OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

JAMP Eutrophication Monitoring Guidelines: Chlorophyll a in Water

(OSPAR Agreement 2012-11)[1]

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[1] Replaces OSPAR Agreement 1997-04

1. Introduction

Nutrient enrichment of transitional, costal and marine waters can give rise to increased primary production and phytoplankton biomass which is part of eutrophication process. The human driven eutrophication process can cause undesirable disturbance of ecosystems and therefore an integrated monitoring programme to characterise the status and changes in aquatic ecosystems with respect to eutrophication is required.

Chlorophyll a is the most used operational indicator for phytoplankton biomass. However, the chlorophyll a content in relation to organic carbon is different for different taxa and can vary, due to the physiological status, as does the cellular carbon compounds. Chlorophyll includes a variety of different pigments like chlorophyll b, c1, c2, divinyl chlorophyll a and divinyl chlorophyll b.

Extracts of algae pigments contain also variable amounts of the chlorophyll precursor pigments and breakdown products like chlorophyllide a, phaeophorbide a and phaeophytin a. Usually, the phaeopigments have been discriminated from chlorophyll a and chlorophyllide a by measuring the pigment extract before and after the addition of acid: the so called 'acidification method'. The acidification causes a conversion of chlorophyll to phaeophytin and the chlorophyll a content can be calculated from the difference of both measurements and should be reported as "active chlorophyll a" and "phaeopigments". But interlaboratory comparisons have shown that the calculated values for phaeopigments are less reliable than the values for chlorophyll a. Therefore the acidification is no longer recommended because it is time consuming and the results are questionable.

The non-acidified methods give an estimate of pigment concentration which is a combination of chlorophyll a, chlorophyllide a, phaeophorbide a and phaeophytin a. Because the standard photometric and fluorometric methods for determining chlorophyll a do not completely separate the different chlorophylls or distinguish between chlorophyll a and chlorophyllide a the term "total chlorophyll a" should therefore be used when reporting results from these methods. For chlorophyll data, analysed by HPLC, which considered the other chlorophyll derivates as well the term "chlorophyll a" should be used.

2. Purposes

The measurement of chlorophyll a concentrations in water is carried out for the following purposes:

1. to quantify the concentration and spatial extension of phytoplankton biomass and the frequency of phytoplankton blooms;
2. to identify seasonal trends, over periods of several years, of phytoplankton biomass, and in primary production, and frequency and duration of phytoplankton blooms;
3. to implement a basic eutrophication survey in the Eutrophication Monitoring Programme.

3. Quantitative objectives

The quantitative objective for assessing eutrophication processes should allow to link long time changes of chlorophyll concentrations to changes of nutrient concentrations. To achieve this, the monitoring programme must take into account the natural seasonal and inter-annual variability in chlorophyll a concentrations and the variability induced by hydrodynamic processes (patches, fronts) as well. The monitoring of reference sampling stations outside the eutrophicated region should be used to identify the effects of other pressures such as climate change.

It is intended that the region-specific temporal trend monitoring programme should have the power (90 %) to detect a change in concentration (50 %) over a selected period (10 years). To clarify the situation and to help define objectives Contracting Parties should undertake statistical analyses of their existing data sets. This would help to determine the representativeness of the monitoring stations and thus the selection of suitable sampling stations and sampling frequencies.

The monitoring programme should enable Contracting Parties to determine the representativeness of their monitoring stations with regard to the spatial and temporal variability in chlorophyll concentrations. This would include a definition of the extent of the monitoring area and selection of representative monitoring stations, supported by a combination of remote sensing and sea-truth measurements.

4. Sampling strategy

In most aquatic systems there is a pronounced seasonal cycle of phytoplankton growth and species succession, and both the timing and magnitude of seasonal events such as the spring bloom or summer standing crops exhibit inter-annual variability. To cover the spatial distribution of chlorophyll concentrations, horizontal and vertical sampling at a sufficient number of appropriate stations is required during the entire growth season. The sampling programme should be supplemented by regular aerial surveys may be carried out using visual and remote sensing devices.

Since chlorophyll is only a proxy for phytoplankton biomass the correlation with phytoplankton biomass estimated using microscopic phytoplankton community analysis with cell counts and cell volume measurements (Olenina et al, 2006) should be combined with chlorophyll analyses for representative samples (in time and space). Data on phytoplankton composition would also provide information on whether the abundance of particular harmful species is increasing and on changes in the structure of the phytoplankton community. To do this a subset of samples covering the whole growing season should be analysed using both techniques every year. For the methods of microscopic phytoplankton community analysis refer to the details of EN 15204 (2006).

Suspended particulate matter (SPM) (see e.g. Yeats and Brügmann, 1990), temperature, salinity, the loadings of nutrients (total nitrogen and total phosphorus) and winter concentrations of dissolved inorganic nutrients and light penetration measured as PAR (Photosynthetically Active Radiation), supplemented by Secchi depth and measurements of SPM should also be measured, as supporting/interpretation variables. In shallow areas also microphytobenthos should be monitored, related to specified tidal modes. Offshore vertical profiling should be combined with estimations of density profiles.

5. Sampling equipment

A suitable water sampler should be used to collect samples for chlorophyll measurements. A non-transparent sampling device is recommended and because chlorophyll is photolabile (is broken down to colourless compounds in the light) extraction procedures and measurements should be carried out in low light.

For the open sea, the standard sampling depths for chlorophyll are in the upper water column the same as for nutrients: 1 m, 5 m, 10 m, 15 m, 20 m, and around thermoclines (changes of temperature, density and/or nutrient concentrations). In coastal waters, without stratification, samples from 1 m or vertically integrated samples (1 – 10 m) should be analysed. Single samples of distinct depths allow a direct correlation to specific gradients and are often less contaminated. For helicopter sampling a single sample from the mixed surface layer should be taken. During monitoring additional sample(s) should be obtained from chlorophyll maxima present at other depths. Such maxima may be detected by profiling probes with chlorophyll fluorometer/CTD. Chlorophyll should be analysed in a sub sample from the samples used for phytoplankton and primary production measurements.

Automated measurement systems and sampling equipment on research vessels and ships of opportunity (Ferry Box systems), instrumented continuous plankton recorders (CPRs) and undulating oceanographic recorders can provide useful supporting information. Instrumented moorings can also be used to provide high frequency measurements of chlorophyll to resolve short term events but are single point measurements. Anti-fouling devices must be used on moored instruments and service intervals and calibration checks must be high enough to make sure that bio fouling does not influence results.

When using in situ chlorophyll fluorometers the effect of photoquenching (i.e. reduced chlorophyll fluorescence signal at high light intensities should be taken into account. One means of doing this is to measure light. For moored systems photoquenching can be avoided by using data recorded during darkness.

6. Storage and pre-treatment of samples

Chlorophyll samples should be filtered immediately after sampling and filtering should be carried out under green or low light conditions. Filters should be extracted immediately, and the extract should be kept deep-frozen. If it is not possible to follow this procedure the filters should be kept frozen at < -20 °C for no longer than 21 days. If stored longer a temperature of < -80 °C should be maintained to avoid degradation of chlorophyll.

7. Analytical procedures

Standard procedures for the determination of chlorophyll are given in Strickland and Parsons (1968), UNESCO (1994), HELCOM (1988) and ISO 10260 (1992). It is important to report the method used. It should be ensured, that the same method of measuring chlorophyll concentrations is used and the same procedure (sample collection, filtration, extraction and storage) is followed during the surveys. Changes must be well documented.

If HPLC is used for chlorophyll analysis the method by Wright et al (1991) should be used. This HPLC method has been commonly used, and it is accepted that it does not distinguish between chlorophyll a and divinyl chlorophyll a derivates. If that level of differentiation is required an interlaboratory comparison of different HPLC methods should be consulted (e.g. Claustre et al 2004).

If in-situ chlorophyll fluorometers are used, they should be calibrated with local natural water samples with a range of chlorophyll concentrations. All measuring instruments should be calibrated with filtered water samples and standard chlorophyll a.

8. Quality assurance

The quality assurance programme should ensure that the data are fit for the purpose for which they have been collected, i.e. that they satisfy the detection limits and levels of accuracy compatible with the objectives of the monitoring programme.

The laboratories should ensure that the minimum performance criteria are based on an uncertainty of measurement of 50 % or below (k = 2) estimated at the level of relevant environmental quality standards and a limit of quantification equal or below a value of 30 % of the relevant environmental quality standards. For methods of calculating uncertainty refer to ISO/DIS 11352 (2011-03).

Because a Certified Reference Material (CRM) for chlorophyll is not available the laboratories should take part in interlaboratory comparisons on a regular basis. Internal methods should be properly validated. As a routine procedure for controlling systematic errors, the use of control charts is recommended. It is common practice in analytical laboratories to run duplicate analyses at frequent intervals as a means of monitoring the precision of analyses and detecting out-of-control situations in R-charts so called Range (control) charts or Precision charts. This is often done for determinants for which there are no suitable control samples or reference materials available. For chlorophyll analyses it is recommended to run at least one duplicate sample within every batch of samples.

Laboratories carrying out chlorophyll analyses have to establish a quality management system according to EN ISO/IEC 17025. An accreditation by a recognized accreditation authority is recommended. The recommendations of EN 14996 (2006) should be kept in mind.

9. Reporting requirements

Data reporting should be in accordance with the requirements for National Comments and with the latest ICES reporting formats, together with information on methods used, the limit of quantification, the uncertainty value, the method of calculating uncertainty and any other comments or information relevant to an ultimate assessment of the data.

10. References

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