*DOI:*
10.1016/j.scitotenv.2022.157652

*Document Version*
Peer reviewed version

**This is the Author's Accepted Manuscript version of the article: Jantharadej, K., Kongprajug, A., Mhuantong, W., Limpiyakorn, T., Suwannasilp, B. B., Mongkolsuk, S., & Sirikanchana, K. (2022). Comparative genomic analyses of pathogenic bacteria and viruses and antimicrobial resistance genes in an urban transportation canal. Science of The Total Environment, 848, 157652. Accepted for publication on 22 July 2022.**

# Comparative genomic analyses of pathogenic bacteria and viruses and antimicrobial resistance genes in an urban transportation canal

**Krittayapong Jantharadej[1], Akechai Kongprajug[2], Wuttichai Mhuantong[3], Tawan Limpiyakorn[1,4,5], Benjaporn Boonchayaanant Suwannasilp[1,4,5], Skorn Mongkolsuk[2,6], Kwanrawee Sirikanchana[2,6*]**

*[1]Department of Environmental Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok, Thailand*

*[2]Research Laboratory of Biotechnology, Chulabhorn Research Institute, Bangkok, Thailand*

*[3]National Center for Genetic Engineering and Biotechnology, Enzyme Technology Laboratory, Pathum Thani, Thailand*

*[4]Center of Excellence on Hazardous Substance Management, Chulalongkorn University, Bangkok, Thailand*

[5]*Biotechnology for Wastewater Engineering Research Group, Chulalongkorn University, Bangkok, Thailand*

[6]*Center of Excellence on Environmental Health and Toxicology (EHT), OPS, MHESI, Bangkok, Thailand*

**Corresponding author. Mailing address: Research Laboratory of Biotechnology, Chulabhorn Research Institute, 54 Kamphaeng Phet 6, Talat Bang Khen, Lak Si, Bangkok, Thailand 10210; E-mail: kwanrawee@cri.or.th*

**Abstract**

Water commuting is a major urban transportation method in Thailand. However, urban boat commuters risk exposure to microbially contaminated bioaerosols or splash. We aimed to investigate the microbial community structures, identify bacterial and viral pathogens, and assess the abundance of antimicrobial resistance genes (ARGs) using next-generation sequencing (NGS) at 10 sampling sites along an 18 km transportation boat route in the Saen Saep Canal,, which traverses cultural, commercial, and suburban land-based zones. The shotgun metagenomic (Illumina HiSeq) and 16s rRNA gene amplicon (V4 region) (Illumina MiSeq) sequencing platforms revealed diverse microbial clusters aligned with the zones, with explicit segregation between the cultural and suburban sites. The shotgun metagenomic sequencing further identified bacterial and viral pathogens, and ARGs. The predominant bacterial pathogens (>0.5% relative abundance) were the *Burkholderia cepacia* complex, *Arcobacter butzleri*, *Burkholderia vietnamiensis*, *Klebsiella pneumoniae*, and the *Enterobacter cloacae* complex. The viruses (0.28%–0.67% abundance in all microbial sequences) comprised mainly vertebrate viruses and bacteriophages, with encephalomyocarditis virus (33.3%–58.2% abundance in viral sequences),

hepatitis C virus genotype 1, human alphaherpesvirus 1, and human betaherpesvirus 6A among the human viral pathogens. The 15 ARG types contained 611 ARG subtypes, including those resistant to beta-lactam, which was the most diverse and abundant group (206 subtypes; 17.0%–27.5%), aminoglycoside (94 subtypes; 9.6%–15.3%), tetracycline (80 subtypes; 15.6%–20.2%), and macrolide (79 subtypes; 14.5%–32.1%). Interestingly, the abundance of ARGs associated with resistance to beta-lactam, trimethoprim, and sulphonamide, as well as *A. butzleri* and crAssphage, at the cultural sites was significantly different from the other sites ($p<0.05$). We demonstrated the benefits of using NGS to deliver insights into microbial communities, and antimicrobial resistance, both of which pose a risk to human health. Using NGSmay facilitate microbial risk mitigation and management for urban water commuters and proximal residents.

**KEYWORDS:** freshwater, virus, bacteria, crAssphage, microbial diversity, antibiotic resistance

## 1. Introduction

Urban canal networks facilitate transportation, flood protection, agriculture, waste management, and human health and well-being (Anceno et al., 2007; Völker and Kistemann, 2011). Notably, inland water transportation has been an integral part of economic development and society in many countries as it is a component of human mobility, tourism, and leisure travel, as well as the transportation of commercial, agricultural and industrial goods and products (Cheemakurthy et al., 2017; Jurkovic et al., 2021; Tanko and Burke, 2017). Inland water transport has been promoted for its sustainability features and eco-friendliness in modern smart cities as an option to avoid land-based transit congestion at an economical cost (Iamtrakul et al., 2018; United Nations Economic Commission for Europe, 2011). Urban ferry and boat transits serve

commuters and passengers via large-scale transit networks such as those in Amsterdam, Brisbane, Hong Kong, and Istanbul, medium-scale transits such as those in Copenhagen, Gothenburg, and Hamburg, and small-scale services such as those in Boston, Oslo, and Rotterdam (Cheemakurthy et al., 2017). Remarkably, such services transported over 70 million passengers in Bangkok in 2019 (Marine Department of Thailand, 2020).

Notwithstanding, many populated urban waterways have microbial pollution problems associated with human sewage contamination (Amin et al., 2020; Shahin et al., 2021; Sirikanchana et al., 2014; Wangkahad et al., 2015; World Health Organization [WHO], 2018). Boat passengers are thus at risk of being exposed to microbially contaminated bioaerosols or splash (Ginn et al., 2021; Pringsulaka et al., 2017). In addition to pathogenic organisms, antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) have emerged as waterborne microbial pollutants that pose a risk to human health (Amarasiri et al., 2020; Verhougstraete et al., 2020). Preventive and curative treatments of ARB-infected patients are immensely difficult. ARBs thus cause increased human illness and death as well as higher treatment costs and longer treatment duration (WHO, 2015). Moreover, antibiotic resistance acquisition of natural bacteria can be promoted via the horizontal gene transfer of ARGs through transformation (free DNA uptake), conjugation (gene transfer from another bacteria), and transduction (genes carried by bacteriophages) (Amarasiri et al., 2020). A number of studies have shown that a polluted surface water acts as an environmental reservoir for ARBs and ARGs contaminated by wastewater sources and stormwater pollution (Karkman et al., 2019; Lee et al., 2020; Makkaew et al., 2021; Stange et al., 2016; Zheng et al., 2021). A health approach that takes into account the interlinkage of the human, animal, and environmental sectors has been

emphasized to tackle the problem of antimicrobial resistance (AMR) (Booton et al., 2021; European Commission, 2017; WHO, 2015), and AMR-related topics pertaining to water and the environment have been a research focus over the last two decades (Luz et al., 2022). It has also been shown that environmental characteristics, such as high suspended solids and low dissolved oxygen (DO) favor pathogen-related genera and ARGs (He et al., 2022; Ott et al., 2021), which increase the risks to public health. However, information regarding microbial contamination, including pathogenic bacteria and viruses, as well as emerging ARG contaminants in urban transportation canals, is limited.

Next-generation sequencing (NGS) can be used to identify microbial communities from a wide range of environments and provides a method by which to capture unculturable organisms and determine the complex associations between such microorganisms in their natural environments (Caporaso et al., 2012; Jin et al., 2018; Lapierre et al., 2019). Specifically, 16S rRNA gene amplicon sequencing can elucidate bacterial and archaeal community structures, while shotgun metagenomic sequencing is able to assign microbial species, as well as genes of interest, such as viral communities and ARGs (Cui et al., 2019; Jantharadej et al., 2021; Ranjan et al., 2016). Consequently, the objectives of this study were (1) to determine the structure and abundance of the microbial communities in 10 transport canal locations in Thailand, (2) to investigate the presence in canal waters of pathogenic bacteria and viruses and ARGs that could pose a risk to human health, and (3) to examine the effects of designated land-based zoning on microbial and ARG diversity. The results of this study may support the application of NGS by elucidating the microbial health risks to boat commuters and proximal residents along polluted urban canals and thus informing measures for microbial risk mitigation and management.

## 2. Materials and methods

### *2.1 Site description and water sampling*

The 53.5-km Saen Saep Canal, which was constructed in 1840, connects Bangkok and Chachoengsao Province, Thailand. Despite its deteriorating water quality and nuisance odors, with a history of $10^4$–$10^8$ CFU/100 mL of total coliforms (Department of Drainage and Sewerage, 2011; Jantharadej et al., 2021; Pollution Control Department (PCD), 2018), the canal serves as one of the main transportation routes for urban commuters, with an average of 70,709 people per day on weekdays and 40,283 people per day on weekends and national holidays in 2019 (Marine Department of Thailand, 2020). In this study, one-time sampling of canal water samples were conducted from 10 sampling sites (sites A–J) along an 18 km transportation boat route in the Saen Saep Canal, Bangkok (Fig. 1; Table S1). The sampling sites were located in three zoning classifications from upstream to downstream, namely, cultural (sites A and B), commercial (sites C–F), and suburban residential (sites G–J), with different land uses according to the Department of City Planning, Bangkok Metropolitan Administration (Table S2) (BMA, 2017). The cultural zone is located in the downtown area with its historical sightseeing attractions and has a population density of 23,667 people per $km^2$ (BMA, 2017, 2018). The commercial area comprises commercial buildings, shopping malls, condominiums, hospitals, schools, and restaurants and has a population density of 5,434–10,097 people per $km^2$. The water samples in both areas had a dark brown color and foul odor. The suburban residential area along the canal serves a large number of residents and includes many facilities, such as villages, condominiums, markets, informal settlements, and department stores. Its population density is 5,148 people per $km^2$. The water samples from this area had a dark green color and slightly foul odor. One-liter water samples were collected at the passenger ports, which intrude 1–2 m into the

10 m wide canal. The samples were captured in sterile containers placed 30 cm below the water surface in accordance with Thailand's standard protocol for microbiological sampling (National Environment Board, 2017). The sample collection period was February 18–26, 2019, which represented the dry season. The samples were transported on ice to the laboratory within 3 h of collection. The physicochemical parameters, including the DO, conductivity, salinity, pH, and temperature, were measured on site using portable meters (YSI Pro2030 and YSI 60, YSI Inc., USA). The water samples were stored at 4°C until further processing.

### ***2.2*** *Sample preparation and DNA extraction*

One liter of water was centrifuged at 4,300 × g for 15 minutes at room temperature, and the pellet was kept in a 2 mL sterile microcentrifuge tube. The supernatant was then pH-adjusted to 3.5 with 2N HCl and filtered through a 0.45 μm pore-size HAWP membrane (Merck Millipore, USA) (A. Kongprajug et al., 2019). The filtered membrane and sediment pellet were combined and the DNA extracted using a FastDNA SPIN kit for soil (MP Biomedicals, USA) in accordance with the manufacturer's instructions. The DNA quality was assessed using agarose gel electrophoresis (2.0% agarose gel, 100V, 30 min), and the DNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). The DNA samples were stored at −20°C until use.

### *2.3 16S rRNA gene sequencing and bioinformatics*

We performed 16S rRNA gene amplicon sequencing (MiSeq, Illumina, USA) for the V4 region using modified primers, namely, modified 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), which improved the coverage of the archaeal

and bacterial 16S rRNA genes (Ding et al., 2015). The 50 µL PCR reaction was composed of each of 1 µM forward and reverse primers, 1.25 U Taq polymerase, 4 mM $MgCl_2$, 5 µL of 10× buffer (Fermentas, Thermo Fisher Scientific, USA), 0.2 µM dNTPs, and 4 µL DNA template. The PCR reactions were run on a thermocycler (Bio-Rad, USA) using the following program: 3 min of denaturation at 95°C; 23 cycles of 30 s at 95°C, 30 s for annealing at 56°C, and 30 s for elongation at 72°C; and a final extension at 72°C for 10 min. The resultant PCR products were checked for quality on agarose gel electrophoresis (2.0% agarose gel, 100V, 30 min) and further purified using AMPure XP beads (Beckman Coulter, USA) and indexed using 5 µL Nextera XT index primer in a 50 µL PCR reaction by following eight cycles of the aforementioned PCR cycling condition. Next, the purified amplicons were pooled in an equimolar proportion and paired-end sequenced at a 6 pM final loading concentration into an Illumina MiSeq sequencer (Illumina, USA) in accordance with the published protocol (Caporaso et al., 2012) at the Omics Sciences and Bioinformatics Center (Chulalongkorn University, Thailand). For the data analysis, the raw sequencing data in FASTQ format were processed to remove the PCR primer sequences. Quantitative Insights Into Microbial Ecology 2 (QIIME 2; version 1.6.0) was used to analyze the sequencing data (Bolyen et al., 2019). The DADA2 algorithm (version 1.10) as a QIIME 2 plugin was applied to merge and denoise the sequences (Callahan et al., 2016). All the amplicon sequence variants (ASVs) with a frequency lower than 0.1% of the mean sample depth were removed, and the rest were grouped into taxa using a naive Bayes approach, which was implemented in the scikit-learn Python algorithms (Pedregosa et al., 2011). The representative sequence of each ASV was used to perform a taxonomy using the SILVA database (Quast et al., 2013).

183 *2.4 Shotgun sequencing and bioinformatics*

184 Sample DNA (100 ng) was subjected to sequencing library preparation using the QIAseq FX
185 DNA Library Kit (QIAGEN, Germany). Briefly, the DNA was fragmented using an enzymatic
186 reaction and cleaned with AMPure XP magnetic beads (Beckman Coulter, USA). An indexed
187 adapter was ligated to the fragmented DNA. The quality and quantity of the indexed libraries
188 were measured using an Agilent 2100 Bioanalyzer (Agilent, USA) and QFX fluorometer
189 (Denovix, USA) and pooled in an equimolar quantity (Caporaso et al., 2012). Cluster generation
190 and paired-end 2×150 nucleotide read sequencing were performed on one lane of the HiSeq 4000
191 sequencer (Illumina, USA) at the Omics Sciences and Bioinformatics Center (Chulalongkorn
192 University, Thailand). The quality and adaptor trimming of the FASTQ files were conducted
193 using Trim Galore! version 0.4.4 (Babraham Bioinformatics, 2020). To profile the abundance of
194 the microbial communities, the filtered sequence reads were classified using Centrifuge version
195 1.0.4 (Kim et al., 2016) with the prebuilt index database and default settings. The sequence reads
196 were assigned a taxonomy using the National Center for Biotechnology Information (NCBI)
197 databases. The profiling of the viral metagenomic communities was completed using the
198 Microbial Community Profiling method (MiCoP) (Lapierre et al., 2019). The MiCoP was set up
199 to use the BWA-MEM mapping method to map the sequence reads (Li, 2013). Finally, the
200 sequence reads were assigned virus species using the full NCBI Virus RefSeq databases, and
201 these results were then filtered and profiled using the compute-abundances.py script. To predict
202 the ARG, all the cleaned shotgun sequences were mapped into the AMR gene sequences from
203 the ResFinder database (version 2022-02-04) (Bortolaia et al., 2020) using k-mer alignment
204 (Clausen et al., 2018). The raw read count was converted to the percent relative abundance
205 before further analysis. The ARG relative abundances from the different sampling sites were

clustered using the unweighted pair group method with the arithmetic mean agglomerative hierarchical clustering method. Hypothetical testing for significant differences was conducted via Welch's *t*-test. The plot and statistical test for the ARG were performed in STAMP (version 2.1.3) (Parks et al., 2014).

### *2.5 Statistical data analysis*

The similarities within the microbial community were assessed using principal coordinate analysis (PCoA) via the Bray–Curtis dissimilarity matrix. The permutational multivariate analysis of variance (PERMANOVA) was computed to determine any significant differences between the land use groups. A comparison of the microbial diversity and abundance between the land use groups was performed using the linear discriminant analysis (LDA) effect size method (Segata et al., 2011) with an LDA score greater than 4.0. We conducted the correlation analysis using Spearman's correlation (R package corrplot, version 0.84) on U-Score rank (R package 'NADA2' version 1.0.1). The agglomeration method ward.D2 was used for the hierarchical clustering (R package stat (R Core Team, 2019)).

## 3. Results and discussion

### *3.1 Microbial community structure and presumptive functions*

The PCoA analysis of the microbial sequencing profiles demonstrated separate clusters in the cultural and suburban zones, as supported by the PERMANOVA test (Fig. 2 and Table S3). Although the microbial diversity data for the shotgun sequencing included eukaryote and virus domains in addition to bacteria and virus domains (Fig. S1), we observed consistent microbial diversities between the two platforms, which was in line with a previous report (Caporaso et al.,

2012). The bacteria domain was deemed predominant by both the shotgun and amplicon sequencing platforms, with the eukaryota and viruses additionally characterized via the shotgun sequencing (Fig. S1). *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Cyanobacteria* were the five most abundant phyla, within the bacterial domain, as determined by both sequencing platforms. *Chordata* phylum of the eukaryote domain was additionally predominant, as ascertained by the shotgun sequencing (Fig. S2). *Epsilonbacteraeota* is a newly established phylum, which was reclassified from a class of the *Proteobacteria* phylum, and was therefore identified as a separate phylum only in the amplicon sequencing due to differences in the databases used for the two sequencing platforms (Waite et al., 2017). These core phyla have also been found in other tropical and subtropical anthropogenically impacted freshwater (Ibekwe et al., 2016; Obieze et al., 2022; Ung et al., 2019; Wang et al., 2016). The shotgun sequencing indicated that the *Pseudomonas* and *Burkholderia* genera of the *Proteobacteria* phylum were most dominant, while the amplicon sequencing showed that C39 was most abundant (Fig. 3). The LDA scores indicated significant differences between the land-based zones for certain taxa (Fig. S3).

Notably, we observed a significant correlation ($p < 0.05$) in the copresence of certain genera, such as *Arcobacter* (nitrogen-fixing bacteria) and *Sulfurospirillum* (nitrate-reducing bacteria), *Acidovorax* (nitrate-reducing bacteria) and *Limnohabitans* (planktonic bacteria), and *Acidovorax* and *Hydrogenophaga* (both nitrate-reducing bacteria) (Fig. S4). The water quality parameters of the canal water samples from sites A to J indicated narrow ranges comprising DO at 0.1–1.6 mg/L, conductivity at 551–1,054 µS/cm, a pH of 7.14–7.56, a temperature of 28.7°C–30.1°C, and salinity of 0.1–0.5 parts per thousand (Table S1), which concurred with the canal's historical

252 record (PCD, 2018). The PCA plot showed the following clusters of top 40 genera as identified
253 by the shotgun sequencing (Fig. S5a) and water quality parameters: DO – *Ralstonia*, pH –
254 temperature – *Allochromatium* – *Cyanobium* – *Synechococcus*, and salinity – conductivity –
255 *Rhodobacter*. The top 40 genera analyzed by the amplicon sequencing (Fig. S5b) were also
256 clustered with the water quality parameters as follows: DO – *Pseudomonas*, salinity –
257 conductivity – uncultured bacterial in family *Rikenellaceae*, and pH – temperature –
258 *Hydrogenophaga* – *Dechlorobacter* – unclassified genus in family *Lentimicroblaceae*.
259
260 The associated microbial functions of the top 40 genera are summarized in Table S4. The nitrate-
261 reducing bacteria group represented the highest relative abundance in the canal water, which
262 could be due to the denitrification process promoted by the low oxygen condition and high
263 nitrate concentrations in the Saen Saep Canal (Jantharadej et al., 2021; PCD, 2018). Another
264 dominant group, the nitrogen-fixing bacteria, can fix the nitrogen gas in the atmosphere and
265 convert it into an ammonia form in water. Moreover, certain sampling points contained low DO
266 and possibly had an anaerobic condition, which resulted in the presence of fermentative bacteria.
267 Pathogenic genera, including *Pseudomonas*, *Mycobacterium*, *Aeromonas*, *Acinetobacter*,
268 *Arcobacter*, and *Bacteroides*, have also been reported in urban lakes and rivers (Cui et al., 2019;
269 Dong et al., 2019; Jin et al., 2018).
270
271 *3.2 Pathogenic bacterial species*
272 The shotgun metagenomic sequencing revealed the 35 most abundant pathogenic bacterial
273 species (Fig. 4). The predominant species comprising more than 0.5% relative abundance in at
274 least one canal water sample were the *Burkholderia cepacia* complex (0.6%–2.3%), *Arcobacter*

*butzleri* (0.03%–1.5 %), *Burkholderia vietnamiensis* (0.1%–1.2%), the *Enterobacter cloacae* complex (0.1%–1.2%), and *Klebsiella pneumoniae* (0.07%–0.7%). The *Burkholderia cepacia* complex and other abundant species in the *Burkholderia* group showed high abundance at canal sites G and H in the suburban area. The *Burkholderia* species are opportunistic pathogens that cause respiratory tract infections, especially in patients with cystic fibrosis. They have been found in municipal wastewater (Chu et al., 2018; LiPuma, 2005; Ragupathi and Veeraraghavan, 2019), and their association with ARGs and high resistance to antiseptics and disinfectants has raised further concerns with respect to public health (Chu et al., 2018; McDonnell and Russell, 1999).

*Arcobacter butzleri* was the second most prevalent pathogenic species in the canal and the most abundant in the cultural zone (1.48% and 0.67% at sites A and B). This species can cause watery diarrhea and bacteremia and has been associated with fecal pollution from wastewater (Shrestha et al., 2022). *A. butzleri* has previously been isolated from canal water in Thailand (Morita et al., 2004; Tomioka et al., 2021). The presence of this bacterial pathogen in environmental water could pose a risk to public health, especially with its relatively higher persistence to high organic matter concentrations and warm conditions compared to other enteric pathogens (Tomioka et al., 2021; Van Driessche and Houf, 2008) and its reported AMR (Ferreira et al., 2019).

The *Enterobacter cloacae* complex and *Klebsiella pneumoniae* showed the highest abundance at sites E and F in the commercial area (Fig. 4). The *E. cloacae* complex has been associated with infections of the urinary tract, respiratory tract, skin, and bloodstream in immunocompromised patients (Brisse et al., 2006; Selenic et al., 2003), and antibiotic resistance has increased the

298 significance of *E. cloacae* as a public health concern (Chen and Huang, 2013; Ebomah and
299 Okoh, 2020) (Fig. 4). *Klebsiella pneumoniae* is an opportunistic pathogen that causes frequent
300 outbreaks in hospitals (Wu and Li, 2015). A study reported closely related clinical and
301 environmental *Klebsiella pneumoniae* isolates from hospital patients, hospital sewage, and the
302 canals surrounding a hospital in Thailand. (Runcharoen et al., 2017). This species has been
303 prioritized for AMR concern and included in the WHO's (2017) global AMR surveillance
304 system, GLASS.
305
306
307
308 *3.3 Viral community structure*
309 The relative abundance of viral communities in the canal water samples, as identified by shotgun
310 metagenomic sequencing, represented 0.28%–0.67% of all the microbial communities (Fig. S1a).
311 Overall, the viral community structures comprised five Baltimore classes, mostly single-stranded
312 RNA (ssRNA) viruses (34.8%–60.4% of all virus sequences) and double-stranded DNA
313 (dsDNA) viruses without an RNA stage (31.5%–58.9%) (Fig. S6a). *Picornaviridae* ssRNA,
314 which are vertebrate-infecting viruses, were the most abundant family in all the canal water
315 samples (33.8%–56.9%) (Fig. S6b). The dsDNA viruses comprised 10 predominant families,
316 namely, *Myoviridae*, *Podoviridae*, *Siphoviridae*, *Baculoviridae*, *Alloherpesviridae*,
317 *Herpesviridae*, *Nudiviridae*, *Phycodnaviridae*, *Polydnaviridae*, and *Poxviridae.* The families
318 *Myoviridae*, *Siphoviridae*, and *Podoviridae*, which belong to the *Caudovirales* order, constituted
319 the major bacteriophages in the canal water (8.8%–41.3%). Previous studies have reported the
320 families *Myoviridae*, *Siphoviridae*, and *Podoviridae* as mainly dominant in freshwater (Gu et al.,

2018; Mohiuddin and Schellhorn, 2015; Tseng et al., 2013). The *Baculoviridae* family, which infects invertebrates (i.e., insects), was also predominant at 0.7%–25.6%, while the *Herpesviridae* family, which infects vertebrates, was noticeable in the samples from sites E and F in the commercial area and sites H, I, and J in the suburban area, at up to 4% relative abundance. Moreover, almost all the dsRNA viruses were sorted into an unclassified family related to fungi-infecting viruses and accounted for 3.0%–12.2% of the total viral sequences. Seven virus groups were classified according to their host types, with a higher abundance of the vertebrate-infecting viruses, bacteriophages, invertebrate-infecting viruses, and fungi-infecting viruses, and a relatively lower abundance of the plant-infecting viruses, algae-infecting viruses, and protozoa-infecting viruses (Fig. S6c).

The 35 most abundant virus species in the canal water samples are shown in Fig. 5. Encephalomyocarditis virus (EMCV), a vertebrate-infecting virus in the *Picornaviridae* family that causes a broad range of infections in mammals and humans, showed the highest relative abundance at 33.3%–58.2% of the total virus sequences. Alcelaphine gammaherpesvirus types 1 and 2, which infect ruminants, were observed in the samples from sites E and F in the commercial area at up to 2% of the total virus sequences. Moreover, fish-infecting virus species such as the piscine myocarditis-like virus, Cyprinid herpesvirus types 1 and 3, and fathead minnow picornavirus were detected in the canal water. With a relative abundance greater than 10% in the samples from sites A, C, E, G, I, and J, the invertebrate-infecting virus Orgyia pseudotsugata multiple nucleopolyhedrovirus, which belongs to the *Baculoviridae* family, was the most dominant of the insect-infecting viruses in all the canal water samples. *Saccharomyces*

343 *cerevisiae* killer virus M1, a fungi-infecting (yeast) virus, was detected in all the samples at a
344 range of 3.0%–12.2% of the total virus sequences.
345
346 Several bacteriophages, including *Bacillus* phage Stitch, were detected in the canal water and
347 found to be highly abundant at sites A, C, D, and G, where they accounted for 9.2%–25.5% of
348 the total virus sequences. A higher abundance of *Planktothrix* phage PaV-LD and *Rhodobacter*
349 phage RcapNL at over 2% relative abundance was observed at sites G, H, I, and J, while
350 *Salmonella* phage SJ46 and *Escherichia* virus P1 were detected at more than 2.5% at sites A and
351 B. The *Aeromonas* phage vB_AsaM-56 had a relative abundance of 5% at site B, and
352 *Staphylococcus* phage Team1 (4%) was dominant at site H. The other bacteriophages included
353 *Burkholderia* virus phiE 125, *Bordetella* virus BPP1, uncultured crAssphage, *Ralstonia* phage
354 RSS30, and various species of *Pseudomonas* phages.
355
356 *3.4 Pathogenic viruses*
357 The main human viral pathogen in the canal water was EMCV (33.3%–58.2%) across all the
358 samples (Fig. 6). EMCV belongs to the *Cardiovirus* genus of the family *Picornaviridae* and can
359 infect a broad variety of vertebrate species, including rodents, pigs, birds, cattle, wild animals,
360 several species of non-human primates, and humans (Hammoumi et al., 2012). EMCV causes
361 acute myocarditis outbreaks in piglets and pregnant sows on pig farms worldwide (Feng et al.,
362 2015). EMCV infection in humans is fairly common via the respiratory and oral routes and is
363 mostly asymptomatic (Carocci and Bakkali-Kassimi, 2012; Oberste et al., 2009). It is likely that
364 the rodents or infected rodent carcasses common in the city's water pipes may be involved in the

spread of EMCV in the canal water. Rodents have been reported as a major source of EMCV outbreaks via water in pig farms (Alexandersen et al., 2019).

Other human viral pathogens (i.e., hepatitis C virus genotype 1, human alphaherpesvirus 1, and human betaherpesvirus 6A) represented less than 1% across all the samples. The hepatitis C virus is a bloodborne pathogen that causes acute or chronic hepatitis. It can be transmitted by drug injection, blood transfusion, hemodialysis, organ transplantation, and less frequently, sexual relations (Modi and Liang, 2008). It is therefore less likely for the hepatitis C virus to be transmitted via contaminated canal water. Human alphaherpesviruses and betaherpesviruses belong to the *Herpesviridae* family, which contains dsDNA (i.e., no RNA stage). They primarily cause infections of the mouth, face, eyes, pharynx, and central nervous system and can be transmitted via skin exposure, oral secretions, and respiratory droplets (Chayavichitsilp et al., 2009; Dockrell, 2003). Although waterborne transmission is not deemed the main route of hepatitis C and human herpesvirus infection, their genomes have been found in wastewater, polluted freshwater (Alexyuk et al., 2017; Corpuz et al., 2020; McCall et al., 2020), and sewage-contaminated aerosols (Brisebois et al., 2018). In addition, the *Herpesviridae* family is persistent in water environments and aerosols at ambient temperatures (Sobsey and Meschke, 2003), which could be a risk factor for environmental transmission.

Notably, the common enteric human pathogenic viruses, namely, noroviruses, hepatitis A and E viruses, rotaviruses, enteroviruses, adenoviruses, astroviruses, and caliciviruses, were not detected by shotgun metagenomic sequencing. CrAssphage, a bacteriophage used worldwide as a human fecal indicator (Sabar et al., 2022), showed the highest abundance at the cultural sites A

388 and B with a relative abundance of 2.14%–2.45%, while the relative abundance at the other sites
389 was lower at 0.16%–0.78%. CrAssphage has been found in human sewage and
390 anthropogenically impacted freshwater and seawater and is thus used as a human-specific fecal
391 source tracker in Thailand (Akechai Kongprajug et al., 2019; Sangkaew et al., 2021).
392
393 *3.5 ARGs*
394 The water samples along the canal revealed a total of 611 ARG subtypes from 15 ARG types
395 (Fig. 7a). Those related to resistance to beta-lactam (206 subtypes) were the most diverse ARG
396 types, followed by the ARG types associated with resistance to aminoglycoside (94 subtypes),
397 tetracycline (80 subtypes), macrolide (79 subtypes), trimethoprim (36 subtypes), phenicol (24
398 subtypes), colistin (22 subtypes), sulphonamide (22 subtypes), quinolone (18 subtypes), and
399 disinfectant (six subtypes), as well as other ARG types, namely, those linked to resistance to
400 fusidic acid, glycopeptide, nitroimidazole, rifampicin, and fosfomycin, with a total of 24
401 subtypes. The six most abundant ARG types were those related to resistance to beta-lactam
402 (17.0%–27.5%), macrolide (14.5%–32.1%), tetracycline (15.6%–20.2%), aminoglycoside
403 (9.6%–15.3%), sulfonamide (6.4%–10.9%), and quinolone (4.6%–13.1%) (Fig. 7a). These
404 groups have been found to be widespread in municipal wastewater even after treatment (Ping et
405 al., 2022; Raza et al., 2022; Zou et al., 2022). Moreover, macrolide-resistant genes had the
406 highest abundance (32.1%) at site D in the commercial zone. The most abundant single gene
407 subtype was the *tlr(c)* gene in the macrolide type, which encodes tylosin-resistance protein, with
408 a 4.4% relative abundance at site B in the cultural zone. The prevalent genes with more than 1%
409 relative abundance at almost all the sites were the quinolone resistance gene *OqxB* and
410 sulphonamide resistance genes *sul1* and *sul2*, while the aminoglycoside resistance genes *aac(3)-*

411 *VIII* and *aac(3)-IIIb*, betalactam resistance genes *bla*$_{SRT-2}$, *bla*$_{PAU-1}$, *bla*$_{OKP-B-8}$, *POM-1*, *bla*$_{OXA-455}$, and *bla*$_{GES-23}$, macrolide resistance genes *srm(B)*, *erm(38)*, *ole(C)*, *car(A)*, and *tlr(C)*, quinolone resistance genes *qepA1*, and *qepA2*, and tetracycline resistance genes *tet(C)*, *tet(M)*, *tcr3*, and *otr(A)* were each present in the water at at least one site (Table S5). The abundance of ARG types was not significantly different between the commercial and suburban sites; however, it was different at the cultural sites (Fig. 7b). The cultural sites contained a higher abundance of trimethoprim resistance genes than the commercial sites and a higher abundance of beta-lactam and trimethoprim resistance genes than the suburban sites, although they had a lower abundance of sulphonamide resistance genes than the suburban sites ($p < 0.05$). As previously reported by Davis et al. (2020) and Liu et al. (2021), the different types and prevalence of anthropogenic activities from the three land-based zones (Table S2) could have contributed to the diverse levels of ARG contamination.

423

424 *3.6 Implications for canal water quality management and public health risk mitigation*

425 In this study, we utilized NGS analyses to characterize the microbial pollutants (i.e., bacterial and viral human pathogens and ARGs) in the canal water in Bangkok, Thailand, to determine the risks to boat commuters. Both 16s rRNA amplicon sequencing and shotgun metagenomic sequencing delivered similar taxonomic classifications at the sampling sites. Consequently, the amplicon sequencing method could serve as a more economical option for this purpose. Shotgun metagenomic sequencing could provide further information on the levels of microbial species, various genes of interest, and the molecular functions encoded in the metagenomes (Ibarbalz et al., 2016; Ranjan et al., 2016). Currently, the use of NGS technologies may present challenges, such as high costs and the need for specialized equipment and data analysis and interpretation

expertise (Garner et al., 2021). However, with the rapid development of new technologies and eventual lower costs, NGS offers considerable potential for an improved understanding of microbial ecology in the fields of water engineering and water quality management (Garner et al., 2021; McDaniel et al., 2021). Furthermore, during the COVID-19 pandemic, the NGS application proved to be useful in monitoring for SARS-CoV-2 and its variants to determine community outbreaks (Agrawal et al., 2022; Martínez-Puchol et al., 2021; Smyth et al., 2022). While NGS could offer a holistic approach to microbial community characterization, its limited resolution for taxonomic identification at a species or strain level needs to be addressed. This limitation could be countered by combining NGS with robust gene-targeted molecular detection method (e.g., quantitative or digital PCR) to monitor microorganisms of concern, such as waterborne pathogens and microbial source tracking markers, in wastewater and polluted water, as well as for SARS-CoV-2 surveillance in wastewater (Ho et al., 2022; Kongprajug et al., 2021a; Sangsanont et al., 2022).

This study demonstrated that the microbial pollutants identified in the canal water were mostly respiratory and gastrointestinal microorganisms, among them, the predominant pathogenic *Burkholderia cepacia*, *Arcobacter butzleri*, and encephalomyocarditis virus, as well as ARG groups related to resistance to beta-lactam, aminoglycoside, tetracycline, and macrolide. Epidemiological surveillance data for Bangkok in 2021 revealed that diarrhea was the leading cause of morbidity at 393.3 per 100,000 population, followed by pyrexia (160.3 per 100,000) and pneumonia (106.1 per 100,000) (Institute for Urban Disease Control and Prevention, 2022). Although no information on the routes of exposure for these morbidity rates was published, the risk of exposure to these pathogens and ARGs could be aggravated by aerosols and airborne

particulate matter (Ginn et al., 2021; Xie et al., 2022). At present, no mitigation measures aimed at preventing aerosol and urban particulate matter exposure are in place; however, such measures could lead to lower public health risks. Furthermore, quantitative microbial risk assessments could be conducted to determine the current risks and evaluate the performance of any mitigation measures (Dada and Gyawali, 2021; Denpetkul et al., 2022; Kongprajug et al., 2021b).

This study further revealed that microbial compositions and ARG profiles can be associated with zoning. The Saen Saep Canal could be polluted by wastewater sources from various residential and communal facilities, 70% of whose untreated and treated effluent exceeds treated effluent standards (PCD, 2016). While the wastewater from a small area is connected through sewer lines to a municipal wastewater treatment plant, most of the areas along the Saen Saep Canal are not connected to sewer lines (PCD, 2016). The Saen Saep Canal reportedly receives approximately 49,000 $m^3$ wastewater per day, which corresponds to biochemical oxygen demand loading of 2,630 kg per day (PCD, 2016). However, pinpointing the wastewater sources that could be contributing to the canal at each sampling site is a challenge due to the complexities of the city plans and the possibility of wastewater inputs from upstream activities and connecting canals. Notably, the cultural zone constitutes the highest population density compared to the other two zones and serves tourists visiting cultural attractions (Table S1). Tourists who are accommodated in the cultural area (sites A and B) could carry intestinal microbiomes that are different from those of the local residents, (Yatsunenko et al., 2012), and they could thus contribute to the differences in microbial diversity in the canal. In particular, crAssphage, which was detected at a higher relative abundance at the cultural sites, has been described as displaying different shedding rates among populations from different geographical regions (Cinek et al., 2018;

Stachler and Bibby, 2014). Furthermore, tourists, who reportedly also carry different patterns of ARB and ARGs, could account for the differences in ARGs at the cultural sites (Benenson et al., 2018; Bokhary et al., 2021). Notwithstanding, contributions of microbial and gene diversity from tourists require further investigation due to tourists' characteristically short-term stays and turnover dynamics. Hospital wastewater may further contribute to the dissimilarities in the canal microbial diversity as in vivo exposure to antibiotics affects the gut microbiome (Liu et al., 2020). Age, lifestyle, and social networks also affect human gut microbiomes (Brito et al., 2019; Obregon-Tito et al., 2015; Xu et al., 2019). Environmental factors, as well as contaminated ARGs, could further influence the regrowth and persistence of contaminated microorganisms (Booncharoen et al., 2018; Dean and Mitchell, 2022; He et al., 2022; Ott et al., 2021; Yang et al., 2022). The cultural sites showed high *Arcobacter butzleri* and crAssphage as well as beta-lactam and trimethoprim resistance genes . Our study thus indicates that further investigation of the wastewater treatment plants and interventions at those facilities may be required at the cultural sites. Furthermore, the effects of stormwater runoff on pollution contamination into the canal should be studied to provide insights into microbial pollution during wet weather conditions.

In summary, similar to other locations worldwide, a better understanding of the microbial risks of polluted canal water in Thailand could facilitate appropriate interventions, such as commuter protective equipment, protective barriers on boats, and wastewater reduction at the source, with the aim of improving the quality of life of boat passengers and residents living near the canal. We supports the recently approved governmental agreement of Thailand's 11-year Saen Saep Canal Environment Rehabilitation Development Plan (2021–2031) on water quality restoration

and the 20-year Thailand National Strategy with its focus on quality-of-life improvements based on green growth.

## 4. Conclusions

This study demonstrated the use of shotgun metagenomic sequencing and 16s rRNA amplicon sequencing to evaluate microbial water quality in an urban transportation canal. The microbial compositions and ARG profiles indicated associations with zoning. The main presumptive microbial functions were involved with anaerobic photosynthesis and fermentation, which corresponded to the low DO conditions in the canal. The main bacterial and viral pathogens identified were the *Burkholderia cepacian* complex, *Arcobacter butzleri*, the encephalomyocarditis virus, and the hepatitis C virus, all of which pose risks to public health. The antibiotic-resistance profiles in this study also indicated a risk of ARG transmission through the environment, with those associated with resistance to beta-lactam, aminoglycoside, tetracycline, and macrolide as the most abundant ARG types. This study emphasized that, while showing certain limitations in its ability to identify common waterborne bacteria and viruses, the application of NGS in elucidating microbial water quality could assist in the development of water quality restoration and mitigation measures to reduce the health risks to water transportation passengers and residents living near polluted canal water.

## CRediT authorship contribution statement

Krittayapong Jantharadej: Formal analysis, Investigation, Writing – original draft, Visualization. Akechai Kongprajug: Formal analysis, Visualization. Wuttichai Mhuantong: Formal analysis, Visualization. Tawan Limpiyakorn: Conceptualization, Resources, Writing – review & editing.

Benjaporn Boonchayaanant Suwannasilp: Conceptualization, Resources, Writing – review & editing. Skorn Mongkolsuk: Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. Kwanrawee Sirikanchana: Conceptualization, Resources, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work was financially supported by the Thailand Science Research and Innovation (TSRI), Chulabhorn Research Institute (grant no. 2536702/43410).

## References

Agrawal, S., Orschler, L., Schubert, S., Zachmann, K., Heijnen, L., Tavazzi, S., Gawlik, B.M., de Graaf, M., Medema, G., Lackner, S., 2022. Prevalence and circulation patterns of SARS-CoV-2 variants in European sewage mirror clinical data of 54 European cities. Water Res. 214, 118162. doi:10.1016/j.watres.2022.118162

Alexandersen, S., Knowles, N.J., Belsham, G.J., Dekker, A., Nfon, C., Zhang, Z., Koenen, F., 2019. Chapter 40 Picornaviruses, in: Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W., Zhang, J. (Eds.), Diseases of Swine, 11th Edition. Wiley, pp. 641–684.

Alexyuk, M.S., Turmagambetova, A.S., Alexyuk, P.G., Bogoyavlenskiy, A.P., Berezin, V.E.,

548 2017. Comparative study of viromes from freshwater samples of the Ile-Balkhash region of
549 Kazakhstan captured through metagenomic analysis. VirusDisease 28, 18–25.
550 doi:10.1007/s13337-016-0353-5
551 Amarasiri, M., Sano, D., Suzuki, S., 2020. Understanding human health risks caused by
552 antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water
553 environments: Current knowledge and questions to be answered. Crit. Rev. Environ. Sci.
554 Technol. 50, 2016–2059. doi:10.1080/10643389.2019.1692611
555 Amin, N., Liu, P., Foster, T., Rahman, M., Miah, M.R., Ahmed, G.B., Kabir, M., Raj, S., Moe,
556 C.L., Willetts, J., 2020. Pathogen flows from on-site sanitation systems in low-income
557 urban neighborhoods, Dhaka: A quantitative environmental assessment. Int. J. Hyg.
558 Environ. Health 230, 113619. doi:10.1016/j.ijheh.2020.113619
559 Anceno, a. J., Ozaki, M., Dang, Y.N.D., Chuluun, B., Shipin, O.V., 2007. Canal networks as
560 extended waste stabilization ponds: fate of pathogens in constructed waterways in
561 Pathumthani Province, Thailand. Water Sci. Technol. 55, 143. doi:10.2166/wst.2007.348
562 Babraham Bioinformatics, 2020. Trim Galore [WWW Document]. URL
563 https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/ (accessed 9.1.20).
564 Benenson, S., Nir-Paz, R., Golomb, M., Schwartz, C., Amit, S., Moses, A.E., Cohen, M.J., 2018.
565 Carriage of multi-drug resistant bacteria among foreigners seeking medical care. Sci. Rep.
566 8, 1–7. doi:10.1038/s41598-018-27908-x
567 BMA, 2018. 2018 Statistical Profiles of Bangkok. Admistrative Strategy Devision. [WWW
568 Document]. URL https://webportal.bangkok.go.th/pipd/page/sub/16726/สถิติกรุงเทพมหานคร-
569 2561
570 BMA, 2017. 2016 Environment Study Report in Bangkok (Water Quality). Department of City

571 Planning. Bangkok Metropolitan Administration.
572 Bokhary, H., Pangesti, K.N.A., Rashid, H., Abd El Ghany, M., Hill-Cawthorne, G.A., 2021.
573 Travel-related antimicrobial resistance: a systematic review. Trop. Med. Infect. Dis. 6, 1–
574 27. doi:10.3390/tropicalmed6010011
575 Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., ..., Caporaso, J.G., 2019. Reproducible,
576 interactive, scalable and extensible microbiome data science using QIIME2. Nat.
577 Biotechnol. 37, 850–852. doi:10.1038/s41587-019-0190-3
578 Booncharoen, N., Mongkolsuk, S., Sirikanchana, K., 2018. Comparative persistence of human
579 sewage-specific enterococcal bacteriophages in freshwater and seawater. Appl. Microbiol.
580 Biotechnol. 102, 6235–6246.
581 Booton, R.D., Meeyai, A., Alhusein, N., Buller, H., Feil, E., Lambert, H., Mongkolsuk, S.,
582 Pitchforth, E., Reyher, K.K., Sakcamduang, W., Satayavivad, J., Singer, A.C.,
583 Sringernyuang, L., Thamlikitkul, V., Vass, L., Avison, M.B., Turner, K.M.E., 2021. One
584 Health drivers of antibacterial resistance : quantifying the relative impacts of human ,
585 animal and environmental use and transmission. One Heal. 12, 100220.
586 Bortolaia, V., Kaas, R.S., Ruppe, E., Roberts, M.C., Schwarz, S., Cattoir, V., Philippon, A.,
587 Allesoe, R.L., Rebelo, A.R., Florensa, A.F., Fagelhauer, L., Chakraborty, T., Neumann, B.,
588 Werner, G., Bender, J.K., Stingl, K., Nguyen, M., Coppens, J., Xavier, B.B., Malhotra-
589 Kumar, S., Westh, H., Pinholt, M., Anjum, M.F., Duggett, N.A., Kempf, I., Nykäsenoja, S.,
590 Olkkola, S., Wieczorek, K., Amaro, A., Clemente, L., Mossong, J., Losch, S., Ragimbeau,
591 C., Lund, O., Aarestrup, F.M., 2020. ResFinder 4.0 for predictions of phenotypes from
592 genotypes. J. Antimicrob. Chemother. 75, 3491–3500. doi:10.1093/jac/dkaa345
593 Brisebois, E., Veillette, M., Dion-Dupont, V., Lavoie, J., Corbeil, J., Culley, A., Duchaine, C.,

2018. Human viral pathogens are pervasive in wastewater treatment center aerosols. J. Environ. Sci. (China) 67, 45–53. doi:10.1016/j.jes.2017.07.015

Brisse, S., Grimont, F., Grimont, P.A.D., 2006. The Genus Klebsiella, in: The Prokaryotes. pp. 159–196.

Brito, I.L., Gurry, T., Zhao, S., Huang, K., Young, S.K., Shea, T.P., Naisilisili, W., Jenkins, A.P., Jupiter, S.D., Gevers, D., Alm, E.J., 2019. Transmission of human-associated microbiota along family and social networks. Nat. Microbiol. 4, 964–971. doi:10.1038/s41564-019-0409-6

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. doi:10.1038/nmeth.3869

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. doi:10.1038/ismej.2012.8

Carocci, M., Bakkali-Kassimi, L., 2012. The encephalomyocarditis virus. Virulence 3, 351–367. doi:10.4161/viru.20573

Chayavichitsilp, P., Buckwalter, J., Andrew, C., Friedlander, S., 2009. Herpes Simplex. Infect. Dis. (Auckl). 30, 119–130.

Cheemakurthy, H., Tanko, M., Garme, K., 2017. Urban waterborne public transport systems: An overview of existing operations in world cities, KTH Royal Institute of Technology. doi:10.13140/RG.2.2.32606.69446

Chen, C.-H., Huang, C.-C., 2013. Risk factor analysis for extended-spectrum β-lactamase-

producing Enterobacter cloacae bloodstream infections in central Taiwan. BMC Infect. Dis. 13, 417. doi:10.1186/1471-2334-13-417

Chu, B.T.T., Petrovich, M.L., Chaudhary, A., Wright, D., Murphy, B., Wells, G., Poretsky, R., 2018. Metagenomics reveals the impact of wastewater treatment plants on the dispersal of microorganisms and genes in aquatic sediments. Applid Environ. Microbiol. 84, e02168-17.

Cinek, O., Mazankova, K., Kramna, L., Odeh, R., Alassaf, A., Ibekwe, M.A.U., Ahmadov, G., Mekki, H., Abdullah, M.A., Elmahi, B.M.E., Hyöty, H., Rainetova, P., 2018. Quantitative CrAssphage real-time PCR assay derived from data of multiple geographically distant populations. J. Med. Virol. 90, 767–771. doi:10.1002/jmv.25012

Clausen, P.T.L.C., Aarestrup, F.M., Lund, O., 2018. Rapid and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics 19, 1–8. doi:10.1186/s12859-018-2336-6

Corpuz, M.V.A., Buonerba, A., Vigliotta, G., Zarra, T., Ballesteros, F., Campiglia, P., Belgiorno, V., Korshin, G., Naddeo, V., 2020. Viruses in wastewater: occurrence, abundance and detection methods. Sci. Total Environ. 104743. doi:10.1016/j.phrs.2020.104743

Cui, Q., Huang, Y., Wang, H., Fang, T., 2019. Diversity and abundance of bacterial pathogens in urban rivers impacted by domestic sewage. Environ. Pollut. 249, 24–35. doi:10.1016/j.envpol.2019.02.094

Dada, A.C., Gyawali, P., 2021. Quantitative microbial risk assessment (QMRA) of occupational exposure to SARS-CoV-2 in wastewater treatment plants. Sci. Total Environ. 763, 142989. doi:10.1016/j.scitotenv.2020.142989

Davis, B.C., Riquelme, M.V., Ramirez-toro, G., Bandaragoda, C., Garner, E., Rhoads, W.J., Vikesland, P., Pruden, A., 2020. Demonstrating an Integrated Antibiotic Resistance Gene

640 Surveillance Approach in Puerto Rican Watersheds Post-Hurricane Maria. Environ. Sci.
641 Technol. 54, 15108–15119. doi:10.1021/acs.est.0c05567
642 Dean, K., Mitchell, J., 2022. Identifying water quality and environmental factors that influence
643 indicator and pathogen decay in natural surface waters. Water Res. 118051.
644 doi:10.1016/j.watres.2022.118051
645 Denpetkul, T., Pumkaew, M., Sittipunsakda, O., Leaungwutiwon, P., Mongkolsuk, S.,
646 Sirikanchana, K., 2022. Effects of face masks and ventilation on the risk of SARS-CoV-2
647 respiratory transmission in public toilets: a quantitative microbial risk assessment. J. Water
648 Health 20, 300–313. doi:10.2166/wh.2022.190
649 Department of Drainage and Sewerage, 2011. 2011 Water Quality in Saen Saep Canal [WWW
650 Document]. URL http://dds.bangkok.go.th/wqmo/data_wqm/data/3_1.htm
651 Ding, K., Wen, X., Li, Y., Shen, B., Zhang, B., 2015. Ammonia-oxidizing archaea versus
652 bacteria in two soil aquifer treatment systems. Appl. Microbiol. Biotechnol. 99, 1337–1347.
653 doi:10.1007/s00253-014-6188-3
654 Dockrell, D.H., 2003. Human herpesvirus 6: Molecular biology and clinical features. J. Med.
655 Microbiol. 52, 5–18. doi:10.1099/jmm.0.05074-0
656 Dong, P., Cui, Q., Fang, T., Huang, Y., Wang, H., 2019. Occurrence of antibiotic resistance
657 genes and bacterial pathogens in water and sediment in urban recreational water. J. Environ.
658 Sci. (China) 77, 65–74. doi:10.1016/j.jes.2018.06.011
659 Ebomah, K.E., Okoh, A.I., 2020. An African perspective on the prevalence, fate and effects of
660 carbapenem resistance genes in hospital effluents and wastewater treatment plant (WWTP)
661 final effluents: A critical review. Heliyon 6, e03899. doi:10.1016/j.heliyon.2020.e03899
662 European Commission, 2017. One Health Action Plan against Antimicrobial Resistance (AMR).

663 Feng, R., Wei, J., Zhang, H., Fan, J., Li, X., Wang, D., Xie, J., Qiao, Z., Li, M., Bai, J., Ma, Z.,
664 2015. National serosurvey of encephalomyocarditis virus in healthy people and pigs in
665 China. Arch. Virol. 160, 2957–2964. doi:10.1007/s00705-015-2591-z
666 Ferreira, S., Luís, Â., Oleastro, M., Pereira, L., Domingues, F.C., 2019. A meta-analytic
667 perspective on Arcobacter spp. antibiotic resistance. J. Glob. Antimicrob. Resist. 16, 130–
668 139. doi:10.1016/j.jgar.2018.12.018
669 Garner, E., Davis, B.C., Milligan, E., Blair, M.F., Keenum, I., Maile-Moskowitz, A., Pan, J.,
670 Gnegy, M., Liguori, K., Gupta, S., Prussin II, A.J., Marr, L.C., Heath, L.S., Vikesland, P.J.,
671 Zhang, L., Pruden, A., 2021. Next Generation Sequencing Approaches to Evaluate Water
672 and Wastewater Quality. Water Res. 194, 116907. doi:10.1016/j.watres.2021.116907
673 Ginn, O., Rocha-Melogno, L., Bivins, A., Lowry, S., Cardelino, M., Nichols, D., Tripathi, S.,
674 Soria, F., Andrade, M., Bergin, M., Deshusses, M.A., Brown, J., 2021. Detection and
675 quantification of enteric pathogens in aerosols near open wastewater canals in cities with
676 poor sanitation. Environ. Sci. Technol. 55, 14758–14771.
677 Gu, X., Tay, Q.X.M., Te, S.H., Saeidi, N., Goh, S.G., Kushmaro, A., Thompson, J.R., Gin,
678 K.Y.H., 2018. Geospatial distribution of viromes in tropical freshwater ecosystems. Water
679 Res. 137, 220–232. doi:10.1016/j.watres.2018.03.017
680 Hammoumi, S., Guy, M., Eloit, M., Bakkali-Kassimi, L., 2012. Encephalomyocarditis virus may
681 use different pathways to initiate infection of primary human cardiomyocytes. Arch. Virol.
682 157, 43–52. doi:10.1007/s00705-011-1133-6
683 He, Yike, Bai, M., He, Yaodong, Wang, S., Zhang, J., Jiang, S., Wang, G., 2022. Suspended
684 particles are hotspots for pathogen-related bacteria and ARGs in coastal beach waters of
685 northern China. Sci. Total Environ. 153004. doi:10.1016/j.scitotenv.2022.153004

686 Ho, J., Stange, C., Suhrborg, R., Wurzbacher, C., Drewes, J.E., Tiehm, A., 2022. SARS-CoV-2
687 wastewater surveillance in Germany: long-term RT-digital droplet PCR monitoring,
688 suitability of primer/probe combinations and biomarker stability. Water Res. 210, 117977.
689 doi:10.1016/j.watres.2021.117977
690 Iamtrakul, P., Raungratanaamporn, I., Klaylee, J., 2018. Contribution on water transportation for
691 resilient and sustainable lowland cities. Lowl. Technol. Int. 20, 341–350.
692 doi:10.0001/ialt_lti
693 Ibarbalz, F.M., Orellana, E., Figuerola, E.L.M., Erijman, L., 2016. Shotgun metagenomic
694 profiles have a high capacity to discriminate samples of activated sludge according to
695 wastewater type. Appl. Environ. Microbiol. 82, 5186–5196. doi:10.1128/AEM.00916-16
696 Ibekwe, A.M., Ma, J., Murinda, S.E., 2016. Bacterial community composition and structure in an
697 urban river impacted by different pollutant sources. Sci. Total Environ. 566–567, 1176–
698 1185. doi:10.1016/j.scitotenv.2016.05.168
699 Institute for Urban Disease Control and Prevention, 2022. 2021 Bangkok Epidemiological
700 Surveillance Report. Department of Disease Control. Ministry of Public Health.
701 Jantharadej, K., Limpiyakorn, T., Kongprajug, A., Mongkolsuk, S., Sirikanchana, K.,
702 Suwannasilp, B.B., 2021. Microbial community compositions and sulfate‐reducing
703 bacterial profiles in malodorous urban canal sediments. Arch. Microbiol.
704 doi:10.1007/s00203-020-02157-7
705 Jin, D., Kong, X., Cui, B., Jin, S., Xie, Y., Wang, X., Deng, Y., 2018. Bacterial communities and
706 potential waterborne pathogens within the typical urban surface waters. Sci. Rep. 8, 1–9.
707 doi:10.1038/s41598-018-31706-w
708 Jurkovic, M., Kalina, T., Morvay, K., Hudcovský, M., Gorzelanczyk, P., 2021. Impacts of Water

709 Transport Development on the Economy and Society. Transp. Res. Procedia 55, 244–251.
710 doi:10.1016/j.trpro.2021.06.028
711 Karkman, A., Pärnänen, K., Larsson, D.G.J., 2019. Fecal pollution can explain antibiotic
712 resistance gene abundances in anthropogenically impacted environments. Nat. Commun.
713 10, 1–8. doi:10.1101/341487
714 Kim, D., Song, L., Breitwieser, F.P., Salzberg, S.L., 2016. Centrifuge: Rapid and sensitive
715 classification of metagenomic sequences. Genome Res. 26, 1721–1729.
716 doi:10.1101/gr.210641.116
717 Kongprajug, A., Chyerochana, N., Rattanakul, S., Denpetkul, T., Sangkaew, W., Somnark, P.,
718 Patarapongsant, Y., Tomyim, K., Sresang, M., Mongkolsuk, S., Sirikanchana, K., 2021a.
719 Integrated analyses of fecal indicator bacteria , microbial source tracking markers , and
720 pathogens for Southeast Asian beach water quality assessment. Water Res. 203, 117479.
721 Kongprajug, A., Chyerochana, N., Somnark, P., Leelapanang Kampaengthong, P., Mongkolsuk,
722 S., Sirikanchana, K., 2019. Human and animal microbial source tracking in a tropical river
723 with multiple land use activities. Int. J. Hyg. Environ. Health 222.
724 doi:10.1016/j.ijheh.2019.01.005
725 Kongprajug, A., Denpetkul, T., Chyerochana, N., Mongkolsuk, S., Sirikanchana, K., 2021b.
726 Human fecal pollution and associated microbial risks in a coastal industrial-residential
727 mixed use watershed. Front. Microbiol. 12. doi:https://doi.org/10.3389/fmicb.2021.647602
728 Kongprajug, Akechai, Mongkolsuk, S., Sirikanchana, K., 2019. CrAssphage as a potential
729 human sewage marker for microbial source tracking in Southeast Asia. Environ. Sci.
730 Technol. Lett. 6, 159–164. doi:10.1021/acs.estlett.9b00041
731 Lapierre, N., Mangul, S., Alser, M., Mandric, I., Wu, N.C., Koslicki, D., Eskin, E., 2019.

MiCoP: Microbial community profiling method for detecting viral and fungal organisms in metagenomic samples. BMC Genomics 20, 1–10. doi:10.1186/s12864-019-5699-9

Lee, S., Suits, M., Wituszynski, D., Winston, R., Martin, J., Lee, J., 2020. Residential urban stormwater runoff: A comprehensive profile of microbiome and antibiotic resistance. Sci. Total Environ. 723, 138033. doi:10.1016/j.scitotenv.2020.138033

Li, H., 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM 00, 1–3.

LiPuma, J.J., 2005. Update on the Burkholderia cepacia complex. Curr. Opin. Pulm. Med. 11, 528–533. doi:10.1097/01.mcp.0000181475.85187.ed

Liu, C., Li, X., Zheng, S., Kai, Z., Jin, T., Shi, R., Huang, H., Zheng, X., 2021. Effects of wastewater treatment and manure application on the dissemination of antimicrobial resistance around swine feedlots. J. Clean. Prod. 280, 123794. doi:10.1016/j.jclepro.2020.123794

Liu, L., Wang, Q., Wu, X., Qi, H., Das, R., Lin, H., Shi, J., Wang, S., Yang, J., Xue, Y., Mao, D., Luo, Y., 2020. Vancomycin exposure caused opportunistic pathogens bloom in intestinal microbiome by simulator of the human intestinal microbial ecosystem (SHIME). Environ. Pollut. 265, 114399. doi:10.1016/j.envpol.2020.114399

Luz, C.F., van Niekerk, J.M., Keizer, J., Beerlage-de Jong, N., Braakman-Jansen, L.M.A., Stein, A., Sinha, B., van Gemert-Pijnen, J.E.W.C., Glasner, C., 2022. Mapping twenty years of antimicrobial resistance research trends. Artif. Intell. Med. 123, 102216. doi:10.1016/j.artmed.2021.102216

Makkaew, P., Kongprajug, A., Chyerochana, N., Sresung, M., Precha, N., Mongkolsuk, S., Sirikanchana, K., 2021. Persisting antibiotic resistance gene pollution and its association

755 with human sewage sources in tropical marine beach waters. Int. J. Hyg. Environ. Health
756 238, 113859. doi:10.1016/j.ijheh.2021.113859
757 Marine Department of Thailand, 2020. 2020 Statistical records for water transport of goods and
758 passengers.
759 Martínez-Puchol, S., Itarte, M., Rusiñol, M., Forés, E., Mejías-Molina, C., Andrés, C., Antón, A.,
760 Quer, J., Abril, J.F., Girones, R., Bofill-Mas, S., 2021. Exploring the diversity of
761 coronavirus in sewage during COVID-19 pandemic: Don't miss the forest for the trees. Sci.
762 Total Environ. 800, 149562. doi:10.1016/j.scitotenv.2021.149562
763 McCall, C., Wu, H., Miyani, B., Xagoraraki, I., 2020. Identification of multiple potential viral
764 diseases in a large urban center using wastewater surveillance. Water Res. 184, 116160.
765 doi:10.1016/j.watres.2020.116160
766 McDaniel, E.A., Wahl, S.A., Ishii, S., Pinto, A., Ziels, R., Nielsen, P.H., McMahon, K.D.,
767 Williams, R.B.H., 2021. Prospects for multi-omics in the microbial ecology of water
768 engineering. Water Res. 205, 117608. doi:10.1016/j.watres.2021.117608
769 McDonnell, G., Russell, A.D., 1999. Antiseptics and Disinfectants: Activity, Action, and
770 Resistance. Clin. Microbiol. Rev. 12, 147–179. doi:10.4135/9781412983907.n399
771 Modi, A.A., Liang, T.J., 2008. Hepatitis C: A clinical review. Oral Dis. 14, 10–14.
772 doi:10.1111/j.1601-0825.2007.01419.x
773 Mohiuddin, M., Schellhorn, H.E., 2015. Spatial and temporal dynamics of virus occurrence in
774 two freshwater lakes captured through metagenomic analysis. Front. Microbiol. 6, 1–13.
775 doi:10.3389/fmicb.2015.00960
776 Morita, Y., Maruyama, S., Kabeya, H., Boonmar, S., Nimsuphan, B., Nagai, A., Kozawa, K.,
777 Nakajima, T., Mikami, T., Kimura, H., 2004. Isolation and phylogenetic analysis of

778 Arcobacter spp. in ground chicken meat and environmental water in Japan and Thailand.
779 Microbiol. Immunol. 48, 527–533. doi:10.1111/j.1348-0421.2004.tb03548.x
780 National Environment Board, 2017. Notification of the National Environmental Board, No.
781 134/288, B.E. 2560 (2017), entitled Coastal Water Quality Standards (in Thai). R. Gov.
782 Gaz. 134, 28–37.
783 Oberste, M.S., Gotuzzo, E., Blair, P., Nix, W.A., Ksiazek, T.G., Comer, J.A., Rollin, P.,
784 Goldsmith, C.S., Olson, J., Kochel, T.J., 2009. Human febrile illness caused by
785 encephalomyocarditis virus infection, peru. Emerg. Infect. Dis. 15, 640–646.
786 doi:10.3201/eid1504.081428
787 Obieze, C.C., Wani, G.A., Shah, M.A., Reshi, Z.A., Comeau, A.M., Khasa, D.P., 2022.
788 Anthropogenic activities and geographic locations regulate microbial diversity, community
789 assembly and species sorting in Canadian and Indian freshwater lakes. Sci. Total Environ.
790 826, 154292. doi:10.1016/j.scitotenv.2022.154292
791 Obregon-Tito, A.J., Tito, R.Y., Metcalf, J., Sankaranarayanan, K., Clemente, J.C., Ursell, L.K.,
792 Zech Xu, Z., Van Treuren, W., Knight, R., Gaffney, P.M., Spicer, P., Lawson, P., Marin-
793 Reyes, L., Trujillo-Villarroel, O., Foster, M., Guija-Poma, E., Troncoso-Corzo, L.,
794 Warinner, C., Ozga, A.T., Lewis, C.M., 2015. Subsistence strategies in traditional societies
795 distinguish gut microbiomes. Nat. Commun. 6, 1–9. doi:10.1038/ncomms7505
796 Ott, A., O'Donnell, G., Tran, N.H., Mohd Haniffah, M.R., Su, J.-Q., Zealand, A.M., Gin, K.Y.-
797 H., Goodson, M.L., Zhu, Y.-G., Graham, D.W., 2021. Developing surrogate markers for
798 predicting antibiotic resistance "Hot Spots" in rivers where limited data are available.
799 Environ. Sci. Technol. 55, 7466–7478. doi:10.1021/acs.est.1c00939
800 Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: Statistical analysis of

taxonomic and functional profiles. Bioinformatics 30, 3123–3124. doi:10.1093/bioinformatics/btu494

PCD, 2016. Sources of pollution along the Saen Saep canal. Pollution Control Department, Ministry of Natural Resources and Environment, Thailand, 1-23.

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., Duchesnay, E., 2011. Scikit-learn: Machine Learning in Python. J. Mach. Learn. Res. 12, 2825–2830. doi:10.1145/2786984.2786995

Ping, Q., Zhang, Z., Ma, L., Yan, T., Wang, L., Li, Y., 2022. The prevalence and removal of antibiotic resistance genes in full-scale wastewater treatment plants: Bacterial host, influencing factors and correlation with nitrogen metabolic pathway. Sci. Total Environ. 827, 154154. doi:10.1016/j.scitotenv.2022.154154

Pollution Control Department (PCD), 2018. Thailand State of Pollution Report 2017 (in Thai).

Pringsulaka, O., Suwannasai, N., Sunthornthummas, S., 2017. Assessment of Indicator Microorganisms and Fungi : Health Risk in the Saen Saeb Canal , Thailand. Chiang Mai J Sci 44, 309–321.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596. doi:10.1093/nar/gks1219

R Core Team, 2019. R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL https//www.R-project.org/.

Ragupathi, N.K.D., Veeraraghavan, B., 2019. Accurate identification and epidemiological characterization of Burkholderia cepacia complex: An update. Ann. Clin. Microbiol.

824 Antimicrob. 18, 1–10. doi:10.1186/s12941-019-0306-0
825 Ranjan, R., Rani, A., Metwally, A., McGee, H.S., Perkins, D.L., 2016. Analysis of the
826 microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing.
827 Biochem. Biophys. Res. Commun. 469, 967–977. doi:10.1016/j.bbrc.2015.12.083
828 Raza, S., Shin, H., Hur, H.G., Unno, T., 2022. Higher abundance of core antimicrobial resistant
829 genes in effluent from wastewater treatment plants. Water Res. 208, 117882.
830 doi:10.1016/j.watres.2021.117882
831 Runcharoen, C., Moradigaravand, D., Blane, B., Paksanont, S., Thammachote, J., Anun, S.,
832 Parkhill, J., Chantratita, N., Peacock, S.J., 2017. Whole genome sequencing reveals high-
833 resolution epidemiological links between clinical and environmental Klebsiella
834 pneumoniae. Genome Med. 9, 1–10. doi:10.1186/s13073-017-0397-1
835 Sabar, M.A., Honda, R., Haramoto, E., 2022. CrAssphage as an indicator of human-fecal
836 contamination in water environment and virus reduction in wastewater treatment. Water
837 Res. 221, 118827. doi:10.1016/j.watres.2022.118827
838 Sangkaew, W., Kongprajug, A., Chyerochana, N., Ahmed, W., Rattanakul, S., Denpetkul, T.,
839 Mongkolsuk, S., Sirikanchana, K., 2021. Performance of viral and bacterial genetic markers
840 for sewage pollution tracking in tropical Thailand. Water Res. 190, 116706.
841 doi:https://doi.org/10.1016/j.watres.2020.116706
842 Sangsanont, J., Rattanakul, S., Kongprajug, A., Chyerochana, N., Sresung, M., Sriporatana, N.,
843 Wanlapakorn, N., Poovorawan, Y., Mongkolsuk, S., Sirikanchana, K., 2022. SARS-CoV-2
844 RNA surveillance in large to small centralized wastewater treatment plants preceding the
845 third COVID-19 resurgence in Bangkok, Thailand. Sci. Total Environ. 809, 151169.
846 doi:10.1016/j.scitotenv.2021.151169

847 Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C.,
848 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60.
849 doi:10.1186/gb-2011-12-6-r60
850 Selenic, D., Dodson, D.R., Jensen, B., Arduino, M.J., Panlilio, A., Archibald, L.K., 2003.
851 Enterobacter cloacae bloodstream infections in pediatric patients traced to a hospital
852 pharmacy. Am. J. Heal. Pharm. 60, 1440–1446. doi:10.1093/ajhp/60.14.1440
853 Shahin, S., Keevy, H., Dada, A.C., Gyawali, P., Sherchan, S.P., 2021. Incidence of human
854 associated HF183 Bacteroides marker and E. coli levels in New Orleans Canals. Sci. Total
855 Environ. 150356.
856 Shrestha, R.G., Tanaka, Y., Haramoto, E., 2022. A Review on the Prevalence of Arcobacter in
857 Aquatic Environments. Water 14, 1–17. doi:10.2174/157340011797183201
858 Sirikanchana, K., Wangkahad, B., Mongkolsuk, S., 2014. The capability of non-native strains of
859 Bacteroides bacteria to detect bacteriophages as faecal indicators in a tropical area. J. Appl.
860 Microbiol. 117, 1820–1829. doi:10.1111/jam.12646
861 Smyth, D.S., Trujillo, M., Gregory, D.A., Cheung, K., Gao, A., Graham, M., Guan, Y.,
862 Guldenpfennig, C., Hoxie, I., Kannoly, S., Kubota, N., Lyddon, T.D., Markman, M.,
863 Rushford, C., San, K.M., Sompanya, G., Spagnolo, F., Suarez, R., Teixeiro, E., Daniels, M.,
864 Johnson, M.C., Dennehy, J.J., 2022. Tracking cryptic SARS-CoV-2 lineages detected in
865 NYC wastewater. Nat. Commun. 13, 1–9. doi:10.1038/s41467-022-28246-3
866 Sobsey, M.D., Meschke, J.S., 2003. Virus survival in the environment with special attention to
867 survival in sewage droplets and other environmental media of fecal or respiratory origin.
868 Rep. World Heal. Organ. Geneva, Switz. 70 pages.
869 Stachler, E., Bibby, K., 2014. Metagenomic Evaluation of the Highly Abundant Human Gut

870 Bacteriophage CrAssphage for Source Tracking of Human Fecal Pollution. Environ. Sci.
871 Technol. Lett. 1, 405–409. doi:10.1021/ez500266s
872 Stange, C., Sidhu, J.P.S., Tiehm, A., Toze, S., 2016. Antibiotic resistance and virulence genes in
873 coliform water isolates. Int. J. Hyg. Environ. Health 219, 823–831.
874 doi:10.1016/j.ijheh.2016.07.015
875 Tanko, M., Burke, M.I., 2017. Transport innovations and their effect on cities: The emergence of
876 urban linear ferries worldwide. Transp. Res. Procedia 25, 3957–3970.
877 doi:10.1016/j.trpro.2017.05.483
878 Tomioka, N., Yoochatchaval, W., Takemura, Y., Matsuura, N., Danshita, T., Srisang, P.,
879 Mungjomklang, N., Syutsubo, K., 2021. Detection of potentially pathogenic Arcobacter
880 spp. in Bangkok canals and the Chao Phraya River. J. Water Health 19, 657–670.
881 doi:10.2166/wh.2021.239
882 Tseng, C.H., Chiang, P.W., Shiah, F.K., Chen, Y.L., Liou, J.R., Hsu, T.C., Maheswararajah, S.,
883 Saeed, I., Halgamuge, S., Tang, S.L., 2013. Microbial and viral metagenomes of a
884 subtropical freshwater reservoir subject to climatic disturbances. ISME J. 7, 2374–2386.
885 doi:10.1038/ismej.2013.118
886 Ung, P., Peng, C., Yuk, S., Tan, R., Ann, V., Miyanaga, K., Tanji, Y., 2019. Dynamics of
887 bacterial community in Tonle Sap Lake, a large tropical flood-pulse system in Southeast
888 Asia. Sci. Total Environ. 664, 414–423. doi:10.1016/j.scitotenv.2019.01.351
889 United Nations Economic Commission for Europe, 2011. White paper on Efficient and
890 Sustainable Inland Water Transport in Europe. ECE/TRANS/SC.3/189. Inland Transport
891 Committee Working Party on Inland Water Transport.
892 Van Driessche, E., Houf, K., 2008. Survival capacity in water of Arcobacter species under

different temperature conditions. J. Appl. Microbiol. 105, 443–451. doi:10.1111/j.1365-2672.2008.03762.x

Verhougstraete, M.P., Pogreba-Brown, K., Reynolds, K.A., Lamparelli, C.C., Zanoli Sato, M.I., Wade, T.J., Eisenberg, J.N.S., 2020. A critical analysis of recreational water guidelines developed from temperate climate data and applied to the tropics. Water Res. 170. doi:10.1016/j.watres.2019.115294

Völker, S., Kistemann, T., 2011. The impact of blue space on human health and well-being - Salutogenetic health effects of inland surface waters: A review. Int. J. Hyg. Environ. Health 214, 449–460. doi:10.1016/j.ijheh.2011.05.001

Waite, D.W., Vanwonterghem, I., Rinke, C., Parks, D.H., Zhang, Y., Takai, K., Sievert, S.M., Simon, J., Campbell, B.J., Hanson, T.E., Woyke, T., Klotz, M.G., Hugenholtz, P., 2017. Comparative genomic analysis of the class Epsilonproteobacteria and proposed reclassification to epsilonbacteraeota (phyl. nov.). Front. Microbiol. 8. doi:10.3389/fmicb.2017.00682

Wang, P., Chen, B., Yuan, R., Li, C., Li, Y., 2016. Characteristics of aquatic bacterial community and the influencing factors in an urban river. Sci. Total Environ. 569–570, 382–389. doi:10.1016/j.scitotenv.2016.06.130

Wangkahad, B., Bosup, S., Mongkolsuk, S., Sirikanchana, K., 2015. Occurrence of bacteriophages infecting Aeromonas, Enterobacter, and Klebsiella in water and association with contamination sources in Thailand. J. Water Health 13, 613–624. doi:10.2166/wh.2014.204

WHO, 2018. Guidelines on sanitation and health, World Health Organization.

WHO, 2017. Global Antimicrobial Resistance Surveillance System (GLASS) Report, Who.

doi:ISBN 978-92-4-151344-9

WHO, 2015. Global Action Plan on Antimicrobial Resistance, World Health Organisation.

Wu, M., Li, X., 2015. Chapter 87 Klebsiella pneumoniae and Pseudomonas aeruginosa, in: Molecular Medical Microbiology: Second Edition. Elsevier Ltd, pp. 1547–1564. doi:10.1016/B978-0-12-397169-2.00087-1

Xie, J., Jin, L., Wu, D., Pruden, A., Li, X., 2022. Inhalable antibiotic resistome from wastewater treatment plants to urban areas: Bacterial hosts, dissemination risks, and source contributions. Environ. Sci. Technol. doi:10.1021/acs.est.1c07023

Xu, C., Zhu, H., Qiu, P., 2019. Aging progression of human gut microbiota. BMC Microbiol. 19, 1–10. doi:10.1186/s12866-019-1616-2

Yang, P., Hao, S., Han, M., Xu, J., Yu, S., Chen, C., Zhang, H., Ning, K., 2022. Analysis of antibiotic resistance genes reveals its important role in influencing the community structure of ocean microbiome. Sci. Total Environ. 153731. doi:10.1016/j.scitotenv.2022.153731

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., Heath, A.C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J.G., Lozupone, C.A., Lauber, C., Clemente, J.C., Knights, D., Knight, R., Gordon, J.I., 2012. Human gut microbiome viewed across age and geography. Nature 486, 222–227. doi:10.1038/nature11053

Zheng, D., Yin, G., Liu, M., Chen, C., Jiang, Y., Hou, L., Zheng, Y., 2021. A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. Sci. Total Environ. 777, 146009. doi:10.1016/j.scitotenv.2021.146009

Zou, Y., Wu, M., Liu, J., Tu, W., Xie, F., Wang, H., 2022. Deciphering the extracellular and intracellular antibiotic resistance genes in multiple environments reveals the persistence of

extracellular ones. J. Hazard. Mater. 429, 128275. doi:10.1016/j.jhazmat.2022.128275

## Figure legends

**Fig. 1.** Map of the sampling sites along the Saen Saep Canal. Sites A and B (green) are located in the cultural zone, sites C–F (orange) in the commercial zone, and sites G–J (yellow) in the suburban residential zone.

**Fig. 2.** Principal coordinate analysis of the microbial communities in the canal water samples from sites A–J when analyzed using shotgun metagenomic sequencing (a) and 16s rRNA amplicon sequencing (b)

**Fig. 3.** The relative abundance of the 40 most abundant microorganisms in the genus levels in the canal water samples from sites A–J analyzed using shotgun metagenomic sequencing (a) and 16S rRNA gene sequencing (b)

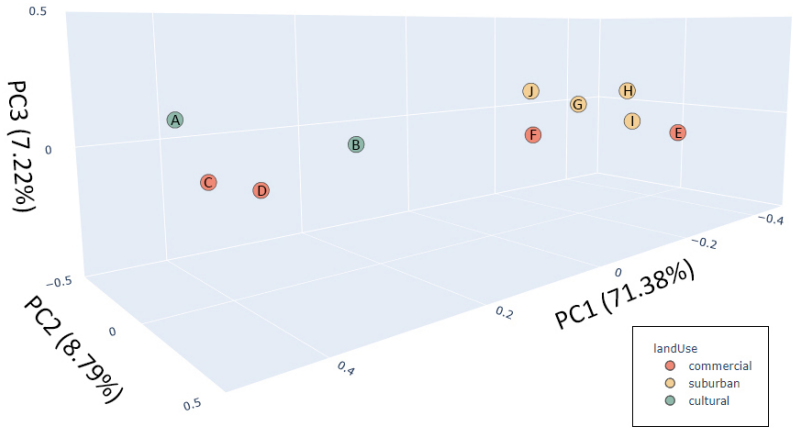
**Fig. 4.** Relative abundance of the 35 most abundant pathogenic bacteria in the canal water samples from sites A–J using shotgun metagenomic sequencing

**Fig. 5**. The relative abundance of the 35 most abundant viruses in the canal water samples from sites A–J using shotgun metagenomic sequencing

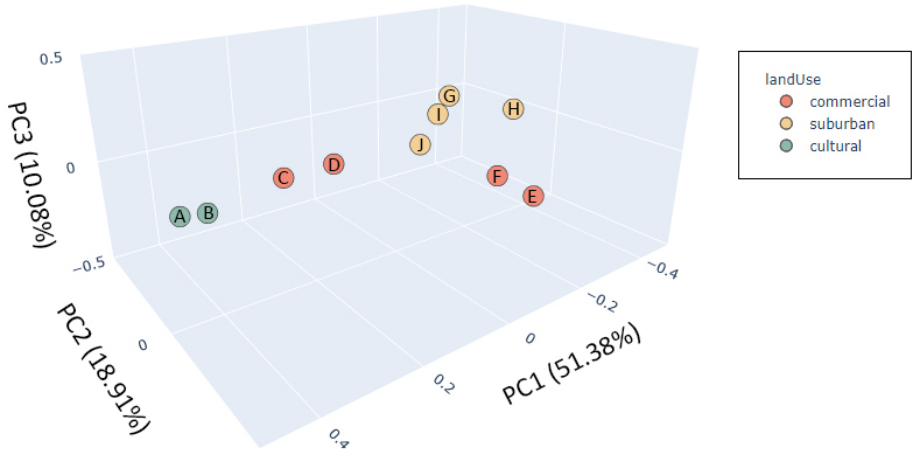
**Fig. 6.** The relative abundance of the human viral pathogens in the canal water samples from sites A–J using shotgun metagenomic sequencing

**Fig. 7**. The relative abundance of the ARG types in the canal water samples from sites A–J using shotgun metagenomic sequencing (a) and the ARG types that showed significant differences in line with the land use sites (b). The other ARG types include those related to resistance to fusidic acid, glycopeptide, nitroimidazole, rifampicin, and fosfomycin.


**Fig. 1.** Map of the sampling sites along the Saen Saep Canal. Sites A and B (green) are located in the cultural zone, sites C–F (orange) in the commercial zone, and sites G–J (yellow) in the suburban residential zone.

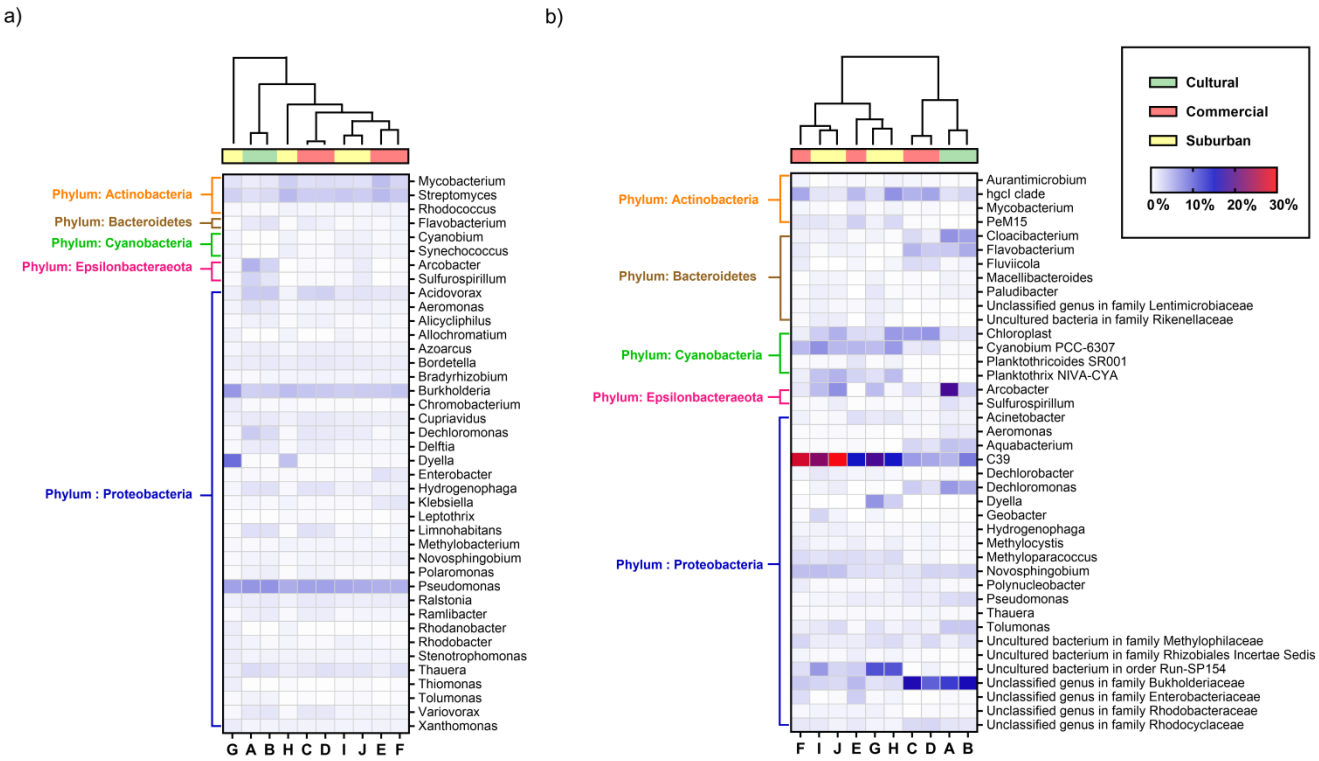



(a)




(b)

**Fig. 2.** Principal coordinate analysis of the microbial communities in the canal water samples from sites A–J when analyzed using shotgun metagenomic sequencing (a) and 16s rRNA amplicon sequencing (b)



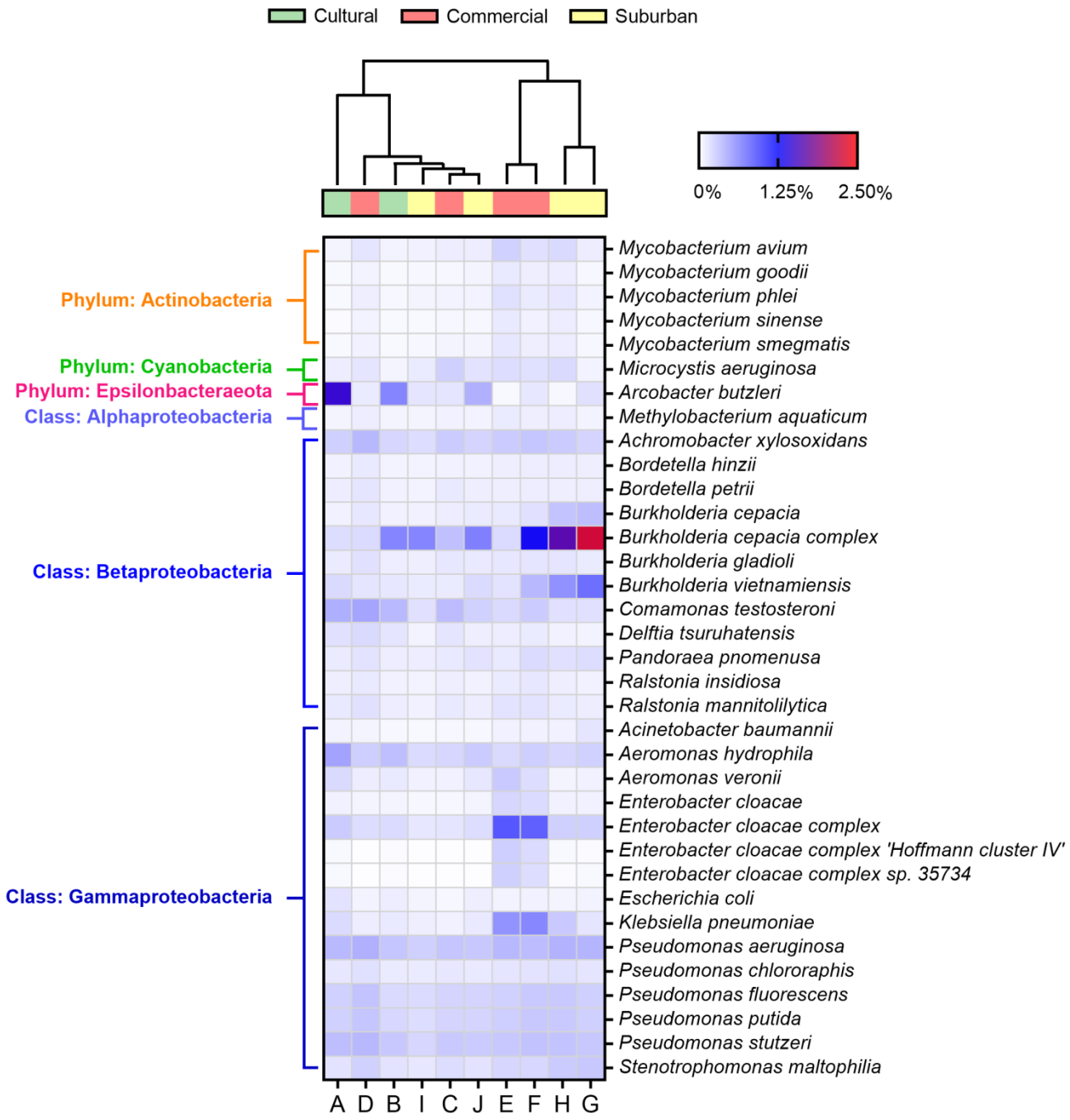

978

979 **Fig. 3.** The relative abundance of the 40 most abundant microorganisms in the genus levels in the

980 canal water samples from sites A–J analyzed using shotgun metagenomic sequencing (a) and

981 16S rRNA gene sequencing (b)

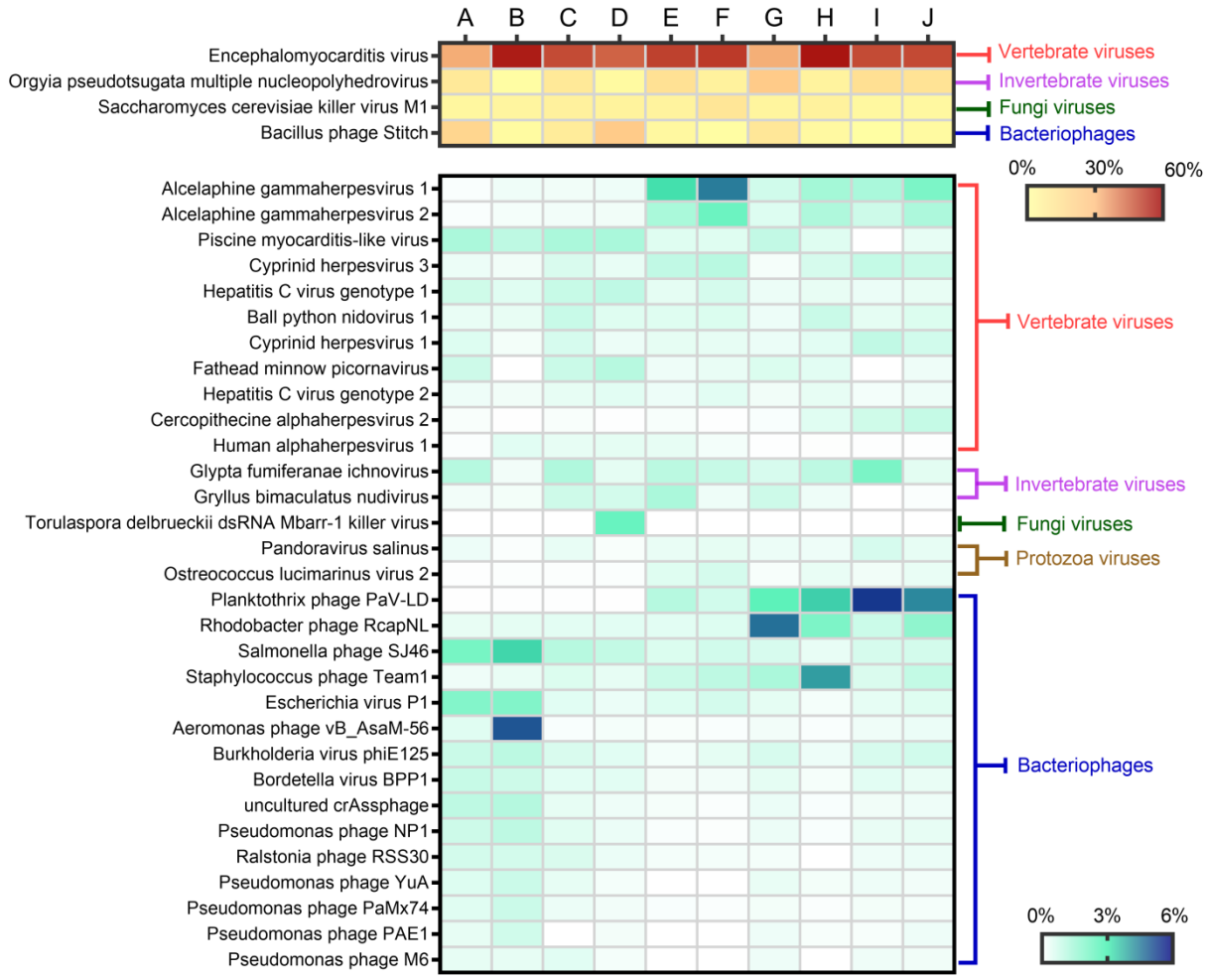
982




983

984 **Fig. 4.** Relative abundance of the 35 most abundant pathogenic bacteria in the canal water

985 samples from sites A–J using shotgun metagenomic sequencing

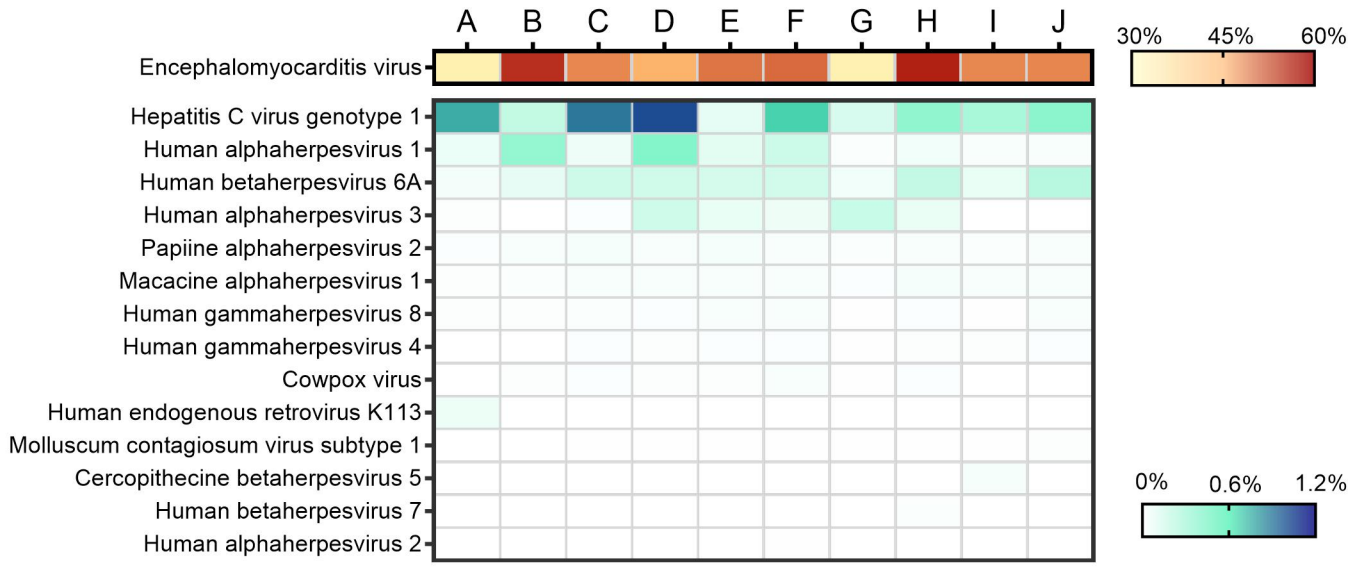



986

987 **Fig. 5**. The relative abundance of the 35 most abundant viruses in the canal water samples from

988 sites A–J using shotgun metagenomic sequencing

989

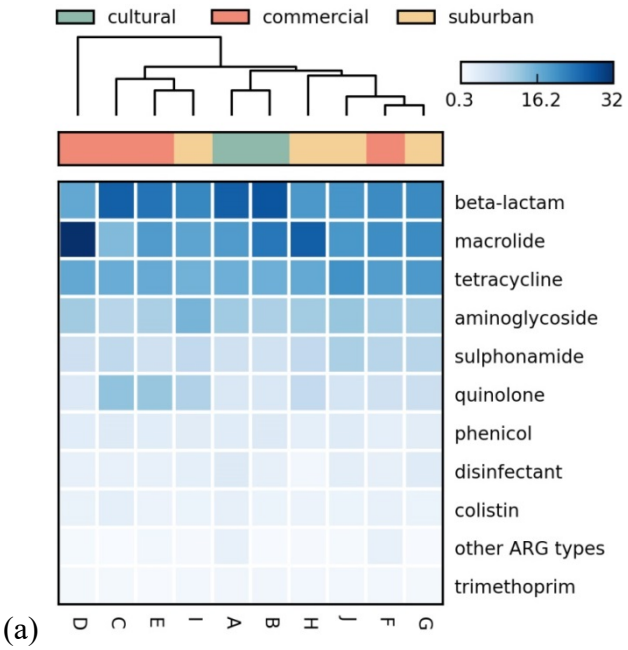



990

991 **Fig. 6.** The relative abundance of the human viral pathogens in the canal water samples from

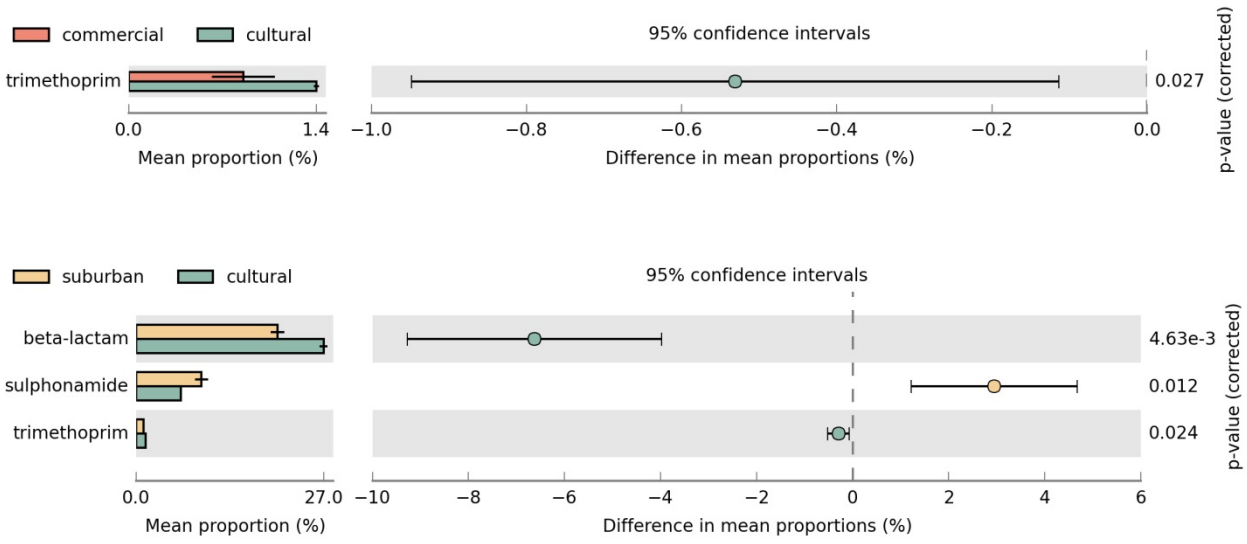
992 sites A–J using shotgun metagenomic sequencing

993




994 (a)

995 (b)




996

997 **Fig. 7**. The relative abundance of the ARG types in the canal water samples from sites A–J using
998 shotgun metagenomic sequencing (a) and the ARG types that showed significant differences in
999 line with the land use sites (b). The other ARG types include those related to resistance to fusidic
1000 acid, glycopeptide, nitroimidazole, rifampicin, and fosfomycin.

1001