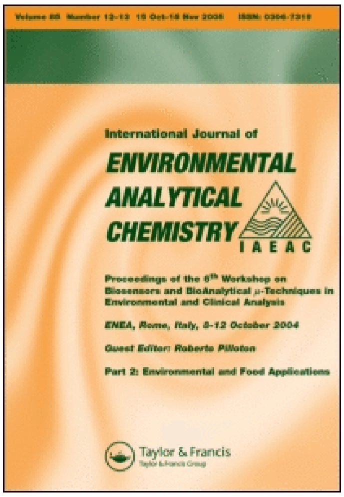

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THREE-DIMENSIONAL, MOLECULAR STRUCTURES OF HUMIC ACIDS AND THEIR INTERACTIONS WITH WATER AND DISSOLVED CONTAMINANTS

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A novel three-dimensional structural concept for humic acids (HAs) in soils and water was developed which was based on comprehensive investigations combining geochemical, wet-chemical, biochemical, spectroscopic, agricultural and ecological data with analytical pyrolysis. Direct, temperature-programmed pyrolysis in the ion-source of the mass spectrometer combined with soft ionization in very high electric fields (Py-FIMS) and Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC-MS) were the principal analytical methods for the proposed humic acid monomer. Emphasis was put on molecular modeling and geometry optimization of humic complexes with biological and anthropogenic substances using modern PC software (HyperChem) in order to determine low energy conformations, space requirements, voids, as well as inter- and intramolecular hydrogen bonds. Initial simulations of molecular model structures of humic acids for trapping and binding of water molecules and humic-contaminant complexes with pentachlorophenol dissolved in water are illustrated. Molecular mechanics calculations are reported (a) to visualize interactions between humic acid and water as well as humic acid and contaminant; (b) to describe the nanochemistry (e.g. bond distances, angles, torsions, bends, van der Waals forces and inter- and intramolecular hydrogen bonds) of humic acids and their complexes in *vacuum* and in *aqueous solution*; and (c) to introduce experiments using periodic boxes containing water molecules with dissolved humic—contaminant complexes.

KEY WORDS: Aquatic humic substances, biocides dissolved organic matter, geometry optimization, humic acid, hydrogen bonds, molecular mechanics, molecular modeling, nanochemistry, pentachlorophenol.

INTRODUCTION

Novel molecular-chemical models for humic substances such as monomer humic acid (HA) have been proposed[1-5] in an integrated approach using a wide variety of analytical methods. The HA model is important because in soils humic substances constitute 70–80% of soil organic matter (SOM) and all chemical reactions of the latter (large surface, voids, high adsorption capacity, good metal complexer, good medium for microorganisms, can store nutrients and especially water) can be explained on the basis of HAs, which are the principal humic compounds in the environment. In the dissolved organic matter (DOM) of most natural waters, HAs are less abundant compared to fulvic acids (FAs) which are richer in oxygen functions such as carboxylic and phenolic groups. Therefore, in the present investigation emphasis was put on interactions between these groups. From the analytical aspect, two independent, complementary techniques:

pyrolysis-mass spectrometry using soft ionization in high electric fields (Py-FIMS) *and* Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC-MS) gave the fundamental structural data[6]. Aquatic humic fractions obtained from surface (river, lake) and ground water[7-11] and dissolved organic matter (DOM) in water extracts from soils[12-14] were successfully investigated by these methods. Moreover, direct analyses of DOM in freeze-dried seepage and ground water from soils[15-17] and organic matter of sewage water for soil irrigation[18] allowed the characterization and identification of the organic molecular constituents by Py-FIMS without further treatment.

Analytical pyrolysis studies so far have been published mostly as two-dimensional (2D) chemical structures and reaction schemes. However, recent progress in powerful, relatively low cost software and personal computers now allows 3D-displays and computer-assisted design (CAD) of structures and model reactions[19]. For molecular modeling, simulation and visualization of complex macromolecules which are often the target of thermal degradation studies, virtually a new dimension is opened up.

Thus, modern techniques of molecular modeling[19] were employed to develop three-dimensional (3D) structures of monomer[20], oligomer[21] and polymer[22] humic acids, organo-mineral soil complexes[23] and soil particles[24]. In particular trapping and bonding of biological (peptides, carbohydrates) and anthropogenic (pesticides, plasticizer) substances[18,25] in the voids of the geometrically optimized structures were investigated. Since all these investigations were performed by calculations in vacuum, it was of interest to evaluate to what extent the gas phase experiments can be transferred to the condensed phase such as water and whether direct modeling of humic substances and their interactions with biocides in water can be performed.

The aims of this study were:

- to illustrate humic acids by 3D structures and molecular mechanics calculations for geometry optimization and thus energy minimization;
- to visualize and simulate interactions between humic acid and water as well as humic acid and contaminant;
- to describe the results of nanochemistry of humic acids and their complexes in *vacuum* and in *aqueous solution;* and
- to report experiments of periodic boxes containing water molecules with dissolved humic—contaminant complexes.

EXPERIMENTAL

The basis for the structural concept of humic acids were geochemical, wet-chemical, reduction-oxidation reactions, ^{13}C-NMR spectroscopic data[5] and results of analytical pyrolysis[4] using the following two complementary methods:

Pyrolysis-field ionization mass spectrometry (Py-FIMS)

For temperature-resolved Py-FIMS, about 100 µg of humic substances such as humic acid (HA), fulvic acid (FA), humin or 5 mg of whole soil samples, respectively, were thermally degraded in the ion source of a MAT 731 (Finnigan, 28127 Bremen, Germany) modified high performance (AMD Intectra GmbH, 27243 Harpstedt, Germany) mass spectrometer. The samples were weighed before and after Py-FIMS (error ± 0.01 mg) to determine the pyrolysis residue and the produced *volatile matter*. The heatable/coolable direct introduction system with electronic temperature-programming, adjusted at the + 8kV potential of the ion source and the field ionization emitter, was used. The slotted cathode plate serving as counter electrode was on –6 kV potential. Thus, at 2 mm distance between the emitter tips and the cathode, in total a potential difference of 14 kV is applied resulting in an extremely high electric field strength which is the essential basis for the described soft ionization method. All samples were heated in high vacuum (1.3 10^{-4} Pa) from 323 K to 973 K at a heating rate of approximately 0.5 K s^{-1}. About 60 magnetic scans were recorded for the mass range m/z 16 to m/z 1,000. In general, at least three replicates were performed for each sample. The total ion intensities (TII) of the single spectra were normalized to 1 mg sample weight, averaged for replicate runs, and plotted versus the pyrolysis temperature, resulting in Py-FIMS thermograms. For the selection of biomarkers and quantitative evaluations, in particular of humic substances whole soils and soil particle-size fractions, detailed descriptions of the method have been published[6].

Curie-point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)

The humic substances and soils were pyrolyzed in a type 0316 Curie-point pyrolyzer (Fischer, 53340 Meckenheim, Germany). The samples were not pretreated except drying and milling. The final pyrolysis temperatures employed were 573 K, 773 K and 973 K, respectively. The total heating time was varied between 3 and 9.9 s.

Following split injection (split ratio 1:3; flow rate 1 ml 20 s^{-1}) the pyrolysis products were separated on a gas chromatograph (Varian 3700, 64289 Darmstadt, Germany), equipped with a 30 m capillary column (DB5), coated with 0.25 µm film thickness and an inner diameter of 0.32 mm). The starting temperature for the gas chromatographic temperature program was 313 K, and the end temperature was 523 K, with a heating rate of 10 K min^{-1}. The gas chromatograph was connected to a, thermoionic nitrogen-specific detector (TSD) and a double-focusing Finnigan MAT 212 mass spectrometer. Conditions for mass spectrometric detection in the electron ionization mode were +3 kV accelerating voltage, 70 eV electron energy, 2.2 kV multiplier voltage, 1.1 s/mass decade scan speed and a recorded mass range between m/z 50 and m/z 500. A detailed description of the principle, potential and limitations of Py-GC-MS of humic fractions and soils has been given[26]. Although Py-GC-MS studies in combination with library searches are time- and labor-consuming, relatively large sample amounts are required, and generally allow only the identification of small, stable pyrolysate constituents, the complementary data are very helpful for the interpretation of Py-FIMS results. In particular, studies of organic nitrogen-containing compounds in soils using the TSD detector were reported and shed some light on the structures of the "unknown" nitrogen[27].

Structural modeling and geometry optimization

The handdrawn humic acid 2D structure[3] was converted to the 3D structural model[20]. For all described 2D and 3D work, model construction, chemical interaction studies and molecular mechanics calculations the HyperChem software (release 4) for Microsoft Windows 95™[19] was used. In the present text some main software commands are indicated in brackets (in *italics*). The original program output in Ångstrom and kcal was given in nm and kJ, respectively. It is noteworthy that all space filling renderings use standard van der Waals radii for the atoms, for instance when the smallest rectangular box (x, y, z) enclosing the molecule is constructed. The employed IBM-compatible personal computer consisted of a tower 486DX2/66, VLB 34 in combination with 32 MB memory, 17" color monitor with AVGA VLB/1MB graphic card, 815 MB disk, and peripheric hardware (e.g. Epson Stylus color printer) plus utility programs.

Isolated gas phase molecules in vacuum are the simplest to treat computationally. For work in the condensed phase, the molecular sytem is placed in a periodic box containing water molecules (*Set Up; Periodic Box*). In both cases, the size and complexity of humic macromolecules require simplifications using a definition of a carbon atom that includes the sp, sp^2 and sp^3 bonded hydrogen atoms (*United atoms*)[19]. In this manner, calculations with the available PC capacity lead to geometry optimization and a low energy conformation. The complete molecule is displayed again with the CH, CH_2 and CH_3 groups upon an alternative command (*All Atoms*).

RESULTS AND DISCUSSION

In view of the molecular size and complexity of humic materials, so far geometry optimizations and nanochemistry investigations by HyperChem were performed in vacuum. The following preliminary studies describe: (i) the interactions of humic acids with isolated water molecules ***in*** vacuum and (ii) calculations of dissolved humic acid and humic acid/contaminant complexes in water boxes.

Interactions of humic acids with water

Figure 1 shows the 3D-structure of a humic complex V which contains the proposed HA structure (I, 738 atoms)[20] with two biological molecules, a sugar (II) and a peptide (III), and ten isolated water molecules (IV) inserted and trapped in the voids. The elemental composition of I was $C_{308}H_{335}0_{90}N_5$ with a molecular mass of 5,547.004 g mol^{-1}. Calculation of the elemental analysis indicated 66.69% C, 6.09% H, 25.96% O, and 1.26% N. Following geometry optimization (Polak-Ribiere algorithm, *United atoms*; terminating gradient < 4.19 kJ $(0.1\ nm)^{-1}$ mol^{-1}) the total energy of the molecular system was calculated to be 1 ,448.0557 kJ $(0.1\ nm)^{-1}$ mol^{-1} at a gradient of 1.03304 kJ $(0.1\ nm)^{-1}$ mol^{-1}. The gradient is defined as the rate of change of the energy of a molecular system as a function of atomic positions[19]. The dimensions of the smallest box enclosing I were: x = 3.0615 nm; y = 2. 1408 nm; and z = 5.0393 nm.

For sugars, the *trisaccharide* (II, 66 atoms) can be regarded as a cellulose subunit that is assumed to be present in organic matter of soils and water. This trisaccharide has the elemental composition $C_{18}H_{32}0_{16}$ with a molecular mass of 504.441 g mol^{-1} and an elemental analysis of 42.86% C, 6.39% H, and 50.75 O. Geometry optimization (and thus energy minimization) was performed by single-point calculation using the algorithm of Polak-Ribiere (*All Atoms*, in vacuum, 660 cycles, 1,480 points) with a convergence limit of 0.419 kJ $(0.1\ nm)^{-1}$ mol^{-1}. For II at a gradient of 0.3874 kJ $(0.1\ nm)^{-1}$ mol^{-1}, the total energy was determined as 144.2888 kJ $(0.1\ nm)^{-1}$ mol^{-1} [6]. The dimensions of the smallest box enclosing II were: x = 0.6954 nm; y = 0.6076 nm; and z = 1.4358 nm.

The *hexapeptide* 1Asp2Gly3Arg4Glu5Ala6Lys (III, 92 atoms) contains characteristic amino acids identified and determined in soils[27,28] and DOM. This peptide has the elemental composition $C_{26}H_{46}O_{10}N_{10}$, elemental analysis of 47.41% C, 7.04% H, 24.29% O, and 21.26% N with a molecular mass of 658.71 g mol^{-1}. Geometry optimization (*All*

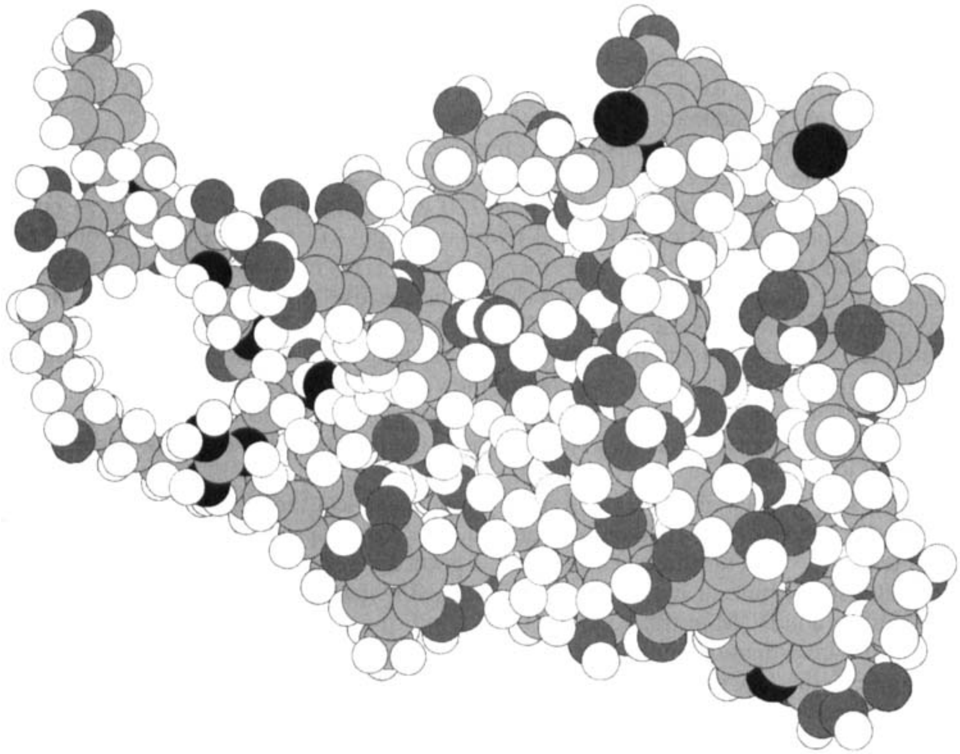


Figure 1 Model 3D-structure of proposed humic acid monomer (I, *All atoms*)[20] with occlusion of a trisaccharide (II) and hexapeptide (III) plus 10 H_2O molecules IV (approx. 3% water content): the resulting humic complex V has 926 atoms. Element colors are: *carbon* = *gray*; hydrogen = white; *nitrogen* = *black*; and *oxygen* = *dark gray*; (HyperChem software commands are given in *italics*). (see Color Plate I at back of journal)

atoms) gave the total energy of III as 2.9095 kJ (0.1 nm)$^{-1}$ mol^{-1} at a gradient of 0.0519 kJ (0.1 nm) mol^{-1}.[6] The dimensions of the smallest box enclosing III were: x = 1.1644 nm; y = 1.0561 nm; and z = 2.1699 nm.

In order to study the interactions between the HA complex with the trapped two biological molecules and water, before geometry optimization ten water molecules were inserted randomly into the workspace (*in vacuum*). At this stage, the distances between each other within the HA structure varied from 0.2 nm to 2.2 nm. The water molecules (IV, 30 atoms, $H_{20}O_{10}$, summed mol. mass = 180.152 g mol^{-1}) were initially distributed in the humic complex of I + II + III at a relatively high total energy level of 7,042.9650 kJ (0.1 nm)$^{-1}$ mol^{-1} and a gradient of 85.4224 kJ (0.1 nm)$^{-1}$ mol^{-1}. Following energy minimization (and approaching an optimal conformation) at a gradient of 4.1698 kJ (0.1 nm)$^{-1}$ mol^{-1}, the total energy dropped to 730.1069 kJ (0.1 nm)$^{-1}$ mol^{-1}.

The HA complex including the water molecules (V) had the elemental composition $C_{352}H_{433}O_{126}N_{15}$ and elemental analysis of 61.36% C, 6.33% H, 29.26% O, and 3.06% N with a molecular mass of 6,890.315 g mol^{-1}. The dimensions of the smallest box enclosing V were: x = 3.0876 nm; y = 1.8311 nm; and z = 4.7574 nm. It is interesting to observe that the occlusion of the two biological compounds and 3% water which is an acceptable value in isolated humic or fulvic fractions, leads to an increase in oxygen by more than 3% and a drop in carbon by more than 5%. Thus, values for the composition of the HA model I and isolated humic fractions from soils and water come to a better agreement, although oxygen deficiency is still a problem if only data from Py-FIMS and Py-GC-MS are considered.

When the HyperChem software is employed[19], hydrogen bonds are formed if the hydrogen-donor distance is less than 0.32 nm and the angle made by covalent bonds to the donor and acceptor atoms is less than 120 degrees. Altogether 11 hydrogen bonds developed between: (a) carboxyl and hydroxy groups (I) 2; (b) carboxyl groups and water (I and IV) 2; (c) carboxyl and ether groups (I) 1; (d) hydroxyl and hydroxyl groups (I) 2; and (e) water—water (IV and IV) 2. Whereas five water molecules were clearly fixed at defined hydrophilic positions in the HA complex, the other five drifted through the molecule during energy minimization with intermediate hydrogen bonds but also by simple trapping in hydrophobic portions of the voids. One has to keep in mind that in this first step geometry optimization (and thus energy minimization) was performed in vacuum by molecular mechanics calculations using the algorithm of Polak-Ribiere (202 cycles, 430 points).

Interestingly even after the occlusion of two biological molecules and the ten water molecules, the HA conformation obtained after geometry optimization still showed more than ten open voids of different size. Thus, a good argument for the network structure as characteristic feature of humic acids[1,3] is obtained which should be valid even more for fulvic acids and DOM in water.

For the investigation and prediction of water-solute interactions of humic substances in Figure 2 the HA monomer I[20] was submerged into a periodic box formed by 5,575 well-defined water molecules using the HyperChem software. The software uses TIP3P models of water molecules[29] equilibrated at 300 K (25°C) and one atmosphere to solvate a molecular system in a cubic box. In total 17,463 atoms are shown (HA, 738 + water 16,725). A summed molecular mass of the water box of 105,983.6 g mol^{-1}, elemental composition of $C_{308}H_{11485}O_{5665}N_5$ and elemental analysis of 3.49% C, 10.92% H, 85.52% O, and 0.07% N were calculated. In the center of the displayed water box a few atoms of the HA structure are discerned, but in general, even in high quality color plots the overall picture of 5578 molecules is difficult to evaluate as such. Therefore, in the following alternative ways to display structural features are selected. The HyperChem programs include the options to select atoms, molecules or residues for closer investigations or to cut slabs of different size and depth (z-clipping tool) from the water box for better visualization and improved characterization.

The clipping of slabs out of the periodic water box in the direction of the z-axis are available in the workspace between the front at 4.0 nm and back at 21.81 nm. The constructed water-humic acid solute system extends from 10.02 nm front to 15.79 nm back according to the dimensions of the water box in the z-direction. The selected section of the HA in water (*All Atoms*) shown in Figure 3a ranges from 13.28 nm front to 16.08 nm back and thus corresponds to a thickness of the clipping slab of 2.8 nm. This slab contains in total 1836 atoms. The corresponding elemental composition of $C_{103}H_{1259}O_{572}N_2$ and an elemental analysis of 10.68% C, 10.08% H, 79.0% O, and 0.24% N were calculated (summed mass 11,584.8 g mol^{-1}).

When focusing on details in this selected slab, Figure 3b illustrates a selected, enlarged section with an *intramolecular* hydrogen bond of the HA between H(49) and O(16). In total 222 atoms were selected including 45 water molecules.The 0(8) and H(49) hydroxyl group is fixed by C(4) on one end and on the other by a carboxyl function (C(14) and 0(16)) which is bound to an aryl carbon moiety with

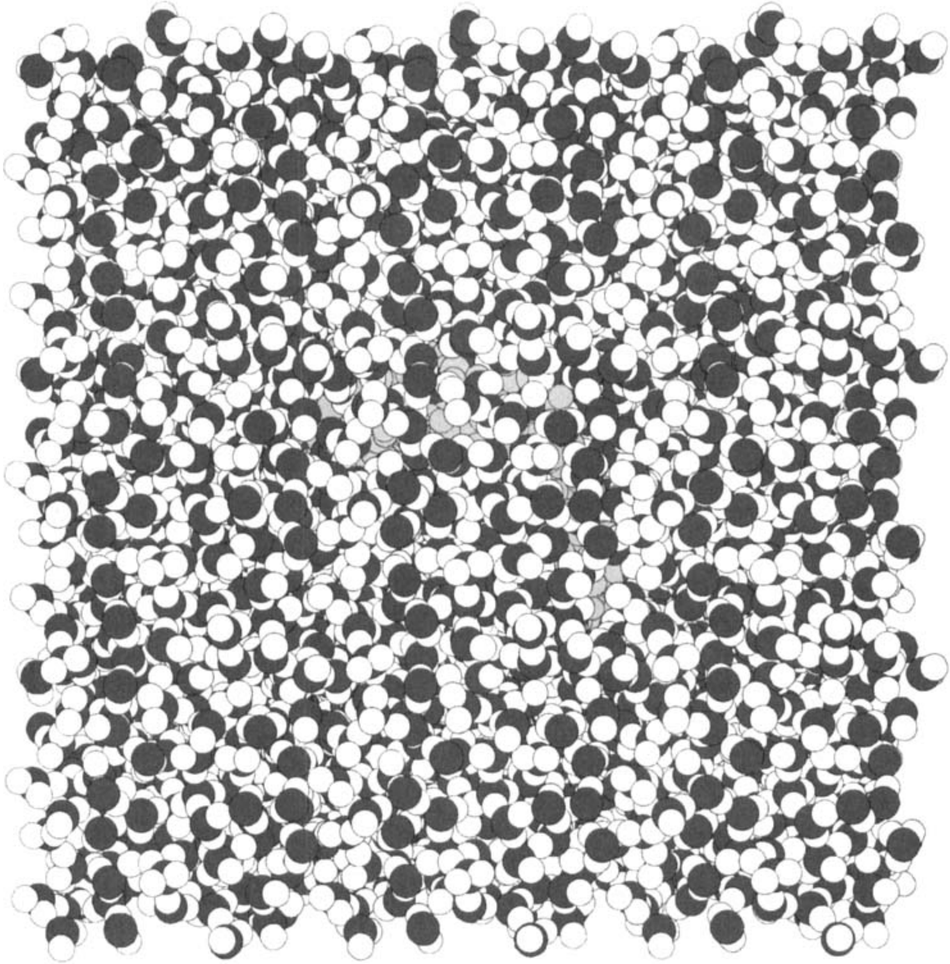
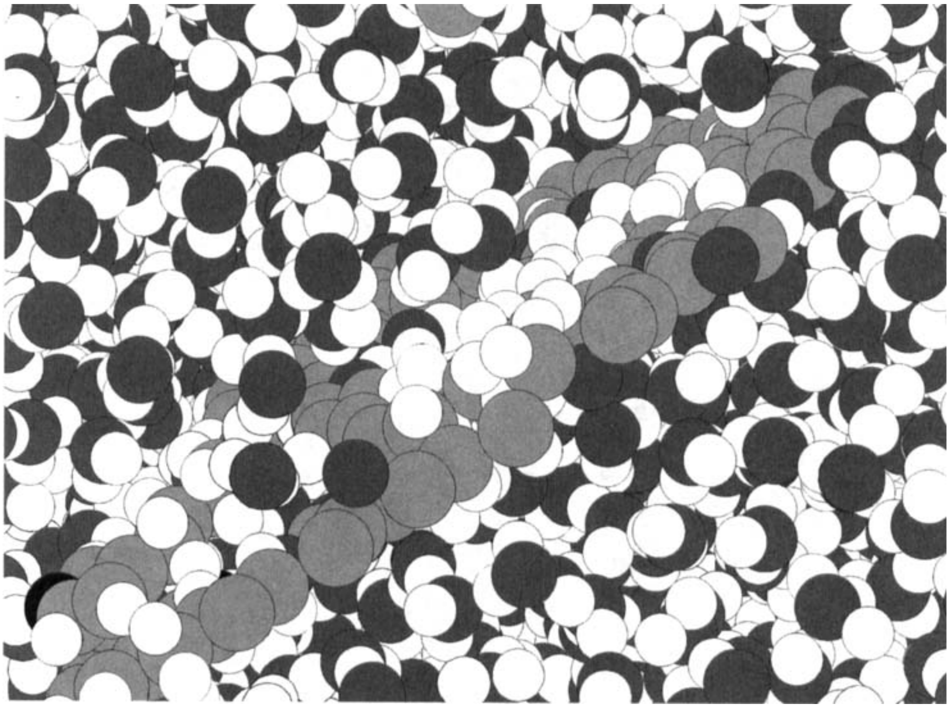


Figure 2 Periodic box of 5,575 water molecules (16,725 atoms) into which the HA (I, 738 atoms, see Figure 1) is submerged. The dimensions of the cubic water box are: x, y, z = 5.6104 nm. The minimum distance between solvent and solute is 0.23 nm. Hydrogen atoms are white, oxygen atoms *dark gray* and HA atoms (*scarcely visible in the center*) *light gray*. (see Color Plate II at back of journal)

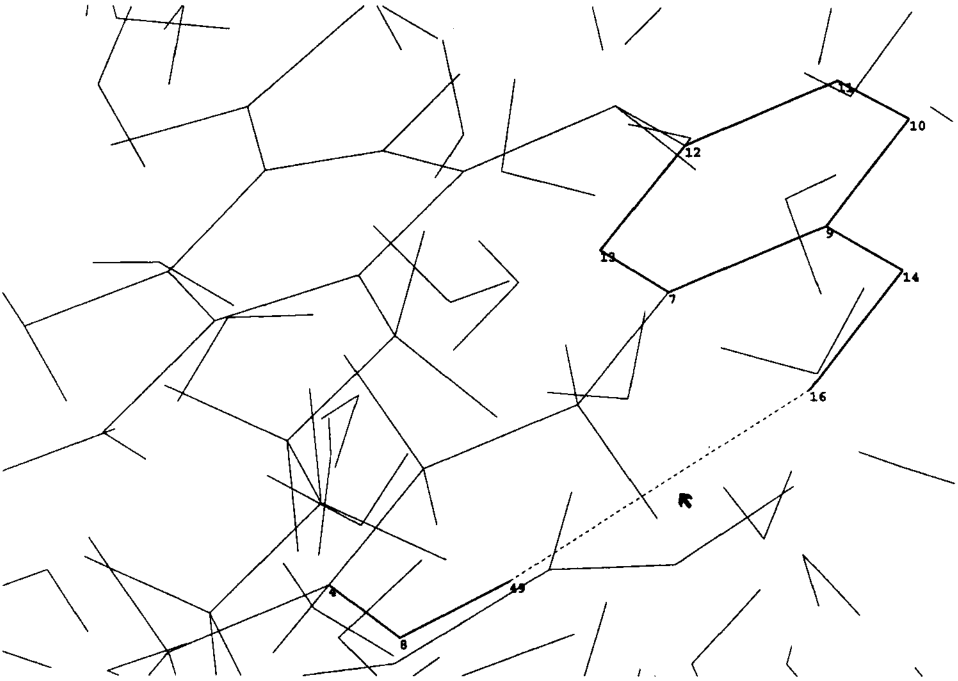
C(7,9,10,11,12,13). The distance between H(49) and 0(16) was calculated to be 0.2735 nm.

Intermolecular hydrogen bonds between the HA and vast majority of water molecules were observed much rarer than expected. For instance in Figure 3c a large section of the clipping slab (see Figure 3a) contains 350 atoms including about 87 water molecules. The corresponding elemental composition of $C_{41}H_{213}O_{96}$ and elemental analysis of 21.95% C, 9.57% H, and 68.47% O were calculated (summed mass 2,243.077 g mol^{-1}). The selected water molecule marked by O(1), H(2) and H(3) is attached via hydrogen bridge to the keto group of the HA (O(38) and C(32)). Without further geometry optimization only this one hydrogen bond is detected in the described slab.

3a



3b



See caption on facing page.

3c

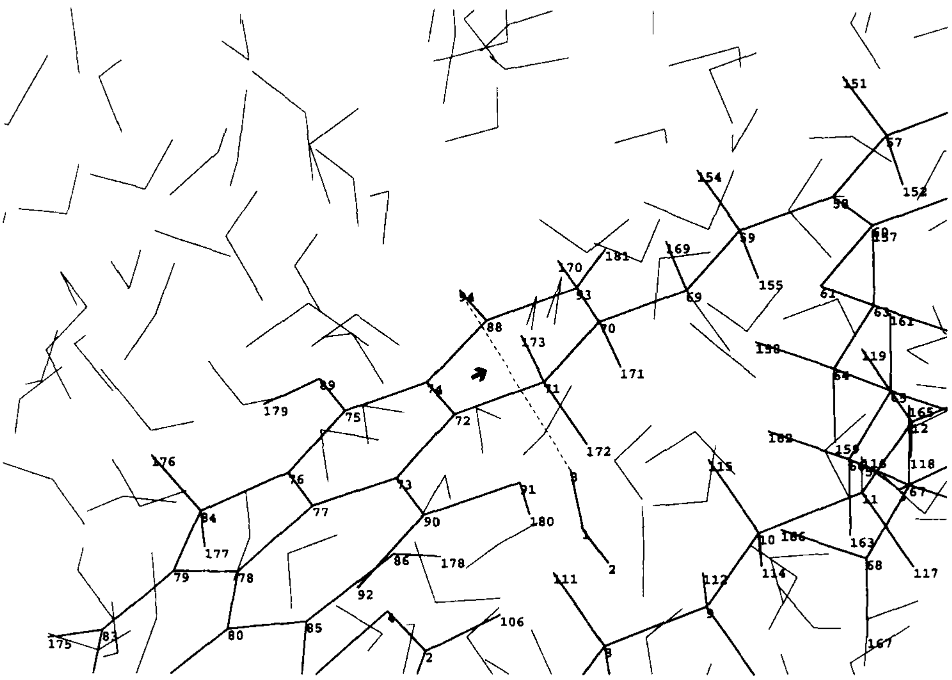


Figure 3 Sections selected with increasing magnification of the HA[20] in a z-clipping slab of the periodic water box: a) disks, 1836 atoms (*All Atoms*); hydrogen (white), carbon (*gray*); oxygen (*dark gray*); nitrogen (*black*); b) *intramolecular* HA hydrogen bond (dotted line between H(49) and O(16): see →); and c) *intermolecular* hydrogen bond (dotted line: see →) between O(94) of the humic acid and H(3) of a water molecule. *Both HA and the reacting water molecule are highlighted by thick lines and labelled by atom* numbers (*Preferences, Thick lines; Display; Labels*). The distance of this bond was determined to be 0.2735 nm. (see Color Plate III at back of journal for Figure 3a)

Interaction of humic acids with contaminants in water

The influence of an anthropogenic compound such as a pesticide on the structure of humic substances was evaluated in two steps: (a) in vacuum and (b) dissolved in water. As an example of a pesticide, pentachlorophenol (VI, 13 atoms) was chosen because of two reasons. Firstly, from the ecological aspect, this compound is of environmental concern for instance in water protection areas. Secondly, from the structural and modeling aspect, it was of interest to investigate a simple and easily discerned molecule which displays a relatively nonpolar moiety of aromatic chlorine atoms and a polar phenolic function. Pentachlorophenol is almost insoluble in water (8 mg in 100 ml) and forms a sodium salt, sodium pentachlorophenate which is soluble in water; and is applied as insecticide for termite control, pre-harvest defoliant, and general herbicide[30]. It has the elemental composition C_6HOCl_5; molecular mass of 266.338 g mol^{-1}; and the elemental analysis of C 27.06%, H 0.38%, O 6.01%, and Cl 66.56%.

In vacuum geometry optimization of VI with the termination gradient of < 0.0419 kJ $(0.1\ nm)^{-1}\ mol^{-1}$, gave a total energy of 33.6579 kJ $(0.1\ nm)^{-1}\ mol^{-1}$ and a convergence gradient of 0.00078 kJ $(0.1\ nm)^{-1}\ mol^{-1}$. The smallest box enclosing VI had the dimensions x = 0.57585 nm; y = 0.00007; and, z = 0.54316 nm which is consistent with a thin, planar molecule with respect to the y-axis and different sizes in the x,y-plane.

Dissolved in a periodic water *box* (*Smallest box enclosing solute:* x, y, z = 1.8701 nm) containing 208 water molecules, neither VI nor its sodium salt showed intermolecular hydrogen bonds with water when geometry optimization (190 calculation cycles, 621 points) was performed at a convergence gradient of 0.3981 kJ $(0.1\ \mathrm{nm})^{-1}\ \mathrm{mol}^{-1}$. The corresponding total energy of solute VI plus the 208 solvent molecules was 2,302.2165 kJ $(0.1\ \mathrm{nm})^{-1}\ \mathrm{mol}^{-1}$.

The complex of pentachlorophenol (VI) with the HA molecule I[20] (without the 12 trapped molecules as described in Figure 1) was constructed (VII, 751 atoms) and geometrically optimized to observe movements and bonding capacities during energy minimization. For complex VII the elemental composition of $C_{314}H_{336}O_{91}N_5Cl_5$, elemental analysis of 64.88% C, 5.83% H, 25.04 % O, 1.2% N, and 3.05% Cl, and molecular mass 5,813.347 g mol^{-1} were determined using HyperChem.

As shown in Figure 4a in the upper right portion of the contaminant-HA complex (VII), before starting the molecular mechanics calculations, the planar pentachlorophenol molecule had been rotated into the x,y-plane and shifted along the z-axis into a void of the HA structure. Three large, light gray disks visualize three chlorine atoms with the adjacent six carbon atoms of the benzene ring of the molecule. The carbon atoms are smaller, dark gray and partially overlapped by three (HA) hydrogens. During progressing geometry optimization, the pentachlorophenol molecules stayed trapped and closely intercalated in the HA void, mainly oriented and moving in small distances in the z-axis. No formation of hydrogen bonds was observed at this stage, although the HA molecule displayed an increasing number of intramolecular hydrogen bonds.

Following molecular mechanics calculation of VII below a gradient level of < 2 kJ $(0.1\ \mathrm{nm})^{-1}\ \mathrm{mol}^{-1}$ resulted in a total energy of the humic-contaminant complex of 804 kJ $(0.1\ \mathrm{nm})^{-1}\ \mathrm{mol}^{-1}$. The dimensions of the smallest box enclosing VII were: x = 3.1219 nm; y = 1.8087 nm; and z = 4.7526 nm. Geometry optimization (and thus energy minimization) led to the formation of an *intermolecular* hydrogen bond between the hydrogen of a free carboxylic group of the HA and the phenolic oxygen of pentachlorophenol as in shown in Figure 4b, the walls of a HA void formed by two long C-C chains were expanded by the trapped contaminant and immobilization occurred through hydrogen bonding (see dotted line). In the vicinity of VI, hydrophobic and hydrophilic portions of the walls of the HA void are observed in accordance with the chemical properties of the contaminant. Continued calculations of VII kept the hydrogen bond intact, whereas calculations of the selected, displayed section in Figure 4b alone resulted in rupture of the H bond.

In Figure 5a the cubic water box filled with 5521 water molecules is shown with the submerged solute VII (751 atoms) which had been geometrically optimized in vaccum as described above. For the whole solute-water system an elemental composition of $C_{314}H_{11378}O_{5612}N_5Cl_5$, elemental analysis of 3.58% C, 10.89% H, 85.29% O, 0.07% N and 0.17% Cl, and summed molecular mass of 105,277.1 g mol^{-1} were determined. In black/white as well as color plots, it is difficult to discern details of the dissolved HA-pentachlorophenol complex, not to speak of intra- and/or intermolecular bonding. Using the tools offered by the HyperChem software, an attempt was made to zoom in on the interactions between VII and the solvent water and to visualize the bonds between the contaminant and HA and water.

In the first step, clipping tool in depth x-axis to delete the water molecules that obscure of the binding slab in front and the back. The depth of the cubic water box is 2.7 nm (*System*: *back* 11.22 nm and *front* 8.6 nm). As shown in Figure 5b, clipping of the

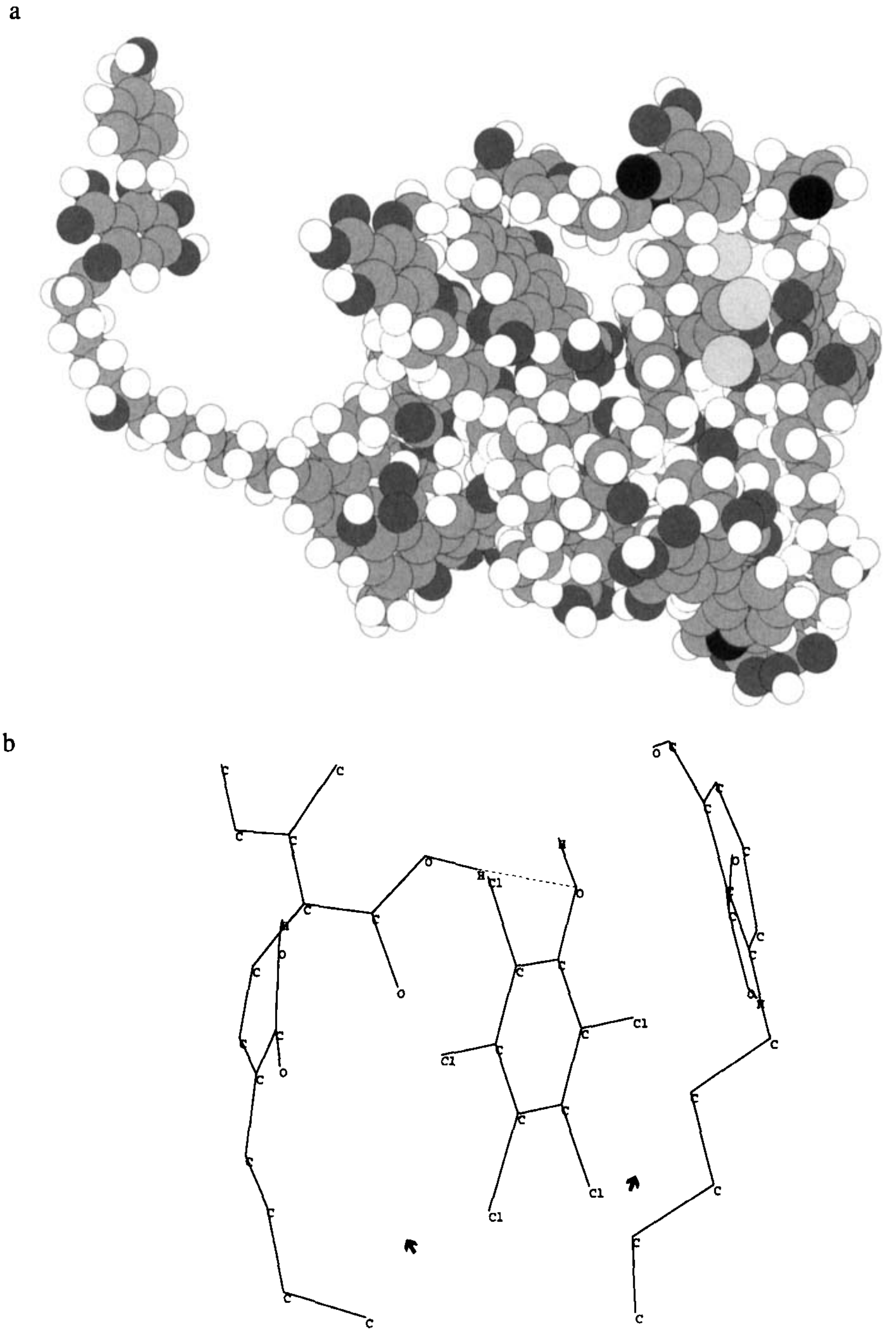


Figure 4 a) 3D model of the humic-contaminant complex (VII, 751 atoms) constructed from HA (I) and pentachlorophenol (VI) following geometry optimization using HyperChem (chlorine atoms in light gray); and b) highly magnified slab (70 atoms) of VII shows the *intermolecular* hydrogen bond between I and VII with a measured distance of 0.2151 nm (*Show/Recalculate hydrogen bonds*). The hydrophobic portions of the voids enclosing VII are marked by two arrows (→). (see Color Plate IV at back of journal for Figure 4a)

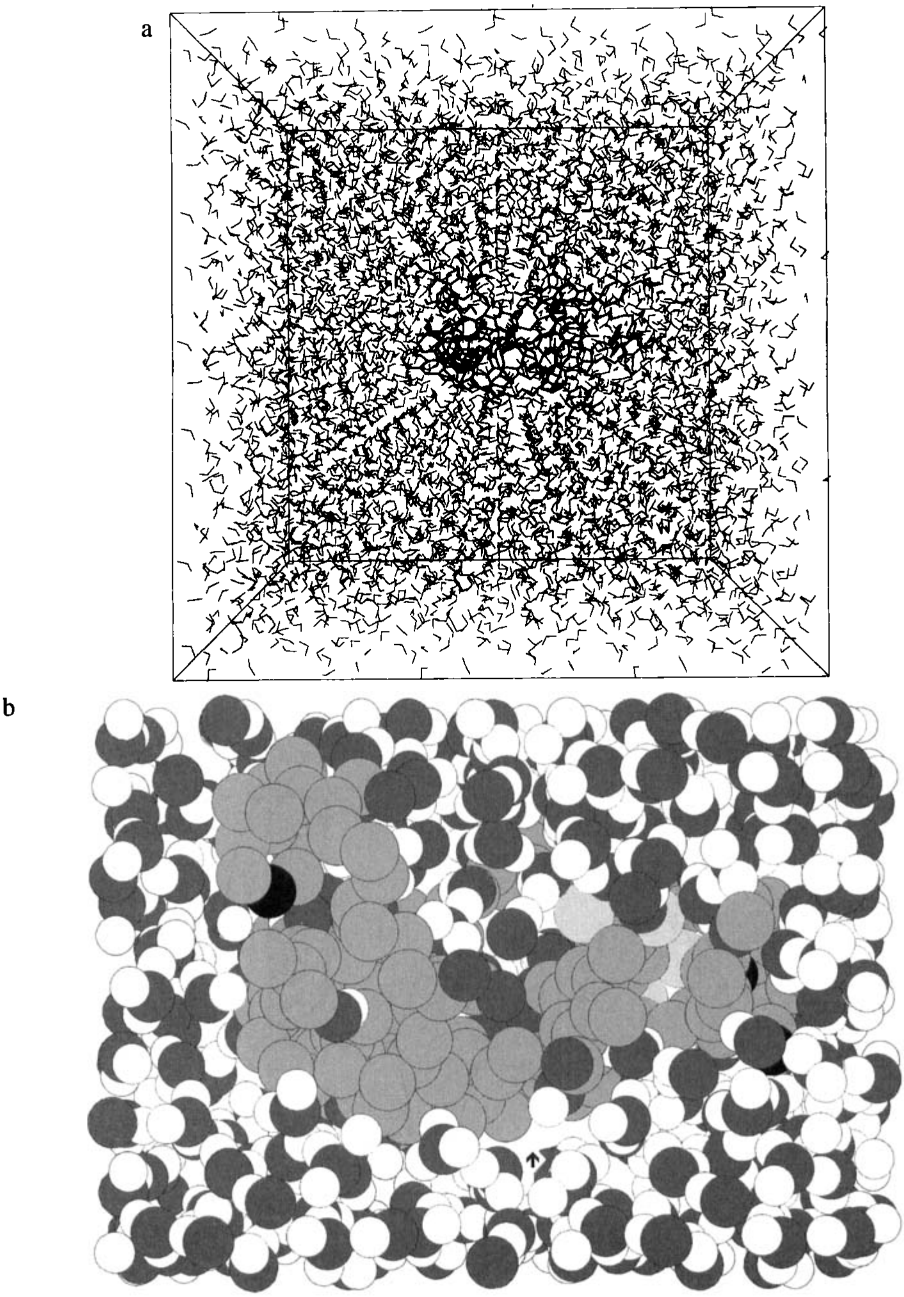


Figure 5 a) Simulation of the dissolving process of the HA-contaminant complex VII in a periodic water box (17,318 atoms; *Display; Perspective*). The dimensions of the water box are: x, y, z = 5.6104 nm. The minimum distance between solvent and solute is 0.23 nm. In the center of the cubic box, the bonds of the humic acid and the hydrogen-bonded pentachlorophenol were selected and displayed in thick lines (*Thick lines*).; and b) following geometry optimization of the solute—water complex, VII was clipped in the z-axis by 10.17 nm and the enlarged slab of the complex was selected (2132 atoms, *Disks, United atoms*). These displayed atoms are: hydrogen (white), oxygen (dark gray), carbon (gray) and chlorine (light gray). The hydrophobic HA surface is marked by arrow (→). (see Color Plate V at back of journal for Figure 5b)

system at 10.17 nm deletes the water molecules behind the complex VII and illustrates only the front slab with a depth of 10.17 nm. Both the humic acid and the contaminant are very tightly surrounded by water molecules, however, the basic HA network structure with the corresponding long-chain aliphatics, aromatic subunits and frequent voids can be evualated. Two point are particularly remarkable: (1) in the vicinity of the aliphatic chains clearly gaps have opened up which are obviously not accessable by water molecules due to hydrophobic HA properties in these areas. On the other hand, areas of high densities of hydroxyl- and carboxylic functions are closely packed by water and indicate hydrophilic HA properties. Thus, micelle formation of humic substances can be examined at nanochemistry level. (2) Although the void that immobilized pentachlorophenol in the HA void (cf. Figure 4a,b) is cut open by the clipping tool, four chlorine atoms are now visible and a hydrophobic cavern with HA is formed as indicated already in Figure 4b. In view of the complexity of the investigated HA/contaminant/water systems it is also clear that without color displays and a more detailed presentation of the structural features, it is difficult to gain a more simple system without loss of relevant information. Therefore, in addition the same visualized portion in the slab (2132 atoms) is shown in Figure 5c the *Sticks* mode and the trapped contaminant VI given in heavy lines. The hydrophobic moieties near IV and HA are marked by arrows (→).

In the second step, a section of 161 atoms was cut out of the slab shown above (cf. Figure 5b). For this section, an elemental composition of $C_{52}H_{73}O_{31}CL_5$, elemental analysis of 45.54% C, 5.37% H, 36.17% O, and 12.93% Cl, and summed molecular mass of 1,371.395 g mol^{-1} were calculated. The selected atoms are labelled by numbers in Figure 6a and by element symbols in Figure 6b. In the center of the slab section, the aromatic carbon atoms C(2), (3), (6), (5), (4) and (1) forming the benzene ring and the five chlorine atoms (8), (9), (10) (11), (12) of pentachlorophenol (VI) are clearly observed. Highly interesting is the formation of an *intermolecular* hydrogen bond between a carboxylic group of the HA (aromatic C(12), carboxyl C(13), keto O(14), hydroxy O(15) and attached hydroxyl H(136) with the hydroxyl O(7) of VI (see dotted line; distance 0.2145 nm). This immobilization of VI by HA is in agreement with the process observed by geometry optimization in vacuum (cf. Figure 4a,b). Despite the fact that there is a high density of water molecules near the hydroxyl function of VI, *no* hydrogen bond with water is produced. In contrast, five *intermolecular* hydrogen bonds between water molecules and two *intramolecular* hydrogen bonds between HA subunits are generated. This observation is supported by Schnitzer[31] who investigated water retention by humic substances and found that the increase in water adsorption with higher relative humidity suggests that water molecules are more tightly hydrogen-bonded to each other than to humic and fulvic acids.

CONCLUSIONS

The capacity of computational chemistry for simulation and visualization of chemical reactions such as water/humic acid interactions, and formation of aquatic humic-contaminant complexes on the basis of analytical pyrolysis results has been demonstrated. Since carboxylic and phenolic groups play the central role, the obtained results can be regarded as representative for HAs, FAs and DOM. Four main facts emerge:

(1) *in the voids*, the network spaces of the proposed dynamic HA model are sufficient to trap and bind biological molecules such as a sugars, peptides and 3% water. The

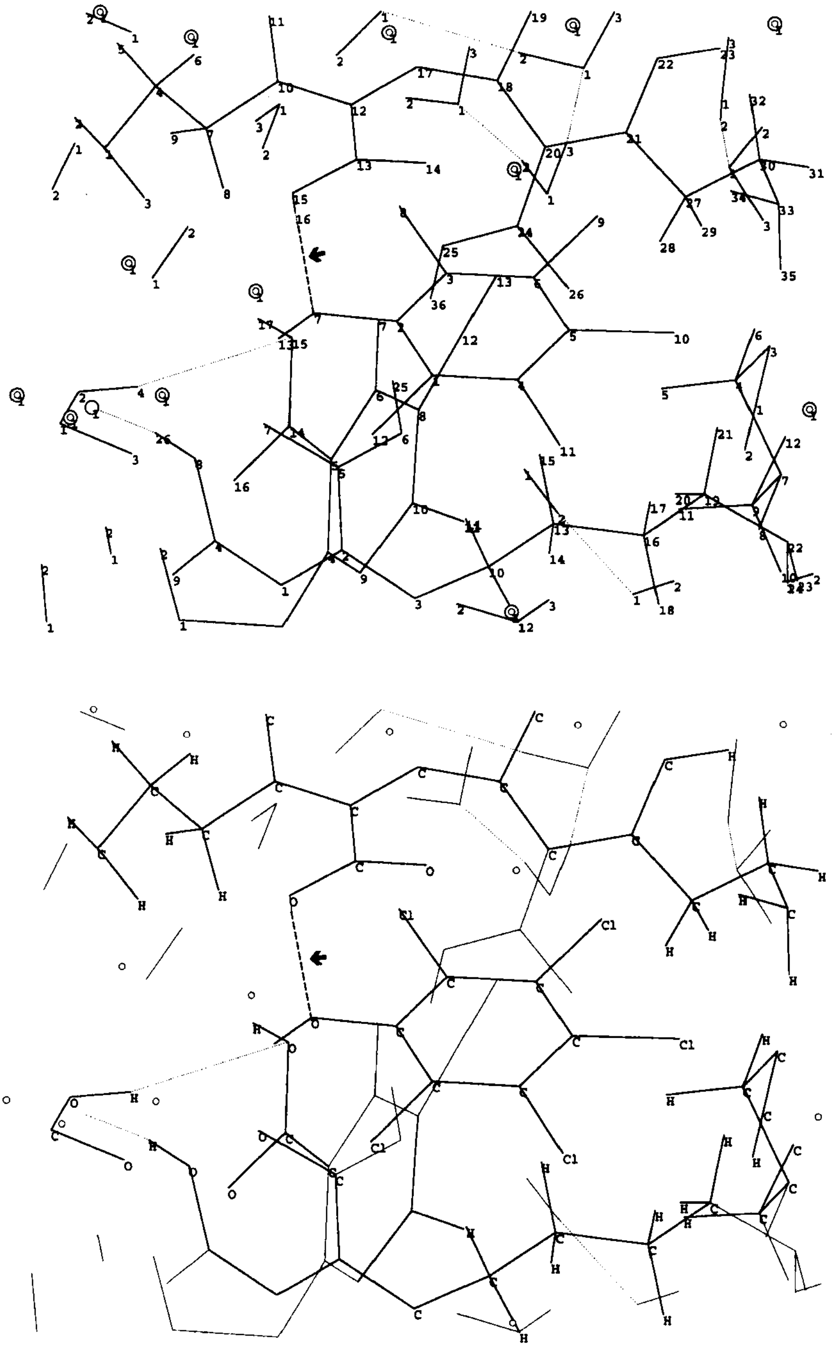


Figure 6 Section (161 atoms) in a slab of the dissolved contaminant-HA complex (VII) in the periodic water box showing the structural environment of the pentachlorophenol binding to the humic structure by adding labels a) of atom numbers; and b) of atom symbols. *The intermolecular* hydrogen bond between HA and VI in water is shown by dotted lines (see: →).

obtained occlusion complexes improve the agreement in elemental analyses between natural HA standards and the hypothetic model;

(2) *in vacuum*, the molecular mechanics calculations of humic substances and DOM (which are faster and require less computer capacity compared to those in condensed phases) are helpful as first approximation to investigate reactions of these complex materials in water. Furthermore, precise data on humic bonds at nanometer level are supplied and simulation and prediction of interactions with biocides are feasible. One should be aware, however, that usually a local (not a global) minimum on the potential surface is reached;

(3) *in water*, geometry optimization (and thus energy minimization) gives insight into hydrophobic and hydrophilic properties, surface tension, micelle formation, and although ionic charges have not been considered at this stage, a more realistic picture of the chemical and ecological potential of humic substances can be offered; and

(4) *in general*, computational chemistry offers relatively economic, powerful tools to observe binding of humic substances to biological molecules and anthropogenic compounds at nanometer level using exact bond distances, bond angles, torsion angles, non-bonded distances, van der Waals forces, ionic charges and intra- and intermolecular hydrogen bonds (nanochemistry). This novel tool should be well suited for fundamental and applied investigations of physical properties, chemical and biological reactivity and molecular dynamics of humic substances and dissolved organic matter in water.

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