RESEARCH ARTICLE

Co-polymerization of penta-halogenated phenols in humic substances by catalytic oxidation using biomimetic catalysis

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Abstract

Introduction A synthetic water-soluble *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrinate of iron(III) chloride, Fe-(TDCPPS)Cl, was employed to catalyze the oxidative co-polymerization of penta-halogenated phenols in two humic materials of different origin.

Materials and methods Co-polymerization of pentachlorophenol (PCP) was followed by high-performance size-exclusion chromatography (HPSEC), the unbound PCP recovered from reacting humic solutions was evaluated by gas-chromatography/electron capture detector, and the oxidative catalyzed coupling of pentafluorophenol (PFP) into humic matter was assessed by liquid-state ^{19}F-NMR spectroscopy. HPSEC showed that the catalyzed oxidative coupling between PCP and humic molecules increased the apparent weight-average molecular weight (M_w) values in both humic substances.

Results and discussion HPSEC further indicated that the co-polymerization reaction turned the loosely bound humic supramolecular structures into more stable conformations, which could no longer be disrupted by the disaggregating effect of acetic acid. The occurrence of covalent linkages established between PCP and humic molecules was also suggested by the very little amount of PCP found free in solution after the catalyzed co-polymerization. ^{19}F-NMR spectroscopy suggested that also PFP could be oxidatively coupled to humic materials. PFP-humic co-polymerization reaction produced ^{19}F-spectra with many more ^{19}F signals and wider chemical shifts spread than for PFP alone or PFP subjected to catalyzed coupling without humic matter.

Conclusions These findings show that biomimetic iron-porphyrin is an efficient catalyst for the covalent binding of polyhalogenated phenols to humic molecules, thereby suggesting that the co-polymerization reaction may become a useful technology to remediate soils and waters contaminated by polyhalogenated phenols and their analogues.

Keywords Humic substances · Biomimetic catalysis · Environmental remediation

1 Introduction

Polyhalogenated aromatic compounds, such as chlorophenols, constitute a major group of environmental pollutants having been extensively used as wood preservatives, pesticides, and herbicides (Puhakka and Järvinen 1992; Armenante et al. 1999). Chlorophenols undergo several natural environmental processes such as biodegradation, chemical and photochemical degradation, volatilization, dispersion, and adsorption (Czaplicka 2004). The transformations of environmental contaminants are often mediated by humic substances (HS), the major component of natural organic matter (Piccolo and Celano 1994; Piccolo et al. 1996; Simpson 2006; Celano et al. 2008). HS are ubiquitous in waters, soils, and sediments and are progressively regarded as supramolecular structures of

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self-associated small biomolecules held together by hydrophobic forces in metastable conformations (Piccolo 2001, 2002; Peravuori 2005; Simpson 2002), which can be disrupted by the action of organic acids (Piccolo et al. 1999).

Oxidative coupling reactions catalyzed by natural oxidoreductase enzymes, such as peroxidase, are believed to degrade chlorophenols in soil. Enzymes catalyze production of highly reactive free radicals by removing an electron and a hydrogen ion from chorophenol hydroxyl groups (Park et al. 1999; Kobayashi et al. 2001). The final products are dechlorinated and oligomerized/polymerized chlorophenols which are less bioavailable, less mobile, and less toxic. Due to the high cost and poor environmental activity of these enzymes, no serious attempts have been conducted to use oxidative enzymes in environmental remediation practices.

An efficient alternative is represented by biomimetic catalysts, such as synthetic metal-porphyrins (Sheldon 1994; Nam et al. 2000), which exert the same enzymatic activity without incurring in the same pitfalls. Water-soluble iron-porphyrins are nontoxic compounds which have been successfully applied as biomimetic catalysts to dechlorinate and detoxify chlorophenols (Fukushima et al. 2003a, b; Hahn et al. 2007) and other organic compounds (Traylor et al. 1984; Song et al. 1997; Meunier and Sorokin 1997). Metal-porphyrins were also found to catalyze polymerization of HS by coupling the humic phenolic moieties present in HS by either chemical oxidation (Piccolo et al. 2005; Šmejkalová and Piccolo 2006; Šmejkalová et al. 2006) or photo-oxidation (Šmejkalová and Piccolo 2005). However, the role of HS in the catalyzed transformation of chlorophenols was so far explained with enhanced oxidation and dechlorination reaction rates (Fukushima et al. 2003a, b; Hahn et al. 2007) and stabilization of iron-porphyrin catalyst on HS (Fukushima and Tatsumi 2006; Fukushima et al. 2007).

In this work, we aimed to show that oxidative reactions catalyzed by synthetic metal-porphyrins in the presence of humic structures inactivate and detoxify chlorophenols through co-polymerization in HS. The study was conducted with pentachlorophenol (PCP) and its fluorinated homologue pentafluorophenol (PFP). The co-polymerization of PCP with humic molecules was assessed by evaluating the molecular dimension of humic matter and the amount of PCP remained free after the catalyzed oxidative reaction with PCP. Due to the large natural abundance and NMR detectability of the ^{19}F nucleus, PFP was selected to obtain more resolved and sensitive ^{19}F-NMR spectra of co-polymerized products than those observable by less abundant ^{13}C nuclei in ^{13}C-NMR spectra of PCP.

2 Materials and methods

2.1 Materials

Pentachlorophenol and pentafluorophenol were acquired from Sigma Aldrich (Germany) at 99% purity and used without further purification. Information on the synthesis, structure, and characterization of the water-soluble meso-tetra-(2,6-dichloro-3-sulfonatophenyl)-porphyrinate of Fe (III) [Fe(TDCPPS)Cl] used here as a biomimetic catalyst (FeP) are reported elsewhere (Piccolo et al. 2005).

The humic acids (HA) were extracted from an oxidized coal (HA-COX) provided by Eniricerche S.p.a. (Italy) and from a North Dakota lignite (HA-LIG) provided by Mammoth, Int. Chem. Co. (USA). The original material was shaken overnight in a 0.5-M NaOH and 0.1-M $Na_4P_2O_7$ solution under N_2 atmosphere. The HA was precipitated from the alkaline extract by adding 6 M HCl until pH 1, purified by three cycles of dissolution in 0.1 M NaOH and precipitation in 6 M HCl, treated with a 0.5% (v/v) HCl–HF solution for 48 h, dialyzed against deionised water until chloride-free (Spectrapore, 3500 Mw cut-off), and freeze-dried. An aliquot of each HA was then suspended in 100 ml of deionised water and titrated (VIT 90 Videotitrator, Radiometer, Copenhagen, Denmark) with a CO_2-free solution of 0.1 M KOH to pH 7. The resulting potassium humate was filtered through a Millipore 0.45 μ, freeze-dried, and homogenized in agate mortar. The chemical and spectroscopic characteristics of both HA-COX and HA-LIG were previously reported (Cozzolino et al 2001).

2.2 Co-polymerization reaction

Control humic solutions of both HA-LIG and HA-COX were prepared by re-dissolving 2.0 mg of potassium humate in 10 ml of deionised water. These solutions were modified as follows: (1) addition of 1 mg of PCP, to obtain a second control; (2) addition of 0.1 mg of FeP (100 μL of a 0.0072 mM FeP water solution) and 50 μL of a freshly prepared 5% (w/v) H_2O_2 solution as oxidizing agent, to obtain a third control of the reaction without PCP; (3) addition of the same amount of FeP catalyst, H_2O_2 and PCP as above, to induce the oxidative co-polymerization reaction. Further HA control solutions were prepared by adding either FeP or H_2O_2 alone, in the amounts described above. The solutions were vigorously stirred and then incubated in daylight. Preliminary high-performance size-exclusion chromatography (HPSEC) experiments indicated that oxidative polymerization was negligible after 24 h at room temperature. Therefore, after 24 h of reaction time, all samples were filtered through a 0.45-μm glass filter (Whatman GF/C) and injected in the HPSEC system. Glacial acetic acid (Carlo Erba, Italy) was added (about

60–70 μL) to both control and reaction solutions until pH-meter readings passed from about 6.5–7 to 3.5, and the solutions were re-analyzed by HPSEC.

PFP was also co-polymerized with HA to acquire ^{19}F-NMR spectra of products. The reaction solution contained 10 mg of either COX or LIG potassium humate, 50 mg of PFP, 5 mg of FeP, and 250 μL of 5% (w/v) H$_2$O$_2$, in 50 mL of deionised water. After 24 h of incubation in daylight, the solution pH was brought to 1 by adding 6 N HCl and the precipitated HA was isolated by centrifugation, washed twice with deionised water, and freeze-dried. The freeze-dried reaction products were dissolved in deuterated DMSO for NMR spectral acquisition.

2.3 HPSEC

The HPSEC system was composed by a Shimadzu LC-10-AD$_{VP}$ pump equipped with a Rheodyne rotary injector and a 100-μL sample loop. A Polysep-GFC-P-3000 600× 7.5 mm i.d. column and a Polysep-GFC-P-3000 75× 7.5 mm i.d. pre-column (Phenomenex, Inc., CA, USA) were used. The elution flow rate was set to 0.6 mL min^{-1} for an eluting solution made of 0.1 M NaH$_2$PO$_4$, buffered at pH 7 with 0.5 M NaOH, filtered through Millipore 0.45 μm, and degassed with He. Detection was achieved at 30°C constant temperature by using a Gilson 116 UV-detector operating at 280 nm and a Unipoint Gilson Software to record each run automatically. The column total (V_t=13.47 mL) and void volume (V_0=6.29 mL) were measured with water and a Blue dextran (M_w=2,000 kDa) aqueous solution, respectively, and calibrated with polystyrene sulphonates of known molecular weights (130,000, 32,000, 16,800, 6,780, and 4,300 Da, Polymer Standard Service, Germany). The apparent weight-averaged molecular weight (M_w) was calculated from triplicate HPSEC chromatograms, as previously reported (Piccolo et al. 2005). The M_w are apparent values because not only molecular associations were measured instead of single molecules, but also because there are no standards which may precisely represent complex humic structures. The relative standard deviation of calculated values varied to a maximum of 7%.

2.4 Quantification of co-polymerized PCP

Different PCP concentrations (0.1, 0.5, 1, 5, and 10 μg mL^{-1}) were prepared in 1 mL of deionised water and added with 0.2 mg of either COX or LIG potassium humates, 1 μg of FeP (100 μL of a 7.2×10^{-5} mM FeP water solution), and 5 μL of a 5% (w/v) H$_2$O$_2$ solution, and incubated for 24 h. Control solutions were prepared with either PCP alone or added with the same HA amount as above. Each reaction and control solution was loaded on a

Strata-X (Sigma Aldrich, Germany) SPE column to separate PCP from HA. The adsorbed PCP was then eluted with methanol, dried under N$_2$, re-suspended in acetonitrile, and sylilated with BSTFA [N,O-bis(trimethylsilyl) trifluoroacetamide, Sigma Aldrich, Germany] at 80°C for 30 min. Sylilated samples were dried under N$_2$ flux and then re-dissolved in 1 mL of n-hexane (MS grade, Merck, Germany) before determination.

Silylated PCP samples were determined with an RtX-5 30-m long, 0.25-mm i.d., fused silica capillary column (Restek Corporation, Bellefonte, PA) using a Clarus 500 gas chromatograph (PerkinElmer, Palo Alto, CA) equipped with an electron capture detector (ECD) and set with a split ratio of 20:1. Helium was the carrier gas (1 mL min^{-1}) and a 96% argon/4% methane gas mixture was the make-up gas (30 mL/min). The injector temperature was set at 250°C and the oven temperature went from the initial 80°C to the final 200°C at 15°C min^{-1} rate. This temperature was kept for 5 min, then at 15°C min^{-1} the oven temperature reached 250°C, which was kept for 3 min more. PCP was determined by an external calibration curve, with relative standard deviation among triplicates of a maximum of 5%.

2.5 ^{19}F-NMR spectroscopy

^{19}F-NMR spectra were acquired with a Bruker UltraShield 500 MHz magnet operating at a proton frequency of 500.13 MHz and at a fluorine frequency of 470.59 MHz, equipped with a Quadruple (QXI) Resonance Probe (5 mm PAQXI Z-grad), and using a Carr-Purcell-Meiboom-Gill sequence to suppress broad lines with proton decoupling. The ^{19}F-NMR chemical shift scale was calibrated using 0.05% trifluorotoluene in CDCl$_3$, resonating at −63.72 ppm.

3 Results and discussion

3.1 Analytical HPSEC

The effects of the catalyst-assisted oxidative co-polymerization of PCP into selected HA were assessed by evaluating the apparent weight-average molecular weights (M_w) of resulting reaction mixtures for both HA-LIG and HA-COX. The co-polymerized PCP-HA solutions were injected into the HPSEC system either before or after addition of acetic acid that was used to lower the mixture pH to 3.5. It has been shown that the hydrophobically stable HA supramolecular conformations are altered when added with organic acids to lower the pH to 3.5, as new and stronger intermolecular hydrogen bonds are formed between complementary humic functions (Piccolo et al. 1999, 2002, 2003; Cozzolino et al. 2001). However, if humic supramolecular conformations are

firmly stabilized by intermolecular covalent bonds induced by catalytic oxidative polymerization, their alteration by pH lowering is significantly reduced (Piccolo et al. 2005; Šmejkalová and Piccolo 2005).

The HPSEC chromatograms for both HA-LIG and HA-COX illustrate the molecular size distribution of two humic solutions subjected to different treatments and after acetic acid addition (Fig. 1). Elution profiles are relative to control HA solutions at pH 6.5 (Fig. 1a), and humic solutions undergone the catalyzed oxidative coupling reaction without and with PCP (Fig. 1b and c, respectively), whereas the modifications induced by acetic acid addition in the respective solutions are shown by chromatograms in Fig. 1a′, b′, and c′. For both control solutions, the diffuse peak at about 9 mL of elution volume in control chromatograms was reduced considerably after acetic acid addition, though more extensively for HA-COX than for HA-LIG. The absorption was shifted to larger elution volumes, whereby two additional peaks appeared in the case of HA-COX, whereas a single peak was shown for HA-LIG at about the column total volume. This behavior further confirms earlier reports that indicated HA signals in HPSEC as due to apparently large, though weakly associated molecular sizes, which are easily disrupted by pH change (Piccolo et al. 1999, 2002; 2003).

The elution profiles of humic solution varied substantially when the materials were treated with FeP catalyst and H_2O_2 oxidant (HA + FeP; Fig. 1b). For both HA, the first peak at about 9 mL increased significantly in absorbance as compared to control, and, in the case of HA-LIG, it even appeared at slightly lower elution volumes. The concomitant presence of PCP in the reaction solution (HA + FeP + PCP) increased further the first peak intensity (Fig. 1c), thereby implying a contribution of PCP to the peak absorbance. Contrary to control, when the HA + FeP and HA + FeP + PCP solutions were treated with acetic acid (Fig. 1b′ and c′), the first peak maintained a similar, if not larger, absorbance, though its maximum was shifted to larger elution volumes. Moreover, while the HPSEC profile of HA-COX added with acetic acid was similar to control, the HA-LIG solution showed two more chromatographic peaks which were not present in the AcOH-treated control solution. The limited action of acetic acid to modify the chromatographic behavior of both HA + FeP and HA + FeP + PCP solutions to the same extent as that of the control suggests that intermolecular covalent bonds must have been formed among humic molecules and/or between these and PCP. This means that the oxidative biomimetic catalysis was successful in stabilizing the previously loose humic conformation, thus implying the occurrence of HA poly-

Fig. 1 HPSEC chromatograms of HA-COX and HA-LIG solutions under different treatments: (a) HA control solution before and (a′) after acetic acid addition; (b) HA + FeP reaction solution without PCP and (b′) after acetic acid addition; (c) HA + FeP + PCP reaction solution and (c′) after acetic acid addition
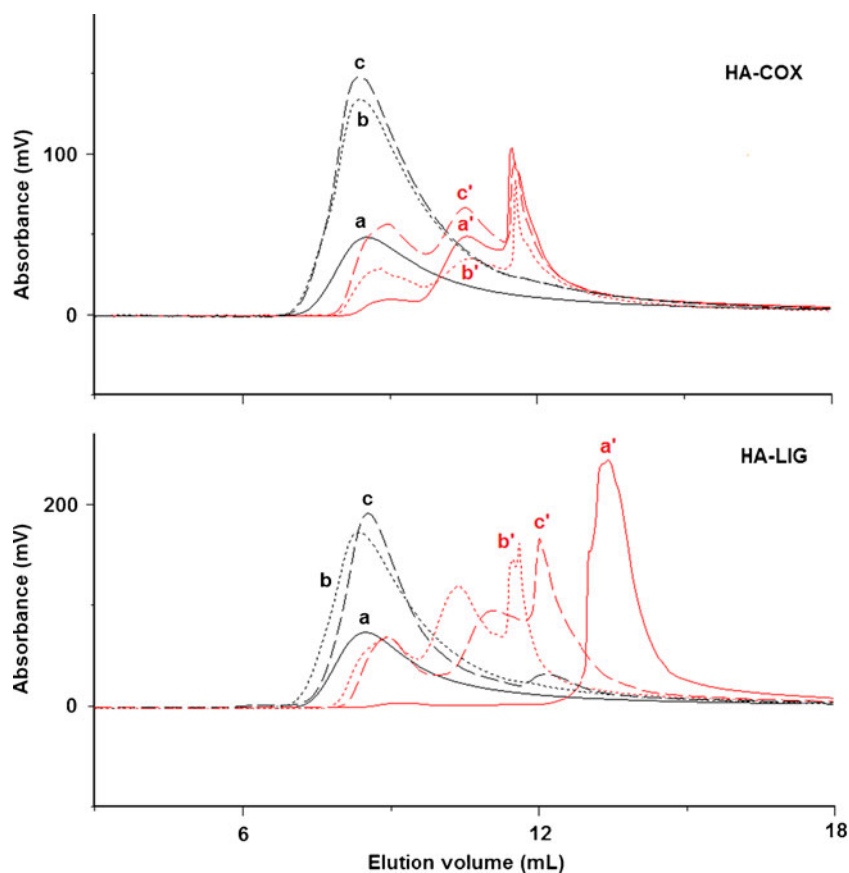
Table 1 Apparent weight-averaged molecular weight (M_w) and their standard deviation (±SD) of humic solutions under different treatments, as measured from HPSEC chromatograms before and after acetic acid (AcOH) addition

The Δ value is referred to M_w difference between the specific M_w and its respective control solution

Solutions	HA-COX	HA-LIG
HA	4,268±238	4,568±159
HA + AcOH	315±20	246±15
HA + PCP	3,031±166 $\Delta=-1,236$	4,178±72 $\Delta=-391$
HA + PCP + AcOH	590±7 $\Delta=+275$	233±13 $\Delta=-12$
HA + FeP	5,844±359 $\Delta=+1,576$	7,380±247 $\Delta=+2,811$
HA + FeP + AcOH	1,700±22 $\Delta=+1,385$	1,580±67 $\Delta=+1,334$
HA + FeP + PCP	5,897±75 $\Delta=+1,629$	6,190±226 $\Delta=+1,622$
HA + FeP + PCP + AcOH	1,484±55 $\Delta=+1,169$	802±14 $\Delta=+556$

merization (Piccolo et al. 2005; Šmejkalová and Piccolo 2005) and PCP-HA co-polymerization.

The M_w values obtained from size-exclusion chromatograms are shown in Table 1. The humic solutions added with PCP alone (not shown in Fig. 1) revealed a decrease in the M_w value, in respect to control solution, more for HA-COX than for HA-LIG. This should be interpreted with the ability of the poorly water-soluble PCP to interact with the hydrophobic domains of humic superstructures, and thus weaken the humic conformational stabilities (Cozzolino et al. 2001). Nevertheless, it appears that PCP established stronger hydrophobic interactions with HA-COX than with HA-LIG and thus limited the conformational disruption of HA-COX following AcOH addition (positive ΔM_w values in respect to control; Table 1). When the humic solution was subjected to the catalyzed oxidative reaction the behavior observed in chromatograms either without or with PCP (Fig. 1) was reflected by M_w values, which were significantly larger than control (Table 1). This may be, respectively, explained with either a self-polymerization among humic molecules or a co-polymerization of PCP with humic molecules. Moreover, the M_w values for both HA-COX and HA-LIG remained significantly larger than for control even after acetic acid addition. This confirmed that humic conformations had been stabilized by the occurrence of covalent bonds either among humic molecules or between PCP and humic molecules (Piccolo et al. 2005; Šmejkalová and Piccolo 2005). However, it must be noted that M_w values for the oxidative coupling either with or without PCP did not show a substantial difference, thus implying that self-polymerization of humic molecules was equally conformationally determinant as PCP co-polymerization.

3.2 GC–ECD

In order to verify whether PCP was really co-polymerized in humic matter, the PCP remaining free in solution after the co-polymerization reaction was isolated and quantified by gas-chromatography/electron capture detector (GC–ECD; Table 2). Free PCP recovered from solution with HA was slightly different from that obtained from the blank solution without HA, the deviations growing larger with greater PCP concentration. The differences should be attributed to a physical adsorption of PCP on the humic structures. Conversely, when PCP was subjected to the catalyzed oxidative reaction in the presence of both HA materials, the contaminant remaining free in solution at all concentrations significantly decreased (Table 2), thus implying that PCP was mostly bound to humic molecules by a covalent coupling promoted by the FeP catalyst.

Oxidized PCP reaction products were not detected by GC–ECD (Fig. 2), thus implying that they, if formed, may have also been co-polymerized in humic matter. Moreover, it was noted that the PCP covalent binding to both humic materials increased with the progressively larger PCP concentration in the reaction mixture. In fact, at the greatest PCP concentration (10 mg L^{-1}), the disappearance of free PCP from solution was nearly 100% for both HA, thereby

Table 2 Added and recovered PCP (mg L^{-1}), as detected by GC/ECD, when present alone in solution (blank), in addition to HA (PCP + HA), and in the catalyzed oxidative co-polymerization reaction (PCP + HA + FeP)

Added PCP	Recovered PCP		
	Blank	PCP + HA	PCP + HA + FeP
HA-COX			
10	9.68±0.20	8.55±0.82	0.76±0.15
5	4.66±0.36	4.54±1.45	0.23±0.05
1	1.28±0.05	1.36±0.07	0.08±0.05
0.5	0.39±0.03	0.56±0.02	0.10±0.04
0.1	0.09±0.00	0.08±0.00	0.01±0.00
HA-LIG			
10	9.68±0.20	9.61±0.42	0.02±0.00
5	4.66±0.36	3.84±0.24	0.21±0.02
1	1.28±0.05	0.21±0.01	0.04±0.01
0.5	0.39±0.03	0.42±0.04	0.22±0.01
0.1	0.09±0.00	0.10±0.01	0.05±0.01

Fig. 2 GC–ECD chromatogram of products after catalyzed co-polymerization reaction between HA-COX and PCP (10 mg L^{-1}) under daylight for 24 h. Attenuation was raised from 0 to 10 after 7 min when sylilating byproducts peaks were not longer eluted
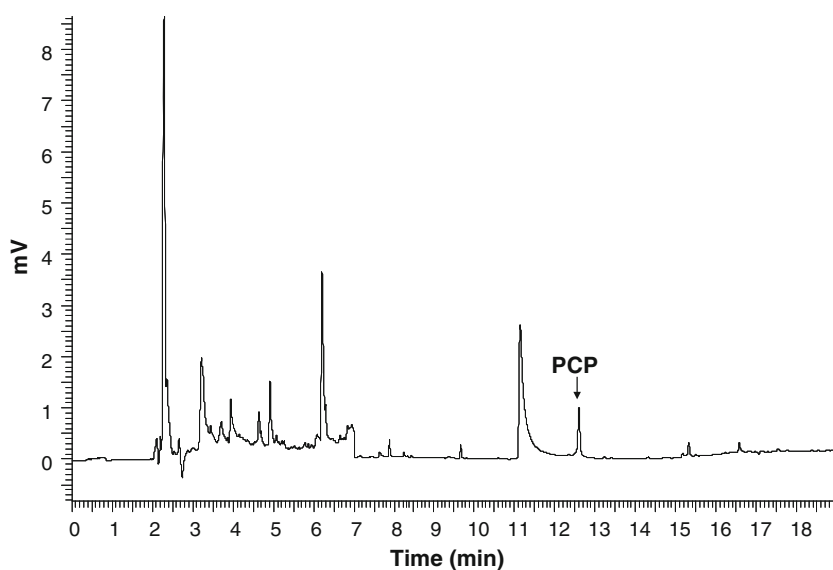
suggesting a large potential of humic matter to co-polymerize this contaminant. While the two HA did not generally behaved substantially different towards PCP catalyzed co-polymerization, PCP addition in no-catalyst conditions seemed to show a trend of larger adsorption on HA-LIG than on HA-COX (Table 2). This may be attributed to a greater presence of flexible long-chain alkyl molecules in HA-LIG (Cozzolino et al 2001), which may be conformationally more efficient in weakly interacting with PCP.

3.3 ^{19}F-NMR spectroscopy

Both liquid- and solid-state ^{13}C-NMR spectroscopy were previously used to determine whether ^{13}C-labeled contaminants were weakly or covalently bound to humic substances (Hatcher et al. 1993; Guthrie et al. 1999). However, unless the contaminant is adequately labeled with ^{13}C nuclei, it is hard to obtain ^{13}C-NMR spectra sufficiently sensitive to show the contaminant ^{13}C signals above those of HA. In this work, we employed pentafluorophenol, a homologue of PCP (Šmejkalová et al. 2009), to exploit the larger detectability of ^{19}F nuclei in liquid-state ^{19}F-NMR spectroscopy than that of natural abundant ^{13}C nuclei in ^{13}C-NMR spectra of PCP. In ^{19}F-NMR spectra of HA solutions added with PFP, only the ^{19}F nuclei are visible and it is thus immediate to verify whether or not PFP is covalently bound to HA. In fact, if PFP did not covalently couple with humic molecules, one would expect the ^{19}F-NMR spectra to be similar to those of PFP alone. Conversely, if the catalyzed oxidative reaction was capable to covalently bind PFP to humic molecules and, thus, change the magnetic environment of PFP molecules, ^{19}F-NMR spectra should show as many additional ^{19}F signals at

different chemical shifts as the new PFP different covalent bonds to humic molecules.

The ^{19}F-spectra obtained after different experiments entailing PFP reactions were almost identical for both HA-COX (not shown) and HA-LIG (Fig. 3). Spectrum A in Fig. 3 was obtained with a solution containing HA, PFP, and H$_2$O$_2$, but without the FeP catalyst. This ^{19}F-spectrum shows only three signals at −161.6, −165.3, and −171.8 ppm for the five fluorine atoms in PFP. Since it is identical to that of PFP alone (not shown), it indicates that no structural modification was induced in PFP by the concomitant presence of humic matter and H$_2$O$_2$.

Conversely, the reaction conducted without HA, but with PFP, H$_2$O$_2$, and the FeP catalyst, provided a different signals distribution in the ^{19}F-spectra (spectrum b in Fig. 3). Though the signals of PFP as such were still visible, a number of additional resonances appeared in spectrum B, mainly in the downfield spectral region. These signals should be attributed to a de-halogenation and a self-oligomerization of PFP under oxidative catalytic conditions, based on signals attribution of similar NMR systems observed under enzymatic catalysis (den Besten et al. 1993; Cnubben et al. 1995; van der Bolt et al. 1997). Though the precise assignment of these signals cannot be provided here, the three signals at −138.1, −149.5, and −160.4 in spectrum b (Fig. 3) may be temptatively attributed to a PFP dimeric structure (Ando and Matsuura 1995). These results clearly show that only the action of the biomimetic catalyst was capable to produce a structural modification of PFP under oxidative conditions.

Furthermore, when the FeP-catalyzed oxidative reaction was conducted in the presence of humic matter, the ^{19}F-spectra showed an even more complex spectral region

Fig. 3 ^{19}F-NMR spectra of reaction solutions: **a** HA-LIG + PFP + H_2O_2, **b** PFP + FeP + H_2O_2 (no HA), and **c** HA-LIG + PFP + FeP + H_2O_2 under exposition to daylight for 24 h
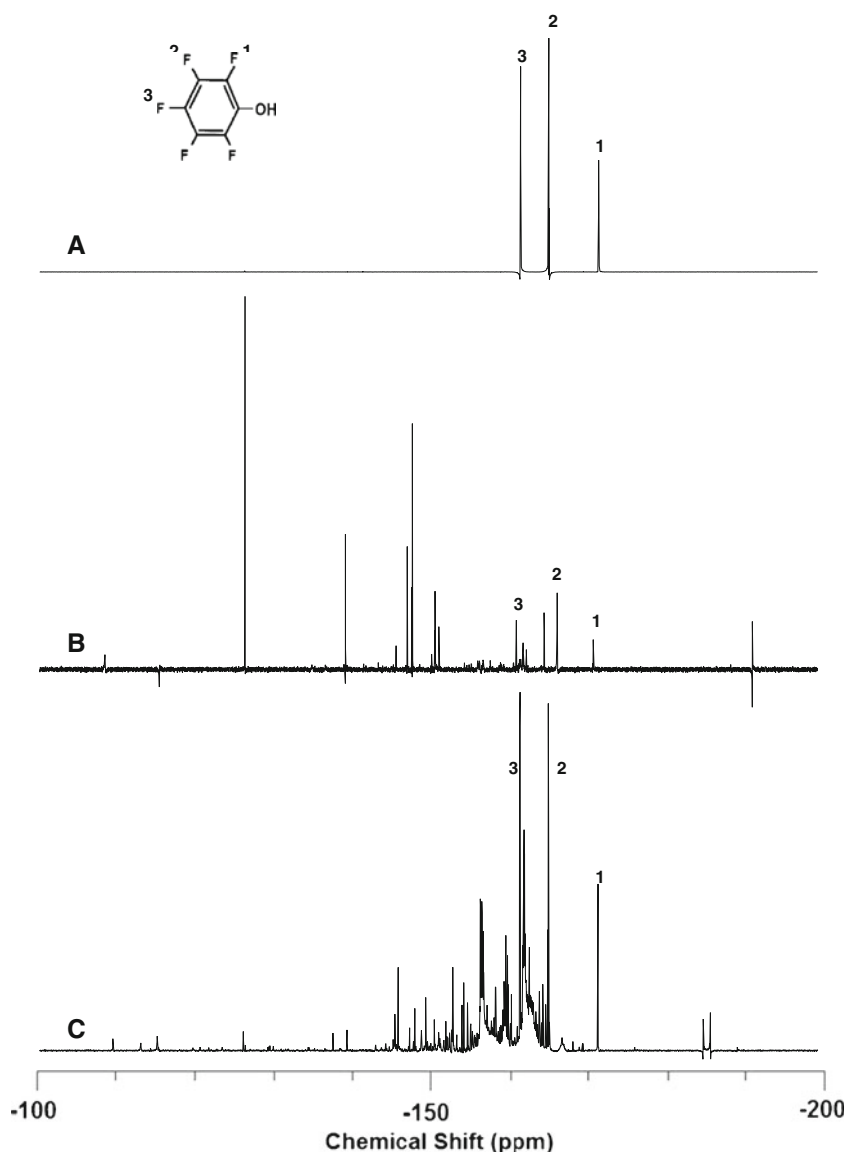
between −140 and −160 ppm (spectrum C in Fig. 3) with many additional signals. However, some of the downfield signals observed in spectrum B for the reaction mixture without HA were not visible in spetrum C. These differences suggest that the presence of HA induced a diversification in the PFP reaction pathways leading to a larger number of ^{19}F signals and chemical shift spread.

In line with previous results using unlabeled and ^{13}C-labeled chlorophenols in catalytic reactions with humic substances (Hatcher et al. 1993; Hahn et al. 2007), the multitude of signals and the chemical shifts complexity in the ^{19}F-spectrum, arising from the FeP oxidative catalysis in the presence of HA, indicates that PFP was mostly covalently bound to humic molecules. This finding combined to earlier results, which have shown that phenolic constituents of humic materials were polymerized in the same oxidative

catalytic conditions of this study (Piccolo et al. 2005; Šmejkalová and Piccolo 2005), suggest that such catalytic reactions have led to the co-polymerization of PFP with both HA.

In conclusion, this work has shown that PCP may be covalently incorporated into humic matter upon an oxidative coupling reaction catalysed by a biomimetic iron-porphyrin. The catalytic co-polymerization of PCP with humic substances was indicated by: 1. the increase in molecular dimension of reaction products as compared to control HA, 2. the nearly complete disappearance of free PCP isolated from reaction solutions, and, 3. the occurrence of multiple ^{19}F-NMR signals whose ^{19}F nuclei become magnetically different due to formation of covalent bonds between humic molecules and PFP. All these results point out that the oxidative biomimetic catalysis can be an

effective technology to co-polymerize polyhalogenated phenols in humic matter and detoxify environmental compartments such as waters, soils, and sediments.

References

Ando S, Matsuura T (1995) Substituent shielding parameters of fluorine 19-NMR on polyfluoroaromatic compounds dissolved in dimethyl sulfoxide-d6. Magn Reson Chem 33:639–645

Armenante PM, Kafkewitz D, Lewandowski GA, Jou CJ (1999) Anaerobic–aerobic treatment of halogenated phenolic compounds. Water Res 33:681–692

Celano G, Spaccini R, Šmejkalová D, Piccolo A (2008) Interactions of three s-triazines with humic acids of different structure. J Ag Food Chem 56:7360–7366

Cnubben NHP, Vervoort J, Boersma MG, Rietjens IMCM (1995) The effect of varying halogen substituent patterns on the cytochrome P450 catalyzed dehalogenation of 4-halogenated anilines to 4-aminophenol metabolites. Biochem Pharmacol 49:1235–48

Cozzolino A, Conte P, Piccolo A (2001) Conformational changes of soil humic substances induced by some hydroxy-, cheto-, and sulphonic acids. Soil Biol Biochem 33:563–571

Czaplicka M (2004) Sources and transformations of chlorophenols in the natural environment. Sci Total Environ 322:21–39

den Besten C, van Bladeren PJ, Duizer E, Vervoort J, Rietjens IM (1993) Cytochrome P450-mediated oxidation of pentafluorophenol to tetrafluorobenzoquinone as the primary reaction product. Chem Res Toxicol 6:674–80

Fukushima M, Tatsumi K (2006) Complex formation of water-soluble iron(III)-porphyrin with humic acids and their effects on the catalytic oxidation of pentachlorophenol. J Mol Catal A: Chem 245:178–184

Fukushima M, Ichikawa H, Kawasaki M, Sawada A, Morimoto K, Tatsumi K (2003a) Effects of humic substances on the pattern oxidation products of pentachlorophenol induced by a biomimetic catalytic system using tetra(p-sulfophenyl)porphyriniron(III) and KHSO$_5$. Environ Sci Technol 37:386–394

Fukushima M, Sawada A, Kawasaki M, Ichikawa H, Morimoto K, Tatsumi K (2003b) Influence of humic substances on the removal of pentachlorophenol by a biomimetic catalytic system with a water-soluble Iron(III)–porphyrin complex. Environ Sci Technol 37:1031–1036

Fukushima M, Tanabe Y, Morimoto K, Tatsumi K (2007) Role of humic acid fraction with higher aromaticity in enhancing the activity of a biomimetic catalyst, tetra(p-sulfonatophenyl)porphineiron(III). Biomacromolecules 8:386–391

Guthrie EA, Bortiatynski JM, Van Heemst JDH, Richman JE, Hardy KS, Kovach EM, Hatcher PG (1999) Determination of [C-13]pyrene sequestration in sediment microcosms using flash pyrolysis GC-MS and C-13 NMR. Environ Sci Technol 33:119–125

Hahn D, Cozzolino A, Piccolo A, Armenante PM (2007) Reduction of 2,4-dichlorophenol toxicity to *Pseudomonas putida* after oxidative incubation with humic substances and a biomimetic catalyst. Ecotox Environ Saf 66:335–342

Hatcher PG, Bortiatynski JM, Minard RD, Dec J, Bollag JM (1993) Use of high-resolution C-13 NMR to examine the enzymatic covalent binding of C-13-labeled 2,4-dichlorophenol to humic substances. Environ Sci Technol 27:2098–2103

Kobayashi S, Uyama H, Kimura S (2001) Enzymatic polymerization. Chem Rev 101:3793–3818

Meunier B, Sorokin A (1997) Oxidation of pollutants catalyzed by metallophtalocyanines. Acc Chem Res 30:470–476

Nam W, Lee HJ, Oh S-Y, Kim C, Jang HG (2000) First success of catalytic epoxidatin of olefins by an electron-rich iron(III) porphyrin complex and H$_2$O$_2$: imidazole effect on the activation of H$_2$O$_2$ by iron porphyrin complex in aprotic solvents. J Inorg Biochem 80:219–225

Park J-W, Dec J, Kim J-E, Bollag J-M (1999) Effect of humic constituents on the transformation of chlorinated phenols and anilines in the presence of oxidoreductive enzymes or birnessite. Environ Sci Technol 33:2028–2034

Peravuori J (2005) NMR spectroscopy study of freshwater humic material in light of supramolecular assembly. Environ Sci Technol 39:5541–5549

Piccolo A (2001) The supramolecular structure of humic substances. Soil Sci 166:810–832

Piccolo A (2002) The supramolecular structure of humic substances. A novel understanding of humus chemistry and implications in soil science. Adv Agron 75:57–134

Piccolo A, Celano G (1994) Hydrogen bonding interactions of the herbicide glyphosate with water soluble humic substances. Environ Toxicol Chem 13:1737–1741

Piccolo A, Celano G, Conte P (1996) Interactions between herbicides and humic substances. Pestic Outlook (R Soc Chem) 7:21–24

Piccolo A, Conte P, Cozzolino A (1999) Effects of mineral and monocarboxylic acids on the molecular association of dissolved humic substances. Eur J Soil Sci 50:687–694

Piccolo A, Conte P, Trivellone E, Van Lagen B, Buurman P (2002) Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by PYR-GC-MS and ^{1}H-NMR spectroscopy. Environ Sci Technol 36:76–84

Piccolo A, Conte P, Cozzolino A, Spaccini R (2003) The conformational structure of humic substances. In: Benbi DK, Nieder R (eds) Handbook of processes and modeling in the soil–plant system. The Haworth Reference Press, New York

Piccolo A, Conte P, Tagliatesta P (2005) Increased conformational rigidity of humic substances by oxidative biomimetic catalysis. Biomacromolecules 6:351–358

Puhakka JA, Järvinen K (1992) Aerobic fluidized-bed treatment of polychlorinated phenolic wood preservative constituents. Water Res 26:765–770

Sheldon RA (1994) Oxidation catalysis by metalloporphyrins. In: Sheldon RA (ed) Metalloporphyrins in catalytic oxidations. Marcel Dekker, New York

Simpson AJ (2002) Determining the molecular weight, aggregation, structures and interactions of natural organic matter using diffusion ordered spectroscopy. Magn Reson Chem 40:S72–S82

Simpson MJ (2006) Nuclear magnetic resonance based investigations of contaminant interactions with soil organic matter. Soil Sci Soc Am J 70:995–1004

Šmejkalová D, Piccolo A (2005) Enhanced molecular dimension of a humic acid induced by photooxidation catalyzed by biomimetic metalporphyrins. Biomacromolecules 6:2120–2125

Šmejkalová D, Piccolo A (2006) Rates of oxidative coupling of humic phenolic monomers catalyzed by a bBiomimetic iron-porphyrin. Environ Sci Technol 40:1644–1649

Šmejkalová D, Piccolo A, Spiteller M (2006) Oligomerization of humic phenolic monomers by oxidative coupling under biomimetic catalysis. Environ Sci Technol 40:6955–6962

Šmejkalová D, Spaccini R, Fontaine B, Piccolo A (2009) Binding of phenol and differently halogenated phenols to dissolved humic

matter as measured by NMR spectroscopy. Environ Sci Technol 43:5377–5382

Song R, Sorokin A, Bernardou J, Meunier B (1997) Metalloporphyrin-catalyzed oxidation of 2-methyl naphtalene to vitamin K3 and 6-methyl-1,4-naphthoquinone by potassium monopersulfate in aqueous solution. J Org Chem 62:673–678

Traylor PS, Dolphin D, Traylor TG (1984) Sterically protected hemins with electronegative substituents: efficient catalysts for hydroxylation and epoxidation. J Chem Soc, Chem Commun 1984:279–280

van der Bolt FJT, van den Heuvel RHH, Vervoort J, van Berkel WJH (1997) ^{19}F-NMR study on the regiospecificity of hydroxylation of tetrafluoro-4-hydroxybenzoate by wild-type and Y385F p-hydroxybenzoate hydroxylase: evidence for a consecutive oxygenolytic dehalogenation mechanism. Biochemistry 36:14192–14201