METHOD STATEMENT

Determinand:
Enumeration of Chlorophyll 'A' in Aquatic Environments. Calculation of Phaeophytin contribution to overall results.

Matrix:
All types of water.

Principle of Method:
This method describes a procedure in which plant material such as Algae is obtained from a water sample by an initial filtration step. Solvent extraction of the pigments is achieved using methanol.(Methanol extraction is particularly useful as it is superior to acetone as an extractant especially when hot). Finally, the chlorophyll 'a' concentration is determined by spectrophotometric evaluation of the extract by carrying out absorbance measurements at two wavelengths: 665nm offers the maximum absorption of chlorophyll 'a', while 750nM compensates for 'background turbidity'. Acidification of the sample will denature and convert chlorophyll to Chlorophyllide and the re-read will relate to the original phaeophytin levels.

Both Chlorophyll and Phaeophytin are pigmented and will contribute to the initial spectrometric result. Rapid Acidification converts Chlorophyll to Chlorophyllide. This is unpigmented. Initial spectrophotometery will evaluate both pigments, while only phaeophytin will remain after acidification.

Sampling and Sample Preparation:
Samples should be collected in a plastic airtight container, which has a minimum capacity of 1 litre. After collection samples should not be exposed to sunlight and must be stored at <10°C during transit. Samples are best analysed on the day of collection or at most, within 24 hours of sampling, if stored in darkness in a refrigerator at a temperature between 2 - 8°C until sample analysis is commenced.

Interferences:
If present in the sample of plant materials, chlorophyll b and c and other pigments such as carotenoids will be extracted by the solvent and will contribute to the absorbance of the extract, even at the wavelength selected for chlorophyll a, thus the chlorophyll content of the sample may not be the true value. When determining phaeophytin there is a very small dilution of the sample upon the addition of acid. A more serious interference effect when phaeophytin is not determined is the presence of degradation products of chlorophyll 'a' (phaeophorbride & phaeophytin) which may be present in appreciable amounts. This method does not distinguish between degraded and undegraded forms of chlorophyll pigment.

Performance of Method:

Determinand	Instrument	LOD (µg/l)	Minimum Reporting Limit (µg/l)	Surface Water	
				% RSD	% Recovery
Chlorophyll 'a' (BU9: Cold Extract)	Genesys20_3426	5.8636	7.0	16.45	66.78
Phaeophytin (MKB: Cold Extract)		3.6847	6.0	15.83	N/A
Chlorophyll 'a' (BUH: Hot Extract)		3.4150	4.0	15.10	75.27
Chlorophyll 'a' (BU9: Cold Extract)	Shimadzu_3049	6.3338	7.0	15.57	70.65
Phaeophytin (MKB: Cold Extract)		5.0709	6.0	20.80	N/A
Chlorophyll 'a' (BUH: Hot Extract)		3.7159	4.0	15.43	80.74

Uncertainty of Measurement

The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2, which gives a level of confidence of approximately 95%.

Determinand	Uncertainty of Measurement %
Chlorophyll 'a' (Cold Extract)	68.87
Phaeophytin (Cold Extract)	37.34
Chlorophyll 'a' (Hot Extract)	51.12

References:

The Determination of Chlorophyll 'a' In Aquatic Environments 1980 – Methods for the Examination of Waters and Associated Materials. SBN 0117516740