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Antioxidant Properties of Humic Substances

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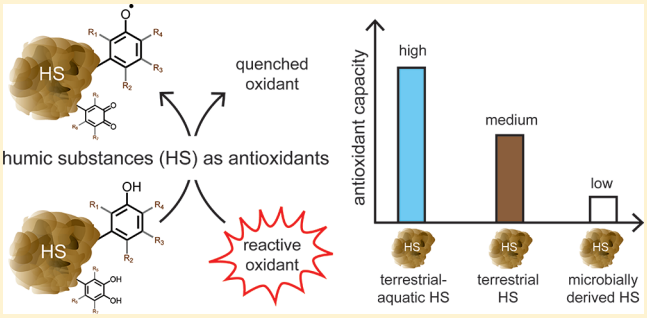
Supporting Information

ABSTRACT: Humic substances (HS) are heterogeneous, redox-active organic macromolecules. While electron transfer to and from HS under reducing conditions is well investigated, comparatively little is known on the electron donating (i.e., antioxidant) properties of HS under oxic conditions. In this work, the electron donating capacities (EDCs) of terrestrial and aquatic HS were quantified by mediated electrochemical oxidation over a wide range of pH values and applied redox potentials (E_h) using 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) as an electron transfer mediator. Electrochemical oxidation of three model humic acids (HAs) was largely irreversible, and the EDCs of these HAs increased with increasing E_h and pH. These results suggest that HS contain a wide variety of moieties that are oxidized at different potentials and that, upon oxidation, release protons and undergo irreversible follow-up reactions. At a given pH and E_h, the EDCs of the HS correlated well with their titrated phenol contents suggesting phenolic moieties as major electron donating groups in HS. Comparing the EDCs of 15 HS with their electron accepting capacities (EACs), aquatic HS had higher EDCs and lower EACs than terrestrial HS of comparable aromaticities. These results indicate that oxidative transformation of HS in the environment results in a depletion of electron donating phenolic moieties with antioxidant properties relative to the electron accepting quinone moieties.



INTRODUCTION

Humic substances (HS) are heterogeneous organic macromolecules that play important roles in biogeochemical and in pollutant redox reactions. As a result, HS redox properties have received considerable interest. Previous studies, including work from our group,[1,2] focused primarily on assessing electron transfer to and from HS under reducing conditions.[3−6] Quinones are considered to be one of the principal reducible moieties present in HS.[3,5,7,8] Evidence in favor of the quinone hypothesis includes largely reversible electron transfer observed when HS are first reduced and subsequently reoxidized in air,[1,9,10] a strong correlation of the electron accepting capacities (EACs) (i.e., the moles of electrons transferable to a given HS mass) with HS aromaticity,[1,3,5] and proton and electron transfer equilibria of HS similar to those of low molecular weight quinones.[2]

Comparatively little is known about the electron donating properties of HS under oxic conditions. This is at least partly due to methodological constraints in earlier studies. Electron donating capacities (EDCs) (i.e., the moles of electrons donated by a given HS mass) were quantified indirectly, either by measuring the reduction of added chemical oxidants (e.g., ferricyanide, Fe^{3+} citrate, and iodine) in batch equilibration experiments or by potentiometric redox titrations.[6,11−14] Phenolic moieties formed from higher plant precursors such as lignin and tannins were suggested as major electron donating moieties, based on the findings of increasing EDCs with increasing solution pH,[11−15] the release of protons upon oxidation of a humic acid (HA),[11] and comparable pH dependencies of the redox titration curves of a HA and of mixtures of low molecular weight phenolic compounds.[11]

Phenolic moieties, which include mono- and poly hydroxylated benzene units, have antioxidant properties.[16,17] Phenolic moieties in HS are therefore expected to affect the concentrations and lifetimes of reactive oxidants in soils and aquatic systems. By quenching reactive oxidants, phenolic moieties may protect other functional groups in HS from oxidation and therefore play an important role in the stability of HS in the environment.[18−20] In surface waters, dissolved HS may decrease indirect photolysis of organic pollutants both by quenching reactive oxygen species and by donating electrons to radical intermediates formed during pollutant degradation, thereby reducing them back to parent compound.[21,22] Similar inhibitory effects of HS are also conceivable in other systems, for instance in oxidative pollutant transformations on manganese dioxide surfaces in soils.[23,24] The antioxidant properties of HS may have beneficial effects on soil-dwelling organisms.[25] In water treatment facilities, electron donation by HS increases the amount of chemical oxidants that are required for water disinfection and pollutant removal.[26−28] Despite the importance of HS as electron donors in these and other

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processes, the electron donating properties of HS remain poorly characterized.

The main objective of this work was to characterize HS antioxidant properties as a function of E_h, solution pH, and HS origin to obtain insight into the reactivity of the electron donating moieties in HS. To this end, we quantified the EDCs of a representative number of terrestrial and aquatic HS and natural organic matter (NOM) samples over wide E_h and pH ranges. We used mediated electrochemical oxidation (MEO), a novel approach that relies on the use of 2,2′-azino-bis(3-ethylbenzothiazoline-sulfonic acid) (ABTS) to mediate electron transfer from the electron donating moieties in HS to working electrodes (WE). We also compared the EDC values of the studied HS to their EAC values[1] to assess the effects of HS origin and transformation history on HS redox properties.

MATERIALS AND METHODS

Chemicals. Diquat dibromide monohydrate (DQ, 99.5%) was obtained from Supelco. Sodium hydroxide, hydrochloric acid, acetic acid, potassium chloride, boric acid, sodium bicarbonate, citric acid monohydrate (all puriss. p.a.), and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (>99%) (ABTS) were obtained from Fluka. Sodium phosphate dibasic dihydrate (puriss. p.a.) was obtained from Sigma.

HS, NOM, and Model Antioxidants. Aldrich HA (AHA) was obtained from Sigma-Aldrich. All other HAs and fulvic acids (FAs) and the NOM samples were acquired from the International Humic Substances Society (IHSS) (St. Paul, MN, USA) and included Suwannee River HA Standard II (SRHA), Elliott Soil HA Standard (ESHA) and FA Standard III (ESFA), Leonardite HA Standard (LHA), Pahokee Peat HA Standard II (PPHAS), HA Reference (PPHAR), and FA Standard II (PPFA), Washkish Peat HA Reference (WPHA), Nordic Aquatic HA (NAHA) and FA (NAFA) References, Pony Lake FA Reference (PLFA), and Suwannee River and Nordic Reservoir NOM (SRNOM and NRNOM). The catalogue numbers of all IHSS samples are given in Tables S1 and S2 of the Supporting Information. Lignin (alkali; average molecular weight ~10 000 Da) and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%) were obtained from Aldrich, and L-ascorbic acid (≥99.0%) was obtained from Fluka. Ascorbic acid and Trolox have well-known antioxidant properties and served as model antioxidants for validation of the MEO method.

Solutions. All solutions were prepared with nanopure water (resistivity >18 MΩ*cm, Barnstead NANOpure System). Solutions for electrochemical experiments contained 0.1 M KCl as the electrolyte and were buffered with 0.1 M acetate at pH 5; citrate at pH 6; phosphate at pH 7, 8, and 12, borate at pH 9 and 10, and carbonate at pH 10 and 11.

Electrochemical Experiments. All experiments were controlled with either 630C or 630D electrochemical analyzers (CH Instruments, Austin, TX) or an Autolab PG302 (EcoChemie B.V., Netherlands). Potentials (E_h) were measured vs Ag/AgCl but are reported vs the standard hydrogen electrode (SHE). Electrochemical experiments in which the WEs were reductively polarized (i.e., cyclic voltammetry of HAs and HA bulk oxidation which involved mediated electrochemical reduction (MER)) were carried out inside a glovebox in an N_2 atmosphere (O_2 < 0.1 ppm; 25 ± 1 °C) to rule out O_2 reduction at the WEs. For measurements inside the glovebox, water, buffer solutions, and diluted acids and bases were made anoxic by purging with argon for three hours under continuous stirring on a stir plate. For the first two hours, the stir plate was heated to 150 °C. The heating was switched off for the third hour. After purging, the solutions were cooled to room temperature prior to transfer into the glovebox. All other solutions were prepared inside the glovebox by dissolving the chemicals in anoxic water or buffer. The EDCs of HS as a function of pH and E_h were quantified under oxic conditions outside the glovebox.

Cyclic Voltammetry. Experiments were conducted in 4−5 mL solutions with a 3 mm diameter glassy carbon disk WE, a platinum wire counter electrode (both from Radiometer Analytical), and an Ag/AgCl reference electrode (Bioanalytical Systems Inc.). The WE was polished and cleaned before each series of scans.[2] Cyclic voltammograms (CVs) were collected in pH-buffered solutions in the absence of ABTS and SRHA, in the presence of either ABTS (concentration C_{ABTS} = 30 μM) or SRHA (C_{SRHA} = 0.1 to 2 g L^{-1}), and in the presence of ABTS (C_{ABTS} = 30 μM) and SRHA (various C_{SRHA}). The cathodic and anodic vertex potentials were at E_h = −0.1 V and +0.9 V respectively, and the scan rate was ν = 10 mV s^{-1}, unless stated differently.

HA Bulk Oxidation Experiments. These experiments served to assess the reversibility of HA oxidation and were conducted in the glovebox. SRHA, LHA, and ESHA were dissolved in pH 7 buffer (0.1 M KCl, 0.1 M phosphate) at concentrations of 2 g L^{-1}. The HA solutions were diluted to 0.4 g L^{-1} in a bulk electrolysis cell to a total volume of 121 mL. The cell contained a high-surface area reticulated vitreous carbon WE polarized to E_h = 0.73 V, an Ag/AgCl reference electrode, and a coiled platinum wire auxiliary electrode in a compartment separated from the WE compartment by a porous glass frit (all from Bioanalytical Systems Inc.). An overhead stirrer (IKA RW16 Basic) with custom-made glass propellers was used to stir the solution inside the electrochemical cell during the oxidation, and the solutions were circulated through a sampling cell (volume 10 mL). Over the course of the oxidation, small volumes of a 5 mM ABTS solution (eight spikes of 60 μL and one spike of 80 μL for SRHA, one spike of 40 μL and five spikes of 30 μL for ESHA, and two spikes of 50 μL and six spiked of 30 μL of LHA) were added to the cell to sustain electron transfer mediation. Decreasing mediation over time likely resulted from slow loss of $ABTS^{\bullet+}$ through reaction with oxidized HS components. The cumulative moles of ABTS spiked were, however, small compared to the moles of electrons, $n_{e^-}^{\text{bulk oxidation}}$ [$mmol_{e^-}$ $(g_{HA})^{-1}$], transferred from the HAs to the WE during bulk oxidation (i.e., 1.6% for ESHA and LHA, and 2.4% for SRHA). $n_{e^-}^{\text{bulk oxidation}}$ were determined by integration of the oxidative currents. No background current correction was required because oxidative currents in solutions without HA were negligible. Over the course of the bulk oxidation, HA aliquots were withdrawn from the sampling cell and analyzed for changes in the UV−vis light absorption (Varian Cary 100 Spectrophotometer; 1 cm Quartz Hellma Suprasil cells), in the EDC by MEO (see below) at E_h = 0.73 V and pH 7 (i.e., the same E_h as used during bulk oxidation), and in the EAC by MER at E_h = −0.49 V and pH 7, as described earlier.[1]

MEO. We used MEO according to Aeschbacher et al.[1] to quantify the EDCs of the model antioxidants Trolox and ascorbic acid (chemical structures in Figure S1 of the Supporting Information), HS, and NOM. MEO was carried out in an electrochemical cell containing pH-buffered solution

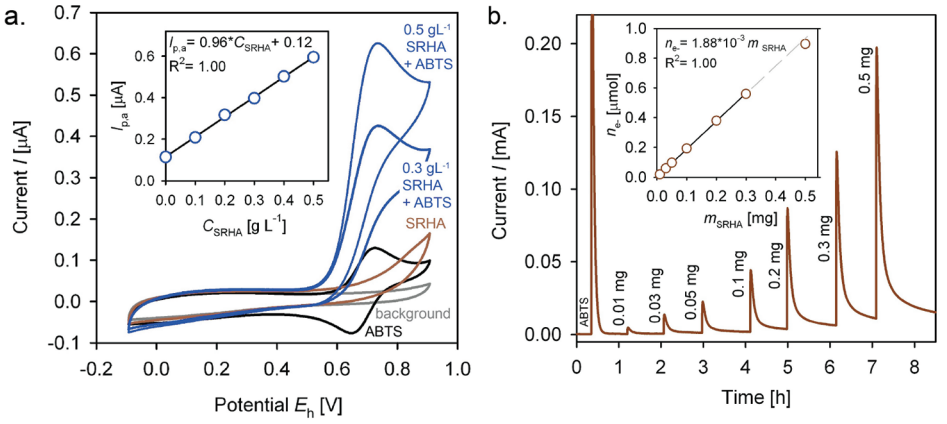

Figure 1. a. Cyclic voltammograms (CVs) collected in solutions containing only background electrolyte (gray trace), only 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, black trace), only Suwanee River Humic Acid (SRHA, brown trace), and both SRHA and ABTS (blue trace at different concentrations of SRHA, C_{SRHA}). Insert: Linear dependency of the oxidative peak current, $I_{p,a}$, on C_{SRHA}. **b.** Oxidative current responses to spikes of ABTS and to spikes of increasing SRHA masses, m_{SRHA}, (E_h = 0.607 V and pH 7). Insert: Plot of moles of electrons, n_{e-}, transferred from SRHA to the working electrode versus m_{SRHA}. The regression line was determined for spiked SRHA masses of m_{SRHA} < 0.5 mg.

with the same electrode setup as used for HA bulk oxidation. First, ABTS was spiked into the cell to a final concentration of C_{ABTS} = 0.13 mM, because lower C_{ABTS} resulted in EDCs that were C_{ABTS}-dependent (Figure S2 of the Supporting Information). The added ABTS was oxidized at the WE, as reflected in oxidative currents that sharply increased, peaked, and then decreased to level off at low and stable baseline currents upon attainment of redox equilibrium between the $ABTS^{\bullet+}$/ABTS couple and the WE. Second, small volumes of HS/NOM/antioxidant solutions were spiked into the electrochemical cell. Electron donation by the HS/NOM/antioxidant resulted in reduction of $ABTS^{\bullet+}$ to ABTS, which was rapidly reoxidized at the WE to $ABTS^{\bullet+}$, resulting in oxidative current peaks. EDCs were obtained by integration of the current peaks. The EDCs of SRHA, ESHA, and LHA were determined over wide E_h and pH ranges (i.e., 0.55 V to 0.73 V and pH 5 to 12). The EDCs of all 15 HS and NOM samples were determined at E_h = 0.61 V and 0.73 V for pH 7 and at E_h = 0.61 V for pH 9. These conditions were selected because the increase by 0.12 V from 0.61 to 0.73 V at constant pH 7 and the increase in two pH units at constant E_h = 0.61 V results in the same increase in thermodynamic driving force for the oxidation of HS moieties with equimolar release of protons and electrons (e.g, phenolic moieties). We did not determine EDCs above E_h = 0.73 V, as preliminary measurements showed large background currents (>100 μA), likely due to ABTS-mediated oxidation of water and/or the WE material.

RESULTS AND DISCUSSION

Validation of MEO Approach. Cyclic voltammetry was used to demonstrate that ABTS mediates electron transfer to the WE from electron donating moieties in SRHA, which was used as a model HS. The CVs collected in solutions containing only ABTS showed fully reversible one electron transfers to ABTS with anodic and cathodic waves centered at E_h = 0.69 V (part a of Figure 1 and Figure S3 of the Supporting Information), in good agreement with the reported standard reduction potential $E_h^0(ABTS^{\bullet+}/ABTS)$ = 0.68 V.[29] The CVs collected in SRHA-containing solutions showed higher oxidative currents during anodic scanning at E_h > 0.6 than the CVs collected in background electrolyte solution (part a of Figure 1), demonstrating that SRHA was directly oxidized at the WE. However, the CVs were featureless, suggesting sluggish electron transfer from the oxidizable moieties in SRHA to the WE. Conversely, the CVs collected in SRHA-solutions in the presence of low ABTS concentrations showed catalytic oxidative currents during anodic scanning. These catalytic currents were much higher than the currents obtained for only ABTS or only SRHA (part a of Figure 1). The catalytic peak currents, $I_{p,a}$, increased linearly with SRHA concentration, C_{SRHA}, up to about 0.5 g_{SRHA} L^{-1} (insert in part a of Figure 1; part a of Figure S4 of the Supporting Information for all CVs). At higher C_{SRHA}, $I_{p,a}$ was no longer proportional to C_{SRHA} (data not shown), possibly due to passivation of the WE by adsorption of SRHA oxidation products. The catalytic current peaks and the linearity between $I_{p,a}$ and C_{SRHA} over a wide C_{SRHA} range demonstrate that ABTS effectively mediated the electrochemical oxidation of SRHA. Mediation is also evident from chronocoulometric analysis of the CVs: for instance, at C_{SRHA}= 0.5 g L^{-1}, each mole of ABTS mediated the transfer of up to 7.5 mols of electrons from electron donating moieties in SRHA to the WE (Figure S4 of the Supporting Information).

Spikes of the model antioxidants Trolox and ascorbic acid to bulk electrolysis cells (E_h = 0.607 V and pH 7) containing ABTS resulted in sharp and baseline-separated oxidative current peaks (Figure S5 of the Supporting Information). Integration of the peaks yielded the numbers of electrons transferred, n_{e-}, which were linearly proportional to the amount of antioxidant added (inserts in Figure S5 of the Supporting Information). The slopes of the regression lines for Trolox and ascorbic acid were 1.95 ± 0.01 and 1.88 ± 0.02 $mmol_{e-}(mmol_{antioxidant})^{-1}$ (±half of the 95% confidence interval) respectively in good agreement with the expected EDC of 2 $mmol_{e-}(mmol_{antioxidant})^{-1}$ for both antioxidants at this E_h.[30] Hence, the experimental setup detected 98 ± 1% and 94 ± 1% of the expected EDC for Trolox and ascorbic acid, respectively.

Part b of Figure 1 and Figure S6 of the Supporting Information show the oxidative current response to increasing spiked mass (m_{HA}) of SRHA and LHA, respectively (E_h = 0.607 V, pH 7). Compared to the model antioxidants, the spikes of HAs resulted in broader current peaks indicating slower ABTS-mediated electron transfer from the HA to the WE than from the model antioxidants. Yet, at $m_{HA} \leq$ 0.3 mg, background currents were reattained within 50 min of spiking. Integration

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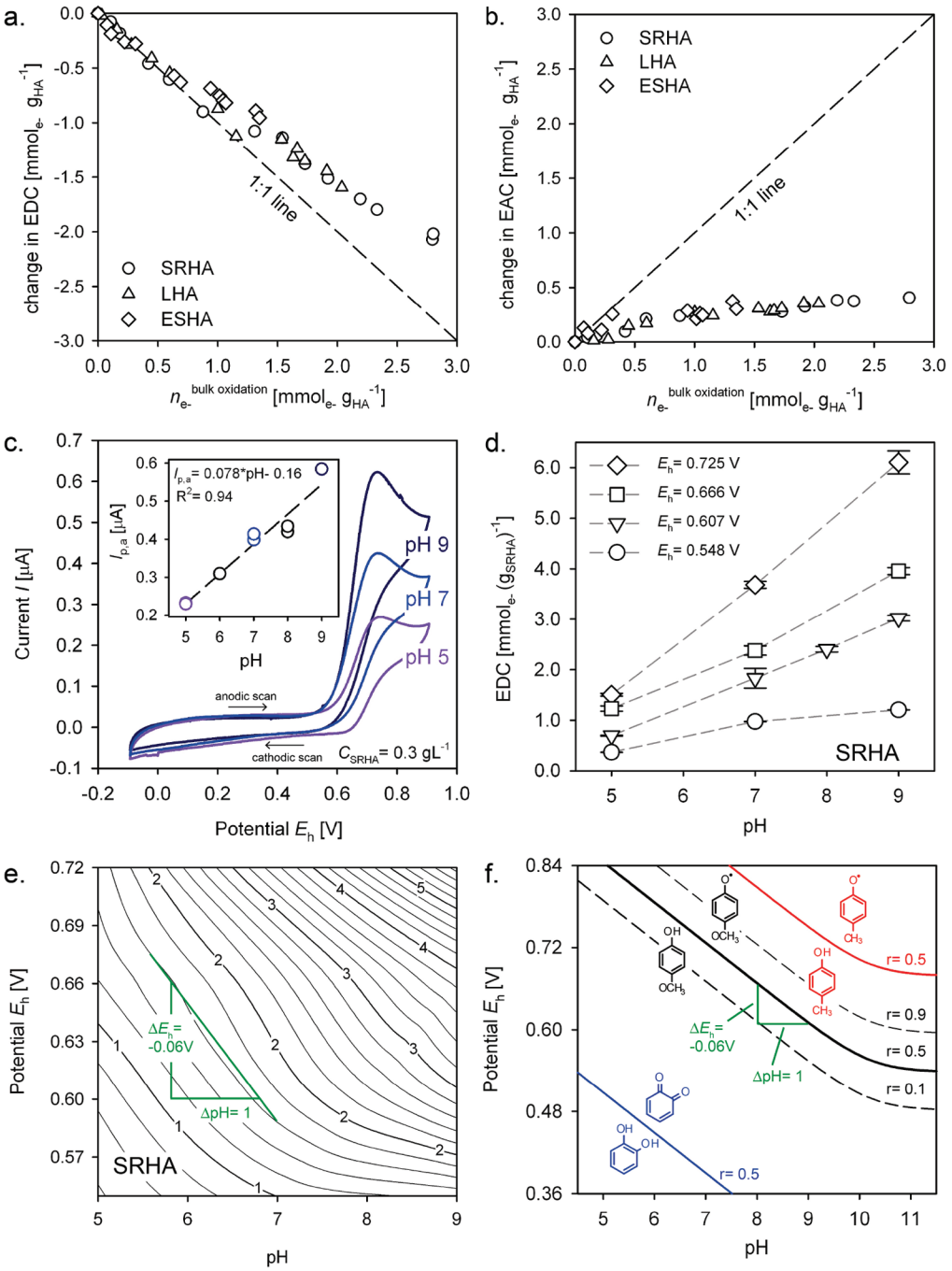

Figure 2. a,b. Decrease in the electron donating capacity (EDC) and increase in the electron accepting capacity (EAC) of Suwanee River Humic Acid (SRHA), Leonardite Humic Acid (LHA), and Elliot Soil Humic Acid (ESHA) as a function of the moles of electrons, $n_{e-}^{bulk\ oxidation}$, transferred from the HAs during their bulk oxidation. The change in EDC corresponds to the difference in EDC at a given $n_{e-}^{bulk\ oxidation}$ and the EDC prior to the bulk oxidation. The change in EAC is defined accordingly. **c.** Cyclic voltammograms of SRHA (concentration C_{SRHA}= 0.3 g L^{-1}) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; concentration C_{ABTS}= 30 μM) collected in solutions at pH 5, 7, and 9 at scan rates of v = 10 mV s^{-1}. Inset: Dependence of the catalytic peak current, $I_{p,a}$, on solution pH. **d.** EDC of SRHA as a function of pH and E_h. **e.** EDC contour plots of SRHA in units of mmol$_{e-}$ (g$_{SRHA}$)$^{-1}$ as a function of E_h and pH. The green reference line has a slope of ΔE_h = −0.06 V per pH. **f.** E_h-pH dependencies of the oxidation of 4-methoxyphenol, 4-methylphenol, and 1,2-dihydroxy-benzene. The solid lines correspond to the standard reduction potentials, E_h^0, at which the degree of oxidation, r, of the compound is 50% (r = 0.5). The dashed lines correspond to r = 0.1 and r = 0.9 for 4-methoxyphenol.

of the baseline-separated peaks showed that n_{e-} was directly proportional to m_{HA} for both SRHA and LHA (inserts in part b of Figure 1 and Figure S6 of the Supporting Information). The slopes of the regression lines of 1.88 ± 0.04 and 1.33 ± 0.02 mmol$_{e-}$(g$_{HA}$)$^{-1}$ (±half of the 95% confidence interval) corresponded to the EDCs of SRHA and LHA respectively under the given E_h and pH conditions. Spikes of SRHA and LHA with m_{HA} > 0.3 mg resulted in n_{e-} values that were slightly smaller than expected based on the linear n_{e-}–m_{HA} dependencies determined at lower m_{HA}. The deviation from linearity likely reflected incomplete oxidation of the HAs at high m_{HA} over the integration interval of 50 min. Given that SRHA and

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LHA had high EDCs among the tested aquatic HS and NOMs and terrestrial HS respectively, we assumed linear response ranges for all tested HS at spiked masses $m_{HA} \leq 0.3$ mg. The quantitative and reproducible detection of the model antioxidants, and the wide linear response range of n_{e^-} to m_{HA} demonstrated that the MEO setup can be used for a systematic assessment of the E_h- and pH-dependencies of the EDCs of HS. We quantified the EDCs of all tested HS by spiking 0.2 mg of the respective HS.

The use of MEO to quantify EDCs has two major advantages over previously employed methods, which include assays that rely on the reductive decolorization of $ABTS^{\bullet+}$ to ABTS,[18,20,31,32] and on potentiometric redox titrations with chemical oxidants.[11−15] First, in MEO, the EDCs are directly quantified by chronocoulometric analysis of oxidative current peaks and not indirectly via changes in the concentration of added oxidants or via changes in the solution E_h. Second, the E_h in MEO is accurately controlled by a potentiostat and remains constant during HS oxidation.

Reversibility of HS oxidation. The reversibility provides information on the chemistry of electron donating moieties in HS and allows for assessment of the likelihood of the depletion of these moieties during chemical and biological oxidation of HS in natural and engineered systems. We determined the reversibility of oxidation for SRHA, ESHA, and LHA, which we selected to represent aquatic and terrestrial HAs.

Parts a and b of Figure 2 show the decrease in the EDCs and the increase in the EACs of aliquots of SRHA, ESHA, and LHA collected over the course of the HA bulk oxidations. During the initial stages of the bulk oxidation, the decrease in the EDCs of the HAs corresponded to the moles of electrons, $n_{e^-}^{\text{bulk oxidation}}$, removed from the respective HAs in the bulk oxidation (part a of Figure 2, data on the 1:1 line at $n_{e^-}^{\text{bulk oxidation}} < 0.8-1$ $\text{mmol}_{e^-}(g_{HA})^{-1}$). The MEO setup therefore quantitatively detected the depletion of electron donating moieties in the HAs during their bulk oxidation. At later stages of the bulk oxidation ($n_{e^-}^{\text{bulk oxidation}} > 0.8-1$ mmol_{e^-} $(g_{HA})^{-1}$), the decrease in the EDCs of the HA aliquots were smaller than expected from the increase in $n_{e^-}^{\text{bulk oxidation}}$. Most likely, this deviation reflected a slight overestimation of $n_{e^-}^{\text{bulk oxidation}}$. It is conceivable that the oxidized species of HA moieties with high reduction potentials (e.g., 1,2- and 1,4-benzoquinones) were unstable under the reducing conditions inside the glovebox. Following ABTS-mediated oxidation, these moieties may have become reduced, and were subsequently reoxidized by ABTS. However, more important for the assessment of reversibility, the EAC values of the HAs aliquots increased only slightly compared to $n_{e^-}^{\text{bulk oxidation}}$ (part b of Figure 2). The EACs of the last samples of SRHA, ESHA, and LHA collected during bulk oxidation were only 14%, 22%, and 17% of their respective $n_{e^-}^{\text{bulk oxidation}}$. The oxidation of all three HAs was therefore largely irreversible. Hydroquinone moieties that are reversibly oxidized therefore contributed little to the measured $n_{e^-}^{\text{bulk oxidation}}$. The irreversibility may have resulted from the oxidation of phenolic moieties to phenoxy radicals, which are known to rapidly undergo irreversible coupling reactions.[30,33,34] Irreversible coupling of phenoxy radicals is consistent with the finding that the free radical content of HAs does not increase upon their oxidation, as shown by EPR spectroscopy.[35,36] The finding of irreversible oxidation strongly suggests that abiotic and biotic oxidations of HS in natural and engineered systems result in an irreversible depletion of HS electron donating moieties.

The bulk oxidation of the three HAs did not alter their UV−vis absorbance spectra (Figure S7 of the Supporting Information) indicating that $ABTS^{\bullet+}$ did not destroy aromatic and quinone moieties in the HAs. This is supported by previous work, which showed that $ABTS^{\bullet+}$ oxidizes aromatic Ar−OH and Ar−NH_2 moieties but leaves aromatic rings intact.[24,37] $ABTS^{\bullet+}$ therefore is a more selective oxidant than ozone and chlorine, which decrease the aromaticity and hence also UV absorbance of HAs.[38−42] The finding that electrochemical oxidation did not alter the UV−vis absorbance spectra of the HAs suggests that charge transfer complexes that give rise to long wavelength absorbance of HAs were not affected by the oxidation.[10,43,44]

pH- and E_h-Dependencies of HS Oxidation. The CVs of ABTS in the absence of SRHA were not affected by pH (Figure S3 of the Supporting Information) consistent with the pH-independent speciation (and hence standard reduction potential (E_h^0)) of the $ABTS^{\bullet+}$/ABTS couple at pH> 2.1[29] (Figure S9 of the Supporting Information). Conversely, CVs collected in solutions containing SRHA and ABTS showed increasing oxidative currents with increasing solution pH (part c of Figure 2). The increase in the catalytic currents with increasing pH must have resulted from more favorable oxidation of electron donating moieties in SRHA at high than low pH as also shown in previous studies.[11−15] We used the MEO approach to systematically investigate the effects of E_h and pH on the EDCs of SRHA, ESHA, and LHA. The EDCs of all three HAs showed comparable E_h and pH dependencies, as shown in parts d and e of Figure 2 for SRHA and in Figures S10 and S11 of the Supporting Information for ESHA and LHA.

At E_h = 0.55 V and pH 7, the EDCs of the three tested HAs ranged from 0.3 to 1.0 $\text{mmol}_{e^-}(g_{HA})^{-1}$. As expected, the EDCs of LHA and ESHA were larger than the reported values of 0.13 and 0.07 $\text{mmol}_{e^-}(g_{HA})^{-1}$ respectively for the two HAs determined with the milder oxidant Fe^{3+}-citrate.[14] The EDCs of SRHA, ESHA, and LHA steadily increased with increasing E_h and pH to values >5 $\text{mmol}_{e^-}\ g_{HA}^{-1}$ at E_h = 0.73 V and pH ≥ 8 (part d of Figure 2, and Figures S10 and S11 of the Supporting Information for SRHA, ESHA, and LHA, respectively). The increase in EDCs with pH and E_h demonstrates that HS oxidation became more feasible with increasing E_h and pH consistent with the results from the CV experiments (part c of Figure 2). The pronounced increase in EDCs with pH suggests that electron donating moieties in HS release protons upon oxidation.

The E_h- and pH-dependencies of EDCs are replotted as contour plots in part e of Figure 2, and Figures S10 and S11 of the Supporting Information for SRHA, ESHA, and LHA, respectively. Each contour line defines E_h and pH conditions with constant EDC. The EDC contour lines had slopes of approximately −0.06 V per pH (i.e., green lines in the Figures) at pH < 8. At higher pH, the slopes for SRHA and LHA tended to become less negative with increasing pH. The E_h−pH dependencies of the EDC contour lines are similar to those of low molecular weight phenolic compounds, as shown in part f of Figure 2 for the one electron oxidation of the monohydroxybenzenes 4-methoxyphenol and 4-methylphenol and for the two-electron oxidation of 1,2-benzohydroquinone. At pH below the acidity constants, $pK_{a\ Ar-OH}$, of the monohydroxybenzenes, their oxidation is coupled to the release of one proton because the formed phenoxy radicals are not protonated at environmentally relevant pH ($pK_a < 0$).[45] The

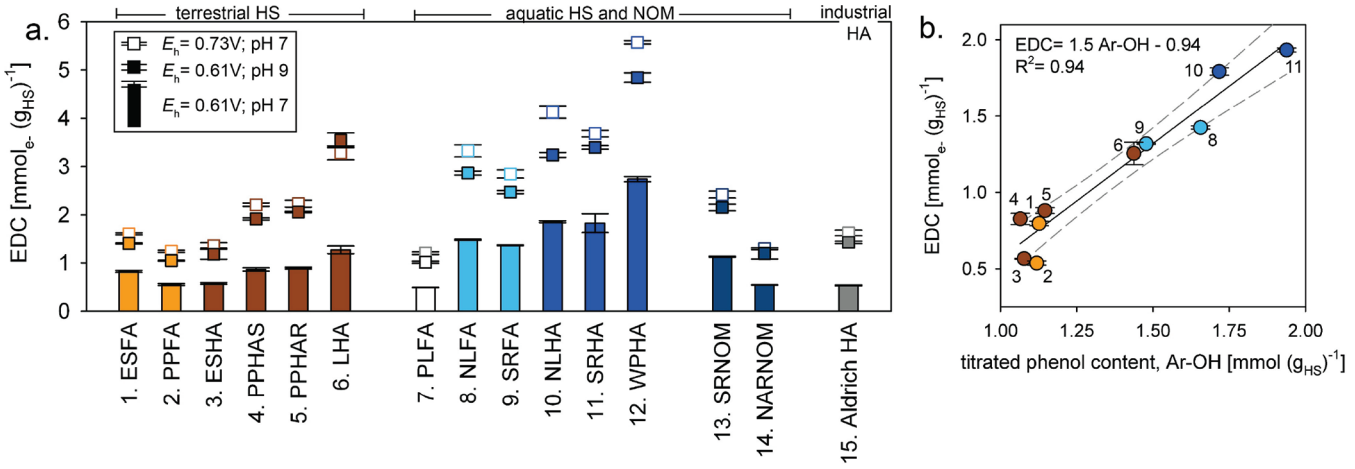

Figure 3. a. Electron donating capacities (EDCs) of 15 humic substances (HS) and natural organic matter (NOM) samples at an applied potential of E_h = 0.61 V at pH 7 and 9 and at E_h = 0.73 V at pH 7. The brown and blue bars represent HS and NOM of terrestrial and aquatic origins, respectively. Aldrich humic acid (AHA, gray bar) is an industrially extracted HA. **b.** Plot of the EDCs (E_h = 0.61 V, pH 7) of ten HS versus their reported titrated phenol contents, Ar−OH.[50]

equimolar release of electrons and protons upon oxidation results in a slope of 0.06 V per pH (T = 25 °C). At pH > $pK_{a\ Ar-OH}$, only one electron is released from the phenolate species to form the phenoxy radical resulting in a pH-independent E_h^0.

Parts e and f of Figure 2 show that the EDCs of the HAs increased over the entire potential range from E_h = 0.55 V to 0.73 V, whereas model monohydroxybenzenes and 1,2-hydroquinones are oxidized over a relatively narrow E_h range (i.e., from 10% oxidized at E_h^0 − 0.059 V to 90% at E_h^0 + 0.059 V, as shown for 4-methoxyphenol in part f of Figure 2). This finding suggests that the electron donating moieties in HAs had a wide distribution of apparent standard redox potentials. Furthermore, parts e and f of Figure 2 show that the majority of the electron donating moieties in the HAs were oxidized at E_h values comparable to those of the monohydroxybenzenes but higher than the E_h^0 for the oxidation of 1,2-benzohydroquinone. Given that the E_h^0 of 1,2-benzohydroquinone lies at the upper end of the E_h^0 range of quinones,[2,46] the EDC-E_h dependence suggests that 1,2- and 1,4-hydroquinone moieties contributed little to the EDCs of the HAs.

The increase in the EDCs of the HAs with E_h and pH likely reflected an increase in the thermodynamic feasibility of oxidation of phenolic moieties.[47−49] Increasing EDC with increasing pH may also have resulted from faster oxidation kinetics of phenolate than phenolic moieties.[48] It has been shown that the oxidation rates of low molecular weight phenols increases linearly with the concentration of the phenolate species.[47,49] Consistently, the EDCs of SRHA, LHA, and ESHA at pH > 7 increased with their titrated charges[50] (q_{TOT}) (Figure S12 of the Supporting Information) and hence their phenolate concentrations.

Dependence of the EDCs of HS and NOM on Their Source Material. Part a of Figure 3 shows the EDCs of eight HAs, five FAs, and two NOM samples of terrestrial, aquatic, and industrial origin, quantified at three selected E_h−pH combinations. All EDC values are provided in Table S1 of the Supporting Information. At E_h = 0.61 V and pH 7, the EDCs ranged from 0.47 ± 0.01 mmol$_{e^-}$(g_{PLFA})$^{-1}$ for PLFA to 2.65 ± 0.05 mmol$_{e^-}$(g_{WPHA})$^{-1}$ for WPHA. With the exception of WPHA, the aquatic HS had higher EDC values than the terrestrial HS. The EDCs of all HS increased by a factor of 2 or more when increasing E_h from 0.61 to 0.73 V (ΔE_h= 0.12 V) while maintaining pH 7. A slightly smaller increase in the EDCs of all HS resulted when the pH was increased from pH 7 to 9 while maintaining E_h = 0.61 V. The electron donating moieties in all tested HS therefore had comparable E_h−pH dependencies.

The EDC values (E_h = 0.61 V; pH 7) of ten of the 12 tested natural HS showed a strong linear correlation to their reported titrated phenol contents Ar−OH[50] (part b of Figure 3, Table S2 of the Supporting Information). PLFA and WPHA were omitted from the correlation analysis, as no titration data was available for these HS. Whereas the linear correlation between EDC and titrated phenol contents supports phenolic moieties as major electron donating moieties, the regression curves have to be carefully interpreted. On the one hand, the reported phenol contents have uncertainties because they were operationally defined as twice the titrated charge density between pH 8 and 10. It is conceivable that the tested HS contain phenolic moieties that deprotonate at much lower pH values (e.g., hydroxy-substituted quinones with acidity constants of $pK_a \ll 8$).[46] Furthermore, the titrated phenol contents may have been affected by acid−base nonequilibria.[50,51] On the other hand, it is possible that, in addition to phenols, nitrogen containing functional groups, such as aromatic amines contributed to the measured EDCs, particularly at higher E_h. Conversely, the Fe, Cu, and Mn contents in all HS, except Aldrich HA, were too small to have significantly contributed to the measured EDCs (Table S2 of the Supporting Information). The uncertainties in the phenol contents and potential contributions from N-containing moieties may explain why some of the EDCs measured at the higher potential E_h = 0.73 V were larger than would be expected based on the reported phenol contents (Table S1 of the Supporting Information).

Abundances of Electron Donating and Accepting Moieties for HS of Different Origin. Part a of Figure 4 and Table S1 of the Supporting Information give an overview of the EDC and EAC values of all tested HS and NOM quantified at pH 7 and E_h = 0.61 V and E_h = −0.49 V, respectively. The data show that terrestrial HS tend to have smaller EDCs and larger EACs than the aquatic HS and NOM samples. This is evident also from part b of Figure 4, in which the EDCs are plotted versus the EACs. Whereas the absolute EDC values and

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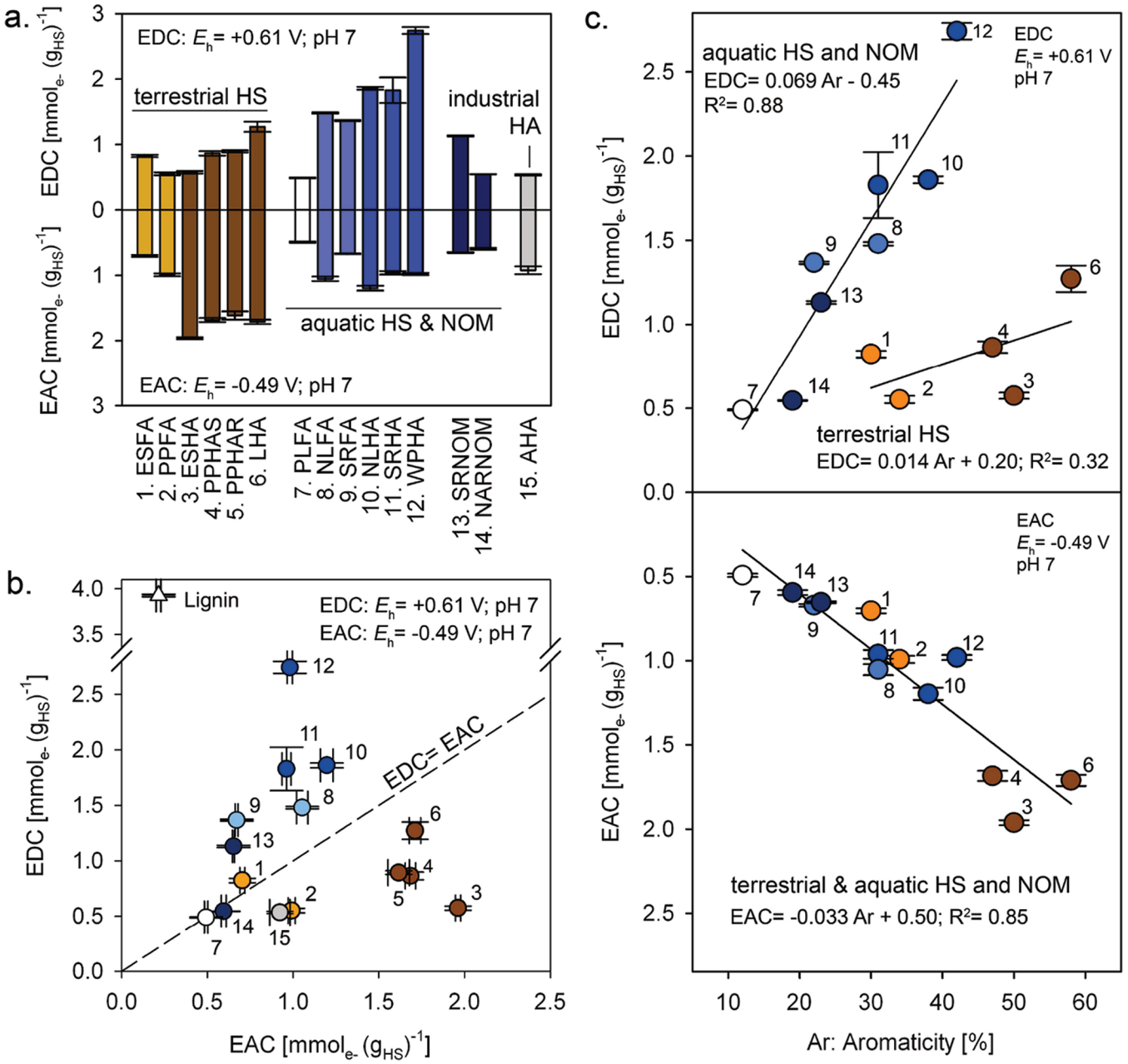

Figure 4. a. Electron donating capacities (EDCs; E_h = 0.61 V, pH 7) and electron accepting capacities (EACs; E_h = −0.49 V, pH 7; data for HS from Aeschbacher et al.[1]) of terrestrial humic substances (HS) (brown bars), aquatic HS and Natural Organic Matter (NOM) (blue bars), and of industrial Aldrich Humic Acid (gray bar). **b.** Data from panel a replotted as EDCs versus EACs. **c.** EDCs and EACs of 13 HS and NOM samples of terrestrial and aquatic origin versus their ^{13}C NMR estimated aromaticities.[52]

therefore the positions of the tested HS and NOMs relative to the 1:1 line in part b of Figure 4 depend on pH and E_h during MEO, these two variables do not affect the overall trend of increasing EDCs and decreasing EACs from terrestrial HS to the aquatic HS and NOMs (Table S1 of the Supporting Information).

In part c of Figure 4 the EDC and EAC values of 13 of the 15 tested samples are compared based on their aromaticities estimated from ^{13}C NMR spectra[52] (Table S2 of the Supporting Information). PPHAR and AHA were not included in the analysis, because their ^{13}C NMR spectra have not been reported. Figure 4c reveals that aquatic HS and NOM have higher EDCs than terrestrial HS of comparable aromaticity. Furthermore, the EDCs of aquatic HS and NOM were strongly correlated (R^2 = 0.88) to their aromaticities, whereas the EDCs of terrestrial HS showed a weaker correlation with aromaticity (R^2 = 0.32). At the same time, the EACs of all 15 HS and NOM samples strongly correlate with aromaticity (R^2 = 0.85) consistent with aromatic reducible moieties such as quinones.

We propose that the systematic effects of HS origin on the EDC and EAC properties of HS reflect the lower extents of oxidative transformations of phenolic precursor materials in aquatic than in terrestrial HS. Aquatic HS are isolated from rivers, streams, and lakes and are commonly much younger than terrestrial HS isolated from soils (i.e., several hundred to several thousand years of age), as shown by ^{14}C dating.[53] WPHA, which had the highest EDC values among the tested HS and NOMs, was isolated from a sphagnum bog containing

poorly degraded, (poly)phenolic plant materials, including lignin and tannins.[54] The poor degradation in bogs and peats has been linked to low activities of phenol oxidases under water-saturated anoxic conditions in such systems.[55] Note that lignin, which is considered an important precursor for aquatic and terrestrial HS, showed very high EDC but low EAC values (part b of Figure 4, Table S1 of the Supporting Information) likely reflecting the high phenol and low quinone contents of lignin.[56] PLFA represents an aquatic end member among the aquatic HS as it originates from a costal pond in Antarctica with organic matter input from lower plants, lichen, and microorganisms without inputs of higher plant (poly)phenolic precursors.[57] Consistently, PLFA showed the lowest EDC and EAC values among the tested HS. Based on the relatively high N and S contents in PLFA,[57] moieties containing these heteroatoms may also have contributed to its EDC and EAC. Suwannee River and Nordic Lake HS and NOM were isolated from inland water systems. HS from such systems typically show higher phenolic content than terrestrial HS, based on ^{13}C NMR data.[53] Ultrahigh resolution mass spectrometry and NMR provided evidence for lignin-derived structures in SRHA.[58−60] Elliott Soil and Pahokee Peat HS originate from agricultural soils and, in contrast to aquatic HS, are expected to be composed of highly degraded precursor materials. Extensive degradation of plant-derived precursors in such systems is supported by recent findings that lignin does not accumulate in the refractory C pool of arable and grassland soils.[61,62] Leonardite HA is extracted from a lignite coal and therefore represents a highly processed aromatic-rich, HA with high EAC and relatively low EDC.

It is unclear whether the increase in EAC from aquatic to terrestrial HS and NOMs of comparable aromaticities resulted from an enrichment of electron accepting quinone moieties that were present already in the source material[56] and/or from the new formation of electron accepting quinone moieties. Quinones may form during the enzymatic oxidation of aromatic moieties.[63,64] Furthermore, quinones may form upon reaction of aromatic and phenolic structures with hydroxyl radicals (·OH),[65] generated, for instance, during HS photolysis,[66] HS redox cycling in the dark,[67] or via enzymatic Fenton chemistry.[68−71]

IMPLICATIONS

The MEO approach presented in this paper allows for accurate and direct quantification of the EDCs of HS as a function of redox potential E_h and pH. MEO is highly sensitive with detection limits of a few μg of HS. This work demonstrates that HS and NOM contain phenolic electron-donating moieties that cover a wide range of oxidation potentials. These moieties may act as antioxidants and thereby affect biogeochemical and pollutant redox reactions in both natural and engineered systems. For instance, this work supports the hypothesis that the inhibitory effect of HS on indirect phototransformation rates of organic pollutants results from electron donation from phenolic moieties in the HS to pollutant oxidation intermediates.[21,22]

The concept of MEO may be used in drinking water facilities to determine the dose of oxidants, such as ozone or chlorine that need to be added to the water for disinfection, pollutant degradation, decolorization, and odor control. At present, drinking water treatment facilities lack suitable approaches to monitor the changes in the EDCs and hence chemical oxidant demand of the incoming water in real time. Measurements of the EDCs of NOM-containing waters may also help to assess their potential for disinfection byproduct formation, as electron-donating moieties in NOM play a role in the formation of these byproducts.[26−28]

This work provides evidence for pronounced and systematic effects of HS source material and age on the relative abundances of electron accepting and donating moieties in HS and NOM. The analysis of the redox capacities of a diverse set of HS and NOM in this work suggests that abiotic and possibly also biotic oxidation reactions of HS and NOM in the environment result in the preferential loss of electron-donating phenolic moieties from higher plant precursors over electron-accepting quinone moieties. As such, this work supports the hypothesis that phenolic moieties with antioxidant properties slow down the oxidative transformation of other moieties in HS and NOM and hence increase their recalcitrance in oxic environments.[18−20] To further test this hypothesis, the EDCs and EACs can be quantified for HS collected along natural gradients of organic matter decomposition, such as soil profiles or an estuary. The depletion of electron donating moieties during oxidative transformation of HS can be further investigated in laboratory studies by quantifying the changes in the EDCs of plant-derived polyphenols, HS, and NOM over the course of abiotic and enzymatic oxidations. We propose that the ratio of electron donating, phenolic moieties to electron accepting quinone moieties in a given HS is a suitable proxy for its extent of the oxidative transformation in the environment.

ASSOCIATED CONTENT

Supporting Information

Electron donating and accepting capacities, and elemental compositions of humic substances; acidic functional group contents and other information regarding humic substances; cyclic voltammograms of ABTS; and additional information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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