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# Multiscale Assessment of Methylarsenic Reactivity in Soil. 2. Distribution and Speciation in Soil

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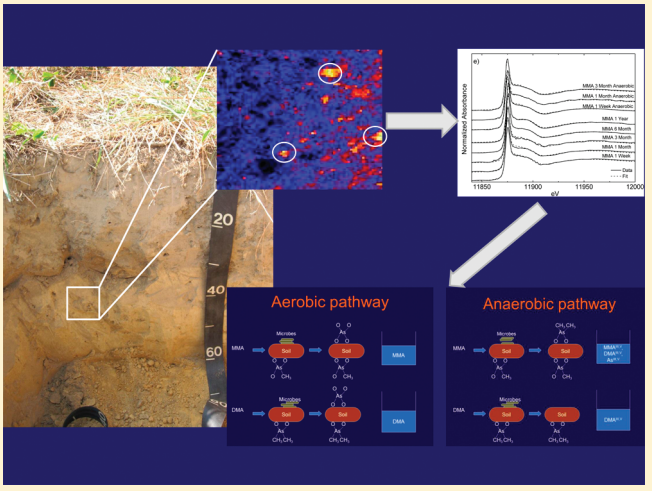
Supporting Information

**ABSTRACT:** Methylated forms of arsenic (As), monomethylarsenate (MMA) and dimethylarsenate (DMA), have historically been used as herbicides and pesticides. Because of their large application to agriculture fields and the toxicity of MMA and DMA, the distribution, speciation, and sorption of methylated As to soils requires investigation. Monomethylarsenate and DMA were reacted with a soil up to one year under aerobic and anaerobic conditions. Microsynchrotron based X-ray fluorescence ($\mu$-SXRF) mapping studies showed that MMA and DMA were heterogeneously distributed in the soil and were mainly associated with iron oxyhydroxides, e.g., goethite, in the soil. Micro-X-ray absorption near edge structure (XANES) spectra collected from As hotspots showed MMA and DMA were demethylated to arsenate over one year incubation under aerobic conditions. Monomethylarsenate was methylated to DMA, and DMA was maintained as DMA over a 3 month incubation under anaerobic conditions. Arsenic−iron precipitation, such as the formation of scorodite ($FeAsO_4 \cdot 2H_2O$), was not observed, indicating that MMA and DMA were mainly associated with Fe-oxyhydroxides as sorption complexes.




## INTRODUCTION

Arsenic (As) occurs in nature both naturally and anthropogenically. The predominate oxidation states for inorganic As species are arsenate ($As^V$, $H_3AsO_4$) and arsenite ($As^{III}$, $H_3AsO_3$). In addition to inorganic forms, organic forms of As also exist in nature; typically occurring in terrestrial environments as MMA ($CH_3H_2As^VO_3$) and DMA ($(CH_3)_2HAs^VO_2$), which have historically been used as herbicides and pesticides in agriculture.[1,2] The primary reason that As has received so much attention is due to its acute toxicity. Arsenic toxicity and the mode of toxin mechanisms depend on its speciation. Monomethylarsenate is known to cause peripheral artery disease related to atherosclerosis, and DMA is known to be a multicancer promoter.[3,4]

The biotransformation of As, particularly, methylation and demethylation, is often controlled by microbes.[5,6] Understanding probable pathways is critical to predicting the fate of As in the environment. A generally accepted As methylation pathway, the Challenger pathway (Figure S2), involves multioxidative methylation and reduction steps.[7] Recently, an alternative As methylation pathway, Hayakawa pathway (Figure S2), has been proposed.[8] The main difference in the newly proposed Hayakawa pathway is 1) the oxidation state of the (3+) species is formed first and oxidized to the analogue (5+) species and 2) MMA and DMA production are not continuous and MMA is also the end product of the methylation pathway. This suggests that the Challenger pathway is not the only route for methylation/demethylation, and there can be multiple pathways.

Previous studies have shown that MMA and DMA sorption maxima on four Ultisol soils are correlated with the clay fraction and Fe-oxyhydroxide contents.[1] The applied MMA and DMA remain in soil for some time. Using a chemical extraction method, MMA and DMA were detected 1−1.5 years after their application to experimental fields, and the half-life of MMA and DMA were 20 and 22 days, respectively.[9] Monomethylarsenate and DMA eventually demethylated to inorganic species in most cases. In an experimental field study, MMA was mainly demethylated to $As^V$ in percolate water,[10] and the additions of cellulose or carbohydrates seem to retard methylation/demethylation.[11,12] However, there is no clear conclusion on the effects of redox potentials on methylation/demethylation. Aerobic conditions can promote demethylation of MMA and DMA to $As^V$. For example,

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43% of MMA and 73% of initial DMA were demethylated to $As^V$ in 70 days.[11] Another study showed that 16% of initial MMA was demethylated to $As^V$ in 30 days.[1] However, anaerobic conditions can promote either methylation or demethylation of MMA and DMA.[11,13] In addition, there are no studies, to our knowledge, on MMA and DMA sorption on soils using X-ray absorption spectroscopy (XAS) and $\mu$-SXRF spectroscopy to determine the speciation and distribution/association of As species. Such studies would provide direct evidence for binding mechanisms that could not be ascertained using other approaches. The objective of this study is to characterize the *in situ* behavior of MMA and DMA speciation, localization, and association with soil components under different redox conditions using $\mu$-SXRF, $\mu$-XAS, and $\mu$-X-ray diffraction (XRD).

## MATERIALS AND METHODS

**Arsenic Compounds.** Reagent grades of $As^V$, MMA, and DMA were used throughout this study. The molecular structures and p$K_a$ values of $As^V$, MMA, and DMA are summarized in Figure S1 and Table S1. Monomethylarsenite and $DMA^{III}$ were provided by Dr. X. Chris Lee from the University of Alberta, Canada.

**Soil Characteristics.** The subsoil (30 cm) of Reybold soil series (Typic Hapludults) was used for this study. The soil was air-dried, ground, and passed through a 2 mm sieve. Soil $pH_{water}$, organic matter content, Al/Fe-oxyhydroxide contents, and various elemental concentrations (Table S2) were measured using standard procedures (details in the SI).

**Soil Incubation Studies.** Soil incubation studies were conducted via batch experiments. For aerobic samples, a soil suspension (250 g/L), containing 0.01 M $NaNO_3$, was equilibrated with 0.2 mM MMA or DMA in Erlenmeyer flasks with vent caps with a 0.22 $\mu$m hydrophobic membrane. The flasks were covered with aluminum foil and placed on an orbital rotating shaker in a growth chamber at 60% humidity. The amount of water was maintained by measuring the total weight of the system weekly. The pH of the system was maintained at pH 6 by adding 0.1 M $HNO_3$ or NaOH. After incubation times of 1 week, 1, 3, 6 months, or 1 year, the suspensions were centrifuged, and supernatants were sampled using syringe filters (Nylon 0.22 $\mu$m pore size). The As concentrations were analyzed using high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The centrifuged soil suspensions were saved as wet pastes for further analysis by $\mu$-SXRF.

Anaerobic samples were prepared with the same concentration of soil and MMA or DMA as for the aerobic samples in a glovebox. pH was measured every two weeks and maintained at pH 6 by adding 0.1 M $HNO_3$ or NaOH. The redox potential of the system was measured before samples were removed for analysis. The wet soil pastes were analyzed at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory, Upton NY. Arsenic concentrations in supernatants were analyzed with HPLC-ICP-MS.

**Micro-SXRF, $\mu$-XANES, and $\mu$-XRD Analysis.** Micro-SXRF, $\mu$-XANES, and $\mu$-XRD measurements of the soil samples were performed at beamline X26A at the NSLS. Anaerobic samples were placed in a sealed sample chamber, and $N_2$ gas was continuously purged to maintain anaerobic conditions during the measurement. Micro-SXRF mapping was conducted with a microfocused beam ($\approx$10 $\mu$m each side) at 13,000 eV. The size of each map was 0.7 mm by 0.7 mm, and scan step size was 10 $\mu$m by 10 $\mu$m at 4 s counting time per pixel. At least three scans of As $\mu$-XANES spectra were collected from each As hotspot. The As K-edge was calibrated as the first derivative of $CaAsO_4$ ($As^V$) = 11874 eV. The data processing and linear combination fitting (LCF) were performed using the Sixpack/Feff program package.[14] The standard spectra used for $\mu$-XANES LCF analysis were $As^V$, MMA, DMA, and $As^{III}$ that were sorbed on the soil and were collected at Beamline 11-2 at the Stanford Synchrotron Radiation Laboratory. The LCF was performed using normalized XANES regions of the spectra. Micro-XRD patterns were collected, using CCD area detectors with exposure times ranging from 60 to 120 s. The detectors were calibrated with a NIST SRM 674a α-corundum crystal using the Fit2D program.[15] Micro-XRD photographs were also integrated using Fit2D to produce 2$\theta$ vs peak intensity graphs. The background spectra of water, mylar, and kapton were subtracted by using XRD-BS before peak analysis. Final peak analysis was performed using the Match! program[16] (details in reference and the SI).

## RESULTS AND DISCUSSION

**Arsenic Distribution Maps.** Arsenic was heterogeneously distributed, and several As hotspots were observed on the $\mu$-SXRF elemental distribution maps (Figure 1, S3). Arsenic distribution in the soil was mainly correlated with Fe distribution, and As hotspots were generally found at Fe hotspots, but Fe hotspots were not necessarily at As hotspots. This could be ascribed to the larger amount of Fe in the soil compared to the amount of added MMA or DMA. The results of chemical extractions suggest a majority of Fe is originated from crystalline Fe oxide phases ($CBD_{Fe}$: 6,742 mg/kg and $Fe_{ox}$: 2,397 mg/kg). The fluorescence counts from Fe were greater than the As counts by an order of magnitude, indicating that the Fe concentration in the soil was much greater than the As concentration (Figure 1). Results from maps from different incubation periods and different starting As species (MMA or DMA) showed no other correlations between As distribution and other elements (Figure 1, S4).

Although not shown, MMA and DMA sorption was also correlated to CDB/oxalate extractable Al-oxyhydroxide concentration in the Reybold subsoil.[17] The soil contains $CBD_{Al}$: 1,912 mg/kg and $Al_{ox}$: 682 mg/kg. It is possible that MMA and DMA distribution is correlated to Al as much as Fe. Aluminum fluorescence signals were too low to detect using the hard X-ray microprobe technique. Also, due to the elemental concentration and linear correlation coefficient value, $R^2$, the effects of Fe-oxyhydroxides on MMA and DMA sorption seem to be greater than Al-oxyhydroxides.[17] In this study, we will mainly focus on Fe-oxyhydroxides as a sorbent for MMA and DMA in the Reybold subsoil.

**Micro-XRD Studies.** Micro-XRD patterns were collected from the As hotspots identified in the $\mu$-SXRF maps (Figure S3). All of the $\mu$-XRD patterns had peaks that corresponded to quartz (Figure S5). Goethite was the major Fe-oxyhydroxide mineral identified (Figure S5). Based on the $\mu$-XANES data (Figure S6), the first derivative peak of 1 month samples showed no significant As speciation changes. Most of the applied MMA or DMA was still MMA or DMA, respectively. It can be concluded that MMA and DMA were mainly sorbed to the Fe-oxyhydroxides. The direct evidence for MMA and DMA sorption to Fe-oxyhydroxides in the soil agrees with the observation that sorption maxima and Fe contents are proportional to each other.[18]

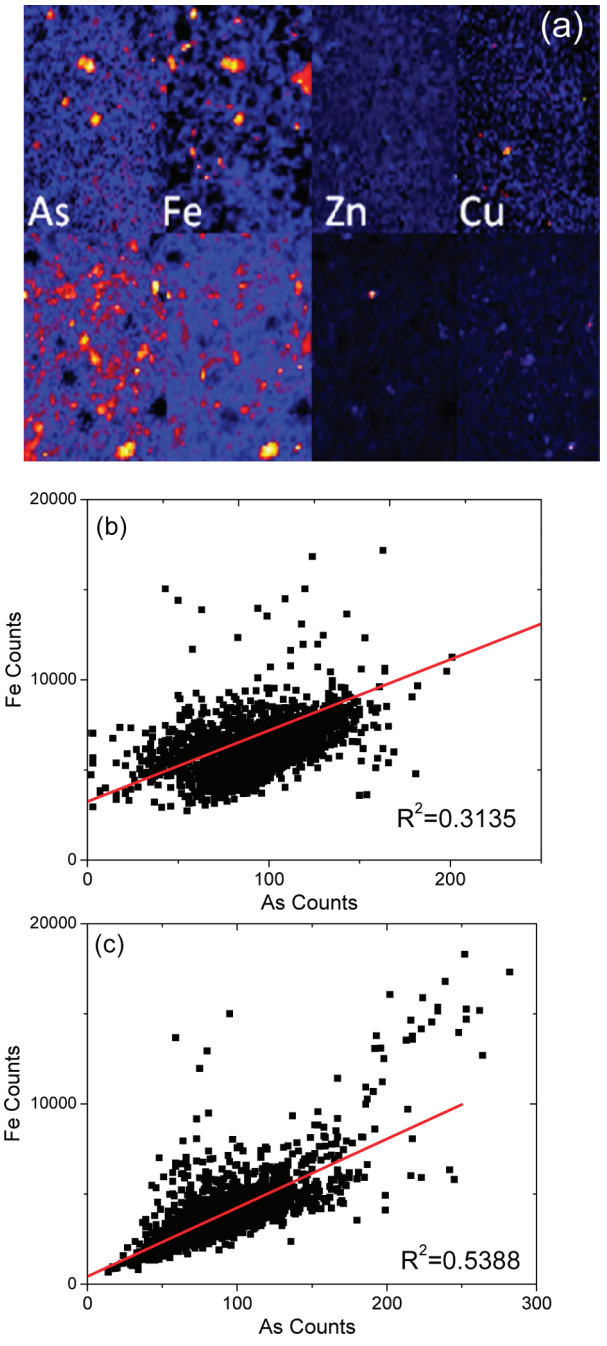


**Figure 1.** Selected $\mu$-SXRF maps for As, Fe, Zn, and Cu, (a) DMA incubated for 3 months under aerobic conditions (top) and MMA incubated for 3 months under anaerobic conditions (bottom) and arsenic and Fe fluorescence count correlation plots from $\mu$-SXRF maps (b) DMA incubated for 3 months under aerobic conditions and (c) MMA incubated for 3 months under anaerobic conditions.

This conclusion can also be supported by the strong sorption of $As^V$, MMA, and DMA to goethite.[19]

New XRD peaks or increasing intensity of existing peaks with longer incubation times were not observed. Peaks corresponding to As−Fe bearing minerals, such as scorodite ($FeAsO_4 \cdot 2H_2O$), were not identified. Scorodite is often observed in environments where As was introduced via mining operations.[20−22] Our results are consistent with a study on $As^V$ sorption to goethite for 1 year.[23] This EXAFS study showed that the $As^V$ was initially sorbed to the surface via bidentate binuclear complexes and the surface complexes did not transform to precipitates, such as scorodite. Based on As solution speciation data, there was no detectable $As^V$ in solution except for one sample. It seemed that most of the $As^V$ produced via demethylation was sorbed to the soil and not available in solution to form any Fe−As crystalline precipitates. There are possibilities that crystalline Fe−As precipitates were below $\mu$-XRD detection limits or poorly crystalline Fe−As precipitates were formed.

**Aqueous As Speciation and As Sorption in Aerobic Samples.** In aerobic samples, only MMA and DMA were detected in the solutions of samples that were reacted with MMA (MMA samples) and DMA (DMA samples), respectively (Figure 2). In the case of the MMA samples, initial MMA sorption was almost 100%. For the 1 week to 1 year incubated samples, the MMA concentration in solution was consistently low, and changes in concentration were not significant. In contrast, DMA concentrations in the DMA samples decreased over time (Figure 2). The DMA concentration in solution was highest for the 1 week incubated sample and lowest for the 1 year sample. After 1 week incubation, the DMA concentration was 3.34 mg/L, which corresponded to approximately 79% sorption. The DMA concentration dropped from 1.48 mg/L (90% sorption) after 1 month of incubation to 0.60 mg/L (96% sorption) after 1 year of incubation.

The low MMA concentration in solution indicates the high sorption affinity of MMA to the soil, particularly to the Fe-oxyhydroxides.[19] The $\mu$-XANES study (Figure 3) showed that the MMA samples were demethylated to produce mainly $As^V$, which was not detected in the solution. As soon as $As^V$ is produced, the $As^V$ is sorbed to the soil due to the strong sorption affinity of $As^V$ to goethite.[19] Possible MMA sorption mechanisms are the formation of inner-sphere and outer-sphere complexes.[2,19] In this study, we consider that inner-sphere complexes are due to ligand exchange and are directly bound to the surface and outer-sphere complexes are due to electrostatic attraction or hydrogen bonding to the surface and are not directly bound to the surface. The formation of inner-sphere complexes, particularly a bidentate binuclear complex formation, was observed between MMA and goethite, which is one of the main MMA sorbents in the soil.[17] Similarly, $As^V$ produced from the demethylation of MMA also forms bidentate binuclear complexes with goethite.[24] Inner-sphere complex formation is often an irreversible process, which can explain the very low concentrations of MMA and $As^V$ in the solution phase of the MMA samples.[25] Outer-sphere complex formation, particularly electrostatic attraction, is observed when two oppositely charged species exist in solution. At pH 6, the Fe-oxyhydroxide surface is positively charged, and MMA is negatively charged ($CH_3AsO_3^-$). It has been reported that DMA and $As^V$ can form outer-sphere complexes with Fe/Al-oxyhydroxides.[26,27] Due to the similar molecular structures between $As^V$, MMA, and DMA, it is possible that MMA also forms an outer-sphere surface complex on the Fe-oxyhydroxide surface, contributing to the high sorption.

The lower sorption of DMA than MMA to the soil (Figure 2) under aerobic conditions is also analogous to the situation of DMA sorption to Al/Fe-oxyhydroxides.[2,19] Due to the weaker sorption affinity of DMA, a ppm level of DMA is consistently detected in the solution of aerobic DMA samples. Possible DMA sorption mechanisms are analogous to the MMA sorption

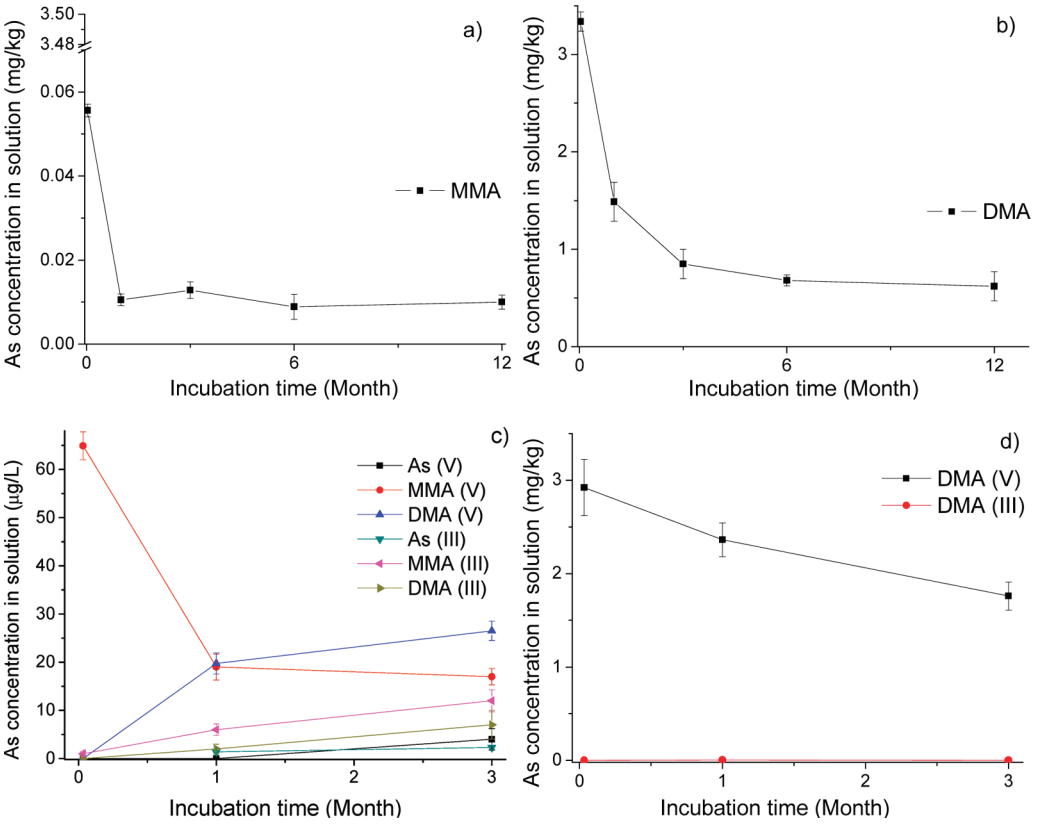


**Figure 2.** As species concentrations in solution. a) MMA samples incubated under aerobic conditions, b) DMA samples incubated under aerobic conditions, c) MMA samples incubated under anaerobic conditions, and d) DMA samples incubated under anaerobic conditions.

mechanisms discussed above. DMA also forms bidentate binuclear complexes with goethite.[17] The weak sorption affinity of DMA, despite forming inner-sphere bidentate binuclear complexes, is likely due to the effect of additional methyl group substitution on the As atom, such as size, charge, and geometry effects.[2] The decreasing DMA concentration in the solution over time is likely a result of DMA demethylation. As the incubation time increased, more DMA was demethylated to $As^V$ by microbes, and this $As^V$ was rapidly sorbed to the soil, similar to the observation from MMA samples. The consumption of DMA by microbes leads to decreasing DMA concentration in solution over time. This hypothesis is also supported by the increasing $As^V$ peaks in the $\mu$-XANES spectra (Figure 3).

**Aqueous As Speciation and As Sorption in Anaerobic Samples.** Anaerobic conditions of the samples were verified by measuring the redox potential of each sample. One week incubated samples showed a redox potential of approximately −50 mV, and the remaining samples, 1 month and 3 months incubated samples, had redox potentials between −300 and −350 mV. In the anaerobic samples, As speciation in solution was more varied. The 1 week incubated MMA samples had only 65 $\mu$g/L $MMA^V$ in solution (Figure 2). After 1 month of incubation, $MMA^V$, $DMA^V$, $As^{III}$, $MMA^{III}$, and $DMA^{III}$ were detected at low $\mu$g/L concentrations. In the 3 month incubated samples, $As^V$, $MMA^V$, $DMA^V$, $As^{III}$, $MMA^{III}$, and $DMA^{III}$ were detected at low $\mu$g/L concentrations. Unlike the MMA samples, DMA samples showed little variation in As speciation and increasing sorption over time (Figure 2). Only $DMA^V$ and $DMA^{III}$ were detected in solution from the 1 week to 3 month samples. The $DMA^V$ concentration in the 1 week incubated sample was 2.82 mg/L (82% sorption), and the $DMA^V$ concentration dropped to 1.76 mg/L after 3 months of incubation. The $DMA^{III}$ concentration was constantly low ppb levels from 1 week to 3 months samples.

In the MMA anaerobic samples, there was a small increase in DMA concentration, and increasing peak intensity was seen in the $\mu$-XANES spectra (Figure 4), demonstrating that methylation, mainly DMA production, was taking place under anaerobic conditions. Increasing DMA concentrations in solution indicates that DMA production is larger than the capacity of the soil to sorb DMA due to DMA's relatively lower sorption affinity on goethite. Sorption of reduced As species, especially $MMA^{III}$ or $DMA^{III}$, seems to be negligible. Due to the instability of these species, which are known to be oxidized to their analogue (5+) species within days, very few sorption studies have been conducted, and the sorption mechanisms are unknown.[28] $MMA^{III}$ and $DMA^{III}$ are known to have very low sorption affinity to goethite.[19] Micro-XANES spectra do not show peaks corresponding to these species. The total amount of $MMA^{III}$ and $DMA^{III}$ detected in the solution is likely equivalent to the total $MMA^{III}$ and $DMA^{III}$ produced. The oxidation of $MMA^{III}$ and $DMA^{III}$ during analysis was minimized by keeping samples in a glovebox.

For DMA samples, the decrease in DMA concentration over time can be attributed to continuous DMA sorption on the soil surface (Figure 2). The effect of carbonate on DMA sorption is uncertain, since some studies show decreasing $As^V$ sorption to Fe-oxyhydroxides, a very small effect on $As^V$ sorption, or enhanced $As^V$ sorption.[29−31] Also, the volatilization of DMA to dimethylarsine or trimethylarsine is uncertain, since the actual

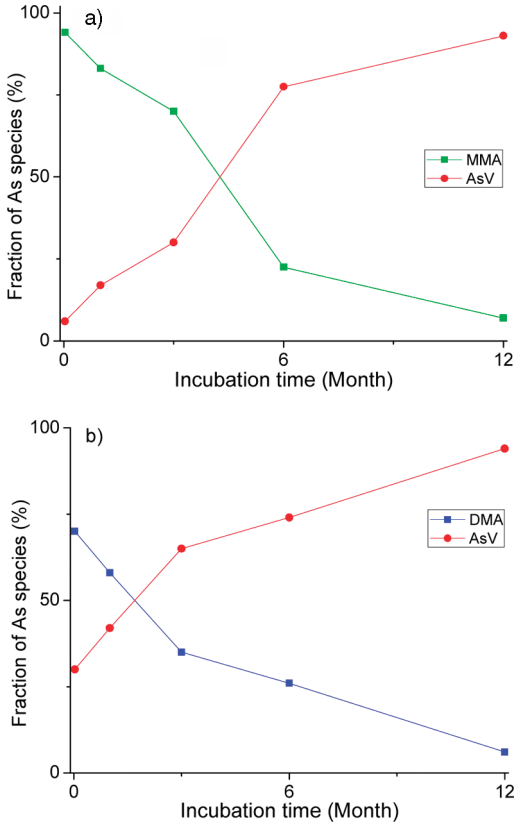


**Figure 3.** Results from fitting the $\mu$-XANES spectra. a) As species in percentage from the LCF for MMA aerobic samples, b) As species in percentage from the LCF for DMA aerobic samples. Uncertainty for all points is within ±5%.

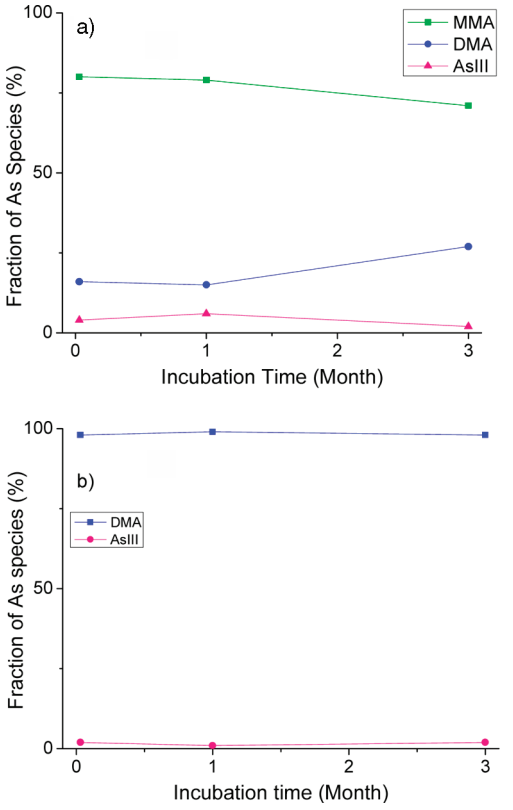


**Figure 4.** Results from fitting the $\mu$-XANES spectra. a) As species in percentage from the LCF for MMA anaerobic samples, b) As species in percentage from the LCF for DMA anaerobic samples. Uncertainty for all points is within ±5%.

measurement of gaseous As species showed very little volatilization.[11,12] High As volatilization rates have been reported in studies, using a mass balance equation and assuming that the missing As in the system is due to volatilization.[32]

**Micro-XANES Studies of Aerobic Samples.** Micro-XANES spectra from As hotspots were collected to determine As speciation. The As speciation was determined by analyzing the peak position of the first derivative of the $\mu$-XANES spectra (Figure S6). From the LCF of the $\mu$-XANES spectra, we determined the percentage of each As species. For fitting the MMA aerobic sample $\mu$-XANES spectra, only MMA and $As^V$ reference spectra were used, since the addition of DMA did not improve the fitting, and the contribution of DMA to the fitting was less than 1%. Based on the fitting, MMA samples showed demethylation as the main As biotransformation product. Monomethylarsenate was gradually demethylated to $As^V$ over one year of incubation (Figure 3). Initially, MMA was stable until 1 month of incubation. The peak due to $As^V$ was hardly recognizable (Figure S6), but the LCF showed that 6% of the total As was $As^V$ for the 1 week incubated samples (Figure 3). However, 6% is barely above the accuracy of data (±5%). It is possible that $As^V$ contribution to the spectra is negligible. After 1 month of incubation, there was a small $As^V$ peak growing at 11,874 eV, and 17% of the total As was $As^V$. The MMA peak gradually became smaller, and the $As^V$ peak increased in intensity. After one year of incubation, $As^V$ was the main species, constituting 93% of the total As.

Dimethylarsenate samples also showed demethylation as the main As biotransformation product. Dimethylarsenate was also demethylated to $As^V$ over one year of incubation (Figure 3). For the LCF for DMA samples, only DMA and $As^V$ spectra were used in the fitting. The MMA spectrum was excluded from the fitting based on a desorption study,[17] which showed that little MMA was produced and sorbed to the soil. The LCF revealed that 30% of the total As was $As^V$ for the 1 week incubated samples (Figure 3, S6). After 1 month of incubation, 58% of the total As was DMA and 42% was $As^V$. After one year of incubation 94% of the total As was $As^V$, and the peak due to DMA was insignificant. As a biological control, the same MMA and DMA samples were incubated for 1 month with an addition of 10 mM sodium azide. Micro-XANES analysis of these samples (data not shown) showed only MMA or DMA peaks indicating that the methylation/demethylation was predominantly a biotic process.

Monomethylarsenate and DMA demethylation to $As^V$ under aerobic conditions agrees with other studies that were conducted under similar conditions.[11,33] These observations confirm that a positive redox potential, indicating an oxidized condition, is the one of the factors that triggers the demethylation. Dimethylarsenate seems to be directly demethylated to $As^V$ (Figure 3), which contradicts the Challenger methylation scheme. The Challenger mechanism (Figure S2) shows that DMA is demethylated to MMA first, and then MMA is transformed to $As^V$. A small amount of MMA could be produced, but this was not evident from the $\mu$-XANES spectra (Figure 3). Assuming MMA is the intermediate of demethylation before the production of $As^V$, the amount of MMA produced should be higher than or equal to the amount of $As^V$ produced, which should be observed from

$\mu$-XANES spectra because MMA has strong sorption affinity to Al/Fe-oxyhydroxides.[2,19] Inconsistency between our observation and the Challenger mechanism can be an indication that there is more than one demethylation pathway that was not identified. For example, the Hayakawa methylation scheme (Figure S2) proposes that DMA can be produced without MMA. There can be other demethylation pathways, in which $As^V$ can be produced without MMA. Monomethylarsenate could have been produced from the methylation of $As^V$ but not from the demethylation of DMA.

The faster rate of DMA demethylation is partially caused by its lower sorption affinity to the soil. The amount of DMA desorption from Al/Fe-oxyhydroxides, using phosphate as a desorbing agent, is higher than desorption studies on $As^V$ or MMA.[2,19] In this study, a part of the applied DMA always stays in solution, which is readily available for microbes to demethylate. Once $As^V$ is produced, $As^V$ can replace the sorbed DMA on the surface that leads to more DMA being available for further demethylation that can accelerate the rate of demethylation. Monomethylarsenate demethylation, on the other hand, is slower. Most of the applied MMA is sorbed to the soil, and very little MMA is in solution initially so that the initial $As^V$ production is small. The small amount of $As^V$ can replace only a small amount of MMA from the surface. More time is required to produce large amounts of $As^V$. In addition, MMA's stronger sorption affinity can make $As^V$ more difficult to replace sorbed MMA than to replace DMA.

**Micro-XANES Studies of Anaerobic Samples.** Under anaerobic incubation, the MMA samples were methylated to DMA over 3 months of incubation (Figure 4). The $As^V$ reference spectrum was not included in the LCF because the addition of the $As^V$ spectrum did not improve the fitting, and the contribution of the spectrum to the fitting was less than 1%. Monomethylarsenite and $DMA^{III}$ spectra were also not included in the fitting since their concentration was too low (Figure 2) and sorption was negligible.[19] According to the peak intensities from $\mu$-XANES spectra, MMA methylation was observed at 1 week (Figure 4, S6). At 1 week, there was an enhanced peak due to DMA, and 16% of the total As was DMA. Dimethylarsenate concentration increased over time, and after 3 months of incubation, 27% of the total As was DMA (Figure 4). Arsenite was consistently observed for all incubation periods, contributing 2−6% of the total As. On the other hand, the DMA samples did not show significant speciation changes. The only peaks recognized from the $\mu$-XANES spectra were DMA and $As^{III}$ (Figure S6). Dimethylarsenate consisted of 98−99% of the total As at all times and $As^{III}$ was about 1−2%. The fitting was performed using DMA and $As^{III}$ standard spectra, since the MMA and $As^V$ standard spectrum did not show significant contribution to the fitting (<1%). As a biological control, the same samples were incubated for 1 month with an addition of 10 mM sodium azide. The solution phase analysis of these samples (data not shown) showed no signs of speciation changes indicating that methylation/demethylation is a biotic process. Due to the limited beam time, $\mu$-XANES analysis was not performed on these samples.

Our study showed that at a lower redox potential, methylation is favored over demethylation similar to the findings from other studies. $As^V$ or MMA methylated to MMA or DMA under anaerobic conditions.[10,13] Although the exact mechanism of methylation is still unclear, two recognized mechanisms favor reduced conditions for methylation to occur. In the Challenger mechanism, pentavalent species have to be reduced at first to undergo further oxidative methylation. Reduced species, such as $As^{III}$ or $MMA^{III}$, are more unstable than oxidized species, such as $As^V$ or $MMA^V$, under normal conditions and can be easily oxidized.[28] Lower redox potential conditions can stabilize reduced species. In the Hayakawa mechanism, the methylation process requires reduced glutathione, which is more stable under reduced conditions.[8] A thermodynamic analysis of the Challenger mechanism also supports methylation being favored under reduced conditions (pe < 0).[34] However, there are studies, showing that demethylation is still observed under anaerobic conditions, especially DMA demethylation to MMA.[13] This variation in the methylation/demethylation of As under anaerobic conditions indicates that this process highly depends on not only environmental conditions but also on the composition of microbial communities in the soils.

**Environmental Significance.** Changes in arsenic speciation are very dynamic, and the methylation/demethylation of As is commonly observed in the environment, yet the exact mechanisms and factors affecting the process are largely unknown. A better understanding of As speciation is essential to predict the fate of As in the environment. Micro-SXRF studies provided direct evidence for MMA and DMA being associated with Fe-oxyhydroxides in soil, similar to $As^V$ association with Fe-oxyhydroxides. Due to the strong sorption affinity of sorbed As species, extraction methods may alter As species on the mineral surface. Micro-SXRF and $\mu$-XANES analyses clearly demonstrated the novel *in situ* qualitative solid-state As speciation under aerobic or anaerobic conditions. Our findings also suggest that under anaerobic conditions, the presence of organoarsenicals, such as MMA and DMA, can be greater than what was previously reported.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Detailed Material and Method section and additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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