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Review Article

Pigment-Based Chemotaxonomy: Review of the Pros and Cons

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Introduction

The basics of pigment-based chemotaxonomy and its application to the study of various microalgal communities (Phytoplankton, epiphytes and periphyton) was presented in a previous OJEES article [1]. Results and discussions on the basis and applicability of this methodology is also well covered in the literature [2-7]. The concept of pigment-based chemotaxonomy relies on the successful extraction [8] of the lipid-soluble pigments (viz. chlorophylls and carotenoids) from microalgal samples followed by the high-performance liquid chromatography (HPLC) separation and photodiode-array (PDA) collection of ultraviolet / visible (UV-Vis) spectra. Retention time on HPLC and the resultant UV-Vis spectra allow a 2D identification of each pigment [2,4,6,8]. Chlorophyll-a (CHLa) is used as a biomass indicator and the CHLa to biomarker pigment or biomarker pigment to CHLa ratios are used for taxon-specific abundance estimations.

Pros and Cons of Pigment-Based Chemotaxonomy

To quote Millie and co-workers [2]; "Past and current efforts at identifying microalgal phylogenetic groups rely largely on microscopic evaluation, which requires a high level of taxonomic skill, may take considerable time, can be variable among personnel, and does not allow characterization of the physiological status of the taxa. High-performance liquid chromatography (HPLC) has proven effective in rapidly separating and distinguishing chlorophylls, chlorophyll-degradation products, and carotenoids within monotypic and mixed algal samples."

PROS

It is the ease and speed of pigment-based chemotaxonomy for routine screening of microalgal communities (Phytoplankton, epiphytes and periphyton) that offers the greatest advantage (aka PRO) to this method. These assessments of taxa and their biomass (viz. CHLa) are then easily cross-plotted to various environmental conditions such as nutrient (N, P, Fe etc.) concentrations, light levels, temperature, tidal changes, rainfall and other drivers.

The identification of biomarker pigments allows detection of various microalgal taxa in a sample. Examples [1-7] include Chlorophyll-b (Chlorophytes), Peridinin (Type 1 Dinoflagellates), Alloxanthin (Cryptophytes), Zeaxanthin (Cyanobacteria), Echinenone (Filamentous cyanobacteria), Canthaxanthin (heterocystous cyanobacteria [9]).

Additionally, pigment-based chemotaxonomy readily detects various nano-and pico-phytoplankton. This includes Alloxanthin for Cryptophytes, Prasinoxanthin for Prasinophytes, 19′-butanoyloxyfucoxanthin and/or 19′-hexanoyloxyfucoxanthin for Haptophytes [10, 11], and aphanizophyll for Nitrogen fixing (diazotrophic) cyanobacteria [6]. These examples are generalizations and are further discussed below under "CONS".

Numerous mathematical methods exist for the conversion of pigment data into taxon-specific estimates of Chlorophyll-a related biomass. This includes simultaneous linear equations, SLEs [1,2,5,6], and various compositional algorithms such as PHYTOCLASS [3], CHEMTAX [4], and a Bayesian estimator [7]. Each method should of course undergo initial and periodic QA/QC (Quality Assurance / Quality Control) methods such as ground truthing versus microscopic and electronic identification / counting [12-14] or with the FlowCAM instrument [15,16].

CONS

Microalgal populations will obviously change in response to nutrients [17], light conditions / depth [18-20], strain variations [21], seasonality [10, 22], wind and sediment resuspension [23-25], and other environmental drivers.

One contrary (CON) attribute of pigment-based chemotaxonomy that is constant is the occurrence of selected biomarker pigments in more than one taxon. For example, Zeaxanthin (ZEA) is usually used to identify the presence and abundance of Cyanobacteria (CYANO). However, ZEA occurs in other taxa as well. Notably ZEA is a photoprotective pigment (PPP) in green algae (Chlorophyta), Therefore, the presence of Chlorophyta, especially in high light conditions where ZEA (PPP) is higher in abundance [18], can indicate a higher amount of Cyanos than reality. Corrections for this can be included in resultant data handling. As an example: Cyanobacteria ZEA equals Total ZEA minus Chlorophyte ZEA. Chlorophyte ZEA would first be estimated from known CHLb/ZEA ratios for green algae, taking the effects of light [18].

Another overlapping taxon biomarker is Alloxanthin (ALLO). ALLO is a commonly used pigment biomarker to indicate the presence of Cryptophytes (Cryptomonads) which are in the nanoplankton size range and often missed or underrepresented in microscopic exams [26, 27]. However, ALLO also exists in Type 5 Dinoflagellates [11]. The presence of high amounts of α -carotene and the presence of monadoxanthin and/or crocoxanthin when ALLO is found does however strengthen the use of ALLO to indicate Cryptophytes [5].

Chlorophyll-b, the widely used biomarker for Chlorophytes, is also found in Type 6 Dinoflagellates [11]. However, the coincidence of lutein usually strengthens the identification of Chlorophytes [5].

Fucoxanthin the main biomarker for diatoms (Chrysophytes), is also found in Type 2, 3 and 4 Dinoflagellates [11]. The presence and amounts of diatoxanthin and diadinoxanthin helps to correlate the fucoxanthin with diatoms.

One must have at least a minor to significant cross-checking QA/QC approach to adequately employ pigment-based chemotaxonomy. For example, a rapid microscopic screening of selected samples can be used to see if diatoms and / or dinoflagellates are present / dominate when fucoxanthin is present [1,2,5,6].

In the case of pigment-based chemotaxonomy using Simultaneous Linear Equations (SLEs), one needs to develop and test these for the environment being assessed. Here (Table 1) is a comparison of the output from four different SLE equations using the same HPLC data from two different sites in a subtropical estuary in Southern Florida. Line 1a is the result of the SLE developed by the present author [1,5,6] for South Florida Marine and Estuarine systems such as where these two samples were collected. Lines 1b, 1c and 1d are the chemotaxonomic outputs for these two sites using the SLEs from other world sites given by Uitz et al. [20], Vidusi et al. [28], and Marrinho and Rodriquez [29] respectively. It is noted here that these SLEs used ratios based on molar [1,5,6] or weight [20,28,29] ratios of CHLa/biomarker [1,5,6] or biomarker / CHLa [20, 28,29] as detailed in the referenced publications. As weight data from HPLC analysis is easily converted to molar data, I choose to use molar ratios in the hope that eventually that will allow physicochemical and / or photochemical (light) conclusions to be revealed [30].

It must be noted here that this comparison (Table 1) does not indicate that the data in lines 1b, 1c and 1d from equations by other researchers is inherently wrong. Rather, this exercise is meant to show that one must tailor the SLE or any mathematical treatment used to the area being investigated. That is, there is no one simple equation that will suffice for all environments.

Table 1: Comparison of Pigment-Based Chemotaxonomic estimation of phytoplankton distributions in two separate sites using four different Simultaneous Linear Equations.

SITE A					
	CYANO	**CHLORO**	**DIAT**	**DINO**	**CRYPTO**
1a	17	32	29	1	21
1b	20	19	48	2	11
1c	18	12	26	2	42
1d	38	20	24	<1	18
SITE B					
	CYANO	**CHLORO**	**DIAT**	**DINO**	**CRYPTO**
1a	16	16	4	60	4
1b	17	7	5	68	3
1c	13	4	3	74	6
1d	54	15	5	21	5

Conclusion

Pigment-based chemotaxonomy is an excellent tool for the fast assessment of microalgal populations, notably phytoplankton. This allows for a more rapid way of monitoring changes over time and environmental changes such as tidal cycles, temperature, light, nutrient pollution, and other factors. That said, one must also have ways to cross-check (QA/QC) the results, usually microscopy and/or FlowCAM analyses.

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Conflict of Interest

No conflict of interest

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