

Bacterial community profiles of select tributaries of Laguna Lake in the Philippines

Arizaldo E. Castro,¹ Andrew D. Montecillo,² Ren Mark D. Villanueva,¹ Marie Christine M. Obusan¹

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT Laguna Lake is known for its ecological, economic, and cultural importance. Effects of urbanization and accumulation of emerging pollutants have been associated with its water quality; however, the microbial ecology of its tributaries remains to be explored. We report bacterial community profiles from shotgun metagenomes of its select tributary waters.

KEYWORDS Laguna Lake, bacterial community, emerging pollutants, microbial ecology, Philippines, metagenomic analysis

Laguna Lake is a class C freshwater body that supports fisheries, agriculture, and livestock within nearby regions. It covers 911.7 km² and holds 3.2 billion cubic meters of water (1). It receives water from about 100 rivers and streams, mainly from the east. Urban rivers and waterways, serving as pollutant carriers from residential and commercial areas, affect the lake. Key tributaries of interest are those in Metro Manila and Laguna due to urbanization. Heavy metals and antibiotic-resistant bacteria (2), along with fecal indicators, have been found in the lake (3, 4). Therefore, analyzing its bacterial communities is crucial due to their role as pollution indicators. Three tributaries were profiled in this study: San Cristobal River ([14.236944 N, 121.113611 E](#)), Mangangate River ([14.430278 N, 121.045 E](#)), and Sapang Malapit Creek ([14.571455 N 121.089539 E](#)). San Cristobal River, one of the most polluted rivers influencing the lake's health, contributes about 5% of Laguna Lake's total water (5, 6). Mangangate River in Muntinlupa City, 10 km long, has high fecal contamination. Sapang Malapit Creek in Pasig City is a highly polluted waterway spanning 160 m in length (7). These tributaries collect waste from nearby agro-industrial and residential areas.

Three samples per site were collected during the 2021 Philippine southwest monsoon. For each sample, 1,000 mL of water from 1 m depth was collected using sterile amber bottles. Samples were settled to remove suspended solids. After this, pre-filtration and vacuum filtration with 0.45 µm nitrocellulose membranes were performed, using 3,000 mL per site. Membrane filters were processed for total eDNA extraction using QIAGEN DNeasy PowerWater Kit immediately after filtration. Extracted DNA was stored at -20°C. Library preparation was performed using the Illumina Nextera XT DNA kit, and DNA sequencing was conducted on a NovaSeq 6000 platform (2 × 100 bp). Reads for all biological replicates per site were quality controlled with fastp (0.23.2), trimming adapters, and discarding low-quality sequences (<Q20). Reads were annotated using mOTUs2 (2.5.1) to determine taxonomic profiles using phylogenetic marker gene-based operational taxonomic units (mOTUs) at ≥97% minimum nucleotide identity. The metagenomes contained 98.46% to 99.56% bacteria and 0.09% to 0.37% archaea. Using the mean relative abundances of the three replicates per site, the major bacterial phyla included Proteobacteria (78.58% to 91.13%), Bacteroidetes (3.72% to 13.01%), Firmicutes (0.54% to 3.58%), and Actinobacteria (0.37% to 2.25%) (Fig. 1). At the species level, 918 mOTUs were identified. Across three sites, nine mOTUs had mean

Editor Frank J. Stewart, Montana State University, Bozeman, Montana, USA

Address correspondence to Marie Christine M. Obusan, mmobusan@up.edu.ph.

The authors declare no conflict of interest.

See the funding table on p. 3.

Received 6 December 2023

Accepted 2 April 2024

Published 17 April 2024

Copyright © 2024 Castro et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](#).

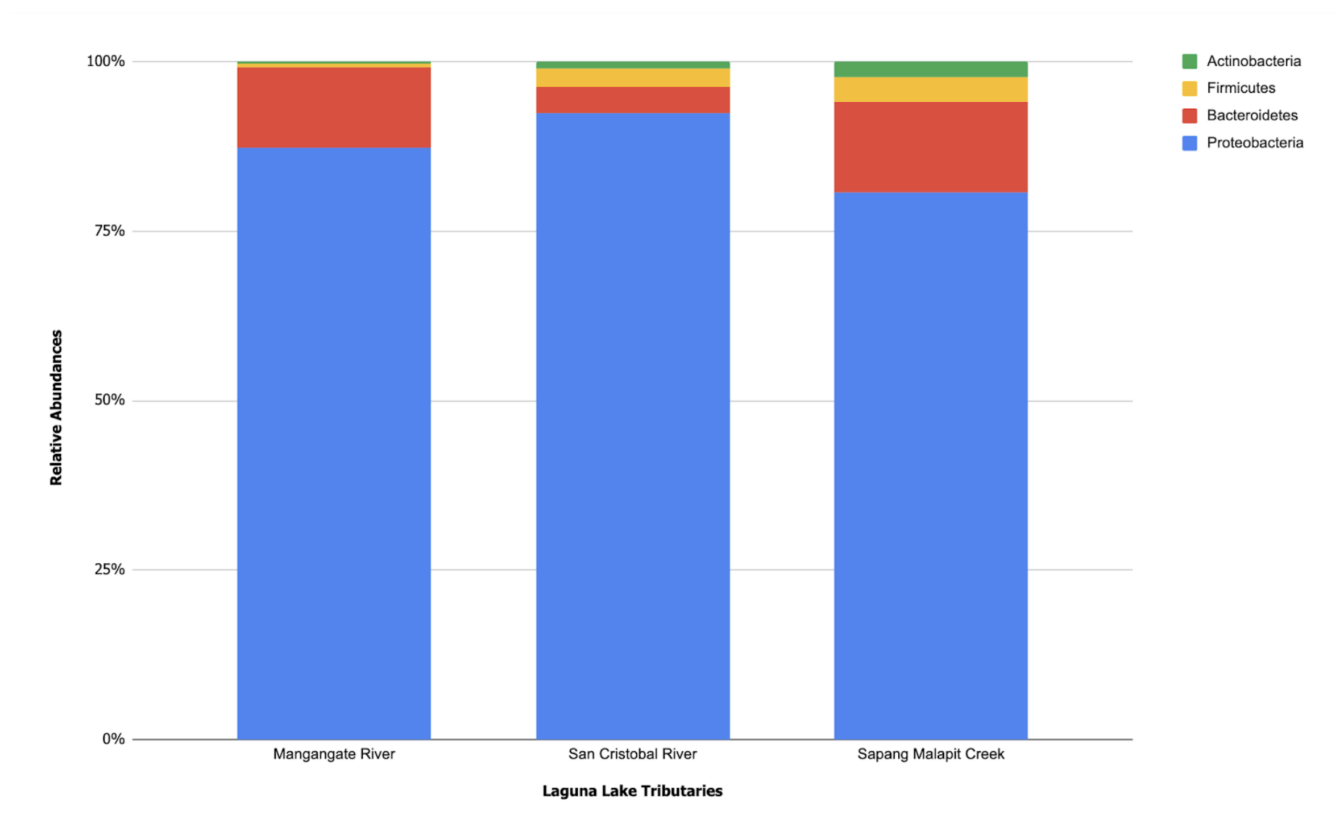


FIG 1 Phylum-level community profiles of Laguna Lake tributaries San Cristobal River, Mangangate River, and Sapang Malapit Creek.

relative abundances over 2.00%: *Acinetobacter towneri* (2.00%), *Acinetobacter harbinensis* (2.09%), *Tolumonas auensis* (2.57%), *Bacteroides graminisolvans* (2.69%), *Parabacteroides chartae* (4.32%), *Sulfurospirillum* sp. (4.34%), *Acidovorax temperans* (5.51%), *Dechloromonas agitata* (11.56%), and *Aeromonas* sp. (12.21%).

The presence and abundance of potentially pathogenic bacteria, specifically *Acinetobacter* and *Aeromonas*, raise concerns about infectious microbes in Laguna Lake, considering its recreational and economic purposes.

ACKNOWLEDGMENTS

This project is made possible by a grant from the Department of Science and Technology-Philippine Council for Health Research and Development (DOST-PCHRD) received by Marie Christine M. Obusan through Project 1 (Microbial Communities As Sentinels of Environmental Pollutants in Metro Manila's Drinking Water Resources) of the research program "Water Assessment of Teratogens and Endocrine Disruptors on Fetal-Maternal Health (WATER-FeMaH)."

Sampling permit from the Laguna Lake Development Authority (LLDA) was secured prior to water sample collection.

AUTHOR AFFILIATIONS

¹Microbial Ecology of Terrestrial and Aquatic Systems Laboratory, Institute of Biology, College of Science, University of the Philippines Diliman, Quezon City, Philippines

²Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Los Baños, Laguna, Philippines

AUTHOR ORCID*s*

Arizaldo E. Castro  <http://orcid.org/0000-0001-6929-5407>

Andrew D. Montecillo  <http://orcid.org/0000-0003-0627-1920>

FUNDING

Funder	Grant(s)	Author(s)
Department of Science and Technology, Philippines (DOST)		Marie Christine M. Obusan

DATA AVAILABILITY

The raw sequences obtained in this project are available in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) under the following accession numbers: [SRR18609915](#) (San Cristobal River), [SRR18609916](#) (Mangagate River), and [SRR26638712](#) (Sapang Malapit Creek).

REFERENCES

1. Delos Reyes M, Martens R. 1994. Geoecology of Laguna de Bay, Philippines I. Techno-commercial impact on the trophic level structure of the Laguna de Bay aquatic ecosystem 1968–1980. *Ecological Modelling* 75–76:497–509. [https://doi.org/10.1016/0304-3800\(94\)90043-4](https://doi.org/10.1016/0304-3800(94)90043-4)
2. Ntabugi KMM, Manegabe BJ, Dewar JB, Simbahan JF, Flavie ME, Sekomo CB. 2021. Synergistic increase in antibiotic resistance with tolerance to cadmium and lead in environmental bacteria isolated from the San Cristobal River, Laguna de Bay, Philippines. *International Journal of Environmental Studies* 78:165–183. <https://doi.org/10.1080/00207233.2020.1834307>
3. Dela Peña LBRO, Labrador KL, Nacario MAG, Bolo NR, Rivera WL. 2021. Microbial source tracking of fecal contamination in Laguna Lake, Philippines using the library-dependent method, rep-PCR. *J Water Health* 19:762–774. <https://doi.org/10.2166/wh.2021.119>
4. Abello JJM, Malajacan GT, Labrador KL, Nacario MAG, Galarion LH, Obusan MCM, Rivera WL. 2021. Library-independent source tracking of fecal contamination in selected stations and tributaries of Laguna Lake, Philippines. *J Water Health* 19:846–854. <https://doi.org/10.2166/wh.2021.058>
5. Piggeries, poultry farms polluting Laguna Lake suspended: DENR. 2019. Philippine News Agency. Available from: <https://www.pna.gov.ph/articles/1060113>
6. Daño AM, Fortus KRM. 2015. San Cristobal watershed vulnerability assessment to soil erosion and water pollution. *Sylvatrop Tech J Philippine Ecosyst Nat Resour* 25:27–50.
7. DENR MEO EAST NAGSAGAWA NG JOINT CLEANUP ACTIVITY SA SAPANG MALAPIT CREEK. 2023 Available from: <https://ncr.denr.gov.ph/index.php/news-events/photo-releases/denr-meo-east-nagsagawa-ng-joint-cleanup-activity-sa-sapang-malapit-creek>