



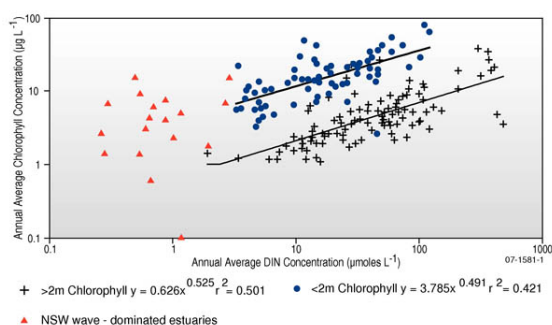
## Measuring Chlorophyll a

### What is chlorophyll a?

Chlorophyll is the green pigment in plants that allows them to create energy from light – to photosynthesize. By measuring chlorophyll, you are indirectly measuring the amount of photosynthesizing plants (such as algae or phytoplankton) found in a sample. Chlorophyll *a* pigment is universal to all plant types, while other chlorophylls (such as *b*, *c1*, *c2*, *d*, *f*) may be specific to certain plants, algae, or cyanobacteria and may be used to identify major algal groups present<sup>1</sup>.

### Why measure chlorophyll a?

The flow of nutrients, such as phosphorous and nitrogen, into inland and coastal waters contributes to enhanced algae growth. The graph below shows how chlorophyll *a* can correlate to dissolved inorganic nitrogen (DIN).<sup>2</sup> Changes to nutrient loadings can also change the phytoplankton species composition and diversity.



High levels of algae often indicate poor water quality and low levels often suggest good conditions. Elevated phytoplankton levels can lead to reduced water clarity, affecting sea grass growth and related fisheries. In extreme cases, eutrophication can lead to hypoxia (oxygen-depleted “dead zones” that kill fish, shellfish, and crustaceans) and harmful and/or toxic algal blooms. From a water quality perspective, chlorophyll *a* is the best available, most direct measure of the amount and quality of phytoplankton and the potential to lead to reduced water clarity and low dissolved oxygen impairments.<sup>3</sup> Chlorophyll *a* is

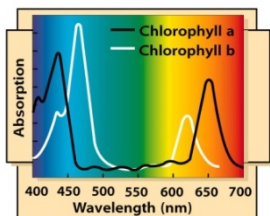
often reported in mass per unit volume, e.g.  $\mu\text{g/L}$ ,  $\text{mg/L}$ . The chlorophyll *a* threshold for impacts on fish is generally considered to be  $100 \mu\text{g/L}$ .<sup>4</sup> The cleanest waters will have chlorophyll-*a* levels of less than  $5 \mu\text{g/L}$ .<sup>5</sup> The specific, applicable water quality standard will depend on the uses and goals for that water body.

### Who measures chlorophyll a?

Drinking water treatment plants with surface water impoundments will measure chlorophyll *a* to manage the source water. This may include predicting and controlling algae blooms or determining where to draw water to avoid algae intake, which can clog filtration systems, increase organic load, cause a public health nuisance, and necessitate extra treatment. In the United States, other agencies analyzing chlorophyll *a* include: US Army Corps of Engineers; US Environmental Protection Agency (US EPA); state environmental agencies and laboratories; Chesapeake Bay Program; US Geological Survey (USGS); National Oceanic and Atmospheric Administration (NOAA); Audubon of Florida; academic institutions, and others.

### How is chlorophyll a measured?

Chlorophyll *a* is measured by filtering a known amount of sample water through a filter, usually a glass fiber filter. The filter is ground up in an acetone solution, which is then processed and analyzed. There are three standard techniques for determining chlorophyll *a* concentrations: spectrophotometry, fluorometry, and high performance liquid chromatography (HPLC). Spectrophotometry is the most commonly used laboratory method. The sample processing time is typically 1-5 minutes, the estimated detection limit (DL) is  $0.08 \text{ mg/L}$ <sup>6</sup> (using a 1 cm cell - use larger for lower DL), and the instrumentation cost is low. The HPLC method is able to differentiate between chlorophyll types and accessory pigments, but is a slower and more demanding technique, e.g. 20-25 minutes of sample processing time. The cost of an HPLC instrument may be 10 times the cost of a spectrophotometer, and there are considerable ongoing consumables costs. Fluorescence is an indirect method for measuring chlorophyll *a*, and is well suited for remote monitoring.



To measure chlorophyll *a* by the spectrophotometric technique, a spectrophotometer with a narrow band width (pass) is used to take measurements at multiple wavelengths. For example, the trichromatic method uses measurements at 750 nm (turbidity correction), 664 nm (chlorophyll *a*), 647 nm (chlorophyll *b* correction), and 630 nm (chlorophyll *c*1, *c*2 correction). See absorption spectra for chlorophyll *a* and *b* to in the image to the left<sup>7</sup>.

An alternate method uses measurements at 750, 664, and/or 665 nm before and after acidification, which corrects for pheophytin *a* and turbidity interferences. The wavelengths and equations used will depend on the method chosen. Examples of accepted chlorophyll *a* testing methods include US EPA 446.0, Standard Methods 10200 H, ASTM D3731, DIN 38412-16, ISO 10260, and others.

### Equipment for testing chlorophyll *a* by spectrophotometer

The Thermo Scientific™ Orion™ AquaMate™ 8000 UV-Vis spectrophotometer is a good choice for this testing and meets the requirements for accepted chlorophyll *a* testing methods, such as listed above. The instrument has the necessary narrow band pass, covers the required wavelengths, accommodates sample cells from 1 to 10 cm, and is designed to offer the accuracy, dependability and ease of use your lab requires.

Product	Description	Part Number
Instrument	Thermo Scientific Orion AquaMate 8000 UV-Vis Spectrophotometer	AQ8000
The following accessories, parts, and consumables can be found at <a href="http://www.unitylabservices.com">http://www.unitylabservices.com</a>		
Accessories, Parts and Consumables	1 cm (10 mm) matched glass cells <i>(other cells available for lower detection limits)</i>	331709-000
	Wavelength accuracy and instrument performance standards filter set	333150
Services	Recalibration service for standards filter set (P/N 333150) above	701-101600
	Annual spectrophotometer performance calibration services	Visit <a href="http://www.unitylabservices.com">www.unitylabservices.com</a>

### Notes and References

1. Method 10200 H. Chlorophyll, Standard Methods for the Examination of Water and Wastewater, [www.standardmethods.org](http://www.standardmethods.org)
2. Chlorophyll *a* concentrations, Ozcoasts, Marine & Coastal Environment Group, Geoscience Australia, [http://www.ozcoasts.gov.au/indicators/chlorophyll\\_a.jsp](http://www.ozcoasts.gov.au/indicators/chlorophyll_a.jsp)
3. Ambient Water Quality Criteria, USEPA April 2003, [http://www.chesapeakebay.net/content/publications/cbp\\_13142.pdf](http://www.chesapeakebay.net/content/publications/cbp_13142.pdf)
4. Chlorophyll-*a* Methodology, [http://www.evergladesplan.org/pm/pm\\_docs/qaot/june\\_2009\\_workshop/062309\\_mtg\\_qaot\\_chlor\\_methods.pdf](http://www.evergladesplan.org/pm/pm_docs/qaot/june_2009_workshop/062309_mtg_qaot_chlor_methods.pdf)
5. Nielson, J. and P. Jernakoff, P. 1996. A review of the interaction of sediment and water quality with benthic communities. Port Phillip Bay Environmental Study. Technical Report No. 25, 1-130.
6. Chlorophyll-*a* in Lakes, <http://www.ecy.wa.gov/programs/wq/plants/management/joymanual/chlorophyll.html>
7. Absorption spectra for chlorophyll *a* and *b*, <https://wiki.bio.purdue.edu/biol13100/index.php/File:Chlorophyll.JPG>

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