



C, G and K) and downstream (Site A) of Selangor River Basin in Peninsular Malaysia. The map above shows all the stations in the study whereas the table below shows the month and year of sampling. Some of the selected sites are also shown in the pictures below.

Sample name	Site	Month	Year
A0120	A	January	2020
C0120	C	January	2020
A0722	A	July	2022
C0722	C	July	2022
G0722	G	July	2022
K0722	K	July	2022
A1122	A	November	2022
G0923	G	September	2023
K0923	K	September	2023
A1023	A	October	2023
C1023	C	October	2023
L1023	L	October	2023

*In situ* physicochemical parameters [temperature, salinity, pH, and dissolved oxygen (DO)], dissolved inorganic nutrients [nitrate (NO<sub>3</sub>), nitrite (NO<sub>2</sub>), ammonium (NH<sub>4</sub>), phosphate (PO<sub>4</sub>), and silicate (SiO<sub>4</sub>)], chemical oxygen demand (COD), biological oxygen demand (BOD) and total suspended solid (TSS) were measured.



Station C



Station G



Station L

*Quantification of selected antibiotic*

Water samples collected were kept frozen, and transported to Center for Marine Environmental Studies (CMES), Ehime University for antibiotics analyses. The concentration of selected antibiotics from six different classes (Macrolides, fluoroquinolones, tetracyclines, sulfonamides, Trimethoprim, beta lactam) were measured using HPLC and LC-MS/MS in CMES.

### *Bacterial community profile*

A volume of 200-500 mL of water samples were filtered on 0.2µm pore size Isopore membrane, depending on the water turbidity. Bacterial DNA extractions were carried out using DNEasy PowerWater kit and checked for concentration using Qubit 3.0 fluorometer before sending for sequencing. DNA samples were sent for whole genome metagenomic analysis via high throughput sequencing (Illumina Novaseq), with library preparation.

### *Quantification of antibiotic resistance genes in the metagenomic reads*

The processed metagenomic reads were also screened for antibiotic resistance genes using bioinformatic tools with the Comprehensive Antibiotic Resistance Database (CARD) database. The quantification of antibiotic resistance genes was carried out using the normalized reads.

### **Results/Activities**

Online meetings via Zoom platform were carried out regularly. Prof Kozo has visited the sampling locations in Malaysia from 5<sup>th</sup> to 7<sup>th</sup> of June, 2023. The Collaborative Research Agreement and a Material Transfer agreement between Ehime University and Universiti Malaya were signed and completed during the visit.

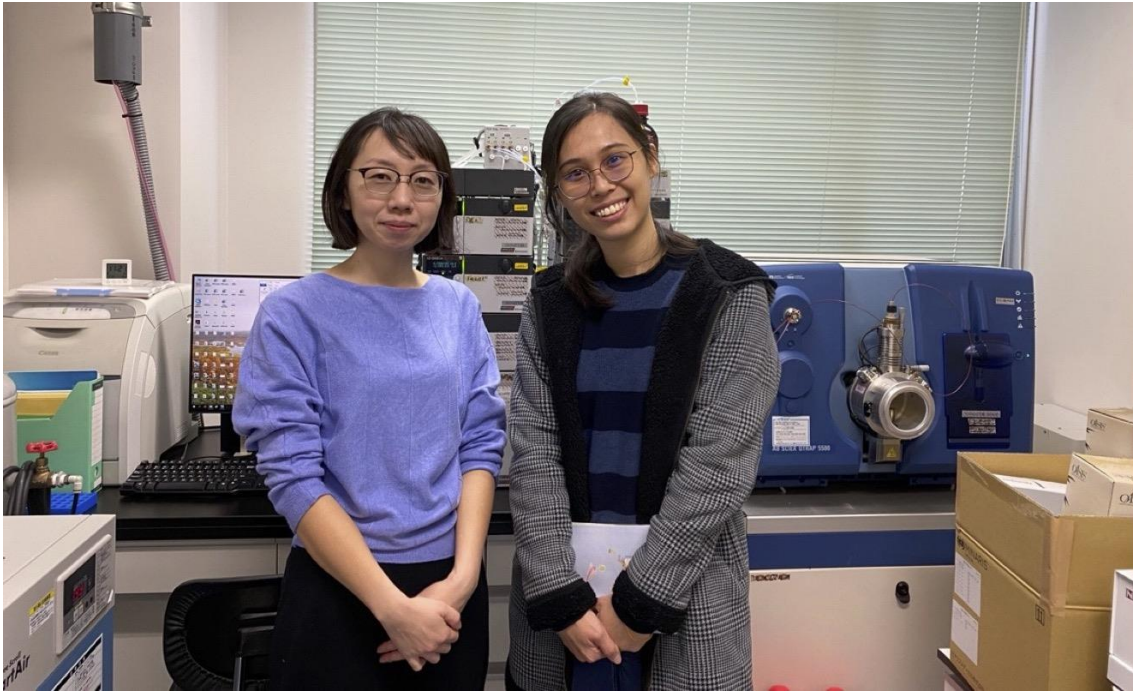


Picture taken in Universiti Malaya, from left: Assoc. Prof. Dr Lee Choon Weng (Universiti Malaya), Professor Dr Kozo Watanabe (Ehime University), Associate Professor Dr Bong Chui Wei (Universiti Malaya), Wong Yi You (Universiti Malaya)

In February 2024, Wong Yi You went to CMES, Ehime University, Japan to learn antibiotic extractions and LC-MS/MS processes to screen for 50 antibiotics, including Macrolides, fluoroquinolones, tetracyclines, sulfonamides, Trimethoprim, beta lactam, from the water samples. The extraction and analyses with LC-MS/MS were carried out with the help of Assistant Professor Dr Rumi Tanoue (Ehime University). A total of eight antibiotics (Trimethoprim, Lincomycin, Clindamycin, Clarithromycin, Ciprofloxacin, Fluconazole, Metronidazole and Florfenicol) ranging from 0.14 – 5 ng/L were detected from the 12 samples.



Pre-treatment before antibiotic extraction.



Picture taken in CMES, Japan, on the left is Assistant Professor Dr Rumi Tanoue (Ehime University).

Raw data from the 12 metagenomic sequencings were successfully received. During the stay in Japan, Wong Yi You also learnt data analyses for the metagenome data available. Currently, analyses using bioinformatic tools for the bacterial community profile and antibiotic resistance genes are ongoing.



### **Future challenges**

The main challenges will be the subsequent analyses of data using different bioinformatic tools. The results from antibiotic detection, and metagenome sequencing will be analysed along with the environmental factors for temporal and spatial variation.