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-10

Chemical analysis of anoxic waters

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ANNEX B-10 CHEMICAL ANALYSIS OF ANOXIC WATERS

1. INTRODUCTION

Anoxic seawater is generally found in enclosed areas with restricted water exchange. In most cases, a physical barrier (sill) as well as a pronounced density stratification will prevent oxygen from reaching the deeper parts of the sea area. Anoxic conditions will occur if the rate of oxidation of organic matter by bacteria is greater than the supply of oxygen. Anoxic waters are a natural phenomenon (Richards, 1965; Sarmiento *et al*., 1988), and anoxic waters have occurred during the geological history of the Baltic Sea (Jerbo, 1972; Hallberg, 1974). Recently, there have been some indications that eutrophication has increased the extent of the anoxic areas in, e.g., the Baltic Sea. Primary factors promoting anoxic conditions are stagnant conditions and density stratification (Gerlach, 1994).

Anoxic conditions result from several factors, for example, stagnation periods, inputs of organic material, and strong thermoclines. The bacterial production of sulphide starts in the sediments, where the bacteria find suitable substrates, and then expands into the water column.

When oxygen is depleted in a basin, bacteria first turn to the second-best electron acceptor, which in sea water is nitrate. Denitrification occurs, and the nitrate will be consumed rather rapidly. After reducing some other minor elements, the bacteria will turn to sulphate. The reduction of sulphate occurs according to the reaction:

$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 53\ SO_4^{2-}\ 53\ CO_2 + 53\ HCO_3^- + 53\ HS^- + 16\ NH_3 + 53\ H_2O + H_3PO_4$

If anoxic sea water becomes reoxygenized, sulphides will be oxidized to sulphate according to:

$HS^- + 2\ O_2\ HSO_4^-$

2. EXPERIMENTAL PROBLEMS ENCOUNTERED

2.1 HYDROGEN SULPHIDE

No ideal method for the determination of hydrogen sulphide in sea water exists today. The presently most widespread method, which is based on the formation of methylene blue and spectrophotometric measurement, although robust and simple to perform in the field, suffers from several weaknesses. The calibration of the reagents is an elaborate procedure requiring, among other things, the availability of oxygen-free water. Another obstacle is that Na_2S, which is used as the sulphide source in the calibration, is not available as a water-free compound of pro analysi quality. Furthermore, the stock and working solutions of sulphide made up for the calibration are extremely unstable, and the working solution will change concentration substantially in a short time (1-2 hours). Sulphides that are commercially available in the pure form generally suffer from extremely low water solubility, and thus are not suitable for this kind of work.

Sampling is carried out using the same technique as for oxygen, and thus is not a general problem for the trained marine scientist. If the samples will not be measured within acceptable time limits, they are generally preserved with zinc acetate (to form zinc sulphide) prior to analysis. The relatively poor precision of the method, often 5-10 %, could probably be attributed to the combined effects of all steps in the sampling and sample pretreatment procedure.

Validation of results is very difficult, since there are no certified reference materials (CRMs) available for sulphide in sea water. The parameter is very rarely included in interlaboratory comparison exercises, mainly due to problems in withdrawing multiple samples with the same sulphide concentration from one sample container.

Very high concentrations of sulphide in certain unusually stagnant areas will cause problems. In some cases, the absorption of the sample will lie outside the working range of the spectrophotometer. Dilution of the sample is possible, but will undoubtedly introduce more uncertainty into the measurement.

2.2 OXYGEN

In cases where sensors are used for measuring the oxygen content of the water column, anoxic layers will poison the sond and quickly deteriorate its performance. The best way to avoid this is not to lower the sond into any anoxic water layers, which will make it rather impractical in many areas of the Baltic Sea.

2.3 SALINITY

The combined effect of mineralization of organic matter and accumulation of nutrients may cause a shift in the salinity measurements by conductivity by no more than 0.02 PSU (Grasshoff, 1975). This difference is caused by differences in ionic composition between the sample and the standard sea water used for calibrating the salinometer. Practical problems may occur, possibly due to particles in the water, causing a certain instability in the conductivity reading. After running a series of anoxic samples, the salinometer has to be rinsed carefully with deionized water and ethanol.

2.4 NUTRIENTS

Of the inorganic nutrients, phosphate is the compound giving rise to special problems in anoxic waters. Both the natural turbidity of anoxic samples and the influence of the sulphide present on the colour reaction may cause biased results or results of low precision. The reduction of the phosphomolybdenum complex to the blue complex is catalysed by antimony. Sulphide could react with the antimony ions to form a yellow-greenish turbidity, which disturbs the photometric measurement (Nehring, 1994). In addition, colloidal sulphur may be formed when the acid molybdate reagent is added (Grasshoff *et al*, 1983). These problems can be overcome by removing the sulphide by oxidation with bromine or degassing under a stream of nitrogen. The wavelength at maximum absorbance of the colour complex also coincides with strong absorption caused by turbidity. The safety limit of interferences has previously been reported to be 2 mg/l of sulphide for phosphorus and ammonia.

In anoxic waters, nitrate will be reduced to ammonia, disappearing rapidly as the oxygen disappears. The presence of small amounts of nitrate in anoxic waters is possible, but only in layers influenced by rapid mixing with overlying water masses (Grasshoff, 1975). In order to find measurable quantities of nitrate in these waters, the speed of mixing has to be higher than the speed of denitrification of the nitrate. The presence of nitrate in anoxic waters should otherwise be treated with care, since it is probably a result of oxidation of ammonia in the sample upon contact with the atmosphere when sampling.

Nitrite is normally not present in detectable amounts in anoxic waters, as it has been reduced to ammonia. However, nitrite has been observed in the presence of large quantities of ammonia in anoxic waters, possibly as a result of rapid oxidation upon contact with the atmosphere. Sulphide has been reported to interfere with the nitrite measurements, and should (if possible) be removed from the sample.

Ammonia accumulates in the anoxic water and remains fairly stable. The oxidizer for the development of the indophenol blue, hypochlorite, is partly consumed by the oxidation of sulphide. It may thus be necessary to increase the amount of hypochlorite added to the sample in strongly sulphidic waters (Nehring, 1994). In all particle-rich waters, including anoxic waters, it is necessary to measure and subtract the seawater blank.

Silicate accumulates in stagnant waters, and the high concentrations make the determination less sensitive to interferences. Sulphide concentrations up to approximately 150 µmol/l will not affect the formation of the colour complex for the determination of silicate, even if the silicomolybdic acid may partly be reduced. At higher concentrations, it may be advisable to remove the sulphide or to dilute the sample.

2.5 TOTAL PHOSPHORUS AND NITROGEN

The hydrogen sulphide is oxidized to elemental sulphur or sulphate by the oxidation reagent used in the analysis of total phosphorus and nitrogen and thus does not interfere directly. In extreme cases, with extraordinarily high sulphide concentrations, all of the sulphide may not be oxidized and may possibly create a problem (see Section I.2.4, above). A high particle content may, as for inorganic phosphorus, give rise to blank problems. In the analysis of total phosphorus, the oxidation and hydrolysis of phosphorus compounds may not be complete, especially when both nitrogen and phosphorus compounds are combusted simultaneously in alkaline media (cf. Koroleff, 1983). It has furthermore been demonstrated that the oxidation of organic phosphorus compounds using potassium peroxodisulphate ($K_2S_2O_8$) is an unsuitable method in the presence of dissolved iron, possibly due to the formation of iron(III) phosphate during the oxidation process (Ichinose *et al*., 1984).

2.6 ORGANIC CARBON AND NITROGEN

The most modern technique for determining the levels of particulate organic carbon or nitrogen (POC/PON) in sea water starts with filtration of the water through 0.45 µm filters. The filters, with their content of particulate matter, are combusted in an oxygen-rich atmosphere to produce gaseous CO_2 and NO_2. The analysis is very straightforward and robust, and there seem to be no problems (theoretical or practical) involved in the analysis of samples originating from anoxic waters. The samples are characterized by high levels of POC/PON, since anoxic waters are rich in particles, detritus, and other non-living organic material.

2.7 HALOGENATED ORGANIC CONTAMINANTS

The methods used for the determination of halogenated organic contaminants in water are based on extraction of the contaminants from the sea water matrix followed by gas chromatographic separation and some kind of detection. For work in the open sea, the electron capture detector (ECD) is the preferred choice due to its selectivity and sensitivity. The electron capture detector is very selective towards elements with large electron-capturing capability, for example, the halogens. However, the detector also has a certain response towards oxygen and sulphur, and will thus be disturbed by the occurrence of compounds containing these elements. This may give rise to great difficulties in detecting and quantifying, in particular, volatile halogenated compounds in anoxic waters (Krysell *et al*., 1994).

Anoxic environments will cause a breakdown of many halogenated compounds, complicating the distribution patterns and lowering their concentrations. Chlorophenolic compounds have been shown to dehalogenate in anoxic sediments (Abrahamsson and Klick, 1989) and the breakdown of carbon tetrachloride has been observed in anoxic waters (Krysell *et al*., 1994; Tanhua *et al*., 1996).

2.8 METALLIC TRACE ELEMENTS

The concentrations of certain metal ions, most importantly copper (Cu), lead (Pb) and zinc (Zn), and to some extent cadmium (Cd), decrease rapidly in anoxic waters due to the low solubility of their corresponding sulphides. The relatively lower concentrations that follow cause problems mainly when it comes to the correction for blanks, since the blanks become disproportionately high.

In basins with very high sulphide concentrations, elemental sulphur may under some circumstances cause problems in the analysis, since it will be extracted into the same fraction as the metals.

Methods involving ion exchangers for sample work-up and concentration may give a very low yield unless the strength of the ion exchange resin can match that of the strongly bound metal sulphides.

2.9 PH

Electrode deterioration may occur, because in sulphidic waters sulphide will react with the Ag/AgCl electrode, considerably shortening the lifetime of the electrode.

2.10 ALKALINITY

There are no experimental problems, but anoxic waters contain an organic fraction which contributes to the alkalinity. The nature of this organic fraction is still under discussion; it has been suggested that it consists of amino acids or humic substances. Since the true nature of the organic fraction has not been determined, there are still doubts about how it fits into the definition of alkalinity and how the data should be treated and normalized. When determining alkalinity in sulphidic waters, it is more reliable to use a titration method with an indicator because sulphide will react with the Ag/AgCl electrode used in potentiometric titration.

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