Manual for Marine Monitoring in the

COMBINE

Programme of HELCOM

Part B

General guidelines on quality assurance for monitoring in the Baltic Sea

Annex B-9
Technical note on the determination of nutrients

ANNEX B-9 TECHNICAL NOTE ON THE DETERMINATION OF NUTRIENTS

The commonly designated nutrients are inorganic nitrogen compounds (NO_3^-, NO_2^-, NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4^{3-}). Total phosphorus (P_{tot}) and total nitrogen (N_{tot}) are also included because of their importance in relation to ecosystem analysis and budgets.

Nutrients in sea water are considered trace determinands and their analysis is liable to various sources of contamination. Sea water for nutrient analysis is usually collected from research vessels or ships of opportunity (e.g., ferry boats, fishing boats, coast guard or navy vessels). The reference method for measuring nutrients in the Baltic Sea (including storage and pretreatment) is Grasshoff (1976) 'Methods of Seawater Analysis'.

1. SAMPLE HANDLING

Special attention must be paid to possible nutrient sample contamination generated by the ship. Wastewater discharged from wash basins, showers and toilets contains significant amounts of phosphorus and nitrogen compounds and, therefore, can contaminate surface waters to be sampled. For this reason, the water sampler must be deployed far from wastewater outlets, even if no sewage is discharged at the time of sampling. Although most modern ships are equipped with special sewage tanks, they are often emptied at sea owing to a lack of appropriate reception facilities in ports. In addition, there are potential problems with kitchen garbage.

Mixing by the ship's propeller can disturb the natural distribution of the determinands in the surface layer, particularly as regards oxygen. These problems, including the exact location of the ship, should be considered along with the natural variability.

Phosphorus and nitrogen compounds are secreted from human skin. However, touching of the sampler and the sample bottles by hands does not cause problems unless the sample comes into contact with the outer surface of the sampler or sample bottle. This is something that should never happen since the outer

surfaces cannot be kept free of contamination on-board a ship. In view of the potential for contamination, the analyst should preferably supervise the collection of samples. The attaching of bottles to a hydrowire or the preparation of a rosette and the subsequent removal and transport of samples to the ship's laboratory should be done by trained personnel.

The written instructions for the collection of samples should include the precautions to be taken when a sub-sample is transferred to the storage container. The instructions must include the details of the essential record of the sample: station location, station code, depth of sampling, date, time, etc., and the identity of the person responsible for sampling.

2. STORAGE OF SAMPLES

The stability of nutrients in seawater samples depends strongly on the season and the location from which the samples were taken. Nutrients in seawater samples are generally unstable. Grasshoff (1976) recommends that ammonia and nitrite are measured no later than one hour after sampling. Samples for nitrate, phosphate and silicate should preferably be analysed within six hours after sampling, and no later than ten hours. If for practical reasons samples cannot be analysed within these time limits, the corresponding data should be flagged if stored in databases, unless the storage method has been validated.

Samples should be stored protected from light and refrigerated. Plastic bottles must be used if silicate is measured. New sample bottles sometimes adsorb nutrients onto their walls. The new bottles, if necessary, should be cleaned with phosphate-free detergent, rinsed generously with distilled/deionized water and left filled with sea water containing nutrients for a few days. Then checks for adsorption of nutrients onto the walls or losses due to transformation to another chemical form should be carried out. Sample bottles should always be rinsed with the seawater sample from the sampler before they are filled. As regards ammonia determination, glassware for ammonia should always be cleaned with dilute hydrochloric acid.

If samples cannot be analysed within the above-mentioned time limits, the following methods of storage can be recommended.

Silicate	0-4 ^{o}C protected from light. **Do** not **freeze (polymerization may occur).**
Nitrite	Freezing or 0-4 ^{o}C protected from light. Do **not** acidify (rapid decomposition).
Ammonia	No known preservation methods are applicable.
Nitrate	Freezing.
Total nitrogen	Freezing or 0-4 ^{o}C protected from light. Do **not** acidify (enhanced risk of contamination).
Phosphate	Freezing or acidification.

Silicate	0-4 °C protected from light. **Do** not **freeze (polymerization may occur).**
Total phosphorus	Freezing or acidification with sulphuric acid with storage at 0-4 °C protected from light.

Addition of mercury or chloroform are alternative preservation methods for all nutrients except ammonia. These chemicals can affect the reaction kinetics, especially with automated methods, and this effect should be evaluated by the laboratory. The same chemical preservation of calibrants and quality controls can compensate for this effect. The use of mercury should be minimized and optimum disposal procedures should be ensured.

These preservation methods are all second choice to immediate analysis. They should, as mentioned, be validated by each laboratory, taking into account the concentration levels, storage time and environment, differences in sample matrices, and the analytical method of the laboratory.

Since no preservation method for nutrients can, at present, be recommended for general use, each laboratory must validate its storage methods for each nutrient before they are used routinely.

3. SAMPLE PRETREATMENT

Sea water contains microorganisms and other suspended matter of different composition. In some cases, these particles bias the measurement of the determinand in the soluble phase. The suspended matter can be removed either by filtration or centrifugation. Unnecessary manipulation of the sample should be avoided, but in particle-rich waters (e.g., coastal waters, during plankton blooms) filtration or centrifugation may become necessary. It is important that the procedure used for filtration/centrifugation has been validated.

For removing algae from the water sample, a GF/C filter is adequate. For work in open oceans with low concentrations of suspended matter, GF/F filters are considered suitable for suspended matter separation from open sea water. Filtration in closed systems with a neutral gas is recommended. Centrifugation is especially advisable for samples destined for ammonia determination.

If a sample containing particles is not filtered, the turbidity causes light scattering which can bias a colorimetric measurement. In this case, a turbidity blank should be carried out by measuring light absorption of the sample before adding the colour-forming reagents.

4. APPROPRIATE CHEMICAL ANALYTICAL METHODS

The choice of an analytical method should be based on the following criteria:

• the method should measure the desired constituent, i.e., be adequately specific, with accuracy sufficient to meet the data needs in the presence of interferences normally encountered in natural samples;
• the method should be sufficiently simple and rapid to permit routine use for the examination of large numbers of samples.

The reference methods used for manual nutrient measurements are described by Grasshoff (1976). Any changes to the reference methodology should be validated before use for routine work (see Annex C).

Apart from manual methods, various automated methods are in use, including different types of continuous flow analysis (CFA, steady state mode, and peak mode) or flow injection analysis (FIA or Reverse Flow Injection). The analyst has to be aware of the effects of the different analytical conditions in automated analysis which might affect accuracy.

5. CALIBRATION AND THE BLANK

Stock standard solutions should be prepared separately for each determinand using analytical grade reagents that can be pretreated to a precise stochiometric composition, e.g., by drying excess moisture. Reagents containing crystal water should be dried at a sufficiently low temperature in order not to remove the crystal water (the drying temperature is compound dependent). Stock standard solutions containing more than 1 mM are stable for long periods (up to one year refrigerated), but working calibration solutions must be prepared daily and used within hours of preparation.

Blank sea water may be prepared from a bulk sample of offshore surface sea water collected in summer, when the nutrients are at low or below-detection concentrations (Kirkwood, 1994). Blank sea water and reagents totally devoid of nutrients are, however, difficult to achieve, especially regarding the content of ammonia. Optimum handling precautions should be taken to minimize the content of nutrients to below approximately 10% of the measuring range. The concentrations of nutrients in the blank and reagents can be assessed by the standard addition method.

For ammonia analysis, the salinity of the samples affects the reaction kinetics, mainly due to the buffer effect of marine water, that results in a sub-optimum end pH. This effect can give biased results, especially with kinetically dependent automated methods. In the Baltic Sea, the salinity ranges from approximately 0 to 30, and therefore the size of this bias will be variable. This kinetic effect should be checked by standard addition, or by checking the pH of the reagent-sample mixture, which should be in the range between 10.5 and 11.

Whenever compensation for this bias is deemed necessary, one of the following methods is suggested:

- If all samples have the same salinity, calibrate using the addition of calibrants to one of the samples. In some situations, low-nutrient sea water can be prepared by aging and filtering natural sea water (as mentioned above).

- Empirical correction in accordance with the measured sample salinity or pH value.

For all photometric nutrient measurements differences in light refraction, caused by differences in the salt concentration, can give rise to shifts in blank/baseline values, especially in light-measuring cells with round windows. This can be compensated by using blanks and calibrants of the same salt concentration as the samples.

Particles can give rise to light-scattering effects that result in interferences in all photometric nutrient analyses. This bias can be avoided by measuring the sample before addition of the colour reagent, or by filtration or centrifugation where this does not cause contamination.

6. REFERENCES

Grasshoff, K. 1976. Methods of seawater analysis. Verlag Chemie, Weinheim, New York.

Kirkwood, D. 1994. Nutrients: Practical notes on their determination in seawater. *In* ICES/HELCOM Workshop on Quality Assurance of Chemical Analytical Procedures for the Baltic Monitoring Programme. Ed. by G. Topping and U. Harms. Baltic Sea Environment Proceedings No. 58: 23-47.