Photoreduction and Stabilization Capability of Molecular Weight Fractionated Natural Organic Matter in Transformation of Silver Ion to Metallic Nanoparticle

Yongguang Yin,[†] Mohai Shen,[†] Xiaoxia Zhou,[†] Sujuan Yu,[†] Jingbo Chao,[‡] Jingfu Liu,^{*,†} and Guibin Jiang[†]

[†]State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

[‡]Chemical Metrology and Analytical Science Division, National Institute of Metrology, Beijing 100013, China

S Supporting Information

ABSTRACT: Photoinduced reduction of silver ion (Ag^+) to silver nanoparticles (AgNPs) by dissolved organic matter (DOM) plays a crucial role in the transformation and transport of engineered AgNPs and Ag^+ in aquatic environments. DOM is a mixture of natural polymers with wide molecular weight (MW) distribution, and the roles of specific components of DOM in the photoreduction of Ag^+ to AgNPs are still not understood. In this study, MW fractionated natural organic matter (M_r -NOM) were investigated for their roles on the photoreduction process and stabilization of the formed AgNPs. This photoinduced reduction process depends highly on pH, concentration of Ag^+ and NOM, light quality, and the MW of M_r -NOM. Monochromatic radiation and light attenuation correction suggested that the difference of M_r -NOM on reduction was mainly ascribed to the differential light attenuation of M_r -NOM rather than the “real” reductive ability. More importantly, compared with low MW fractions, the high MW M_r -NOMs exhibit drastically higher capability in stabilizing the photosynthesized AgNPs against Ca^{2+} -induced aggregation. This finding is important for a better understanding of the differential roles of M_r -NOM in the transformation and transport of Ag^+ and engineered AgNPs in DOM-rich surface water.

INTRODUCTION

With the progress of nanotechnology, more and more nanotechnology-based consumer products are involved into people's daily life. According to the data from a recent report, “Project on Emerging Nanotechnologies,” the nanotechnology consumer products inventory contained 1628 products or product lines in 2013, in which consumer products containing silver nanomaterials accounted for over 23%.¹ Silver nanoparticles (AgNPs), well-known for their broad-spectrum antimicrobial properties, have many applications in consumer products, including textiles, food packaging, cosmetics, medical devices, and household appliances.² In producing, using, washing, and disposing of AgNPs and their products, AgNPs could inevitably enter surrounding environments.^{3–7} As demonstrated recently, AgNPs are toxic to microorganisms, algae, plants, invertebrates, vertebrates and human cell lines.⁸ Thus, the widespread use and potential toxicity of AgNPs to various organisms and humans have led to concerns about their safety.

The transformation of different silver species in natural environments plays a crucial role on the stability, transport, and toxicity of AgNPs.⁸ Unlike other persistent toxic substances, AgNPs are highly dynamic and prone to transform into other

silver species in natural environments.^{9–12} It is known that both metallic silver and silver ion (Ag^+) are not thermodynamically stable under environmental conditions.⁸ Metallic AgNPs could be oxidized by dissolved O_2 and release Ag^+ in aquatic environments, a process that is highly dependent on the size of AgNPs and the pH of the solution.¹³ Although the effect of size also plays an important role in the uptake and toxicity of AgNPs,^{14,15} the acute toxicity of AgNPs has been mainly ascribed to the released Ag^+ .¹⁶ In natural waters, dissolved Ag^+ could additionally be rereduced to metallic AgNPs by dissolved organic matter (DOM)^{17–20} or extracellular polymer substances.^{21,22} Our previous study demonstrated that in natural surface water Ag^+ could convert to AgNPs under sunlight by DOM.¹⁸ In this process, a phenol group in DOM transfers one electron to dissolved O_2 to form a superoxide radical,²³ and this superoxide subsequently reduces Ag^+ to AgNPs.²⁴ Another study suggested Ag^+ –NOM complexation also plays a crucial role in this reduction process.²⁰ The reduction of Ag^+ to AgNPs

Received: April 24, 2014

Revised: July 16, 2014

Accepted: July 22, 2014

Published: July 22, 2014

by DOM possibly improves the persistence of AgNPs in sunlit DOM-rich aqueous environments¹⁸ and has crucial impact on the toxicity of AgNPs to organisms.²¹ However, DOM is a complex polydispersed mixture of natural polymers, with molecular weights (MW) ranging from less than 100 Da to over 300 kDa,²⁵ and it is still unknown which components of DOM are mainly responsible for the reduction of Ag⁺ and stabilization of the resulting AgNPs. Different MW fractionated DOM differ greatly in chemical composition,²⁶ acidity,²⁷ adsorption,²⁸ binding,^{29,30} and reaction behaviors.^{31–33} Carboxylic groups were found to be concentrated in low MW fractions of humic acids (HA), while polysaccharides and peptides were in high MW fractions.^{22,30} The difference among MW fractions of HA could further induce the difference of photochemical properties.^{34–36} For example, the photoreduction of Fe(III) and Cr(VI) highly depends on the MW of DOM, with low MW DOM is the major driver for photoreduction.^{33,36}

The overall objective of this study was to investigate the effect of MW fractionated natural organic matter (M_F-NOM) on the reduction of Ag⁺ into AgNPs in water and to understand the effects of M_F-NOM on the morphology and stabilization of photosynthesized AgNPs in the presence of Ca²⁺. To this end, Suwannee River Natural Organic Matter (SRNOM) was selected as model DOM, and the reductive kinetics of Ag⁺ by M_F-NOMs and pristine SRNOM were studied and compared under simulated sunlight and heating. This work is helpful to understand the fate of highly dynamic AgNPs and other ionic silver species and their interaction with DOM in surface waters.

MATERIALS AND METHODS

Materials. SRNOM (Lot No. 1R101N) was obtained from the International Humic Substance Society (St. Paul, MN). Stock solutions were prepared at 1.0 g L⁻¹ in deionized water and shaken on a rotator overnight at room temperature. AgClO₄ was purchased from Beijing Chemicals (Beijing, China). Amicon Ultra-15 centrifugal filter (with 3 kDa, 10 kDa, 30 kDa, and 100 kDa nominal MW cutoff, respectively) from Millipore (Darmstadt, Germany) were used for fractionation of SRNOM.

UV films, purchased from Energy-Film (Portland, Oregon), were used as UV-block film. Mylar films, purchased from United States Plastic Corp. (Lima, Ohio), were defined as UV-B-block film. Aluminum foil was purchased from Cleanwrap (Shanghai, China). Transmittance of light through these films or aluminum foil is shown in Supporting Information (S) Figure S1.

NOM Fractionation. The Amicon Ultra-15 centrifugal filters were initially rinsed with deionized water thoroughly to remove glycerol. Next, the stock solution of SRNOM was loaded into the 100 kDa filter and centrifuged for 30 min at 3743g by a Sigma 3–18 K centrifuge (St. Louis, MO). The filtrate was collected and then further filtered sequentially by 30 kDa, 10 kDa and 3 kDa filters. The centrifuging rates for 30 kDa, 10 kDa and 3 kDa filter were 5095, 6654, and 8422 g, respectively. The retentate was then washed with deionized water and collected as M_F-NOM (>100 kDa, 30–100 kDa, 10–30 kDa, 3–10 kDa, respectively). The filtrate after 3 kDa filter treatment was collected as <3 kDa M_F-NOM.

Characterization of Pristine and M_F-NOM. Concentrations of pristine SRNOM and M_F-NOM were measured on a Teledyne Tekmar Total Organic Carbon (TOC) Fusion Analyzer (Mason, OH). The MW distribution of M_F-NOM fraction is shown in SI Figure S2. The total recovery of TOC

was 69.2%. Potential losses of NOM could have occurred during the washing procedure or by adsorption onto the filter membrane.³⁰ Five mg L⁻¹ dissolved organic carbon (DOC) of pristine- or M_F-NOMs were prepared in deionized water and adjusted to pH 8.0 with 5 mmol L⁻¹ borate buffer for UV-vis and fluorescence spectroscopic characterization. UV-vis spectra were recorded on a Shimadzu UV-vis-NIR Spectrophotometer (UV-3600, Japan). Fluorescence excitation–emission matrices were measured by a Horiba spectrophotometer (FluoroMax-4, Edison, NJ) with a 1 cm quartz cuvette. Emission was measured from 280 to 600 nm in 2 nm increments, at excitation wavelengths of 200 to 450 nm in 5 nm increments, with both excitation and emission slit widths 5 nm. The fluorescence inner-filtering effect was corrected by UV-vis absorbance spectra of the identical NOM solution.

Photoinduced Reduction of Ag⁺ to AgNPs. The experiments were performed in 100 mL quartz glass bottles with quartz caps. The simulated sunlight was provided by a solar simulator (TEMI880, Beifang Lihui, Beijing, China) equipped with three wind-refrigerated 2500 W Xe lamp. The spectrum of the simulated sunlight was measured by a miniature fiber optic spectrometer (USB2000, 2E7909, Ocean-Optics Inc., Dunedin, FL) with wavelength from 339 to 1027 nm (SI Figure S3a). The intensity of the light was set as 400 W m⁻². During the experiment, the reaction temperature was maintained at ~35 °C. The total volume of the solution was 100 mL. Five mmol L⁻¹ borate was used to buffer the solution. Three mL solution was sampled at specific time intervals for UV-vis spectrometry and transmission electron microscope (TEM) characterization. Considering the repeatability and reproducibility of the photoreduction process and UV-vis spectrometry, we performed each test in one sample without duplication for UV-visible spectroscopy characterization. The pH, concentrations of Ag⁺ and NOM, and concentration of Ca²⁺ (as Ca(ClO₄)₂) were investigated during photoreduction of Ag⁺ to AgNPs.

The effect of light quality was investigated by covering the quartz bottle with aluminum foil, UV-block film, and UV-B-block film, and the resulting solutions were defined as “dark” (covered with aluminum foil), “Full radiation” (without covering), “UV-A+ Visible” (covered with UV-B-block film) and “Visible only” (covered with UV-block film).

The essential reductive abilities of M_F-NOMs on Ag⁺ to AgNPs were further evaluated at a high concentration of NOM (0.4 mmol L⁻¹ AgClO₄, 20 mg L⁻¹ DOC NOM). The monochromatic irradiation (366 nm) was provided with a rotating turntable, merry-go-round photoreactor (XPA, Xujiang Electromechanics, Nanjing, China) adapted with glass filters to isolate the 366 nm light from the Hg lamp and with a medium pressure Hg vapor lamp (100 W) in the center. The spectrum of light source was also measured by a miniature fiber optic spectrometer (USB2000, 2E7909, OceanOptics Inc., Dunedin, FL) (SI Figure S3b and c). In addition, the transmittance of light through glass filter is shown in SI Figure S4, demonstrating that the glass filter could effectively separate 366 nm light from Hg lamp. The experiments were performed in 50 mL quartz tubes with quartz caps (50 mL, 2.2 cm diameter) and reaction temperature was maintained at ~10 °C by thermostatic water-bath (CHD-0515, Boyuan Analytical Instrument, Changzhou, China).

Ultrafiltration coupled with inductively coupled plasma mass spectrometry (ICP-MS) (7700ce, Agilent, Santa Clara, CA) was used to determine Ag⁺ and AgNPs in the photosynthesized

AgNPs solution. Briefly, 1 mL photosynthesized AgNPs solution, 4 mL deionized water and 5 mL of saturated Na₂EDTA, were added into the Amicon Ultra-15 centrifugal filter (with 3 kDa nominal MWCO) (Millipore, Darmstadt, Germany). After centrifugation at 9600 rpm for 40 min, the filtrate was diluted by 5% HNO₃ for Ag⁺ determination by ICP-MS. The concentration of AgNPs was calculated by subtracting Ag⁺ concentration from the total silver concentration, which was determined by ultrafiltration-ICP-MS. Here, Na₂EDTA was added to complex with Ag⁺ and facilitate the separation of Ag⁺ and AgNPs. According to previous studies, the dissolution rate of AgNPs by EDTA is quite low and should not influence the determination of Ag⁺ and AgNPs.^{37–39} The recovery of Ag⁺ in ultrafiltration process in the presence of 5 mg L^{−1} SRNOM was determined as 98.1 ± 1.4%. For Ag⁺ and AgNPs determination, a minimum of two samples were measured.

The pseudo-first-order rate equation of ionic Ag photo-reduction is

$$\frac{d[\text{AgNP}]}{dt} = k_{\lambda}[\text{Ag}^+] \quad (1)$$

The apparent formation rate constant k_{λ} in the presence of pristine and M_F-NOM, corrected for light screening effect, is⁴⁰

$$k_{\lambda, \text{corrected}} = \frac{k_{\lambda}}{S_{\lambda}} = (2.303k_{\lambda}\alpha_{\lambda}l)/(1 - 10^{-\alpha_{\lambda}l}) \quad (2)$$

where $k_{\lambda, \text{corrected}}$ is the corrected formation rate constant considering the light screening effect, S_{λ} . The light screening factor, α_{λ} , is the decadic absorption coefficient of the solution at 366 nm, and l is the light path length of the reaction cell (2.2 cm).

Heat-Induced Reduction of Ag⁺ to AgNPs. The experiments were performed in 100 mL pressure-tight brown glass bottles. The reaction mixture of AgClO₄ and NOM solution, was incubated at 4 °C (in a refrigerator), room temperature (RT, ~25 °C), 45 °C, and 60 °C (heated in water thermostatic bath). The reaction was monitored at regular intervals by UV–vis spectroscopy. A 5 mmol L^{−1} borate (pH 8.0) was used to buffer the solution.

Characterization of AgNPs. UV–vis spectra were recorded from 200 to 800 nm on a Shimadzu UV–vis-NIR Spectrophotometer (UV-3600, Japan). Transmission electron microscopy (TEM) images, coupled with an energy dispersive spectrometer (EDS) and selected area electron diffraction (SAED) were obtained from a TEANAI G² 20–200 kV (FEI, Hillsboro, OR) transmission electron microscope. The specimen for HRTEM was prepared by slow evaporation of solution onto an ultrathin carbon-coated copper grid (T11032, Xinxing Bairui, Beijing, China).

Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) according to suggested protocol. Briefly, the detector employed a laser source of 633 nm and detection angle of 173°. Aqueous solutions of AgNPs (1.5 mL) were analyzed using disposable plastics sizing cuvettes (DTS0012) at 25 °C. Equilibration time was set as 120 s. Three measurements were performed for each sample, with 11 subruns and a 10 s run duration. The measurement position was optimized automatically by the ZetaSizer.

RESULTS AND DISCUSSION

Characteristics of Pristine and M_F-NOM. The prepared M_F-NOMs were initially characterized by UV–vis spectroscopy and fluorescence excitation–emission spectroscopy (SI Figure S5 and S6). Generally, UV–vis spectra revealed that with increasing MW, the molar absorptivity (ϵ) at 280 nm (SI Table S1) increased significantly, indicating increased abundances of aromatic moiety in higher MW M_F-NOMs.⁴¹ The Ex/Em and maximum intensity of fluorescence peaks for pristine- and M_F-NOMs are shown in SI Table S1. The Ex/Em of spectra peaks indicate humic-like structures in pristine NOM and M_F-NOM.⁴² Additionally, the maximum intensity of fluorescence peaks increased as the MW of M_F-NOM decreased, suggesting increased abundances of carboxylic moiety in lower MW M_F-NOMs.³⁵

Photoreduction of Ag⁺ to AgNPs by Pristine and M_F-NOM. The photoreduction of Ag⁺ to AgNPs was initially examined with 0.2 mmol L^{−1} AgClO₄ and 5 mg L^{−1} DOC of pristine or M_F-NOM in borate buffer (pH 8.0) under simulated sunlight (Xe lamp). In 1 h, the color of solution within <3 kDa M_F-NOM turned to a yellow or pale-red color, while solutions within other NOM did not change significantly. This result indicates that the formation rate of AgNPs in <3 kDa M_F-NOM is much higher than those of others in this initial stage. An apparent slow induction stage was observed for higher MW M_F-NOMs. However, with increasing irradiation time, the color deepened for all these samples. The UV–vis spectra (SI Figure S7) showed that a peak was evident at ~400 nm, corresponding to the surface plasma resonance (SPR) absorption of AgNPs. The SPR peak of AgNPs in <3 kDa M_F-NOM was much broader than others and associated with a longer wavelength, indicating polydispersed and larger AgNPs formed in the solution. It should be noted that although UV–vis spectrum can be used as a convenient indicator for the formation and concentration of AgNPs, the accuracy of quantifying the AgNPs by UV–visible spectroscopy is difficult because of the effect of AgNPs size on their SPR. Thus, we further used ultrafiltration-ICP-MS to determine the concentration of Ag⁺ in the photoirradiated solution, indicating no significant difference between various NOM for reduction of Ag⁺ (72.8%–80.2% reduction of Ag in pristine and M_F-NOM after 29 h irradiation). The fast reduction of Ag⁺ by <3 kDa M_F-NOM in the first several hours (SI Figure S7) is possibly ascribed to the fast nucleation of AgNPs.

Comprehensive characterizations, including TEM, SAED, and EDS, were also performed to validate the formation of AgNPs (SI Figure S8). All characterizations provided conclusive evidence for the formation of AgNPs through the photoreduction of Ag⁺ by pristine and M_F-NOM. TEM images (SI Figure S8a) also revealed a dual-mode distribution of size of AgNPs with large (average diameter ~10.9 nm) and small (average diameter ~3.7 nm) particles, which have previously been observed in the heat-induced formation of AgNPs in NOM solution.¹⁹ DLS further confirmed the occurrence of dual-mode distribution of size of nanoparticles in the reaction solution (SI Table S2). From the high resolution TEM (HRTEM), we clearly observed single crystal small AgNPs and polycrystalline larger AgNPs (SI Figure S8b), suggesting a mechanism of reduction-nucleation-growth and fusion.^{11,43,44} The TEM images shown in SI Figure S9 revealed that the AgNPs formed in <3 kDa M_F-NOM have larger size than those in >100 kDa M_F-NOM, which is consistent with UV–vis

Figure 1. Effect of pH on the formation of AgNPs from Ag^+ in the presence of pristine or M_f -NOM under simulated sunlight irradiation. (a) pH 7.4, (b) pH 8.0, (c) pH 9.0. The absorbance was the maximum absorbance at ~ 400 nm. Background absorption of NOM was deducted from the prepared AgNPs solution. Solution conditions: $0.2 \text{ mmol L}^{-1} \text{ AgClO}_4$, 5 mg L^{-1} DOC pristine or M_f -NOM, 5 mmol L^{-1} borate buffer.

Figure 2. Effect of NOM concentration on the formation of AgNPs from Ag^+ in the presence of pristine or M_f -NOM under simulated sunlight irradiation. (a) pristine NOM, (b) $>100 \text{ kDa}$ M_f -NOM, (c) $30\text{--}100 \text{ kDa}$ M_f -NOM, (d) $10\text{--}30 \text{ kDa}$ M_f -NOM, (e) $3\text{--}10 \text{ kDa}$ M_f -NOM, (f) $<3 \text{ kDa}$ M_f -NOM. The absorbance was the maximum absorbance at ~ 400 nm. Background absorption of NOM was deducted from the prepared AgNPs solution. Solution conditions: $0.2 \text{ mmol L}^{-1} \text{ AgClO}_4$ and 5 mmol L^{-1} borate buffer (pH 8.0).

results. This finding suggests that low MW M_f -NOM has weaker stabilization capability for AgNPs compared to high MW M_f -NOM in the formation process of AgNPs.

Effect of pH on Photoreduction of Ag^+ to AgNPs.

Overall, pH greatly influenced the photoreduction of Ag^+ to AgNPs by pristine and M_f -NOM, as shown in Figure 1. This pH-dependent photoreduction has been observed in previous studies,^{18,20} mainly caused by the pH-dependent reductive potential of NOM. With an increase of pH, the reductive potential of NOM increases linearly, resulting in a higher formation rate of AgNPs.¹⁸ Also, the SPR peak shifts to a shorter wavelength (from 426 nm at pH 7.4 to 402 nm at pH 9.0 for $<3 \text{ kDa}$ M_f -NOM, and from 415 nm at pH 7.4 to 408 nm at pH 9.0 for $>100 \text{ kDa}$ M_f -NOM) (SI Figure S10), indicating the formation of smaller AgNPs.^{45,46} This impact of

pH on the size of AgNPs is more significant for low MW M_f -NOM. TEM images (SI Figure S11) clearly show that with the increase of pH from 7.4 to 8.0, the size of AgNPs formed in $<3 \text{ kDa}$ M_f -NOM solution significantly decreased. The smaller size of formed AgNPs at higher pH is ascribed to the dissociation of phenolic and carboxyl group,⁴⁷ which result in better dispersion of AgNPs by NOM. The more significant impact of pH on AgNPs size formed in low MW M_f -NOM solution is possibly due to the abundance of carboxylic moiety in lower MW M_f -NOMs.

Effect of Concentration of Ag^+ and NOM on Photoreduction of Ag^+ to AgNPs. Effect of Ag^+ concentration on the formation of AgNPs was investigated in the concentration range of $0\text{--}0.5 \text{ mmol L}^{-1} \text{ Ag}^+$ (SI Figure S12). When there is only NOM in the solution (without Ag^+), no formation of

AgNPs was observed (SI Figure S13). In addition, no significant photodegradation of pristine and M_F -NOM was evident (SI Figure S13). With increasing Ag^+ concentration, the formation of AgNPs increased accordingly (SI Figure S12), consisted with a pseudo-first-order reaction kinetics to Ag^+ .¹⁸

The AgNPs formation rate was further examined as a function of NOM concentration ranging from 1 to 20 $mg\ L^{-1}$ DOC (Figure 2 and SI Figure S14). No formation of AgNPs was observed in $AgClO_4$ solution without NOM. With NOM present, increased formation of AgNPs was observed, but no linear relationship was found between the formation rate of AgNPs and concentration of NOM, especially at higher NOM concentration. As NOM has intense light absorption in the UV-vis region, this observed leveling-off phenomenon of the formation rate was possibly caused by the light attenuation of NOM.²⁰ This speculation was further supported by photo-reduction of Ag^+ by M_F -NOM at different NOM concentrations. At 1 $mg\ L^{-1}$ DOC of M_F -NOM, the difference in the reductive ability of M_F -NOM was not significant (SI Figure S14). However, with increased M_F -NOM concentration to 20 $mg\ L^{-1}$ DOC, reduction of Ag^+ by pristine and low MW M_F -NOM increased significantly expand the difference (SI Figure S14), owing to the minor light attenuation of pristine and low MW M_F -NOM than high MW M_F -NOM.

The light attenuation effect of NOM on AgNP formation was further demonstrated by using a monochromatic radiation (366 nm). The UV-vis spectra (SI Figure S15) showed a stronger and broader SPR absorption of AgNPs in <3 kDa M_F -NOM solution than in other M_F -NOMs, suggesting enhanced AgNPs formation in <3 kDa M_F -NOM solution. Ag^+ and AgNPs determination by ultrafiltration-ICP-MS also confirmed that the formation rate of AgNPs in <3 kDa M_F -NOM solution (0.200 h^{-1}) is much higher than other M_F -NOMs (0.101–0.118 h^{-1}) (Table 1). However, as shown in Table 1, after corrected the

Table 1. Formation Rate Constants of AgNPs (k_i) and Corrected Formation Rate Constants of AgNPs ($k_{i,corrected}$) under 366 nm Irradiation^a

M_F -NOM	α_i	S_i	$k_i(h^{-1})$	$k_{i,corrected}(h^{-1})$
>100 kDa	0.680	0.281	0.101	0.360
30–100 kDa	0.583	0.321	0.109	0.340
10–30 kDa	0.475	0.378	0.118	0.313
3–10 kDa	0.373	0.449	0.116	0.258
<3 kDa	0.226	0.595	0.200	0.335

^aSolution conditions: 0.4 $mmol\ L^{-1}$ $AgClO_4$, 20 $mg\ L^{-1}$ DOC M_F -NOM, 5 $mmol\ L^{-1}$ borate buffer (pH 8.0) under 366 nm irradiation.

light attenuation effect of NOM, the corrected formation rate constants of AgNPs ($k_{i,corrected}$) in the presence of M_F -NOM are quite similar, demonstrating the lower apparent formation rate constants of AgNPs (k_i) in higher MW M_F -NOM solutions are mainly ascribed to their stronger light attenuation effect than that of the lower MW M_F -NOMs.

Effects of Light Quality on the Photoreduction of Ag^+ to AgNPs. Effects of simulated light quality on the photoreduction of Ag^+ to AgNPs are shown in SI Figure S16. While there is minor formation of AgNPs under dark conditions, significant formation of AgNPs was observed in NOM solutions exposed to full simulated sunlight, “UV-A+Visible” and “Visible only”. Considering the transmittance of UV-block film in visible region is ~80% (SI Figure S1), the difference of formation rate of AgNPs between “Full radiation” and “UV-A

+Visible” is not significant (SI Figure S16), indicating UV-B in Xe lamp plays a minor role in the reduction of Ag^+ to AgNPs. SI Figure S16 also showed that both UV-A and visible light play important roles in the photoreduction, with visible light as the primary driver in this reduction process.

Effect of Ca^{2+} on Photoreduction of Ag^+ to AgNPs and the Stability of Photoinduced AgNPs. Ca^{2+} is ubiquitous in natural waters, with concentration ranging from several $mg\ L^{-1}$ to over 100 $mg\ L^{-1}$. It has been well demonstrated that Ca^{2+} could induce the aggregation of AgNPs while NOM could enhance the stability of AgNPs against aggregation.^{48,49} Recently, study further revealed that high MW M_F -NOM provided significantly better stability of gold nanoparticles than low MW M_F -NOM.³⁰ We then assumed that M_F -NOM would also behave differently on the aggregation of AgNPs in the presence of Ca^{2+} . Thus, it is expected that the difference of AgNPs aggregation in different M_F -NOM solutions will influence the formation and growth of AgNPs. Figure 3

Figure 3. Photography of photoinduced AgNPs solution in the presence of pristine or M_F -NOM and Ca^{2+} . Reaction conditions: 0.2 $mmol\ L^{-1}$ $AgClO_4$, 5 $mg\ L^{-1}$ DOC pristine or M_F -NOM, 5 $mmol\ L^{-1}$ borate buffer (pH 8.0) under simulated sunlight for 29 h.

shows the impact of 0–150 $mg\ L^{-1}$ Ca^{2+} on the photoreduction of Ag^+ to AgNPs in pristine and M_F -NOM solutions at the end of a 29-h-exposure. It is clear that the tint of AgNPs solution in the presence of identical NOM turned from yellow to red, then to black, with increasing Ca^{2+} concentration. High concentration of Ca^{2+} also induced sedimentation of AgNPs in low MW M_F -NOM solution. The change of color and formation of precipitation is indicative of the aggregation and formation of larger AgNPs in the presence of Ca^{2+} . In addition, the tint of AgNPs in lower MW M_F -NOM solution changed more significantly than that in higher MW M_F -NOM solution, indicating high instability of AgNPs in lower MW M_F -NOM solution. This assumption was further supported by the UV-vis spectra (Figure 4). With increasing Ca^{2+} concentration, the wavelength of the SPR peak shifted to the red-region and the peak intensity decreased. Comparably, the impact of Ca^{2+} coexisting with high MW M_F -NOM on AgNPs formation was not significant than that with low one. The TEM image (Figure 5) further confirmed the result of the UV-vis observation. The AgNPs in the >100 kDa fraction was still well dispersed in 30 $mg\ L^{-1}$ Ca^{2+} (Figure 5b). However, significant aggregation of AgNPs, especially larger AgNPs, was observed in pristine NOM and <3 kDa fraction solution (Figure 5a, c, and d). Figure 5d also highlights an oriented attachment between two adjacent nanoparticles. The abundance of larger AgNPs (~20 nm size) in pristine and <3 kDa NOM was much higher than that in >100 kDa M_F -NOM. In addition, AgNPs over 100 nm in size

Figure 4. Impact of Ca^{2+} on UV-vis spectra of AgNPs formed in pristine and M_f -NOM under simulated sunlight. (a) pristine NOM, (b) >100 kDa M_f -NOM, (c) 30–100 kDa M_f -NOM, (d) 10–30 kDa M_f -NOM, (e) 3–10 kDa M_f -NOM, and (f) <3 kDa M_f -NOM. Background absorption of NOM was deducted from the prepared AgNPs solution. Solution conditions: 0.2 mmol L^{-1} AgClO_4 and 5 mg L^{-1} DOC pristine or M_f -NOM in 5 mmol L^{-1} borate buffer (pH 8.0) with 0, 30, 80, 150 mg L^{-1} Ca^{2+} (as $\text{Ca}(\text{ClO}_4)_2$), respectively.

Figure 5. TEM images of AgNPs photosynthesized in pristine NOM (a), >100 kDa M_f -NOM (b) and <3 kDa M_f -NOM (c and d) in the presence of 30 mg L^{-1} Ca^{2+} . The scale bars are 20 nm, 20 nm, 100 nm, and 5 nm for (a), (b), (c), and (d), respectively. Reaction conditions: 0.2 mmol L^{-1} AgClO_4 , 5 mg L^{-1} DOC pristine or M_f -NOM, 30 mg L^{-1} Ca^{2+} , 5 mmol L^{-1} borate buffer (pH 8.0) under simulated sunlight for 29 h.

were observed in <3 kDa M_f -NOM solution. Ultrafiltration-ICP-MS revealed that the percent of Ag^+ in total Ag in the photoirradiated solution increased from ~20% (without Ca^{2+}) to ~40% with 150 mg L^{-1} Ca^{2+} , indicating Ca^{2+} may inhibit the reduction of Ag^+ to AgNPs by NOM. SI Figure S17 shows that the co-occurrence of 150 mg L^{-1} Ca^{2+} slightly increased the light absorption of NOM in the wavelength from 250 to 500 nm, indicating an interaction between Ca^{2+} and NOM. The decreasing reduction of Ag^+ to AgNPs by Ca^{2+} might be ascribed to the competitive binding of NOM by Ag^+ and Ca^{2+}

and suggest that the binding of Ag^+ to NOM plays an important role in this reduction process.²⁰

Electrostatic and steric stabilization provided by NOM adsorbed on the surfaces of AgNPs may prevent aggregation.⁵⁰ This dispersion effect of NOM on AgNPs could inhibit the fusion-induced growth of AgNPs. In the presence of Ca^{2+} , increasing aggregation was observed with decreasing MWs of NOM, possibly due to different interactions between M_f -NOM with varying MWs and nanoparticles.³⁰ The aggregation of AgNPs induced by Ca^{2+} could reduce the distance between AgNPs and consequently fuse “old” small AgNPs to “new” larger AgNPs with the reduction of Ag^+ to elemental Ag between adjacent “old” AgNPs. This assumption is supported by a TEM image of AgNPs in <3 kDa fraction (Figure 5d), where we observe larger AgNPs formation through aggregation and oriented-attachment-fusion of smaller AgNPs.

Comparison of Photo- and Heat-induced Reduction of Ag^+ to AgNPs. The heat-induced reduction of Ag^+ to AgNPs by pristine NOM and M_f -NOM was also investigated at 4 °C, room temperature (~25 °C, data not shown), 45 °C, and 60 °C (SI Figure S18). At 4 °C, no formation of AgNPs was observed in 35 h. With increasing incubation temperature, no significant effect on the size of the AgNPs was observed, and the formation rate of AgNPs increased markedly. When comparing the effects of heating with light irradiation for AgNPs formation, we find that heat-induced AgNPs have a much narrower half peak width and homogeneous size distribution compared to photoinduced AgNPs. Under light irradiation, oscillation of electrons at resonant conditions resulted in a nonuniform surface charge distribution in AgNPs and the creation of an oscillating dipole.⁵¹ This dipole–dipole attraction between particles was strong enough to drive the aggregation of nanoparticles.⁵² The aggregation of

small AgNPs and further reduction Ag^+ to Ag^0 could induce the fusion and growth of nanoparticles and result in formation of larger AgNPs. Therefore, the photo- and heat-induced AgNPs result in varying size-distribution patterns. The TEM images (SI Figure S19) show a comparison of AgNPs in >100 kDa M_F -NOM solution and those in <3 kDa M_F -NOM solution, indicating that the <3 kDa M_F -NOM solution contains larger and more polydispersed AgNPs and suggesting varying stabilization capabilities of M_F -NOM on AgNPs with heating.

Environmental Implications. Findings in this study reveal that different M_F -NOM show differential photoreduction activity toward Ag^+ and has significantly different effects on the stability of photoinduced AgNPs against aggregation in the presence of Ca^{2+} . Factors considered in this study, including simulated sunlight, heating, and other parameters (pH, DOM concentration, Ca^{2+} concentration), are environmentally relevant, suggesting that the differential roles of M_F -NOM on photoreduction and stabilization in transformation of Ag^+ to AgNPs may have significant implications in real environments. As environmental waters with different origins could have different DOC concentrations, NOM MW distributions, and chemical compositions, the photoreduction of Ag^+ to AgNPs in these aquatic environments may be highly site-specific. For example, in DOM-rich blackish water, the effects of light attenuation could decrease the photoreduction of Ag^+ significantly. Furthermore, this difference might also have drastic impacts on the transport, adsorption, and sedimentation of the photoinduced AgNPs in the aquatic environment. The selective adsorption of M_F -NOM on minerals could also change the MW distribution of NOM in natural waters,^{53,54} which could further influence the dispersion and aggregation of AgNPs. Considering the drastic difference of M_F -NOM in the photoreduction of Ag^+ to AgNPs and the stabilization of the photosynthesized AgNPs, especially in the presence of Ca^{2+} , further studies are strongly suggested to reveal the roles of M_F -NOM in the stabilization of engineered AgNPs and their effects on the phototransformation of engineered AgNPs in sunlight water under environmental conditions.

■ ASSOCIATED CONTENT

Supporting Information

Additional results are provided in Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +86-10-62849192; fax: +86-10-62849192; e-mail: jfliu@rcees.ac.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB14020101), the National Natural Science Foundation of China (21025729, 21337004, and 21207124), and Young Scientists Fund of RCEES (RCEES-QN-20130028F). We thank Miss Mingfang Wang in the Beijing National Center for Electron Microscopy for TEM characterizations and Dr. Yanbin Li from Ocean University of China for providing the UV and mylar film and discussion on this work. Dr. Adina Chuang Howe from

Michigan State University is thanked for enhancing the quality of English of this paper. The authors would also like to express their sincere thanks to the anonymous reviewers for their valuable comments and suggestions on this paper.

■ REFERENCES

- (1) Project on Emerging Nanotechnologies, 2014\, Consumer Products Inventory. <http://www.nanotechproject.org/cpi> (accessed 4/10/2014).
- (2) Reidy, B.; Haase, A.; Luch, A.; Dawson, K. A.; Lynch, I. Mechanisms of silver nanoparticle release, transformation and toxicity: A critical review of current knowledge and recommendations for future studies and applications. *Materials* **2013**, *6*, 2295–2350.
- (3) Benn, T.; Cavanagh, B.; Hristovski, K.; Posner, J. D.; Westerhoff, P. The release of nanosilver from consumer products used in the home. *J. Environ. Qual.* **2010**, *39*, 1875–1882.
- (4) Benn, T. M.; Westerhoff, P. Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol.* **2008**, *42*, 4133–4139.
- (5) Kaegi, R.; Sinnet, B.; Zuleeg, S.; Hagendorfer, H.; Mueller, E.; Vonbank, R.; Boller, M.; Burkhardt, M. Release of silver nanoparticles from outdoor facades. *Environ. Pollut.* **2010**, *158* (9), 2900–2905.
- (6) Quadros, M. E.; Marr, L. C. Silver nanoparticles and total aerosols emitted by nanotechnology related consumer spray products. *Environ. Sci. Technol.* **2011**, *45*, 10713–10719.
- (7) Quadros, M. E.; Pierson, R.; Tulve, N. S.; Willis, R.; Rogers, K.; Thomas, T. A.; Marr, L. C. Release of silver from nanotechnology-based consumer products for children. *Environ. Sci. Technol.* **2013**, *47*, 8894–8901.
- (8) Levand, C.; Hotze, E. M.; Lowry, G. V.; Brown, G. E. Environmental transformations of silver nanoparticles: Impact on stability and toxicity. *Environ. Sci. Technol.* **2012**, *46*, 6900–6914.
- (9) Wiesner, M. R.; Lowry, G. V.; Casman, E.; Bertsch, P. M.; Matson, C. W.; Di Giulio, I. R. T.; Liu, J.; Hochella, M. F. Meditations on the ubiquity and mutability of nano-sized materials in the environment. *ACS Nano* **2011**, *5*, 8466–8470.
- (10) Glover, R. D.; Miller, J. M.; Hutchison, J. E. Generation of metal nanoparticles from silver and copper objects: Nanoparticle dynamics on surfaces and potential sources of nanoparticles in the environment. *ACS Nano* **2011**, *5*, 8950–8957.
- (11) Yu, S. J.; Yin, Y. G.; Chao, J. B.; Shen, M. H.; Liu, J. F. Highly dynamic PVP-coated silver nanoparticles in aquatic environments: Chemical and morphology change induced by oxidation of Ag^0 and reduction of Ag^+ . *Environ. Sci. Technol.* **2014**, *48*, 403–411.
- (12) Li, Y.; Zhang, W.; Niu, J. F.; Chen, Y. S. Surface-coating-dependent dissolution, aggregation, and reactive oxygen species (ROS) generation of silver nanoparticles under different irradiation conditions. *Environ. Sci. Technol.* **2013**, *47*, 10293–10301.
- (13) Liu, J.; Sonshine, D. A.; Shervani, S.; Hurt, R. H. Controlled release of biologically active silver from nanosilver surfaces. *ACS Nano* **2010**, *4*, 6903–6913.
- (14) Zhao, C. M.; Wang, W. X. Size-dependent uptake of silver nanoparticles in *Daphnia magna*. *Environ. Sci. Technol.* **2012**, *46*, 11345–11351.
- (15) Liu, W.; Wu, Y.; Wang, C.; Li, H. C.; Wang, T.; Liao, C. Y.; Cui, L.; Zhou, Q. F.; Yan, B.; Jiang, G. B. Impact of silver nanoparticles on human cells: Effect of particle size. *Nanotoxicology* **2010**, *4*, 319–330.
- (16) Xiu, Z. M.; Zhang, Q. B.; Puppala, H. L.; Colvin, V. L.; Alvarez, P. J. J. Negligible particle-specific antibacterial activity of silver nanoparticles. *Nano Lett.* **2012**, *12*, 4271–4275.
- (17) Akaighe, N.; MacCuspie, R. I.; Navarro, D. A.; Aga, D. S.; Banerjee, S.; Sohn, M.; Sharma, V. K. Humic acid-induced silver nanoparticle formation under environmentally relevant conditions. *Environ. Sci. Technol.* **2011**, *45*, 3895–3901.
- (18) Yin, Y. G.; Liu, J. F.; Jiang, G. B. Sunlight-induced reduction of ionic Ag and Au to metallic nanoparticles by dissolved organic matter. *ACS Nano* **2012**, *6*, 7910–7919.

- (19) Adegboyega, N. F.; Sharma, V. K.; Siskova, K.; Zboril, R.; Sohn, M.; Schultz, B. J.; Banerjee, S. Interactions of aqueous Ag^+ with fulvic acids: Mechanisms of silver nanoparticle formation and investigation of stability. *Environ. Sci. Technol.* **2013**, *47*, 757–764.
- (20) Hou, W.-C.; Stuart, B.; Howes, R.; Zepp, R. G. Sunlight-driven reduction of silver ions by natural organic matter: Formation and transformation of silver nanoparticles. *Environ. Sci. Technol.* **2013**, *47*, 7713–7721.
- (21) Juganson, K.; Mortimer, M.; Ivask, A.; Kasemets, K.; Kahru, A. Extracellular conversion of silver ions into silver nanoparticles by protozoan *Tetrahymena thermophila*. *Environ. Sci.: Processes Impacts* **2013**, *15*, 244–250.
- (22) Kang, F. X.; Alvarez, P. J.; Zhu, D. Q. Microbial extracellular polymeric substances reduce Ag^+ to silver nanoparticles and antagonize bactericidal activity. *Environ. Sci. Technol.* **2014**, *48*, 316–322.
- (23) Rose, A. L.; Waite, D. Role of superoxide in the photochemical reduction of iron in seawater. *Geochim. Cosmochim. Acta* **2006**, *70* (15), 3869–3882.
- (24) Jones, A. M.; Garg, S.; He, D.; Pham, A. N.; Waite, T. D. Superoxide-mediated formation and charging of silver nanoparticles. *Environ. Sci. Technol.* **2011**, *45* (4), 1428–1434.
- (25) Mostofa, K. M. G.; Liu, C. Q.; Mottaleb, M. A.; Wan, G. J.; Ogawa, H.; Vione, D.; Yoshioka, T.; Wu, F. C. Dissolved organic matter in natural waters. In *Photobiogeochemistry of Organic Matter: Principles and Practices in Water Environments*; Mostofa, K. M. G.; Yoshioka, T.; Mottaleb, M. A.; Vione, D., Eds.; Springer, 2013.
- (26) Richard, C.; Guyot, G.; Rivaton, A.; Trubetskaya, O.; Trubetskoy, O.; Cavani, L.; Ciavatta, C. Spectroscopic approach for elucidation of structural peculiarities of Andisol soil humic acid fractionated by SEC-PAGE setup. *Geoderma* **2007**, *142* (1–2), 210–216.
- (27) Falzoni, A.; Seeber, R.; Tonelli, D.; Ciavatta, C.; Gessa, C.; Montecchio, D. Dependence on molecular weight of acid-base properties of humic and fulvic acids. *Analyst* **1998**, *26* (5), 214–219.
- (28) Ohno, T.; Chorover, J.; Omoike, A.; Hunt, J. Molecular weight and humification index as predictors of adsorption for plant- and manure-derived dissolved organic matter to goethite. *Eur. J. Soil Sci.* **2007**, *58*, 125–132.
- (29) Chen, G. A.; Lin, C.; Chen, L. A.; Yang, H. Effect of size-fractionation dissolved organic matter on the mobility of prometryne in soil. *Chemosphere* **2010**, *79* (11), 1046–1055.
- (30) Louie, S. M.; Tilton, R. D.; Lowry, G. V. Effects of molecular weight distribution and chemical properties of natural organic matter on gold nanoparticle aggregation. *Environ. Sci. Technol.* **2013**, *47*, 4245–4254.
- (31) Dong, M. M.; Mezyk, S. P.; Rosario-Ortiz, F. L. Reactivity of effluent organic matter (EfOM) with hydroxyl radical as a function of molecular weight. *Environ. Sci. Technol.* **2010**, *44*, 5714–5720.
- (32) Kitis, M.; Karanfil, T.; Wigton, A.; Kilduff, J. E. Probing reactivity of dissolved organic matter for disinfection by-product formation using XAD-8 resin adsorption and ultrafiltration fractionation. *Water Res.* **2002**, *36* (15), 3834–3848.
- (33) Chen, S. Y.; Huang, S. W.; Chiang, P. N.; Liu, J. C.; Kuan, W. H.; Huang, J. H.; Hung, J. T.; Tzou, Y. M.; Chen, C. C.; Wang, M. K. Influence of chemical compositions and molecular weights of humic acids on Cr(VI) photo-reduction. *J. Hazard. Mater.* **2011**, *197*, 337–344.
- (34) Aguer, J. P.; Trubetskaya, O.; Trubetskoy, O.; Richard, C. Photoinductive properties of soil humic acids and their fractions obtained by tandem size exclusion chromatography-polyacrylamide gel electrophoresis. *Chemosphere* **2001**, *44* (2), 205–209.
- (35) Richard, C.; Trubetskaya, O.; Trubetskoy, O.; Reznikova, O.; Afanas'eva, G.; Aguer, J. P.; Guyot, G. Key role of the low molecular size fraction of soil humic acids for fluorescence and photoinductive activity. *Environ. Sci. Technol.* **2004**, *38* (7), 2052–2057.
- (36) Meunier, L.; Laubscher, H.; Hug, S. J.; Sulzberger, B. Effects of size and origin of natural dissolved organic matter compounds on the redox cycling of iron in sunlit surface waters. *Aquat. Sci.* **2005**, *67* (3), 292–307.
- (37) Hartmann, G.; Hutterer, C.; Schuster, M. Ultra-trace determination of silver nanoparticles in water samples using cloud point extraction and ETAAS. *J. Anal. Atom. Spectrom.* **2013**, *28* (4), 567–572.
- (38) Hartmann, G.; Baumgartner, T.; Schuster, M. Influence of particle coating and matrix constituents on the cloud point extraction efficiency of silver nanoparticles (Ag-NPs) and application for monitoring the formation of Ag-NPs from Ag^+ . *Anal. Chem.* **2014**, *86* (1), 790–796.
- (39) Chappell, M. A.; Miller, L. F.; George, A. J.; Pettway, B. A.; Price, C. L.; Porter, B. E.; Bednar, A. J.; Seiter, J. M.; Kennedy, A. J.; Steevens, J. A. Simultaneous dispersion-dissolution behavior of concentrated silver nanoparticle suspensions in the presence of model organic solutes. *Chemosphere* **2011**, *84* (8), 1108–1116.
- (40) Zepp, R. G.; Schlotzhauer, P. F.; Sink, R. M. Photosensitized transformations involving electronic energy transfer in natural waters - Role of humic substances. *Environ. Sci. Technol.* **1985**, *19*, 74–81.
- (41) Peuravuori, J.; Pihlaja, K. Molecular size distribution and spectroscopic properties of aquatic humic substances. *Anal. Chim. Acta* **1997**, *337* (2), 133–149.
- (42) Coble, P. G. Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy. *Mar. Chem.* **1996**, *51* (4), 325–346.
- (43) Taubert, A.; Wiesler, U. M.; Mullen, K. Dendrimer-controlled one-pot synthesis of gold nanoparticles with a bimodal size distribution and their self-assembly in the solid state. *J. Mater. Chem.* **2003**, *13* (5), 1090–1093.
- (44) Yin, Y. G.; Yu, S. J.; Liu, J. F.; Jiang, G. B. Thermal and photoinduced reduction of ionic Au(III) to elemental Au nanoparticles by dissolved organic matter in water: Possible source of naturally occurring Au nanoparticles. *Environ. Sci. Technol.* **2014**, *48*, 2671–2679.
- (45) Lin, X. Z.; Teng, X. W.; Yang, H. Direct synthesis of narrowly dispersed silver nanoparticles using a single-source precursor. *Langmuir* **2003**, *19* (24), 10081–10085.
- (46) Peng, S.; McMahon, J. M.; Schatz, G. C.; Gray, S. K.; Sun, Y. G. Reversing the size-dependence of surface plasmon resonances. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107* (33), 14530–14534.
- (47) Atalay, Y. B.; Carbonaro, R. F.; Di Toro, D. M. Distribution of proton dissociation constants for model humic and fulvic acid molecules. *Environ. Sci. Technol.* **2009**, *43* (10), 3626–3631.
- (48) El Badawy, A. M.; Scheckel, K. G.; Suidan, M.; Tolaymat, T. The impact of stabilization mechanism on the aggregation kinetics of silver nanoparticles. *Sci. Total Environ.* **2012**, *429*, 325–331.
- (49) Huynh, K. A.; Chen, K. L. Aggregation kinetics of citrate and polyvinylpyrrolidone coated silver nanoparticles in monovalent and divalent electrolyte solutions. *Environ. Sci. Technol.* **2011**, *45* (13), 5564–5571.
- (50) Lau, B. L. T.; Hockaday, W. C.; Ikuma, K.; Furman, O.; Decho, A. W. A preliminary assessment of the interactions between the capping agents of silver nanoparticles and environmental organics. *Colloid. Surf., A* **2013**, *435*, 22–27.
- (51) Cheng, Y. W.; Yin, L. Y.; Lin, S. H.; Wiesner, M.; Bernhardt, E.; Liu, J. Toxicity reduction of polymer-stabilized silver nanoparticles by sunlight. *J. Phys. Chem. C* **2011**, *115* (11), 4425–4432.
- (52) Tang, Z. Y.; Kotov, N. A.; Giersig, M. Spontaneous organization of single CdTe nanoparticles into luminescent nanowires. *Science* **2002**, *297* (5579), 237–240.
- (53) Gu, B. H.; Schmitt, J.; Chen, Z.; Liang, L. Y.; McCarthy, J. F. Adsorption and desorption of different organic-matter fractions on iron-oxide. *Geochim. Cosmochim. Acta* **1995**, *59* (2), 219–229.
- (54) Wang, L. L.; Chin, Y. P.; Traina, S. J. Adsorption of (poly)maleic acid and an aquatic fulvic acid by goethite. *Geochim. Cosmochim. Acta* **1997**, *61* (24), 5313–5324.