Chlorophyll Measurements at Sagres, PORTUGAL

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Content of this presentation

• Comparison of spectrophotometric methods with High Pressure Liquid Chromatographic (HPLC) method.
• Comparison of pigment data obtained by all methods at Sagres.
• Some odd & sods at the end:
  – Summary of match-ups at Sagres to date
  – Calibration of TACCs;
  – Introduction to Ocean Colour Portugal web site.
  – Comparison of sun photometers;

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Spectrophotometry Methodology

• **Lorenzen et al. (1967) (Lor.):**
  Total Chlα: the method also enables estimates of phaeopigments:

  \[
  [\text{Chl} \alpha](\mu g/l) = \frac{26.7(E_{664} - E_{664}') \times v}{V \times l}
  \]

  Where \( E \) is absorbance, \( o \) and \( a \) are before and after acidification, \( v \) is volume of acetone extract (ml), \( V \) is volume of water filtered (l) and \( l \) is the path length of the cuvette (cm).

• **Jeffrey and Humphrey (1975) or “Trichromatric equation” (J&H):**
  Total Chlα: corrections are made for other chlorophylls

  \[
  [\text{Chl} \alpha](\mu g/l) = 11.85E_{664} - 1.54E_{647} - 0.08E_{630}
  \]

  J&H is more accurate e.g. correction for interference
  Lor. is sometimes more useful e.g. degradation products!
HPLC Methodology

• **Method:** Jeffrey et al. (1997)

• **LC System:**
  – Agilent 1200 quaternary pump equipped with temperature-controlled autosampler (maintained at 4ºC);
  – Agilent 1200 diode array detector G1315B, scanning at 436 and 450 nm;
  – Alltech Altima C18 column, 15cm length, 4.6 um of internal diameter and 3um of particle size.

• **Elution System** – Tertiary gradient elution system:
  – Solvent A: 80:20 (v/v) Methanol:0.5M Ammonium Acetate
  – Solvent B: 90:10 (v/v) Acetonitrile:Water
  – Solvent C: Ethyl Acetate

• **Calibration:**
  – Pigment Standards were purchased from DHI;
  – 19 different pigment standards are tested for individual standard response factors. Samples concentrations are calculated with the response factors determined using single point calibration (3 replicate injections of the same compound);
  – The linearity of the system is tested and LOD&LOQ is determined using 3 multipoint calibration curves – 1 calibration curve for a mixture of 11 carotenoids, 1 calibration curve for a mixture of chlorophylls and 1 calibration curve for Chlα alone (performance metrics have been determined for the latter).
Spectrophotometry vs HPLC

\[ R^2 (\text{HPLC}_{\text{NIVA}}) = 0.56 \]
\[ R^2 (\text{HPLC}_{\text{Portugal}}) = 0.75 \]

\[ R^2 (\text{HPLC}_{\text{NIVA}}) = 0.55 \]
\[ R^2 (\text{HPLC}_{\text{Portugal}}) = 0.67 \]
All data (2 Spect. Methods vs HPLC$_{\text{NIVA and Portugal}}$)

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Spectrophotometry vs HPLC

• Spectrophotometric at Sagres overestimates API1 relative to HPLC data – 30% (average);
• Several papers show overestimation of Chlα by spectrophotometric methods
  - Lorenzen (1967)
  - Sartory (1985)
  - Dos Santos et al. (2003)
• Jeffrey et al. (1997) – Good correlations between the 2 methods for pure pigment mixtures, but much lower correlations for natural seawater samples or for mixed standards of chlorophylls and degradation products;

Note chlorophyllide a and phaeopigments cannot be distinguished directly by spectrophotometry.
API1 versus API2

**2008-09**

- **Station A**

**2010-11**

- **Station A**

**Station B**

**Station B**

**Station C**

**Station C**

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Odds and Sods

• Match-ups to date at Sagres: SA 7 days; SB 19 days; SC 23 days.

• Calibration of the Portuguese TACCS together with the Portuguese and Swedish Solar Light sun photometers with Bio-Optika January 2012

• www.ocportugal.org up and running
Simple calibration setup…

Gerald Moore
Bio-Optika
Comparison between “hand-held” sun photometers and CIMEL at Sagres
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