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Microbial mediated arsenic biotransformation in wetlands

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1 **Abstract**

2 Arsenic (As) is a pervasive environmental toxin and carcinogenic metalloid. It ranks

3 at the top of the US priority List of Hazardous Substances and causes worldwide

4 human health problems. Wetlands, including natural and artificial ecosystems (i.e.

5 paddy soils) are highly susceptible to As enrichment; acting not only as repositories

6 for water but a host of other elemental/chemical moieties. While macro-scale

7 processes (physical and geological) supply As to wetlands, it is the micro-scale

8 biogeochemistry that regulates the fluxes of As and other trace elements from the

9 semi-terrestrial to neighboring plant/aquatic/atmospheric compartments. Among these

10 fine-scale events, microbial mediated As biotransformations contribute most to the

11 element's changing forms, acting as the 'switch' in defining a wetland as either a

12 source or sink of As. Much of our understanding of these important microbial

13 catalyzed reactions follows relatively recent scientific discoveries. Here we document

14 some of these key advances, with focuses on the implications that wetlands and their

15 microbial mediated transformation pathways have on the global As cycle, the

16 chemistries of microbial mediated As oxidation, reduction and methylation, and future

17 research priorities areas.

20

1 Introduction

Arsenic (As) exists in four oxidation states (-III, 0, +III, and +V), and is present in both inorganic and organic forms. Commonly found in highly auriferous regions, As also associates with the mineral ores of other metals such as iron, copper, and lead [1]. Speciation in addition to concentration is fundamental to the understanding of As toxicity, mobility and fate in the environment [2]. In the biosphere, arsenate (As^V) and arsenite (As^{III}) are the most abundant inorganic As species. Under aerobic conditions, As^V dominates, is typically strongly absorbed to iron oxy-hydroxide minerals, and exhibits limited mobility/bioavailability [3]. Conversely, under anaerobis, a shift in As speciation to the trivalent form means the element is both more toxic and inherently more labile [3].

Common organic As species found in our bodies as well as in waters, soils, feeds and foods include monomethylarsenate ($MMAs^V$), dimethylarsenate ($DMAs^V$), trimethylarsine oxide (TMAsO), arsenosugars and arsenobetaine (AsB) (Table 1). They can be introduced into the environment due to microbial mediated biomethylation [4] and via anthropogenic activities, such as pesticide/fertilizer applications. Historically, the organic arsenical Roxarsone (2-nitrophenol-4-arsonic acid), has been added to poultry feeds as a growth promoter, however this has been largely phased out due to both animal and environmental health concerns [5]. Most, but not all, of the organic As species are considerably less toxic compared to their inorganic As counterparts. Typically, they are found **with** high concentrations in marine organisms. Arsenosugars constitute the main organo-As species found in algae,

1 while in other marine dwellers, from lobsters to white fish, the toxin is packaged as

2 the inert and very stable/unreactive AsB [6]. In freshwaters and soils/sediments,

3 generally MMAs^V, DMAs^V and volatile TMAsO are the most commonly detected

4 organic As species [7].

5   As the interface between terrestrial and aquatic ecosystems, wetlands occupy

6 between 5.3 and 12.8 million km^2 of the earth's surface [8]. Defined by their

7 inundation by water and constant transition between wet and dry states, oxygen

8 availability is typically very heterogeneous resulting in regions of water and sediment

9 where anaerobism is commonplace [9]. Because of their wide distribution, high

10 productivity, and rich biodiversity, wetlands have been viewed as having a major

11 influence on many global biogeochemistries, including the carbon and nitrogen cycles

12 [10, 11] in addition to impacting on As transfer [2].

13   Owing to their depositional characteristics, wetlands are also highly susceptible

14 to contamination by heavy metals and other toxic trace elements. Macro-scale

15 processes (physical and geological events), which include anthropogenic activities,

16 wet, dry deposition, erosion, volcanism, and weathering are essential in determining

17 As loading in these environments (Figure 1). Even seemingly 'pristine' wetland

18 habitats/ecosystems, with Yellowstone National Park being a prime example [12], can

19 be 'naturally' enriched in As. However, anthropogenic activities such as mining,

20 smelting, urban waste and wastewater discharges, fertilizer and pesticide use also

21 contribute significantly to the build-up of As stores in wetland [13]. Often,

22 disconnected from the surrounding geology, these human induced redistributions of

As can be the hardest to predict and hence pose perhaps the greatest hazard to human health.

Despite the importance of macro-scale processes in determining As loading into wetlands, it is micro-scale biogeochemistry, which includes desorption, dissolution [14] and microbial mediated As^V reduction, As^{III} oxidation and methylation [2] that mainly regulate the fluxes of As from the semi-terrestrial to either aquatic or atmospheric compartments (Figure 1). Recent advances in molecular methods have enabled great leaps in our understanding of microbial mediated As biotransformations. This review summarizes the role of wetland microbiology within the global As cycle, and the distribution and behavior of As in water submerged soils and sediments. Moreover, it provides a detailed overview of the key transformation **biochemistries**, ranking the events in order of priority and discussing their interactions within a chemically complex and heterogeneous localized environment. Finally, an outlook for future research areas and introduction to emerging new technologies for measuring As cycling in wetlands is presented.

2   Distribution and behavior of As in wetlands

2.1 Overview

Wetlands can be broadly classified as either '*natural*', which includes marine, **coastal** zone and inland wetlands, or '*artificial*' wetlands such as paddy soils. Although, the true state of most systems is perhaps as a mixture/intermediary of the two. The definition of what constitutes, as a 'contaminated sediment' is similarly quite open to

interpretation, as the acceptable quality criteria for As in wetland soils varies between countries (Table 2). For example, the Canadian Council of Ministers of the Environment (CCME), have set interim quality guidelines (ISQGs) for marine/estuarine sites at 7.24 mg kg^{-1} dry weight [15], whereas trigger thresholds in China are marked slightly higher, so a site is only given contamination status when concentrations of 20 mg kg^{-1} are breached [16]. An alternative method to evaluate the quality of a wetland, is to consider the As concentration in extractable waters. Recently reported incidences where detected As in overlying surface waters exceeded the US Environmental Protection Agency's (EPA) maximum containment level of As in drinking water (10 μg L^{-1}) are summarized in Table 3.

Perhaps the focal point of As contamination in wetlands, where to date most of the scientific research attention has been directed lies in southeast Asia [17-21]. Here the As source originally derived from eroded Himalayan rock, now resides as buried lens of sediment making up the Bay of Bengal Delta. These near-surface alluvia are the cause of As release to aquifers, and threaten tens of millions of people consuming these waters [22]. In samples collected from fifteen selected Bhaluka wetlands in Bangladesh, the concentration of As in the waters ranged from 7 to 80 μg L^{-1}, with 93% found exceeding WHO recommended permissible limits [23]. For further information on the Bangladesh As crisis, refer to reviews by Meharg *et al*. [24] and Meharg & Zhao [25].

Paddy field contamination in the region is also a serious problem, here high soil As concentrations are linked to the quality of the groundwaters used for irrigation [20].

1   This problem was uncovered in 2003, when seventy-one soils collected from three

2   districts, revealed a range of As concentrations in soils spanning 3.1 mg kg^{-1} (*baseline*)

3   up to 42.5 mg kg^{-1} [20]. While a later study by Stroud *et al*. [26], reported an even

4   greater variation in total As concentrations in twelve paddy soils from Bangladesh,

5   again similar baselines of 4 mg kg^{-1}, but this time the inputs of As from irrigation

6   caused soils to reach 138 mg kg^{-1}. This highlights the ease in which As can

7   accumulate in agricultural-wetland soils, which then leads to the direct transfer to

8   food supply chains [20, 27].

9   China, meanwhile, is another country that has extensively studied the problem of

10 As build-up in wetlands [28-31]. Here though the main inputs derive not from

11 groundwaters, but instead take many different forms/guises. Although, often difficult

12 to characterize due to the mixing of multiple and varying sources, they do share a

13 commonality, in that inputs have intensified as the country had modernized. For

14 example, freshwater and salt marsh sediments in **the** Yellow **river** delta, before the

15 Xiaolangdi reservoir was built, were already As enriched, recording values of 30 mg

16 kg^{-1}. However, after it was constructed, changes to the sediment flows coupled with

17 increased human settlement and activity in the area, resulted in further As deposition

18 taking the concentration up to 45 mg kg^{-1} [32], equivalent to a 4.5-fold increase from

19 baseline levels for soils in the Yangtze river delta [33].

20 Like Bangladesh, paddy field contamination is also a topic of importance in

21 China, with numerous studies highlighting this as a prominent issue, see Table 3

22 [28-31, 34]. However, total As concentrations only tell part of the story. Here As

1   species are essential to understand the bioavailability and uptake of As in rice. Based

2   on soil pore waters collected from Bangladesh and Chinese paddy soils, As^{III} is the

3   dominant species, accounting for 66-100% of the total As [26]. Methylated As species

4   ($DMAs^V$ and $MMAs^V$) are also commonly detected in rice soils [34]. Although the

5   uptake of organic As into rice is not as rapid as As^{III}, once inside roots they quickly

6   translocate to the above-ground tissues [25]. Volatile $TMAs^{III}$ has also been recently

7   detected in field studies of paddy soils from Spain and Bangladesh, albeit only at

8   trace-concentrations [35].

9   Far from just being confined to Asia, the problem of As contamination in

10   **wetlands** is a feature on other continents as well. For example, concentrations of total

11   As in twenty-five surface sediments (0-4 cm) collected from wetlands in

12   Massachusetts in the U.S., ranged from 20 to 2100 mg kg^{-1} [36]. In southwest Spain,

13   the contamination by As in the Guadalquivir marshes, arising from the mine-tailing

14   spill in Aznalcollar, led to severe wetland soil pollution, with far reaching biological

15   effects for local wildlife, a subject covered in depth by a number of related

16   publications (Table 3) [37-39].

17

18   **2.2 Biogeochemistry of As transformation and transport in wetlands**

19   The predominant form of As in soil prior to flooding is As^V. Bound commonly to iron

20   (oxy)hydroxides, due in equal measure to it's high affinity for the species and

21   abundance in most soils, the labiality and subsequently toxicity of As^V is relatively

22   low [40]. Saturating the system with water causes soil redox potentials to rapidly

1   decrease, in response to the depletion of electron acceptors, such as oxygen, nitrate,

2   manganese oxides and iron oxides/hydroxides. When Eh drops to below -200 mV

3   porewater As^V concentrations decline as the species is transformed to As^{III} [25]. In the

4   short-term, buffering can occur, because in these highly reducing conditions iron

5   oxides/hydroxides are solubilized, releasing As^V. However, As^{III} development will

6   dominate overtime [25, 41, 42]. This phenomenon can be further enhanced, by the

7   direct reduction of soil reservoirs of As^V. Due to As^{III} being less strongly bound to soil

8   particle surfaces than As^V, it partitions more readily into solution phase, increasing the

9   overall bioavailability of the toxin [40, 43].

10   Organic carbon (OC) has also been shown to mobilize As in aquifers in south

11   Bangladesh [44], while high concentrations of dissolved organic matter (DOM) in soil

12   porewaters can complete with surface absorbed As^V and As^{III} displacing them into

13   solution [21, 25]. Other important chemistries that control As transport, include the

14   grouping of interactions that As has with sulfur rich minerals [45]. Under highly

15   reducing conditions, and in the presence of dissolved sulfide, As^{III} can form stable

16   complexes, for example, orpiment (As_2S_3) or realgar (As_4S_4) [36]. Immobilizing As

17   by controlling redox conditions, to favor As-S precipitation, is a prac**tice** used in

18   constructed **wetlands** for reducing As bioavailability [46]. However, the sites still

19   require careful management, as oxidation of the sediment can result in the dissolution

20   of sulfides and re-mobilization of As^{III} back into the environment [47, 48].

21

22

3   Mechanisms of microbial mediated As biotransformation in wetlands

Compared to chemical change, microbial mediated As redox reactions occur far more rapidly [49]. Study has showed As^V reduction in soils to be significantly suppresses when γ-irradiated, highlighting the dominant role the microbiota play in this in solum As transfer step [40]. Microbial activity can be increased/simulated by enhancing the availability of OC sources, a response that differs between species/communities. This in turn contributes to different rates and forms of As biotransformation, impacting on both inorganic and methylated As trends [28]. Indeed, most methylated As species detected in wetlands are originally derived from microbial mediated As reactions [31, 35], because plants are not efficient at methylating inorganic As [50]. These different As species with their ranging properties/characteristics define the manner that As is transferred back and forth from the main land and aquatic stores [51-54]. Some volatile As is released into the atmosphere, in the form of AsH_3, CH_3AsH_2 ($MeAsH_2$), $(CH_3)_2AsH$ (Me_2AsH), and $(CH_3)_3As$ ($TMAs^{III}$). However compared to the land and water exchanges the fluxes are relatively minor [28, 35].

The evolution of such effective and diverse microbial mediated As biotransformation systems arose because they protect against As toxicity. The pathways of how microbes deal with As have been extensively studied in the last couple of decades, revealing various genes and enzymes responsible for As biotransformation and its biotransportation out of cells [50, 55-57]. The main systems used in As biotransformation by microbes in wetlands are further summarized below.

3.1 As^{III} oxidation in wetlands

Rapid As^{III} oxidation in the environment is mediated by metabolic microbial processes [1, 58], which are catalyzed by As^{III} oxidase (Aio). This enzyme is encoded by *aioA* and *aioB* genes for the two subunits AioA (AoxB) and AioB (AoxA) [55, 59]. Microbial mediated As^{III} oxidation is considered as one of the primary As detoxification mechanisms for microbes because it can oxidize As^{III} to the less toxic As^V [55]. The high abundance of *aioA* genes in paddy soils under flooding conditions further suggests the importance of this pathway [31, 60]. As^{III} oxidizing bacteria in paddy soils mainly assign to the following family groupings: *Phyllobacteriaceae*, *Bradyrhizobiaceae*, *Methylobacteriaceae*, *Rhizobiales*, *Burkholderiales* and *Comamonadaceae* [60, 61].

In other types of wetland, for example coastal sediments, a high diversity of *aioA*-like genes have also been detected. The most abundant *aioA*-like genes derived from *Roseobacter litoralis* Och 149 [62]. As^{III} oxidizing bacteria isolated from natural and constructed wetlands in the Republic of Korea showed that they were all able to grow in the presence of high concentrations of As^{III} (10 mM). The bacterium identified as *Pseudomonas stutzeri* strain GIST-BDAN2 showed an especially high activity of As^{III} oxidation (completely oxidized 1 mM As^{III} to As^V within 25-30 h) and possessed both the *aoxB* and *aoxR* genes [63]. Three As^{III} oxidizing bacteria including *Agrobacterium tumefaciens*, *Pseudomonas fluorescens*, and *Variovorax paradoxus*-like organisms were also isolated from the same Madison River Valley soils that also supported large populations of As^V reducing bacteria [64]. This

11

1 highlights the close overlay of the microbial catalyzed oxidation and reduction

2 pathways, and how this dichotomy functions as the principal 'switch' which controls

3 As fate in a specific site or zone within a site.

4

3.2 As^{V} reduction in wetlands

6 Microbial mediated As^{V} reduction segregates into two functional schemes, the

7 respiratory and the detoxification pathway. The respiratory pathway is catalyzed by

8 the As^{V} respiratory reductase (ArrA) complex, which consists of a large catalytic

9 subunit (ArrA) and a small subunit (ArrB) [65-67]. It can mediate anaerobic

10 dissimilatory As^{V} reduction and couple this with energy production [68-70]. A more

11 universal system of As^{V} detoxification reduction, is catalyzed by the cytoplasmic As^{V}

12 reductase (ArsC), which is present in both aerobic and anaerobic microbes [71]. The

13 *arsC* gene occurs in the *ars* operons next to the *arsB* gene that functions as an As^{III}

14 membrane pump in most bacteria [56], controlling cytoplasmic As^{V} reduction, and

15 thereafter As^{III} efflux.

16 In wetland sediments, microbial mediated As^{V} reduction is considered to be a

17 crucial mechanism controlling As^{V} mobilization [72], and contributing factor leading

18 to As contamination of ground water, as has happened in south and southeast Asia [22,

19 73]. Due to the anaerobic conditions encountered in wetlands, dissimilatory As^{V}

20 respiring bacteria are quite commonplace. The dissimilatory As^{V} reduction gene *arrA*

21 has been detected in various wetlands, including paddy soils [60, 61], coastal

22 sediments from south China [62], and estuarine sediments from Chesapeake Bay in

the U.S. [74]. Zhang *et al.* [34] revealed that *arrA* sequences detected in paddy soils were analogous to those found in *Geobacter* species, which have been frequently found in As rich sediments before [72, 75]. Similarly, *Geobacter* are prominent in Japanese paddy soil [54]. Observations of *Geobacter* species OR-1 have revealed that in addition to As, ferrihydrite is also being used as an electron acceptor, thus catalyzing the dissolution of As from As^V-absorbed ferrihydrite, by promoting ferrous iron formation [54]. Moreover, As K-edge X-ray absorption near-edge structure analysis demonstrated the OR-1 can also reduce soil/solid bound As^V directly. In addition to *Geobacter*-related bacteria, other microbiota, such as *Shewanella* species, that utilize the coupling/uncoupling of iron and As reduction as a means of acquiring energy, are also capable of dissimilatory As^V reduction [76, 77].

Similar to *arrA, arsC* has also been frequently detected in paddy soils [60, 61] and coastal sediments [62]. The As^V reducing microbes with the most active detoxification pathways in paddy soils mostly belonged to typically rhizospheric bacteria groups, such as *Rhizobiales* and *Pseudomonadales* [61]. Study of pure culture bacteria from Madison River Valley soils revealed five isolates, *Agrobacterium tumefaciens*, *Flavobacterium* sp., *Microbacterium* sp. and two *Arthrobacter* sp.-like organisms capable of rapidly reducing As^V under aerobic conditions, with the later found to possess new putative *arsC* genes [64]. Seventeen As-resistant bacteria isolated from Mandovi and Zuari estuarine water systems revealed *arsA* (ATPase), *arsB* and *arsC* genes on their plasmid DNA. While, *arsC* genes were individually detected in thirteen bacterial isolates, including the genera's *Halomonas* and

13

1 *Acinetobacter* [78]. Using genome sequencing of *Bacillus* bacterium isolated from

2 Andean wetlands in northern Chile, the *arsC* gene function was found as

3 detoxification As^V reduction [79]. Together with the As^V respiring bacteria, these

4 detoxification As^V reducing microbes catalyze the reduction of both the soil absorbed

5 As^V and dissolved As^V in solution into As^{III}.

6

3.3 As^{III} methylation and volatilization in wetlands

8 Organic As is introduced primarily into the environment via the microbial catalyzed

9 methylation of As^{III}. The As^{III} *S*-adenosylme-thionine methyltransferase (ArsM),

10 which is responsible for As^{III} methylation has recently been found to be common to

11 many different microbes [12, 80-82]. It is encoded by *arsM* genes, and **catalyzes** the

12 generation of less toxic organic As species, such as $MMAs^V$, $DMAs^V$ and TMAO.

13 The volatile As gas including $MeAsH_2$, Me_2AsH and $TMAs^{III}$ could also be generated

14 during the biomethylation processes. To date, $TMAs^{III}$ is the most commonly detected

15 volatile As species in the natural environment [28, 35] and under *in vitro* conditions

16 during pure culture [81-83].

17 In microcosm experiments using As contaminated paddy soils, Huang *et al*. [28]

18 and Jia *et al*. [29] found evidence of both microbial mediated As^{III} methylation and

19 volatilization. Recently discovered universal primers for the amplification of *arsM*

20 genes have uncovered *arsM* sequences as being widespread in numerous paddy soils

21 [29, 61]. Continued investigation reveals these *arsM* sequences mainly derive from

22 the phyla *Gemmatimonadales*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* and the

domain *Archaea* [61]. By applying a metagenomic approach, Cai *et al.* [62] also found *arsM* genes in coastal sediments, but the dominant bacteria species involved in As^{III} methylation were not identified in this study. Furthermore the ability to methylate and volatilize As^{III} has been discovered in three cyanobacteria species that are common in paddy soils [82], and a small free-living eukaryote *Ostreococcus tauri* found in coastal waters [83]. In general, to the best of our knowledge, the study of As^{III} methylation and volatilization in wetlands is still rather limited, but given the rapid advance in our understanding of the importance of this aspect of the As biogeochemical cycle, this is an area that will likely be of increasing interest in the future.

3.4 Environmental factors affecting As biotransformation microbes

Various environmental factors affecting the diversity, behavior and metabolism of As biotransformation microbes have been observed in wetlands. One of the most important is the As concentration of the soils/sediments with As biotransformation microbe abundance found to correlate positively with As concentration in paddy soils [61]. Similarly, *arsM* gene abundance and the concentration of methylated As in paddy soil solutions also exhibit a strong positive correlation [29]. The amount and form of OM in wetlands is also key, for example, several experiments carried out in microcosms using paddy soils have demonstrated that the amendment of OM promotes the activity of As^{III} oxidizing bacteria [28]. The application of rice straw can increase the community diversity of As^{III} oxidizing bacteria [60], and the activity of

1   As^{III} methylation microbes in paddy soils [28, 29]. Straw also has an impact on As^V

2   detoxification reducing microbes, acting to moderately simulate their activity, but

3   greatly enhance the total abundance of As^V reducers [28].

4   The concentration of SO_4^{2-} in paddy soils strongly influences microbial

5   community compositions, modifying both As^{III} oxidation and As^V detoxification

6   reduction activity. It is postulated that this is due to the possible sharing of microbial

7   groups of sulfur oxidation and As redox [61]. The microbial community composition

8   of bacteria/archaea involved in As biotransformations is also particularly sensitive to

9   iron   concentrations.   This   is   reasonable,   especially   considering   that   the

10   absorption-desorption dynamics of iron /oxyhydroxides and As^V influence the

11   concentration and species of bioavailable As in wetlands [41, 42]. For the microbial

12   communities carrying out As^{III} methylation, it is the available NO_3^--N, NH_4^+-N, that

13   appears to be the most sensitive environmental parameter influencing community

14   composition [61]. Other factors, such as pH, redox potential, alkalinity, temperature

15   and dissolved oxygen, also contribute and influence As biotransformation microbial

16   abundance and activity [46]. However, investigation into this topic is still in its

17   infancy. Much remains to be done to improve our understanding of how we can

18   optimize the function of microbial mediated As biotransformations by changing the

19   management of local wetland environments. A significant hurdle, blocking this goal

20   has been the lack of suitable methods to observe and measure the dynamic

21   environmental conditions, at scales appropriate to the biota driving the change.

22   However, with the development of new multi-parameter, visualization tools such as

1   diffusive gradients in thin films (DGT)/planar optode sandwich sensors [84, 85] and

2   advanced radiography and isotope imaging, *in situ* mapping of As and other key

3   elements is now possible. Employing these technologies concurrently with 2D

4   enzyme activity plots (zymography) and microbial imaging, i.e. fluorescence *in situ*

5   hybridization (FISH) or oligonucleotides labeled probes with light emitting

6   chromophores [86], offers further exciting possibilities to unravel the complex

7   environment-biota interactions impacting on wetland As release.

8

9   **4   Concluding remarks and perspectives**

10   Microbes act as the 'switch' turning a wetland from an As sink to source through As^{V}

11   reduction, As^{III} oxidation, As^{III} methylation, and As volatilization processes (Figure 1).

12   As^{V} reducing bacteria/archea can enhance As mobilization from sediments to water

13   [54, 75], while As^{III} oxidizing microbes act to decrease As mobility and bioavailability

14   [3]. The two groups of As^{V} reducers and As^{III} oxidizers act in tandem, controlling the

15   direction/path of the inorganic As cycle. While wetland As^{III} methylaters create

16   organic As species ($MMAs^{V}$, $DMAs^{V}$, $TMAs^{III}$), they also generate volatile As,

17   promoting its release into the atmosphere [2].

18   Considering the key role wetlands play in delivering essential ecosystems

19   services and the large area they occupy, the microbial mediated As biotransformations

20   they host/support remain indispensable components of a 'safe' global As

21   biogeochemical cycle, one that functions in the best interests of humankind. Failure to

22   live in balance with As' complex biogeochemistry can have devastating consequences,

from the release of As from near-surface wetland sediments to underground drinking water supplies [72], to its transfer into rice plants [41].

Better management of wetland resources for optimized microbial control of As could potentially yield huge gains in public and environmental health. However more research is needed to improve our understanding of how to make best use of these microbial mediated reactions. We recommend research attention is immediately directed at gaps in our understanding of wetland As cycling, such as microbial organic-As degradation, as the literature available on this topic is scant. Furthermore, continued improvements to the technologies available to study *in situ*, both the chemistry and biology of the mercurial wetland environment is needed.

References

1.   Oremland R S,J F Stolz. The ecology of arsenic. Science, 2003, 300(5621): 939-944.

2.   Zhu Y-G, M Yoshinaga, F-J Zhao,B P Rosen. Earth abides arsenic biotransformations. Annual Review of Earth and Planetary Sciences, 2014, 42(0): 443-467.

3.   Bhattacharya P, A H Welch, K G Stollenwerk, M J McLaughlin, J Bundschuh,G Panaullah. Arsenic in the environment: Biology and Chemistry. Science of the Total Environment, 2007, 379(2-3): 109-120.

4.   Bentley R,T G Chasteen. Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbiology and Molecular Biology Reviews, 2002, 66(2): 250-271.

5.   Silbergeld E K,K Nachman. The environmental and public health risks associated with arsenical use in animal feeds. Annals of the New York Academy of Sciences, 2008, 1140(1): 346-357.

6.   Murray L A, A Raab, I L Marr,J Feldmann. Biotransformation of arsenate to arsenosugars by Chlorella vulgaris. Applied organometallic chemistry, 2003, 17(9): 669-674.

7.   Moore J W,S Ramamoorthy. Heavy metals in natural waters: applied monitoring and impact assessment. 2012: Springer Science & Business Media.

8.   Zedler J B,S Kercher. Wetland resources: status, trends, ecosystem services, and restorability. Annu. Rev. Environ. Resour., 2005, 30: 39-74.

9.   Keddy P A. Wetland ecology: principles and conservation. 2010: Cambridge University Press.

10.   Chmura G L, S C Anisfeld, D R Cahoon,J C Lynch. Global carbon sequestration in tidal, saline wetland soils. Global biogeochemical cycles, 2003, 17(4).

11.   Wang S, Y Wang, X Feng, L Zhai,G Zhu. Quantitative analyses of ammonia-oxidizing Archaea and bacteria in the sediments of four nitrogen-rich wetlands in China. Applied microbiology and biotechnology, 2011, 90(2): 779-787.

12.   Qin J, C R Lehr, C Yuan, X C Le, T R McDermott,B P Rosen. Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga. Proceedings of the National Academy of Sciences, 2009, 106(13): 5213-5217.

13.   Bhakta J N,Y Munekage. Spatial distribution and contamination status of arsenic, cadmium and lead in some coastal shrimp (*Macrobrachium rosenbergii*) farming ponds of Viet Nam. Pacific Journal of Science and Technology, 2009, 11: 606-15.

14.   Wang S,C N Mulligan. Occurrence of arsenic contamination in Canada: sources, behavior and distribution. Science of the Total Environment, 2006, 366(2-3): 701-721.

15.   ENVIRONMENT C C O M O T. Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Summary Tables. Canadian Environmental Quality Guidelines, 1999. 2001, Canadian Council of Ministers of the Environment Winnipeg.

16.   Li Q, Z Wu, B Chu, N Zhang, S Cai,J Fang. Heavy metals in coastal wetland sediments of the Pearl River Estuary, China. Environmental Pollution, 2007, 149(2): 158-164.

17.   Huq S I, A Rahman, N Sultana,R Naidu. Extent and severity of arsenic contamination in soils of Bangladesh. Fate of arsenic in the environment. Dhaka: Bangladesh University of Engineering and Technology, 2003: 69-84.

18.   Huq S I,J U M Shoaib. Soils and humans. in The Soils of Bangladesh. 2013, Springer. p. 125-129.

19.   Lu Y, E E Adomako, A Solaiman, M R Islam, C Deacon, P Williams, G Rahman,A A Meharg.

Baseline soil variation is a major factor in arsenic accumulation in Bengal Delta paddy rice. Environmental science & technology, 2009, 43(6): 1724-1729.

20. Meharg A A,M M Rahman. Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. Environmental Science & Technology, 2003, 37(2): 229-234.

21. Williams P N, H Zhang, W Davison, A A Meharg, M Hossain, G J Norton, H Brammer,M R Islam. Organic Matter　Solid Phase Interactions Are Critical for Predicting Arsenic Release and Plant Uptake in Bangladesh Paddy Soils. Environmental science & technology, 2011, 45(14): 6080-6087.

22. Polizzotto M L, B D Kocar, S G Benner, M Sampson,S Fendorf. Near-surface wetland sediments as a source of arsenic release to ground water in Asia. Nature, 2008, 454(7203): 505-508.

23. Alam M, M Ali, N A Al-Harbi,T R Choudhury. Contamination status of arsenic, lead, and cadmium of different wetland waters. Toxicological & Environmental Chemistry, 2011, 93(10): 1934-1945.

24. Meharg A. Venomous earth: How arsenic caused the world's worst mass poisoning. 2005.

25. Meharg A A,F-J Zhao. Biogeochemistry of Arsenic in Paddy Environments. in Arsenic & Rice. 2012, Springer. p. 71-101.

26. Stroud J L, M A Khan, G J Norton, M R Islam, T Dasgupta, Y-G Zhu, A H Price, A A Meharg, S P McGrath,F-J Zhao. Assessing the labile arsenic pool in contaminated paddy soils by isotopic dilution techniques and simple extractions. Environmental science & technology, 2011, 45(10): 4262-4269.

27. Williams P N, A Villada, C Deacon, A Raab, J Figuerola, A J Green, J Feldmann,A A Meharg. Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. Environmental Science & Technology, 2007, 41(19): 6854-6859.

28. Huang H, Y Jia, G X Sun,Y G Zhu. Arsenic speciation and volatilization from flooded paddy soils amended with different organic matters. Environmental science & technology, 2012, 46(4): 2163-2168.

29. Jia Y, H Huang, M Zhong, F-H Wang, L-M Zhang,Y-G Zhu. Microbial arsenic methylation in soil and rice rhizosphere. Environmental science & technology, 2013, 47(7): 3141-3148.

30. Williams P N, M Lei, G Sun, Q Huang, Y Lu, C Deacon, A A Meharg,Y-G Zhu. Occurrence and partitioning of cadmium, arsenic and lead in mine impacted paddy rice: Hunan, China. Environmental science & technology, 2009, 43(3): 637-642.

31. Zhao F-J, E Harris, J Yan, J Ma, L Wu, W Liu, S P McGrath, J Zhou,Y-G Zhu. Arsenic methylation in soils and its relationship with microbial *arsM* abundance and diversity, and As speciation in rice. Environmental science & technology, 2013, 47(13): 7147-7154.

32. Bai J, R Xiao, K Zhang,H Gao. Arsenic and heavy metal pollution in wetland soils from tidal freshwater and salt marshes before and after the flow-sediment regulation regime in the Yellow River Delta, China. Journal of Hydrology, 2012, 450: 244-253.

33. Gorenc S, R Kostaschuk,Z Chen. Spatial variations in heavy metals on tidal flats in the Yangtze Estuary, China. Environmental Geology, 2004, 45(8): 1101-1108.

34. Zhang S-Y, F-J Zhao, G-X Sun, J-Q Su, X-R Yang, H Li,Y-G Zhu. Diversity and abundance of arsenic biotransformation genes in paddy soils from Southern China. Environmental science & technology, 2015, 49(7): 4138-4146.

35. Mestrot A, J Feldmann, E M Krupp, M S Hossain, G Roman-Ross,A A Meharg. Field fluxes and speciation of arsines emanating from soils. Environmental science & technology, 2011, 45(5): 1798-1804.

36. Wilkin R T,R G Ford. Arsenic solid-phase partitioning in reducing sediments of a contaminated wetland. Chemical Geology, 2006, 228(1): 156-174.

37. Grimalt J O, M Ferrer,E Macpherson. The mine tailing accident in Aznalcollar. Science of the Total Environment, 1999, 242(1): 3-11.

38. Kraus U,J Wiegand. Long-term effects of the Aznalcóllar mine spill—heavy metal content and mobility in soils and sediments of the Guadiamar river valley (SW Spain). Science of the Total Environment, 2006, 367(2): 855-871.

39. Mateo R, M A Taggart, A J Green, C Cristòfol, A Ramis, H Lefranc, J Figuerola,A A Meharg. Altered porphyrin excretion and histopathology of greylag geese (Anser anser) exposed to soil contaminated with lead and arsenic in the Guadalquivir Marshes, southwestern Spain. Environmental Toxicology and Chemistry, 2006, 25(1): 203-212.

40. Yamaguchi N, T Nakamura, D Dong, Y Takahashi, S Amachi,T Makino. Arsenic release from flooded paddy soils is influenced by speciation, Eh, pH, and iron dissolution. Chemosphere, 2011, 83(7): 925-932.

41. Xu X, S McGrath, A Meharg,F Zhao. Growing rice aerobically markedly decreases arsenic accumulation. Environmental science & technology, 2008, 42(15): 5574-5579.

42. Cummings D E, F Caccavo, S Fendorf,R F Rosenzweig. Arsenic mobilization by the dissimilatory Fe (III)-reducing bacterium Shewanella alga BrY. Environmental Science & Technology, 1999, 33(5): 723-729.

43. Takahashi Y, R Minamikawa, K H Hattori, K Kurishima, N Kihou,K Yuita. Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. Environmental science & technology, 2004, 38(4): 1038-1044.

44. Harvey C F, C H Swartz, A Badruzzaman, N Keon-Blute, W Yu, M A Ali, J Jay, R Beckie, V Niedan,D Brabander. Arsenic mobility and groundwater extraction in Bangladesh. Science, 2002, 298(5598): 1602-1606.

45. Bostick B C, C Chen,S Fendorf. Arsenite retention mechanisms within estuarine sediments of Pescadero, CA. Environmental science & technology, 2004, 38(12): 3299-3304.

46. Lizama K, T D Fletcher,G Sun. Removal processes for arsenic in constructed wetlands. Chemosphere, 2011, 84(8): 1032-1043.

47. Morse J W. Interactions of trace metals with authigenic sulfide minerals: implications for their bioavailability. Marine Chemistry, 1994, 46(1): 1-6.

48. Saulnier I,A Mucci. Trace metal remobilization following the resuspension of estuarine sediments: Saguenay Fjord, Canada. Applied Geochemistry, 2000, 15(2): 191-210.

49. Kirk G. The biogeochemistry of submerged soils. 2004: John Wiley & Sons.

50. Ye J, C Rensing, B P Rosen,Y G Zhu. Arsenic biomethylation by photosynthetic organisms. Trends in Plant Science, 2012, 17(3): 155-162.

51. Maguffin S C, M F Kirk, A R Daigle, S R Hinkle,Q Jin. Substantial contribution of biomethylation to aquifer arsenic cycling. Nature Geoscience, 2015.

52. Drahota P, L Falteisek, A Redlich, J Rohovec, T Matoušek,I Čepička. Microbial effects on the release and attenuation of arsenic in the shallow subsurface of a natural geochemical anomaly. Environmental Pollution, 2013, 180: 84-91.

53. Mumford A C, J L Barringer, W M Benzel, P A Reilly,L Young. Microbial transformations of arsenic: mobilization from glauconitic sediments to water. Water research, 2012, 46(9): 2859-2868.

54. Ohtsuka T, N Yamaguchi, T Makino, K Sakurai, K Kimura, K Kudo, E Homma, D T Dong,S Amachi. Arsenic dissolution from Japanese paddy soil by a dissimilatory arsenate-reducing bacterium *Geobacter* sp. OR-1. Environmental science & technology, 2013, 47(12): 6263-6271.

55. Slyemi D,V Bonnefoy. How prokaryotes deal with arsenic†. Environmental microbiology reports, 2012, 4(6): 571-586.

56. Silver S,L T Phung. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Applied and environmental microbiology, 2005, 71(2): 599-608.

57. Rosen B P,Z Liu. Transport pathways for arsenic and selenium: a minireview. Environment International, 2009, 35(3): 512-515.

58. Mukhopadhyay R, B P Rosen, L T Phung,S Silver. Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiology Reviews, 2002, 26(3): 311-325.

59. Stolz J F, P Basu, J M Santini,R S Oremland. Arsenic and Selenium in Microbial Metabolism*. Annu. Rev. Microbiol., 2006, 60: 107-130.

60. Jia Y, H Huang, Z Chen,Y-G Zhu. Arsenic uptake by rice is influenced by microbe-mediated arsenic redox changes in the rhizosphere. Environmental science & technology, 2014, 48(2): 1001-1007.

61. Zhang S-Y, F Zhao, G Sun, J Su, X Yang, H Li,Y-G Zhu. Diversity and abundance of arsenic biotransformation genes in paddy soils from Southern China. Environmental science & technology, 2015.

62. Cai L, K Yu, Y Yang, B-w Chen, X-d Li,T Zhang. Metagenomic exploration reveals high levels of microbial arsenic metabolism genes in activated sludge and coastal sediments. Applied microbiology and biotechnology, 2013, 97(21): 9579-9588.

63. Chang J-S, I-H Yoon, J-H Lee, K-R Kim, J An,K-W Kim. Arsenic detoxification potential of aox genes in arsenite-oxidizing bacteria isolated from natural and constructed wetlands in the Republic of Korea. Environmental geochemistry and health, 2010, 32(2): 95-105.

64. Macur R E, C R Jackson, L M Botero, T R Mcdermott,W P Inskeep. Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. Environmental science & technology, 2004, 38(1): 104-111.

65. Afkar E, J Lisak, C Saltikov, P Basu, R S Oremland,J F Stolz. The respiratory arsenate reductase from Bacillus selenitireducens strain MLS10. FEMS microbiology letters, 2003, 226(1): 107-112.

66. Saltikov C W,D K Newman. Genetic identification of a respiratory arsenate reductase. Proceedings of the National Academy of Sciences, 2003, 100(19): 10983-10988.

67. Krafft T,J M Macy. Purification and characterization of the respiratory arsenate reductase of Chrysiogenes arsenatis. European Journal of Biochemistry, 1998, 255(3): 647-653.

68. van Lis R, W Nitschke, S Duval,B Schoepp-Cothenet. Arsenics as bioenergetic substrates. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2013, 1827(2): 176-188.

69. Malasarn D, W Saltikov, K M Campbell, J M Santini, J G Hering,D K Newman. *arrA* is a reliable marker for As(V) respiration. Science, 2004, 306(5695): 455-455.

70. Hoeft S E, T R Kulp, J F Stolz, J T Hollibaugh,R S Oremland. Dissimilatory arsenate

reduction with sulfide as electron donor: Experiments with mono lake water and isolation of strain MLMS-1, a chemoautotrophic arsenate respirer. Applied and Environmental Microbiology, 2004, 70(5): 2741-2747.

71. Bhattacharjee H,B P Rosen. Arsenic metabolism in prokaryotic and eukaryotic microbes. Molecular microbiology of heavy metals., ed. S.S. Nies DH. Vol. 6. 2007, Heidelberg, Germany: Springer. 371-406.

72. Islam F S, A G Gault, C Boothman, D A Polya, J M Charnock, D Chatterjee,J R Lloyd. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. Nature, 2004, 430(6995): 68-71.

73. Fendorf S, H A Michael,A van Geen. Spatial and temporal variations of groundwater arsenic in South and Southeast Asia. Science, 2010, 328(5982): 1123-1127.

74. Song B, E Chyun, P R Jaffé,B B Ward. Molecular methods to detect and monitor dissimilatory arsenate‐respiring bacteria (DARB) in sediments. Fems Microbiology Ecology, 2009, 68(1): 108-117.

75. Héry M, B Van Dongen, F Gill, D Mondal, D Vaughan, R Pancost, D Polya,J Lloyd. Arsenic release and attenuation in low organic carbon aquifer sediments from West Bengal. Geobiology, 2010, 8(2): 155-168.

76. Oremland R S,J F Stolz. Arsenic, microbes and contaminated aquifers. Trends in microbiology, 2005, 13(2): 45-49.

77. Tufano K J, C Reyes, C W Saltikov,S Fendorf. Reductive processes controlling arsenic retention: revealing the relative importance of iron and arsenic reduction. Environmental science & technology, 2008, 42(22): 8283-8289.

78. Sunita M S L, S Prashant, P B Chari, S N Rao, P Balaravi,P K Kishor. Molecular identification of arsenic-resistant estuarine bacteria and characterization of their ars genotype. Ecotoxicology, 2012, 21(1): 202-212.

79. Vilo C, A Galetovic, J E Araya, B Gómez-Silva,Q Dong. Draft genome sequence of a Bacillus bacterium from the Atacama Desert wetlands metagenome. Genome announcements, 2015, 3(4): e00955-15.

80. Qin J, B P Rosen, Y Zhang, G Wang, S Franke,C Rensing. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. Proceedings of the National Academy of Sciences, 2006, 103(7): 2075-2080.

81. Wang P-P, G-X Sun,Y-G Zhu. Identification and characterization of arsenite methyltransferase from an archaeon, *Methanosarcina acetivorans* C2A. Environmental science & technology, 2014, 48(21): 12706-12713.

82. Yin X, J Chen, J Qin, G Sun, B Rosen,Y Zhu. Biotransformation and volatilization of arsenic by three photosynthetic cyanobacteria. Plant Physiology, 2011, 156(3): 1631-1638.

83. Zhang S-Y, G-X Sun, X-X Yin, C Rensing,Y-G Zhu. Biomethylation and volatilization of arsenic by the marine microalgae *Ostreococcus tauri*. Chemosphere, 2013.

84. Williams P N, J Santner, M Larsen, N J Lehto, E Oburger, W Wenzel, R N Glud, W Davison,H Zhang. Localized flux maxima of arsenic, lead, and iron around root apices in flooded lowland rice. Environmental science & technology, 2014, 48(15): 8498-8506.

85. Guan D-X, P N Williams, J Luo, J-L Zheng, H-C Xu, C Cai,L Q Ma. Novel precipitated zirconia-based DGT technique for high-resolution imaging of oxyanions in waters and

sediments. Environmental science & technology, 2015, 49(6): 3653-3661.

86.   Oburger E,H Schmidt. New methods to unravel rhizosphere processes. Trends in plant science, 2016, 21(3): 243-255.

87.   Chapman P M, F Wang, C Janssen, G Persoone,H E Allen. Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. Canadian Journal of Fisheries and Aquatic Sciences, 1998, 55(10): 2221-2243.

16. National Standard of PR China, 2002. National Standard of PR China Marine Sediment Quality (GB 18668-2002) Standards Press of China, Beijing (2002) (in Chinese).

Table 1. Structure of prevalent As species in the environment.

Species	Structure of As speciation
Arsenite, As^{III}	
Aesenate, As^{V}	
Methylarsonate, MMAs^{V}	
Dimethylarsinate, DMAs^{V}	
Thrimethylarsine oxide, TMAO	
Aesenosugars	
Arsenobetaine, AsB	

Table 2. The quality guidelines for As contamination in wetlands from different countries.

Sediment quality guidelines in various countries	Countries	Level	Arsenic concentration (mg kg^{-1})	References
Hongkong ISQVs	China	ISQV-low	8.2	[87]
		ISQV-high	70	
Sediment Quality Criteria	China	Class I	20	[16]
		Class II	65	
Canadian Environmental Quality Guideline	Canada	ISQGs	7.24	[15]
		PEL	41.6	

ISQV, interim sediment quality value; ISQGs, interim sediment quality guidelines; PEL: probable effect level.

Table 3. Summary of recently detected arsenic contaminated wetlands.

Wetland types	Country	Sampling Location	As concentration (mg kg^{-1}/ μg L		References
Coastal wetlands	China	Yellow River delta	38		[32]
			45		
		Yangtze River delta	10		[33]
Inland wetlands	U.S.	Massachusetts	20-2100		[36]
	Spain	Guadalquivir	20		[38]
		Fatehpur	72-114		
Paddy soils	Bangladesh	Dhumrakandi	62-138		[26]
		Paranpur	73-77		
		Faridpur	34		
	India	De Ganga	17		
	China	Chenzhou	60		[61]

		Qiyang	79	
		Anqing	19	
		Jiaxing	20	
		Yingtan	16	
		Jingzhou	19	
		Changde	16	
		Jiangmen	25	
		Guilin	21	
		Guiyang	21	
		Zhanjiang	18	
Wetland waters	Bangladesh	Malahar	60	[23]
		Gerajan	80	
		Dohuria	11-14	

Behi	11
Porabait	26
Barakhaillah	20
Jora	11
Uhila	18
Barakuri	11
Jerukuri	11
Bigaira	11

Figure caption

Figure 1. Sources of As introduced to wetland, microbial mediated As biotransformation in wetland, and genes responsible for As^{V} respiratory reduction, As^{V} detoxification reduction, As^{III} oxidation, and As^{III} methylation.