ALGAL PRESERVATION AND ENUMERATION TECHNIQUES FOR USE IN MICRO- AND MESOCOSMS STUDIES

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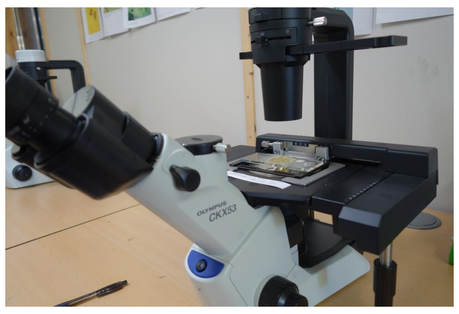
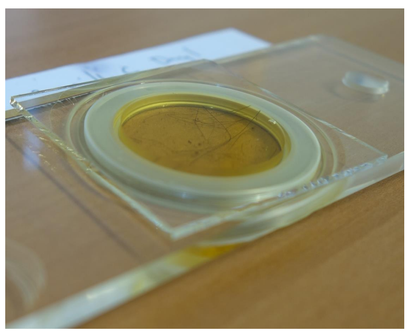
Experimental systems such as micro/mesocosms offer a more comprehensive analysis of the effects of Plant Protection Products (PPP's) on aquatic communities in comparison to single species studies. Whilst definitively more representative of natural systems, this increase in complexity brings further challenges. Freshwater algal communities are very diverse, the taxonomic identification of these species is difficult, and requires trained taxonomists.

When designing micro- and mesocosm studies, consideration needs to be given to the methods employed for sampling, preservation and enumeration to ensure all steps are fit for purpose. The sampling processes need to offer a true representation of the algal assemblages present. One of the major difficulties in sampling freshwater algae is preservation. The need for preservation at all should be considered as they will damage algal morphology over time, however this step is often a necessity in larger, long-term studies. Following sample collection, enumeration is necessary to collect the data needed to derive ecotoxicological endpoints, e.g. MDD's, EC_x and NOEC's. As with the sampling procedures, this process needs to use sub-samples that accurately reflect the whole test system.





THE ALGAL SAMPLE "LIFE STAGES"

Collection → Preservation → Enumeration → Analysis

	Collection		Preservation			Enumeration	
	Filtering	Depth Integrated Water Sampler (DIWS)	70% Ethanol	Lugol's	10% Formaldehyde	Utermöhl Chamber	Siphon
+	- Quick and simple - Dense, mixed algae sample. - Can dilute if needed.	- Representative of the whole water body - Known volume of sample collected.	- Rapidly preserves cells - Remain preserved for a long time period if stored correctly	- Rapidly preserves cells - Easy to use. - When applied in the right concentration, it can make some organelles more visible. - Adding acetic acid helps preserve the flagella.	- Rapidly preserves cells - Does not degrade cells over time. - Cells are a good representation of when they were "alive".	- Clear sample slide for manual count using a microscope. - Samples can range from 10 mL to 100 mL. 	- Can be completed in advance for counting.
−	- Cannot sample a known amount of water when "sweeping" through the water body. - Hard to clean for use on multiple samples.	- Sample could be very dilute and not represent a true reflection of the algal true community. - Not currently feasible to analyse entire DIWS sample.	- Will rapidly evaporate if stored incorrectly. - Can cause cell damage, hindering identification	- If concentration is too high, the algal cells are darkly stained making identification near impossible. - 6-month storage life span due to cell degradation.	- High human health risks - Fast evaporation and very flammable.	- Requires 24 hours to settle the sample before counting. - Requires multiple chambers to settle multiple samples.	- Time consuming. - Potential loss of algae cells when discarding supernatant. - Samples need to be preserved in Lugol's to help cells sink.

What works best at CEA

The are multiple ways to completed the sample life from collection to disposal. There are many variables that contribute what route to take. The sample process we use at CEA for mesocosm studies:

Collection: DIWS method. This gives a full representation of the mesocosm algae community.

Preservation: 1% Lugol's and acetic acid. We process the samples as quickly as we can after collection. All samples need to be processed within the 6-month sample expiry. In some cases, we can preserve a duplicate sample in formaldehyde as a back-up for algal species that are difficult to identify.

Enumeration: The Utermöhl method. To concentrate the sample for counting we settle an appropriate volume of the Lugol's preserved sample in one of our multiple Utermöhl chambers and leave them to settle for 24 h. Once the Supernatant is removed our trained algal taxonomists count 50 random views of a Whipple grid located in the microscope eyepiece. The volume of sample settled will depend upon the density of the algal population within each sample.