their properties are confusing from the viewpoint of monodisciplinary sciences — chemistry, biology, and Earth sciences. Humic substances can be regarded as one of the chemical anomalies. This attitude is evident even from the definition of humic substances (Stevenson, 1982). The most common definition is (Humic Substances, 1985): "Humic substances are a general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight, and refractory. Humic substances can be divided in three fractions:

- Humin is the fraction of humic substances that is not soluble in water at any pH

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- Humic acid (HA) is the fraction of humic substances that is not soluble in water under acidic conditions (below pH 2), but becomes soluble at greater pH
- Fulvic acid (FA) is the fraction of humic substances that is soluble under all pH conditions

The main forms of organic carbon (and humic substances) in aquatic environments are dissolved and particulate (colloidal) organic matter. The division between dissolved organic matter (DOM) and particulate organic matter (POM) is generally made on the basis of whether it passes through a 0.45-µm filter (Figure 4). The generation of these forms proceeds during the genesis and transformation of organic matter and is linked with processes such as decomposition of living matter, association of organic molecules with inorganic particulate matter and aggregation of smaller particles.

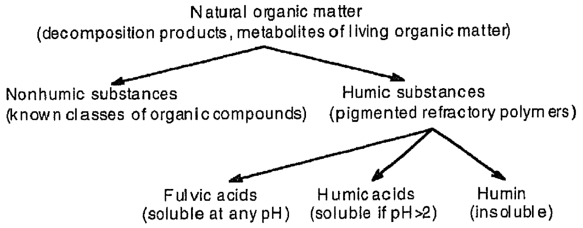


FIGURE 1. Classification of humic substances.

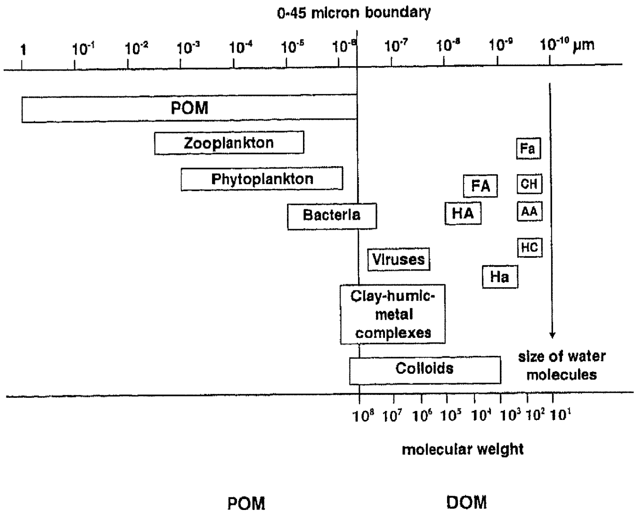


FIGURE 2. Size range of particulate and dissolved organic matter and carbon compounds in natural waters. AA, amino acids; CH, carbohydrates; DOM, dissolved organic matter; POM, particulate organic matter; Fa, fatty acids; Ha, hydrophilic acids; HC, hydrocarbons. (Adapted from Thurman, 1985a.)

Aquatic humic substances originate mostly from three processes:

1. Allochthonous aquatic humic substances derived from terrestrial organic matter
2. Autochthonous aquatic humic substances derived from *in situ* biological production
3. Humic substances derived from agricultural, domestic, and industrial activities (Degens, 1982)

Contemporary structural models considers humic substances as polifunctional macromolecules (Figure 3).

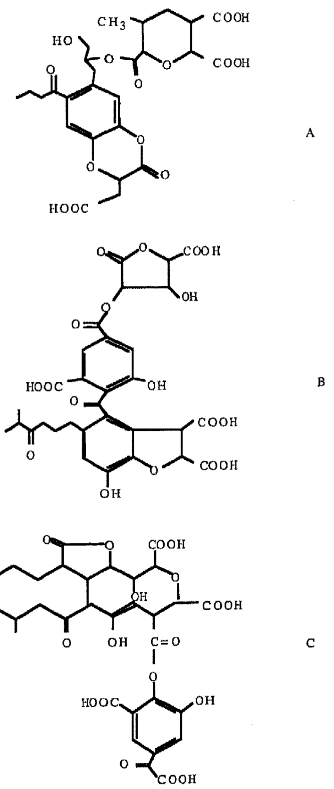


FIGURE 3. Three structural models (A-C) of fulvic acid molecules from the Suwannee River (Leenheer et al., 1995).

The use of a wide variety of analytical methods, including degradative and nondegradative approaches, to characterize Suwanee River fulvic acids, led to a model proposed by Leenheer et al. (1989, 1995b). This model shows the most possible average structural formulas (Figure 3) and also considers the possible precursors in the humification process. The aromatic and aliphatic structures in the proposed formulas indicate hypothetical residues of parent compounds, such as lignins, tannins, carbohydrates, and lipids, which are incorporated into the structure of humic substances. The multitude of possible links of these structural units and their biodegradation degree can explain the large diversity of structural formulas for the possible humus structure. The proposed structures at best can explain the actually found physical and chemical properties of humic substances isolated from the given environments, but the coexistence of HS as complexes with mineral particles is neglected.

Despite the great role of aquatic humic substances in the environment, many aspects of their functions and properties are still unclear, including the analytical determination of AHS.

II. PRINCIPLES FOR ANALYSIS OF HUMIC SUBSTANCES

A commonly required water characteristic is the concentration of aquatic humic substances. Despite comparatively high concentrations in natural waters and yet higher concentrations of humic substances in waste waters, their analytical determination is, however, problematic. This is surprising, regarding the importance of humic substances in geochemical cycles of elements and metals, in other xenobiotic speciation, and as indicators of biological processes in waterbodies. In the early period of humus investigations, AHS has been characterized by spectrophotometrical determination of water color, chemical oxygen demand (COD), and biological oxygen demand (BOD). Improved instrumental techniques and organic carbon analysers allows the evaluation of the concentrations of humic substances as organic carbon content (TOC, total organic carbon, DOC, dissolved organic carbon, POC, particulate organic carbon) in waters. Contemporary recommended methods are based on such approaches.

However, all of these analytical methods provide only indirect measures of humus concentration in water.

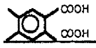
The problems of analytical determination of concentrations of aquatic humic substances in natural waters are closely related to their properties and the lack of strict boundaries between humic and nonhumic substances. The fundamental properties that may be used for analytical determination of AHS are (Table 1):

TABLE 1

Major Functional Groups and Building Blocks of Aquatic Humic and Associated Substances

Loosely bound substances

Metal ions: (Ca^{+2}, Fe^{+3}, Na^{+} etc.), adsorbed inorganic particulate materials, low molecular weight organic substances

Functional groups	
–COOH	Carboxyl groups
–OH	Hydroxylgroups
>C=O	Carbonylgroups
–O–	Ether groups
$-OCH_3$	Methoxylgroups
$-CH_3$	Methylgroups
–C(N	Cyanogroups
–SH, $-SO_3H$	Sulphur containing groups
$-NH_2$, >N–	Amino groups
Major structures of aquatic humic substances	
$-CH_2-CH_2-$, $-CH = CH-$, $-CH_2$ $-NH_2$	Aliphatic structures
	Phenoles, phenolecarboxylic acids
	Benzenecarboxylic acids
	Aromatic and polyaromatic structures
	Quinones
	Oxygen containing heterocycles
	Nitrogen containing heterocycles
	Sulphur containing heterocycles
Pentoses and hexoses	Carbohydrates

- Organic carbon as a building element of humic matter (they are organic substances)
- Their acidic character (humic substances are polycationites)
- Ability of humic substances to form insoluble salts with some cations
- The presence of chromophores and fluorophores in their structure
- Presence of hydrophobic structural units in their structure

The most common method for the analysis of humic substances in surface waters is the determination of organic carbon (COD, BOD, TOC, DOC, POC). This is a very powerful and widely used method to study organic matter in natural waters (Datsko, 1959; Degens, 1982; Coel et al., 1984 Agren, 1991), but it neglects the differences between low molecular organic substances and humic substances.

III. CONTEMPORARY ANALYTICAL METHODS OF HUMIC SUBSTANCES

The sorption of humic substances in the UV-Vis spectral region allows this characteristic and water color to be used for their analytical determination. Water color has been determined for many years and hence may be used for trend analysis of humus concentrations in surface waters. Associated analytical problems are sensitivity and interference, particularly from iron and polyphenolic substances (lignin). A more powerful method is the fluorimetric method, which allows the determination of lignin sulfonates and humic substances simultaneously in waters. The fluorimetric method has been used to measure fulvic acids in seawater, after preconcentration on DEAE cellulose and subsequent desorption and final fluorimetric determination (Ivanova et al., 1986). Also, simultaneous absorbance measurements at two wavelengths can be used to determine humic acid and iron concentrations in natural waters (Carpenter and Smith, 1984). This method requires sample volumes of less than 15 ml and is sensitive enough for direct application to most natural waters (limit of detection is 0.01 mg/l for humic acid).

To isolate humic matter from a pool of organic and inorganic substances and to separate humic acids from fulvic acids, chromatographic methods are most successful. Their application is based on the use of polymeric anionites (containing tertiary amino groups or diethylaminoethylgroups) or on the use of hydrophobic sorbents (styrene-divinylbenzene or acrylic acid copolymers). Functional groups in these sorbents may interact with carboxylgroups or hydrophobic structural units in humic substances, allowing their selective isolation from aquatic environments and their separation from accompanying substances. An example of this method is based on the isolation of humic substances from waters by their chromatographic sorption on DEAE cellulose, elution precipitation of humic acids at pH 2, and spectrophotometric determination of both humic and fulvic acids (Krasiukov and Lapin, 1988). Nonionic macroreticular sorbents (Amberlite XAD resins) have been proposed for analysis of humic substances (Sorouradin et al., 1993). Analytical determination of humic substances has been achieved by sorption and desorption with subsequent spectrophotometric analysis. To achieve quantitative desorption, elution of polymers with sodium dodecyl sulfate solution in strongly alkaline media was used.

Methods based on use of DEAE or XAD sorbents for analytical determination of AHS are now standard methods (Standard Methods, 1992). Aquatic humic substances are concentrated by column chromatography on DEAE cellulose or XAD resin, then desorbed and measured as dissolved organic carbon concentrations (Figure 4). The estimated detection limit is 1.1 mg/l for a 50-ml sample. A recommended analytical method based on the use of sorbents is included in the appendix.

Thin layer chromatography on silica gel plates has also been used successfully to analyze humic substances in surface waters after their pre-concentration on Amberlite XAD 4 column (Katalan-Macan et al., 1992).

Later developed method is based on the oxidation of humic acid with hydrogen peroxide in the presence of formaldehyde in alkaline solution (Kitano et al., 1994). This method is sensitive and selective (detection limit 50 ppb and relative standard deviation for five measurements 1.8%) for the determination of HA in natural waters with little interference from substances common in

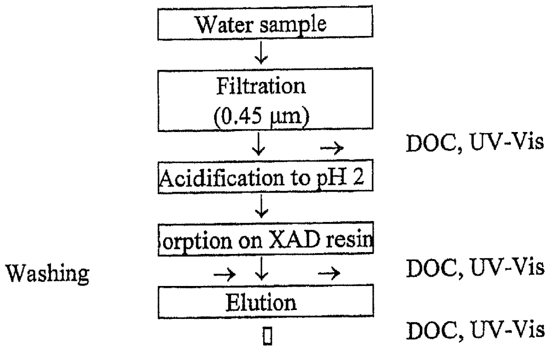


FIGURE 4. Sequence of steps for analysis of aquatic humic substances.

natural waters. The method can also be realized continuously. Humic acids in natural waters have been analysed also by chemiluminiscence produced by the reaction between humic acid and NaBrO in alkaline solution (Hu et al., 1994).

Enzyme-linked immunoassay for the determination of humic acids in natural waters has been proposed (Ulrich et al., 1993) using humic acid coupled to snail *Helix pomatia* hemocyanin and a humic acid horseradish peroxidase conjugate as a tracer. This detection method has achieved high sensitivity (detection limit was estimated to be 0.5 μg/l) and allowed to elucidate structural differences among humic substances of different origin.

Because suspended particles in surface waters are covered by humic substances, it is important determine their amount. This can be achieved by selective desorption of humic substances from particulate matter (Hiraide et al., 1988). It has been found that the major part of metals on or in suspended particles exist as humic complexes (Hiraide, 1992).

IV. CONCLUSIONS

Unless substantial efforts to develop analytical methods for determination of humic substances in surface waters, the problem still is open and versatile, efficient, and unified methods are needed. As the most promising approaches can be regarded use of chromatographic techniques, as well as different approaches based on preconcentraiton of humic substances.

REFERENCES

Agren, S. (1991) A study on unfractioned DOC and its aluminum binding. *Finnish Humus News*, **3(1)**, 291–297.

Carpenter, P.D., Smith, J.D. (1984) Simultaneous spectrophotometric determination of humic acid and iron in water. *Anal. Chim. Acta*, **159**, 299–308.

Coel, J.J., McDowell, W.H., Likens, G.E. (1984) Sources and molecular weight of "dissolved" organic carbon in an oligotrophic lake. *Oikos*, **42**, 1–9.

Datsko, V.G. (1959) *Organic substances in waters of southern seas of USSR*, Izd.AN SSSR: Moscow (in Russian).

Degens, E.T. (1982) *Transport of carbon and minerals in major world rivers*. Part 1. Proceedings of a workshop arranged by Scientific Committee on problems of the Environment and UNEP. Hamburg: Hamburg University.

Degens, E.T. (1982) *Transport of carbon and minerals in major world rivers*. Part 1. Proceedings of a workshop arranged by Scientific Committee on problems of the Environment and UNEP. Hamburg: Hamburg University.

Hiraide, M. (1992) Heavy metals complexed with humic substances in fresh waters. *Anal. Sci.*, **8**, 453–459.

Hiraide, M., Arima Y., Mizuike, A. (1988) Selective desorption and analysis of humic substances on suspended particles in river water. *Microchim. Acta.*, **111**, 231–238.

Hope, D., Billet, M.F., Cresser, M.S. (1994) A review of the export of carbon in river water: fluxes and processes. *Environ. Pollut.*, **84**, 301–324.

Hu, X., Kitano, M., Takenaka, N., Bandow, H., Maeda, Y., Zhang, D. (1994) Sensitive determination of humic acid in natural water by chemiluminiscence-flow-injection method. *Bunseki Kagaku*, **43(12)**, 1077–82.

Humic substances in soil, sediment, and water. Geochemistry, Isolation and Characterization. (1985) (Eds. Aiken, G.R., McKnight, D.M., Wershaw, R.L.) N.Y.: Wiley.

Ivanova, E.K., Pershina, I.V., Polenova, T.V., Cherniak, C.M. (1986) Fluorimetric analysis of marine fulvic acids. *Zh. Anal. Chim.*, **61**, 7–12, (in Russian).

Katalan-Macan, M., Cerjan-Stafanovic, S., Jalsovec, D. (1992) TLC determination of aquatic humic acids. *Water Sci. Technol.*, **26(9-11)**, 2567–2570.

Kitano, M., Ogasawara, Y., Hu, X., Norimichi, T., Bandow., Maeda, Y. (1994) Continuous determination of trace amounts of humic acid in natural water by chem-iluminiscence method. *Microchem. J.*, **49**, 265–274.

Klavins, M. (1998) Aquatic humic substances, Riga:LU, 235 p.

Krasiukov V.N., Lapin, I.A. (1990) Investigation of humus substances in solution by means of gel-exclusion chromatography on porous glasses. *Pochvovedenije*, (7), 48–57 (in Russian).

Krasiukov, V.N., Lapin, I.A. (1988) Method for analysis of humic substances in surface waters. *USSR patent N 1385041* (in Russian).

Krasiukov, V.N., Lapin, I.A. (1988) Method for analysis of humic substances in surface waters. *USSR patent N 1385041* (in Russian).

Leenheer, J.A., Brown, P.A., Noyes, T.I. (1989a) Implications of mixture characteristics on humic-substance chemistry. In: *Aquatic humic substances. Advances in Chemistry,* vol. 219, (Eds. Suffet, I.H., McCarthy, P.) Washington ACS Series: ACS, 25–40.

Leenheer, J.A., Wershaw, R.L., Reddy, M.M. (1995a) Strong acid, carboxyl-group structures in fulvic acid from the Suwannee River, Georgia. 1. Minor structures. *Environ. Sci. Technol.*, **29(2)**, 393–398.

Leenheer, J.A., Wershaw, R.L., Reddy, M.M. (1995b) Strong acid, carboxyl-group structures in fulvic acid from the Suwannee River, Georgia. 2. Major structures. *Environ. Sci. Technol.*, **29(2)**, 399–405.

Sorouradin, M.H., Hiraide, M., Kawaguchi, H. (1993) Spectrophotometric determination of humic substances in water by sorption on XAD-2 resin followed by desorption with sodium dodecyl sulfate. *Anal. Sci.*, **9**, 863–865.

Standard methods. For examination of water and wastewater. (1992) (Eds. Greenberg, A.E., Clesceri, L.S., Eaton, A.D.), Washington: APHA.

Stevenson, F.J. (1982) Humus chemistry. N.Y.: J. Wiley.

Thurman, E.M. (1985a) *Organic geochemistry of natural waters.* Netherlands: Martinus Nijhoff/Dr. W. Junk Publishers.

Ulrich, P., Weller, M.G., Knopp, D., Niessner, R. (1993) Enzyme-linked immunosorbent assay for humic acids. *Anal. Sci.*, **9**, 795–797.

II

Removal of Humic Substances During Treatment of Drinking Water Using Sorbents

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REMOVAL OF HUMIC SUBSTANCES DURING TREATMENT OF DRINKING WATER USING SORBENTS

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Abstract

The removal of humic substances during drinking water preparation by artificial recharge of groundwaters using synthetic sorbents, activated carbon and anthracite was assessed. Humic (HA) and fulvic (FA) acids isolated from soil, peat, water, filters, from the infiltration basin for artificial groundwater recharge, as well as commercial humic acid, were used in study. It was found that adsorption is determined by the sorbent properties, and the nature of the humic substance. The sorption process is slow, controlled by pH and ionic strength (sorption increases in acidic and high ion strength conditions), and depends on the porosity of the used sorbents and the character of their functional groups. The tested sorbents proved their efficiency in pilot scale experiments.

Key words – humic substances, sorption, drinking water, artificial groundwater recharge.

Introduction

Water treatment aimed at drinking water preparation is the removal of undesired water ingredients. Numerous techniques can be used for these purposes, including coagulation, filtration and sorption approaches (Water Quality and Treatment… 1990). Humic substances are among the different groups of substances present in natural waters which adversely influence the quality of drinking water (Thurman 1985, Kļaviņš 1998). These high molecular weight polycationites form carcinogenic and mutagenic organochlorines upon chlorination (Loper 1980, Kronberg and Vartiainen 1988). Humic substances also form stable complexes with metal ions and persistent organic xenobiotics, and thus are responsible for the transport of many contaminants during the drinking water preparation process. The presence of humic substances also gives an unpleasant odour, colour and taste to drinking water. Substantial efforts have been made to develop drinking water treatment technologies to reduce the concentration of humic substances during drinking water treatment process (Eikebrokk 1989). The sorption of humic substances from the aqueous phase on solid surfaces occurs as a result of attractive interactions due to Van der Waals forces, hydrogen bonding and electrostatic forces (Kļaviņš 1998). As the molecular mass of humic substances can reach up to several thousands Daltons, high porosity of the sorbents (Thurman and Malcolm 1981) is of importance. More effort should be paid to the development of efficient sorbent systems for preparation of drinking water. The main problems related to removal of humic substances are comparatively high concentrations in surface waters and major differences in their properties depending on their origin. In surface waters, use of sorbents for removal of HS may be economically unacceptable, but their concentrations in groundwater are much lower and use of sorbents may be practical. There is a need to develop techniques for removal of organic substances after artificial recharge of groundwater – a sustainable, energy saving method used in water supply in Rīga (Juhna et al. 1999). This process consists of filtration of lake waters (Lake Baltezers) through the surface sediment layer mostly consisting from loose quaternary sediments, composed from silica, clays, minor amounts of organic sediments and iron oxides, thus allowing to remove bacteria, fungi, algae, particulate matter from treated water and to reduce the concentration of organic substances (from 15 mg O_2 l^{-1} COD_{Mn} in infiltration ponds, to 7 mg O_2 l^{-1} in groundwater). However, the achieved organic matter concentration can be considered as too high for further use without purification but too low to efficiently use the common water treatment methods (coagulation etc.)

The objectives of the study were to assess in respect to humic substances the sorption properties of different sorbents and to evaluate the possibilities to use sorbents for drinking water treatment with additional cleaning of water after artificial recharge.

Table 1. *Elemental and functional composition, of humic substances used for adsorption experiments.*

Humic substance	Elemental composition, %			-COOH	ArOH	Aromaticity
	C	H	N	mmol g^{-1}		%
Aquatic FA from Lake Islienas (FA-I)	56.41	3.85	0.87	3.87	1.43	22.4
Commercial HA (Aldrich) (HA-A)	49.89	4.76	2.30	2.15	1.17	53.5
Soil FA (FA-So)	46.13	4.09	2.48	3.10	1.65	28.7
Soil HA (HA-So)	54.05	5.21	3.71	2.10	1.56	34.3
Peat HA (*Sphagnum peat*) (HA-Sp)	51.39	4.08	2.25	—	—	—
Peat HA (pine peat) (HA-P)	52.34	4.28	3.86	1.84	0.96	65.3

Materials and methods

Isolation and analyses of humic substances

Humic (HA) and fulvic (FA) acids were isolated from soil and peat by extraction with 0.1M NaOH (Methods of Soil Analysis, 1989), but from water of Lake Islienas by a method described by Kļaviņš and Cinis (1990). The elemental composition of isolated humic substances was determined on a Perkin Elmer 240 B CHN analyzer. The content of carboxyl groups was determined using $Ba(OH)_2$ titration, and the content of hydroxyl groups after acetylation (Methods of Soil Analysis, 1989). Aromaticity of the humic matter was calculated using the following equation: $\text{aromaticity} = 0.05\ \backepsilon + 6.74$ where: $\backepsilon$- molar absorptivity (at 280 nm) of humic substances ($l(mol\ cm)^{-1}$) (Chin et al. 1994). Commercial (Aldrich) humic substances were used for comparison. Properties of used humic substances are summarised in Table 1. Concentrations of humic substances in water were determined spectrophotometrically (410 nm) using a HACH DR 2000 spectrophotometer.

Adsorption Studies

Sorbents used in this study were used as obtained from Dow Chemicals, Enola Ltd., Lachema, Biolar, Chemviron Carbon, Reachim, Rohm&Haas (Table 2). Before use the sorbents were conditioned and washed. Adsorption studies of humic substances on different sorbents were conducted as batch experiments performed in 100 ml sealed glass bottles on a rotary shaker table 24 h at 20°C. 100 ml of either 5, 10, 25, 50, 75, 100, 250 mg l^{-1} solutions of humic substances with 1 g of sorbent were shaken until equilibrium occurred (24 hrs.). After separation of phases by filtration, the concentrations of humic matter in the supernatant phase were determined spectrophotometrically (HACH 2000, glass cell with path length 2.5 cm) as absorption at 410 nm (values obtained from initially prepared calibration graph). The quantity of humic substances adsorbed to different phases was determined from the difference of the initial aqueous phase concentration and the amount in solution at equilibrium. The type of humic substance, pH of the solution, and sorption time were varied. The sorp-

Table 2. *Properties of sorbents used for testing of sorption of humic substances.*

Name	Type*	Matrix**	Porosity***	Exchange capacity meq g^{-1}
Activated carbon (GAC)	Hydrophobic	Carbon	MR	—
Amberlite IRA-405	Strong anion*	St-Dvb	MR	4.2
AB-17-8	Strong anion*	St-Dvb	Gel-type	4.4
Spheron DEAE	Weak anion	Ma	MR	0.34
Amberlite IR-120	Strong cation	St-Dvb	Gel-type	4.3
Amino propyl silica	Weak anion	Silica	MR	0.18
Diaion PA-17-8	Weak anion	St-Dvb	MR	—
Amberlite IRA-95	Weak anion	St-Dvb	MR	4.7
Amberlite IR-124	Strong cation	St-Dvb	MR	4.3
Anthracite	Hydrophobic	Carbon	—	—
Polisorb-1	Hydrophobic	St-Dvb	MR	—

* Strong anion – strong anion exchange resin (commonly containing functional groups such as $-N(CH_3)_3^+$; Weak anion – weak anion exchange resin (commonly containing functional groups such as $-NH_2$ or $-N(C_2H_5)_2$; Strong cation – strong cation exchange resin (contain functional groups $-SO_3H^-$).

** St-Dvb – styrene-divinylbenzene; Ma – Methacrylic acid derivatives.

*** MR – macroreticular.

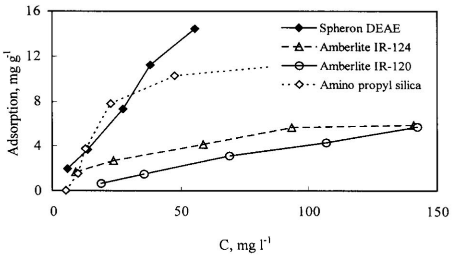

Figure 1. *Sorption of humic acid (Aldrich) onto ion exchange resins.*

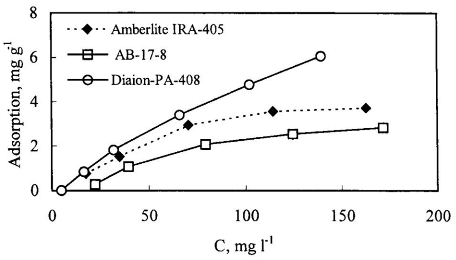

Figure 2. *Sorption of humic acid (Aldrich) onto ion exchange resins.*

tion test was performed in a laboratory scale column (30 × 250 mm) filled with sorbents (GAC, Polysorb-1, Amberlite IRA-95). A humic acid solution (Aldrich, 25 mg l^{-1}) was percolated through the column with a velocity 6 ml min^{-1}, sampling fractions of 10 ml with a "Pharmacia Fine Chemicals" fraction collector and analysing concentrations of the remaining humic acids in solution. The sorption test in a pilot scale column was performed taking groundwater from a depth of 15 m at the artificial recharge plant "Baltezers". A pilot scale column (820 × 200 mm; volume – 25 dm^3) was filled with GAC or anion exchange resin AB-17-8 and tested (flow rate 100 l h^{-1}, volume of sorbent 5 liters, COD_{Mn} of ingoing water 8.5 mgO_2 l^{-1}). In the outlet, COD_{Mn}, TDS, N_{tot}, and P_{tot}, were estimated for 2 months, taking weekly samples.

Results and discussion

Since humic substances are anionic polyelectrolytes, macroporous anion exchange resins can be considered to be well suited for their removal. However, the preference of the resin type (strong base resins *vs.* weak base resins) and the rigidity of the resin matrix are highly controversial (Fu and Symons 1990, Hand et al. 1994). In real processes the interaction with other ions in treated water should be considered (Eikebrokk 1989). Also carbonaceous resins have been recommended for drinking water treatment (Hand et al. 1994), which seems yet more prospective as such polymers are used for isolation of aquatic humic substances (XAD-8). Other resins comparatively widely studied for treatment include granulated activated carbon (GAC), obtained from wood and cleaned for use in water purification, and more recently, anthracite (Removal of humic substances... 1999). Sorption of humic substances on carbon-based sorbents proceeds due to Van der Waals interaction and depends on the pore size of the sorbent. In the case of GAC, macropores (~ 40 % of total pore volume) and micropores (> 1 nm) dominate, but the specific surface area is 1000–1400 m^2 g^{-1}. In comparison, the specific surface area of anthracite is ~ 5 m^2 g^{-1} and it does not contain micro and mesopores, but only a relatively increased (eroded) surface (Removal of humic substances... 1999)

Humic substances present in the groundwater after its artificial recharge can originate from aquatic, soil, peat and sedimentary HS. As the properties of HS depend on their origin, we have separately studied the sorptive behaviour of these substances, using as a surrogate commercially available humic acid (Table 1). The properties of the studied HS fall into the range common for humic substances (Orlov 1990, Kļaviņš, 1998). The humic substances differ in their aromaticity, since the dominant structures of humic substances are benzene- and phenolecarboxylic acids (Kļaviņš 1998) (aromatic structural units), and residues of carbohydrates and mono- and dicarboxylic acids (aliphatic structural units). The aromaticity is highest for peat and soil humic substances, and lowest for aquatic humic substances (Kļaviņš et al. 1999).

The sorptive properties of studied humic substances were determined in batchwise test conditions, in a laboratory scale column and in a pilot scale column. Among the studied sorbents (Figures 1–3), the macroreticular weak anion exchange resins (DEAE Spheron, Amino propyl silica, Diaion PA-408) were the most efficient. Porosity appeared to prevail over functional group con-

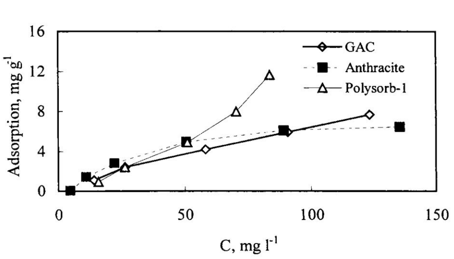

Figure 3. *Sorption of humic acid (Aldrich) onto GAC, anthracite and Polysorb-1.*

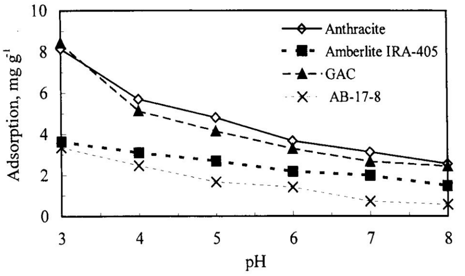

Figure 4. *Sorption of humic acid onto sorbents depending on pH* (sorbent: 1 g, sorption conditions and pH adjustment as in experimental part: sorption time 24 hrs).

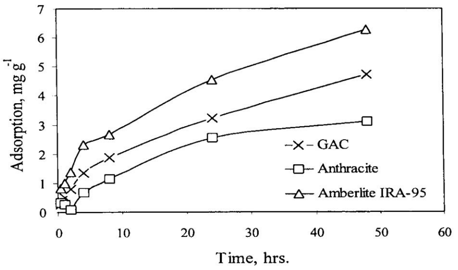

Figure 5. *Kinetic curves of sorption.*

centration and type in determining sorption efficiency: weak anion exchangers with a low concentration of functional groups were more efficient than strong anion exchange resins with nearly ten times higher concentrations of functional groups.

Sorption on anthracite led to lower equilibrium concentrations (<90 mg l^{-1}), which is to be expected in water treatment, and the sorption was improved over that on GAC which is more commonly used for water purification. These differences show that the micropores in GAC are not easily accessible to humic substances. The sorption efficiency was high on carbonaceous (hydrophobic) resins (Polysorb-1) and moderate on cation exchange resins, implying that there are several mechanisms involved in retention of humic substance. The observed differences in sorption, depending on the sorbent properties, shows the importance of optimisation of sorbent selection or even a need to design special resin types for removal of humic substances.

The sorption of humic substances on the sorbents (Figure 4) is highest at low pH of the humic matter solution. At pH that is more common in the real environment, sorption of humic matter is much lower. The dependence on pH is higher for hydrophobic sorbents, but less expressed for ion exchangers. However, the sorption is low at pH where the humic substances as polycationites are thermodynamically less stable and tend to precipitate, which means that the sorption is dominated not so much by the forces of adsorption of humic matter onto sorbents, but rather by the intermolecular association forces between humic matter.

Study of sorption kinetics (Figure 5) shows that the sorption process is slow (especially for GAC and Anthracite). The observed pore size values for nitrogen adsorption (BET method) were much higher than size of humic macromolecule. The sorption kinetics indicate that the sorption process is diffusion controlled. The sorption of humic substances much depends also on the type and properties of humic substances (Figure 6). Depending on their humic substance origin, the sorption efficiency could be optimal on hydrophobic sorbents (GAC) or ion exchangers (Amberlite IRA-95). Therefore, the optimal sorbent for each case, depending on the dominant sources of humic substances can differ.

To study the potential for practical groundwater purification, the most perspective sorbents were tested first in laboratory scale columns (Figure 7), but afterwards in a pilot scale system (Figure 8).

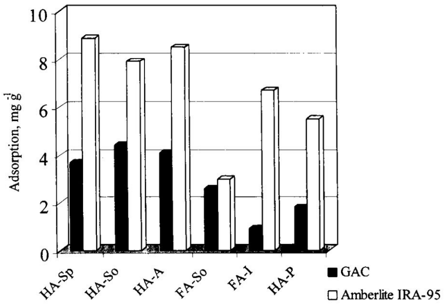

Figure 6. *Sorption depending on type of humic substances.*

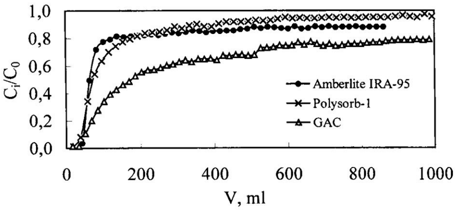

Figure 7. *Breakthrough curves for three types of resins in laboratory scale column.*

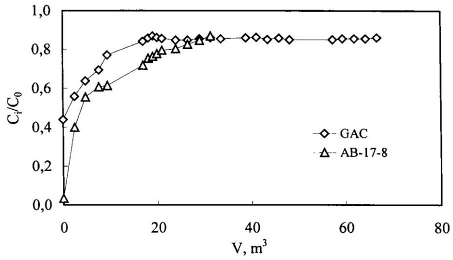

Figure 8. *Breakthrough curves for two types of resins in pilot scale column.*

The use of the tested sorbents increased the quality of treated water (Figure 7, 8). Anion exchange resins and GAC proved to be more efficient for removal of organic matter. The sorption curves show comparatively rapid saturation of the used sorbents and achievement of equilibrium with organic matter in aqueous phase. A possible explanation for this may be that humic substances comprise up to 60–80 % of the total dissolved organic matter in groundwater (and this is in general agreement with data from literature) (Kļaviņš 1998). To confirm this suggestion, biologically degradable organic carbon (BDOC) was estimated (Volk et al. 1997). In water of lake Baltezers, BDOC comprised 30–40 % of the total DOC, compared to 15–25 % of total DOC in groundwater. The experimental pilot scale experiments showed that the major part of HS are removed during the test period, while low molecular weight organic substances are not retained, but those may be efficiently removed using known biodegradation methods or other approaches.

Conclusions

Humic substances may be efficiently removed from groundwater using different types of sorbents. Macroreticular anion exchange resins and GAC were found to be the most efficient. The sorption depends also on the properties of HS.

References

Chin, Y.-P., Aiken, G., O'Loughlin, E. (1994) Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ. Sci. Technol., **28**(**11**), 1853–1858.

Eikebrokk B. (1989) Fjerning av humus. Vatten, 45, 297–315.

Fu, P. L. K., Symons J. M. (1990) Removing aquatic organic substances by anion exchange resins. J.Amer.Water Works Assoc., 82, 10, 70–77.

Hand D. W., Herlevich, J. A., Perram, D. L., Crittenden, J. C. (1994) Synthetic adsorbent versus GAC for TCE removal. J.Amer. Water Works Assoc., 86, 64–72.

Juhna T. M. Kļaviņš, J. Sproģis. (1998) Retention of humic substances by filter-sand from infiltration ponds for artificial groundwater recharge. Latv. Chem.J., 4, 87–93.

Kļaviņš, M., Apsīte, E., Parele, E. (1997) Humic substances in surface waters of Latvia. *Proc. Latv. Acad. Sci., Ser. B*, **51** (3/4), 143–153.

Kļaviņš, M. (1998) *Aquatic humic substances: Characterisation, Structure and Genesis.* Rīga: LU, 286 pp.

Kļaviņš, M., Cinis U. (1990) Isolation of humic substances from surface waters. Izv. AN Latv. SSR, Khim.Ser., 3, pp. 360–364 (in Russian).

Kļaviņš M., Seržāne, J., Supe, A. (1999) Properties of soil and peat humic substances from Latvia. Proc. Latv. Acad. Sci, ser B, 53(5), 249–256.

Kronberg, L., Vartiainen, T. (1988) Ames mutagenicity and concentration of the strong mutagen 3-chloro-4-(dichloromethyl)-4-oxo-butenoic acid in chlorine-treated tap waters. Mutation Res., 206, 177–182.

Loper, J. C. (1980) Mutagenic effects of organic compounds in drinking water. Mutation Res., 76, 241–268.

Methods of Soil Analysis. (1989) American Society of Agronomy Inc. Publisher, Madison, Wisc. pp. 1247.

Orlov, D.S. (1990) Soil humic acids and general humification theory. Moscow:MGU, 324 p. (in Russian).

Removal of humic substances from water. (1999) (Ed. H. Odegaard) International IAWQ-IWSA Conference, Norges forskningsrad., Trondheim, Norway, 280 p.

Thurman, E. M. (1985) *Organic Geochemistry of Natural Waters.* Martinus Nijhoff/Dr. W. Junk Publishers, Wageningen, 234 pp.

Thurman, E. M., Malcolm, R. L. (1981) Preparative isolation of aquatic humic substances. Environ. Sci. Technol., **15**, 463–466.

Volk, C. J., Volk, K. D., Kaplan, L. A. (1997) Chemical composition of biodegradable dissolved organic matter in streamwater. Limnol. Oceanogr., 42(1), 39–44.

Water Quality and Treatment: A Handbook of Community Water Supply (1990) American Waterworks Association, Ed. F. W. Pontius, McGrawHill: N.Y., 1193 p.

III

Immobilisation of Humic Substances

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Immobilisation of humic substances

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Abstract

Attempts were made to immobilise humic substances (HS) by grafting them onto different carriers (styrene-divinylbenzene copolymers, cellulose and silica) as well as by their crosslinking with formaldehyde. Reaction with Merrifield resin was used for the immobilisation of HS, coupling by means of water-soluble carbodiimides to carriers containing amino-groups. Crosslinking of HS with formaldehyde (also in presence of other substances able to enter polycondensation reactions with formaldehyde) was shown to be an efficient method for their insolubilisation. Properties of the obtained immobilised HS were studied, including their potential use as sorbents for several metal ions and organic substances. The adsorption efficiency was found to depend both on the sorbate used, type of humic substance immobilised and character of the polymeric matrix. Immobilised HS can be regarded as a prospective sorbents for the removal of different organic molecules and metals ions from the aquatic environment. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Humic substances; Sorbents; Immobilisation

1. Introduction

Humic substances (HS) are among the many factors, which affect soil fertility, and they play a major role in organic carbon turnover. HS form the major organic component of soil, peat and natural waters, and as resources they are abundant. About 60–85% of the organic matter in surface waters derived from natural sources consists of HS [1]. Regarding their function in ecosystems, HS are able to complex heavy metals and persistent organic xenobiotics [2]. Interaction of HS with xenobiotics may modify the uptake and toxicity of these compounds to living organisms, and affect the fate of pollutants in the environment. The immobilisation of HS on solid carriers is an interesting approach for investigation of their interaction with various substances, as shown by Klavins [3] Szabo and Bulman [4] and Koopal et al. [5,6]. Although, immobilisation has shown its usefulness for investigation of the structure, catalytic activity and other properties of proteins, nucleic acids and other macromolecules [7], in the case of HS, there are few publications [3–6]. Also, immobilised HS can serve as sorbents for organic and inorganic substances, enabling study of their interactions. Since, HS are mostly present in immobilised form (bound to mineral or organic matrixes) in natural environments, they

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are likely to be important in the fate of different xenobiotics.

The aim of the present study is to compare different methods for immobilisation of HS and their subsequent use as sorbents.

2. Materials and methods

Merrifield resin (chloromethylated styrene–divinylbenzene copolymer) (100–200 mesh, –CH_2Cl group content 1.6 mmol g^{-1}, Bapeks Ltd) and aminopropylsilica (particle size 0.1–0.25 mm, content of aminogroups 130 μmol g^{-1}, Enola Ltd) were used as a carriers. Analytical grade metal salts (excepting in the case of Cu and Co sulphates, Mn chloride and Cd nitrate) were used to prepare 0.01 M stock solutions (pH 6.5). Commercial humic acid (HA_C) was obtained from Aldrich, and *N*-ethyl-*N*′ (3-dimethyl-aminopropyl)-carbodiimide hydrochloride from Sennchemicals. For comparison, humic acids (HA) were isolated from soil and peat by extraction with 0.1 M NaOH [8], and from water by Thurman and Malcolm method [9]. Concentrations of functional groups were determined by standard methods [10]. IR spectra of finely ground polymers in nujol or hexachlorobutadiene were recorded on a Perkin–Elmer 58B apparatus for suspensions. UV spectra were recorded on an Unicam SP 1800 spectrophotometer. Elemental analysis was carried out using a Carlo Erba EA-1108 analyser.

2.1. Immobilisation of humic substances on Merrifield resin (polymer I)

Merrifield resin 10.00 g (1.60 mmol g^{-1} of –CH_2Cl) was added to a solution of 0.50 g humic acid (Aldrich) in 100 ml, 0.05 M NaOH and 100 ml 50% dimethylformamide–water. The mixture was stirred at 80 °C for 20 h. The polymer was filtered off, washed with dimethylformamide, 0.1 M Na_2CO_3, water, 0.1 M HCl, water, acetone and dried at 50 °C to give 10.43 g of immobilised humic substance I.

Found: C 86.17; H 7.29; Cl 0.32%, concentration of immobilised HS 20.5 mg g^{-1}.

2.2. Immobilisation of humic substances on epoxypropylsilica (polymer II)

To 5.00 g of epoxypropylsilica (concentration of epoxygroups 0.18 mmol g^{-1}, Enola Ltd) were added 0.60 g HA (Aldrich) dissolved in 50 ml of 0.25 M NaOH, and gently stirred for 10 h at 40 °C. The resulting polymer was filtered off, washed with water, 0.1 M HCl, water, acetone and dried at 50 °C to give 5.02 g of polymer II.

Found: C 12.32; H 3.74; N 0.12%, concentration of HA: 57 mg g^{-1}.

2.3. Immobilisation of humic substances on epoxypropylcellulose (polymer III)

To 5.00 g of cellulose in 50 ml of 0.3 M NaOH at 40 °C was added 10 ml of epichlorohydrine dropwise within 1 h. The mixture was stirred at 60 °C for 2 h. Then, 0.60 g HA were dissolved in 50 ml of 0.025 M NaOH, and 5 ml of epichlorohydrine (dropwise) was added and stirred at 40 °C for 4 h. The polymer was filtered off, washed with acetone, water, 0.1 M HCl, water, acetone and dried at 50 °C to give 4.65 g of polymer III.

Found: C 42.20; H 6.13; N 0.10%, concentration of carboxylgroups 0.098 mmol g^{-1}; concentration of HA: 87 mg g^{-1}.

2.4. Immobilisation of humic substances by means of carbodiimides (polymer IV)

To 10.00 g of aminopropylsilica were added 100 mg of humic acid dissolved in 50 ml of 0.1 M $NaHCO_3$, and the pH of the reaction mixture were adjusted to pH 4.6 by means of 0.5 M HCl. After addition of 500 mg of *N*-ethyl-*N*′ (3-dimethyl-aminopropyl)-carbodiimide hydrochloride, the mixture was stirred, maintaining the reaction pH 4–5 by addition of small portions of 0.1 M HCl. After stirring for 4 h, the mixture was kept overnight. The polymer was filtered off, washed with acetone, water, 0.1 M HCl, water, acetone and dried at 50 °C to give 10.05 g of polymer IV.

Found: C 4.67; H 0.59; N 0.31%, concentration of HA: 7 mg g^{-1}.

2.5. Immobilisation of humic substances by polycondensation reaction with formaldehyde (polymer V)

Paraformaldehyde (13.6 g) was added in small portions to a stirred suspension of 10.00 g of humic acid in 20 ml formic acid at 100 °C, stirring being continued until gelation of the reaction mixture began (approximately 3 h). The stirring pad was removed and the heating prolonged for a further 5 h. The obtained polymer was cut into large pieces and crushed. The product was carefully washed with water, 0.1 M NaOH, water, dimethylformamide, water, 0.5 M HCl, water and extracted with acetone. The yield of polymer V_A was 5.35 g.

Found: C 53.93; H 3.59; N 1.33%. Calculated content of HS 373 mg g^{-1}.

Other immobilised HS (V_B–V_D) were prepared similarly, and their characteristics are given below.

2.5.1. Polymer V_B

Polymer V_B was obtained as polymer V_A, with addition of 10.0 g of phenol during the polycondensation process into the reaction mixture. Found: C 66.24%, H 6.39. Calculated content of HS 340 mg g^{-1}.

2.5.2. Polymer V_C

Polymer V_C was obtained as polymer V_A, with addition of 10.0 g of toluene during the polycondensation process into the reaction mixture. Found: C 58.60%, H 6.51. Calculated content of HS 185 mg g^{-1}.

2.5.3. Polymer V_D

Polymer V_D was obtained as polymer V_A, with addition of 10.0 g of resorcinol during the polycondensation process into the reaction mixture. Found: C 60.98, H 6.30, N 0.78%. Calculated content of HS 430 mg g^{-1}.

2.6. Determination of immobilised humic matter content

Samples of 1.00 g of polymer containing immobilised HS were treated by stirring at 80 °C for 6 h with 50 ml of 5 M NaOH. After filtration, the filtrate was diluted to 100 ml with 3 M HCl. Absorbance of the solution was measured at 410 nm and the content of HS was calculated from a calibration graph, prepared by hydrolysing a known amount of the same humic substance under the same conditions.

2.7. Determination of sorption on immobilised humic substances

One hundred milligram of the respective sorbent (Table 2) was suspended in 25 ml of metal salt (1×10^{-3} M) or *p*-aminoazobenzene (1×10^{-6}–1×10^{-2} M). After shaking for 24 h, the polymer was filtered off and the concentration of metal (by flame AAS Perkin Elmer 403) or *p*-aminoazobenzene (spectrophotometrically) was determined in liquid phase.

3. Results and discussion

The HS used in this study (Table 1) were isolated from peat, soil and water according to

Table 1
Properties of HA from soil, peat and water

Humic acids		Elemental composition, (%)			–COOH, (mmol g^{-1})	ArOH, (mmol g^{-1})
		C	H	N		
Water	HA_W	57.21	3.94	1.03	4.15	1.42
Soil	HA_S	54.05	5.21	3.71	2.10	1.56
Commercial	HA_C	49.89	4.76	2.30	2.15	1.17
Peat	HA_P	52.34	4.28	3.86	1.84	0.96

Table 2
Properties of immobilised HS

Immobilised HS	C (%)	H (%)	N (%)	Content of HS (mg g^{-1})	Swelling with water (ml g^{-1})	Swelling with benzene (ml g^{-1})
I-HA$_C$	86.17	7.29	–	20	0.87	1.85
I-HA$_W$	76.06	8.95	1.95	223	0.54	1.03
I-HA$_S$	76.12	9.16	0.97	228	0.59	1.06
I-HA$_P$	77.03	8.21	0.43	208	0.48	1.01
II-HA$_C$	12.32	3.74	0.12	57	0.11	0.35
II-HA$_S$	12.31	4.76	0.32	85	0.12	0.45
III-HA$_S$	43.86	7.30	1.50	83	0.18	0.56
III-HA$_C$	42.20	6.13	0.30	87	0.22	0.64
IV-HA$_C$	4.67	0.59	0.31	7	0.12	0.12
V_A-HA$_C$	53.93	3.59	1.33	373	1.15	2.35
V_B-HA$_C$	66.24	6.39	0.05	340	0.73	1.87
V_C-HA$_C$	58.60	6.51	0.13	185	0.82	1.84
V_D-HA$_C$	60.98	6.30	0.78	430	1.14	2.40

HA type is explained in Table 1 and method of immobilisation is numbered similar as in Fig. 2.

known methods [11]. HA$_C$ (Aldrich) was used for comparison. The main characteristics of the studied HS were found to be within the range of values common for humic matter of similar origin [12]. The most probable structures in HS that can be used for their immobilisation, according to hypothetical structural models, are residues of phenolic structures in their core, and functional groups such as phenolic hydroxylgroups, carboxylgroups, and aminogroups (Fig. 1) in the peripheral part of the humic macromolecules. Alkylation, acylation and condensation are potential reactions for use in immobilisation by linking of HS via these structures to the solid phase of the polymer. For effective sorption, the responsible functional groups should be present in sufficient quantities.

Five different methods were used for the immobilisation of HS (Fig. 2):

1. Immobilisation by grafting on chloromethylated styrene–divinylbenzene copolymer;
2. Immobilisation on epoxypropylsilica;
3. Immobilisation on epoxypropylcellulose;
4. Immobilisation on aminopropylsilica using coupling by means of water soluble carbodiimide;
5. Polycondensation of HS with formaldehyde and molecules able to enter in condensation reactions (phenols and alkylaromatic substances).

Immobilisation of HS on chloromethylated styrene-divinylbenzene copolymers (Merrifield resin) was conducted in a mixture of water with organic solvents (under conditions similar to those used in solid phase organic synthesis) in the presence of alkaline reagents, yielding stable polymers with high concentrations of immobilised HS.

Hydroxylgroups dominate among the functional groups in HS, and they are sufficiently reactive to react in the presence of catalysts in alkaline media with epoxygroups. This reaction was used to immobilise HS on cellulose or silica matrix via carriers containing epoxygroups. The conditions used for immobilisation of HS, selected as those commonly used for immobilisation of similar biomolecules [7], allowed to obtain immobilised HS with high yield.

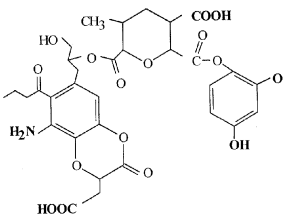
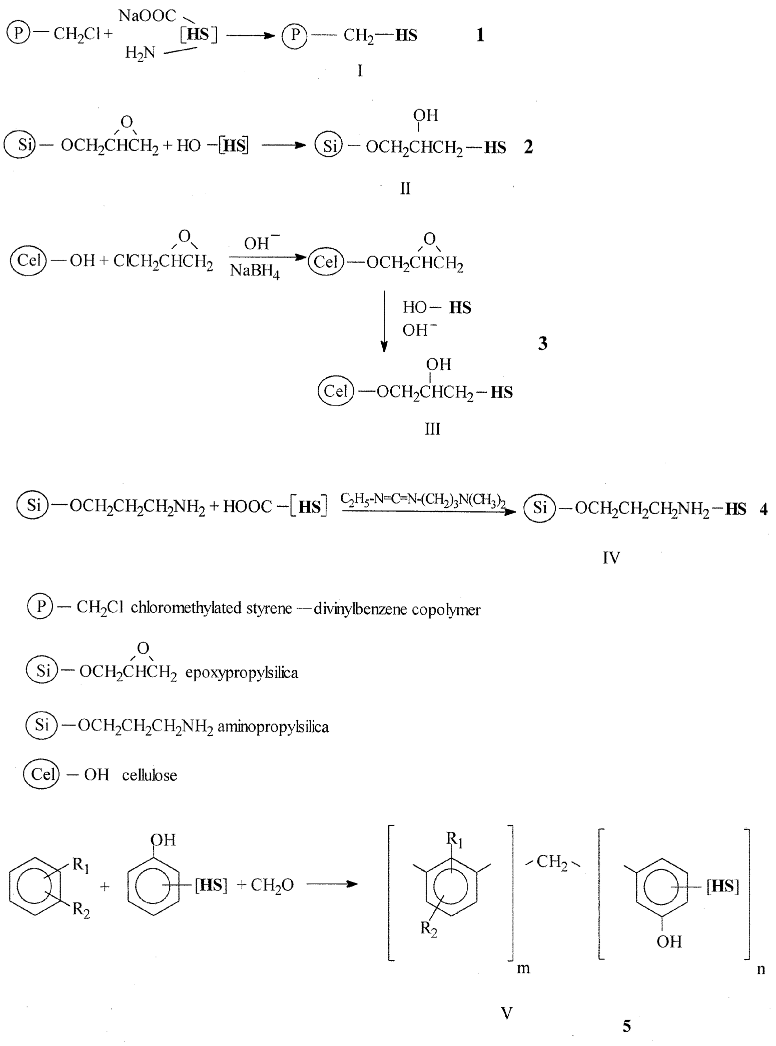


Fig. 1. Structures in hypothetical molecules [11] of HS for potential use in their immobilisation.



R_1, R_2 – H; OH, CH_3

V_A – polycondensation of humic acid

V_B – R_1 = H; R_2 = OH

V_C – R_1 = H; R_2 = CH_3

V_D – R_1, R_2 = OH

Fig. 2. Methods and carriers used for immobilisation of HS.

Carboxylgroups can be used for immobilisation using approaches widely adopted in peptide synthesis and solid phase organic synthesis—coupling by means of carbodiimides [7]. Also, immobilised HS can be obtained using as a matrix silica carrier containing amino groups removed from the surface of the polymer via a spacer and water-soluble carbodiimide.

The basic properties of the humus core can be utilised for immobilisation, by virtue of the presence of phenolic units in the structure of HS. As phenols and ethers can enter in polycondensation reactions with formaldehyde, HS can form polycondensation polymers. Phenolic groups in the HS are in constrained positions and thus the corresponding sites in the aromatic core of HS are of low reactivity, yields of polycondensation polymers using pure HS are low, and the mechanical stability of the obtained polymers is poor. If other low molecular weight substances (phenols, alkylaromatic substances) capable of entering in condensation reactions are added to the reaction mixture, the yields of the obtained polymers increase greatly and it is possible to obtain polymers with high mechanical stability.

The proposed immobilisation methods allow to immobilise not only commercial HS, but also HS from water, peat and soil with different properties (Table 1). The immobilisation efficiency of different HS tested was found to differ only slightly.

The obtained polymers were characterised by weight gain during reaction, elemental analysis and IR spectra of polymers after the reaction (Fig. 3). The IR spectra of immobilised HS show general features common for HS, sometimes with overlapping of lines from the polymer matrix used for immobilisation of HS. Absorption bands in the spectral region 3600–2800 cm^{-1} are very broad and due to the presence of –OH groups. Sorption at wavelengths 2920 and 2860–2850 cm^{-1} indicates the presence of CH_3– and CH_2– groups. Typical intensive sorption lines are common for the region around 1700 cm^{-1} (1725–1700 cm^{-1}), characteristic for carbonylgroups.

The total content of immobilised HS was determined by alkaline hydrolysis followed by spectrophotometric analysis. All immobilised HS were coloured polymers, and the porosity and particle size of polymer matrix during the immobilisation process decreased minimally. The content of immobilised HS varied between 7 and 430 mg HS g^{-1} sorbent. The immobilised HS were hydrolytically stable (Table 3).

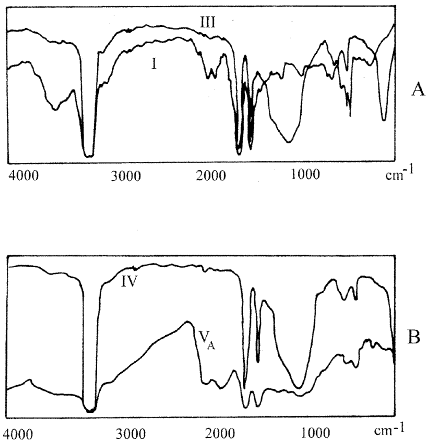


Fig. 3. IR spectra if immobilised HS I, III, IV, V_A.

The observed uptake of metal ions (Cu^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}) by obtained sorbents is presented in Table 4. The uptake of metals was relatively rapid, and commonly the equilibrium conditions were reached within less than 30 min. The initial polymer matrix did not absorb appreciable quantities of metals. The uptake capacity for different metals depended on the polymer matrix and type of humic matter bound to the

Table 3
Hydrolytic stability of immobilised HS

Immobilised HS	Hydrolytic stability (% of HS desorbed after 24 h)		
	0.05 N HCl	0.05 N NaCl	0.05 N NaOH
I-HA_C	1	0	3
I-HA_S	2	0	4
I-HA_P	2	0	5
II-HA_S	6	0	3
III-HA_S	4	0	2
V_B-HA_C	0	0	5
V_B-HA_C	5	0	8

Table 4
Sorption of metal ions on immobilised HS

Polymer	Cu^{2+} (μmol g^{-1})	Co^{2+} (μmol g^{-1})	Mn^{2+} (μmol g^{-1})	Cd^{2+} (μmol g^{-1})
I-HA$_{\mathrm{C}}$	39	23	44	80
II-HA$_{\mathrm{S}}$	39	27	34	80
IV-HA$_{\mathrm{C}}$	34	33	33	80
V_{A}-HA$_{\mathrm{C}}$	–	–	104	120
V_{B}-HA$_{\mathrm{C}}$	72	38	50	85
V_{C}-HA$_{\mathrm{C}}$	108	61	60	99
V_{D}-HA$_{\mathrm{C}}$	187	166	183	177

polymer. Among the tested HS, the highest sorption capacity of immobilised HS obtained using polycondensation reaction was found for polymer V.

The obtained immobilised HS, can also be used as sorbents for hydrophobic organic chemicals, as demonstrated by sorption of *p*-aminoazobenzene on the obtained polymers (Fig. 4). The sorption was rapid, especially in the case of macroporous sorbents. For polymers obtained using polycondensation, the sorption was slower. The sorption of hydrophobic organic chemicals was dependent on the presence of other dissolved substances (NaCl); and increased as the dissolved substance concentration was raised. This effect could be explained by the decreasing solubility of *p*-aminoazobenzene in solutions with high ionic strength. The sorption efficiency of *p*-aminoazobenzene differed greatly between sorbents, being highest for polycondensation polymers and lowest for HS immobilised onto silica carriers (possibly due to low concentration of immobilised HS).

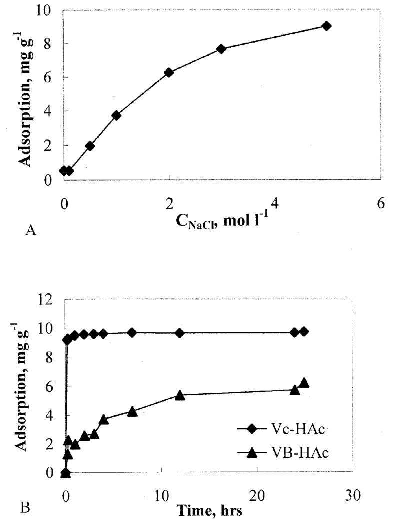


Fig. 4. Sorption of *p*-aminoazobenzene onto immobilised HS (A) depending on concentration of NaCl; (B) kinetics of sorption.

4. Conclusions

The described methods allow to obtain HS immobilised on different carriers. The obtained immobilised HS can be regarded as prospective sorbents for both hydrophobic organic substances and metal ions.

References

[1] E.M. Thurman, Organic geochemistry of natural waters, Martinus Nijhoff/Dr W. Junk, Wageningen, 1985.
[2] I.H. Suffet, P. MacCarthy, Aquatic humic substances, Am. Chem. Soc., Washington, DC, Adv. Chem. Ser., 1988, 212–219.
[3] M. Klavins, Immobilization of humic substances, Latv. Chem. J. 1 (1993) 96.
[4] G. Szabo, R.A. Bulman, J. Liquid Chromatogr. 17 (1994) 2593.
[5] L.K. Koopal, Y. Yang, A.J. Minnard, P.L.M. Theunissen, W.H. Van Riemsdijk, Coll. Surf. A 141 (1988) 385.
[6] Y.H. Yang, L.K. Koopal, Coll. Surf. A151 (1999) 201.

[7] K. Mosbach (Ed.), Methods in Enzymology, Academic Press, New York, 1976.

[8] M. Klaviņš, J. Seržāne, A. Supe, Proc. Latv. Acad. Sci. Ser. B 53 (5) (1999) 249.

[9] E.M. Thurman, R.L. Malcolm, Environ. Sci. Technol. 15 (1981) 463.

[10] A.L. Page (ed.). Methods of Soil Analysis, Part 2, Second Edition, Soil Sciences Society of America, Madison, 1982.

[11] M. Klavins, Aquatic Humic Substances: Characterisation, Structure and Genesis, University of Latvia, Riga, 1998.

[12] D. Orlov, Soil humic acids and general humification theory, MGU, Moscow, 1990.

IV

Sorption of Humic Substances on Aquifer Material at Artificial Recharge of Groundwater

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Sorption of humic substances on aquifer material at artificial recharge of groundwater

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Abstract

Experiments in batch equilibrium system were carried out to evaluate the importance of physical and chemical factors determining the sorption efficiency of humic substances (HS) on aquifer material, which has been used for artificial recharge of groundwater (ARG) in drinking water production. Results showed that an increase of the amount of clay in the aquifer material and a decrease of pH in water increased the sorption efficiency. The sorption of higher molecular weight, more hydrophobic and aromatic HS (Aldrich and forest soil humic acids) were greater than the sorption of acidic HS (river fulvic acids), either on the aquifer material or to its representative sorbing phases, clay and organic matter. The sorption on the aquifer material was largely due to physical sorption (hydrophobic attractions). This study showed the importance of HS composition on their removal during ARG and contributed to an understanding of the HS sorption mechanisms in this process.

Keywords: Humic substances; Sorption; Artificial recharge of groundwater; Aquifer material; Drinking water

1. Introduction

Artificial recharge of groundwater (ARG) is achieved by putting surface water in basins or other facilities where it infiltrates into the ground and moves through porous media downwards to recharge aquifers. In this process, without adding any reagents, suspended particles and human pathogens are effectively removed from the water (Huisman and Olsthoorn, 1983). Therefore, in areas where permeable aquifers are available, ARG has been used for drinking water production for more than 150 years. Nowadays, it is recognized that removing humic substances (HS) (humic acids (HA) and fulvic acids (FA)) from drinking water is important because they impart a yellow colour to water and may lead to the formation of carcinogenic by-products during disinfections (Rook, 1974) as well as undesirable bacterial biofilm growth (Ellis et al., 2000) in distribution systems. The removal efficiently of HS during ARG varies from place to place; the causes of these variations are often not understood. Thus, more knowledge about the mechanisms involved in removing HS is of interest.

Due to partially colloidal properties (Jones and Bryan, 1998) and its ability to associate with inorganic colloids (Burba et al., 2001), straining and interception in porous media both in solid–water and gas–water interface may be involved in HS retention during the ARG. However, sorption to aquifer material (hereafter filtersand) (McCarthy et al., 1993) followed by microbial degradation are the most important mechanisms.

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Because HS are relatively resistant to degradation whereas attachment to surfaces may increase their availability for bacteria (Camper, 2002), sorption is most likely a limiting step for HS removal during ARG. The mechanisms of HS sorption to filtersand have not been widely studied. However, given the similarity in composition of material, these mechanisms are assumed to be similar to those occurring in soils (Jacks and Frycklund, 1995), river sediments, and natural aquifer materials. Among the different mechanisms proposed, ligand exchange (chemisorption), hydrophobic attraction, and electrostatic attraction (physical sorption) are considered the most important for removal of HS by surfaces (Stumm, 1992) because HS has both acidic functional groups (carboxyl and phenol) and hydrophobic moieties. Due to the carboxyl groups, HS may participate in ligand exchange or electrostatic interaction with oxide surfaces, whereas hydrophobic properties allow HS to accumulate on surfaces because of their incompatibility with water, which is more polar than the surface (Lai and Chen, 2001). The importance of these mechanisms can be evaluated by comparing the sorption of FA (i.e. more acid fraction of HS) with HA (i.e. more hydrophobic fraction of HS). In addition to the type of HS, the physical–chemical properties of sorbing material (e.g. texture, surface areas, amount of oxides groups) and of water (e.g. pH, ion strength, concentration of divalent metals) govern the sorption mechanisms.

In the present study the sorption of FA and HA on the filtersand and its major composing phases participating in the sorption (i.e. iron and aluminium oxides, organic matter) was evaluated in laboratory batch equilibrium systems. The objective of the study was to investigate how the composition of the filtersand and HS influence sorption efficiency of HS on the filtersand, and to evaluate importance of the sorption mechanisms in removal of HS during ARG.

2. Materials and methods

2.1. Isolation and analyses of humic substances

HA and FA from typical forest soil (podzolic) in Latvia and filtersand from the infiltration basin used for drinking water production at Baltezers ARG plant in Riga, Latvia were isolated by extraction with 0.1 M NaOH (Thurman, 1985). HA and FA from water of Lake Baltezers (used for ARG) and the Salaca River were isolated by the Thurman and Malcolm method (Thurman and Malcolm, 1981), which was modified by Klavins and Cinis (1990). Commercial (Aldrich Chemical Company—Milwaukee, WI) HA isolated from coal was used for comparison. The elemental composition of isolated HS was determined on a Perkin–Elmer 240 B CHN analyser. Ash content was determined by ignition of samples at 500 °C for 5 h. The content of carboxyl groups was determined using $Ba(OH)_2$ titration, and the content of hydroxyl groups was done after acetylation (APHA, 1988). Aromaticity of HS was determined based on measurement of their ultraviolet light absorption and calculated according to Chin et al. (1994). The $(O+N)/C$ atomic ratio was used to establish hydrophilicity/hydrophobicity of HS as suggested by De Paolis and Kukkonen (1997). The higher is this ratio the more hydrophilic is the substance. Molecular weight distribution was determined with gel filtration on Sephadex G-100. Properties of the HS used are summarised in Table 1. Concentrations of HS in water were measured colorimetrically at 410 nm wavelengths using a HACH DR 2000 spectrophotometer and calculated from calibration graphs initially prepared for each HS.

2.2. Sampling, preparation and analysis of the filtersand

Filtersand was sampled at the Baltezers plant where groundwater is artificially recharged by infiltration of

Table 1
Elemental and functional composition of HS from filtersand of infiltration basins at Baltezers plant (Filtersand HA); HA from water of Lake Baltezers (Lake HA); HA from deposits of Lake Baltezers (Sediment HA); FA from Salaca River (River FA); podzolic soil HA (Soil HA) and Aldrich HA

HS	Elemental composition (%)				Atomic ratio		[a]C_{-COOH} (mmol/g)	[b]C_{ArOH} (mmol/g)	[c]$C_{aromaticity}$ (%)
	C	H	N	O	H/O	$(N+O)/C$			
Filtersand HA	54.6	3.7	0.9	40.8	1.45	0.57	4.88	1.18	32.5
Lake HA	57.2	3.9	1.0	40.2	1.57	0.63	4.15	1.42	19.3
Sediment HA	52.1	6.0	4.3	37.5	2.58	0.61	2.43	–	–
River FA	46.3	3.7	2.9	47.1	1.26	0.82	4.45	0.98	16.3
Soil HA	49.8	4.7	2.1	43.3	1.75	0.69	2.21	–	65.3
Aldrich HA	49.9	4.7	2.3	43.1	1.76	0.69	2.15	–	53.5

[a] Carboxylic groups.
[b] Phenolic hydroxyl groups.
[c] Aromaticity.

water from Lake Baltezers through the infiltration basins. The basins were excavated in quaternary deposits at the end of the 1950s. In 1997, samples were collected from the bottoms of three infiltration ponds numbered accordingly 3, 4, and 8. Samples from each basin were taken close to the periphery (A), the centre (C) of the basins and in between these sampling points (B). The surfacial filtersand layer (0–4 cm) was sampled by Ekman drag, and deeper layers (4–24 cm) were taken using a core sampler and dissected into segments of 4 cm. The samples were air dried, sieved (<2 mm), and subsequently used for analyses and sorption experiments.

Particle size was estimated by dry sieve analysis or by the hydrometer method. BET surface area for minerals was determined by Micrometric FlowSorb II 2300 (30% H_2, 70% He) or Coulter SA 3100. The mineral composition was estimated using X-ray diffraction (diffractometer DRON-3). The filtersand chemical composition was determined by standard methods (APHA, 1988). For chemical analyses iron was extracted from filtersand samples (~10 g) with 25 ml conc. HNO_3, together with 5 ml 60% $HClO_4$. The content of organic carbon (OC) in the filtersand was determined by $K_2Cr_2O_7$ digestion (Methods of Soil Analysis, 1989).

2.3. Filtersand with organic matter removed

The organic matter in filtersand samples was removed by oxidation in acidic media: 500 g of the filtersand treated with a mixture of 375 ml 15% H_2O_2 and 10% CH_3COOH (55 ml) at 75 °C for 4 h, after which a new portion (100 ml) of 30% H_2O_2 was added with heating for an additional 2 h. Next, the samples were washed with 0.01 M NaCl to remove the entrained salts, then by distilled water, and dried at 105 °C (24 h).

2.4. Oxides, clays and lake sediments

To determine more specifically what phases contributing to HS sorption on aquifer material, phases representing those found in aquifer material were obtained for the study. Reanal supplied Alumina (α-Al_2O_3). Goethite (α-FeOOH) was synthesised accordingly (Lattimer and Jones, 1992). Clays were collected in Latvia; their characteristics are given in Table 2. Surfacial sediments layer (67% OC, BET of 1.51 m^2/g, 7.37 mg/g Fe) were collected by Ekman drag from the bottom of Lake Baltezers and analysed as per the methods cited above.

2.5. Sorption experiments

Sorption studies for HS on different phases were conducted as batch experiments performed in 50 ml sealed glass bottles on a rotary shaker table for 16 h at 20 °C. In 50 ml glassware, 5, 10, 25, 50, 75, 100, and 250 mg/l solutions of HS were equilibrated with 0.5 g of sorbent until equilibrium occurred. After separation of the sorbent by filtration through filter paper, the concentration of HS in the filtrate was analysed. The quantity of HS sorbed to different phases was determined from the difference between the initial aqueous phase concentration and the concentration in solution at equilibrium. Due to the release of organic matter from the filtersand and lake sediments, the results of the sorption experiments could not be interpreted by Freundlich or Langmuir isotherms. When initially sorbed (background) organic matter needs to be considered, the initial mass approach (Nodvin et al., 1986) is useful. This approach was also used in this study. In the initial mass approach, the concentration of a substance retained or released (normalized to sorbent mass) is plotted as a function of the initial concentration of the substances (also normalized to sorbent mass).

3. Results

3.1. Composition of the filtersand

Mineralogical analysis using X-ray diffraction spectroscopy revealed that the filtersand, regardless the sampling location and depth, was dominantly composed of SiO_2 (quartz) with some admixture of aluminium silicate (labradorite plagioclase). An absence of iron oxide minerals (e.g. celadonite and glauconite) indicated that iron, detected by chemical analyses, (Table 3) presumably infiltrated in via surface water. The texture of the aquifer material ranged from clay to fine sand. Typical concentrations of clay in filtersand ranged between 1% and 3% (basins no. 8 and 3), whereas it was as

Table 2
Sorption (mg/g) of different HA and FAs (FA) of different origins on clay (pH 3.4, temperature 20 °C and 100 mg of clay)

HS	Clay sample no. 1[a]	Clay sample no. 2[b]	Clay sample no. 3[c]
Aldrich HA	27.1	29.2	16.0
Soil HA	26.3	28.4	13.5
River FA	12.1	14.2	6.4

[a] Greenish gray, calcareous clay, dominantly smectite.
[b] Reddish brown fat clay, dominantly smectite.
[c] Black, lean clay, dominantly kaolinite.

Table 3
Physical and chemical characteristics of aquifer material (filtersand) recovered from surfacial layers (0–4 cm) of infiltration ponds used for artificial groundwater recharged from Lake Baltezers compared to composition of soil analysed by Jardine et al. (1989)

Sampling site	BET (m^2/g)	OC (wt.%)	Particle size analyses (dry wt.%)			Fe (mg/g)
			Sand	Silt	Clay	
8B[a]	n.d.	0.5	77.0	22.0	1.0	0.64
3A[a]	0.49	0.6	65.7	31.0	3.3	0.71
4A[a]	0.85	0.5	40.8	37.2	22.0	0.69
Reference soil[b]	n.d.	0.2	32.2	44.5	23.6	16.82[c]

n.d.: not determined, BET: surface area and OC: percent of organic matter.

[a] Number represents the no. of basin, letter represents sampling locations in the basin (see part 2.1 in the text).

[b] Jardine et al. (1989).

[c] Dithionite-citrate-bicarbonate extractable Fe.

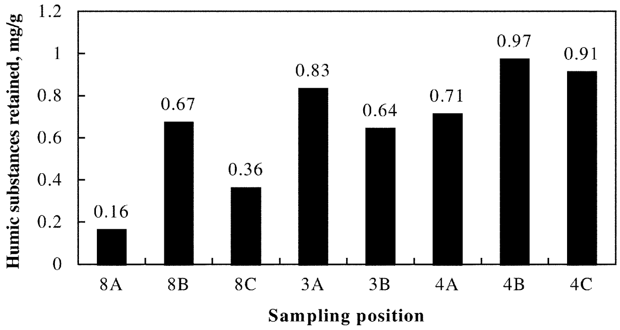

Fig. 1. Sorption of Aldrich HA (100 mg/l) on filtersand samples from different infiltration basins (sample size 500 mg; pH 6.5; sorption time 16 h). All samples were taken from the uppermost layer, 0–4 cm, but at different positions (A, B, C) in the basin. See composition of filtersand in Table 3.

higher in basin no. 4 close to that found in soils, see Table 3. The amount of OC was about 0.5%.

3.2. Composition of humic substances

Soil HA and commercially available (Aldrich) HA, were more aromatic and contained less from the carboxyl groups than river FA, Table 1. The (O + N/C) atomic ratios for Aldrich HA and soil HA were significantly lower than for river FA (Table 1) and indicated that both HAs were more hydrophobic than the FA. The molecular mass of the aquatic FA was lower (500–1000 Da) than for both HAs (500–3000 Da).

3.3. HS sorption on filtersand and related phases

Sorption of Aldrich HS increased with increased amounts of clay and decreased with increased amount of OC in the filtersand, Figs. 1 and 2. Sorption was heterogeneous among samples, but generally increased with the depth below the basins, Fig. 2. Sorption experiments of different HS on clays revealed that HS sorption exceeded the FA sorption, while it was better on smectite (the expandable clays) compared to koalinite, Table 2. Sorption to the filtersand from which organic matter was chemically removed was low or negative. At the same pH,

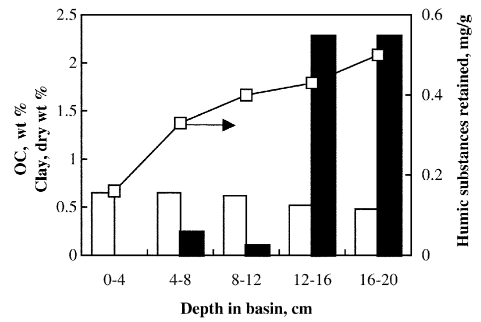

Fig. 2. Relationship between sorption of Aldrich HA (100 mg/l) to filtersand and the different depths below infiltration basin no. 8A (sample size 500 mg; pH 6.5; sorption time 16 h). Legend: *left y-axis*, OC, organic carbon (white bars); clay (black bars); *right y-axis*, adsorbed HS (open squares).

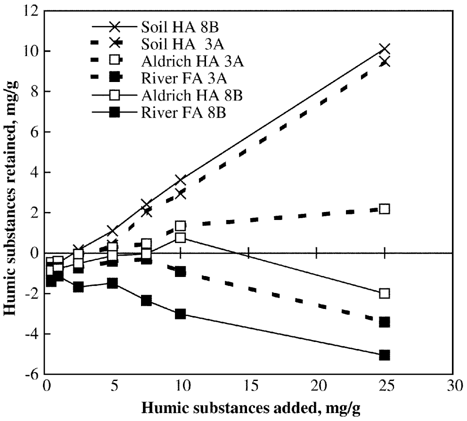

Fig. 3. Sorption of different HS on the upper layer of filtersand from basin 8A (—) and 3A (- - -) (sample size 500 mg; pH 6.5; sorption time 16 h).

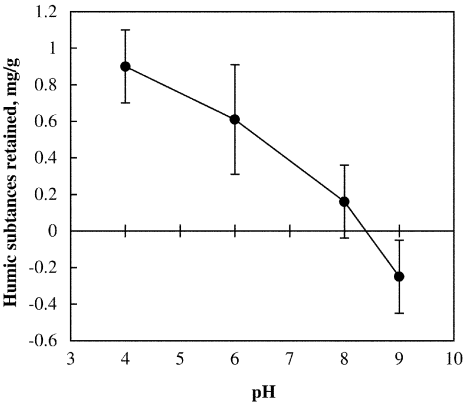

Fig. 4. Relationship between the sorption of HS and pH of filtersand samples from the infiltration basins. Each data point represents mean values of filtersand collected from all basins at different depths and positions. Vertical error bars represent the standard deviation from mean values.

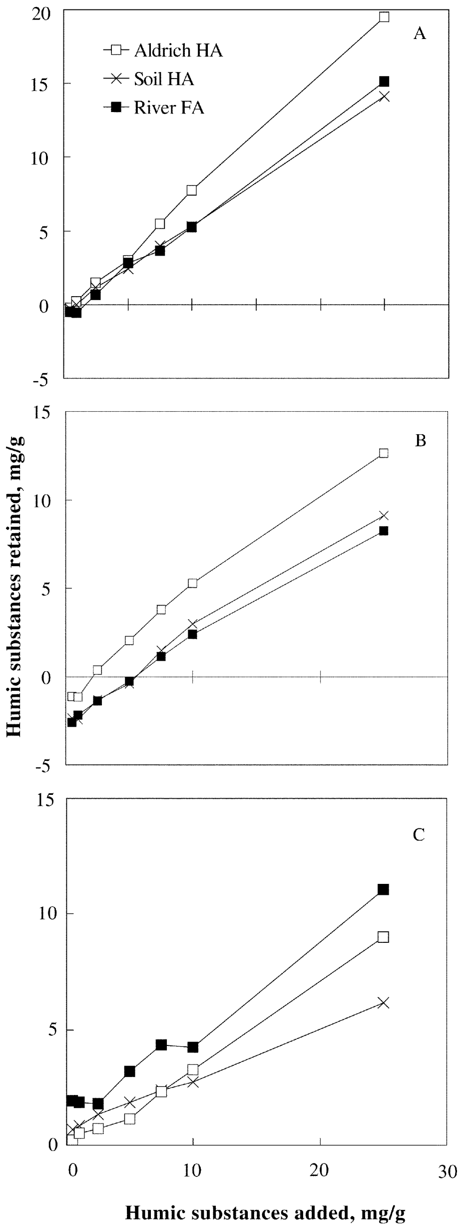

Fig. 5. Sorption (sample size 500 mg; pH 6.5; sorption time 16 h; temp 20 °C) of FA and different HA on (A) crystalline Alumina (Al_2O_3), (B) sediments from Lake Baltezers (13.3% OC) and (C) crystalline goethite (α-FeOOH).

Aldrich and soil HA were sorbed better than river FA to typical filtersand (basins no. 3 and 8) from Baltezers plant, Fig. 3. Regardless of the filtersand composition, the lowering of pH significantly increased the sorption efficiently of HS, Fig. 4. To understand the influence of different phases composing the filtersand, the sorption of HA and FA to iron, aluminium oxides, and organic rich sediments were tested. HA were always better or similarly sorbed to all filtersand components except iron oxide, which instead better sorbed FA (Fig. 5).

4. Discussion

Shen (1999) suggested that chemisorption (ligand exchange) is the dominant mechanism for sorption of HS to soil. Jardine et al. (1989), after observing temperature independency of HS sorption to soil, suggested that physical sorption driven by entropy changes (hydrophobic attraction) is more important. Gu et al. (1994) argued that due to fact that most of positively charged sites (e.g iron oxides, broken edges of clay surfaces) are occupied with organic matter, the ligand exchange is unlikely to be a dominant interaction in the soil system.

The composition of the filtersand used for ARG systems was similar to that of soils but with lower amounts of clay and iron oxide (Table 3), the surfaces bearing major binding sites for sorption of HS. A relatively high amount of OC (Table 3) indicated that a large proportion of these sites had already been occupied with organic matter making their surfaces negatively charged (Day et al., 1994), and thus unavailable for ligand exchange. The quartz was a major constituent of the filtersand. Ligand exchange of HS with quartz surfaces is minor, as proven in experiments by Spark et al. (1997), in which no proton consumption was observed in the interaction between the quartz and HS. Thus, under conditions where filtersand has a limited number of binding sites, ligand exchange is probably not the dominant process for removing HS during ARG.

The results showed that sorption of HS increases with an increase the fraction of clay in the filtersand. Due to small size the clay particles have a large specific surface area that, depending of chemical and physical properties of clay (not identified herein), can be conductive for either physical or chemical sorption to occur. The results from this study showed that HA, more aromatic and with higher molecular mass fraction of HS were better removed than FA, more acidic fraction with higher amounts of functional groups, Fig. 3. This might indicate that physical interaction rather than ligand exchange between HS and the filtersand are important. However, this should be carefully considered since lower sorption efficiency of HS with a higher amount of functional groups (e.g. FA) is not always indicative of a lack of coordinative interaction (ligand exchange). HS are macromolecules which behave like polyelectrolytes; thus their sorption is determined not only by the availability of sites, but also by lateral interaction between themselves and the steric arrangements of the macromolecule (Kaiser and Zech, 1997; Vermeer et al., 1998). Due to a high amount of functional groups, HS stretches (due to lateral repelling between the functional groups) and when becomes sorbed on the surfaces occupies a large area that makes overall sorption efficiency of FA lower than of HA. This is also demonstrated by the finding that HS, with high amounts of aromatic groups and lower amounts of carboxyl groups, can be preferably sorbed to the surface with high amounts of binding sites such as iron or aluminium oxides (Evanko and Dzombak, 1999; Meier et al., 1999). This occurs even though ligand exchange is the dominant mechanism of interactions of HS with those surfaces (Tipping, 1981; Day et al., 1994; Gu et al., 1994; Edwards et al., 1996; Tombácz et al., 2000).

In the sorption process HS replaced nearly all previously retained HS before any sorption could occur, Fig. 3. This indicates that HS were weakly sorbed on the bare filtersand, perhaps as a result of physical interaction. Elemental analyses showed an insignificant increases of oxygen and carboxyl groups in the filtersand HS compared to HS of the water used for recharge. Thus, chemisorption, which generally contributes to higher level of theses moieties in the sorbed HS, was not significant.

A change of pH affected both HS and filtersand properties. A decrease of pH should increase the positive charge of oxides, thereby making an electrostatic attraction with negatively charged HS molecules possible. However, at acidic pH HS become more hydrophobic, thus increasing the possibility for hydrophobic attraction. Since the filtersand contained low amounts of iron and clay, the strong positive effect of sorption (Fig. 4) that was due to a lowering of pH, was perhaps also due to a hydrophobic effect rather than an increase of electrostatic attraction. Therefore, the sorption may be dominated not so much by the forces between the filtersand surface and HS, but rather by the intermolecular association forces between HS and its incompatibility with water.

However, we observed that background organic matter also plays an important role. HS is better attracted to organic rich sediments than to filtersand, which had a relatively lower amount of OC. Elemental analyses revealed that filtersand showed significantly lower H/O ratio compared to sediments, Table 1. The H/O ratio indicates the humification degree of HS (De Paolis and Kukkonen, 1997). Thus, the higher ratio indicated that HS in sediments were more humified than the filtersand HS, which apparently also increases the ability to remove HS from water.

From this discussion, we conclude that due to low amounts of binding sites in filtersand, a hydrophobic fraction such as HA is preferably removed over more acidic fraction such as FA. This may lead to a fractionation of HS during ARG where large hydrophobic and aromatic molecules are removed in the earlier in the process, though FA are conserved unless the aquifer is rich with iron oxides or clay. The conclusion reached from this laboratory scale study is consistent with earlier findings from full-scale studies presented by McCarthy et al. (1993) and Schwarzenbach et al. (1983).

The aromatic fractions of HS are a major source of by-products and colour, thus ARG decreases the risk of carcinogenicity and improves the aesthetic quality of surface water. If filtersand, through which ARG is accomplished, is poor in iron oxide minerals or clay, the removal of FA would be low even after long infiltration distances.

5. Conclusion

Sorption experiments showed that clay content increased whereas organic matter decreased the sorption of HS to filtersand at ARG. Organic matter was released because it was not chemically bound and completely stabilized. More aromatic, hydrophobic fractions of HS (HA) were to a higher extend sorbed than acidic fractions of HS (FA). Chemisorption appears to be less important than physical sorption (hydrophobic sorption enhanced by polyelectrolytic effect) for HS removal during ARG.

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References

APHA, 1988. Standard methods for analysis waters and wastewaters. APHA, NY.

Burba, P., van den Bergh, J., Klockow, D., 2001. On-site characterization of humic-rich hydrocolloids and their metal loading by means of mobile size-fractionation and exchange techniques. Fresenius J. Anal. Chem. 371 (5), 660–669.

Camper, A.K., 2002. Involvement of humic substances in regrowth. Presented at the NSF International World Health Organization Symposium on HPC Bacteria in Drinking Water, April 22–24, 2002, Geneva, Switzerland.

Chin, Y.-P., Aiken, G., O'Loughlin, E., 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ. Sci. Technol. 28 (11), 1853–1858.

Day, G.M., Hart, B.T., McKelvie, I.D., Beckett, R., 1994. Adsorption of natural organic matter onto goethite. Colloid Surf. A 89 (1), 1–13.

De Paolis, F., Kukkonen, J., 1997. Binding of organic pollutants to humic and fulvic acids: influence of pH and the structure of humic material. Chemosphere 34 (8), 1693–1704.

Edwards, M., Benjamin, M.M., Ryan, J.N., 1996. Role of organic acidity in sorption of natural organic matter (NOM) to oxide surfaces. Colloids Surf. A 107, 297–307.

Ellis, B.D., Butterfield, P., Jones, W.L., McFeters, G.A., Camper, A.K., 2000. Effects of carbon source, carbon concentration, and chlorination on growth related parameters of heterotrophic biofilm bacteria. Microb. Ecol. 38, 330–347.

Evanko, C.R., Dzombak, D.A., 1999. Surface complexation modelling of organic acids sorption to goethite. J. Colloid Interface Sci. 214 (2), 189–206.

Gu, B., Schmitt, J., Chen, Z., Liang, L., McCarty, J.F., 1994. Adsorption and desorption of natural organic matter on iron oxide: mechanisms and models. Environ. Sci. Technol. 28 (1), 38–46.

Huisman, L., Olsthoorn, T.N., 1983. Artificial Groundwater Recharges. Pitman Books Limited, Boston.

Jacks, G., Frycklund, C., 1995. Removal of organic matter during groundwater formation. Vatten 51 (1), 66–69.

Jardine, P.M., Weber, N.L., McCarty, F.M., 1989. Mechanisms of dissolved organic carbon adsorption on soil. Soil. Sci. Soc. Am. J. 53, 1378–1385.

Jones, M.N., Bryan, N.D., 1998. Colloidal properties of humic substances. Adv. Colloid. Interface Sci. 78 (1), 1–48.

Kaiser, K., Zech, W., 1997. Competitive sorption of dissolved organic matter to soils and related mineral phases. Soil. Sci. Soc. Am. J. 61, 64–69.

Klavins, M., Cinis, U., 1990. Isolation of humic substances from surface waters. Izv. AN Latv. SSR, Khim. Ser. 3, 360–364 (in Russian).

Lai, C.H., Chen, C.Y., 2001. Removal of metal ions and humic acids from water by iron-coated filter media. Chemosphere 44 (8), 1177–1184.

Lattimer, L.V., Jones, V.P., 1992. Sorption of cadmium and copper onto iron oxides. Inorg. Chem. 23, 326–332.

McCarthy, J.F., Williams, T.M., Liang, L., Jardine, P., Jolley, L.W., Taylor, D.L., Palumo, A.V., Cooper, L.W., 1993. Mobility of natural organic matter in a sandy aquifer. Environ. Sci. Technol. 27 (4), 667–676.

Meier, M., Namjesnik-Dejanovic, K., Maurice, P.A., Chin, Y.-P., Aiken, G.R., 1999. Fractionation of aquitic natural organic matter upon sorption to goethite and kaolinite. Chem. Geol. 157 (3–4), 275–284.

Methods of Soil Analysis, 1989. American Society of Agronomy Inc. Publisher, Madison, Wisconsin.

Nodvin, S.C., Driscoll, C.T., Likens, G.E., 1986. Simple partitioning of anions and dissolved organic carbon in a forest soil. Soil Sci. 142 (1), 27–35.

Rook, J.J., 1974. Formation of haloforms during chlorination of natural waters. J. Water Treat. Exam. 23, 234–243.

Shen, Y.-H., 1999. Sorption of natural dissolved organic matter on soil. Chemosphere 38 (7), 1505–1515.

Spark, M.K., Wells, J.D., Johnson, B.B., 1997. Characteristics of the sorption of humic acids by soil minerals. Aust. J. Soil. Res. 35, 103–112.

Schwarzenbach, R.P., Giger, W., Hoehn, E., Schneider, J.K., 1983. Behaviour of organic compounds during infiltration of river water to groundwater. Field studied. Environ. Sci. Technol. 17 (8), 472–479.

Stumm, W., 1992. Chemistry of the Solid–Water Interfaces. John Wiley & Sons. Inc., USA.

Thurman, E.M., 1985. Organic geochemistry of natural waters. Martinus Nijhoff, Dr. W. Junk Publishers, Wageningen.

Thurman, E.M., Malcolm, R.L., 1981. Preparative isolation of aquatic humic substances. Environ. Sci. Technol. 15 (2), 463–466.

Tipping, E., 1981. The adsorption of aquatic humic substances by iron oxide. Geochim. Cosmochim. Acta 45, 191–199.

Tombácz, E., Dobos, Á., Szekeres, M., Narres, H.D., Klumpp, E., Dékány, I., 2000. Effect of pH and ion strength on the interaction of humic acid with aluminium oxide. Colloid. Polym. Sci. 278 (4), 337–345.

Vermeer, A.W.P., Riemsdijk, W.H., Koopal, L.K., 1998. Adsorption of humic acid to mineral particles. 1. Specific and electrostatic interactions. Langmuir 14 (10), 2810–2819.

V

Comparative Study for Separation of Aquatic Humic-Type Organic Constituents by DAX-8, PVP and DEAE Sorbing Solids and Tangential Ultrafiltration: Elemental Composition, Size-Exclusion Chromatography, UV–vis and FT-IR

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Comparative study for separation of aquatic humic-type organic constituents by DAX-8, PVP and DEAE sorbing solids and tangential ultrafiltration: elemental composition, size-exclusion chromatography, UV–vis and FT-IR

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Abstract

Aquatic humic-type solutes were separated in parallel from the same fresh water source by four different procedures: non-ionic polymethyl methacrylate (DAX-8) and functional cross-linked polyvinylpyrrolidone (PVP) resins, functional diethylaminoethyl cellulose (DEAE) and tangential ultrafiltration completed with a weakly basic anion exchange resin (IRA-67). The similarity–dissimilarity between the quantities and qualities of the different humic samples is discussed, especially in the light of the original dissolved organic matter (DOM). During the past two decades, a significant progress has occurred in the aquatic humic research due to the so-called hydrophobic–hydrophilic properties possessed by certain non-ionic sorbing solids. As a result of many coincidences, it may be justifiable to examine critically the prevailing isolation techniques of aquatic humic solutes and to try to update their complicated definitions. For that reason, it is reasonable to summarize the leading principles of different isolation techniques in Section 1 of this article. The results of the present study strongly support the applicability of the PVP resin, alone or completed in sequence with a suitable non-ionic sorbing solid, for isolation of aquatic humic-type solutes from both quantitative and qualitative points of view. In certain cases, the DEAE cellulose gives a useful alternative for conventional sorbing solids in the isolation of the bulk of aquatic humic solutes. The base-catalyzed ester hydrolysis of the HM during the chromatographic isolation of the DOM seems to be relative minor.

Keywords: Humic substances; DOM; DAX-8; PVP; DEAE; SEC; FT-IR

1. Introduction

The importance of the macromolecular, unidentifiable and very complicated heterogenous mixture of natural organic matter (NOM) in all environmental systems can be considered as a consensus of opinion. These scattered mosaic-like organic constituents are most frequently extracted from the solid NOM with aqueous bases (e.g. [1]). These extracts of so-called humic substances are further partitioned into functional humic (HA) and fulvic (FA) acids based on their solubilities in aqueous acids and bases. This humic–fulvic acid fractionation at strongly acidic conditions (pH ≈ 1) is believed [2] to be useful and meaningful for characterization and separation of humic substances. The nomenclature and definition of these humic substances are not easy-to-understand and they have features of scientifically hair-splitting [3]. The common denominator in the humus chemistry is that all classifications and definitions of humic substances, regardless of the nature of the sample (dissolved or solid), are only operational based on the procedure used for their isolation and no ideal system is available that would satisfy each scientist.

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The carbon cycling in aquatic ecosystems is extremely complicated and the origin of aquatic humic solutes can be dated back to many complex interacting sources (e.g. [4]). The formation of aquatic humic solutes occurs as the result of several processes in the aquatic environment and it is a dynamic process with no unidirectional vector, as has been pointed out in the literature (e.g. [5]). In general, the dissolved organic matter (DOM) in natural water is classified [6] roughly into two groups: (i) non-humic solutes, consisting of compounds belonging to the well-known classes of organic substances such as amino acids, hydrocarbons, carbohydrates, fats, waxes, resins, low-molecular acids, etc. and (ii) very complicated heterogenous humic solutes. These two groups are not completely, neither physically nor chemically, distinguishable from each other, because some natural non-humic solutes, such as carbohydrates, can be an integral part in the structural composition constructing humic solutes.

Nevertheless, the phrase of aquatic humus is very popular in water chemistry, this term is as indefinite as that of the humic substances, and it remains open what is dealt with: DOM, humic solutes in full without partition or something else. The use of specific terms of fulvic and humic acids is based on an assumption that they represent real entities of organic constituents. On the contrary, the abbreviation of humic matter (HM) refers to the generic term of all humic solutes regardless of certain specific isolation–fractionation procedure. The HM, which may, at best, account for as much as 90% of the DOM, has an essential role in the carbon cycle of the dissolved organic carbon (DOC). The ability of the HM, e.g. to inactivate various pesticides and other organic pollutants via complexation–copolymerization [7], to influence transport processes of organic and inorganic pollutants [8], to lower bio-availability of harmful heavy metals via complexation [9], to act as precursors for the formation of several mutagenic organic chemicals during chlorine treatment of natural waters [10] in addition to the structural chemistry, especially in the light of its environmental impact (e.g. [11]), have led to world-wide interest in research of this natural organic material.

The modern humus chemistry has progressed strongly recently, thanks to modern analytical techniques. However, the major problem in the aquatic humus chemistry is still how to separate the HM selectively from other organic and inorganic solutes for obtaining a representative sample. Because of the dilute solutions of the aquatic NOMs, they must be concentrated for further studies. Several techniques, with their advantages and disadvantages, for concentrating and also simultaneously for isolating the aquatic HM from the DOM are available [12], including freeze-drying, chemical precipitation, solvent extraction, reverse osmosis, ultrafiltration and adsorption to solids.

The most frequently applied procedures for simultaneous concentration and fractionation of aquatic humic solutes from most other dissolved constituents are at present the column chromatographic methods by non-ionic sorbing solids (such as XAD resins or analogues). Unfortunately, the manufacture of the reliable XAD-8 resin (Amberlite®) was ceased some years ago. It has been reported [13,14] that XAD-8 resin possibly can be substituted by Supelite™ DAX-8 resin. The potential of the DAX-8 resin as a research tool in the humic sciences has been also tested using several analyses of structural fine-chemistry [15–17]. Despite some promising attempts, it seems, however, to be troublesome and uncertain to substitute the workable XAD-8 resin for a comparable one. In the light of this problem, it may be justifiable to try to adopt a more physical definition for humic-type constituents, not solely based on some fictional hydrophobic–hydrophilic interactions at certain acidity but more real and distinctive functionalities.

The leading principle in using non-ionic sorbing solids is that the method classifies organic solutes in a water sample at preadjusted acidity (pH ≈ 2) into fictional hydrophobic and hydrophilic fractions (in fact, to be dissolved, the original DOM at natural acidities must in reality be quite hydrophilic anyhow). According to certain preadjusted hydrophobic–hydrophilic interactions between organic solutes and the non-ionic sorbing solid, the relatively most hydrophobic macromolecular organic acids (humic substances [18]) are retained onto the adsorbent. This primary organic fraction is most frequently partitioned at strongly acidic conditions (pH ≈ 1) into humic- and fulvic-type acids. The background of the multi-stage non-ionic sorbing solid technique is thoroughly reported and discussed in the literature [18,19]. It has been underlined [20,21] that the utilization of this technique alone may include certain risks for uncontrolled fractionation, reactions and conclusions.

The most peculiar characteristic for different kinds of humic-type constituents is the occurrence of acidic functional (mainly carboxylic) groups which render them into polyelectrolytes. This quality permits the isolation of practically all humic-type solutes in one step from water by certain anion exchange resins. The most popular material for this purpose has been the DEAE cellulose (Sigma, [25249-54-1]) which is a weak anion exchanger with tertiary amine functional groups bound to a hydrophilic matrix ($-OC_2H_4N(C_2H_5)_2$). The quantities of ionized organic humic solutes isolated with this procedure have generally been relatively high (about 80% to nearly 100% of the DOM) in fresh water as compared to the so-called XAD technique [22–27]. The optimum recovery of organic acids of the DOC occurs between pH 4 and 6, and it is possible to isolate almost all organic acids from a fresh water sample without any pH adjustments. On the other hand, it has been reported [28] by studying aquatic marine HM that even the relatively low salinity of, e.g. brackish water will decrease the retention of acidic solutes, especially those with lower molecular sizes, onto the DEAE cellulose. The utilization of anion exchangers in the humus chemistry has been discussed more closely in the literature (e.g. [19]).

Other peculiar characteristics of humic-type organic constituents are their relatively high content of phenolic functional groups, in addition to those of acidic groups, and the abundance of aromatic C=C moieties. These properties permit the utilization of the cross-linked PVP for the fractionation of the DOM into humic-type constituents and other organic residues (primarily comprising carbohydrates, proteins, amino acids and uronic acids) under acidic conditions (pH ≈ 2). Although PVP in its insoluble form has been used in several fields of research, including also the HM beginning in 1968 [29], its utilization in the humus chemistry is lesser-known. However, this does not prove the unsuitability of PVP resins for the isolation–fractionation of the HM [30–35]. The PVP procedure is similar to that of non-ionic sorbing solids, concerning the acidity of the original water, which must first be acidified to about pH 2. The PVP resin forms strong hydrogen bonds with phenolic, hydroxyl and carboxyl groups of the DOM (e.g. [33]), while non-ionic macroporous copolymers classify at a given preadjusted acidity organic humic solutes in a water sample according to their relative hydrophobic–hydrophilic interactions between the surface of the sorbent bed.

Tangential-flow membrane-ultrafiltration provides a method for concentrating the original DOM according to its molecular size. In addition, this procedure (continuous operation) will minimize (e.g. [36,37]), as recently verified [38–40], a number of problems connected (e.g. [41–43]) with the bath operation (dead-ended) even though a slight fouling of the membranes cannot be avoided (e.g. [44]), and large volumes of water can be easily processed for obtaining gram quantities of DOM concentrates with different molecular sizes, e.g. for freeze-drying.

Molecular weight-size distributions are essential properties for estimating physical and chemical characteristics of dissolved humic-type organic constituents. The most extensively used technique for this purpose is apparently the high-performance size-exclusion chromatography (HPSEC) by UV-detection. In all, the UV–vis spectroscopy has its own function in the study of aquatic organic solutes, e.g. UV 254 nm is the most commonly utilized wavelength for monitoring water quality changes, but the absorptivity of smaller molecular size fractions at this wavelength is somewhat reduced. In summary, determining molecular sizes of DOM by different HPSEC applications is not a simple task as reported and reviewed previously (e.g. [39,45]). Worth of considering in this context, is the view stressed in literature that a chromatographic column of the TSK G3000SW-type and 10 mmol sodium acetate buffer at pH 7 as the eluent is the only system being able to separate efficiently different molecular size fractions of the aquatic very heterogenous NOM [46–48].

In the present study, three different sorbing solids (namely DAX, PVP and DEAE) and tangential ultrafiltration (UF, 1 kDa of nominal molecular size, NMW, cutoff), followed with XAD-8/2 (65/35 (w/w)), weakly basic anion (IRA-67) and strongly acidic cation (Dowex 50W x-8) exchange resins, were applied for isolation–fractionation of humic-type organic solutes from the same lake water sample. Several humic isolates separated previously by XAD-8 and DEAE resins from different fresh water sources were utilized as reference samples. Principal basic analyses (molecular size distribution, elemental organic analyses, UV–vis and Fourier-transform infrared (FT-IR) characteristics) were performed to illustrate the general ability of different sorbing solids in isolating humic constituents in comparison with the original DOM.

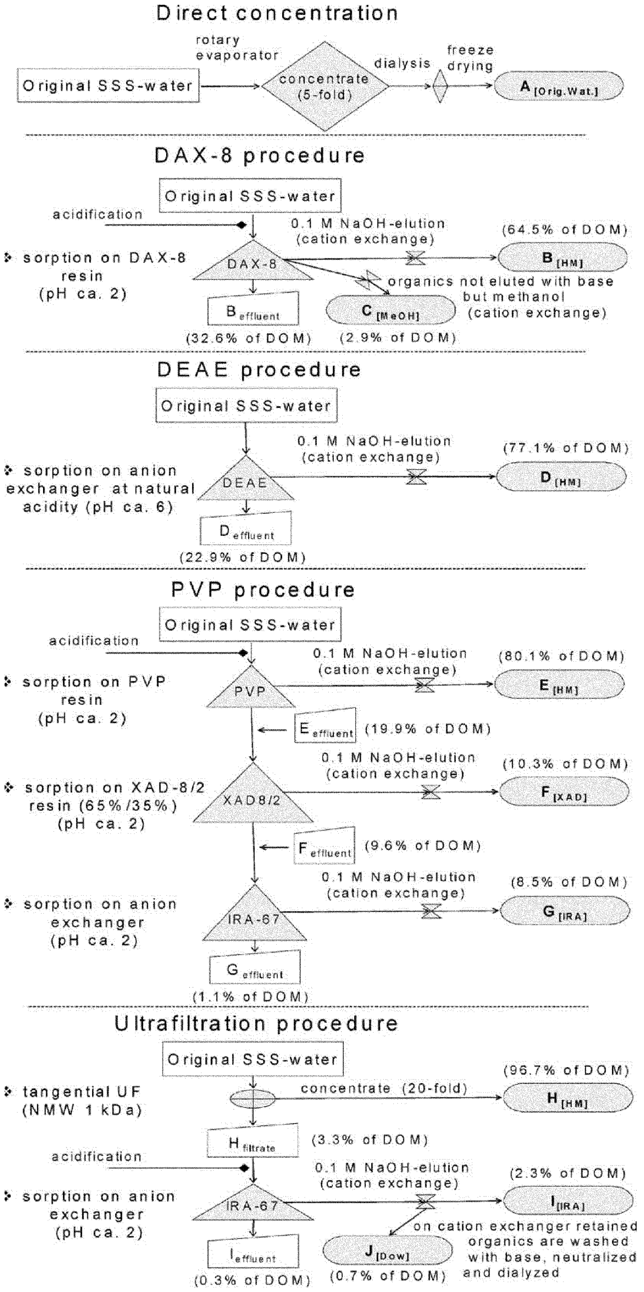
2. Experimental

2.1. Origin and isolation of samples

A natural fresh water sample was collected from Lake Savojärvi (SSS) situated in a marshy region in the south-western part of Finland, in autumn 2002. Lake Savojärvi has very brown water (colour as cobalt–platinum units about 150 mg Pt l^{-1}; DOC 20 mg C l^{-1}; conductivity at 25 °C, about 6 mS m^{-1} and pH 5.8) [39]. The SSS-water sample (about 150 l) was collected 1 m below the surface into glass containers. The original water sample was at first prefiltrated (0.2 μm, Nuclepore polycarbonate filter cartridge, no. 611101) directly after sampling and, thereafter, stored in hermetic containers in the dark at 4 °C during the analysis and isolation procedures. The International Humic Substances Society reference samples of Nordic aquatic fulvic acid (No. FA, code IR105F) and humic acid (No. HA, code IR105H) were isolated by the conventional XAD-8 technique in summer 1986 from the runoff water (colour about 200 mg Pt l^{-1} and DOC 20 mg C l^{-1}) of a Norwegian mire (symbols R8 and R9 in Table 1, respectively). The reference samples R1–R7 were isolated in 1994 from Lake Savojärvi (SS, [26]) by an enlarged XAD technique, XAD-8 → cation exchanger → weakly basic anion exchanger, and in 1989, by the conventional XAD-8 technique from Lake Mekkojärvi (M3, [38]), also a highly coloured lake (colour about 200 mg Pt l^{-1} and DOC about 22 mg C l^{-1}, [39]).

The different analytical procedures applied in this study for isolation and fractionation of the DOM are shown in Scheme 1. A part of the prefiltrated original SSS-water (5 l) was first directly concentrated with a rotary evaporator to 1 l and then dialysed (from about 1.3 mS cm^{-1} to 70 μS cm^{-1}, 20 °C) with Spectra/Por 6 dialyse tube (1 kDa of NMW cutoff) against distilled water and finally freeze-dried (fraction $A_{[Orig.Wat.]}$), in order to obtain a point of reference for comparisons.

The chromatographic isolation methods of HMs by DAX-8 resin and DEAE cellulose in Scheme 1 have been thoroughly reported previously [15–19,26,27]. The organic constituents not eluted from the DAX-8 resin with base (so-called [18] hydrophobic neutrals being relatively too hydrophobic to be eluted with base) were eluted with methanol and labelled as [MeOH] (fraction $C_{[MeOH]}$). It is notable that the alkaline extracts of the primary HM solutes (hydrophobic humic substances) eluted from the DAX-8 (fraction $B_{[HM]}$) and DEAE (fraction $D_{[HM]}$) sorbents were not, in the present study, further divided at strongly acidic



Scheme 1. Analytical procedures for classification of the DOM into different humic-type fractions. For symbols of samples, see Table 1.

Table 1
Major elemental organic analyses and some compositional characteristics for the different humic-type isolates obtained by several isolation procedures[a]

Method[b]	Symbol[b]	Sample[b]	Ash (%)	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\in$[c]	E_2/E_3[d]	Elemental analysis (%)					Atomic ratios				Average molecular formula: $C_vH_wN_xO_yS_z$						
								C	H	N	S	O	H/C	O/C	N/C	S/C	C_v	H_w	N_x[e]	O_y	S_z[e]	ϕ_{total}[f]	Ar. (%)[g]
F.D.	$A_{[\text{Orig.Wat.}]}$	$\text{SSS}_{\text{concentrate}}$	2.6	2750	4.20	331	4.75	51.1	6.4	2.3	1.7	38.4	1.494	0.564	0.038	0.013	117	175	4	66	1	12	22
DAX-8	$B_{[\text{HM}]}$	$\text{SSS}_{\text{isolate}}$	3.1	2630	3.70	325	4.77	53.1	4.7	1.7	0.8	39.7	1.064	0.561	0.028	0.005	116	124	3	65	*1.6*	22	22
DAX-8	$C_{[\text{MeOH}]}$	$\text{SSS}_{\text{isolate}}$	3.4	760	8.23	145	6.20	56.2	6.9	1.6	0.7	34.7	1.455	0.463	0.025	0.004	36	52	*1.1*	16	*6.5*	15	11
DAX-8	B_{effluent}	$\text{SSS}_{\text{effluent}}$		950	4.01	182	5.85																13
DEAE	$D_{[\text{HM}]}$	$\text{SSS}_{\text{isolate}}$	1.9	2740	4.43	329	4.74	53.1	4.3	1.0	0.9	40.8	0.954	0.577	0.017	0.006	121	116	2	70	*1.4*	24	22
DEAE	D_{effluent}	$\text{SSS}_{\text{effluent}}$		370	3.81	35	6.88																5
PVP	$E_{[\text{HM}]}$	$\text{SSS}_{\text{isolate}}$	3.1	2570	3.36	320	4.83	52.1	4.7	1.2	1.3	40.6	1.076	0.585	0.020	0.009	112	120	2	65	1	21	21
PVP	E_{effluent}	$\text{SSS}_{\text{effluent}}$		1260	2.62	220	5.56																16
PVP	$F_{[\text{XAD}]}$	$\text{SSS}_{\text{isolate(XAD8/2)}}$	2.3	1350	4.52	227	5.51	55.4	5.4	1.7	1.1	36.4	1.171	0.494	0.026	0.007	62	73	2	31	*2.2*	20	16
PVP	F_{effluent}	$\text{SSS}_{\text{effluent(XAD8/2)}}$		930	3.22	175	5.88																13
PVP	$G_{[\text{IRA}]}$	$\text{SSS}_{\text{isolate(IRA-67)}}$	3.8	560	3.71	105	6.44	37.4	2.9	1.7	1.4	56.7	0.915	1.138	0.039	0.014	17	16	*1.5*	20	*4.1*	19	9
PVP	G_{effluent}	$\text{SSS}_{\text{effluent(IRA-67)}}$		320	3.42	30	6.86																5
UF	$H_{[\text{HM}]}$	$\text{SSS}_{\text{concentrate}}$	2.2	2860	4.04	336	4.69	51.7	5.8	2.4	1.6	38.5	1.337	0.559	0.040	0.012	123	165	5	69	1	15	22
UF	H_{filtrate}	$\text{SSS}_{\text{filtrate}}$		970	2.30	182	5.85																13
UF	$I_{[\text{IRA}]}$	$\text{SSS}_{\text{isolate(IRA-67)}}$	3.7	660	3.05	126	6.26	36.0	2.7	1.8	1.2	58.4	0.888	1.219	0.042	0.012	20	18	*1.2*	24	*4.2*	19	10
UF	$J_{[\text{Dow}]}$	$\text{SSS}_{\text{isolate(Dowex)}}$	3.6	770	8.04	152	6.15	44.5	6.4	4.6	2.7	41.9	1.703	0.706	0.088	0.023	29	49	3	20	*1.5*	8	12
UF	I_{effluent}	$\text{SSS}_{\text{effluent(IRA-67)}}$		320	3.71	21	6.90																4
DEAE	$R1_{[\text{HM}]}$	SS.[DEAE]	2.2	2840	2.43	354	4.79	53.9	4.2	1.0	0.7	40.2	0.920	0.560	0.016	0.005	127	117	2	71	*1.6*	25	23
XAD-8	$R2_{[\text{HM}]}$	SS.FA	2.4	2232	2.61	332	4.58	54.9	4.4	0.7	0.7	39.5	0.944	0.540	0.010	0.004	102	96	1	55	*2.2*	25	22
XAD-8	$R3_{[\text{HM}]}$	SS.HA	3.1	4451	4.98	407	3.51	55.9	4.2	1.7	0.9	37.3	0.890	0.501	0.026	0.006	207	185	5	104	1	27	26
XAD-8	$R4_{[\text{MeOH}]}$	SS.[MeOH]	3.9	654	4.72	152	6.18	56.3	6.7	1.4	1.1	34.6	1.413	0.462	0.021	0.007	31	43	*1.5*	14	*4.5*	16	12
XAD-8	$R5_{[\text{IRA}]}$	SS.[IRA]	2.1	660	4.99	246	6.41	45.0	1.9	0.4	0.5	52.2	0.505	0.870	0.008	0.004	25	13	*5.1*	22	*10.8*	30	11
XAD-8	$R6_{[\text{HM}]}$	M3.FA	3.8	4475	2.37	356	4.33	56.9	4.3	0.6	1.0	37.2	0.901	0.490	0.009	0.007	212	191	2	104	1	26	23
XAD-8	$R7_{[\text{HM}]}$	M3.HA	5.8	6508	5.28	452	3.70	59.0	4.6	1.2	1.1	34.1	0.925	0.435	0.018	0.007	320	296	6	139	2	27	29
XAD-8	$R8_{[\text{HM}]}$	No.FA	1.3	4750	1.56	410	4.08	53.1	4.6	0.8	0.8	40.7	1.032	0.575	0.013	0.005	210	217	3	121	1	22	26
XAD-8	$R9_{[\text{HM}]}$	No.HA	2.2	6204	3.77	466	3.47	54.6	4.5	1.0	0.9	39.0	0.988	0.536	0.016	0.006	282	279	5	151	2	24	30

[a] Elemental composition is given on ash- and moisture-free basis.

[b] Different [-HM] fractions obtained from the SSS-water were not divided into so-called humic- (HA) and fulvic- (FA) acid subfractions, thus representing their combined mixtures; F.D., freeze-drying; R1–9, reference samples; for other symbols, see Scheme 1.

[c] $\in$, molar absorptivity at 280 nm (1 mol^{-1} cm^{-1} of OC).

[d] E_2/E_3, quotient of absorbances at 250 and 365 nm.

[e] The decimal numbers in italics indicate frequency of occurrence, i.e. one per stated number of molecules with a given molecular weight.

[f] ϕ_{total}, estimated total amount of unsaturation in mmol g^{-1}.

[g] Ar. (%), estimated aromaticity.

conditions into functional HA- and FA-type fractions. The amounts of the original SSS-water used for the DAX-8 and DEAE procedures were 10.0 and 44.9 l, respectively. All cation exchanged concentrates $B_{[HM]}$ (1.00 l), $C_{[MeOH]}$ (0.25 l) and $D_{[HM]}$ (1.50 l) were finally freeze-dried.

As shown in Scheme 1, the PVP (Sigma [25249-54-1]) resin was first washed with distilled water, dilute NaOH, 10% HCl, distilled water until free of Cl^-, acetone, distilled water, and thereafter, suspended in 0.01 M HCl (pH 2). Pretreated slurry of PVP resin (160 g dry weight) was placed in a glass bottle (10 l) and the original SSS-water samples (7–9 l per an isolation cycle, in all, 45.0 l), acidified (HCl) to pH 2, were added and the mixture was stirred for 16 h (batch method). The suspension was filtered and the resin slurry washed with distilled water before eluting (0.1 M NaOH) the retained humic-type organics (fraction $E_{[HM]}$). The combined acidic (pH 2) filtrates ($E_{effluent}$, in all, 40.0 l) were further treated by the bath method, first with XAD-8/2 resin (fraction $F_{[XAD]}$) and next with IRA-67 anion exchanger (fraction $G_{[IRA]}$) for obtaining the rest of humic-type macromolecular organic acids. All cation exchanged concentrates $E_{[HM]}$ (4.43 l), $F_{[XAD]}$ (0.21 l) and $G_{[IRA]}$ (0.60 l) were finally freeze-dried.

The UF procedure in Scheme 1 was carried out using a tangential-flow membrane-filtration system (4 GPM Pellicon Cassete System by Millipore). The original SSS-water sample (51.16 l) was concentrated (concentration degree, 20; the consistency of the molecular size distribution with that of original water was verified by HPSEC during the concentration process) by a membrane of 1 kDa NMW (filter code: PCAC, cellulosic material by Millipore). The dialysed (from about 290 to 85 μS cm^{-1}) UF concentrate $H_{[HM]}$ (1.00 l) was freeze-dried for obtaining solid HM/DOM for analyses. The UF filtrate ($H_{filtrate}$, 46.65 l) was further acidified (HCl) to pH 2 and treated with weakly basic anion exchanger (IRA-67) followed with cation exchanger for characterizing the nature of remained humic-type solutes with NMW of $\leq$1 kDa. The basic extract from the IRA-67 resin was cation exchanged for obtaining the actual macromolecular organic acids/humic-type constituents of smaller molecular sizes (fraction $I_{[IRA]}$). Onto the cation exchanger retained various organic constituents (fraction $J_{[Dow]}$) were eluted with NaOH solution. The basic extract of the fraction $J_{[Dow]}$ was neutralized to pH 7 with HCl and the salt (NaCl) formed was removed by dialysis. The purified concentrates $I_{[IRA]}$ (0.76 l) and $J_{[Dow]}$ (0.87 l) were finally freeze-dried.

2.2. *Chemical, chromatographic and spectroscopic analyses*

The determination of the moisture and ash contents of the freeze-dried HMs as well as their elemental compositions (carbon, hydrogen, nitrogen and sulphur; the content of oxygen was taken as a difference from 100%) has been discussed previously [15]. Table 1 shows the results of the elemental organic analyses on ash- and moisture-free basis for the different humic isolates.

The number- ($\bar{M}_n$) and weight-averaged ($\bar{M}_w$) molecular weights before freeze-drying the different HM-type concentrates (diluted about 10-fold with the sodium acetate eluent solution) were determined by HPSEC using a silica-based TSK G3000SW column (7.5 mm × 300 mm with a 7.5 mm × 75 mm precolumn) and 10 mmol sodium acetate solution (pH was adjusted to 7.0 with acetic acid) as an eluent at 20 °C [39,46–48]. The flow rate of the eluent was 0.80 ml min^{-1} and the injection volume, 80 μl. The pumping system was an L-6200A Intelligent Pump (Merck Hitachi) and the eluted solute was detected at 254 nm (L-4250 UV–vis Detector, Merck Hitachi). The void (V_o, 6.67 ml) and total permeation ($V_o + V_i$, 15.98 ml) volumes were determined using Blue Dextran 2000 and acetone, respectively; the total volume (V_t) of the gel bed was 16.57 ml. Elution parameters were calculated as a distribution coefficient [39]: $k'_D = (V_e - V_o)/((V_o + V_i) - V_o)$, where V_e, elution volume of the solute. Averaged $\bar{M}_n$ and $\bar{M}_w$ values for the heterogenous humic mixtures were calculated by the equations: $\bar{M}_n = \Sigma n_i/\Sigma n_i/\mathrm{MW}_i$ and $\bar{M}_w = \Sigma n_i\mathrm{MW}_i/\Sigma n_i$, where n_i is the number/weight (in this case, relative response at 254 nm) of a solute i with the molecular weight MW_i. Distribution of MW_i values for sample solutes, at some eluted volume *i*, were estimated by the calibration equation presented previously [39] for this HPSEC column system. The maximum absorbance-scale for each SEC chromatogram in Fig. 1 is the same, thus making the results more comparable with each other. Relative UV_{254}-absorbances {(UV abs at 254 nm/OC in the original solution, mg l^{-1}) × 100} were calculated for the chromatograms, irrespective of the fact that the real OC concentration of the eluted sample was not exactly known but the injection volumes were constant, in order to bring the samples into the same line. The quotient $\bar{M}_w/\bar{M}_n$ is a coarse estimate for the degree of polydispersity of the mixture (the value 1 is given for homogenous polymers). The previous results obtained by different aromatic acids implied that the charge exclusion effect of the applied HPSEC system is quite insignificant, i.e. the charged aquatic humic solutes have a fair chance to permeate into the stationary phase pores while using a 10 mmol sodium acetate, at pH 7.0, as the eluent [39].

The UV–vis spectrophotometric analyses for the different HM-type concentrates (diluted about 10-fold with the sodium acetate eluent solution) were performed on a dual-beam Spectrometer Lamda 12 (Perkin-Elmer) for the calculation of the E_2/E_3-ratio (absorbances at 250 and 365 nm) and $\in$ (molar absorptivity at 280 nm, l mol^{-1} cm^{-1} of OC). Samples were placed in a 1 cm quartz window cuvette and scanned (60 nm min^{-1}; data interval, 0.20 nm) from 670 to 200 nm. The previously isolated reference samples have been also dissolved in corresponding sodium acetate eluent solution.

Fourier-transform infrared (FT-IR) spectra for different solid samples in Fig. 2 were collected in the transmission mode using a Galaxy 6030 FT-IR spectrophotometer (Matt-

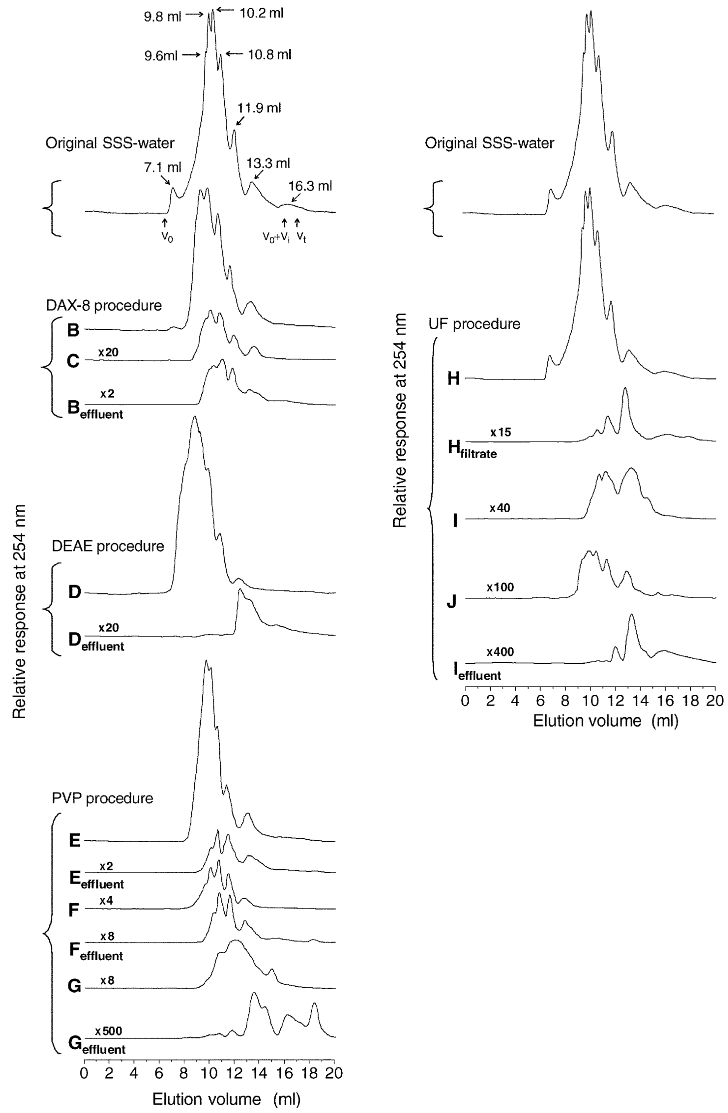


Fig. 1. Molecular size distributions for the organic solutes in the original water sample and different fractions obtained by several separation procedures. TSK G3000SW, 7.5 mm × 300 mm with a 7.5 mm × 75 mm guard column; 10 mmol sodium acetate; pH 7.0; 20 °C; sample loop, 80 μl; flow rate, 0.80 ml min^{-1}; $V_o = 6.67$; $V_o + V_i = 15.98$ and $V_t = 16.57$ ml. Some chromatograms are enlarged 2- to 500-fold for better visualizing. For symbols of samples, see Table 1.

son Instruments) equipped with a DTGS detector. The original spectral bandwidth was 4 cm^{-1}. About 1.5 mg of the freeze-dried sample (desiccator dried) and 200 mg of KBr powder (dried in an oven at 100 °C) were ground together and hydraulically pressed into a small pellet (−13 mm in diameter, −0.5 mm thick). The pelletized KBr samples were further dried overnight in a desiccator prior to analysis to minimize interferences from absorbed water. All samples were measured at a constant temperature (20 °C). The intensities of the recorded FT-IR spectra were standardized

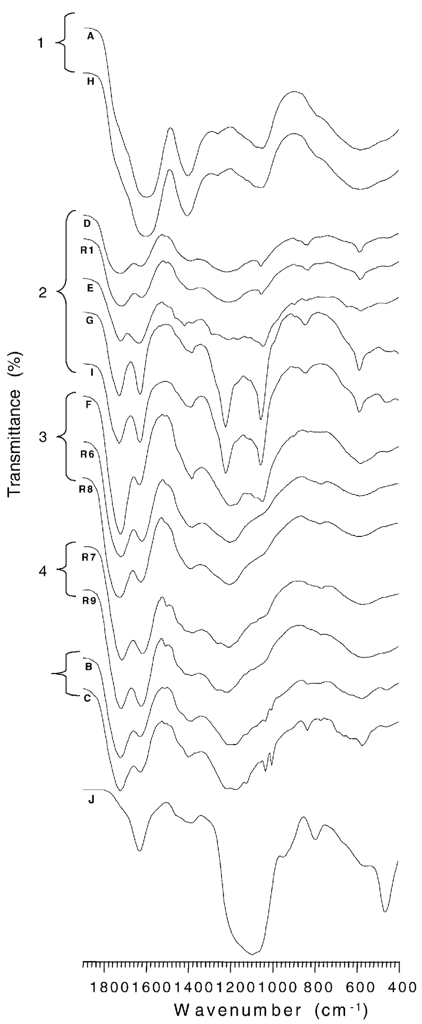


Fig. 2. FT-IR spectra in the zone 1900–400 cm^{-1} for the original water sample and different humic-type fractions obtained by several separation procedures. The numbers of the regrouped spectra point to the clusters in Fig. 4. For symbols of samples, see Table 1.

against the OM contents of the samples to reduce their weighing differences and ash contents for obtaining more semi-quantitative spectra. Although the FT-IR spectra were scanned between 4000 and 400 cm^{-1}, Fig. 2 shows only the region 1900–400 cm^{-1} where the major differences were observed. The transmittance-scale for each spectrum is the same thus making the results more comparable with each other.

3. Results and discussion

3.1. Effects of different isolation methods on the quantities of humic-type organic solutes, molecular size distributions of separated mixtures

Scheme 1 sums up the efficiency of a given isolation method (percent of the original DOM) in dividing original organic solutes into different specific subfractions. Likewise, Fig. 1 illustrates the molecular size distributions of obtained mixtures in relation to their quantities. The applied HPSEC system separated the heterogenous mixture of the original SSS-water sample into eight different molecular size groups, i.e. humps of the chromatogram at the elution volumes of about 7.1, 9.6, 9.8, 10.2, 10.8, 11.9, 13.3 and 16.3 ml corresponding to molecular sizes of about 115300, 11200, 9300, 6400, 3660, 1300, 360 and <100 Da, respectively. The exclusion volume of the last hump at 16.3 ml was clearly greater than the total permeation volume ($V_o + V_i$, 15.98 ml) indicating that some interactions occur between these small-molecular size constituents and the gel matrix.

3.1.1. DAX-8 procedure

The ability of the non-ionic DAX-8 resin (in Scheme 1) to divide the original DOM at pH 2 into so-called hydrophobic acids ($B_{[HM]}$, "humic substances"), hydrophobic neutrals ($C_{[MeOH]}$) and less hydrophobic effluent ($B_{effluent}$) was of the same magnitude as reported previously (e.g. [15–17,26]). The quantitatively significant and relatively more hydrophilic organic constituents remaining in the $B_{effluent}$ are occasionally further divided by weakly basic anion exchangers or certain suitable non-ionic sorbing solids into a so-called "transphilic" fraction (hydrophilic acids, about 14–25% of the fresh water DOC [19,26]) and hydrophilic neutrals. It has been recently [16] demonstrated that the substitute DAX-8 resin can, at best, isolate about 20% more hydrophobic HM-type organic constituents than the previous XAD-8 resin. However, it appears that certain failures occur in the manufacturing of the DAX-8 resin thus perverting the reliability of the isolation. This, among other thinks, speaks for arguments to test also other isolation techniques.

Fig. 1 indicates that the molecular size distribution of different humic-type constituents in fraction $B_{[HM]}$ resembles closely to that of the original SSS-water. This single HPSEC analysis verifies the previous statement, obtained [27,40] by complicated fine-structural analyses, that HM-type solutes obtained by the DAX–XAD procedure must play a role as certain definite entities in the original DOM and they cannot be merely accidental products of the isolation process. However, during the isolation procedure, some aggregation–rearrangement of certain constituents were taken place which is also visible from the decreased $\bar{M}_w/\bar{M}_n$ value in Table 1, i.e. the molecular size distribution became narrower and the fingerprint humps in the chromatogram shifted slightly towards higher molecular sizes. The shapes of the elution profiles in the chromatograms obtained for the more hydrophobic $\mathrm{C}_{[\mathrm{MeOH}]}$ fraction and transphilic effluent (B_{effluent}) are rather similar to those of the original SSS-water and the fraction $\mathrm{B}_{[\mathrm{HM}]}$. Most noteworthy is the continuous existence of the relatively high-molecular size constituents at the elution volumes of 9.8 and 10.8 ml. The loss of these constituents during the conventional single isolation procedures is a notable drawback because previous studies [49,50] prove that the organic constituents belonging to this, so-called non-humic, category contain qualitatively the same principal building blocks and the most powerful discriminating factor appears to be their relative content.

3.1.2. DEAE procedure

Scheme 1 proves the high retaining capacity of the DEAE cellulose for HM-type organic macromolecular acids (about 17% more HM, fraction $\mathrm{D}_{[\mathrm{HM}]}$, was obtained than with the DAX-8 procedure) analogously to previous studies [15,16,26]. It has been recently verified [11,26,27], by fine-structural analyses, that the integrated whole of macromolecular organic acids isolated by the DEAE technique resembles both quantitatively and qualitatively very closely to an average combination of the four different acidic fractions obtained by the multi-stage DAX–XAD procedure, i.e. so-called: (1) hydrophobic FA- and (2) HA-type acids, (3) hydrophobic neutral solutes ([MeOH]) and (4) hydrophilic acids (a transphilic fraction). This is an essential advantage from the structural chemistry point of view and shows that the DEAE isolate represents a clear-cut average HM for fresh waters. From a practical point of view, it should be emphasized that a complete back-elution of the adsorbed humic solutes from the DEAE cellulose is somewhat more difficult than in the case of DAX-8 resin or its homologues.

Fig. 1 proves that the same fingerprint information as shown in the chromatogram of the original SSS-water is partly retained in that of the fraction $\mathrm{D}_{[\mathrm{HM}]}$; namely, two humps at the elution volumes of about 9.8 and 10.8 ml and one more slightly after 11.9 ml (at 12.2 ml, equal to 990 Da). In contrast, the maximum elution of organic solutes appears now, as early as, at about 8.7 ml (equal to 26,000 Da). This discrepancy shows that certain rearrangements take place among the macromolecular constituents during the isolation procedure. However, the averaged $\bar{M}_n$ and $\bar{M}_w/\bar{M}_n$ values in Table 1 speak for that these compositional changes are not, in reality, so drastic as compared to the situation predominating in the original water. On the other hand, the effluent of the DEAE column ($\mathrm{D}_{\mathrm{effluent}}$) was mainly composed of organic constituents with relative small molecular sizes, about 87% of organics were eluted after 12.2 ml contributing to the benefits of the DEAE technique.

3.1.3. PVP procedure

Scheme 1 demonstrates that the ability of the PVP resin to retain HM-type solutes at pH 2 (fraction $\mathrm{E}_{[\mathrm{HM}]}$), even by a simple batch technique, was particularly effective corresponding to the results of Chen et al. [34,35]. The content of organic solutes in the $\mathrm{E}_{\mathrm{effluent}}$ was of the same order of magnitude (19–23% of the original DOM) than in the case of the DEAE procedure. The DAX–XAD technique (in this case, XAD-8/2 resin) was able to isolate a significant part (ca. 52%) of organic solutes from the $\mathrm{E}_{\mathrm{effluent}}$ as HM-type constituents (fraction $\mathrm{F}_{[\mathrm{XAD}]}$). The weakly basic IRA-67 anion exchanger further retained from the $\mathrm{F}_{\mathrm{effluent}}$ very effectively (ca. 89%) certain kinds of macromolecular HM-type acids (fraction $\mathrm{G}_{[\mathrm{IRA}]}$, which corresponds, according to the XAD terminology, to a so-called transphilic fraction). The final content of organic solutes in the last $\mathrm{G}_{\mathrm{effluent}}$ was comparatively low (ca. 1% of the original DOM).

Fig. 1 confirms that PVP is a workable resin for retaining HM-type constituents, which are found in the original water. Three elution humps of the fraction $\mathrm{E}_{[\mathrm{HM}]}$ positioned at about 9.8, 10.2 and 10.8 ml and two humps at 11.3 and 13.0 ml (2300 and 470 Da, respectively). However, the distinct high-molecular size hump at the elution volume of about 7.1 ml (115,300 Da), which was visible in the chromatogram of the original SSS-water, was now missing. Likewise, the molecular size distribution was narrower for the fraction $\mathrm{E}_{[\mathrm{HM}]}$ than that obtained for the original SSS-water (cf. $\bar{M}_n$ and $\bar{M}_w/\bar{M}_n$ values of $\mathrm{A}_{[\mathrm{Orig.Wat.}]}$ in Table 1) speaking for the fact that certain compositional rearrangements take place during the isolation procedure. The chromatogram of the $\mathrm{E}_{\mathrm{effluent}}$ contained almost the same elution humps, yet with somewhat improved resolution power, which were also obtained for the fraction $\mathrm{E}_{[\mathrm{HM}]}$ (namely at 10.2, 10.8, 11.3 and 13.0 ml, only the elution hump for the largest molecular size at 9.8 ml was absent). This outcome is apparently a result of the slightly different retaining capacity of the PVP resin. The fraction $\mathrm{F}_{[\mathrm{XAD}]}$ contained the three elution humps at 10.2, 10.8 and 11.3 ml also characteristic for the $\mathrm{E}_{[\mathrm{HM}]}$ and $\mathrm{E}_{\mathrm{effluent}}$ fractions. However, the relative proportion of the elution hump at about 10.2 ml was significantly higher, compared with the situation in the original $\mathrm{E}_{\mathrm{effluent}}$ fraction, and the small elution hump at about 9.6 ml, specific for the original DOM, was now visible in the fraction $\mathrm{F}_{[\mathrm{XAD}]}$, in addition to the molecular size of the latest elution hump being slightly increased from about 470 to 620 Da (13.0 versus 12.7 ml). The $\mathrm{F}_{\mathrm{effluent}}$ contained again the same four molecular size fractions (namely those at 10.2, 10.8, 11.3 and 12.7 ml) which were specific for the preceding $\mathrm{F}_{[\mathrm{XAD}]}$ fraction but the molecular size distribution was now narrower (cf. Table 1). On the other hand, the fine structure of the chromatogram for the $\mathrm{G}_{[\mathrm{IRA}]}$ fraction was fully different, only a small shoulder was visible at about 10.8 ml indicating a possible similarity with the previous $\mathrm{F}_{\mathrm{effluent}}$. The dissimilarity may be caused by the aggregation degree of these small-molecular size organic macromolecular HM-type acids being too high for the complete penetration into the gel matrix. The remaining $\mathrm{G}_{\mathrm{effluent}}$ fraction was split into several scattered elution humps with small molecular sizes. The intensive elution humps at about 16.3 ml and especially at 18.3 ml (total volume of the gel bed (V_{t}) was ca. 16.6 ml) indicate strong interactions with the gel matrix. The sequence of the six HPSEC chromatograms given for the enlarged PVP application demonstrates that the isolation of HM-type organic solutes is not a straightforward and very simple task, especially since after a certain separation phase the remaining organic constituents seem to attain again a certain compositional equilibrium state resembling those of the previous fractions in the isolation flow chart. Similar re-configuration of humic solutes after separation has been postulated earlier [39,51].

3.1.4. UF procedure

Scheme 1 shows that the 20-fold concentrate using a membrane of 1 kDa cutoff includes about 97% organic solutes of the original DOM with molecular size greater than the applied NMW value (fraction $H_{[HM]}$). The cutoff range of the applied tangential UF membrane was in accordance with the HPSEC results (cf. Table 1) and previous findings [39]. The ability of the weakly basic IRA-67 resin to retain small-molecular size constituents (organic acids, etc.) from the $H_{filtrate}$ was very effective (ca. 91% of the remaining organic solutes were removed from the parent solution). Cation exchanger retained about 21% of miscellaneous organic constituents from the basic extract of the IRA-67 resin (fraction $J_{[Dow]}$; amines, amino acids, peptides, etc.), and the rest (ca. 70%) consisted of certain kinds of macromolecular HM-type acids (fraction $I_{[IRA]}$). The content of organic constituents in the final $I_{effluent}$ was negligible and thus it represents practically mere water.

Fig. 1 proves that a controlled concentration of the original water sample does not increase the relative content of small-molecular size constituents, as stated [52] previously, but the fingerprint information of the chromatogram $H_{[HM]}$ was totally identical with that obtained for the original SSS-water (cf. $\bar{M}_n$ and $\bar{M}_w/\bar{M}_n$ values of $A_{[Orig.Wat.]}$ in Table 1). The chromatogram of the $H_{filtrate}$ possessed three new elution humps at about 10.5, 11.3 and 12.8 ml (4850, 2300 and 570 Da, respectively), which significantly differed from the corresponding candidates obtained for the previous $H_{[HM]}$ sample, one minor hump at 16.3 ml (<100 Da) being common with the previous head samples and a new very marginal one at about 17.8 ml ($\lll$100 Da) indicating possibly slight interactions with the gel matrix. This outcome speaks for the tendency, occurring after removing of the DOM, that the remaining organic solutes take readily a new re-configuration, i.e. find a new equilibrium state. The molecular size distribution of organic constituents in the fraction $I_{[IRA]}$ was quite different from that obtained for the previous parent solutes in the $H_{filtrate}$. In accordance with this outcome, the molecular size distribution of the $J_{[Dow]}$ fraction, obtained during the purification procedure of the fraction $I_{[IRA]}$ by a cation exchanger, had nothing in common with those obtained in the whole isolation flow chart. On the other hand, as a peculiarity, the molecular size distribution of the final $I_{effluent}$ fraction resembled very closely to the parent $H_{filtrate}$ fraction. This irregularity illustrates that certain kinds of organic constituents of the original heterogenous DOM do not behave as definite entities but after they are removed from their original equilibrium state they form new aggregates.

3.2. Spectroscopic behaviours of organic solutes during different isolation procedure

Each isolation procedure in Scheme 1 divided the original DOM into a specific main fraction and an effluent-filtrate, which was further divided into several subfractions. The different fractions of each isolation procedure, weighted with their relative contents, were computationally combined for estimating how well the sum functions of these individual pieces correspond to the molecular size distribution of the original state of the DOM, and what was the effect of the isolation procedure on the absorbance-abilities of chromophores at 254 nm. The combined chromatograms correlated surprisingly well, in the case of DAX-8, DEAE and PVP procedures, with that obtained for the original water sample ($r = 0.943 \pm 0.001$, $P_{0.95}$), and the decrease in the total chromatogram area was only 8 ± 1% which was, considering experimental errors, almost insignificant. This recalculation indicates that the absorbance-ability of different chromophores remains reversible in the different chemical treatments and each isolation method gives, to a certain degree, a representative candidate for the HM model, especially in the light of the molecular size distribution.

3.3. Differentiation of solid isolates obtained by applied procedures

In addition to experimental results in Table 1, some values were derived by certain theoretical or experimental equations. Molar absorptivities ($\in$, $1\,\mathrm{mol}^{-1}\,\mathrm{cm}^{-1}$ of OC) were measured at 280 nm. A good correlation was observed, by means of equations presented [39] previously, between $\in$, total aromaticity (Ar. (%)) and $\bar{M}_n$ values being also consistent with the results of Chin et al. [53]. It has been stated [54] that the absorbance of humic waters at 365 nm will increase relatively more with increasing molecular size than that at 250 nm thus permitting an estimate (E_2/E_3-ratio) for the relative degree of humification. The correlation obtained in the present study between the quotient E_2/E_3 and $\in$ was acceptable ($r = 0.89$, $P_{0.95}$) demonstrating that when the E_2/E_3-ratio increased, the estimated Ar. (%) and $\bar{M}_n$ values decreased. The total amount of unsaturation (ϕ_{total}) in $\mathrm{mmol\,g}^{-1}$ was calculated, for better describing the nature of different isolated fractions, by means of the method presented in [55] specially for the complex mixture of humic solutes: $\phi_{total} = \mathrm{C}_{total} + \mathrm{N}_{total}/2 - \mathrm{H}_{total}/2 + 1000/\bar{M}_n$, where C_{total}, N_{total} and H_{total} are the contents of carbon, nitrogen and hydrogen in $\mathrm{mol\,g}^{-1}$ and $\bar{M}_n$ is the number-averaged molecular size. It is notable that ϕ_{total} is the sum of carboxyl (ϕ_{COOH}), carbonyl ($\phi_{\mathrm{C=O}}$, a pi-bond), aromatic (ϕ_{Ar}) and aliphatic (ϕ_{al}, cycloalkyl groups) unsaturations and unsaturation due to alkenes, esters, amides, etc. (ϕ_{xs}). The different ϕ_{total} values obtained in the present study for HM-type isolates were in good accordance with those reported [11,27,40] previously for various HM-type mixtures. The average molecular formulas for different solid isolates in Table 1 were calculated, by means of unprocessed number-averaged $\overline{M}_n$ values estimated by HPSEC, for better visualizing the composition of a sample. The use of more reliable $\overline{M}_n$ values, obtained by vapor–pressure osmometry (VPO), as a standard for comparison is a common practice in humus chemistry. The relationship between the $\overline{M}_n$ values obtained by the VPO and the applied HPSEC was [39]: $\overline{M}_{n\mathrm{VPO}} = 591.249 + 0.049 \times \overline{M}_{n\mathrm{HPSEC}} + 7.531\mathrm{E}^{-6} \times \overline{M}_n^2$, ($r^2 = 0.984$, $n = 19$, $P_{0.95}$), which was also previously adopted [11,27,40] for calculating structural characteristics per an average humic molecule.

Table 1 shows that although some variation occurred, e.g. between the elemental compositions of different actual HM-type isolates, the parameters were fairly similar with each other. Likewise, the discriminating power between the hydrogen-to-carbon (H/C) and oxygen-to-carbon (O/C) ratios (van Kravelens diagram [56], whose application is still under intensive studies [57]) is not very easy to realize. For better extracting the small differences among the nineteen different isolates and to find a possible similarity–dissimilarity between samples, the original data was slightly manipulated. The multi-dimensional dataset of Table 1 was examined closer with a statistical–graphical principal components analysis (PCA). The fundamental idea of the PCA is to reduce the numerous variables and to seek for linear combinations of those variables explaining most of the variability. Accordingly, PCA (eigenanalysis) and subsequent inspection of the eigenvector plots is one of the first and foremost procedures that can be done when tackling a multi-dimensional dataset. The credit of multivariate methods is that a huge amount of data can be presented in a graphical form, which would be very hard to do using tables of numbers or univariate statistics. For the PCA, from the dataset of Table 1, a 19 × 6 matrix (19 objects (samples) and 6 variables: $\overline{M}_n$, ∈, H/C, O/C, N/C and E_2/E_3) was generated for calculating variances and covariances. From this, eigenvectors and eigenvalues have been extracted.

Fig. 3 shows the biplot (scatterplot) of the six variables for the different samples (19 cases) isolated and processed with different techniques and chemical treatments (level of statement, 86% for the first two PC). The six arrowhead lines intersecting at (0,0) represent the weights of the variables. The direction and length of each vector (variable) are proportional to its contribution to the principal components, and the angle between any two is inversely proportional to the correlation between them. According to the statistical–graphical analysis of Fig. 3, the actual HM-type isolates ($\mathrm{B_{[HM]}}$, $\mathrm{D_{[HM]}}$, $\mathrm{E_{[HM]}}$, $\mathrm{R1_{[HM]}}$ and $\mathrm{R2_{[HM]}}$) formed a compact cluster I, regardless of the applied isolation procedure. In other words, the averaged values of the studied parameters for these samples were almost similar. The original DOM ($\mathrm{A_{[Orig.Wat.]}}$) and its UF concentrate ($\mathrm{H_{[HM]}}$) formed, as expected, also a compact cluster II quite close to that of I (the only significant discriminating factor between clusters I and II was the H/C-ratio). The so-called hydrophobic neutral isolate $\mathrm{C_{[MeOH]}}$ and its reference $\mathrm{R4_{[MeOH]}}$ fell into cluster III, and the strongest discriminating parameters in respect of their HM homologues (cluster I) were $\overline{M}_n$, ∈ and E_2/E_3. The same three structural parameters discriminated also the HA-type $\mathrm{R3_{[HM]}}$ sample from its FA-type $\mathrm{R2_{[HM]}}$ counterpart and the HA-type $\mathrm{R7_{[HM]}}$ and $\mathrm{R9_{[HM]}}$ samples from their FA-type $\mathrm{R6_{[HM]}}$ and $\mathrm{R8_{[HM]}}$ counterparts (respectively). The environmental impact was also revealed clearest by these three parameters, i.e. the FA-type $\mathrm{R6_{[HM]}}$ and $\mathrm{R8_{[HM]}}$ samples became clearly separated from their $\mathrm{R2_{[HM]}}$ homologue, and the HA-type $\mathrm{R7_{[HM]}}$ and $\mathrm{R9_{[HM]}}$ samples were positioned far from their $\mathrm{R3_{[M]}}$ homologue. The discriminating effect of these three parameters prevailed also for the $\mathrm{F_{[XAD]}}$ sample, i.e. the E_2/E_3-ratio was slightly greater and $\overline{M}_n$ as well ∈ values smaller than

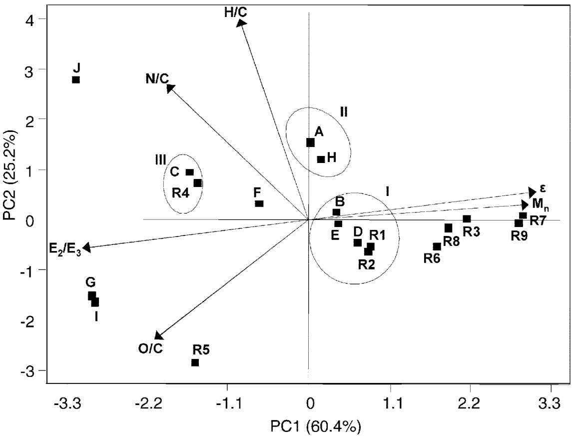


Fig. 3. Graphical two-dimensional perspective of projections (biplot) of the six basic structural variables on the first two principal components for the distribution of the different humic-type isolates obtained by several procedures. For symbols of samples and variables, see Table 1.

those obtained for the corresponding main $D_{[HM]}$ fraction. The O/C-ratio was exceptionally high for $G_{[IRA]}$, $I_{[IRA]}$ and $R5_{[IRA]}$ samples resulting, together with other parameters, in their positioning far from other samples. The divergence of $R5_{[IRA]}$ from $G_{[IRA]}$ and $I_{[IRA]}$ is due to their different isolation procedures. The special $J_{[Dow]}$ sample formed its own group, far from other samples, particularly owing to very high N/C- and H/C-ratios, in addition to other parameters. The statistical–graphical analysis of Fig. 3 confirms that the molecular size distribution and certain simple spectroscopic properties are very powerful discriminating parameters between different natural organic isolates.

3.4. FT-IR analysis

Infrared spectroscopy has been widely used for gross characterization of humic-type constituents and can provide valuable information on the structural and functional properties of NOMs, e.g. oxygen-containing functional groups, occurrence of protein and carbohydrate moieties and relative proportions of aromatic versus aliphatic moieties. However, the unambiguous assignments of different spectral bands are not possible for humic-type very heterogenous organic material.

All spectra in Fig. 2 are characterized by a number of absorption bands, exhibiting variable relative intensities, typical of humic-type materials [32,34,58–61]:

- A band around 1724 cm^{-1}, typically associated to the C=O stretching of carbonyl functions, particularly aldehydes, ketones and carboxyl groups (COOH);
- The range of 1650–1600 cm^{-1}, C=O stretch (amide), aromatic C=C, hydrogen bonded C=O, double bond conjugated with carbonyl and COO^{-} vibrations, COO^{-} symmetrical stretch;
- A discrete small band at about 1510 cm^{-1}, possible ascribed to C–C stretching of aromatic rings (lignin indicator), to conjugated C=N systems and amino functionalities;
- A broad band around 1400 cm^{-1} describing several functionalities, aliphatic C–H, CC–H$_3$, C–H stretching of methyl groups, C–H bending, O–H deformation and C–O stretching of phenolic groups, COO^{-} antisymmetrical stretch, salts of COOH;
- Near 1224 cm^{-1}, C–O stretching and O–H deformation of COOH groups, aromatic and ester linkage C–O, phenolic C–OH;
- Around 1054 cm^{-1}, C–C, C–OH, C–O–C typical of glucosidic linkages, Si–O impurities, especially C–O stretches of carbohydrates and peptides;
- A small band at 840 cm^{-1}, aromatic C–H vibrations;
- For low-energy vibrations a broad band at about 600 cm^{-1}, specific for inorganic and organometallic compounds.

3.4.1. Differentiation of samples according to FT-IR analysis

Fig. 2 verifies that it is practically impossible to draw definite conclusions about the influence of the isolation procedure on the structural compositions of separated fractions directly from their multidimensional functionalities. The only IR spectrum which definitely differed from other was obtained for the special $J_{[Dow]}$ faction which is, according to the XAD nomenclature, equal to the so-called hydrophilic bases. The broad absorbance bands positioned at 1633, 1395, 1100 and 802 cm^{-1} speak for the high contents of carbohydrates and peptides in this fraction, in addition to small amounts of aromatics. The functionality of this $J_{[Dow]}$ fraction was surprisingly similar to the IR spectrum reported earlier for an analogous hydrophilic base-fraction [18]. The coarse structural similarity–dissimilarity, in the light of applied isolation procedures, of the other fourteen IR spectra of Fig. 2 was proved in Fig. 4 using the statistical–graphical analysis.

Fig. 4 proves the power of the PCA in solving complicated problems. For the PCA, from the IR dataset, a 14 × 300 matrix (14 objects (samples) and 300 variables, intensities of IR spectra between 1900 and 400 cm^{-1}; bandwidth, 5 cm^{-1}) was generated for calculating variances and covariances. From this, eigenvectors and eigenvalues have been extracted. Fig. 4 shows the three-dimensional scatterplot of projections (referred also to scores) of the multi-dimensional IR dataset on the first three principal components (arbitrary units). The overall level of the statement of the structural functionalities was 95% on the first three PCs. The discriminating effect of the PC1 between different samples/isolation procedures was very high (level of the statement was near 76% of the total variance). The most effective on the PC1 were the multiple functionalities between 1760 and 1350 cm^{-1} and two more specific functionalities centred on near 1224 and 1054 cm^{-1}. The most effective remaining unexplained specific variations between the samples on the PC2 were the functionalities arising from the intensities around 1700, 1500 and 1200 cm^{-1} and from a distinct intensity centred on near 1054 cm^{-1}. The remaining unexplained variation between certain samples on the PC3 was quite specific attached to the

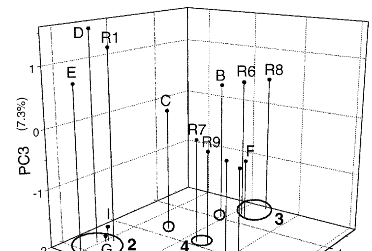


Fig. 4. Graphical three-dimensional perspective of projections of the multidimensional FT-IR dataset (in the zone, 1900–400 cm^{-1}) on the first three principal components for the distribution of the different humic-type isolates obtained by several isolation procedures. For symbols of samples, see Table 1.

single functionalities centred on near 1724, 1630, 1383, 1224 and 1554 cm^{-1}.

Fig. 4 demonstrates that the PCA divides the different samples according to their IR functionalities into distinct clusters. The greatest dissimilarity appears between the cluster 1 (samples $A_{[Orig.Wat]}$ and $H_{[HM]}$) and cluster 2 (samples $D_{[HM]}$, $E_{[HM]}$, $R1_{[HM]}$, $G_{[IRA]}$ and $I_{[IRA]}$). In view of the total reliability (95%, including the PC3), the structural functionality of the samples $D_{[HM]}$, $E_{[HM]}$ and $R1_{[HM]}$ is quite similar but totally different from that obtained for the special samples $G_{[IRA]}$ and $I_{[IRA]}$. This is in agreement with the situation in Fig. 3 (cluster I). On the other hand, the structural similarity–dissimilarity in Fig. 4 among the three actual HM-type isolates and the samples $A_{[Orig.Wat]}$ and $H_{[HM]}$ is clearly different from that obtained by means of elemental analyses and other simple parameters in Fig. 3. This is a natural result of the chemical, even relative mild, treatment of the original DOM. Figs. 3 and 4 verify also that it is possible to isolate by the same procedure from the same fresh water sampling source reasonably similar HM-type isolates ($D_{[HM]}$ versus $R1_{[HM]}$) and that the seasonal variation is quite marginal. The variation of the many functionalities dominating the PC1 was quite minor between the samples positioned in the clusters 3 and 4, and certain specific functionalities typifying the PC2 were the most significant difference between these samples. The samples $R6_{[HM]}$ and $R8_{[HM]}$, representing so-called FA-type fractions of the XAD-8 technique, formed their own cluster 3, thus indicating similar uniformity as that obtained in Fig. 3. On the other hand, the sample $F_{[XAD]}$, representing a specific fraction separated by the XAD-8/2 resin, also positioned in cluster 3 unlike in Fig. 3 where its most important discriminating factor was the molecular size distribution. This is a quite sensible result because the isolation mechanism of the XAD resins (or analogues) is based on certain functionalities of the DOM prevailing in the preadjusted conditions. The variation among the $R6_{[HM]}$, $R8_{[HM]}$ and $F_{[XAD]}$ samples was practically attached to certain single minor functionalities dominating the PC3. The HA-type samples $R7_{[HM]}$ and $R9_{[HM]}$ also formed their own cluster 4 thus being consistent with the results in Fig. 3. The sample $B_{[HM]}$, representing a total amount (HA + FA) of so-called hydrophobic humic substances isolated by the DAX-8 resin, formed its own group quite close to the combined cluster 3 (FA-type isolates). The structural similarity of the $B_{[HM]}$ sample with these FA-type isolates is in agreement with that in Fig. 3 ($B_{[HM]}$ versus $R2_{[HM]}$), with the environmental impact on the quality of structural composition as well as with the previous results [15–17]. The sample $C_{[MeOH]}$, which, according to the XAD nomenclature, represents the so-called very hydrophobic fraction, also formed a separate cluster but without any close relationship to other clusters or samples. On the other hand, the IR spectrum of the $C_{[MeOH]}$ sample resembles, excepting certain minor functionalities, those obtained for FA- or HA-type isolates, or their primary mixture, separated by XAD-8 or DAX-8 resins. This is quite evident since it has been previously verified [49,50] that the different hydrophobic FA-, HA- and MeOH-type functional fractions contained qualitatively the same principal building blocks and only the relative content of which was the most powerful discriminating factor.

3.4.2. Possible ester hydrolyses during isolation by FT-IR analysis

The FT-IR technique has been intensive adapted for determining the carboxylic acid content of humic-type constituents and analogues [62–64]. Nevertheless the carboxylate groups generate different absorption bands within a large frequency region and also other functional groups will absorb at the same wavelengths (overlapping), the main interest is directed to spectral ranges at about 1580–1630 and 1710–1730 cm^{-1} generally assigned to the carboxylate anion ($-COO^{-1}$) and protonated $-COOH$ groups, respectively. It is noteworthy that traces of carboxylate bands are present in all FT-IR spectra of humic-type constituents, even those at pH ≈ 2, indicating the presence of very strongly acidic groups—as also remarked by Cabaniss [64]. Maurice et al. have recently reported [65] that a small shoulder being comprised in the absorption band of the protonated $-COOH$ groups, at higher frequency with a maximum near 1770 cm^{-1}, corresponds to the ester carbonyl groups ($-COOR$). The separation of this hardly visible small shoulder (cf. Fig. 2) as a distinct absorption band from the spectral profile is not an easy task requiring different kind of data manipulation, e.g. linear Gaussian/Lorentzian band shapes and/or special multi-point method for baseline correction [66,67]. All chromatographic isolation techniques contain the basic extraction (pH ≈ 13) of the organic matter retained onto the sorbing solid thus permitting a possibility for potential base-catalyzed ester hydrolyses of the HM [65,68,69].

Table 2 proves that the loss of ester groups ($-COOR$) in concentrating (20-fold) the original water by the tangential UF technique (1 kDa of NMW cutoff) was quite minor ($A_{[Orig.Wat.]}$ versus $H_{[HM]}$). The $I_{[IRA]}$ fraction obtained from $H_{filtrate}$ (cf. Scheme 1) contained still a significant amount of $-COOR$ groups (ca. 2% of original), and it was possible to find even some traces from the $J_{[Dow]}$ fraction after the two-fold base extraction of OM indicating the relatively permanent nature of some $-COOR$ groups. It was possible to obtain about 70% of the original $-COOR$ content by the DAX-8 technique ($B_{[HM]}$ + $C_{[MeOH]}$) and 72% by the DEAE technique ($D_{[HM]}$) thus speaking for the fact that the effect of the acidic pretreatment of the original water on the ester hydrolysis is practically negligible. The PAP technique alone separated from the original SSS-water about 81% of the original $-COOR$ content and its enlarged application ($E_{[HM]}$ + $F_{[BAD]}$ + $G_{[IRA]}$) as much as about 92%. The above mass balance estimation expresses that the base-catalyzed ester hydrolysis of the DOM is not as disadvantageous factor in the chromatographic isolation of the HM as speculated (e.g. [65,68,69]).

Table 2 also indicates that only about 3% of carboxylic groups of the DOM were in their protonated form in the orig-

Table 2
Selected FT-IR data for different isolated humic-type samples[a]

Sample[b]	Method	–COOR (cm^{-1})[c]	–COOH (cm^{-1})[c]	$-COO^{-1}$ (cm^{-1})[c]	–COOR (rel. area)[d]	–COOH (rel. area1)[d]	$-COO^{-1}$ (rel. area2)[d]	–COOR (percent of $A_{[Orig.Wat.]}$)	–COOH (percent of Σrel. areas 1 and 2)
$A_{[Orig.Wat.]}$	F.D.	1770	1715	1585	237.2	33.3	1189.8	100.0	2.7
$H_{[HM]}$	UF	1770	1715	1585	229.9	29.9	1179.1	96.9	2.5
$I_{[IRA]}$	UF	1770	1725	1630	4.3	21.3	5.5	1.8	79.6
$J_{[Dow]}$	UF	1770	1735	1630	0.1	0.3	11.0	0.02	3.0
$B_{[HM]}$	DAX-8	1780	1720	1615	163.1	358.5	214.9	68.8	62.5
$C_{[MeOH]}$	DAX-8	1780	1720	1620	3.1	9.1	18.2	1.3	33.3
$D_{[HM]}$	DEAE	1785	1710	1605	170.0	408.4	126.7	71.7	76.3
$E_{[HM]}$	PVP	1780	1725	1625	191.4	721.7	108.8	80.7	86.9
$F_{[XAD]}$	PVP	1775	1715	1620	19.1	99.7	73.9	8.1	57.5
$G_{[IRA]}$	PVP	1770	1725	1630	7.4	98.6	29.1	3.1	77.2

[a] For symbols of samples, see Table 1.

[b] All isolates were separated in parallel from the same SSS-water.

[c] Maximum frequency of the absorption band.

[d] Relative areas of estimated distinct absorption bands are standardized proportional to their quantities in the original DOM.

inal acidity (pH 5.8) of the water. This outcome is in line with the heterogeneity of different acid constants (pK_a) of HM reported in the literature (e.g. [26,70,71]). All chromatographic HM isolates were finally treated by strongly acidic cation exchanger before freeze-drying for protonating the $-COO^{-1}$ groups. Despite that the frequency range between 1580 and 1630 cm^{-1} does not merely represent the absorption of carboxylic groups, it is well justifiable to attribute, at least, a small part to remaining carboxylate anions.

4. Conclusions

It is evident that no ideal system is available for isolating pure hypothetical humic substances from a water sample, and during each isolation procedure based on a chemically assisted sorption–desorption technique, certain changes in the structural composition of the DOM take place, as shown in this study and also verified [11] previously. Most important is that researchers choosing between different isolation procedures do consider the purpose of the isolation, i.e. whether humic fraction is desirable, as it has also been emphasized [65]. According to this study, the following conclusions can be drawn:

- The DEAE cellulose serves as a practicable choice for fresh water studies. The overwhelming advantage of this sorbing solid is that any adjustments of the original acidity of the water are not needed, and it is possible to isolate a significant amount (about 75–80% of DOM) of organic solutes as humic matter. A minor disadvantage is the somewhat tedious back-elution of the adsorbed organic solutes.
- The PVP resin seems to be very useful in isolating humic-type constituents from the fresh water sample, especially in relation to their original form. The ability of the PVP resin to retain organic humic solutes was exceptionally high (≥80% of DOM), and when connected in sequence with a non-ionic sorbing solid about 90% of the DOM was retained. The critical disadvantage of the PVP procedure is that the original acidity of the water sample must be adjusted to the pH 2 for protonating the acidic functional groups of the DOM.
- The mass balance estimation of ester carbonyl carbons speaks for the fact that the base-catalyzed ester hydrolysis of the DOM, possibly taking place during the chromatographic isolation phases of the HM, is not as critical disadvantage as speculated.
- To better characterize the different isolated humic-type samples in relation to applied isolation procedures more sensitive analyses (e.g. ^1H and ^{13}C NMR and thermal degradation) are in progress.

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References

[1] F.J. Stevenson, Humus chemistry: genesis, composition, reactions, Wiley, NY, 1982.

[2] R.L. Malcolm, Geochemistry of stream fulvic and humic substances, in: G.R. Aiken, D.M. McKnight, R.L. Wershaw, P. MacCarthy (Eds.), Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation and Characterization, Wiley, NY, 1985, pp. 181–209.

[3] R.L. Malcolm, Anal. Chim. Acta 232 (1990) 19.

[4] C. Steinberg, U. Muenster, Geochemistry and ecological role of humic substances in lakewater, in: G.R. Aiken, D.M. McKnight, R.L. Wershaw, P. MacCarthy (Eds.), Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation and Characterization, Wiley, NY, 1985, pp. 105–145.

[5] F.H. Frimmel, R.F. Christman (Eds.), Humic Substances and their Role in the Environment, Wiley, NY, 1988, p. 271.

[6] E.M. Thurman, Organic Geochemistry of Natural Waters, Martinus Nijhoff/W. Junk Publisher, Dordrecht, 1985.

[7] J.-M. Bollag, Environ. Sci. Technol. 26 (1992) 1876.

[8] D. Klotz, D. Lazik, Isot. Environ. Health Stud. 31 (1995) 61.

[9] M. Wolf, G. Teichmann, E. Hoque, W. Szymczak, W. Schimmack, Fresenius J. Anal. Chem. 363 (1999) 596.

[10] V.-A. Långvik, B. Holmbom, Water Res. 28 (1994) 553.

[11] J. Peuravuori, Anal. Chim. Acta 429 (2001) 75.

[12] G.R. Aiken, Isolation and concentration techniques for aquatic humic substances, in: G.R. Aiken, D.M. McKnight, R.L. Wershaw, P. MacCarthy (Eds.), Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation and Characterization, Wiley, NY, 1985, pp. 363–385.

[13] J.J. Farnworth, IHSS Newslett. 12 (1994) 2.

[14] J.J. Farnworth, IHSS Newslett. 13 (1995) 8.

[15] J. Peuravuori, P. Ingman, K. Pihlaja, R. Koivikko, Talanta 55 (2001) 733.

[16] J. Peuravuori, R. Koivikko, K. Pihlaja, Water Res. 36 (2002) 4552.

[17] J. Peuravuori, T. Lehtonen, K. Pihlaja, Anal. Chim. Acta 471 (2002) 219.

[18] J.A. Leenheer, Environ. Sci. Technol. 15 (1981) 578.

[19] J. Peuravuori, K. Pihlaja, Humic substances in water, in: L.M.L. Nollet (Ed.), Handbook of Water Analysis, Marcell Dekker Inc., NY, 2000, pp. 387–408.

[20] F.H. Frimmel, Characterization of organic acids in freshwater: a current status and limitations, in: E.M. Perdue, E.T. Gjessing (Eds.), Organic Acids in Aquatic Ecosystems, Wiley, NY, 1990, pp. 5–23.

[21] M.S. Shuman, Carboxyl acidity of aquatic organic matter: possible systematic errors introduced by XAD extraction, in: E.M. Perdue, E.T. Gjessing (Eds.), Organic Acids in Aquatic Ecosystems, Wiley, NY, 1990, pp. 97–109.

[22] R.F. Packham, Proc. Soc. Water Treat. Exam. 13 (1964) 316.

[23] C.J. Miles, J.R. Tuschall, P.L. Brezonik, Anal. Chem. 55 (1983) 410.

[24] C. Pettersson, I. Arsenie, J. Ephraim, H. Borén, B. Allard, Sci. Total Environ. 81–82 (1989) 287.

[25] C. Pettersson, J. Ephraim, B. Allard, Org. Geochem. 21 (1994) 443.

[26] J. Peuravuori, K. Pihlaja, N. Välimäki, Environ. Int. 23 (1997) 453.

[27] J. Peuravuori, K. Pihlaja, Anal. Chim. Acta 363 (1998) 235.

[28] C. Pettersson, L. Rahm, Environ. Int. 22 (1996) 551.

[29] G.D. Swincer, J.M. Oades, D.J. Greenland, Aust. J. Soil Res. 6 (1968) 211.

[30] L.E. Lowe, Can. J. Soil Sci. 60 (1980) 219.

[31] C. Ciavatta, M. Govi, L.V. Antisari, P. Sequi, J. Chromatogr. 509 (1990) 141.

[32] A. Watanabe, S. Kuwatsuka, Soil Sci. Plant Nutr. 38 (1992) 31.

[33] C. Ciavatta, M. Govi, J. Chromatogr. 643 (1993) 261.

[34] J. Chen, B. Gu, E.J. LeBoeuf, H. Pan, S. Dai, Chemosphere 48 (2002) 59.

[35] J. Chen, E.J. LeBoeuf, S. Dai, B. Gu, Chemosphere 50 (2003) 639.

[36] A.S. Michaels, Ultrafiltration, in: E.S. Perry (Ed.), Progress in Separation and Purification, Wiley, NY, 1968, pp. 297–334.

[37] V.M.T. Ganzerli, L. Maggi, R. Stella, G. Ciceri, Chem. Ecol. 1 (1983) 279.

[38] J. Peuravuori, K. Pihlaja, Environ. Int. 23 (1997) 441.

[39] J. Peuravuori, K. Pihlaja, Anal. Chim. Acta 337 (1997) 133.

[40] J. Peuravuori, K. Pihlaja, Anal. Chim. Acta 364 (1998) 203.

[41] J. Buffle, P. Deladoey, W. Haerdi, Anal. Chim. Acta 101 (1978) 339.

[42] G.R. Aiken, R.L. Malcolm, Geochim. Cosmochim. Acta 51 (1987) 2177.

[43] K.J. Howe, M.M. Clark, Environ. Sci. Technol. 36 (2002) 3571.

[44] S. Lee, Y. Shim, S. Kim, J. Sohn, S.K. Yim, J. Cho, Water Sci. Technol.: Water Suppl. 2 (2002) 151.

[45] E. Hoque, M. Wolf, G. Teichmann, E. Peller, W. Schimmack, G. Buckau, J. Chromatogr. A 1017 (2003) 97.

[46] T. Vartiainen, A. Liimatainen, P. Kauranen, Sci. Total Environ. 62 (1987) 75.

[47] T.K. Nissinen, I.T. Miettinen, P.J. Martikainen, T. Vartiainen, Chemosphere 45 (2001) 865.

[48] T. Myllykangas, T.K. Nissinen, P. Rantakokko, P.J. Martikainen, T. Vartiainen, Water Res. 36 (2002) 3045.

[49] J. Peuravuori, N. Paaso, K. Pihlaja, Anal. Chim. Acta 39 (1999) 331.

[50] T. Lehtonen, J. Peuravuori, K. Pihlaja, Anal. Chim. Acta 424 (2000) 91.

[51] P.J. Shaw, R. Jones, H. De Haan, Environ. Technol. 15 (1994) 765.

[52] J. Aho, O. Lehto, Arch. Hydrobiol. 101 (1984) 21.

[53] Y.-P. Chin, G.R. Aiken, E. O'Loughlin, Environ. Sci. Technol. 28 (1994) 1853.

[54] H. De Haan, G. Werlemark, T. De Boer, Plant Soil 75 (1983) 63.

[55] E.M. Perdue, Geochim. Cosmochim. Acta 48 (1984) 1435.

[56] S.A. Visser, Environ. Sci. Technol. 17 (1983) 412.

[57] S. Kim, R.W. Kramer, P.G. Hatcher, Anal. Chem. 75 (2003) 5336.

[58] F. Claret, T. Schäfer, A. Baue, G. Buckau, Sci. Total Environ. 317 (2003) 189.

[59] R.M.B.O. Duarte, E.B.H. Santos, A.C. Duarte, Water Res. 37 (2003) 4073.

[60] C. Cocozza, V. D'Orazio, T.M. Miano, W. Shotyk, Org. Geochem. 34 (2003) 49.

[61] M. Kurková, Z. Klika, C. Kliková, J. Havel, Chemosphere 54 (2004) 1237.

[62] M. Starsinic, Y. Otake, P.L. Walker, P.C. Painter, Fuel 63 (19841002).

[63] J.V. Ibarra, Sci. Total Environ. 81–82 (1989) 121.

[64] S.E. Cabaniss, Anal. Chim. Acta 255 (1991) 23.

[65] P.A. Maurice, M.J. Pullin, S.E. Cabaniss, Q. Zhou, K. Namjesnik-Dejanovic, G.R. Aiken, Water Res. 36 (2002) 2357.

[66] R.J. Marshall, Comput. Biomed. Res. 19 (1986) 319.

[67] R.J. Marshall, A.J. Bleasby, R. Turner, E.H. Cooper, Chemometr. Intell. Lab. 1 (1987) 285.

[68] G.R. Aiken, A critical evaluation of the use of macroporous resins for the isolation of aquatic humic substances, in: F.H. Frimmel, R.F. Christman (Eds.), Humic Substances and Their Role in the Environment, Wiley, NY, 1988, pp. 15–30.

[69] G.R. Aiken, D.M. McKnight, K.A. Thorn, E.M. Thurman, Org. Geochem. 18 (1992) 567.

[70] J. Peuravuori, N. Paaso, K. Pihlaja, Talanta 56 (2002) 523.

[71] J.H. Ephraim, H. Borén, C. Pettersson, I. Arsenie, B. Allard, Environ. Sci. Technol. 23 (1989) 356.

VI

Immobilized Humic Substances as Sorbents

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Immobilized humic substances as sorbents

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Abstract

A new method was developed for the immobilization of humic substances. Humic acids (HA) immobilized onto different carriers were studied as sorbents for organic and inorganic substances. The sorption isotherms of 4-aminoazobenzene, Crystal Violet, Methylene Green, and flavine mononucleotide on immobilized HA show that pH and salt concentration have a significant effect on the sorption process, largely depending on the properties of polymeric matrix. Humic acids from different sources showed differing sorption capacity for the studied groups of substances.

Keywords: Humic acids; Sorbents; Immobilization

1. 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 their 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. At the interactions both organic (usually detritus particles) and inorganic (mineral matter) substances become covered with humic substances and thus contaminant movement can be largely modified by interaction with humic substances (De Paolis and Kukkonen, 1997). 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 and the structure of the HS depend on their origin (Ritchie and Perdue, 2003).

To offer new areas of use and to study the interaction between xenobiotics and humic substances, their immobilization on solid carriers (Bulman and Szabo, 1991; Klavins, 1993; Klavins and Eglite, 2000) has been suggested. Considering the wide application of immobilized enzymes and low molecular biologically active molecules in chromatography and biotechnology, immobilization of HS has received substantial interest. Several approaches for immobilization of HS have been suggested. Immobilized HS were used to study the interactions of HS with metal ions and organic substances (Koopal et al., 1998; Yang and Koopal, 1999; Laor et al., 2002; de la Rosa et al., 2003; Prado et al., 2003), and as models of humate-coated minerals to study migration of ^{241}Am around nuclear waste repositories (Szabo et al., 1998).

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Considering immobilized HS as prospective sorbents for organic and inorganic substances, the aim of the present study is to study the sorption character of organic substances and metal ions onto humic substances isolated from different environments and immobilized onto differing polymeric matrixes.

2. Materials and methods

The properties of commercial humic acid (Aldrich Chemical Co. Ltd.) and obtained humic acids isolated from soil, peat and water as are described earlier (Klavins and Eglite, 2000). Elemental analysis was carried out by a Perkin–Elmer 240B analyzer.

The specific surface areas of the prepared solid phases were determined by the Brunauer–Emmet–Teller (BET) method using a EMS-61 Sorptometer. Adsorption isotherms of N_2 were determined volumetrically at 100 °C. Prior to the measurements each sample was dried in vacuum at 383 K for 1 h. The maximum error in these measurements was about 5%.

Humic substances were immobilized as described earlier (Klavins and Eglite, 2000) and their basic properties are summarized in Table 1.

Adsorption of *p*-aminoazobenzene (4-amino-1,1′-azobenzene, *p*-AAB) on immobilized humic acids was conducted as batch experiments in 100 ml sealed glass bottles on a rotary shaker table 24 h at 20 °C. 30 ml of either 10, 30, 50, 70, 90, 120 mg l^{-1} solutions of *p*-aminoazobenzene with 100 mg of sorbent were shaken until equilibrium was reached (24 h). After separation of the phases by filtration through Watman paper filter, the concentrations of *p*-AAB in the supernatant phase were determined spectrophotometrically (using a HACH 2000, glass cell with path length 2.5 cm) as absorption at 470 nm (values obtained from initially prepared calibration graph). The quantity of *p*-AAB adsorbed to different phases was determined from the difference of the initial aqueous phase concentration and the amount in solution at equilibrium. Sorption of Crystal Violet (CrV), Methylene Green (MethG), flavine mononucleotide (FMN) and metal ions on immobilized humic substances was performed similarly. Concentrations of metal ions were determined using atomic absorption spectrometry in an air–acetylene flame (Perkin–Elmer 2380 with double beam and deuterium background corrector). pH of solution has been adjusted using citric acid—phosphate buffers.

The sorption test was performed in a laboratory scale column (10 × 250 mm) filled with humic acid immobilized onto aminopropylsilica (IV). A *p*-AAB solution (1; 2; 3 mg l^{-1}) was percolated through the column with a velocity 6 ml min^{-1}, sampling fractions of 18 ml with a "Pharmacia Fine Chemicals" fraction collector and analyzing concentrations of the humic acids remaining in solution.

3. Results and discussion

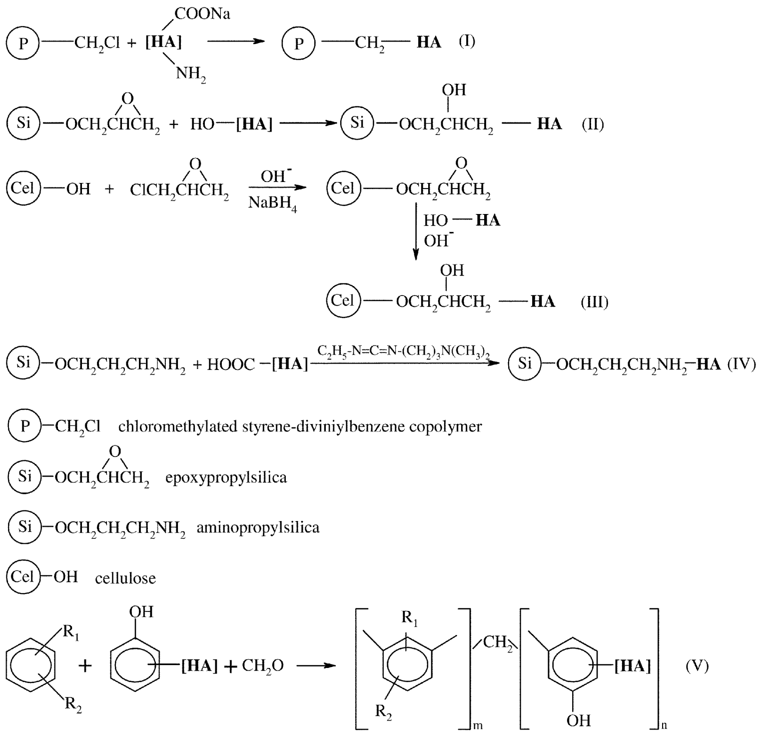
Considering that the properties of humic substances depend on their origin, humic acids of commercial origin (HA_C) as well as HA isolated from the major sources, soil (HA_S) and peat (HA_P), were used to cover the variability in properties of humic acids.

The following methods (Klavins and Eglite, 2000) have previously been used for the immobilization of HS (Fig. 1):

(I) Immobilization by grafting on Merrifield resin or chloromethylated macroreticular styrene–divinylbenzene copolymer;
(II) Immobilization on epoxypropylsilica;
(III) Immobilization on epoxypropycellulose;

Table 1
Properties of immobilized humic acids

Immobilized HA	C (%)	H (%)	N (%)	COOH (mmol g^{-1})	Content of HA (mg g^{-1})	Swelling with water (ml g^{-1})	Swelling with benzene (ml g^{-1})
I-HA_C	86.17	7.29	0.21	0.185	120	0.87	1.85
I-HA_S	76.12	9.16	0.97	0.153	228	0.59	1.06
I-HA_P	77.03	8.21	0.43	0.131	208	0.48	1.01
II-HA_C	12.32	3.74	0.12	0.072	57	0.11	0.35
II-HA_S	12.31	4.76	0.32	0.065	85	0.12	0.45
III-HA_S	43.86	7.30	1.50	0.098	83	0.18	0.56
III-HA_C	42.20	6.13	0.30	0.86	87	0.22	0.64
IV-HA_C	4.67	0.59	0.31	–	7	0.12	0.12
V_A–HA_C	53.93	3.59	1.33	0.723	373	1.15	2.35
V_B–HA_C	66.24	6.39	0.05	1.180	340	0.73	1.87
V_C–HA_C	58.60	6.51	0.13	0.943	185	0.82	1.84
V_D–HA_C	60.98	6.30	0.78	1.322	430	1.14	2.40



R_1, R_2 - H;OH; CH_3
V_A - polycondensation of humic acid
V_B - R_1=H, R_2=OH
V_C - R_1=H, R_2=CH_3
V_D - R_1, R_2=OH

Fig. 1. Methods used for immobilization of humic substances: (I) by grafting on Merrifield resin; (II) on epoxypropylsilica; (III) on epoxypropycellulose; (IV) on aminopropylsilica; (V) by polycondensation with formaldehyde (V_A); phenol (V_B); toluene (V_C) and resorcinol (V_D).

(IV) Immobilization on aminopropylsilica using coupling by means of water soluble carbodimide;
(V) Polycondensation of HA with formaldehyde and molecules able to enter in condensation reactions (phenols).

The suggested approaches allow to remove the immobilized humic macromolecule from the surface of polymer chain and to increase the flexibility of interactions of immobilized humic substances with sorbates.

The obtained immobilized HS were characterized by elemental and functional analysis, as well as by the amount of immobilized HS (Table 1). The differing matrices used in the study and the variability of humic substances employed allow to obtain a range of sorbents with different hydrophobicity of the polymeric matrix, concentration of immobilized humic substances, and also porosity. The surface area of polymers obtained using polycondensation (V_A–V_D, Fig. 1) is low (0.326–0.580 $m^2\,g^{-1}$), higher for sorbents (I) on macroreticular styrene–divinylbenzene copolymer (51.6 $m^2\,g^{-1}$), and for sorbents on silica (II, IV) it can reach even 106 $m^2\,g^{-1}$.

The sorption kinetics (Fig. 2) on the immobilized humic acids depends on porosity of the polymeric

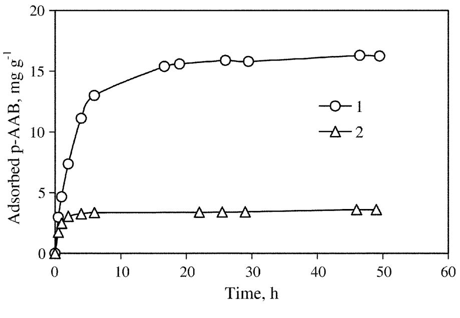


Fig. 2. Sorption kinetics of *p*-aminoazobenzene (*p*-AAB) on immobilized humic acids: (1) I-HA_S, (2) IV-HA_C.

matrix and the hydrophilicity/hydrophobicity balance of the sorbates, being fast for sorption on macroreticular polymers, but comparatively slow on gel-type polymers (immobilized humic substances on styrene–divinylbenzene copolymers or polycondensation polymers).

The character of the sorption process depends on the salt concentration in the solution (Fig. 3). Also in this case the sorption capacity depends on the porosity of the polymer structure which is insignificant for gel-type polymers and substantial for sorbents on aminopropylsilica. The *p*-AAB sorption ability on immobilized humic substances depends also from pH, and with increase of pH in the solution the sorption capacity is increasing (Fig. 4).

The sorption isotherms of *p*-aminoazobenzene (*p*-AAB) on immobilized humic substances are shown in Fig. 5. The adsorption of *p*-aminoazobenzene on immobilized humic substances differs substantially depending on the properties of the original polymeric carrier. On silica polymers the sorption capacity is low (Yang and Koopal, 1999), while on humic substances immobilized on Merrifield resin it can be quite high. However, for polymers obtained using polycondensation with formaldehyde, the specific polymeric matrix as such does not exist. To compare the obtained immobilized humic substances, the sorption was expressed as mg of *p*-AAB on 1 g of sorbent. The experimental data were fitted to empirical Freundlich and Langmuir adsorption equations. We found that the Langmuir isotherm gave the

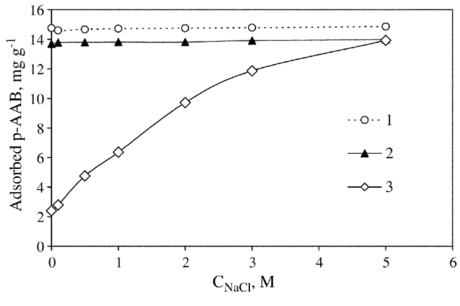


Fig. 3. Sorption of *p*-aminoazobenzene on immobilized humic acids: (1) V_C–HA_P, (2) I-HA_S, (3) IV-HA_C depending on the ionic strength of the solution.

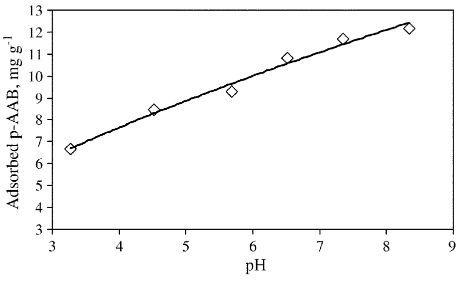


Fig. 4. Sorption of *p*-aminoazobenzene on immobilized humic substances V_B–HA_{coal} (size of polymer particles 0.063–0.160 mm) depending on the pH of the solution.

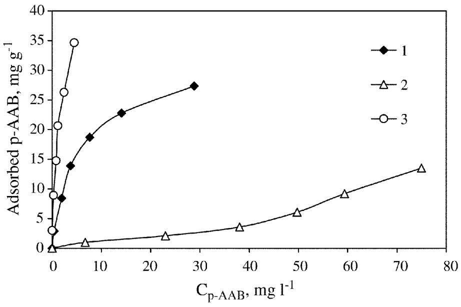


Fig. 5. Sorption of *p*-aminoazobenzene on immobilized humic acids: (1) I-HA_S, (2) IV-HA_C, (3) V_C–HA_P depending on the type of polymeric matrix.

Table 2
Langmuir constants (*K* and *b* are empirical constants related to bonding energy and sorption maximum, respectively) for the sorption of *p*-aminoazobenzene on immobilized humic acids

Immobilized HA	*K*	*b*	r^2
I-HA_S	3.226	0.066	0.88
II-HA_C	0.021	0.01	0.91
III-HA_S	0.085	0.018	0.94
IV-HA_C	0.043	0.031	0.98
V_A–HA_C	0.121	0.039	0.99
V_C–HA_C	6.599	0.055	0.95
V_D–HA_C	0.024	0.028	0.97

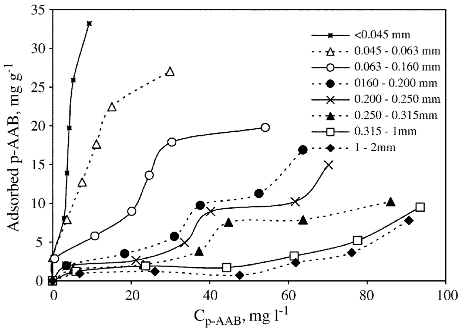


Fig. 6. Sorption of *p*-aminoazobenzene on immobilized humic substances V_B–HA_{coal} depending on the size of polymer particles.

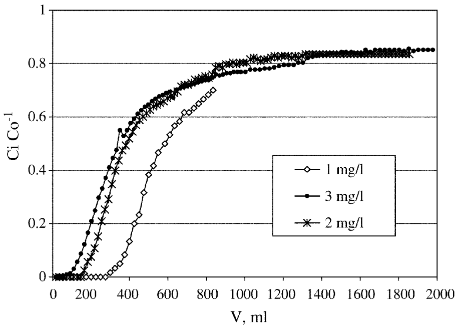


Fig. 7. Sorption of *p*-aminoazobenzene on humic acid immobilized on aminopropylsilica (IV-HA_C) in a laboratory scale column.

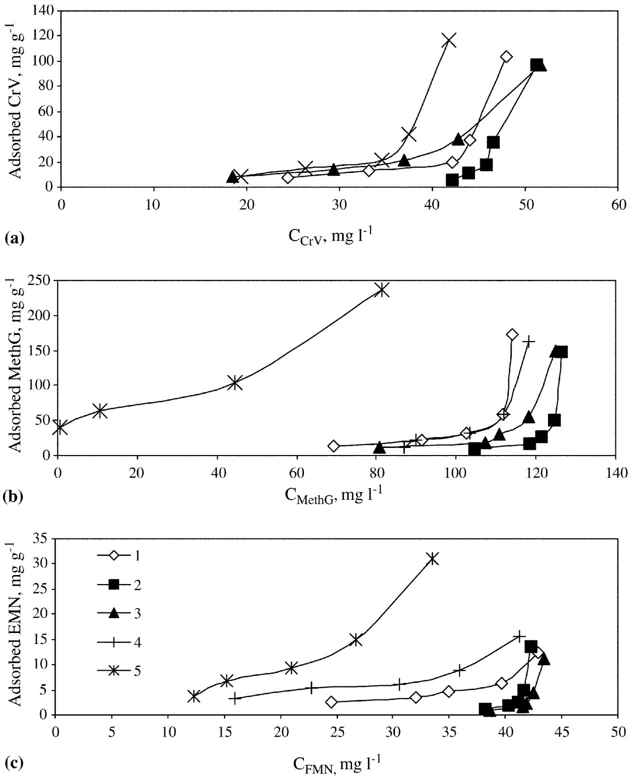


Fig. 8. Sorption of (a) Crystal Violet (CrV), (b) MethyleneGreen (MethG) and (c) flavine mononucleotide (FMN) on immobilized humic acids: (1) I-HA_S, (2) V_B–HA_P, (3) III-HA_C, (4) IV-HA_C, (5) V_C–HA_P depending on the type of polymeric matrix.

Table 3
Sorption of metal ions and organic molecules on immobilized humic substances

IM HV	Cu^{2+} (mg g^{-1})	Co^{2+} (mg g^{-1})	Ni^{2+} (mg g^{-1})	Mn^{2+} (mg g^{-1})	Cd^{2+} (mg g^{-1})
I-HA$_C$	2.5	1.3	1.3	2.4	9.0
I-HA$_P$	10.6	2.8	1.7	3.7	0.0
II-HA$_S$	2.5	1.6	0.3	1.9	9.0
III-HA$_C$	4.6	2.3	1.6	2.7	9.5
IV-HA$_C$	11.9	9.8	5.9	10.1	19.8
V_A–HA$_C$	2.2	1.9	1.3	1.8	9.0
V_B–HA$_C$	6.9	3.6	2.3	3.3	11.0
V_C–HA$_C$	3.1	1.7	1.1	2.8	7.3
V_D–HA$_C$	4.3	1.9	1.7	5.7	13.5

best fit for the description of the *p*-aminoazobenzene sorption. The r^2 values together with Langmuir empirical constants are given in Table 2. Comparison of adsorption isotherms shows substantial differences between the obtained polymers and, possibly also the major impact of the original polymeric matrix: for polymer obtained by grafting humic acid onto Merrifield resin the sorption capacity is higher and can be described by the Langmuir model, while the sorption capacity on humic substances immobilized onto silica is lower. The Langmuir constants *K* and *b*, indicating bonding energy and the maximum sorption capacity, respectively differed from sample to sample, suggesting that various mechanisms of sorption may occur.

The sorption on HS immobilized on a carrier especially on a polymer carrier obtained using polycondensation much depends on the size of the polymeric particles (Fig. 6). The sorption takes place faster for small polymer particles and follows the Langmuir model. However, on large polymer particles obtained using polycondensation (V_A–V_D, Fig. 1), the sorption follows the so-called S-type form, indicating a low surface affinity that increases with surface saturation with sorbate. The polycondensation polymers are gel-type, their swelling in aquatic media much depends on the polymer composition, the sorption in this case is evidently lower than on macroreticular polymers, it takes place on the surface layers of sorbent, and it is diffusion controlled.

The obtained immobilized humic acids demonstrated good sorption properties for column processes (after Fig. 7). For column processes, the most effective sorbents are obtained using more rigid matrices (silica, crosslinked styrene–divinylbenzene copolymer with a high degree of crosslinking) and the retention processes much depend on the concentration of sorbate.

In comparison to the sorption process of *p*-aminoazobenzene, the character of sorption differs for higher molecular-weight polar sorbates, such as the dyes Crystal Violet, Methylene Green, and flavine mononucleotide (Fig. 8). All of these isotherms clearly exhibited evidence for a sequence of adsorption steps and a relatively low affinity of immobilized humic substances with respect to hydrophilic cationic sorbates.

The first stage of the adsorption is characterized by relatively low sorption efficiency, but is followed with a significant increase of the sorbed amount. The affinity of selected sorbates towards the sorbent significantly increases after surface saturation of sorbents is reached. However, immobilized humic substances cannot be considered as good sorbent for sorption of hydrophilic cationic sorbates.

As it is well known, humic substances in solution can form stable complexes with metal ions as well as with organic molecules (after Fig. 8). The same is true also for immobilized humic substances, as shown by the observed sorption of some metal ions and some organic substances (Table 3). Immobilized HS have rather good sorption capacity for a wide range of substances. They are prospective for the investigation of humus properties and can serve as a potential inexpensive sorbents for removal of metal ions and both hydrophobic and hydrophilic organic substances during waste water treatment and drinking water purification procedures.

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References

Bulman, R.A., Szabo, G., 1991. Investigations of the interactions of transuranic radionuclides with humic and fulvic acids immobilized on silica gel. Lect. Notes Earth Sci. 33, 329–336.

de la Rosa, G., Gardea-Torresday, J.L., Peralta-Videa, J.R., Herrera, I., Contreras, C., 2003. Use of silica-immobilized humin for heavy metal removal from aqueous solution under flow conditions. Bioresour. Technol. 90, 11–17.

De Paolis, F., Kukkonen, J., 1997. Binding of organic pollutants to humic and fulvic acids—influence of pH and the structure of humic material. Chemosphere 34, 1693–1704.

Klavins, M., 1993. Humic substances in surface waters: their isolation and structure. Latv. Chem. J. 1, 83–95.

Klavins, M., Eglite, L., 2000. Immobilisation of humic substances. Coll. Surf. A: Physicochem. Eng. Asp. 203, 47–54.

Koopal, L.K., Yang, Y., Minnaard, A.J., Theunissen, P.L.M., Van Riemsdijk, W.H., 1998. Chemical immobilization of humic acid on silica. Coll. Surf. A: Physicochem. Eng. Asp. 181, 385–395.

Laor, Y., Zolkov, Ch., Armon, R., 2002. Immobilizing humic acid in a sol–gel matrix: a new tool to study humic-contaminants sorption interactions. Environ. Sci. Technol. 36, 1054–1060.

Leenheer, J.A., Wershaw, R.L., Brown, G.K., Reddy, M.M., 2003. Characterization and diagenesis of strong-acid carboxyl groups in humic substances. Appl. Geochem. 18, 471–482.

MacCarthy, P., 2001. The principles of humic substances. Soil Sci. 166, 738–751.

Prado, A.G.S., Miranda, B.S., Jacintho, G.V.M., 2003. Interaction of indigo carmine dye with silica modified with humic acids at solid/liquid interface. Surf. Sci. 542, 276–282.

Ritchie, J.D., Perdue, E.M., 2003. Proton-binding study of standard and reference fulvic acids, humic acids and natural organic matter. Geochim. Cosmochim. Acta 67, 85–96.

Szabo, G., Guczi, J., Koblinger Bokori, E., Bulman, R.A., 1998. An examination of the sorption characteristics of ^{241}Am(III) and Eu(III) bound to humic substances chemically immobilized on silica gel. Radiochim. Acta 82, 355–359.

Yang, Y.H., Koopal, L.K., 1999. Immobilization of humic acids and binding of nitrophenol to immobilized humics. Coll. Surf. A. Physicochem. Eng. Asp. 151, 201–212.

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