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NATURE AND ABUNDANCE OF ORGANIC RADICALS IN NATURAL ORGANIC MATTER - EFFECT OF pH AND IRRADIATION

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Dissolved natural organic matter (NOM) plays an essential role in freshwater geochemical and biochemical processes. A major property, its redox behavior, can be attributed to the chinone building blocks, which can form stable radicals. However, electron paramagnetic resonance (EPR) data indicating free radicals on solid NOM are sparse. Here we present EPR-spectra of 23 NOM from European surface waters isolated by reverse osmosis. The organic radical concentrations of NOM ranged from 5×10^{15} to 1.84×10^{17} spins g^{-1}, and g-values from 2.0031 to 2.0045. Number and type of organic radicals in solid NOM are significantly influenced by the pH of raw water. EPR-experiments

indicate the presence of semiquinone-type radicals in coexistence with carbon-centred "aromatic" radicals, with the semiquinone-type radicals dominating at alkaline pH. Basically these processes are reversible. Organic radical concentrations in NOM adjusted to pH 6.5 before freeze-drying correlate with iron and aluminum contents.

UV- and VIS-irradiation of solid NOM can lead to more than 10-fold increase of the concentration of organic radicals. These radicals were long-lived and had the same g-value as the original radical. Similar effects were not observed with isolated humic- and fulvic acids, demonstrating the limited reflection of environmental properties of organic carbon by the classical isolation procedure.

Humic substances, reverse osmosis-isolations, EPR, FTIR, pH, irradiation

INTRODUCTION

Dissolved natural organic matter (NOM) play an essential role in most freshwater geochemical processes; e.g. photochemical processes (*i*), redox reactions (*ii*), interaction with traces of metal ions (*iii*) and hydrophobic compounds (*iv*).

Humic substances (HS), comprising the largest proportion (50–80%) of NOM, are complex organic molecules with polyphenolic and quinoid units as well as semiquinoid radicals as integral parts of the humic structure. These semiquionoid radicals in HS are important as they play an essential role in most of the processes mentioned above. Such stable organic free radicals can be studied by electron paramagnetic resonance (EPR) spectroscopy (*v*).

Usually there is only between 1 and 100 mg L^{-1} of NOM in freshwater ecosystems (*vi*). NOM can be concentrated by evaporation, captured by ultra-filtration, or isolated by reverse osmosis (*vii*). Humic substances themselves can be separated from the other NOM constituents by hydrophobic adsorption, typically on XAD. Evaporation and ultra-filtration are generally not practical for large NOM quantities,

45 and the main problems with XAD-adsorption are that it may exclude important organic water

46 constituents. Reverse osmosis-isolation is a preferred up-concentration technique as re-dissolution of

47 the freeze-dried material resembles the original freshwater NOM to a large extend (*viii*).

48 Stable organic radicals of isolated HS fractions (*i.e.* humic and fulvic acids) have been studied

49 extensively, and correlations with aromaticity and humification have been established for spin densities

50 in humic acids (*ix,x,xi,xii*). However, EPR-data on NOM are basically lacking. Little is therefore known

51 about the free organic radicals in NOM.

52 Here we present a comparative study on free organic radical concentrations in 23 NOM samples. This

53 study includes 19 well characterized NOM from the NOM-typing project (*viii*) and from the NOMiNiC

54 project (*xiii*). EPR-data on the NOM are compared with those for standard humic- and fulvic acids

55 provided by the International Humic Substance Society (IHSS).

56 The organic radicals in humic- and fulvic acids are reported to be sensitive to various physical

57 (radiation, x-ray (*xii*) or heat (*xi*)) and chemical influences (reducing and oxidizing agents, acid and

58 bases (*ii,xii,xiv*)). Increasing numbers of free organic radicals in humic matter as well as in tannins and

59 lignins in solution at higher pH was first reported by Steelink (*xv*). This increase is mainly caused by

60 electron transfer reactions between quinone and phenolic groups. Further mechanisms include

61 polymerization reactions (*xii*), or the generation of semiquinone radicals by autooxidation of

62 hydroquinone at alkaline pH (*xvi*). Studies on pH-effects on solid samples of humic or fulvic acids are

63 rare (*iv,xvii*). The effect of pH in NOM-solutions prior to freeze-drying on the resulting dry NOM and

64 its spin density has not yet been investigated. FTIR-spectroscopy was used to obtain further information

65 on the protonation of carboxyl groups and thus on structural properties of solid NOM.

66 Another parameter that affects the concentration of organic radicals in solid NOM is irradiation. No

67 previous information has been given on irradiation mediated enhancement of radicals in solid samples.

68 Here we present evidence for radical formation in solid NOM after both UV- and VIS irradiation.

EXPERIMENTAL SECTION

Natural organic matter. Dissolved natural organic matter (NOM) from German and Fennoscandic surface waters were up-concentrated by reverse osmosis and later freeze-dried. Inorganic cations in the raw water were exchanged for Na^+ prior to the reverse osmosis. Two of the samples are German NOM that are previously described (*xviii*). The 19 NOM-samples from Fennoscandic surface waters have been thoroughly characterized, either as a part of the NOM-typing project (*viii*) or the NOMiNiC-project (*xiii*). Humic- and fulvic acids were provided by the IHSS (*xix*) and are from Suwannee River, Florida peat, Summit hill and Elliott soil and Leonardite. Materials from Laurentian soil are provided by the former Fredriks Research Products, Amsterdam, the Netherlands.

Preparation of NOM from pH-adjusted solutions. The pH of NOM solutions (1 g L^{-1}) was adjusted by a 702 SM Tritrino (Metrohm Switzerland) titrator using either 0.1 N HCl or 0.1 N NaOH. The added aliquots of HCl or NaOH, respectively, were recorded and used to correct the weight of the samples for EPR and FTIR readings. In general, the fraction of the titration agent comprised less than 3 % (w/w) of the final freeze-dried NOM sample. For freeze-drying of 50 mL aliquots of pH-adjusted solutions, a Gamma 1-20 device (Christ, Germany) was used.

EPR-spectroscopy. Cw X-band EPR-spectra of pH-adjusted and freeze-dried samples were recorded at room temperature using a spectrometer of the type ERS300 (Centre of construction of scientific devices/Magnettech GmbH, Berlin-Adlershof, Germany) at a microwave power (P_{MW}) 2 mW. The samples were measured in quartz glass sample tubes with an inner diameter of 3 mm. For the determination of g-values, MgO/Cr^{3+} (g = 1.9796) was placed inside the cavity and measured simul-taneously with the samples. Spin concentrations (used to label the number of unpaired spins per gram of the actual sample) were determined using DPPH (α,α-diphenyl-picrylhydrazyl) in toluene as reference (*xx*). In order to minimize errors, all quartz glass tubes were adjusted to the same position inside the cavity. DPPH solutions were checked photometrically for stability and were used within 2 h after

94 preparation. Absolute spin concentrations were calculated after double integration of the spectra in

95 relation to those of DPPH.

96 **FTIR-spectroscopy.** FTIR-spectra were recorded on the freeze-dried material dispersed in KBr pellets

97 in the 4000 to 400 cm^{-1} range using a Shimadzu Hyper IR spectrometer. The KBr pellets were obtained

98 by pressing a mixture of 3 mg NOM and 800 mg KBr (99.99 pure, Sigma). The spectrometer was

99 blanked with KBr prior to use. The absorbance bands in the spectral range 1800-1500 cm^{-1} were fitted

100 by a non-linear curve fit using ORIGIN6.1.

101 **Gel permeation chromatography (HPSEC).** Results of size-exclusion-chromatography differ widely,

102 depending strongly on the methods and the calibration substances used (*xxi*). Therefore, all samples had

103 to be investigated under the same conditions in order to obtain comparable results. HPSEC was

104 performed by custom-designed automated size-exclusion-chromatography as described previously

105 (*xxii*). The chromatograms were used to detect molecular weights (M_p) at the peak maximum of the

106 humic matter fraction. In addition, the specific UV-absorbance (sUVa) of humic matter and NOM,

107 which is defined as the ratio of the spectral absorption coefficient at 254 nm and the organic carbon

108 concentration of the HS-fraction, was determined. This parameter can be used as a measure for aromatic

109 and unsaturated structures.

110 **Irradiation of solid NOM and HS.** UVC and UVA/B: 0.02 g of solid NOM and HS, equally

111 distributed on an area of 15 cm^2, were UVC and UVA/B irradiated by a low pressure mercury lamp

112 (HNU6, 0.032 W cm^{-2}), or by a fluorescence tube (Cosmedico, Arimed B, UVA: 1.5 W m^{-2}, UVB: 43.4

113 mW m^{-2}), respectively. For visible (VIS) radiation exposure, samples were irradiated inside the EPR-

114 tubes by a tungsten lamp (250 mW cm^{-2}).

115

116 **RESULTS AND DISCUSSION**

117 **EPR-spectra of NOM.** The EPR-spectra of NOM are characterized by a narrow single line at g ≈

118 2.004 which can be attributed to organic radicals. Fig. 1A-C shows the EPR-spectra for Aurevann,

119   Trehørningen, and Birkenes spring, as examples. The typical sextet of lines, assigned to Mn^{2+}-ions with

120   a splitting constant a $\approx$ 0.9 mT, can be found at g $\approx$ 2. Furthermore, all spectra of NOM showed

121   asymmetrical lines at an effective g-value (g′) $\approx$ 4.2, which is assigned to Fe^{3+}-ions (*xxiii*). The latter is

122   often accompanied by a second, broad resonance (*xxiv*) at g′ $\approx$ 2. Some spectra exhibited an apparently

123   unresolved broad feature at g′ = 3 − 2, which probably consist of an envelope of several resonances

124   arising from various magnetically-interacting ions, such as {Fe^{2+}….Fe^{3+}}-, Cu^{2+}-, Mn^{2+}- and V^{4+}-ions as

125   described in Senesi et al. (*xxv*).

126   The EPR-parameters (spin concentration, linewidth (ΔB) and g-values) of the free organic radicals of

127   both NOM and IHSS humic- and fulvic acids are summarized in Tab. S1. Previous and present

128   measured spin concentrations for the free organic radicals in Suwannee River IHSS-standard (*xxv*) show

129   a variation from 0.64×10^{17} spins g^{-1} to 3.93×10^{17} spins g^{-1} (this work: 1.16×10^{17} spins g^{-1}) and

130   0.54×10^{17} spins g^{-1} to 5.41×10^{17} spins g^{-1} (this work: 1.01×10^{17} spins g^{-1}) for the humic- and fulvic

131   acids, respectively. The determination of absolute spin densities bears a certain degree of experimental

132   error due to geometrical arrangement, differences in microwave power, cavity couplings and use of

133   standards (*xxvi*). However, the ratio between spin concentrations in humic and fulvic acids remains

134   about 1.16, which is in good agreement with the data published in (*xxvi*, 1.18). Therefore, despite

135   differences in the absolute data, the relative values of spin concentrations can be used for further

136   interpretation.

137   Comparison of averaged values for ΔB, g-values, and spin concentrations of humic acids, fulvic acids

138   and NOM reveals that: NOM give broader lines (p < 0.001, ΔB_{NOM} = 0.62 $\pm$ 0.18 mT, ΔB_{HA} = 0.43$\pm$

139   0.065 mT), higher g-values (p < 0.001, g_{NOM} = 2.0038 $\pm$ 0.0005, g_{HA} = 2.0034$\pm$0.0002, g_{FA} =

140   2.0034$\pm$0.0003), and lower spin concentrations (J_{NOM} = 4×10^{16} spins g^{-1}, J_{HA}= 3.8×10^{17} spins g^{-1}, J_{FA}

141   =1.7×10^{17} spins g^{-1}, p<0.003). Inhomogeneous broadening effects may be caused by unresolved

142   hyperfine structure and broadening by statistical distribution of spin-coupling parameters. The latter

143   effect may point to higher heterogeneity of the contributing radical species in NOM. Higher g-values

144   (i.e. ~ 2.0044) are typical for semiquinone radicals, whereas smaller g-values (i.e. ~ 2.0035), as

145 measured for humic- and fulvic acids, indicate that organic radicals are part of aromatic subsystems of

146 the samples.

147 Organic radicals could be detected in all samples, except in those from Birkenes, which are

148 characterized by intense lines from Mn^{2+}-ions. Birkenes is an acidified site with comparatively low

149 organic carbon concentrations (*viii,xiii*). The structures of NOM from Birkenes appear to be simpler

150 (lowest molecular weight), less aromatic, and composed essentially of polysaccharide residues (*xiii*).

151 Because of differing ash contents among NOM and humic- and fulvic acids, spin concentrations were

152 related to gram carbon (instead of the mass of the samples). High spin concentrations, comparable to the

153 aquatic Suwannee River humic acid, were measured only in material from lakes with circum neutral pH

154 (i.e. Trehørningen, Lake Schwarzer See, and Hietajärvi). Low spin concentrations were found in the rest

155 of the investigated NOM, which are all from acid surface waters (*cf.* Tab. S1).

156 In order to assess which other factors might influence EPR-parameters in NOM, a correlation analysis

157 was conducted on g-values, spin concentration and ΔB with the physico-chemical data of the NOM as

158 well as their catchment characteristics from the NOMiNiC-project (*xiii*). A number of the characteristics

159 of NOM were found to be strongly correlated with the g-value. The best correlation (R = -0.95, p =

160 0.000) was found between g-value and H^+ activity in the original water sample. A slightly weaker

161 correlation was found between the g-value and the H^+ activity with the re-dissolved NOM solution. We

162 found a similar trend of increasing g-values (R = 0.80, p = 0.000) as well as increasing spin densities (R

163 = 0.75, p = 0.000) with pH of raw water, when including the German and NOM-typing samples into the

164 analysis (Fig. 2A and 2B). From these data we conclude that the pH-value of the raw water is an

165 important factor that bears a strong influence on the organic radicals in solid NOM. In addition to this,

166 the g-values of the NOMiNiC samples were found to correlate with the spectral absorption, both in the

167 UV and visible range (R = -0.91 and R = -0.86, respectively). These parameters are often used as

168 proxies for the amount of TOC in solution. Vogt et al. (*xiii,xxvii*), studying key site variables governing

169 the functional characteristics of NOM in Nordic forested watersheds, found that both H^+ and TOC

170 concentration were significant empirical factors explaining much of the variation in the physico-

171 chemical characteristics of NOM. The co-variation among these parameters may therefore give an

172 indication why g-values are correlated with a large number of NOM descriptors. The complete set of

173 significant correlations is given in the supplementary information (*cf.* Tab. S2).

174 Furthermore, using all NOM and HS samples, we investigated whether EPR-parameters are correlated

175 with M_p, and sUVa (*cf.* Tab. S1). The spin concentration was found to be correlated both to M_p ($r =$

176 0.76, $p = 0.000$) and sUVa ($r = 0.81$, $p = 0.000$), but no correlation were found for g-values and ΔB.

177 Furthermore, excluding humic- and fulvic acids from the linear regression analysis rendered no

178 significant correlation for NOM alone.

179 **EPR-spectra of NOM equilibrated at different pH.** In order to study the influence of pH on the

180 organic radicals in NOM the pH was adjusted from 3 to 10, in steps of one pH unit, in four solutions of

181 NOM Skjervatjern fall, Lake Fuchskuhle, Hietajärvi fall and Lake Schwarzer See comprising a wide

182 range of spin concentrations. Darker solutions were obtained for all samples with increasing pH. These

183 changes were preserved in the freeze-dried samples as well. This is likely caused by an increasing

184 amount of anionic structures due to the de-protonation of weak organic acids. Such anionic structures

185 are additionally stabilized by the added Na^+-ions (from the pH titration adding NaOH), as well as by

186 further aggregation and/or polymerization of the HS.

187 At low pH nearly no organic radicals were detected in the EPR-spectra (*cf.* Fig. S1). This is in

188 agreement with the general observation of enhancement of subsequent reactions of free radicals by

189 protonation (yielding a smaller net effect). With increasing pH a line at g $\approx$ 2.004 increases in all pH-

190 adjusted samples (Fig. 2C) and is accompanied by increasing g-values (Fig. 2D). No trend of ΔB-

191 changes was observed. This may be caused by structural reorganization induced by pH-dependent

192 changes of the H bonds as well as further ionic interactions. Increasing spin concentrations of organic

193 radicals in humic matter with increasing pH were reported by Sposito et al. (*xvii*), and Martin-Neto et al.

194 (*iv*). However, some of their samples also exhibited no effect or even decreasing spin concentrations

195 with increasing pH.

196   Parallel to these effects on the organic radicals, a decrease of the sextet of lines assigned to Mn^{2+}-ions

197   occurs. This probably displays the pH-dependent oxidation to antiferromagnetic MnO_2. Moreover, the

198   formation of $Mn(OH)_2$, which will be partially oxidized to Mn(III), when exposed to oxygen, and

199   formation of Mn(II)-complexes with organic rests (particularly with carboxylic and phenolic groups)

200   characterized by large zero-field splittings, must also be considered. No changes, except an increase at

201   pH = 10, were found for the resonances at $g' = 4.3$, (and $g' = 2$) assigned to the Fe^{3+}-ions.

202   In order to check whether the observed pH-effects are reversible, another three samples of Lake

203   Schwarzer See which had previously been adjusted to low pH, were re-dissolved and titrated to pH 9,

204   and another three samples, previously adjusted to high pH, were titrated to pH 3. In both experiments,

205   independent of the starting pH, the expected spin densities were obtained and essential features of the

206   Mn^{2+}-pattern recovered or vanished as described above. In addition, the fact that the changes are

207   reversible exclude effects conceived as irreversible, such as polymerization. It must be pointed out,

208   however, that even at pH = 10, the original high spin concentrations of NOM from Hietajärvi and Lake

209   Schwarzer See were not reached (Fig. 2A and C).

210   Investigation of samples adjusted to circum-identical pH provides the possibility to separate effects due

211   to different sample pH and those of metal ions in NOM. For that purpose 12 NOM samples were

212   adjusted to pH 6.5 before freeze drying and then analyzed. The resulting EPR-parameters were tested

213   for correlations with the concentration of the paramagnetic ions of copper, iron, and manganese and,

214   with the non-paramagnetic aluminum, zinc, calcium, magnesium, and potassium, which are abundant in

215   NOM as well. Due to the presence of paramagnetic ions (*xxviii, xi*) spin exchange can decrease the

216   concentration of organic radicals and change the microwave saturation behavior. Furthermore, effects

217   on the generation and stabilization of organic radicals could be expected from the participation of metal

218   ions in redox processes. The results presented in Tab. S4 point to an influence of iron and aluminum on

219   the spin concentration, and potassium on the g-values of the organic radicals in NOM. These results are

220   in line with Novotny and Martin-Neto 2002 (*xxviii*) and show that the influence of metals on spin

221 concentration in NOM has been masked by the much stronger pH-dependent effect and was therefore

222 not found in the original statistical analysis.

223 **EPR-spectroscopy on NOM irradiated by UV/VIS.** The NOM samples from Lake Fuchskuhle and

224 Schwarzer See were irradiated by UV and visible light (VIS) in order to check whether this may

225 influence spin concentrations in solid NOM. After 40 min of UVC-irradiation between 17 and 40-fold

226 higher spin concentration were observed at constant values of g. The EPR-lines assigned to Fe^{3+}- and

227 Mn^{2+}-ions remained unchanged, indicating that these ions are probably not directly involved in the

228 observed effect. The enhanced radical concentrations remained stable over weeks. UVC-irradiation of

229 NOM was accompanied by a browning of the samples, caused by subsequent reactions of a part of

230 radicals formed. It is known that most aromatic systems form radicals upon UVC-irradiation: however,

231 here the structural relaxation into the radical ion state is in general not so pronounced as in the case of

232 the semiquinone-type radicals. Therefore, they easily can react back to the original state or react with

233 oxygen. It is important to note that in our experiments humic matter and NOM provide the possibility to

234 stabilize such irradiation-induced radicals over weeks. The identical g-values found in irradiated and

235 non-irradiated samples indicate that only a particular type of deep traps exist for the radicals where they

236 can be protected from subsequent reactions. We suppose that the necessary condition for the protection

237 of irradiation-induced radicals is related to the structural arrangement of NOM, which in that case is

238 pH-dependent.

239 Moderate enhancement of radical concentrations in NOM was also observed after both VIS- and low-

240 intensity UVA/B-irradiation (*cf.* Tab. S3). It should be noted that the irradiation power during UVA/B-

241 exposure was less than 10% of that of solar UV-irradiation determined for Lund, Sweden (*xxix*).

242 In contrast to the intense EPR-lines on irradiated NOM, only a small increase was found after UVC-

243 irradiation of solid humic matter (i.e. Soil-FA, Summet Hill-HA, and Peat-(R)-HA, with 2-, 3.9, 1.5 fold

244 increase, respectively). This is basically in agreement with former investigations of Senesi (*xxiv*), who

245 reported that UV-irradiation of solid FA had no affect on the organic radicals. The comparable low

246 enhancement of spin concentration in humic and fulvic acids could be seen in connection with the

247 deeper brown of these samples compared with NOM, which would reduce the penetration depth of light

248 into the sample. This was proven not to be the case by UVC-irradiation of the synthetic HS (HS1500)

249 (*xxx*). Despite that this material has the same dark-brown color as the investigated humic acids, it

250 exhibited a 14-fold increase of the EPR-line upon UVC-irradiation. Therefore, the darkness of the

251 samples can not account for the observed differences. A major difference between NOM and humic and

252 fulvic acids is that the latter are leaving out about 20–50% of the organic material which is referred to as

253 "hydrophilic acids" (*xxxi*). It may therefore be this fraction that accounts for the huge enhancement of

254 radical concentration in NOM found in this study. Although isolated humic and fulvic acids provide

255 valuable standards and models for natural humic matter, these isolations are limited in their reflection of

256 certain properties of NOM.

257 These indicate that exposure of solid NOM to intense solar irradiation can enhance spin concentration.

258 This process takes place on the surface of Earth. The irradiation-induced enhanced radical concentration

259 may be connected to the photo-production of singlet oxygen and other reactive species which Gohre et

260 al. (*xxxii*) observed on soil surfaces. In analogy to the processes known to occur upon irradiation in

261 dissolved NOM and HS (*i*), the increase of radicals then would be connected to secondary radicals,

262 which are formed during oxidation reactions of HS components by reactive oxygen species.

263 **FTIR-spectroscopic investigation of solid NOM at different pH.** FTIR-spectroscopy was applied to

264 gain further insight into the pH-mediated effect on certain functional groups in NOM. Although infrared

265 spectra of HS and NOM are characterized by broad overlapping bands, which complicate their

266 interpretation, a number of peaks (*cf.* Fig. 3 and Fig. S2) can be identified and attributed to functional

267 groups (*xxxiii,xxxiv*). With increasing pH, some spectral changes can be observed, which are basically

268 related to the formation of carboxylate anions, for example to the increase of the bands at 1614–1627

269 cm^{-1} (asymmetric stretching), and 1145 cm^{-1}, (symmetric vibration). In contrast, the bands at 2615 cm^{-1}

270 (OH-vibration of the carboxylate group), at 1712–1723 cm^{-1} (C=O stretching) and the shoulder at 1200-

271 1288 cm^{-1} (C-O stretching vibrations of esters, ethers and phenols), are only present in freeze-dried

272 NOM from solutions with pH 3 to pH 5.

273 In order to quantify the ratio of protonated and deprotonated carboxylic groups, the 1800–1500 cm^{-1}

274 region in a FTIR-spectra of NOM material from FUKU was deconvoluted by Gaussian line profiles.

275 From pH 3 to pH 5 the FTIR-spectra could be fitted by using a minimum of 4 Gauss curves (*cf.* Fig.

276 S3). For spectra of freeze-dried NOM with original solution $\geq$ pH 6 an additional Gauss curve at 1571–

277 1573 cm^{-1} was necessary in order to obtain an acceptable fit result (X^2 below 10^{-5}). Nonlinear curve fit

278 of FTIR-spectra of NOM from HIET and SKJ yielded similar results.

279 The peak areas (A_i) of the Gauss curves in Fig. S4 can be used as a measure for the abundance of

280 functional groups. Based on a conceptual interpretation of the pH-dependency of A_2 and A_3 it is

281 possible to assign those to the COOH- and COO$^-$-groups, respectively. A_4, which had to be introduced

282 at pH $\geq$ 6, increased with pH and Na$^+$ concentration. For all NOM, a strong decrease of the ratios $A_2/(A_3$

283 $+ A_4)$ to a constant value of about 0.13 at pH 6 was obtained, i.e. the increase in A_4 appears to be on the

284 expense of A_3. This fourth peak may therefore represent Na-carboxylate (COONa).

285 **What do the EPR-and FTIR-data tell?** Linear regression analysis was performed in order to check

286 whether pH-dependent EPR- and FTIR-data were correlated. This analysis gave only one significant

287 correlation, namely between A_4 (COONa) and spin concentration (R^2 = 0.81, 0.77 and 0.76 for Lake

288 Fuchskuhle, Hietajärvi and Schwarzer See, respectively). Already in 1966, Tollin and Steelink (*v*)

289 reported a 10–100-fold increase in spin content when humic acids were converted to their solid sodium

290 salts. However, these authors also reported on increased spin contents of these compounds after acid

291 hydrolysis. According to Dudarchik et al. NaCl drastically reduces spin concentration in a peat humic

292 acid (*xxxv*). They concluded that the formation of sodium-salts of HS breaks hydrogen bonds and

293 therefore brings about structural changes of the inter and intra molecular assembly of HS.

294 The observed correlation between COONa and spin concentration may as well reflect the added [OH$^-$]

295 along with Na$^+$ causing a de-protonization of the weak acid functional groups. Additional evidence that

296 the concentration of Na^+-ions in solid NOM does not account for the increase of radical concentration

297 arises from the back-titration experiments when reversibility was tested. It is therefore assumed that we

298 observe a net-effect, where OH^--ions favor the formation of semiquinone radicals, and Na^+-ions on the

299 other hand, hamper stabilization of radicals by competing with H-bonding. The latter obviously provide

300 certain spatial arrangements, so-called "cavities" within humic matter and NOM, which favor the

301 formation of long-lived radicals. In such cavities the partial pressure of oxygen is reduced and spin

302 exchange (*xxxvi*) between the paramagnetic centers takes place yielding the observed single EPR-lines.

303 Furthermore, the cavities shield the radicals from external paramagnetic species like oxygen gas (it is

304 well known that radicals derived from phenolic structures are sufficient sensitive to oxygen) as

305 evidenced in a series of experiments. The presence of such cavities in humic matter is in line with

306 Sposito et al. (*xvii*), and Martin-Neto et al. (*iv*), who concluded from power saturation experiments that

307 at low pH a broader distribution of local effective magnetic sites exist. These environments were

308 discussed to include protected sites of significant hydrophobicity that disappear at higher pH because of

309 conformational changes. Investigations of Myneni et al. (*xxxvii*) revealed that pH may induce changes in

310 humic matter from globular ring-like structures in acidic solution to uniform, small aggregates in

311 alkaline solution. Recent calculations of Alvarez-Puebla and Garrido (2005) (*xxxviii*), on gray humic

312 acids, indicate that stretched linear arrangements dominate at alkaline pH, due to coulomb-repulsion

313 forces of negatively charged groups. Upon acidification the charge of ionized groups is neutralized, and

314 inter- and intra-molecular hydrogen bonding causes shrinkage with exclusion of solvent from the

315 matrix. In our experiments we found larger g-values above 2.004 and broader lines at high pH. This is

316 typical for the spin-orbital interaction of unshared electrons with oxygen and a hyperfine interaction

317 with protons of the aromatic ring, as would be expected for semiquinone-type radicals. At lower pH the

318 concentration of paramagnetic centers and g-values are decreased, which is typical for aromatic

319 polyconjugated systems (*xxxix*). As indicated by lower spin concentration and g-values at low pH,

320 carbon centered radicals are the remaining fraction from the original radical pool. This is considered to

321 be a result of H-bonding, which leads to structural/sterical changes of humic matter. The possibility of a

322 delocalization of the electron spin density from O-atoms to aromatic C-atoms through the aromatic

323 network has been proposed by Senesi et al. (*xl*). Consequently, the measured data on spin concentration

324 and g-value for the organic radicals in HS must be considered as sum parameters for various slightly

325 differing paramagnetic species, implying the presence of semiquinone-type free radicals in

326 coexistence/cooperation with aromatic systems. The state of the carboxylic groups (and other pH-

327 sensitive groups) determines the structural state of NOM, which gives the basis for the stabilization of

328 certain type of organic radicals.

329 The importance of spatial arrangements on the type of radicals formed in NOM is additionally

330 supported by the results of irradiation experiments, where we found that the newly formed radicals

331 exhibited always the same g-value as found for the initial radicals. Despite the generation of a wide

332 variety of transient radical species it appears that only certain "types" of radicals can be stabilized.

333

338

339 SUPPORTING INFORMATION available:

340 Four Figures illustrating for pH-adjusted NOM samples: S1) weight-normalized ESR spectra, S2)

341 FTIR-spectra, S3) and S4) results of band-deconvolution of FTIR-spectra.

342 Four tables: S1) EPR-parameters for all NOM, humic- and fulvic acids investigated, S2) results for

343 EPR-parameter of the correlation matrix performed in the NOMiNiC data, S3) enhancement of spin

344 concentration in solid NOM after UV/VIS-exposure, and S4) correlations for NOM adjusted to pH6.5.

345

346   Brief:

347   EPR-measurements revealed that the concentration of semiquinone-type radicals in solid NOM is

348   influenced by both the pH-value of the original solution and the metal content.
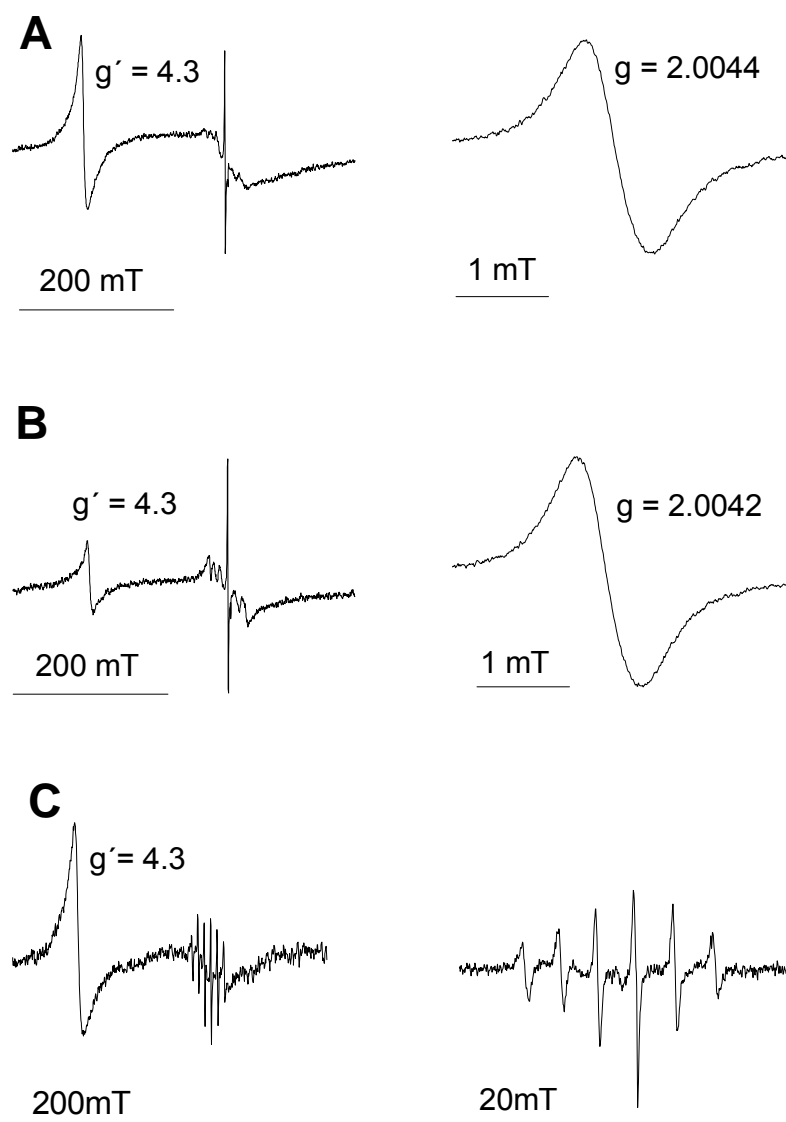
**Figure 1**. Typical EPR-spectra NOM (solids): A) Aurevann, B) Trehorningen, and C) Birkenes spring. Left hand side: wide scan range ESR spectra (500 mT), right hand side: spectra of organic radicals for SZS and Trehørningen and spectrum of Mn^{2+}-ions for Birkenes (10 mT, baseline corrected). The higher intensity of the forth manganese line in Figure 1C, arises from the superimposition with the reference sample $MgO:Cr^{3+}$ fixed in the cavity. Spectra were measured at a microwave power (P_{MW}) 2 mW, and a modulation amplitude of 0.125 mT in quartz glass sample tubes with an inner diameter of 3mm at 291K.

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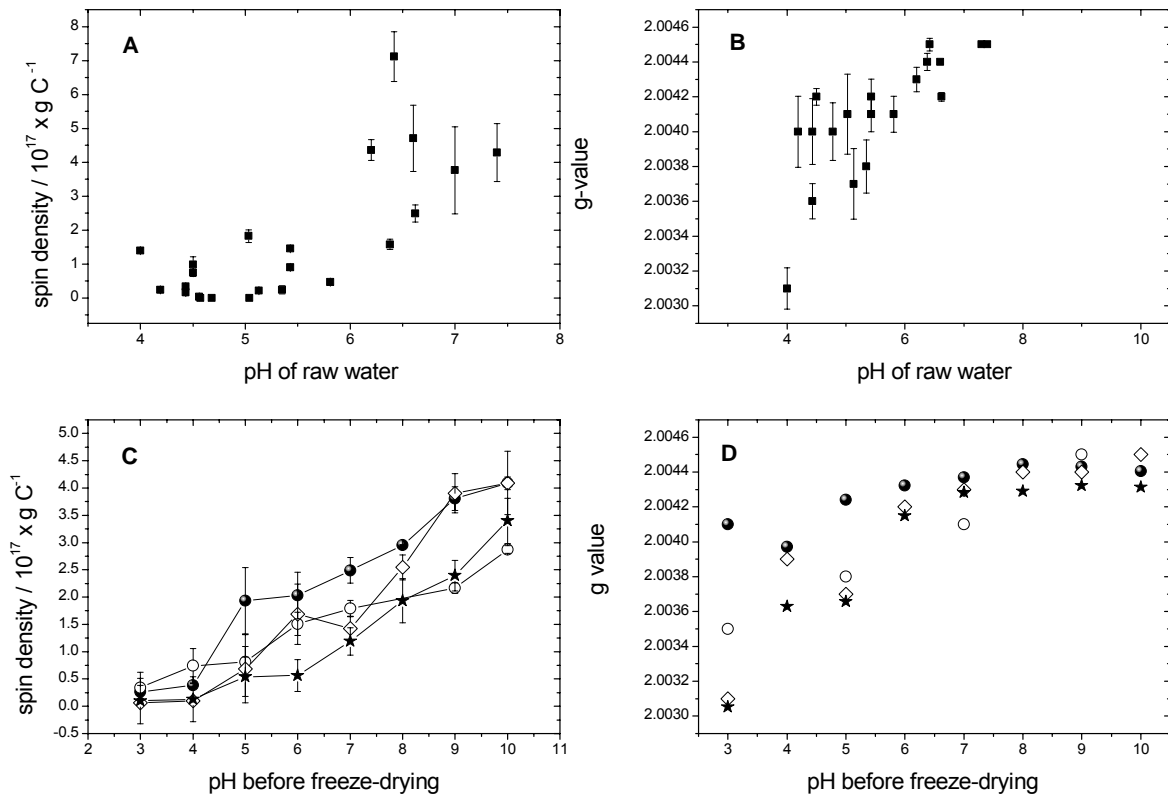
Figure 2. (A) Plot of spin densities normalized to gram carbon, and (B) g-values for NOM vs. pH of raw water taken from literature, (C) spin densities and (D) g-values of pH-adjusted NOM (● Fuchskuhle, ○ Lake Schwarzer See, ◇ Hietajärvi, ★ Skjervatjern).

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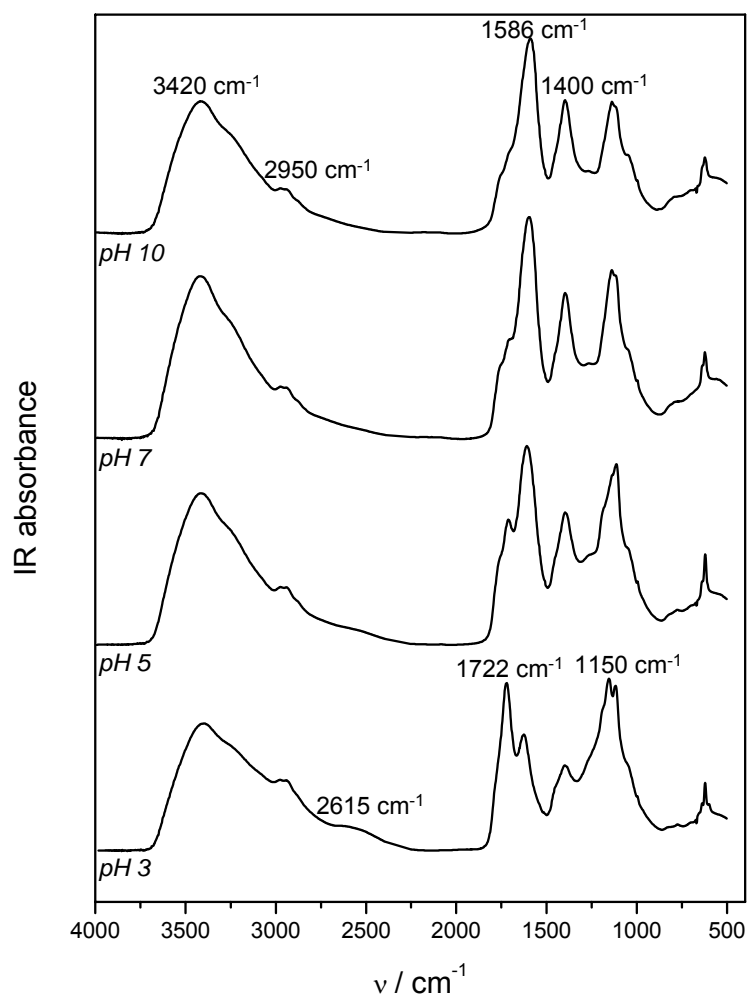
Figure 3. FTIR-absorbance spectra of pH-adjusted solid NOM from Lake Fuchskuhle illustrating the decrease of the absorbance at 1722 cm^{-1} and 2615 cm^{-1} assigned to the protonated form of the carboxylic groups in favor of the absorbance at 1586 cm^{-1} and 1400 cm^{-1}, which are assigned to the carboxylate anion of humic matter with increasing pH.

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