Impacts of Redox Conditions on Arsenic and Antimony Transformation in Paddy Soil: Kinetics and Functional Bacteria

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Abstract
Arsenic (As) and antimony (Sb) are known carcinogens and are present as contaminants in paddy soils. However, the complicated dynamics of the mobility of these metalloids have not been well understood due to changing redox conditions in paddy soils. Herein, the kinetics of dissolved As and Sb, and functional bacteria/genes were examined in a paddy soil cultured under aerobic and anaerobic conditions. Under aerobic condition, dissolved As(V) and Sb(V) increased constantly due to sulfide oxidation by O_2 and bound As and Sb were released. Under anaerobic condition, the reduction of As(V) and Sb(V) occurred, and the mobility of As and Sb were affected by soil redox processes. The bacteria with functional genes *aioA* and *arrA* were responsible for the direct As/Sb transformation, while Fe- and N-related bacteria had an indirect effect on the fate of As/Sb via coupling with the redox processes of Fe and N. These findings improve understanding of the mobility of As and Sb in paddy soil systems under different redox conditions.

Paddy soils were contaminated with heavy metals or metalloids as a result of mining activities. Among the metalloids, As and Sb are considered carcinogens and toxic to human health (National Research Council 2001; Sundar and Chakravarty 2010), and have similar chemical properties,

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often co-existing in soils. Both As(III) and Sb(III) are more toxic to organisms than As(V) and Sb(V). However, the mobility of As and Sb remains controversial (Smedley and Kinniburgh 2002), with As(III) and Sb(V) being the main species in soils (Yamaguchi et al. 2011) as determined by the redox conditions.

Paddy soils frequently experience periodic drainage and flooding processes and, consequently, redox conditions can change substantially. During flooding periods, O_2 is sequestered and consumed, and anaerobic conditions are established; following drainage, O_2 is introduced resulting in aerobic conditions (Noll et al. 2005). Because of the alternating drainage and flooding of paddy soils, changes in redox conditions have significant effects on the transformation of As and Sb (Liesack et al. 2000; Leuz et al. 2006). Firstly, redox conditions can directly influence the valence states of As and Sb in soils. As(V) is the major valence state under aerobic conditions and As(III) is the dominant species under anaerobic conditions. Differing from As, Sb(V) is more stable, even under reducing conditions (Wilson et al. 2010). Secondly, changes in redox conditions can influence microbial communities and directly induce the transformation of As and Sb through expressing putative functional gene, including As/Sb oxidizing gene (*aioA*) and As reducing gene (*arrA*) (Zheng et al. 2013; Li et al. 2016). Conversely, As and Sb

species determine toxicity, which affects the composition and diversity of microbial communities. Thirdly, microbially-mediated Fe/N/S/C cycles indirectly affect the mobility and transformation of As and Sb (Xu et al. 2019). Specifically, As and Sb can be released via the reductive dissolution of Fe (hydroxide)oxides, while As and Sb could be immobilized by Fe(II) oxidation coupling with nitrate reduction or precipitated with sulfide under anaerobic conditions (Noll et al. 2005; Wilson et al. 2010). The content of organic matter also influences the mobility of Sb via biotic and abiotic processes (Tella and Pokrovski 2008; Han et al. 2017). Despite this understanding, most studies have focused on the transformation of As or Sb related to functional microbial communities under either aerobic or anaerobic conditions (Stroud et al. 2011; Yamaguchi et al. 2011; Okkenhaug et al. 2012), whereas the impacts of different redox conditions on As and Sb transformation during periodic drainage and flooding have not been reported. Besides, due to mining activity, acid mine drainage (AMD) is closely linked with the paddy soil downstream. AMD has a seasonal fluctuation, and the humidity of AMD could be affected by rainy and dry seasons. Therefore, the fluctuation of oxygen concentration provided different conditions for Fe(II)-induced phase transformation of iron minerals, and subsequently changed the bioavailability of As and Sb (Johnston et al. 2010; Karimian et al. 2018). Related research is insufficient on these similar processes in paddy soil.

In this study, microcosm experiments were conducted under either aerobic or anaerobic conditions to simulate the drainage and flooding conditions of paddy systems. The aims of this study were to (i) examine the impact of redox conditions on the mobility of As and Sb, and (ii) distinguish the physiochemical and biological factors affecting the mobility of As and Sb. The findings of this study will be beneficial for the interpretation of the behavior of As and Sb in variable paddy soil conditions and help improve soil remediation strategies for As- and Sb-contaminated paddy soils.

Materials and methods

Paddy soil samples were collected from a depth of 0–20 cm adjacent to the Xihuangshan mining area in Hunan Province (27°48′24″N, 111°29′31″E) and stored at 4°C in plastic containers. The samples were air-dried at room temperature and ground before passing through a 2 mm nylon sieve. The chemical analyses were performed as described by Lu (2000). Briefly, pH (5.94) was determined in a 0.01 M $CaCl_2$ solution using a pH meter (Seven Compact, Mettler Toledo, Switzerland); the concentrations of SO_4^{2-} (1.26 g kg^{-1}) and NO_3^- (260 mg kg^{-1}) were determined using ion chromatography (IC, ICS-600, DioneX, USA); total Fe content (1.26 g kg^{-1}) were determined by inductively coupled plasma optical emission spectrometry (ICP–OES, Optima 8000, PerkinElmer, USA); and total As (17.8 mg kg^{-1}) and Sb (282 mg kg^{-1}) were determined by high-performance liquid chromatography–atomic fluorescence spectroscopy (HPLC–AFS, AFS-9710, Haiguang, China). The detection limits of As and Sb were less than 0.02 ng mL^{-1}, and the concentrations of standard curves were 5, 10, 20, 50, 100, and 200 μg L^{-1} ($R^2 = 0.998$ for As and 0.999 for Sb).

Aerobic and anaerobic microcosms were subsequently established in serum bottles (50 mL) containing 2 g of air-dried soil and 20 mL of phosphate-buffered mineral medium (Mu and Scow 1994). Note that PO_4^{3-} in the phosphate-buffer mineral medium has minimal effect on As and Sb adsorption, because As was less affected by PO_4^{3-}, and only 10–20% of Sb(V) was adsorbed by soil at pH 7 (Xi et al. 2010; Arco-Lázaro et al. 2016). Since Sb(III) was not affected by pH, the PO_4^{3-} could only increase the Sb adsorption by 5–10% (Xi et al. 2014). A total of four treatments included two non-sterile and two sterile treatments. For non-sterile treatments, aerobically treated microcosms were sealed with 0.22-μm film and cultured in a shaker at 180 rpm, and anaerobically treated microcosms were flushed with N_2 for 40 min before being sealed and incubated under anaerobic conditions. The microcosms were incubated at 28°C in the dark in triplicate. Sub-samples were taken from each microcosm five times (on day 0, 10, 18, 36, and 60). Sterile treatments were sterilized at 121°C for 20 min, then incubated as above methods under aerobic and anaerobic conditions respectively, these samples were taken from three times (on day 0, 7, and 60). As and Sb were measured using HPLC–AFS.

Microbial communities and related putative functional genes, such as the As oxidation gene (*aioA*) and As reduction gene (*arrA*), were also analyzed. For this, soil extracts following centrifugation were used for DNA-based microbial community analysis. Soil DNA was extracted using the Power Soil DNA kit (Mo Bio laboratory Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The v3 and v4 16 S rRNA regions were amplified and DNA sequencing was performed using the Illumina Miseq platform. The 16 S rRNA, *aioA*, and *arrA* genes were quantified using the CFX 384 Real-Time polymerase chain reaction (PCR) detection system (Bio-Rad Laboratories, USA). The 16 S rRNA gene was amplified using EUB338f/518r primers by program described (Muyzer et al. 1993); the *aioA* gene were amplified using *aioA*95f/*aioA*955r primers and by program described (Inskeep et al. 2007); and the *arrA* gene was amplified using *arrA*-CVF1/*arrA*-CVR1 primers by program described (Mirza et al. 2017). All of the samples were run in triplicate. Each 10 μL reaction volume contained 5 μL of 2 × IQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules,

CA, USA), 0.2 µM of each primer, and 1 µL of DNA (1–10 ng) (Qiao et al. 2019).

Results and Discussion

The kinetics of the dissolved As and Sb species under the different redox conditions are shown in Fig. 1. Under the aerobic condition (Fig. 1a), the concentration of dissolved As(III) was 7.39×10^{-4} mg L^{-1} at the initial stage and then slightly increased to a terminal concentration of 2.90×10^{-4} mg L^{-1}. In comparison, the initial concentration of As(V) was 3.96×10^{-2} mg L^{-1} and rapidly increased to 0.357 mg L^{-1}. Under the anaerobic condition (Fig. 1b), the concentrations of dissolved As(III) and As(V) increased during the first ten days of incubation and subsequently decreased and stabilized. Dissolved Sb(V) was the dominant Sb species in all the samples. Under aerobic conditions (Fig. 1c), the initial concentration of Sb(V) (0.930 mg L^{-1}) increased during the first 18 days then slightly decreased and, finally, increased again to a final concentration of 6.95 mg L^{-1}. Dissolved Sb(III) was not detected during the entire aerobic period. Under the anaerobic condition (Fig. 1d), Sb(V) was more stable, but decreased slightly during the first 36 days before increasing to 0.830 mg L^{-1} by day 60. The concentration of Sb(III) increased from 9.83×10^{-3} to 3.26×10^{-2} mg L^{-1}

during the first 10 days, after which concentrations progressively fell so that no Sb(III) was detected by the end of the incubation period. For sterile treatments, either under aerobic or anaerobic conditions, no As(III) and Sb(III) was detected. Although As(V) was detected under both aerobic and anaerobic conditions, and Sb(V) was only detected under anaerobic condition The concentrations of As(V) and Sb(V) were much lower than those in the non-sterile treatments. These results indicat that the chemical oxidation of As(III) and Sb(III) by the soil components had very small contributions to the overall oxidation of As(III) and Sb(III).

Under aerobic conditions, although As(III) can be oxidized to As(V) and become bound to solid phases (Myers et al. 1973; Kim and Nriagu 2000), colloidal and dissolved As can be mobilized by soil flooding (Xia et al. 2018; Rinklebe et al. 2020). The equilibrium of adsorption–desorption is the thermodynamic process, which was mainly affected by pH. Since the pH in this system was stable due to the pH-buffer, the adsorption-desorption process would not change obviously under aerobic conditions. For Sb, in addition to mobilization by soil flooding, Sb(III) can be oxidized to more soluble Sb(V) (Mitsunobu et al. 2006), thereby enhancing Sb mobility. As a result, dissolved Sb(V) was the dominant species detected. This likely explains why both As and Sb mobility increased under the aerobic condition while also showing different mobility under the different valences.
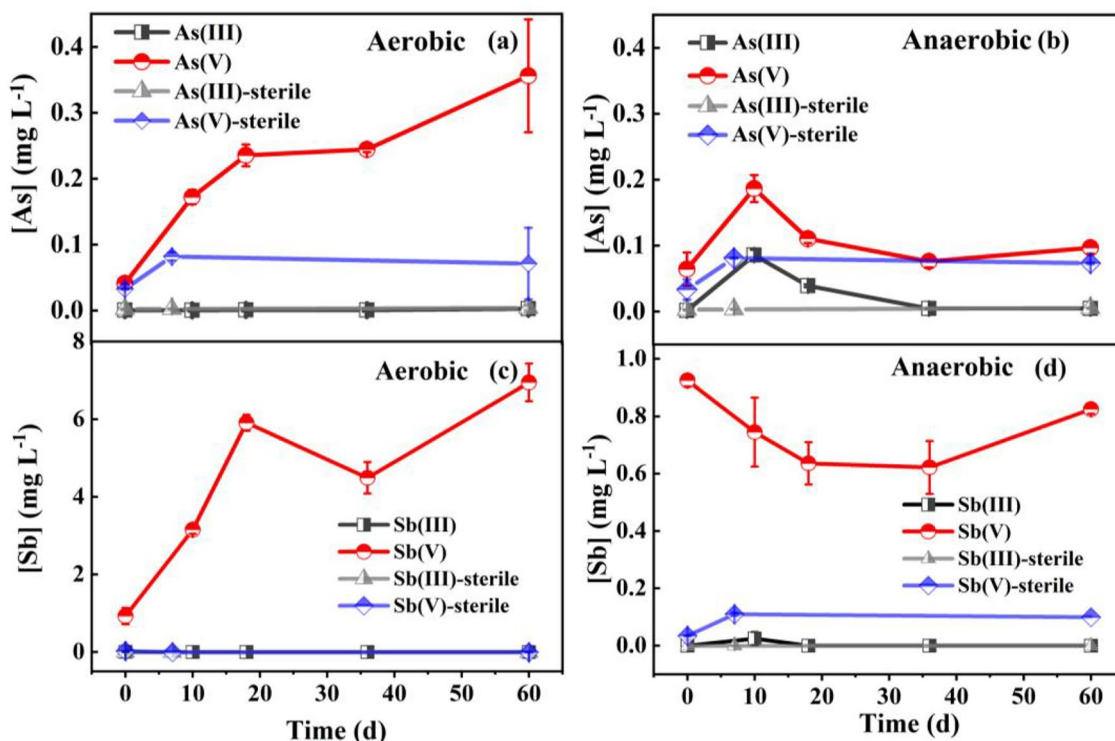

Fig. 1 Kinetics of dissolved As species under **a** aerobic and **b** anaerobic conditions; and the kinetics of Sb species under **c** aerobic and **d** anaerobic conditions. Sterile treatments were conducted as abiotic control. Data are mean ± SD (n = 3)

Under anaerobic conditions, the transformation of dissolved As was affected by different processes during the different experimental stages. During the first ten days, dissolved As increased via the reduction dissolution of minerals, mainly Fe(oxyhydr)oxides (Ohtsuka et al. 2013), which can release Fe (oxyhydr)oxide-bound As into solution. The anaerobic reduction of As(V) to labile As(III) can also increase dissolved As because the mobility of As(III) is much higher than that of As(V) (Zheng et al. 2013). During the remaining 50 days of anaerobic incubation, As could be co-precipitated with the secondary Fe minerals generated from biotic and abiotic processes involving dissolved Fe(II) (Islam et al. 2005), resulting in a decrease in dissolved As. For Sb, the observed decrease in dissolved Sb(V) and the slight increase in dissolved Sb(III) indicated that Sb(V) reduction occurred. However, the constant decrease in dissolved Sb with incubation time suggested that the transformation of Sb differed from As under anaerobic conditions. The mobility of As is likely controlled by iron oxide (Smendley and Kinniburgh 2002) while the dominant controls on the immobilization of Sb could be the availability of organic matter and sulfide (Ashley et al. 2003; Craw et al. 2004; Tella and Pokrovski 2008). For example, Qasim and Motelica (2014) found the highest Sb ratios in non-residual fractions in association with organic/sulfide fractions. Thus, the reduction of sulfate can supply S^{2-} and precipitate with dissolved Sb. Therefore, the main mechanisms of As and Sb immobilization under anaerobic conditions were different. Moreover, the changes in As and Sb concentrations in the sterile control were not significant, indicating the importance of microorganisms, which can either directly oxidize and reduce As/Sb (Ohtsuka et al. 2013; Herath et al. 2017) or indirectly alter As/Sb by altering the redox cycling of other soil elements.

To identify the microorganisms involved in direct As/Sb transformation, putative As oxidation genes (*aioA*) (Fig. 2a) and reduction genes (*arrA*) (Fig. 2b) were detected, which are reported to be related to the transformation of As and Sb (Li et al. 2016). The relative abundances of *aioA* and *arrA* genes were calculated based on *aioA/*16S rRNA and *arrA/*16S rRNA. Under the aerobic condition, the initial relative abundance of the *aioA* gene was 33.3%, which then increased to 51.1% on day 18, and finally decreased to 34.6% at the end of the aerobic stage. This pattern indicates that the active oxidation of As and Sb prevailed under aerobic conditions and consequently resulted in high concentrations of As(V) and Sb(V). Under the anaerobic condition, the abundance of the *aioA* gene had decreased to 8.94% on day 10 and then stabilized. Furthermore, relative to the *arrA* gene, the abundance of the *aioA* gene was much higher overall and was also higher under aerobic conditions (Fig. 2a). This suggests that the redox conditions directly influenced the functional genes involved in As/Sb biotransformation (Yamaguchi et al. 2011). Furthermore, the abundance of *arrA* gene decreased over time under both aerobic and anaerobic conditions (Fig. 2b), indicating that bioreduction of As/Sb was not the dominant process after 18 days of incubation.

To fully understand the direct and indirect roles of microorganisms responsible for As/Sb transformation under aerobic and anaerobic conditions, the relative abundances of microbial communities were examined at the genus level. Species belonging to *Bacillus* (7.42%), *Singulisphaera* (4.56%), and *Defluviicoccus* (5.05%) were found in high relative abundances under both aerobic and anaerobic treatments (Fig. 3). However, the dominant genus in both treatments differed, indicating that redox conditions substantially affect microbial communities. *Massilia* was the dominant genus, increasing in abundance between day 0 (0.160%) to 36 (19.0%) of aerobic incubation (Fig. 3a). *Brevundimonas* increased in abundance from day 10 (4.58%) to the end of aerobic incubation (9.58%). *Singulisphaera, Massilis* and *Brevundimonas* spp. are known to be related to As mobility via As resistance and reduction reactions (Blum et al. 1998; Du et al. 2011; Singh et al. 2016; Saleem et al. 2018). Under the anaerobic condition (Fig. 3b), the relative abundance of *Bacillus* spp. Remained high during the entire incubation period (4.64–8.20%). *Geobacter* spp. was also detected with a high relative abundance, increasing between day 0 (1.14%) and 18 (18.3%) of the anaerobic stage before decreasing. *Bacillus* spp. is identified as a Sb-reducing genus of bacteria

Fig. 2 Relative abundances of functional genes under aerobic and anaerobic conditions: **a** *aioA* gene and **b** *arrA* gene. Data are mean ± SD (n = 3)
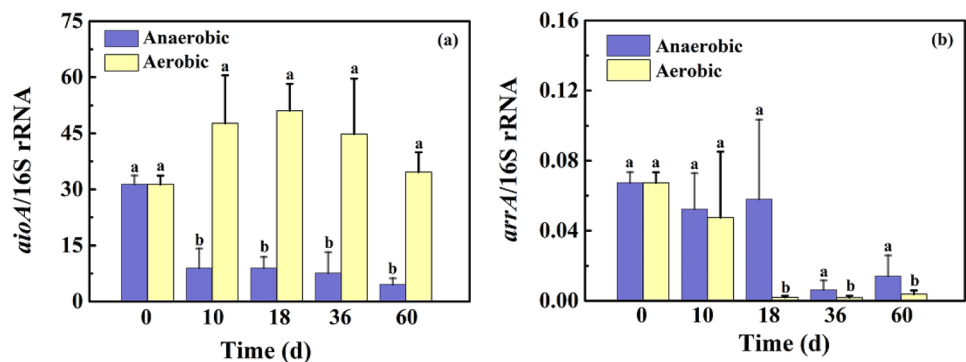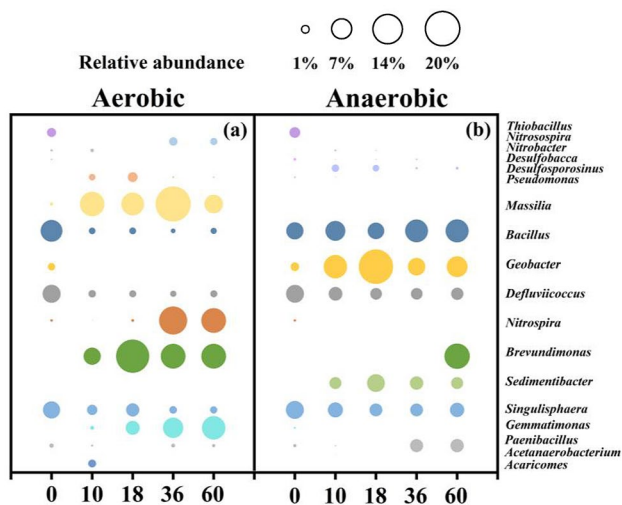
Fig. 3 Changes in the relative abundance of microbial communities at the genus level under aerobic and anaerobic conditions. Only relative abundances higher than 1% and putative Fe/N/S-cycling genera are shown. Data are mean of three triplicate

and is found in metal contaminated sites (Singh et al. 2016; Zhang et al. 2019). *Geobacter* genus is reported to play an important role in As reduction under anaerobic conditions, it could be also reduce other toxic metal ions (Qiao et al. 2019; Shi et al. 2020; He et al. 2020).

In addition to the As/Sb functional bacteria, the relative abundances of microbial communities having an indirect influence (Zhang et al. 2019) were analyzed. The relative abundance of *Nitrospira* spp. increased sharply over the 36 days (from 0.110 to 12.1%). This genus contains aerobic chemolithoautotrophic nitrite-oxidizing bacteria, which can convert ammonia to nitrite and, finally, nitrate (Daims and Wagner 2018). It has also been noted that an increase in available Sb can stimulate urease activity (An and Kim 2009), and N redox cycling is closely related to the transformation of As and Sb (Noll et al. 2005). The relative abundance of putative sulfate reducing genera (*Desulfosporosinus* and *Desulfobacca* spp.) increased from 0.130% on day 0 to 0.690% on day 18, and then decreased in the late period of incubation. This reducing process could produce S^{2-}, which could be precipitated with As or Sb. Moreover, *Geobacter* spp. are typical of Fe-reducing bacteria, and can be involved in the release of As and Sb from Fe (oxyhydr)oxide-bound fractions under anaerobic conditions (Yuan et al. 2019). The results indicated that the direct As/Sb transformation by putative microorganisms was favored under aerobic conditions, with these species accounting for a maximum of 35.3% of the community compared to 13.1% for those species indirectly involved (13.1%). However, the putative microbial species indirectly involved in As/Sb transformation when coupled with Fe/N/S redox processes were much

equal to direct bioprocesses, having a maximum relative abundance of 18.9% compared to 23.2% for those directly involved in As/Sb transformation.

Under aerobic conditions, concentrations of As(V) and Sb(V) increased via the oxidation of As and Sb, respectively, and the simulated soil flooding resulted in the release of colloidal and dissolved As and Sb species. Under anaerobic conditions, As(V) and Sb(V) were reduced. The release of immobilized As might be attributed to the reduction dissolution of Fe (oxyhydr)oxides, dissolved As was then re-immobilized via co-precipitation with microbially mediated secondary minerals. In comparison, the decrease of Sb(V) and the slight increase of soluble Sb(III) could be due to the reduction dissolution of Sb. The relative abundance of *aioA* genes was higher in aerobic treatment than in anaerobic treatment, indicating that the microbial As/Sb oxidation was promoted under aerobic conditions. Besides, the relative abundance of *arrA* genes was lower than *aioA* genes, indicating that the reduction of As and Sb was not the dominant process during the whole period. The microorganisms should have the detoxification capacity by oxidizing the As/Sb into less toxic valence under high concentration of metalloid. Functional gene analysis indicated that direct microbial oxidation was dominant for As and Sb over direct bioreduction effects both under aerobic and anaerobic conditions. Moreover, the microbial community analysis revealed that N, S, and Fe redox cycling can indirectly affect the mobility of As and Sb. This study provides information that links the variation of paddy soils and the effects caused by the seasonal fluctuation in AMD. These findings would provide fundamental understanding of the As/Sb biogeochemical processes and be helpful for improving soil remediation strategies for As- and Sb-contaminated paddy soils.

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