

Supplement of Biogeosciences, 15, 1415–1424, 2018
<https://doi.org/10.5194/bg-15-1415-2018-supplement>
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Supplement of

Revisiting chlorophyll extraction methods in biological soil crusts – methodology for determination of chlorophyll *a* and chlorophyll *a* + *b* as compared to previous methods

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Supplement

Table S1: Average Chl_{a+b} content [mg m^{-2}] and standard deviation obtained by the DMSO and ethanol extraction method.

Sample size $n = 5$

biocrust type	DMSO		Ethanol	
	AM	SD	AM	SD
green algae	597.97	210.82	181.76	50.63
cyanolichen	546.77	258.97	109.98	33.67
green algal lichen	771.62	329.90	124.71	35.55
moss	903.28	169.70	367.28	67.49

5 AM = arithmetic mean

SD = standard deviation

Table S2: Comparison of relative standard deviations (RSD) for the DMSO and ethanol method for all four biocrust types.

biocrust type	RSD (%)	
	DMSO	Ethanol
green algae	35.26	27.86
cyanolichen	47.36	30.61
green algal lichen	42.75	28.51
moss	18.79	18.38

10 Table S3: Reproducibility of chlorophyll extraction with dimethyl sulfoxide (DMSO). Chlorophyll content [$\mu\text{g g}^{-1}$] of eight replicates of homogenized green algae-dominated samples.

green algae sample	Chl_{a+b} [$\mu\text{g g}^{-1}$]
1	40.75
2	37.80
3	38.50
4	32.30
5	36.44
6	44.49
7	33.36

8	44.93
AM	38.57
SD	4.66
RSD (%)	12.08

AM = arithmetic mean

SD = standard deviation

RSD = relative standard deviation (%)

Table S4: Average Chl_{a+b} content [mg m⁻²] and standard deviation for the DMSO method with and without a preparatory grinding step.

Sample size n = 5

biocrust type	grinding		non-grinding	
	AM	SD	AM	SD
green algae	161.66	63.68	219.53	94.57
cyanolichen	224.00	58.38	348.96	88.98
green algal lichen	391.56	93.71	797.70	144.50
moss	557.11	135.16	739.16	201.10

AM= arithmetic mean

SD= standard deviation

Table S5: Average Chl_{a+b} content [mg m⁻²] and standard deviation for the DMSO method with and without an intermediate shaking.

Sample size n = 5

biocrust type	shaking		non-shaking	
	AM	SD	AM	SD
green algae	429.57	246.91	263.49	154.59
cyanolichen	122.53	29.65	32.70	7.99
green algal lichen	149.86	32.48	86.73*	31.24*
moss	363.42	96.76	376.19	103.89

AM = arithmetic mean

SD = standard deviation

* only 4 samples

S6: Instructions for chlorophyll extraction using the DMSO method plus additional shaking

Note: All processing should be done in minimal light to help prevent degradation of chlorophylls.

Preparatory steps:

1. Determination of the dry weight of soil samples (samples dried to constant weight at 60°C)
2. Moisten soil samples slightly with distilled water the day before chlorophyll extraction

Extraction:

3. Heat the water bath to 65°C
4. Place soil samples (+ one blank sample) in 15 mL screw-cap vials
5. Add spatula tip of MgCO₃/ CaCO₃ to the samples
6. Add 6 mL DMSO to the samples
7. Boil samples for 90 minutes in a water bath. Screw caps half tightened to allow expansion of sample, but prevent evaporation.
8. Remove vials from water bath, tighten screw caps and shake samples for 20 minutes on horizontal shaker.
9. After shaking let samples rest upright for a couple of minutes until soil has settled.
10. Pour supernatant into separate labeled vials
11. Repeat steps 6-9
12. Reunite both supernatants per sample
13. DMSO chlorophyll extracts can now be stored at 4°C for about one week or frozen for a longer time period.
14. Before photometric determination the samples must be centrifuged for 10 min at 3000x g and 15°C.
15. Measure extinction at 700, 665 and 648 nm wavelength, adjust zero point using the blank sample.
16. In case extinction is higher than 0.8, the samples have to be diluted with DMSO (1:1) and extinction measurements need to be repeated.

Calculation of Chl_a and Chl_{a+b} contents according to formulas presented in the manuscript.