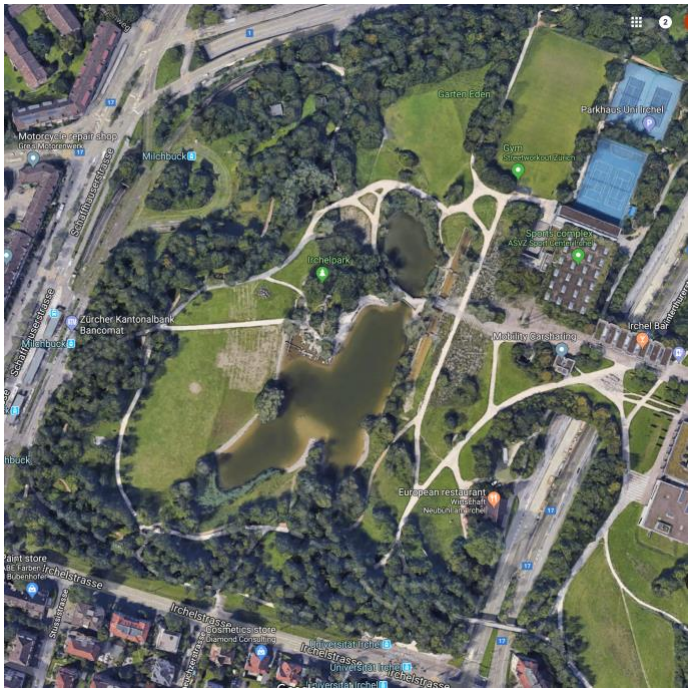
Experiment II. Group soil sampling

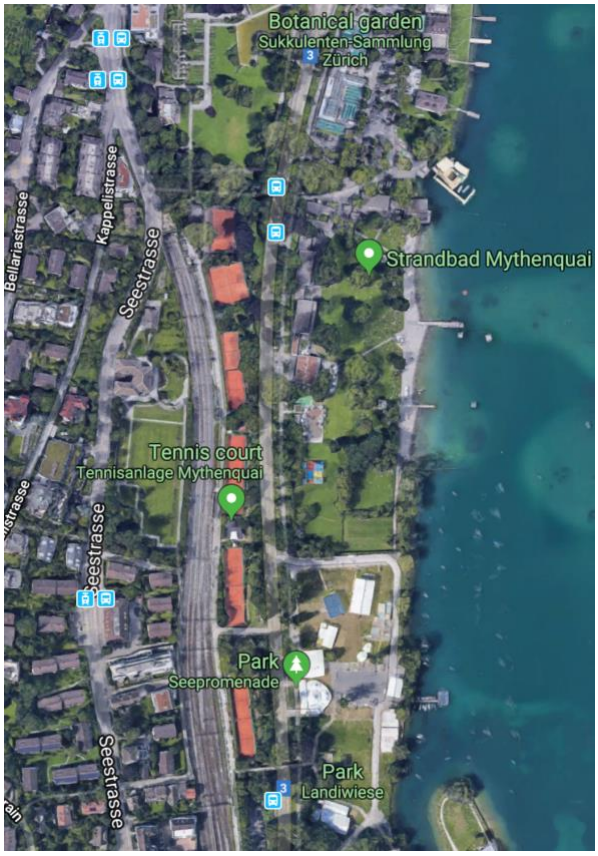
The designated locations:

1) Irchelpark

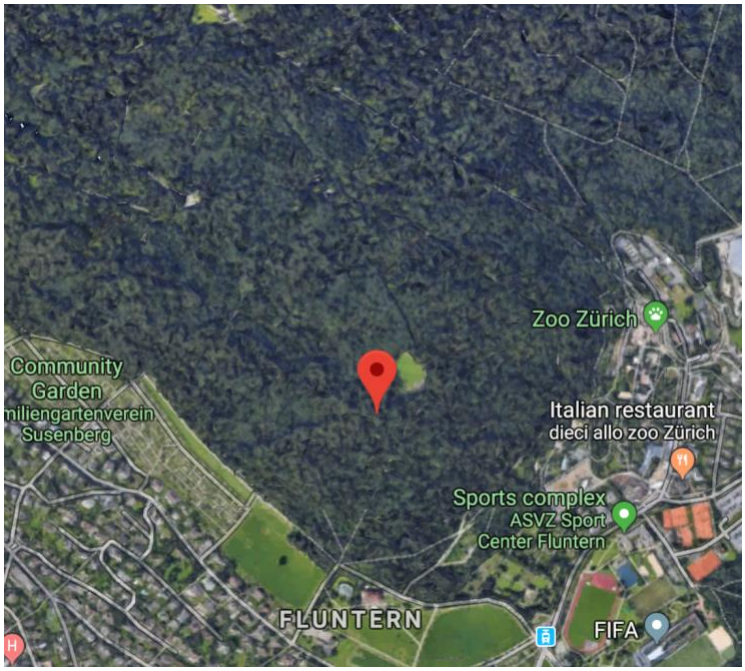


2) ETH - Hönggerberg

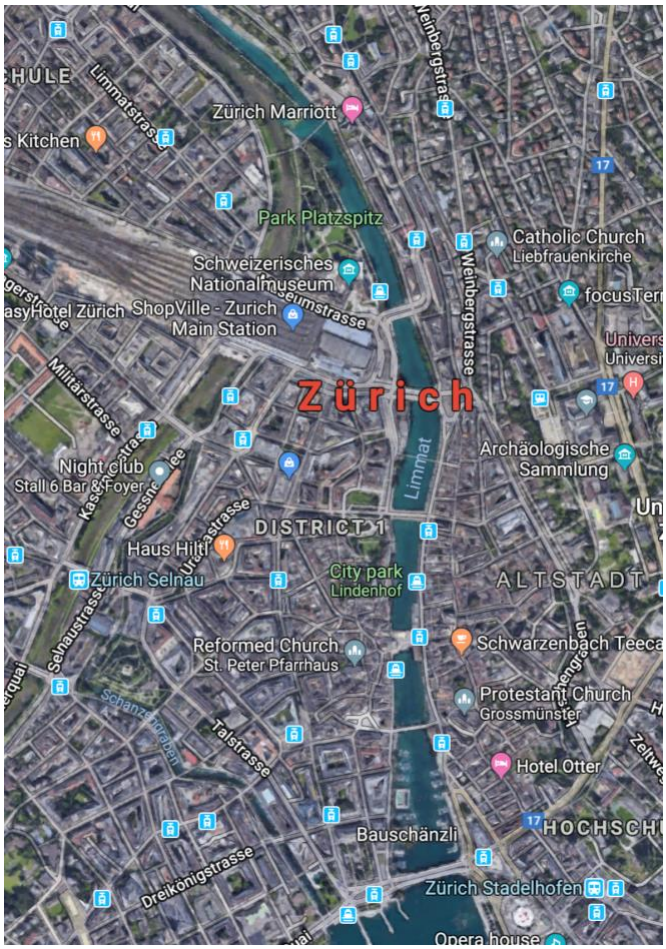
3) Mythenquai area (Lake Zurich)



4) Zurichberg



5) Downtown Zurich



Materials

For the field trip:
2 syringes (labeled A and B) and 1 pair of gloves

For the experiments in the lab:
PBS buffer
2 50 ml Falcon tubes
10 Eppendorf tubes
8 R2 agar plates without antibiotics (no making on the side of petri-dish)
6 R2-Ampicillin plates (I) with one red line marking on the side of petri-dish
6 R2-Chloramphenicol plates (II) with two red lines marking on the side of petri-dish
6 R2-Ciprofloxacin plates (III) with three red lines marking on the side of petri-dish
6 R2-Kanamycin plates (I) with one black line marking on the side of petri-dish
6 R2-Rifampicin (II) with two black lines marking on the side of petri-dish
6 R2-Vancomycin (III) with three black lines marking on the side of petri-dish

The working concentration of each antibiotic used is listed below:
Ampicillin (20 μg/ml), Chloramphenicol (10 μg/ml), Ciprofloxacin (1 μg/ml)
Kanamycin (20 μg/ml), Rifampicin (2.5 μg/ml) and Vancomycin (10 μg/ml)

All agar plates contain the antifungal reagents Cycloheximide (50 μg/ml) and Nystatin (17.9 μg/ml) to eliminate fungi and small eukaryotes in the soil samples.

Procedures

May 10. 2023

> Note: Practice common sense on the field trip for safety measures. Do not wear gloves or carry the syringes in public areas. Don't leave anything behind the sampling sites.

I. Field trip

Students will work together as a team for one designated area. Each student will receive an envelope containing two syringes as sampling devices. Each student will take one soil sample at the same focal point (location **A**) of the designated area and another sample at a second location 50-100 m away (location **B**) from the focal point. All group samples from the same location need to be taken in the spots as close as possible within a "square shoe" area.

1. Discuss, decide, describe and record the GPS coordinates of the focal point (location **A**) for sampling site.
2. Remove materials covering the soil (e.g., grass, leaves, stones, etc.).
3. Remove the sterile syringe from the package and gently "wiggle" it into the soil until the soil reaches the "10 ml" mark of the syringe.
4. Pluck the syringe out and place it back to the plastic package.
5. Take images of the surrounding area of the sampling site (**A**).
6. Decide the direction and the distance of the "away "site (**B**). Describe the location and record the GPS coordinates.
7. Repeat steps 2 to 4.
8. Take images of the surrounding area of the sampling site (**B**).
9. **Return the soil samples to your own lab bench at CHN D53.2 by 12:15 pm today**

   Question of the day: *What is the significance of collecting multiple soil samples at the same site?*

II. Preparation of soil microbial suspensions @ **D53.2 at 14:15**

1. Squeeze out the soil samples (down to 5 ml mark on the syringe) into the 50 ml Falcon tubes labeled A and B. Measure the weight of the soil samples using a weight scale.

   Sample **A** (focal point): _____g.

   Sample **B** (away): _____g

   Note: Remove any dirt debris from the bench surface and clean the surface with Ethanol before the next step.

   ----------**Start with Sample A first**----------

2. Add autoclaved PBS buffer to the 20 ml mark of each Falcon tube. Make sure the Falcon tube cap is securely closed before mixing. **Alternate vertexing and shaking vigorously every 30 seconds for 5 min.**
3. Put the Falcon tubes back in the rack and wait until the aqueous /muddy phases in soil mass separate (5-10 min). Remove at least ~1 ml (= 1000 µl) of the aqueous suspension from samples **A** into a sterile Eppendorf tube labeled **A**.
4. While waiting for the soil samples to settle, label the **bottom** (not the lid) of agar plates with Lab ID, location and the dilution number (see below). There are 22 plates for each sample; **four R2 plates without antibiotics** and **three plates with each type of six different antibiotics**. The antibiotics examined for this experiment include Ampicillin (RED-I), Chloramphenicol (RED-II), Ciprofloxacin (RED-III), Kanamycin (Black-I), Rifampicin (Black-II) and Vancomycin (Black-III)

   For example,

   Four R2 non-antibiotic plates for sample **A** dilution plating should be labeled as follows:

   1A-1 (Lab ID, location-dilution number)

   1A-2

   1A-3

   1A-4

   Three R2-antibiotic plates of each type should be labeled as follows:

   1A-1 (Lab ID, location and dilution number)

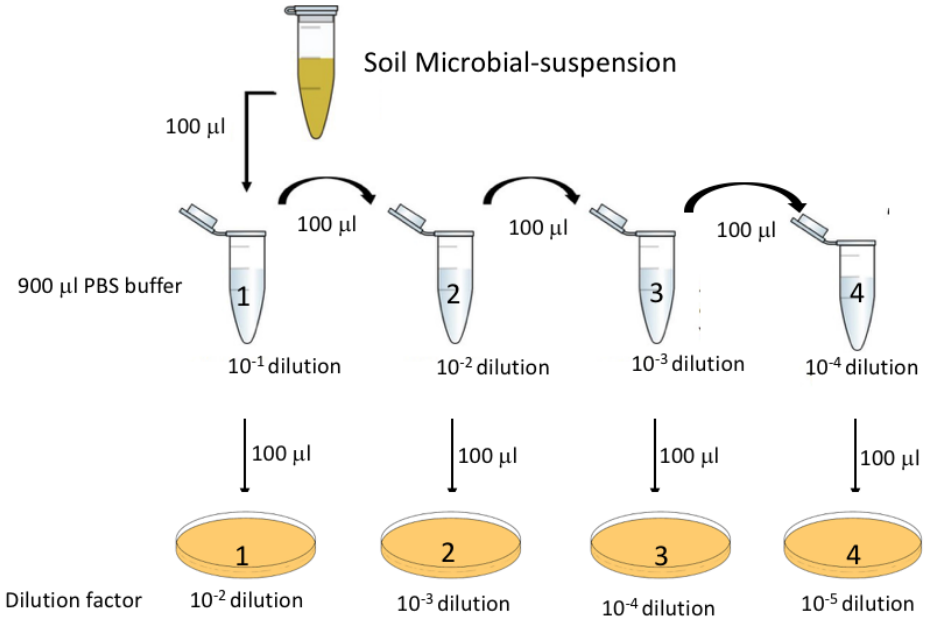
1A-2

1A-3

III. ***Making serial dilutions***

Note: use Eppendorf tubes for serial dilution and change the pipette tip between each dilution step. Avoid contamination.

1. Label the four Eppendorf tubes with dilution numbers 1, 2, 3, and 4. Add 0.9 ml (= 900 µl) PBS buffer into each Eppendorf tube.
2. Use a sterile pipette tip to mix the contents of Eppendorf tube A by pipetting up and down a few times and transfer 0.1 ml (= 100 µl) of the suspension to the first 0.9 ml (= 900 µl) PBS of Eppendorf tube number 1 and discard the tip.
3. Repeat this dilution step three times, each time with 0.1 ml (= 100 µl) from the previous dilution to the next 0.9 ml PBS tube 2, 3 and 4 in a sequential order. This results in serial dilutions of 10^{-1} through 10^{-4} per ml.



IV. Dilution plating and spreading suspension onto R2 agar plates

1. Take four pre-labeled R2 **non-antibiotic** agar plates and set them on the bench in a row. **Starting from the dilution tube number 4**, mix the contents by gently pipetting up and down, transfer 0.1 ml (= 100 µl) of the contents and distribute it evenly at least 5-6 droplets onto **plate no. 4**. **Immediately** spread the liquid droplets with **a sterile blue loop** in a "skating" motion both side-to-side and up-and-down. Repeatedly turn the plate slightly counterclockwise/clockwise and

spread by "skating" and until the droplets are well distributed all over the agar surface and all the liquid is fully absorbed onto the plate.

Note: By plating 0.1 ml which is 1/10 of 1 ml, this changes the dilution factor further by a factor of ten (1 = 10^{-2}, 2 = 10^{-3}, 3 = 10^{-4}).

2. Use the same procedure to plate and spread 100 µl of the diluted bacterial suspension from dilution tube no. 3 to plate no. 3, from dilution tube no. 2 to plate no. 2 and from dilution tube no. 1 to plate no.1 in a sequential order **using the same blue loop**.
3. Take 3 pre-labeled **R2-antibiotic** plates of one antibiotic type and set them in a row. **Starting from the dilution tube number 3**, mix the contents by gently pipetting up and down a few times, transfer 0.1 ml (= 100 µl) of the contents and distribute it evenly in at least 5-6 droplets onto **plate no. 3**. **Immediately spread** the liquid droplets with **a new** sterile blue loop
4. Use the same procedure to plate and spread 100 µl of the diluted bacterial suspension from dilution tube no. 2 on plate no. 2 and from dilution tube no. 1 to plate no.1 in a sequential order using the same blue loop.
5. Repeat steps 3 and 4 for the other 5 kinds of R2-antibiotic plates. Use a new blue loop for a different set of R2-antibiotic plates.

*V. Now repeat the procedure with sample **B***

While waiting for the soil samples to settle, label the **bottom** (not the lid) of agar plates with Lab ID, location and the dilution number (see below). There are 22 plates for each sample; **four R2 plates without antibiotics** and **three plates with each type of six different antibiotics**. The antibiotics examined for this experiment include Ampicillin (RED-I), Chloramphenicol (RED-II), Ciprofloxacin (RED-III), Kanamycin (Black-I), Rifampicin (Black-II) and Vancomycin (Black-III)

For example, if your lab ID is 1.

Four R2 non-antibiotic plates for sample **B** dilution plating should be labeled as follows:

1B-1 (Lab ID, location-dilution number)

1B-2

1B-3

1B-4

Three R2-antibiotic plates of each type should be labeled as follows:

1B-1 (Lab ID, location and dilution number)

1B-2

1B-3

VI. Repeat steps *III* and *IV* for sample **B**

Incubate the bacteria plates at room temperature until next Monday (May 13). Make sure the plates are inverted during the incubation to prevent condensation from falling onto the agar surface.

May 15, 2023

- Record the bacteria CFUs and morphology from AB-plates and non-AB plates.
- Compare CFUs from AB vs non-AB plate, compare frequencies of ABr between focal point A and away site B
- Select two bacteria/group for "the most interesting bacterium" pageant for ABr2023

May16, 2023

VII. Data analysis (See Labnote II) and Labnote II is due by 6 pm

May 17. 2023 at 14:15 pm

VIII. Presentation of Experiment II result

Appendix

Antibiotics

Table 1. Antibiotic classes, targets and drug examples

Antibiotic category	Target	Drugs
Beta-lactam	Cell wall	Penicillin, Ampicillin cephalosporin
Fluoroquinolones	DNA replication	levofloxacin or ciprofloxacin
Aminoglycosides	Protein synthesis	kanamycin, gentamicin or streptomycin
Tetrahydrofolate AB	RNA/DNA synthesis	trimethoprim, rifampicin, chloramphenicol
Colistin	Outer membrane	Colistin or polymyxin
Glycopeptides	G-positive cell wall	Vancomycin

Antibiotics tested in this IP course are highlighted in yellow.

MICs of ABs according to EUCAST (Version 7.1)

Table 2. Antibiotic classes, MICs and working concentration

Antibiotics		MIC breakpoint EUCAST [mg/L]	Working concentration [mg/L]
Penicillin	Ampicillin	8	20
Cephalosporins	Cefotaxime	2	4
Carbapenems	Imipenem	8	16
Fluoroquinolones	Ciprofloxacin	0.5	1
Aminoglycosides	Kanamycin	4	20
Chloramphenicol	Chloramphenicol	8	10
rifampicin	rifampicin	1	2.5
Lipopeptides	Colistin	2	4
Glycopeptides	Vancomycin	4	10