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Protocol status: Working We use this protocol and it's working

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Microbiome and eDNA sampling of sediment

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ABSTRACT

Protocol for field based sampling of bacterial biomass or environmental DNA (eDNA) in freshwater sediment.

SAFETY WARNINGS

Wastewater samples should be treated as a category 2 biohazard. Familiarise yourself with the risks associated with working with wastewater before starting.

https://www.hse.gov.uk/pubns/indg198.pdf

https://www.hse.gov.uk/biosafety/blood-borne-viruses/controlling-riskssewage-workers.htm

sewage-workers.nun

 $\underline{\text{https://support.fitforwork.org/app/answers/detail/a_id/367/} \sim \text{/working-with-sewage}$

 $\underline{https://www.cdc.gov/healthywater/global/sanitation/workers_handlingwaste.}\\ html$

Read the UKCEH risk assessment for handling sewage samples, and ensure you have the appropriate PPE before starting any work.

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Sample collection

- To collect the sediment sample, you will require a sampling pole with a 4 50 mL falcon tube slot on the end, a 4 50 mL falcon tube, and a pair of gloves.
- Put the gloves on and unscrew the lid of the L 50 mL falcon tube. Ensure you keep the lid of the tube in a safe place while you are taking the sample.
- 3 Attach the falcon tube to the end of the sampling pole by placing it in the slot, and make sure it is secure.
- 4 Carefully walk to the river, and once in the area of the river where you would like to take the sediment sample, reach the pole with the falcon tube inside down to the river base.

Safety information

CAUTION: Ensure you are careful when entering and leaving the water to collect the sample.

- Use the open falcon tube at the end of the pole to scoop up the sediment by dragging it through the sediment. You want as much sediment and as little water as possible. You may need to drag the pole/tube several times.
- Once the sediment is in the falcon tube, carefully lift the pole and remove the tube from it. Replace the lid back on the falcon tube and ensure it is secure.

7 Optional preservation buffer step - if you do not require this step, skip to Step 8.

If you would like to add a preservative buffer to the sediment, put a small amount (less than 2 0.5 g) into a 2 mL sterile Eppendorf microcentrifuge tube and add 2 1 mL of Zymo DNA/RNA shield to the tube. Shake the tube to thoroughly mix the sample with the buffer. The sample will then be relatively stable at room temperature for days-weeks, however, it is best to get the sample into cold storage, or preferably frozen at 3 -20 °C as soon as possible once returning to the laboratory. Step 8 is not required if you have followed Step 7, unless you require extra samples to be collected or want spares/backups.

Store the sediment in the 50 mL falcon tube at 5-20 °C, or at the very least keep at 4 °C and out of direct sunlight, until returning to the laboratory. Once in the laboratory, store at 3-20 °C until analysis.