**Supplementary Information**

# Metagenomic Characterization of Microbial Pollutants and Antibiotic- and Metal-Resistance Genes in Sediments from the Canals of Venice

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**Supplementary Figures S1, S2, S3, S4, S5**

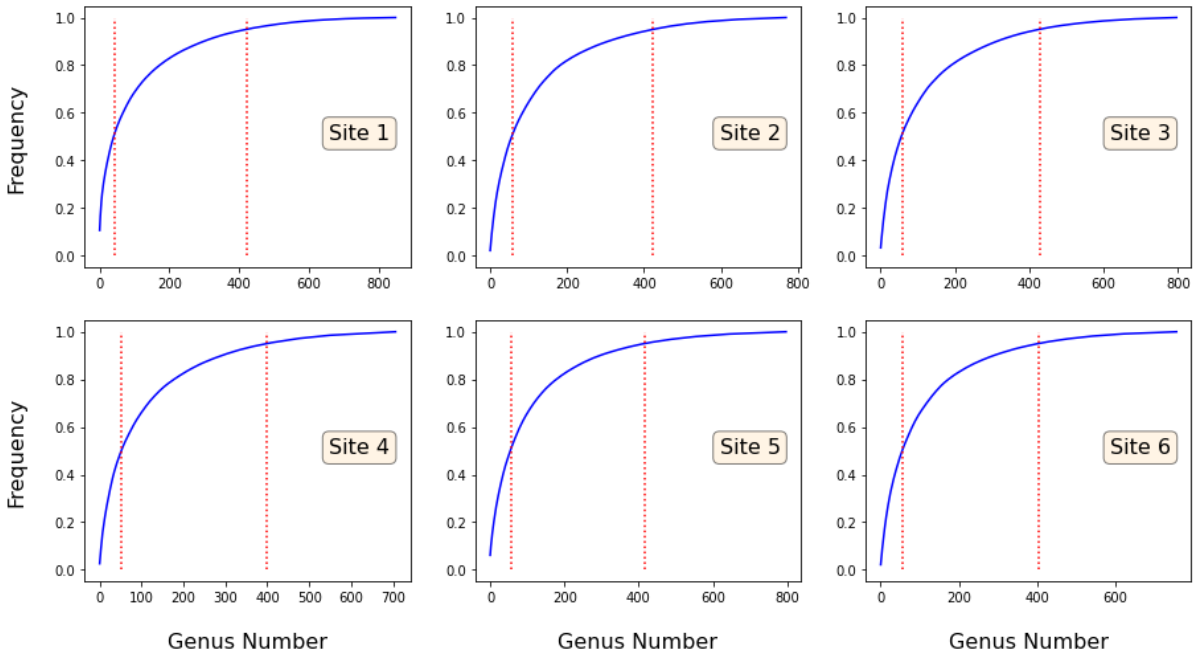



**Figure S1.** Genus frequency accumulation curves. For each site, genera were rank-ordered by decreasing frequency, and their accumulated totals plotted vs. genus number. The dotted vertical lines mark the x-coordinate for G50 and G95, the minimum numbers of genera required to contain 50% and 95% of classified sequences, respectively.

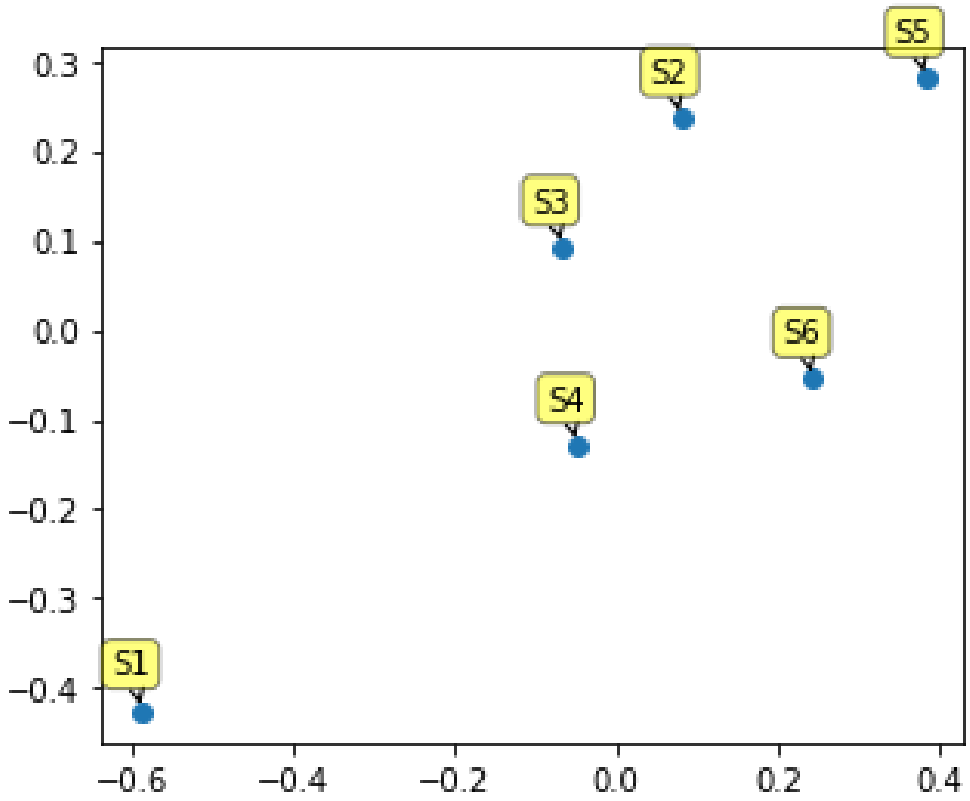



**Figure S2.** NMDS plots optimized for Bray-Curtis dissimilarities based on taxon frequencies.

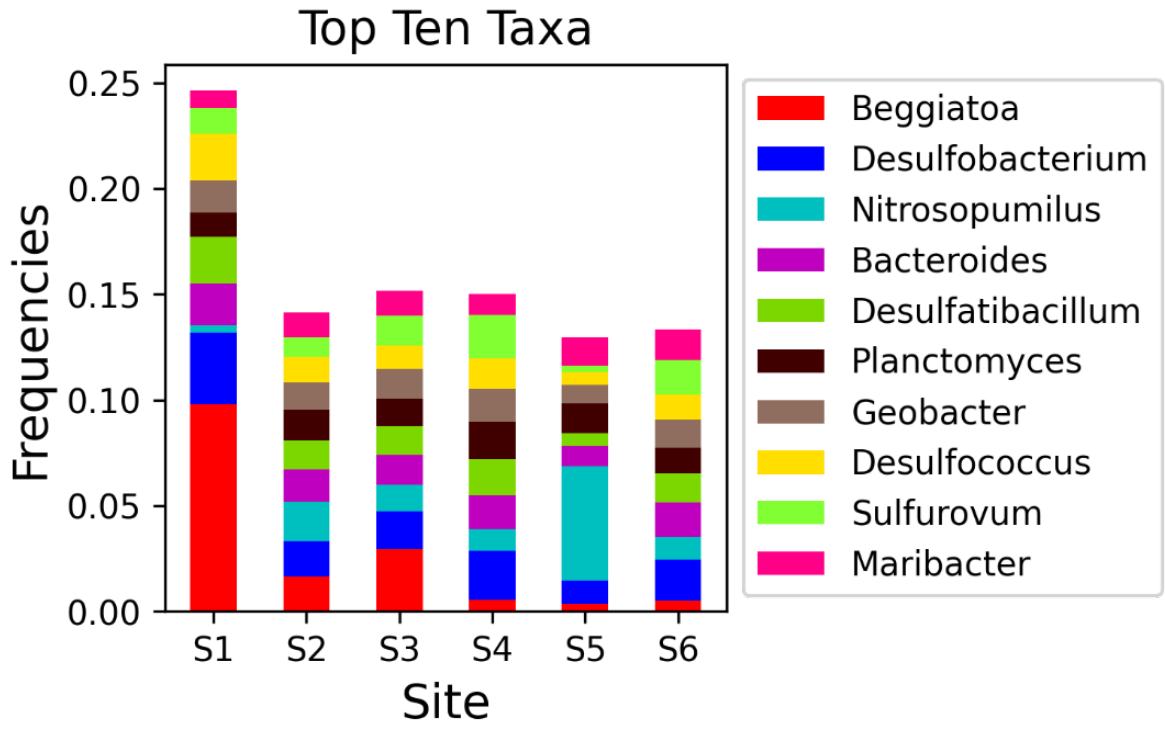



**Figure S3.** Frequencies of the top ten genera from each site. The top ten were selected based on the average frequencies of each genus over all six sites. Barplots show genera ranked bottom-to-top based on average frequency. For the denominator in calculations of taxon frequency at each site, we summed the numbers of sequences in the top 410 genera in that site; the value of 427 was chose as the value including at least 95% of the sequences in each sample (see also G95 value in Table 1).

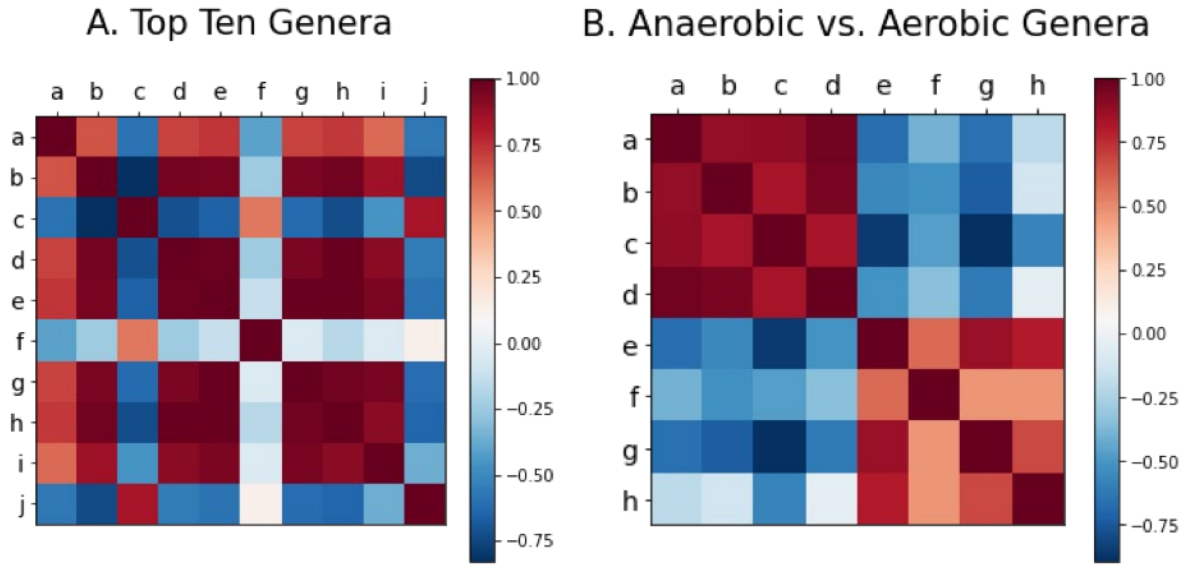



**Figure S4.** Correlation Matrices. This analysis indicates which classes (for Proteobacteria) and/or genera vary among sites in unison or in opposition. The plots are symmetrical with taxa ordered on both axes as follows: Panel A: a, Beggiatoa; b, Desulfobacterium; c, Nitrosopumilus; d, Bacteroides; e, Desulfatibacillum; f, Planctomyces; g, Geobacter; h, Sulfurovum, i, Desulfococcus; j, Maribacter. Panel B: a, Anaerolinea; b, Bacteroides; c, Clostridium; d, Syntrophus; e, Maribacter; f, Mycobacterium; g, Pseudomonas; h, Shewanella. Red indicates positive, and blue negative correlation, and color intensity indicates strength of relationship.

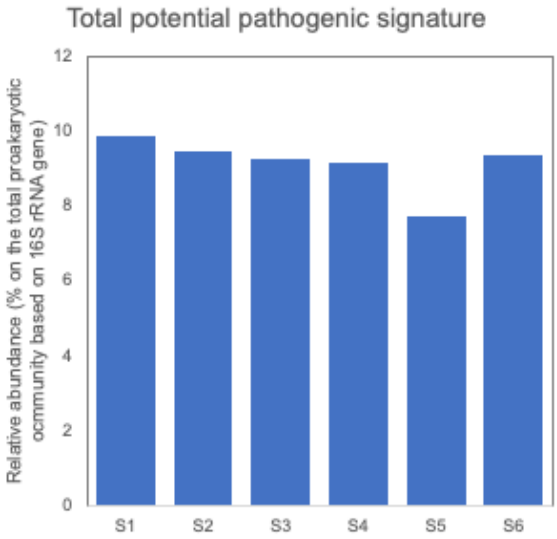



**Figure S5.** Total pathogenic community (i.e., traditional and alternative fecal indicator bacteria, sewage-associated bacteria and other common potential pathogens) in the six samples analyzed, as deriving from the 16S rRNA gene dataset.

## Supplementary Tables S1, S2, S3, S4

**Table S1.** Environmental parameters measured within this study.

| Station | Site Description | Lat N | Long E | Depth | Temp (°C) | ORP | DO (mg/L) | Sal (PSU) |
|---|---|---|---|---|---|---|---|---|
| S1 | Rio de le Toresele at Ponte del Formager; Corner pub | 45.430389 | 12.330759 | BOTTOM | 14.7 | -62 | 8.79 | 28.89 |
| | | | | SURFACE | 15 | -52.8 | 8.84 | 28.85 |
| S2 | Casa Artom deck-Canal Grande at Casa Artom | 45.430962 | 12.331975 | BOTTOM | 14.66 | -72.5 | 8.84 | 29.57 |
| | | | | SURFACE | 14.5 | -32.6 | 8.93 | 29.49 |
| S3 | Casa Artom east bridge - Rio de le Toresele at Campiello Barbaro | 45.430564 | 12.332156 | BOTTOM | NA | NA | NA | NA |
| | | | | SURFACE | 14.1 | -13.8 | 8.93 | 29.11 |
| S4 | Fondamenta de l'Arsenal - Rio dell'Arsenale at Campo de la Tana | 45.433436 | 12.349938 | BOTTOM | 14.7 | -48.9 | 8.85 | 29.2 |
| | | | | SURFACE | 14 | -13.3 | 8.96 | 29.32 |
| S5 | Riva dei Sette Martiri at the entrance of Viale Trento | 45.430169 | 12.354801 | BOTTOM | 14.7 | -33.8 | 8.87 | 29.25 |
| | | | | SURFACE | 14.3 | 10.1 | 8.95 | 29.31 |
| S6 | Rio dei Giardini - Paludo S. Antonio | 45.43025 | 12.359383 | BOTTOM | 15 | -49.9 | 8.53 | 28.71 |
| | | | | SURFACE | 14.6 | -24.7 | 8.65 | 28.76 |

**Table S2.** Keywords used to search for antibiotic resistance genes in our dataset. Antibiotics are grouped by Class, and the totals for each class at all sites are reported in the last column as "Total occurrences for the class". All searches were performed on lower case copies of the VDD database. The keywords used for searches and the number of times they were found in the samples are reported in the columns "Keyword" and "Occurrences", respectively. When none of several related keywords were found, we list only the smallest word or fragment (e.g., if "cipro" was not found, because this fragment is included within "ciprofloxacin", the latter was also not found).

| Antibiotic Class | Keywords | Occurrences | Total occurrences for the class |
|---|---|---|---|
| **Topoisomerase inhibitor** | gyrase | 1408 | **2191** |
| | topoisomerase | 783 | |
| | quinolone, cipro, aminocoumarin | Not found | |
| **Acriflavine** | acriflavin | 1419 | **1419** |
| **Beta-lactamase** | beta-lactamase | 479 | **479** |
| | cillin | 134, all also found within the beta-lactamase search | |
| | cepha, | 2, both also found within the beta-lactamase search | |
| | cefa | 1, also found within the beta-lactamase search | |
| | penem, bactam, clavu | Not found | |
| **Multidrug efflux transporter** | multidrug | 125 | **125** |
| | multidrug efflux | 114 | |
| | multidrug resistance transport | 8 | |
| | multidrug transport | 2 | |
| | multidrug-efflux | 1 | |
| **Macrolide** | macrolide | **83** | **83** |
| | erythromycin, azithromycin, clarithromycin, roxithromycin | Not found | |
| **Aminoglycoside** | myxin | 7, all within "polymyxin" | **7** |
| | aminoglycoside, gentamycin, neomycin, | Not found | |

| | | | |
|---|---|---|---|
| | streptomycin | | |
| **Vancomycin (cell wall synthesis inhibitor)** | vancomycin | 3 | **5** |
| | fosfomycin | 2 | |
| **Folate synthesis inhibitors** | trimethoprim, bactrim, sulfadiazine, sulfamethoxazole sulfanilamide, sulfasalazine, sulfisoxazole, zonisamide | Not found | **0** |
| **Tetracycline** | tet, doxy, cycline | Not found | **0** |
| **Rifampicin** | rifam | Not found | **0** |
| **Nitrofuran** | furan | Not found | **0** |
| **Linezolid** | linez | Not found | **0** |
| **Nitroimidazole** | azole | Not found | **0** |
| **Lincosamide** | lincomycin, clindamycin | Not found | **0** |

**Table S3.** Keywords used to search for heavy metal resistance genes in our dataset. Metal resistance types are listed in the first column, and the totals for each type at all sites are reported in the last column. All searches were performed on lower case copies of the VDD database. The keywords used for searches and the number of times they were found in the samples are reported in the columns "Keyword" and "Occurrences", respectively. When searching for resistance to these individual metals, we excluded genes that also listed more than one.

| Metal Resistance Type | Keywords | Occurrences | Total Occurrences for resistance type |
|---|---|---|---|
| **Cobalt, Zinc, Cadmium transporter** | cobalt-zinc-cadmium resistance protein | 2283 | **2522** |
| | co/zn/cd | 193 | |
| | cobalt/zinc/cadmium | 46 | |
| **Copper transporter** | copper | 1069 | **1069** |

| | | | |
|---|---|---|---|
| **Arsenic transporter** | arsenic | 389 | **389** |
| **Zinc transporter** | zinc | 351 | **381** |
| **Cobalt transporter** | cobalt | 102 | **102** |
| **Mercuric ion reductase** | mercuric | 50 | **50** |
| | mercury | Not found | |
| **Chromate transporter** | chromate | 34 | **34** |
| | chromium | Not found | |
| **Cadmium transporter** | cadmium | 25 | **25** |

**Table S4.** Bray-Curtis dissimilarity matrix for communities from the six canal sample sites.

| Sample | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|
| **S1** | 0 | 0.2482 | 0.2176 | 0.2112 | 0.3836 | 0.2474 |
| **S2** | 0.2482 | 0 | 0.1065 | 0.1546 | 0.2049 | 0.1246 |
| **S3** | 0.2176 | 0.1065 | 0 | 0.1511 | 0.234 | 0.1243 |
| **S4** | 0.2112 | 0.1546 | 0.1511 | 0 | 0.2819 | 0.1453 |
| **S5** | 0.3836 | 0.2049 | 0.234 | 0.2819 | 0 | 0.2363 |
| **S6** | 0.2474 | 0.1246 | 0.1243 | 0.1453 | 0.2363 | 0 |