**Supplementary Information**

**Microbial community structure in aquifers associated with arsenic: analysis of 16S rRNA and arsenite oxidase genes**

Prinpida Sonthiphand1\*, Pasunun Rattanaroongrot1, Kasarnchon Mek-yong1, Kanthida Kusonmano2,3, Chalida Rangsiwutisak2, Pichahpuk Uthaipaisanwong2, Srilert Chotpantarat4,5,6, Teerasit Termsaithong7,8

1Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

2Bioinformatics and Systems Biology Program, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bang Khun Thian, Bangkok, Thailand

3Systems Biology and Bioinformatics Research Laboratory, Pilot Plant Development and Training Institute, King Mongkut’s University of Technology Thonburi, Bangkok, Thailand

4Department of Geology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

5Research Program on Controls of Hazardous Contaminants in Raw Water Resources for Water Scarcity Resilience, Center of Excellence on Hazardous Substance Management (HSM), Chulalongkorn University, Bangkok, Thailand

6Research Unit of Green Mining (GMM), Chulalongkorn University, Bangkok, Thailand

7Learning Institute, King Mongkut’s University of Technology Thonburi, Bangkok, Thailand

8Theoretical and Computational Science Center (TaCS), King Mongkut’s University of Technology Thonburi, Bangkok, Thailand

\*To whom correspondence should be addressed.

E-mail: prinpida.son@mahidol.ac.th

Phone: (+66) 2201-5250

Fax: (+66) 2354-7161

a) b)

D:\GW สุพรรณ\@draft GW manuscript\Figure and Table\Fig. S1 well.tif

Fig. S1. a) Deep groundwater (tube well) and b) shallow groundwater (ring well)

D:\GW สุพรรณ\@PeerJ\@ revised version (submission_V1)\Figure (R1)\Fig. 3.tif

Fig. S2. Rarefaction curves based on the number of OTUs of the bacterial 16S rRNA gene in six deep groundwaters (DW1-DW6), six shallow groundwaters (W1-W6), and surface water (SW).

D:\GW สุพรรณ\@draft GW manuscript\Figure and Table\Fig. S2 phylum.tif

Fig. S3. Relative abundance of microbial compositions at the phylum level in deep groundwater (DW), shallow groundwater (W), and surface water (SW)

Table S1. Summary of diversity indices

|  |  |  |
| --- | --- | --- |
| ID | Diversity indices | |
| Chao1 | Shannon |
| DW1 | 1199.75 | 4.57 |
| DW2 | 704.77 | 1.66 |
| DW3 | 1484.40 | 3.56 |
| DW4 | 906.01 | 3.59 |
| DW5 | 1329.72 | 2.96 |
| DW6 | 2590.69 | 5.85 |
| W1 | 4307.03 | 6.45 |
| W2 | 860.13 | 2.53 |
| W3 | 2324.33 | 2.37 |
| W4 | 2096.52 | 4.22 |
| W5 | 3109.16 | 3.42 |
| W6 | 1485.85 | 1.56 |
| SW | 1455.24 | 4.89 |

Table S2. Pearson’s correlation coefficients (*r*) between geochemical parameters and alpha diversity indices

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Alpha diversity indices | Geochemical parameters | | | | | | | | | | |
| DO  (mg l-1) | pH | ORP (mV) | EC  (µs cm-1) | Temp (°C) | TKN (mg l-1) | NO3--N (mg l-1) | TP  (mg l-1) | TC  (mg l-1) | Total As (µg l-1) | As3+ (µg l-1) |
| Chao1 | -0.201 | -0.010 | 0.046 | 0.060 | **-0.670** | 0.351 | 0.006 | -0.055 | 0.014 | -0.095 | -0.079 |
| Shannon | 0.044 | 0.491 | -0.176 | 0.207 | -0.438 | **0.605** | -0.093 | 0.068 | 0.234 | 0.339 | 0.419 |

Signiﬁcant differences (p < 0.05) are shown in bold.

Table S3. The abundances of *aioA* and 16S rRNA genes estimated by qPCR

|  |  |  |
| --- | --- | --- |
| ID | Abundance of *aioA* gene  (copies per ng of DNA) | Abundance of 16S rRNA gene  (copies per ng of DNA) |
| DW1 | 3.7x103±2.2x102 | 4.3x105±6.1x104 |
| W2 | 3.8x104±3.1x103 | 1.1x106±8.2x104 |
| W3 | 1.7x105±4.8x103 | 4.5x105±1.5x104 |
| W5 | 1.3x104±9.2x102 | 6.4x105±6.3x104 |
| W6 | 1.0x104±3.0x102 | 8.0x105±1.3x105 |
| SW | 2.9x104±7.3x102 | 5.6x105±1.1x105 |

Table S4.Pearson’s correlation coefficients (*r*) between each geochemical parameter

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | DO  (mg l-1) | pH | ORP  (mV) | EC  (µs cm-1) | Temp  (°C) | TKN (mg l-1) | NO3--N (mg l-1) | TP  (mg l-1) | TC  (mg l-1) | Total As (µg l-1) | As3+ (µg l-1) |
| DO  (mg l-1) | 1.00 | **0.70** | 0.30 | -0.05 | 0.36 | -0.10 | 0.04 | 0.09 | -0.16 | 0.26 | 0.34 |
| pH | **0.70** | 1.00 | 0.07 | 0.35 | 0.10 | 0.28 | -0.26 | 0.23 | 0.12 | **0.64** | **0.73** |
| ORP  (mV) | 0.30 | 0.07 | 1.00 | -0.32 | -0.06 | -0.09 | 0.18 | 0.06 | -0.50 | -0.08 | -0.07 |
| EC  (µs cm-1) | -0.05 | 0.35 | -0.32 | 1.00 | 0.15 | 0.11 | -0.34 | -0.38 | 0.28 | 0.03 | 0.10 |
| Temp  (°C) | 0.36 | 0.10 | -0.06 | 0.15 | 1.00 | -0.30 | 0.07 | 0.08 | 0.07 | 0.13 | 0.10 |
| TKN (mg/l) | -0.10 | 0.28 | -0.09 | 0.11 | -0.30 | 1.00 | 0.19 | 0.13 | **0.74** | 0.37 | 0.39 |
| NO3--N (mg l-1) | 0.04 | -0.26 | 0.18 | -0.34 | 0.07 | 0.19 | 1.00 | -0.16 | 0.30 | -0.37 | -0.29 |
| TP  (mg l-1) | 0.09 | 0.23 | 0.06 | -0.38 | 0.08 | 0.13 | -0.16 | 1.00 | -0.02 | **0.82** | **0.74** |
| TC  (mg l-1) | -0.16 | 0.12 | -0.50 | 0.28 | 0.07 | **0.74** | 0.30 | -0.02 | 1.00 | 0.21 | 0.23 |
| Total As (µg l-1) | 0.26 | **0.64** | -0.08 | 0.03 | 0.13 | 0.37 | -0.37 | **0.82** | 0.21 | 1.00 | **0.98** |
| As3+  (µg l-1) | 0.34 | **0.73** | -0.07 | 0.10 | 0.10 | 0.39 | -0.29 | **0.74** | 0.23 | **0.98** | 1.00 |

Signiﬁcant differences (*p* < 0.05) are shown in bold.