

Arsenite and Arsenate Binding to Dissolved Humic Acids: Influence of pH, Type of Humic Acid and Aluminum

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Manuscript submitted to
ENVIRONMENTAL SCIENCE AND TECHNOLOGY
May 2006

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Abstract

The fate of arsenic in the aquatic environment is influenced by dissolved natural organic matter (DOM). Using an equilibrium dialysis method, conditional distribution coefficients (D_{om}) for As(III) and As(V) binding onto two commercial humic acids (terrestrial and aquatic) were determined at environmentally relevant As/dissolved organic carbon (DOC) ratios and as a function of pH. At all pH values, As(V) exhibited stronger binding than As(III). Maximum binding was observed around pH 7, which is consistent with H^+ competition for binding sites at low pH values and OH^- competition for the arsenic centre at high pH. For both oxidation states, D_{om} values were higher the lower the As/DOC ratio was. D_{om} values as a function of the As/DOC ratio were fitted for As(III) and As(V). Compared to the aquatic humic acid, the terrestrial humic acid showed a higher affinity for arsenic binding with 1.5–3 times higher D_{om} values under the same conditions. Aluminum(III) cations in excess to arsenic were shown to be competitors for strong binding sites at very low As/DOC ratios. Under environmentally relevant conditions ($[DOC] = 5 \text{ mgL}^{-1}$, $pH = 7$, $I = 0.05$, $[As(V)] = 67 \text{ nM}$ ($5 \text{ }\mu\text{gL}^{-1}$)), 10% of total As(V) may be bound to DOM, whereas >10% of As(III) is bound to DOM at very low As/DOC ratios only ($<1.32 \text{ nmol As/mg DOC}$).

Introduction

According to the WHO drinking water guideline value for arsenic ($10 \mu\text{gL}^{-1}$), relevant groundwater resources in Argentina, Cambodia, Chile, Mexico, the United States, Vietnam, and West Bengal are to be considered as contaminated (*1-4*). The adverse health effects of arsenic are arsenicosis, various forms of cancer and blackfoot disease (BFD) (*5*). Arsenate complexed by humic material has been shown to be a significant factor in the etiology of BFD (*6*). The bioavailability, toxicity and mobility of As depend on its speciation. In the aquatic environment, inorganic As occurs as As(III), H_3AsO_3 , or as As(V), H_2AsO_4^- and HAsO_4^{2-} . The ratio of As(III)/As(V) actually found in the environment is influenced by microbial activity (*7*), by abiotic redox reactions (*8,9*) and by binding to mineral surfaces (*10*).

Natural organic matter (NOM) is ubiquitous to aquatic systems. The environmental fate of As could be affected by NOM. Firstly, microbial degradation of NOM leads to reductive dissolution of As coated iron(hydr)oxides (*11*). Secondly, dissolved organic material (DOM) is known to act as a competitor for As with respect to binding onto surfaces such as alumina, goethite or hematite (*12,13*). Thirdly, complexation of As by DOM in the presence of metal cations suggest that DOM has an impact on As mobility (*14*). Despite the known relevance of NOM on As speciation, information on the binding behavior of As(III) and As(V) onto dissolved humic substances is scarce (*15,16*).

Moreover, binding constants under environmentally relevant conditions are not available, so the distribution of As(III) and As(V) based on calculations cannot be predicted properly.

In this study, we determined conditional distributions coefficients (D_{om}) for As(III) and As(V) and two commercially available humic acids (terrestrial and aquatic) by a dialysis method. The $[\text{As}]_0/\text{DOC}$ ratio was varied within an environmentally relevant range, focussing on very low ratios such as $0.13 \text{ nmol As/mg dissolved organic carbon (DOC)}$. The D_{om} values were fitted as a function of the As/DOC ratios for As(III) and As(V). The influence of the pH between 4.6 and 8.4 was determined. Additionally, the influence of aluminum in excess compared to As was investigated at very low $[\text{As}]_0/\text{DOC}$ ratios. Finally, the competition between phosphate and arsenate at equimolar concentrations was

investigated. Probable binding mechanisms are proposed and results are discussed with respect to environmental relevance.

Experimental Section

Reagents. All aqueous solutions were prepared with analytical grade Milli-Q water (Millipore). As_2O_3 , $\text{NaH}_2\text{AsO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Na}_3\text{Citrate} \cdot 2\text{H}_2\text{O}$, ascorbic acid, NaBH_4 , NH_4NO_3 , NaN_3 , p.a., NaCl solution (5 M; heavy metals < 0.0001 %), KH_2PO_4 ($\text{p}K_{\text{a}1-3} = 1.96; 7.21; 12.32$), sodium acetate ($\text{p}K_{\text{a}} = 4.75$), 2-morpholino-ethanesulfonic acid (MES, $\text{p}K_{\text{a}} = 6.15$), piperazine-1,4-bis(2-ethane-sulfonic acid) (PIPES, $\text{p}K_{\text{a}} = 6.80$), 3-morpholino-propanesulfonic acid (MOPS, $\text{p}K_{\text{a}} = 7.20$), and N-[Tris(hydroxy-methyl)methyl]-3-aminopropanesulfonic acid (TAPS $\text{p}K_{\text{a}} = 8.40$) were obtained from Fluka. $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was bought from Merck.

Humic Acids. Suwannee River humic acid standard II (SRHA) (Cat. No. 2S101H) was received from the International Humic Substance Society (IHSS, 1991 Upper Buford Circle, St. Paul, MN 55108, USA). Aldrich humic acid (AHA), sodium salt, tech. (No. 101816-104) (AHA) was purchased from Aldrich (Germany). The treatment of SRHA and AHA is described in detail elsewhere (17).

Analytical methods. As(III) and As(total) concentrations ($>13 \text{ nmolL}^{-1}$) were quantified with an atomic fluorescence spectrometer (AFS), Millenium Excalibur. The quantification limit was $0.2 \mu\text{gL}^{-1}$ (2.7 nmol L^{-1}). As(III) was selectively detected by hydride generation in a pH 5 citrate buffer using a procedure described elsewhere (8). As(V) concentrations were calculated as the difference of As(total) and As(III) concentrations. The influence of dissolved organic carbon (DOC) on the AFS response signal was determined for As(III) samples and As(V) samples in the presence of AHA and SRHA, respectively (Figures S1 and S2). DOC had no significant influence on the As(III) response, whereas As(V) spiked DOC solutions showed a significant decrease in response with increased DOC (18). Thus, response signals were corrected for DOC content in the case of As(V).

As(total) concentrations ($<13 \text{ nmolL}^{-1}$) were quantified with high resolution ICP-MS (ICP MS Element 2). The quantification limit was $0.01 \mu\text{gL}^{-1}$ (0.13 nmol L^{-1}). Samples were diluted 1:10, and 100 μL of distilled HNO_3 was added to a total of 10 mL.

Dialysis experiments. In order to determine the conditional distribution coefficients of As(III) and As(V) binding to dissolved humic acid, equilibrium dialysis was used. The equilibrium concentrations of total As(III) and total As(V), respectively, were determined in the outside and in the inside of a dialysis tube (Spectra/Por® Biotech Cellulose Ester (CE) membrane (500 Da)) fixed at the top of a 0.5 L polypropylene bottle. The exact procedure is described elsewhere (17). The inside solution contained humic acid or the ionic medium (blank). The solution inside and outside were spiked with the same As(V) concentrations at the beginning. In the case of As(III), it was spiked only outside in order to prevent photoinduced oxidation of As(III) by humics (19). Organic buffers were used at 1 mM concentrations. Ionic strength was 0.05 (NaCl) if not otherwise stated. The addition of 0.15 mM NaN₃ to all experiments prevented microbial growth. For the aluminum experiments, [Al(III)] was 0.5, 1, 2 and 4 µM. Sorption isotherms were determined at total As concentrations of 13 nM, 67 nM, 0.13 µM, 0.4 µM, 4 µM and for some experiments of 12 µM. Concentrations of DOC were 100 mgL⁻¹ and 50 mgL⁻¹ for AHA and SRHA, respectively. Conditional distribution coefficients for the DOM-water distribution, D_{om} , were calculated using eq 1:

$$D_{om} = \frac{C_{s+w} - C_w}{C_w \cdot [HA] \cdot \{C\}} \quad [\text{LkgDOC}^{-1}] \quad (\text{eq. 1}),$$

where C_w = total [As(III)] or [As(V)] outside the tube
 C_{s+w} = total [As(III)] or [As(V)] inside the tube
 $[HA]$ = concentration of humic acid [kgL⁻¹]
 $\{C\}$ = carbon content of humic acid [kgkg⁻¹].

The characteristics of AHA are summarized in a previous study (17). Due to lack of characterization data of SRHA standard II, similar characteristics as SRHA standard I are assumed (17).

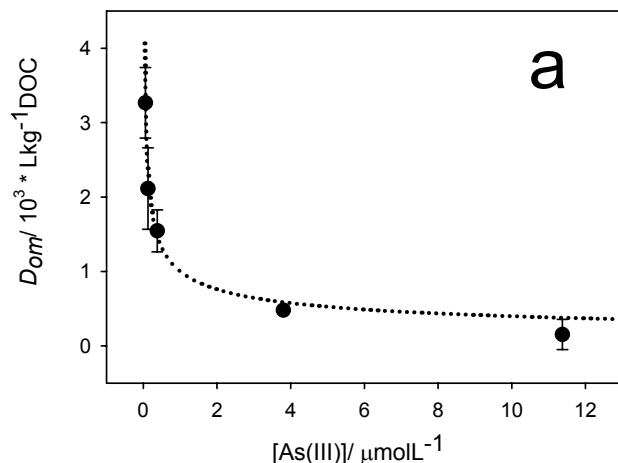
Diffusion kinetics and equilibration time. Figure S3 a,b shows that the diffusion of arsenic through the membrane is complete after 1 d (As(III)) and 8 days (As(V)) in the absence of humic acid. Qualitatively, experiments in the presence of humic acid showed

that As(III) sorption is faster compared to diffusion through the membrane, whereas As(V) binding to humic acid is slower compared to diffusion through the membrane. Hence, dialysis cells with As(III) were shaken at least for 1 d, whereas As(V) batches were shaken at least for 40 days before analysis (120 rpm, 25°C, dark), which guaranteed equilibrium to be achieved (data not shown). For all experiments, recovery of initially spiked As was at least 80% and varied within 15%.

Blanks. Each step of blank runs (without As) was analysed for As contamination. Neither buffer solutions nor membrane tubes, pH meter nor bottles showed any As release ($<0.01 \mu\text{g L}^{-1}$). Moreover, the “procedure blank” carried out at pH 4.6, 7.2 and 8.4, respectively, released less than $0.01 \mu\text{g L}^{-1}$ As. Matrix-adjusted standards (in buffer and in buffer with DOC) had smaller responses than aqueous standards in the ICP-MS analysis. As the decrease in signal was the same for buffer solutions (outside the tube) and buffer with DOC (inside the tube), D_{om} values calculated were not affected.

Results and Discussion

Binding to humic acids. Conditional distribution coefficients for binding of As(III) and As(V) onto AHA (or SRHA), D_{om} , were determined at four different pH values (4.6; 6.1; 7.2 and 8.4) and 4–6 different As/DOC ratios. Both, for As(III) as well as As(V), D_{om} values were higher the lower the As/DOC ratio was. Moreover, for all pH values tested, As(V) exhibited stronger binding than As(III) as illustrated for pH 8.4 and AHA in Figure 1a,b. Binding of As(III) and As(V) onto SRHA showed an analogous pattern with D_{om} values being 1.5–3 times smaller compared to AHA (S4 a,b).



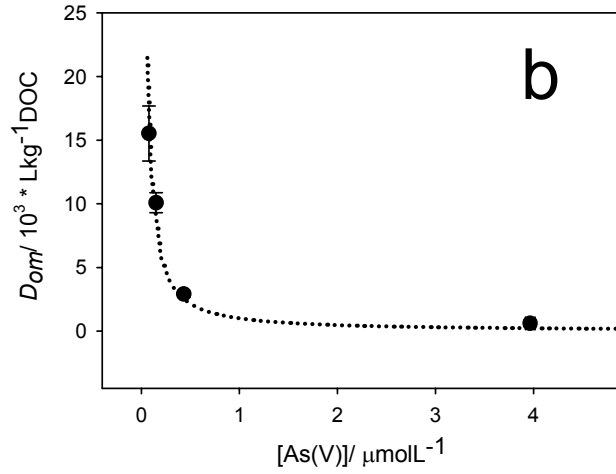


Figure 1 a,b: Distribution coefficients for AHA (D_{om}) as a function of (a) nonbound As(III) and (b) nonbound As(V) at $[DOC] = 100 \text{ mgL}^{-1}$, pH 8.4, 25 °C. Dotted lines represent fitted values: for As(III) $D_{om}/1000 = 1/([As(III)]/\mu\text{M})^{0.4}$ and for As(V) $D_{om}/1000 = 1/([As(V)]/\mu\text{M})^{1.1}$. For D_{om} values as a function of $[As]/DOC$ ratio, see text. Error bars indicate standard deviations of two replicates.

For AHA, the D_{om} values increase by a factor of 6 (As(III)) and 25 (As(V)) when the As/DOC ratio is decreased by a factor of 60 (from 4000 to 70 nmolL^{-1}). Stronger binding sites seem to be involved at lower As/DOC ratios. Distribution coefficients as a function of the As/DOC ratio can be calculated with simple equations. Minimization of

$\sum_i (D_{om,i,calculated} - D_{om,i,measured})^2$ was done by adjusting one parameter (the power in the

denominator) using a curve fit procedure of Microsoft Excel (eq. 2 and 3 for conditions used in Fig. 1a,b with $n=5$ and 4 for As(III) and As(V), respectively):

$$\frac{D_{om}}{1000} = \frac{1}{([As(III)]_{total} / DOC)^{0.12}} \quad (\text{eq. 2})$$

and

$$\frac{D_{om}}{1000} = \frac{1}{([As(V)]_{total} / DOC)^{0.35}} \quad (\text{eq. 3}),$$

where

D_{om}	= distribution coefficient [$\text{Lkg}_{\text{DOC}}^{-1}$]
$[\text{As(III)}]_{\text{total}}$	= total As(III) concentration [μM]
$[\text{As(V)}]_{\text{total}}$	= total As(V) concentration [μM]
DOC	= DOC concentration [mgL^{-1}].

These equations provide a tool for estimation of D_{om} values at a given $[\text{As}]_{\text{total}}/\text{DOC}$ ratio.

Quantifying the occupation of binding sites for AHA results in 17 μmol of As(III)/mol total functional groups and 40 μmol of As(V)/mol total functional groups for a low $[\text{As}]_0/\text{DOC}$ ratio of 0.67 nmol/mg DOC, meaning that 26% (As(III)) and 62% (As(V)) of total As is bound to humics. Under these conditions, less than 0.1‰ of all functional groups are occupied. For As/DOC ratios of >100 nmol As/mg DOC, the concentration of As bound to humic acid remains constant. At this ratio, about 10% of total As(V) is bound to humics resulting in an occupation of $\approx 1\%$ of all binding sites.

Under the same conditions, D_{om} values for As binding to SRHA are smaller by a factor of 1.5–3, although SRHA exhibits more functional groups per gram of DOC (factor ≈ 2) and smaller molecules (factor ≈ 2), which should lead to higher D_{om} values assuming a specific complexation mechanism. The findings of lower distribution coefficients may be partly due to other binding mechanisms such as hydrophobic binding (see section Binding mechanisms below). It is known that neutral pollutants such as alkanes exhibit 10–20 times smaller D_{om} values with SRHA than with AHA (20,21). In contrast to As(III), Sb(III) binding to SRHA showed 10–15 times higher D_{om} values compared to binding onto AHA (17). The centre of Sb(OH)_3 exhibits a more cationic character than the As(III) centre and therefore might preferably be bound by specific complexation.

Influence of pH. The pK_a values of As(OH)_3 and H_3AsO_4 are 9.22, 12.11, 13.41 and 2.25, 6.75, 11.60, respectively (10). Figure 2 a,b shows D_{om} values as a function of pH at two moderate to low As/DOC ratios (1.32 nmol/mg DOC and 0.67 nmol/mg DOC) for As(III) and As(V), respectively. Under these conditions, As(V) binding is 6 to 10 times stronger compared to As(III) binding onto AHA, which may be due to the higher formal charge at

the As(V) centre and/ or additional chelation and stabilizing effects (see section Binding mechanisms). For both arsenic species, maximum binding at pH>7 is observed. However, between pH 4.6 and 7.2 D_{om} values increase only by a factor of 2.5 and 5 for As(III) and As(V), respectively. This pH dependence is in agreement with H^+ competition for functional groups at low pH values and OH^- competition for the As centre at high pH values (see section Binding mechanisms).

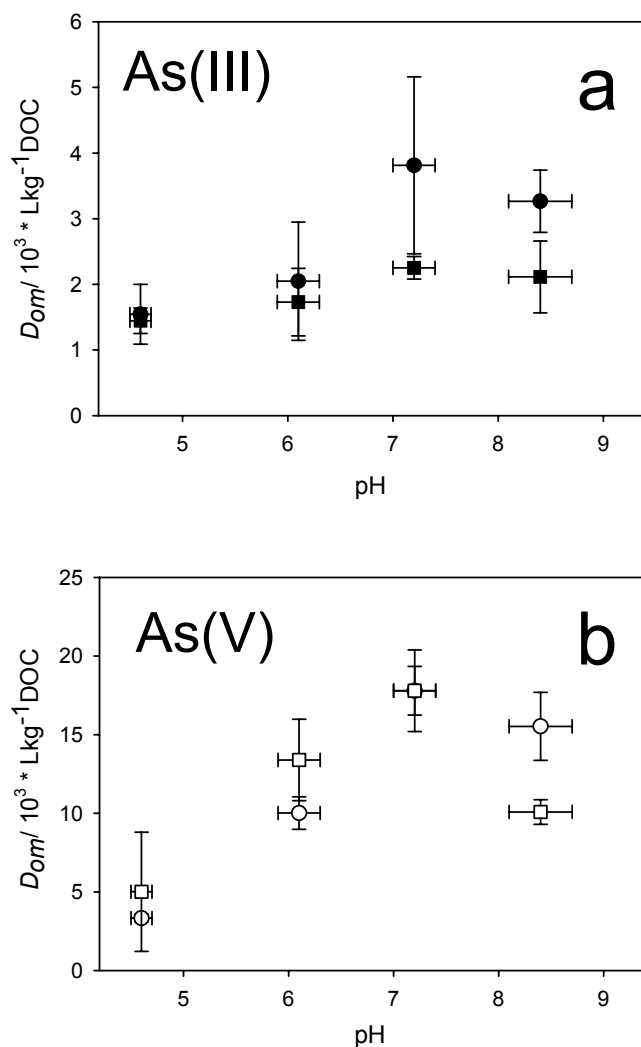


Figure 2a,b: Distribution coefficients for AHA (D_{om}) as a function of pH for (a) As(III) at 0.67 nmol As(III)/ mg DOC (●) and 1.32 nmol As(III)/ mg DOC (■) and (b) As(V) at 0.67 nmol As(III)/ mg DOC (○) and 1.32 nmol As(III)/ mg DOC (□), [DOC] = 100 mgL⁻¹, 25 °C. Note: D_{om} values for As(V) are 6 to 10 times higher than for As(III).

In contrast to moderate As/DOC ratios, at low As/DOC ratios (0.13 nmol/mg DOC), the influence of the pH on the D_{om} values is different. Here, no obvious binding maximum is found (Figure 3). It may be, however, that binding is stronger at lower pH values for both, As(III) and As(V), respectively. Such a behavior could be explained by traces of iron or aluminum forming cation bridges between As and the humic acid (12), which is favoured at lower pH values due to speciation of these metal cations (22).

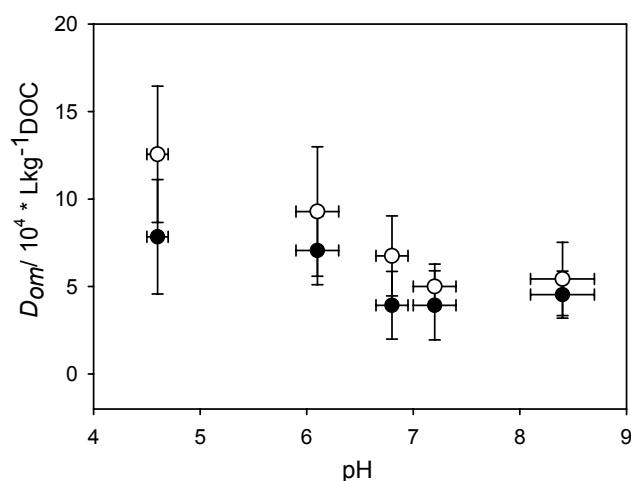
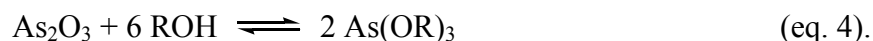
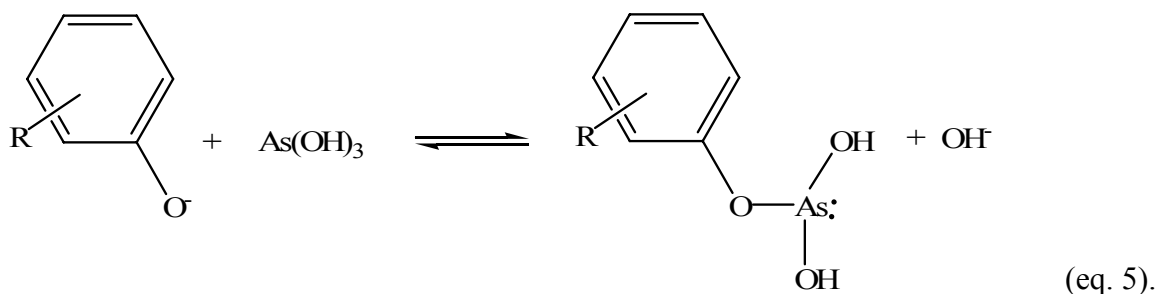


Figure 3: Distribution coefficients for AHA (D_{om}) as a function of pH at 0.13 nmol/mg DOC, As(III) (●), As(V) (○), [DOC] = 100 mgL⁻¹, 25 °C.

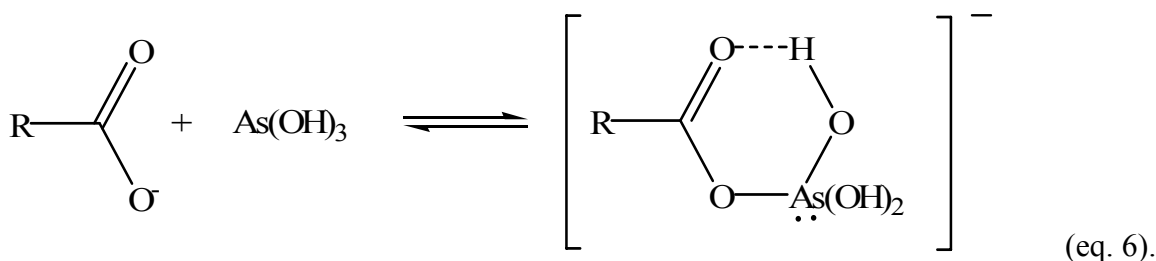
Binding mechanisms. A) Arsenic(III). Up to pH 9, As(III) forms stable neutral hydroxo complexes, As(OH)₃. It is well known that As(III) forms stable “ethers” in the presence of alcohols (eq 4):



Because humic acids exhibit phenolates as functional entities, a ligand exchange reaction could occur (eq. 5):



As phenolates are better Π -donors than carboxylates, equation 5 does probably not occur for carboxylates. It is suggested that carboxylic functional groups bind As(III) by forming a negatively charged adduct (no HO^- release) where H-bridges may be stabilizing (eq. 6):



Such specific complexation mechanisms are in agreement with the pH dependence observed for As(III) binding onto humics. The overall binding trends are influenced by (i) H^+ competition for humic functional groups at low pH values and (ii) OH^- competition for the As(III) centre at high pH values. At circumneutral pH values, As(III) binding is maximum.

The observed decrease of D_{om} values at higher As(III)/DOC ratios can be explained by assuming a small number of strong binding sites and a large number of weaker binding sites. The decrease of D_{om} values at higher As(III)/DOC ratios was also found for Sb(III) (17) and cations such as Hg^{2+} (23). In another study on the photoinduced oxidation of As(III) in the presence of humic acids we speculated whether As(III) bound to SRHA was involved in the rate determining step (19). The pseudo-first-order rate coefficients in this oxidation reaction were higher the smaller the As(III)/DOC ratio was (19). Because we find the same dependence for the D_{om} values of As(III) and SRHA in this study,

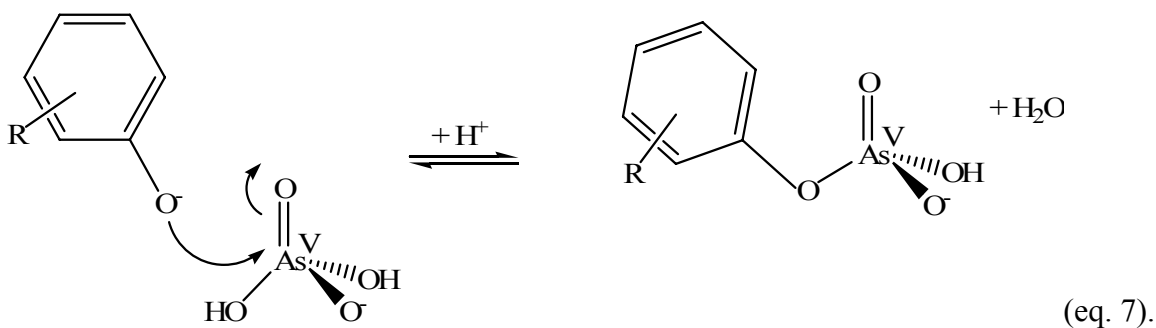
namely higher D_{om} values for smaller As(III)/DOC ratios (S4 a,b), the suggestion of an As(III)-humic acid complex involved in the rate determining step of the photoinduced oxidation of As(III) is supported.

In addition to a specific binding mechanism at functional groups, the neutral As(OH)₃ may partly be bound by hydrophobic interaction, which supports the fact that D_{om} values found are rather small and that binding of As(III) onto SRHA is weaker than onto AHA, although it exhibits more functional groups than AHA (20,21).

B) Arsenic(V). In contrast to As(III), the inorganic As(V) species $H_2AsO_4^-$ and $HAsO_4^{2-}$ are negatively charged in the pH range studied here (4.6 to 8.4). Because humic acids are overall negatively charged, too, only weak As(V) binding would be expected (22).

However, we find stronger As(V) binding compared to As(III) binding and so do other authors (15,16).

According to Huheey, for coordination numbers <6, an associative ligand exchange mechanism at positively charged metal centres may occur (24). As the arsenate centre has a formal charge of +V, an addition of a phenolate entity at the electrophilic centre followed by protonation and water release might take place (eq. 7):



Although the overall charge is negative for both reactants, the driving force may be stabilisation through phenolate donor characteristics, additional chelation by other functional groups and/or H-bridges. For comparison, $SiCl_4$ is hydrolysed in water although the centre is sterically hindered and the four chloride ligands are negatively polarized. Ligand exchange occurs by octet expansion and subsequent HCl elimination.

Competitors for strong binding sites. In natural aquatic systems, cations such as Ca^{2+} , Mg^{2+} and Fe^{3+} are present besides NOM. Such cationic metals may act as competitors or as promoters (cation bridging) for the association of arsenic with NOM (12). In order to evaluate the influence of cations onto As binding to humics, experiments with different aluminum concentrations were performed at pH 4.6. We choose a low As/DOC ratio of 0.13 nmol/mg DOC. Aluminum(III) was chosen because it is not redox active compared to, for example, Fe(III). Moreover, it is small and highly positively charged, which favours binding onto negatively charged humics. Therefore, it is an ideal cation to study the influence of metal cations on arsenic binding onto humics. As a hypothesis, the formation of humic-Al(III)-As bridges should lead to higher D_{om} values with increased Al(III) concentration (a detailed description of this binding mechanism is provided in the SI). However, we found significantly smaller D_{om} values for As(V) and similar D_{om} values for As(III) in the presence of Al(III) (Figure 4). Moreover, in contrast to binding studies without aluminum, D_{om} values in the presence of Al(III) were in most cases significantly smaller for As(V) compared to As(III). Hence, the influence of Al(III) on As(V) binding to humics is different from that on As(III) binding. Under the conditions studied, Al(III) could either act as a competitor for As(V) with respect to strong binding sites on the humic acid or reduce the binding affinity for As(V) by inducing conformational changes of the humic macromolecule, which renders some strong binding sites inaccessible for As(V). Obviously, Al(III) does not serve as a cation bridge for As(V). For As(III), no significant influence of Al(III) on D_{om} values was observed, which agrees with the suggestion of a (at least) partly hydrophobic binding mechanism of As(III) onto humics (see section Binding mechanisms). Like in the case of As(V), Al(III) seems not to form cation bridges for As(III).

Moreover, the formation of AlAsO_4 colloids suspended by DOC should be negligible (25) because Al(III) was equilibrated with the humics for 12 h before As spiking and therefore Al(III) binding to high affinity sites on the humic acid is favoured over colloid formation (22). This Al speciation is unambiguously supported by model calculations with WHAM (Windermere Humic Acid Model (22)). They show that in the presence of 100 mgL^{-1} DOC and $4 \text{ }\mu\text{M}$ Al(III) at pH 4.6 ($I = 0.05$, NaCl), >99% of all Al(III) is bound to the humic acid ($1.999 \times 10^{-5} \text{ mol}_{\text{HA}}^{-1}$), whereas only a small fraction is dissolved as $\text{Al}^{3+}(\text{aq})$

($3 \times 10^{-11} \text{ molL}^{-1}$), $\text{AlOH}^{2+}(\text{aq})$ ($4.5 \times 10^{-12} \text{ molL}^{-1}$), $\text{Al}(\text{OH})_2^+(\text{aq})$ ($5.6 \times 10^{-13} \text{ molL}^{-1}$) and $\text{Al}(\text{OH})_4^-(\text{aq})$ ($4.7 \times 10^{-16} \text{ molL}^{-1}$).

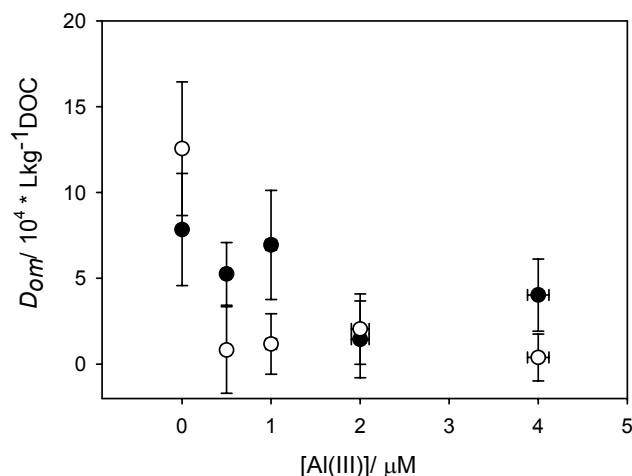


Figure 4: Distribution coefficients for AHA (D_{om}) as a function of Al(III) concentration at pH 4.6, 0.13 nmol/mg DOC, As(III) (\bullet), As(V) (\circ), $[\text{DOC}] = 100 \text{ mgL}^{-1}$, 25 °C.

Experiments with phosphate and arsenate at a molar ratio of 1:1 and at an $[\text{As}]_0/\text{DOC}$ ratio of 0.67 nmol/mg DOC showed that D_{om} values were smaller by a factor of ≈ 2 at pH 6.1 and 7.2 compared to experiments without phosphate (data not shown). Even though phosphate was not used in excess over arsenic, it may act as a competitor for strong binding sites on the humic acid. Phosphate/ arsenate competition was also found by other authors (15). As phosphate is usually present in excess over arsenic under environmentally relevant conditions (1), it may prevent arsenic from binding to humic material.

Arsenic complexation by humics - a survey of the literature. In contrast to cation binding by humic substances (22), anion binding by humics has not been studied comprehensively so far (26). A selection of publications dealing with inorganic and organic As complexation is given in the following and evaluated with respect to As binding by humic acids.

At environmentally relevant conditions, hydroxyl complexes of As are prevailing in aqueous solution. Arsenic complexes with hard inorganic ligands such as chloride or carbonate are rather weak (27,28), whereas soft ligands such as sulfide form strong arsenic complexes (29). The high affinity of sulfhydryl functional groups for As(III) is also reflected by the stable glutathione complexes with As(III) (30). Small organic ligands such as NTA or EDTA are known to form stable As(III) chelate complexes ($[[\text{As}(\text{OH})_2\text{HNTA}]^-]/[\text{As}(\text{OH})^{2+}][\text{HNTA}^{2-}] = 10^{15.3}$ (31); $[[\text{As}(\text{OH})_2\text{HEDTA}]^{2-}]/[\text{As}(\text{OH})^{2+}][\text{H}_2\text{EDTA}^{2-}] = 10^{19.3\pm0.1}$ (32)), and in the case of EDTA also stable As(V) complexes (32). Therefore, our finding of As(III) and As(V) binding to humic acids is plausible because they also exhibit carboxylic, sulfhydryl and phenolic functional groups. In batch experiments using AHA in high concentrations (1500 mgL^{-1}), Warwick *et al.* determined conditional distribution ratios for As(III) and As(V) at different ionic strengths and various pH values (16). They determined conditional stability constants ($\log K = 1.97 \pm 0.02$ for As(V) and $\log K = 1.58 \pm 0.07$ for As(III)) based on a 1:1 stoichiometry for the As-AHA interaction. Increased pH values resulted in increased binding and increased ionic strength in decreased binding due to competition for binding sites. Thanabalasingam and Pickering pointed out the role of ammonium functional groups in As sorption by humic acids (15). Similar to our findings but in contrast to the study of Warwick *et al.* (16), they found maximum binding around pH 6 and weaker binding at higher pH values at similar As/AHA ratios. At $10 \text{ }\mu\text{M}$ free As and 1500 mgL^{-1} AHA (6.6 nmol/mg DOC and pH 5.5), conditional distribution coefficients were 3300 Lkg^{-1} and 4200 Lkg^{-1} for As(III) and As(V), respectively, similar to our findings of 540 Lkg^{-1} and 2000 Lkg^{-1} for As(III) and As(V) (4.0 nmol/mg DOC and pH 6.2). Redman and Macalady determined a great variability in complexation behavior of As(III) and As(V) onto diverse NOM samples and pointed out that cationic metals may be involved in a ternary complexation mechanism: humic acid-cation-arsenic (12). Such a binding mechanism is also supported by the findings of Lin *et al.* demonstrating that As(V) binding to a water extract of compost, WEC, containing mainly fulvic acids and metals such as Fe, Al, Mn, Ca and Mg was 30% to 50%, whereas purified WEC (without metals) did not form any As(V) complexes within 48 h of equilibration (14). Moreover, they showed that Fe, Al and Mn involved in As(V) binding cannot be ion-exchanged by a

XAD-8 resin ion exchanger, whereas K that does not form metal bridges with arsenate was completely ion-exchanged. Our experiments with Al(III) in excess to As(V) at very low As/DOC ratios showed, however, that Al(III) decreased D_{om} values rather than promoted binding.

Shaw *et al.* discuss the binding mechanism of phosphate onto humic acids in the presence of Fe(III) and claim that the interaction is rather complex, including partly ternary humic acid-Fe-PO₄³⁻ complexes and partly association of humic acids with inorganic colloids containing iron and phosphate (25).

Low solubility of various arsenate-containing minerals such as FeAsO₄ ($pK_{s0} = 20.24$), AlAsO₄ ($pK_{s0} = 15.80$) Mn₃(AsO₄)₂ ($pK_{s0} = 28.72$), Ca₃(AsO₄)₂ ($pK_{s0} = 18.17$) and Mg₃(AsO₄)₂ ($pK_{s0} = 19.68$) (33) facilitates the formation of colloids suspended by NOM and should be considered in speciation discussions. Moreover, binding of As onto colloids such as gibbsite (34), FeS (35), goethite and HFO (10), ferrihydrite (36) are known to influence the mobility of arsenic significantly.

Environmental Considerations. When studying arsenic speciation in the aquatic environment, As binding to dissolved humic acids should be taken into account. Under environmentally relevant conditions ([DOC] = 5 mgL⁻¹, pH = 7, I = 0.05, [As(V)] = 67 nM (5 µgL⁻¹)), 10% of total As(V) is bound to dissolved humic acids. At higher DOC concentrations, even a higher percentage of As(V) is bound to DOC. Thus, in oxic groundwaters with As mainly found as As(V) as for example in La Pampa, Argentina, binding of As to humic material should be considered. The binding of As(III), however, seems to be relevant at very low As(III)/DOC ratios only, implying that As(III) mobilization due to binding onto dissolved organic material may be important at high DOC concentrations only, as found for example at margins of peat lenses in anoxic aquifers (37). Nevertheless, As(III)-humic acid complexes seem to play an important role in the photoinduced oxidation of As(III) in the presence of humic acids (19). Such processes are important when anoxic groundwater is pumped up and gets in contact with air and light. Moreover, in anoxic aquifers DOC has a strong potential to mobilize As(III) from metal(hydr)oxides by acting as a competitor for the sorption on surfaces (13).

Acknowledgements

We thank Thomas Rüttimann for his help with HG-AFS measurements. Adrian Ammann is acknowledged for analysis with ICP-MS. We are highly indebted to the Wolfermann-Nägeli foundation, Switzerland, for financial support. We thank Silvio Canonica and the reviewers for their recommendations.

Supporting Information Available

Arsenic recoveries for different DOC concentrations (AHA and SRHA) by HG-AFS, diffusion kinetics and D_{om} values for SRHA as a function of As(III) and As(V) concentration, respectively, are given in Figures S1–S4. The binding mechanisms for cation bridges are discussed.

Literature Cited

- (1) Smedley, P. L.; Kinniburgh, D. G. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem.* **2002**, *17*, 517-568.
- (2) Berg, M.; Tran, H. C.; Nguyen, T. C.; Pham, H. V.; Schertenleib, R.; Giger, W. Arsenic contamination of groundwater and drinking water in Vietnam: a human health threat. *Environ. Sci. Technol.* **2001**, *35*, 2621-2626.
- (3) Polya, D. A.; Gault, A. G.; Diebe, N.; Feldman, P.; Rosenboom, J. W.; Gilligan, E.; Fredericks, D.; Milton, A. H.; Sampson, M.; Rowland, H. A. L.; Lythoge, P. R.; Jones, J. C.; Middleton, C.; Cooke, D. A. Arsenic hazard in shallow Cambodian groundwaters. *Mineral. Mag.* **2005**, *69*, 807-823.
- (4) Welch, A. H.; Lico, M. S.; Hughes, J. L. Arsenic in groundwater of the Western United-States. *Ground Water* **1988**, *26*, 333-347.
- (5) Ratnaik, R. N. Arsenic in Health and Disease. In *Managing Arsenic in the Environment*; Naidu, R., Smith, E., Owens, G., Bhattacharya, P., Nadebaum, P., Eds.: Collingwood VIC 3066, **2006**.
- (6) Hseu, Y. C.; Yang, H. L. The effects of humic acid-arsenate complexes on human red blood cells. *Environ. Res.* **2002**, *89*, 131-137.
- (7) Akai, J.; Izumi, K.; Fukuhara, H.; Masuda, H.; Nakano, S.; Yoshimura, T.; Ohfuji, H.; Anawar, H. M.; Akai, K. Mineralogical and geomicrobiological investigations on groundwater arsenic enrichment in Bangladesh. *Appl. Geochem.* **2004**, *19*, 215-230.
- (8) Hug, S. J.; Leupin, O. Iron-catalyzed oxidation of arsenic(III) by oxygen and by hydrogen peroxide: pH-dependent formation of oxidants in the Fenton reaction. *Environ. Sci. Technol.* **2003**, *37*, 2734-2742.
- (9) Chiu, V. Q.; Hering, J. G. Arsenic adsorption and oxidation at manganite surfaces. 1. Method for simultaneous determination of adsorbed and dissolved arsenic species. *Environ. Sci. Technol.* **2000**, *34*, 2029-2034.
- (10) Dixit, S.; Hering, J. G. Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: Implications for arsenic mobility. *Environ. Sci. Technol.* **2003**, *37*, 4182-4189.
- (11) McArthur, J. M.; Ravenscroft, P.; Safiulla, S.; Thirlwall, M. F. Arsenic in groundwater: Testing pollution mechanisms for sedimentary aquifers in Bangladesh. *Water Resour. Res.* **2001**, *37*, 109-117.
- (12) Redman, A. D.; Macalady, D. L.; Ahmann, D. Natural organic matter affects arsenic speciation and sorption onto hematite. *Environ. Sci. Technol.* **2002**, *36*, 2889-2896.
- (13) Bauer, M.; Blodau, C. Mobilization of arsenic by dissolved organic matter from iron oxides, soils and sediments. *Sci. Total Environ.* **2006**, *354*, 179-190.
- (14) Lin, H. T.; Wang, M. C.; Li, G. C. Complexation of arsenate with humic substance in water extract of compost. *Chemosphere* **2004**, *56*, 1105-1112.
- (15) Thanabalasingam, P.; Pickering, W. F. Arsenic sorption by humic acids. *Environmental Pollution Series B-Chemical and Physical* **1986**, *12*, 233-246.
- (16) Warwick, P.; E., I.; Evans, N. Arsenic's interaction with humic acid. *Environ. Chem.* **2005**, *2*, 119-224.

- (17) Buschmann, J.; Sigg, L. Antimony(III) binding to humic substances: Influence of pH and type of humic acid. *Environ. Sci. Technol.* **2004**, *38*, 4535-4541.
- (18) Hasegawa, H.; Matsui, M.; Okamura, S.; Hojo, M.; Iwasaki, N.; Sohrin, Y. Arsenic speciation including 'hidden' arsenic in natural waters. *Appl. Organometallic Chem.* **1999**, *13*, 113-119.
- (19) Buschmann, J.; Canonica, S.; Lindauer, U.; Hug, S. J.; Sigg, L. Photoirradiation of humic acids induces arsenic(III) oxidation. *Environ. Sci. Technol.* **2005**, *39*, 9541-9546.
- (20) Chin, Y. P.; Aiken, G. R.; Danielsen, K. M. Binding of pyrene to aquatic and commercial humic substances: The role of molecular weight and aromaticity. *Environ. Sci. Technol.* **1997**, *31*, 1630-1635.
- (21) Chiou, C. T.; Kile, D. E.; Brinton, T. I.; Malcolm, R. L.; Leenheer, J. A.; Maccarthy, P. A comparison of water solubility enhancements of organic solutes by aquatic humic materials and commercial humic acids. *Environ. Sci. Technol.* **1987**, *21*, 1231-1234.
- (22) Tipping, E. *Cation binding by humic substances*; Cambridge University Press: Cambridge, **2002**; Vol. 12.
- (23) Haitzer, M.; George, R. A.; Ryan, J. N. Binding of Mercury(II) to Dissolved Organic Matter: The Role of the Mercury-to-DOM Concentration Ratio. *Environ. Sci. Technol.* **2002**, *36*, 3564-3570.
- (24) Huheey, J.; Keiter, E.; Keiter, R. *Anorganische Chemie* New York, **1995**.
- (25) Shaw, P. J.; Jones, R. I.; De Haan, H. The influence of humic substances on the molecular weight distributions of phosphate and iron in epilimnetic lake waters. *Freshwater Biology* **2000**, *45*, 383-393.
- (26) Plant, J. A.; Kinniburgh, D. G.; Smedley, P. L.; Fordyce, F. M.; Klinck, B. A. Arsenic and selenium. In *Environmental Geochemistry*; Lollar, B. S., Ed.; Elsevier: New York, **2005**; Vol. 9, pp 17-66.
- (27) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum Press: New York, **1976**; Vol. 4: Inorganic Complexes.
- (28) Neuberger, C. S.; Helz, G. R. Arsenic(III) carbonate complexing. *Appl. Geochem.* **2005**, *20*, 1218-1225.
- (29) Spycher, N. F.; Reed, M. H. As(III) and Sb(III) sulfide complexes - an evaluation of stoichiometry and stability from existing experimental-data. *Geochim. Cosmochim. Acta* **1989**, *53*, 2185-2194.
- (30) Kobayashi, Y.; Cui, X.; Hirano, S. Stability of arsenic metabolites, arsenic triglutathione As(GS)₃ and methylarsenic diglutathione CH₃As(GS)₂, in rat bile. *Toxicology* **2005**, *211*, 115-123.
- (31) Elenkova, N. G.; Tsoneva, R. A. Polarographic and potentiometric investigation of the system As(III)-nitrilotriacetic acid. *J. Inorg. Nucl. Chem.* **1973**, *35*, 841-847.
- (32) Elenkova, N. G.; Veleva, M. G.; Tsoneva, R. A. Polarographische und potentiometrische Untersuchung über die Zusammensetzung und Stabilität der Arsen/EDTA- Komplexe. *J. Inorg. Nucl. Chem.* **1972**, *34*, 191-196.
- (33) Weast, R. C. *Handbook of Chemistry and Physics 1st student edition* Florida, **1988**.
- (34) Halter, W. E.; Pfeifer, H. R. Arsenic(V) adsorption onto alpha-Al₂O₃ between 25 and 70 degrees C. *Appl. Geochem.* **2001**, *16*, 793-802.

- (35) Wolthers, M.; Charlet, L.; Van der Weijden, C. H.; Van der Linde, P. R.; Rickard, D. Arsenic mobility in the ambient sulfidic environment: Sorption of arsenic(V) and arsenic(III) onto disordered mackinawite. *Geochim. Cosmochim. Acta* **2005**, *69*, 3483-3492.
- (36) Mohapatra, D.; Singh, P.; Zhang, W.; Pullammanappallil, P. The effect of citrate, oxalate, acetate, silicate and phosphate on stability of synthetic arsenic-loaded ferrihydrite and Al-ferrihydrite. *J. Hazard. Mater.* **2005**, *124*, 95-100.
- (37) McArthur, J. M.; Banerjee, D. M.; Hudson-Edwards, K. A.; Mishra, R.; Purohit, R.; Ravenscroft, P.; Cronin, A.; Howarth, R. J.; Chatterjee, A.; Talukder, T.; Lowry, D.; Houghton, S.; Chadha, D. K. Natural organic matter in sedimentary basins and its relation to arsenic in anoxic ground water: the example of West Bengal and its worldwide implications. *Appl. Geochem.* **2004**, *19*, 1255-1293.